Compiled Request for Information (RFI): Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research

Guide Notice Number: NOT-OD-23-140

June 12, 2023 – September 5, 2023

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Submit date: 6/13/2023

I am responding to this RFI: On behalf of myself

Name: Anthony Grace

Name of Organization: University of Pittsburgh

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   The current focus on methodology in my opinion is hurting scientific discovery. Emphasis on novel methods in study sections that reinvent the wheel to the detriment of novel exciting ideas using standard methodology is preventing discoveries that can lead to better disease understanding and treatment.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The current focus on methodology in my opinion is hurting scientific discovery. Emphasis on novel methods in study sections that reinvent the wheel to the detriment of novel exciting ideas using standard methodology is preventing discoveries that can lead to better disease understanding and treatment.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   The current focus on methodology in my opinion is hurting scientific discovery. Emphasis on novel methods in study sections that reinvent the wheel to the detriment of novel exciting ideas using standard methodology is preventing discoveries that can lead to better disease understanding and treatment.
   There needs to be a balance between novel methods and new approaches to discovery; otherwise we will be lost in a technological wasteland.

Submit date: 6/13/2023

I am responding to this RFI: On behalf of myself

Name: Christina M Williams

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Iowa is a joke

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Iowa is a joke

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Iowa is a joke

Submit date: 6/15/2023

I am responding to this RFI: On behalf of myself

Name: Thierry DECELLE

Name of Organization: DCL Solutions

Type of Organization: Other

Type of Organization-Other: International consultancy

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Methods are complementary. It's vital to use any models to address a scientific question, not to oppose methods. NAMs are not alternative. They are scientific methods. They have been used for decades; now the technology developments accelerate the availability of new models.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Qualification of methods is key, which precludes that the model is well-defined and stabilized.
   In 90% of cases, there is no need for validation. Furthermore, comparison between in vivo models and NAMs is not relevant as the read-outs are different, the pathways are different... Looking for replacement is not the way science moves.
   After science, the most limiting factor is the change acceptance. Scientists are very conservationists and should be challenged about the value of their scientific models (the ones they have used for decades).

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   3 thoughts:
   - The starting point is about the posture and attitude of scientists. Very conservative, not open to changes. Eg, the shortage of primates is a chance to think about models in a different manner and look for alternatives to limit the use of primates to the key questions. The current response to the shortage is how to get money to set our own breeding colonies, how to secure the supply chain, to increase the use of feral monkeys...
   - A fair assessment of all methods (including vivo) is required. A model remains a model, ie not 100% of the real world. Knowing the limits and the pros is key to select the best model.
   - Inclusion of rodents in the Animal Welfare Act will put more pressure on biomedical centres which use only rodents and could accelerate NAMs.

Email: thierry@dcl-solutions.com

Submit date: 6/19/2023

I am responding to this RFI: On behalf of myself

Name: Heather Patisaul

Name of Organization: NC State University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   I suggest caution. Particularly in toxicology there is a movement to move to NAMS simply for the sake of using NAMS and minimizing animals in animal research. Although humane use of animals is a laudable goal, the rush to NAMS risks compromising the best available science and, in toxicology at least, making our children the animals upon we then experiment upon and risk our future health. The systems are nowhere near ready to model the brain-gut axis, the fetal-placental axis, and similar systems with even the most basic of functional parallels. Development of these systems should be encouraged and supported, but short of creating real embryos in a dish from stem cells (which people are now close to doing) how are these highly artificial constructs going to model the full complexity of a real biological system? It's tricky. Plus I have seen instances where NAMS can be manipulated to yield the desired result. There need to be guard rails. And they need to model both sexes, multiple ages, multiple genetic backgrounds, etc.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   They need to be validated. Most have not. And they shouldn't be proprietary. Science needs to know how they work. There needs to be a framework for their proper use so they are not misused or extended beyond the purpose for which they were designed.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   There needs to be convincing evidence that they are truly modeling real aspects of human biology. Reporting guidelines for their development and use, akin to the ARRIVE guidelines for animal studies, need to be created and their use enforced. There needs to be greater transparency about their strengths and limitations. They should model biological variability rather than seeking to minimize all forms of variability to the degree possible. The newly published NAS Report on NAMS should be used as a guide.

Submit date: 6/20/2023

I am responding to this RFI: On behalf of myself

Name: Sungatek Oh

Name of Organization: Johns Hopkins University School of medicine

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   An equivalence or Comparability test should be provided.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Prepare the quality control standard to compare or normalize the replicability, reproducibility and reliability.
   There is a lot of variance between samples, including technical variation.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   An explicit standard protocol should be established.

Description: I am happy to discuss about this. please feel free to contact me.

Email: soh52@jhmi.edu

Submit date: 6/21/2023

I am responding to this RFI: On behalf of myself

Name: Pranjali Dhawal

Type of Organization: Not applicable

Role: Select one

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   The novel alternative methods, which are easier to use and have significance in early decision-making, cannot fully replicate the complexity of processes and responses found in living organisms. Nevertheless, these methods, known as NAMs, offer the advantage of quick data acquisition and ensure reproducibility. In addition to their applications in biomedical research, NAMs are also utilized for testing cosmetic raw materials and finished products. Numerous patented models and processes, labeled as NAMs, are now emerging in the market. By studying the interactions of human tissues, biochemical processes, and signaling in vitro, these dynamic models serve as valuable tools for detecting and selecting potential pharmaceutical or cosmeceutical agents in the initial stages.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The factors typically important for limiting the integration of NAMs across research, I believe are, the type of cells/biomolecules utilized for developing these models. Geographical variability and cultural differences have an effect on human physiology, behavioral patterns, and response to particular diseases. For example, in cosmetic research, many of these models are made of one or two skin types making it difficult to apply these models across the globe. NIH can approach national/private laboratories or set up one in various countries to conduct research on tissues/processes/diseases of their population and use that data to create NAMs which are population specific. These variabilities/similarities can further be considered while developing alternative methods that are most applicable for studying diseases or treatments.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Email: dhawal.pranjali5@gmail.com

Submit date: 6/26/2023

I am responding to this RFI: On behalf of myself

Name: Nolan Shaffer

Name of Organization: University of Chicago

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Submit date: 6/26/2023

I am responding to this RFI: On behalf of myself

Name: Risa M Mandell

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Only humans can give consent and only human biological processes can address human variability; moreover, quoting Albert Schweitzer,
   "I am life that wants to live, in the midst of life that wants to live." All beings who metabolize are sentient, including octipuses and fish. Each one is a unique individual with inherent dignity, value, preferences, attachments and relationships. Noble ends, such as curing disease, do not obviate the means by which the end is pursued.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Email: rmm0535@gmail.com

Submit date: 6/26/2023

I am responding to this RFI: On behalf of an organization

Name: Ari Gargir

Name of Organization: RedC Biotech Ltd.

Type of Organization: Biotech pharmaceutical company

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   At this point, the use of animals to test safety or efficacy of our developed product is questionable.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Most likely extensive in vitro testing will teach us more about the product than animal testing.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   I believe that the methodologies have to be tailored to a product or therapy being developed.

Email: ari.gargir@redcbiotech.com

Submit date: 6/26/2023

I am responding to this RFI: On behalf of myself

Name: Susan Fabrican RN

Type of Organization: Not applicable

Role: Select one

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Non-animal methods are superior, provide more accurate information and of course are more humane.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Submit date: 6/27/2023

I am responding to this RFI: On behalf of myself

Name: Shannon Jacobs

Type of Organization: Not applicable

Role: Select one

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   These alternatives could be more valuable to helping humans than animal studies.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Email: sherman1971@yahoo.com

Submit date: 6/28/2023

I am responding to this RFI: On behalf of myself

Name: Rudy J. Richardson

Name of Organization: University of Michigan

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

   1. Past and current alternative methods.

   What we now refer to as “NAMs” have been under development for some time. These alternative approaches include molecular and PBPK modeling, cell and tissue culture, organoids and other 3D culture methods, organs or organisms on a chip technology, high-throughput in vitro screening, and various whole-organism models such as C. elegans, planarians, Drosophila, and zebrafish. Such methods have already enhanced our ability to gain knowledge and understanding about human biology, pathology, and therapeutics. Obvious advantages include the “three Rs” of refinement, reduction, and replacement of vertebrate animal models. In addition, these methods enable considerable savings in time and cost compared to the use of vertebrate animal models.

   2. Progress and limitations.

   When I started out in scientific research, my MD colleagues were reluctant to accept findings pertaining to human disease that had been obtained through the use of even “conventional” animal models rather than from human studies. Now, many of my physician colleagues have embraced models that would seem far removed from humans or human populations. These “unconventional” models include computational molecular modeling, cell and tissue culture, organoids, and non-mammalian organisms such as avian species, C. elegans, planarians, zebrafish, and Drosophila.

   The use of such unconventional models has brought about valuable insights into diseases and therapeutics pertaining to, for example, various neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.

   Limitations include the problem of capturing human variability. However, this shortcoming has applied to conventional animal models as well and can be addressed through the application of modern molecular biology techniques to humanize alternative models and incorporate mutations that give rise to variability. Moreover, by adopting diversified alternative models, we stand a better chance of finding models that mimic various human variants.

   3. Revolutionary prospects.

   The growing list of alternative approaches coupled with high-throughput capabilities is creating an information explosion of unprecedented magnitude. However, the recent advanced in machine learning (ML) and artificial intelligence (AI) make possible the discovery of patterns in the data that elude the human mind. In addition, the application of ML and AI to data analysis can generate new hypotheses for testing.

   Structural biology has already recently witnessed a revolution with the advent of the AlphaFold project, capable of predicting protein structures approaching the accuracy of X-ray diffraction. This

advance needs to be taken further to take into account not only the backbone structures of proteins, but their interactions with ligands, lipids, other proteins, and nucleic acids.
Models that at first might seem highly irrelevant to human disease need to be explored in order to discover why there appears to be a disconnect between the model and human disease so that these differences can be utilized for the development of effective and safe treatments. An example is the use of planarians to model neurodegenerative disease, given that planarians can regenerate a new head following their decapitation. This remarkable regenerative capacity arises from planarians having a substantial population of pluripotent stem cells. Inactivating these stem cells disables regenerative capacity, enabling degenerative processes to be observed. Conversely, preventing the deactivation of the stem cells once again permits regeneration. Thus, here we have a model that initially seemed unsuitable for studying neurodegenerative diseases that can nevertheless be tapped as a potential source of revolutionary insights.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Challenges.
   It can be difficult for researchers to find reliable sources for novel organisms.
   It can be difficult for researchers to find reliable and affordable sources of specialty reagents that might require custom synthesis.
   It can be difficult for non-specialists to integrate technologies across disciplines. For example, a biologist creating large databases might not be equipped to apply AI or ML to find patterns in the data or to generate hypotheses from analyses of the data.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Strategies.
   Provide clearing houses and repositories for easy, reliable, and inexpensive access to novel organisms.
   Provide clearing houses and repositories for easy, reliable, and inexpensive access to specialty reagents and custom syntheses.
   Establish “recruitment and placement” agencies so that researchers in need of colleagues with specialized expertise can locate the people they need for a given project.

Email: rjrich@umich.edu

Submit date: 6/28/2023

I am responding to this RFI: On behalf of myself

Name: Byung Eui Kim

Name of Organization: National Jewish Health

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Organotypic skin (3-dimensional) models could be novel alternative methods to study skin diseases such as atopic dermatitis, psoriasis, contact dermatitis, and skin cancers. We may spare in vivo experiments such as animal and human models by using organotypic skin models with keratinocytes and fibroblasts from subjects with atopic dermatitis, psoriasis, contact dermatitis, wound healing, and skin cancer.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   I and other researchers have published several papers using organotypic skin cultures (PMID: 36609802, PMID: 35189126, PMCID: PMC8021104) to study skin lipid profiles and skin barrier function. We may study various skin diseases including inflammatory skin diseases, wound healing, and skin cancer without limitations such as extreme environmental conditions (high temperatures, toxic chemicals, and pollutants).
   The limitation of the organotypic skin culture model (3D skin) is longevity since the 3D skin can survive up to 4-6 weeks. However, we may potentially increase the lifespan by using stem cells.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   1) We may save animal and human studies by developing standard protocols for 3D skin to study skin diseases and wounds.
   2) We can potentially increase the longevity of 3D skin by using stem cells for keratinocytes, thus allowing us to study the longer-term effects of agents or irritants on keratinocytes and skin
   3) We may develop skin graft models for chronic non-healing wounds using 3D skin models.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/KIM-TRPV1-JACI-2022.pdf

Description: A published paper related to 3D skin model

Email: kimb@njhealth.org

Submit date: 6/28/2023

I am responding to this RFI: On behalf of an organization

Name: Brian Cooley

Name of Organization: Humane Outlook

Type of Organization: Other

Type of Organization-Other: Consultancy

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   My work as a technology journalist has convinced me that the time is here for novel alternative methods to rapidly improve and start delivering results that are better for humans, sooner than we've achieved in the past. Sensor, material, and data techniques have improved radically in the last 10 years.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   This is an aspect of research that is largely dominated inertia and tradition. Leadership by the NIH will give air cover to a wide array of researchers to conduct novel alternative methods of testing rather than using animal tests. This can take decades off the the timeline for improving and perfecting novel alternative methods.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   The techniques that comprise the majority of novel alternative methods have an interesting aspect to them : Some commonality with other areas of advanced technology research, i.e., materials science, machine learning, artificial intelligence. By moving to novel alternative methods, medical research should enjoy a compounding effect by being able to leverage advances in other fields that also use these techniques, as opposed to animal research which is largely a silo that gets little lift from other areas of technology and engineering.

Email: cooleybrian@gmail.com

Submit date: 6/28/2023

I am responding to this RFI: On behalf of myself

Name: Edwin S Monuki

Name of Organization: University of California Irvine

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   I am also a clinician (clinician-scientist) in the neurosciences. Perhaps the broadest and most general challenge I can think of for basic and translational sciences is the visualization/measurement of fluids other than blood (air, lymph, cerebrospinal fluid) to complement solids (tissues/organs).

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Fluid dynamics and the associated universal laws are the domain of physics and mathematical modelling. Thus, for biological fluid visualization, the major challenges are the team science and systems biology requirements.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Submit date: 6/28/2023

I am responding to this RFI: On behalf of myself

Name: Lana Simon

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Please be innovative and use human cell modelling, organs on a chip, 3D printing

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   We need to push for state of the art technology. Stop using animals as their physiology doesn't match humans.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   What is taking so long? Animal studies aren't reliable so move forward with new innovative technology using human cell based approaches, organs on a chip, etc.

Submit date: 6/29/2023

I am responding to this RFI: On behalf of myself

Type of Organization: Not applicable

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   We need to be careful not to put all our eggs in one basket and hope that human cell-based models will be the answer to all biomedical research needs. It is important to look beyond human cell-based models as these alone cannot replace animal research; this is especially true for neurotoxicity and developmental neurotoxicity testing, neurodegenerative diseases, and other neuroactive drug development. Alternative (invertebrate) models are key to filling the gap and providing systems-level information and metabolism. Examples for such complementary models are nematodes, fruit flies, developing zebrafish, and planarians.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Financial and infrastructure support for the development of new NAMs is indispensable for bringing new technologies to fruition and understanding how they fit with other systems. Programs specifically aimed at supporting the development of novel alternative methods that use outside the mainstream approaches are especially important, as these likely face more difficulties competing for standard grants given the current emphasis on human cell based models.
   A major challenge for building in robustness, replicability, reproducibility and reliability of the technologies and the ensuing datasets is that most of the development happens in isolated labs, in parallel, instead of under the umbrella or in collaboration with governmental agencies. Publications and peer review of work are not guarantee for ensuring high quality standards and reproducibility. Different analysis pipelines and data treatment strategies create a patchwork of data that is difficult to integrate across labs and systems.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   The current expectations for the development of a new NAM (especially if it is non-human cell based) are extremely high without sufficient support. This creates a barrier that may block new technologies before they can gain enough traction to be noticed. Small business development, moving NAM systems from academia into industry should be supported through specific programs, as this promises to generate systems that will fulfill the high quality standards necessary for future regulatory applications.

Submit date: 6/30/2023

I am responding to this RFI: On behalf of myself

Name: Zachary Danziger

Name of Organization: Florida International University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   My group is developing two separate NAMs that are unlocking the ability to model elusive dynamics that have impeded basic biological and clinical discoveries.
   The first is a model of an invasive brain-computer interface, a system where electrodes penetrate a (typically severely paralyzed) user's brain and the recorded neural signals are "decoded" into the control of, say, a computer cursor. Designing the decoding algorithms is a bottleneck because the small subject pools limits the experimental rigor that is possible to compare them. Our "human-in-the-loop" model generates synthetic neural data in closed-loop by having healthy (unimplanted) subjects interact with generative computer models, allowing us to evaluate and prototype new decoders. We have validated this method (10.1088/1741-2552/ac97c3) and are releasing the first rigorous decoder comparison study shortly (R01NS109257).
   The second model is purely in silico; however, it merges modern deep learning technology with traditional mathematics. In typical systems physiology there are so many tissues and organs in play that we cannot describe them all and their interactions in sufficient details to accurately simulate the overall behavior. Appealing to AI is problematic because we normally lack enough training data, and even in successful cases the resulting model is uninterpretable and non-generalizable. Our approach is to start with the mathematical biophysics that we understand and only use AI to fill in the gaps, thus constraining the AI approximations and retaining maximum interpretability (critical for computational experiments). We have demonstrated the basic mathematical framework (10.1109/EMBC46164.2021.9631038) and are applying this to computationally develop new nerve stimulation therapies (R01DK133605).

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   If we want to move beyond traditional models, we must engage interdisciplinary teams. It isn't that a computer model expert or an animal model expert don't want to invent new classes of models, it is that they can't undertake the effort with only their expertise. They simply do not know what is feasible in other domains.
   To entice the development and proliferation of NAMs, we must, therefore, demand: 1) That experts in the target system (e.g., a physiologist or clinician) have a fundamental role in (and perhaps even lead) the team that is developing the model. 2) Experiments should be *part of the same project* as the model development, not (only) using a publicly available dataset or borrowing a collaborator's

data. This pushes the group toward well-considered validation and guarantees the model will be scientifically relevant (especially when paired with demand 1).

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Two separate mechanisms could be considered to maximize NAM value. 1) *After* a NAM has been validated, a separate grant mechanism (not during the development project) should be available to support dissemination via developing outwardly accessible software, training, and GUIs. 2) Grant mechanisms allowing or requiring the study of “species translation” should be available. This includes studying explicitly how an important physiological mechanism works in, say, rats and humans *in the same project*, or how a computer model developed using rodent parameters can be morphed in a principled way to represent the corresponding human physiology.

Submit date: 7/7/2023

I am responding to this RFI: On behalf of myself

Name: Asim Ejaz

Name of Organization: University of Pittsburgh

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Our lab is developing a novel full thickness human skin perfusion model as an alternative and a superior model to animals for research applications focusing on skin and adipose tissue. In particular the research application our and collaborators are working actively are melanoma modeling and therapeutics, breast cancer modeling, radiation, chemical, burn, and incisional wounds, skin allergies, cosmetics and skin products development, GVHD, adipose tissue metabolomics, and vaccines development. We believe that the possibility to work with human tissues representing diverse genetic background is the key feature that makes this model not only an alternative rather a superior alternative to animal model and maximizes its scientific utility. In our own experience the adaptation of this model has been revolutionary in understanding the disease state in humans due to the anatomical and physiological relevance.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Based on my personal experience with developing such technologies, I believe that there has to be more awareness to the scientific community about the new technologies and models. Scientific conferences focused on such models across the fields must be organized with promotional benefits. In addition, combined. well defined and distinct funding mechanisms incorporating different NIH institutes should be developed sponsoring different stages of the model development. Special emphasis review panels must be formed with the reviewers getting special training understanding the goals of the funding mechanisms, stage and life cycle of technology development.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   New and well-defined funding mechanisms need to be established to adopt a focused approach at differnt stages of product development and adaptations. NIH institutes must take the initiatives to start incorporating these technologies in internal research and program directors need to take lead in establishing small working groups of the developers, end users and regulatory bodies so that robustness, replicability and reliability issues can be addressed during the development. NIH funding mechanisms need to be designed to address these distinct phases of technology development.

Email: ejaza@upmc.edu

Submit date: 7/12/2023

I am responding to this RFI: On behalf of myself

Name: Randolph Ashton

Name of Organization: Neurosetta

Type of Organization: Biotech pharmaceutical company

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Organoid-based NAMs designed to recapitulate aspects of human CNS development and physiology are not comparable to 'adult' scenarios and are more akin to embryonic tissues. However, this does not mean their utility is limited, as many CNS disorders have neurodevelopmental origins and such NAMs can provide a window into phenotypes that simply cannot not be clinically assessed. For example, Neurosetta has developed a RosetteArray NAM for neurodevelopmental risk assessment that models the earliest stages of neural tube formation, the embryonic structure from which all brain and spinal cord tissues are derived. We are currently marketing this product for general DNT screening, but we are also generating data showing that it can detect Autism Spectrum Disorder, Spina Bifida, and other neurodevelopmental disorder phenotypes. Other's have even used a similar assay to demonstrate a Huntington's disease phenotype, which is a neurodegenerative disorder. Thus, even though organoid-based NAMs may not reach 'adult' levels of maturation, they could still be useful for modeling a disease phenotype and screening for compounds that could revert that disease state. Such molecules may even be useful for preventing the disease state through use as a prophylactic, instead of modern medicine's approach of trying to treat a disease after the pathology is present.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   To catalyze NAM development, areas of human physiology need should be identified, funding should be provided for all aspects of technology development(i.e., discovery, scale-up, validation, translation), and regulatory stakeholders should be gathered under one organization ready to evaluate and integrate technologies as biotech start-up companies are created for the NAMs translation. This framework should be explicit and have a single entry point for regulatory evaluation and adoption.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   The major limitation for using most NAMs as research tools is the complexity of the NAM itself. Funding should be available to take validated NAMs and make them a simplistic, off-the-shelf products for research use. If a NAM cannot be made to fit this purpose, then it should remain an in-house technology of the respective company.

Email: randolph@neurosetta.com

Submit date: 7/14/2023

I am responding to this RFI: On behalf of myself

Name: Albert Folch

Name of Organization: University of Washington

Type of Organization: Not applicable

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Cancer drug testing – a central process in cancer drug development and personalized oncology – is often inaccurate and inefficient because it has traditionally relied on studies in cell cultures or animals that lack the human tumor microenvironment (TME). In the last decade, functional drug testing technologies such as patient-derived organoids (PDOs) (also termed "tumor spheroids") and organs-on-chips (OOCs) have revealed the power and clinical relevance of ex vivo testing of cancer patient tissue. In addition to PDOs and OOCs, several other functional approaches to drug testing have been proposed, each with advantages and limitations. Microdissected tumors ("µDTs" – also termed "ex vivo tumor fragments" or "organotypic multicellular spheroids") have been used since the 1990s to preserve the tumor and its TME (with its native immune cells) within manually-cut small (~1-3 mm-wide) pieces. Implantable or needle microdelivery devices locally deliver small doses of (up to 16) drugs to the tumor in vivo, with maximal preservation of the TME, but issues of tumor accessibility, low throughput, and patient safety limit their applicability. PDX mouse models permit the study of drug responses in an intact organism, however in PDXs most of the TME is from the host mouse and PDX from individual patients grow too slow to inform initial post-operative therapeutic decisions.
   Only 10 years to come to market at a total cost of >$1 billion. To help expedite this vastly inefficient process, Congress recently lifted the requirement of animal testing in pre-clinical studies, however it is difficult to keep up with the pace of progress: immunotherapy clinical trials, especially in combination with other therapies, are exponentially rising in number. Hence more efficient, human TME-preserving drug testing approaches that also deliver high throughput are critically needed to help oncology both keep pace with rapidly evolving treatments and make therapy more affordable.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Microtechnologies such as microfluidics and micromanipulation methods have been proposed to address the bioinspired design and a more physiological, high-throughput control of the TME. Microengineered systems based on patient-derived cells have attempted to provide a more rational approach to the microscale design of the TME for disease modeling and high-throughput drug testing. These microsystems should be sub-divided into those that use a reconstituted TME (PDOs and OOCs) and those that use an intact TME (slices, µDTs, and in-vivo microdelivery). Reconstituted-TME systems offer more versatility for disease modeling (but the faithful replication of in vivo tissues remains a challenge), whereas intact-TME systems offer a high-fidelity approach for drug testing (but engineering certain physiological states can be difficult).

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Reconstituted-TME systems (patient-derived organoids [PDOs] and Organs-on-Chips [OOCs]) offer more versatility for disease modeling, however in these systems the faithful replication of in vivo tissues remains a challenge, mainly the integration of patient-derived immune cells and patient-faithful ECM and microstructures such as vasculature.
   On the other hand, intact-TME systems (slices, micro-dissected tissues [µDTs] such as "cuboids", and in-vivo microdelivery with microneedles) offer a high-fidelity approach for drug testing because they maximally respect the TME. However, in these systems, engineering certain physiological states (for disease modeling) can be challenging.

Email: afolch@uw.edu

Submit date: 7/16/2023

I am responding to this RFI: On behalf of myself

Name: Joseph Wu

Name of Organization: Sttanford University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Technologies such as organ-on-a-chip and engineered tissues, when combined with pluripotent stem cells (PSC)-derived cell types, offer potent tools for mimicking human physiology and disease. A pivotal innovation in this field is the use of CRISPR-Cas9 genome editing to generate isogenic lines from induced PSCs (iPSCs) derived from diverse patient populations. These isogenic lines, genetically identical bar a single specific genetic variation, empower researchers to investigate the pathogenicity of human mutations within a tightly controlled environment. This uncovers the crucial role of genetic diversity in human disease development, progression, and responses to drugs and stressors. This approach is especially vital for understanding rare diseases, which often feature unique, non-conserved mutations that are challenging to accurately replicate in animal models. Similarly, mutations within introns, the non-coding regions of genes that are less conserved and more difficult to model in non-human organisms, can be better understood through this approach. Nevertheless, challenges persist, such as replicating mature human tissues or integrating immune components into these systems, which sometimes necessitate the continued use of whole animal models. Despite these hurdles, the synergistic use of iPSC-derived cells and CRISPR-Cas9 technologies represents a revolutionary pathway to explore the genetic basis of human variability, deepening our understanding of human biology and potentially enhancing health outcomes.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The development and validation of novel alternative method (NAM) technologies, including organ-on-a-chip models with iPSC-derivatives, face numerous challenges in terms of robustness, replicability, reproducibility, and reliability. Among these challenges, the longevity, referring to the duration for which they can be effectively cultured and maintain functionality, is often overlooked. Yet, it is crucial for studying and developing treatments for chronic diseases like diabetes or aging, which require long-term observations. The emphasis on novelty and the development of new platforms in the field often sidelines the need for system sustainability and reproducibility. Addressing this will involve significant research and development investment, not only for generating novel insights but also for understanding and improving the limitations of these technologies, especially their longevity. Enhancements could stem from interdisciplinary collaborations, transparent platforms for sharing methodologies and data, comprehensive training programs, and targeted grant programs. The future of these technologies hinges on the development of standardized protocols and more durable, long-lasting systems, crucial for deepening our understanding of chronic diseases and improving health outcomes.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Maximizing the research value of novel alternative method technologies is contingent on their robustness, replicability, reproducibility, and reliability. Challenges arise from the inherent complexity and variability of biological systems, leading to data heterogeneity and non-reproducible results, and difficulties in standardizing and scaling these technologies across different labs. Enhancing readiness and reliability requires concerted research and development efforts, fostering interdisciplinary collaborations, and early engagement with regulatory bodies. Crucially, transparency in sharing methodologies and datasets is essential, and these should be made publicly available and accessible even to non-bioinformatics experts to ensure broad usability across the scientific community. Barriers to successful integration, including a lack of familiarity and technical expertise within the scientific community, high initial investment requirements, and difficulties in comparing results due to each technology's unique complexities, can be addressed through comprehensive training programs, targeted grant programs, public-private partnerships, and the development of standardized protocols and rigorous validation studies. These efforts aim to facilitate comparability of results across different labs, thereby maximizing the potential of these technologies in advancing our understanding of biological systems and improving health outcomes.

Email: joewu@stanford.edu

Submit date: 7/21/2023

I am responding to this RFI: On behalf of an organization

Name: Joseph Bayana

Name of Organization: Bayana Corp.

Type of Organization: Other

Type of Organization-Other: Women- and Minority-Owned Small Business

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   In artificial intelligence, machine learning, and deep learning (AI, ML, DL), most models are based on so-called generative AI or more specifically Large Language Models (LLMs). Growing acceptance of AI, ML, DL are heralded. However, no actual, factual, real-world, academically substantiated, and reproducible, person-centered big data exists to advance Biomedical Research. Instead, LLMs to advance Biomedical Research are simply based on the limitless permutations and combinations of more than 1.5 million words in the English language, including verbs, adverbs, adjectives, nouns, pronouns, prepositions, conjunctions, articles, et al., that can broadened exponentially by using more than 6,500 other known languages in the world today. Thus, academic journals published to catalyze the development and use of novel alternative methods to advance biomedical research, by using AI, ML, DL, are predominantly based on exchanges of ideas as a result of these limitless permutations and combinations rather than evidence-based, person-centered big data.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   If the objective is catalyzing the development and use of novel alternative methods to Advance Biomedical Research, then the focus should be on harnessing and utilizing the person-centered big data coming from their original, direct, and real-world sources, rather than an over-reliance on the growing body of academic literature made available by LLMs. The goal is to collect, collate, and distribute big data sources that can be used decades or even centuries from now. The biomedical research industry keeps throwing tens of billions of dollars each year on equipment and software that becomes obsolete, based on an interpretation of Moore's Law, after a period of about two years. Evidence-based, person-centered big data are more readily available, and less costly to harvest and curate, than current biomedical research practices and methods.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Strategies for maximizing the research value of novel alternative method technologies should now use the paradigm of big/massive/humungous data, and should avoid LLMs or word search engines on steroids that most academic journals and researchers use nowadays. Compounded big data from LLMs over compounded big data on LLMs, regardless of the scale, will never replace the value of

actual, factual, real-world, academically substantiated, and reproducible, person-centered big data that exists to advance Biomedical Research.

The robustness, replicability, reproducibility and reliability of the technologies and the ensuing datasets of current biomedical research that are based on present-day LLMs will be put into question during the age of big/massive/humungous data. For example, products, parts, and materials used by the National Aeronautics and Space Administration (NASA), including all manufacturers of automobile and aerospace products and materials, can be reverse engineered today. Ironically, using AI, ML, DL in biomedical research cannot be reproduced because the real-world sources are not available. The findings, conclusions, and observations from biomedical research are made available, but inevitably end up as part of LLMs.

From Bayana Corp.'s own research, strategies for bolstering technology readiness and reliability of biomedical technologies can be realized within the indoor environment, where the vast majority of human life is spent. Biomedical researchers continue to ignore the human home, in spite of fact that the highest concentration of actual, factual, real-world, academically substantiated, and reproducible, person-centered big data that exists to advance Biomedical Research are found inside the average human home. Bayana Corp. has identified that big/massive/humungous data is the most significant factor, actually limiting the successful integration of person-centered and big data technologies across research approaches and potential solutions.

Description: Focusing on the Person-Centered Big/Massive/Humungous Biomedical Research Data

Email: sephbay@bayanacorp.com

Submit date: 7/22/2023

I am responding to this RFI: On behalf of myself

Name: Christian Schürch

Name of Organization: University Hospital Tübingen, Germany

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   I use 3D tissue perfusion bioreactor models to study intact human tissue from cancer patients. These models incorporate the full microenvironment of tissue and therefore better recapitulate diseases than "simple" in vitro models or mice (for certain purposes). However, it is difficult to obtain fresh human tissues from surgeries for this research, due to various constraints.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Big challenges are the lack of tissue availability and the diversity of patients to represent different groups of patients. Also, there is lack of funding opportunities, and many grants I submit get rejected because reviewers do not understand the models and their importance.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   One important factor should be the education of clinicians and surgeons (and patients) of the immense value of their tissues, that they should be collected as freshly as possible and processed for different studies (live cell freezing, live tissue culture, xenotransplants, fresh-frozen, RNA later and FFPE, as well as microbiome cultures). Hospitals should have the resources to implement these tissue procurement workflows. Most often, it is structural problems that prevent the implements of these workflows, like long distances between operating room and pathology lab, lack of sterile workbenches in pathology lab, lack of appropriately trained personnel etc.

Email: christian.schuerch@med.uni-tuebingen.de

Submit date: 7/22/2023

I am responding to this RFI: On behalf of an organization

Name: Syed Mukhtar Ahmed

Name of Organization: Greenstone Biosciences, Inc.

Type of Organization: Biotech pharmaceutical company

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

   1. Current Development and Successful Usage:

   Induced pluripotent stem cells (iPSCs): These are cells derived from adult tissues that are genetically reprogrammed to an embryonic stem cell-like state. Their use enables researchers to model human diseases in vitro.

   Features that maximize scientific utility: Ability to generate patient-specific cell lines, potential for disease modeling, drug screening, and personalized medicine.

   2D and 3D models: While traditional 2D cell cultures have been instrumental for biological research, 3D models (like organoids) provide a more in vivo-like environment, mimicking tissue architecture and function.

   Features that maximize scientific utility: Enhanced cell-to-cell interactions, better representation of in vivo conditions, and improved drug response profiling in 3D models.

   Engineered heart tissues (EHTs): These are 3D cardiac tissue constructs that enable the study of cardiac physiology and drug responses.

   Features that maximize scientific utility: Ability to mimic human cardiac physiology, utility in drug testing, and potential in regenerative medicine.

   Microphysiological systems (MPS): Also known as “organs-on-chips”, these are devices that contain cell types arranged to simulate tissue- and organ-level functions.

   Features that maximize scientific utility: High-throughput capability, mimicking organ-level responses, and potential for predicting human-specific responses.

   2. Advancements in Understanding Biological Processes:

   iPSCs: These cells have advanced our understanding of developmental biology and the intricacies of cell fate decisions. They're also pivotal in disease modeling, especially for neurological, cardiovascular, and metabolic disorders.

   Limitations: Concerns related to genomic stability, incomplete reprogramming, and variability among iPSC lines.

   2D and 3D models: While 2D models provide rapid insights, 3D models (like cerebral organoids) have revolutionized our grasp on organ development, tissue regeneration, and disease processes.

   Limitations: Challenges in standardizing 3D culture protocols and ensuring reproducibility.

   EHTs: They've enhanced our understanding of cardiac tissue mechanics, disease processes like cardiomyopathies, and drug-induced cardiotoxicity.

   Limitations: Constructing a completely mature and vascularized cardiac tissue is still challenging.

   MPS: By simulating organ-level functions, they offer insights into organ interactions, pharmacokinetics, and pharmacodynamics.

Limitations: Complexity in integrating multiple organ systems and scaling.
3. Revolutionary Impacts on Human Health:
iPSCs: They hold the promise for regenerative medicine, where patient-specific cells can be used to repair damaged tissues or organs, and in drug discovery, where patient-derived cells can predict drug responses.
2D and 3D models: 3D models, especially, could revolutionize drug testing and toxicology studies, making them more accurate and reducing the need for animal testing.
EHTs: Apart from understanding heart diseases, they can potentially be used in cardiac tissue transplantation.
MPS: By simulating multi-organ interactions, they could greatly enhance our understanding of systemic diseases and provide a platform for precision medicine.
Underserved Areas: Despite these advancements, several areas remain underserved, such as modeling complex diseases like neurodegenerative disorders or systemic diseases, understanding the microbiome's role in health and disease, and studying the aging process at the cellular and system levels.
In summary, while these novel methods provide unparalleled advantages in modeling human biology and diseases, challenges remain. Overcoming these will depend on interdisciplinary collaboration, technological advancements, and continuous validation of these models against human data.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
1. Challenges for Robustness, Replicability, Reproducibility, and Reliability:
Technical Variability: Different labs may use varying protocols, materials, or equipment, leading to inconsistent results.
Biological Variability: Inherent differences between cell lines, especially patient-derived cells like iPSCs, can affect results.
Data Interpretation: Inconsistent methodologies in data analysis or lack of standardized benchmarks can lead to varied interpretations.
Scale-up Challenges: Techniques successful at a small scale may not necessarily yield similar results when scaled up.
Strategies to Address these Challenges:
Standardized Protocols: Encourage the development of universally accepted protocols for creating and maintaining models.
Quality Control: Implement rigorous quality control measures to ensure the consistency of materials, cell lines, and reagents.
Open Science: Promote open access to research methods, raw datasets, and analysis tools to allow for external validation and replication.
Training: Organize workshops and training programs to ensure consistent technical expertise across labs.
2. Strategies for Bolstering Technology Readiness and Reliability:
Collaboration: Foster interdisciplinary collaboration to benefit from a diverse set of expertise.
Iterative Feedback: Encourage a closed feedback loop between researchers and technology developers, ensuring tech developments align with research needs.

Validation: Introduce multi-stage validation processes, using both in vitro and in vivo systems, to ensure technology readiness.
Funding and Grants: Establish dedicated grants for the development and refinement of these technologies.
Regulatory Partnerships: Collaborate with regulatory bodies to ensure that technologies meet safety and efficacy criteria from early stages.
3. Factors Limiting Successful Integration and Potential Solutions:
Cost: High initial investment for some of these technologies can be prohibitive for many labs.
Solution: Provide subsidies or shared facility models to spread out costs.
Complexity: Advanced technologies might require specialized knowledge, potentially hindering their widespread adoption.
Solution: Enhance training programs and simplify user interfaces.
Interoperability: Integration issues can arise when combining novel methods with existing tools or workflows.
Solution: Develop modular and open-source technologies with community input for compatibility.
Skepticism: Some researchers might be hesitant to adopt new methodologies due to doubts about their advantages or applicability.
Solution: Demonstrate the benefits through case studies, publications, and conferences. Engage early adopters as advocates.
In conclusion, while the journey to widespread adoption of novel technologies in biomedical research poses challenges, a strategic and collaborative approach can catalyze their development and validation. Addressing concerns of robustness, reliability, and integration head-on, while also leveraging the strengths of the global research community, will pave the way for these technologies to reshape the future of biomedical research.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
1. Challenges for Robustness, Replicability, Reproducibility, and Reliability:
Complexity of Biological Systems: Biological systems are inherently complex and multi-faceted, which may lead to discrepancies when trying to replicate findings using novel methods.
Variability in Techniques: Different laboratories may adopt varied techniques or protocols, affecting consistency.
Lack of Standards: Absence of unified standards may hamper the consistent use and interpretation of technologies and data.
Resource Limitations: High costs or resource-intensive requirements can hinder the widespread use of novel methods.
Strategies to Address these Challenges:
Consensus-driven Protocols: Engage the community to agree upon and adopt standardized protocols for technology use.
Centralized Resources: Establish centralized repositories or resources where researchers can access validated tools, protocols, and datasets.
Training and Workshops: Promote regular training sessions to ensure researchers are updated with the best practices and technical know-how.

Cross-laboratory Collaborations: Encourage collaborations where multiple labs can validate and reproduce findings using shared methodologies.

2. Strategies for Bolstering Technology Readiness and Reliability:

Pilot Studies: Before wide-scale adoption, conduct comprehensive pilot studies to assess the effectiveness and reliability of technologies.

Peer Review: Engage in rigorous peer review processes to identify potential issues and rectify them before broader implementation.

Feedback Mechanisms: Establish mechanisms for continuous feedback from end-users, enabling iterative improvements.

Engage Industry Partners: Collaboration with industry can accelerate technological refinements and ensure they're tailored to real-world research needs.

3. Factors Limiting Successful Integration and Potential Solutions:

Resistance to Change: Established researchers might be hesitant to adopt novel methods due to comfort with traditional techniques.

Solution: Conduct awareness campaigns showcasing the advantages and successes of the new technologies.

Technical Challenges: Integration of new technologies with existing setups might pose technical challenges.

Solution: Develop plug-and-play or modular systems that are easily integrable with existing infrastructure.

Data Overload: Novel methods might generate vast amounts of data, potentially overwhelming researchers.

Solution: Invest in data management solutions and training programs focused on data interpretation and analysis.

Interdisciplinary Barriers: Some technologies might require expertise from diverse fields, which some labs may lack.

Solution: Promote interdisciplinary collaborations and training to bridge this knowledge gap.

In essence, to maximize the research value of novel alternative method technologies, the scientific community needs to collaboratively address challenges, continuously refine and validate tools, and ensure that they are seamlessly integrable into the broader research landscape. This collaborative approach will ensure that these technologies not only meet the current research needs but also anticipate and evolve according to future demands.

Email: mukhtarahmed@greenstonebio.com

Submit date: 7/23/2023

I am responding to this RFI: On behalf of myself

Name: Edward D. Levin, PhD

Name of Organization: Duke University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   These should be termed complementary models rather than alternative models because their best use is as a complement to the existing animal models which will still be essential for biologic discovery and progress into the future.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   We should develop bridges between different models in our toolbox to enhance the value of all to promote more complete biologic understanding.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   These new models and the integrated organism models complement each other and should be used that way.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/The-need-for-animal-models-Levin.docx

Description: The Need for Animal Models

Email: edlevin@duke.edu

Submit date: 7/24/2023

I am responding to this RFI: On behalf of an organization

Name: Ed Butler

Name of Organization: Rise for Animals

Type of Organization: Other

Type of Organization-Other: Nonprofit Organization

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Please see attached letter

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Please see attached letter

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Please see attached letter

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/2023-07-NIH-Comment-re_-NAMS.pdf

Email: ed@riseforanimals.org

Submit date: 7/26/2023

I am responding to this RFI: On behalf of an organization

Name: Katherine Groff

Name of Organization: PSCI

Type of Organization: Other

Type of Organization-Other: secondary research and funding

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Affinity reagents such as antibodies are essential tools used across biomedical research, and there are opportunities to use modern technologies to improve their quality. A growing concern about the lack of quality and reproducibility of traditional animal-derived antibodies, which often show poor specificity or fail to recognize their targets, is evident in the literature. For this reason and their lack of batch to batch consistency, antibodies have been labeled as a major driver of the 'reproducibility crisis' in research. The development of animal-free, sequence-defined recombinant antibodies presents an opportunity to increase the scientific rigor of NIH-funded research.
   Scientific benefits
   In a 2015 Nature commentary, 111 academic and industry scientists called for an international shift to the use of recombinant antibodies for reasons that include increased reliability and reduced lot-to-lot variability in affinity reagents (Bradbury and Plückthun 2015; see attachment for references). Numerous peer-reviewed publications have highlighted the consequences of using animal-derived antibodies, including generating misleading data that can have considerable public health implications. In fact, a systematic analysis of 185 commercially available hybridoma monoclonal antibodies found that one-third were not reliably monospecific, and the authors recommended replacing the use of animal-derived monoclonal antibodies with sequence-defined recombinant antibodies as a straightforward and cost-effective solution to this serious problem (Bradbury et al. 2018). Another researcher has indicated how research builds upon research using faulty antibodies leading to an avalanche of junk research (Goodman 2018).
   Animal-free recombinant antibodies can be made without batch variation, can be optimized to better perform their intended function, and can be used in all applications in which traditional antibodies are used. They are commercially available and, with appropriate resources, can be developed by researchers in their own laboratories. The numerous scientific advantages of non-animal affinity reagents over animal-derived antibodies include high affinity and specificity, shorter generation time, reduced immunogenicity, the ability to control selection conditions, and the ability to be generated against unstable, toxic, immunosuppressant, and non-immunogenic antigens.
   Economic benefits
   There are no industry-wide standards for antibodies, and the use of faulty antibodies can cost laboratories thousands of dollars a year. An estimated $800 million is spent annually to purchase poorly characterized antibodies, almost half of which is within the domestic market (Bradbury and Plückthun 2015).

The further development and use of non-animal antibodies is an opportunity to address the financial drain of producing and using questionable-quality animal-derived antibodies. In addition, non-animal affinity reagents are much faster to make than animal-derived antibodies. Once an antibody library is established, it takes approximately two to eight weeks to produce a recombinant antibody. In contrast, it takes four or more months to produce an antibody using animals.

2. Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:

"Given the scientific, economic, and ethical benefits of animal-free recombinant antibodies and the ability to rapidly respond to emerging diseases, other countries are moving forward with efforts to transition away from animal-derived antibodies. In 2020, the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM, a division of the European Commission's Joint Research Centre) recommended that "animals should no longer be used for the development and production of antibodies for research regulatory diagnostic and therapeutic applications…EU countries should no longer authorise the development and production of antibodies through animal immunisation where robust legitimate scientific justification is lacking" (European Commission Joint Research Center 2020). Since then, EU member countries have been exploring how to implement this recommendation.

In the US, NIH provides funding for antibodies to be purchased from commercial companies or produced as part of research proposals and through core facility funding. There are multiple pathways to transition to recombinant antibodies.

1. Address antibodies in NIH-funded research. Any existing or newly generated antibody should be detailed in funding applications and be developed and produced using non-animal methods or sequenced so animals are not used moving forward. Robust, legitimate scientific justification should be provided for the use of animals to generate and produce antibodies if it is requested. This justification should include specifics about the researchers' searches for existing non-animal and sequenced antibodies, non-animal antibody production techniques attempted, trouble shooting, experts consulted, and why the approaches tried do not work. See the Appendix for specific questions to address antibodies in applications.

2. Increase funding opportunities for the development and use of non-animal antibodies, creating incentive for their use. This funding may be within existing research grants and be directed to sequence antibodies, to purchase animal-free antibodies, or to develop new antibodies using animal-free methods. Several thousands of animal-derived antibodies exist against the same popular targets. Sequencing existing antibodies is economical and ensures that they are made without batch variations, and without animal use, moving forward.

3. Implement a policy to transition NIH funds away from supporting the development and use of ascites-derived antibodies. The recent EURL ECVAM assessment stated that antibody production using the ascites method should no longer be acceptable under any circumstances. Further, a 1999 NIH commissioned National Research Council report stated that the ascites method was only justified in 3-5% of cases (National Research Council 1999). In the decades since its publication, significant technological advances have been made and a wealth of research has demonstrated that there are no scientific justifications for the use of ascites-derived antibodies.

These steps would help to overcome the propensity for the status quo of using animal-derived antibodies by providing researchers opportunities for more exposure to animal-free antibodies and further developing the infrastructure for animal-free antibody development.

Appendix

Questions to address antibodies in applications

- Did the applicant search for existing or non-animal derived antibodies for their project from third party suppliers to confirm if the antibody was already available? Could an existing antibody be sequenced and converted to recombinant format?

- Did the applicant have access to non-animal derived antibody resources either in their own laboratory or in collaboration with an expert third party, i.e., in vitro display platforms such as phage display libraries?

- What methods did the applicant use to attempt to develop non-animal antibodies for their project, and why were they not successful? Did the applicant provide full characterization and validation of their display platform against a range of targets and evidence that it is of suitable engineered format for the intended application? For each new antigen, did the applicant describe the intended application of the antibody and provide data demonstrating how the library failed? Did the applicant provide a troubleshooting record, such as assessing reagents used during the panning rounds, panning conditions, antigen characteristics and immobilization, and measures taken to improve functional limitations, if relevant?

- Who did the applicant consult about the use of non-animal technologies for the development of antibodies for their project? Did the applicant provide supporting information from an expert in non-animal antibody production demonstrating that an antibody candidate could not be produced using an independent library?

- Did the applicant provide information on measures for quality control and application-relevant validation?

- Does the applicant plan to transform the animal-derived antibody to recombinant format to ensure reproducibility, and if possible, publish or enter the sequence into a public database?

- Could other methods of analysis than an antibody, such as mass spectrometry, PCR, or a reporter assay be used in the project?"

3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/References.pdf

Description: References

Email: katherineg@thepsci.eu

Submit date: 8/1/2023

I am responding to this RFI: On behalf of an organization

Name of Organization: Federation of American Societies for Experimental Biology (FASEB)

Type of Organization: Professional org association

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

Dear Working Group Members,

The Federation of American Societies for Experimental Biology (FASEB) appreciates the opportunity to provide comments on the Request for Information (RFI) (NOT-OD-23-140) regarding the development and use of Novel Alternative Methods (NAMs). As a coalition of 26 member societies across a broad range of scientific disciplines, we recognize the value of developing cutting-edge tools and resources to propel biomedical research forward, including both animal and non-animal models. Considering the rapid pace at which this field is advancing, FASEB appreciates the National Institutes of Health (NIH) Advisory Committee to the Director's (ACD) forward-thinking approach to investing and using NAMs in future biomedical research studies. However, given the many gaps and challenges associated with NAMs—further explained in our comments below—it is essential for the Working Group to emphasize in its final recommendations that animal models remain the premier method for numerous areas of research. Clarifying the research contexts in which NAMs may be appropriate and highlighting their role in supplementing work with animals is an important step in adjusting end-user and public expectations until validation strategies, metrics, and regulatory pathways become more defined.

FASEB has identified three central themes that can aid the Working Group and NIH in outlining future areas of investment:

- Establish uniform validation guidelines and consider parallel investments in validation studies when funding NAMs to ensure new technologies are well-characterized with clear endpoints and metrics.
- Develop and/or endorse NAM-specific reporting and data-sharing guidelines that acknowledge the rigor, reproducibility, and translatability challenges inherent in non-animal models.
- Strengthen partnerships and collaborations between federal agencies, industry, scientific societies, and animal researchers to exchange best practices, minimize regulatory burden, and ensure equitable and feasible implementation.

1a. For NAMs to be effective, it is crucial to have ample evidence demonstrating their validity, replicability, and capacity to accurately reflect human biology and disease. While the field is in its infancy, NAMs have enabled fields such as toxicology to make remarkable advances. For example, the Environmental Protection Agency (EPA) is developing several in silico, in chemico, and in vitro approaches to evaluate skin sensitization, eye irritation, and inhalation risk assessments. Additionally, researchers at the Food and Drug Administration (FDA) recently developed a recirculating, in vitro flow loop system for thrombogenicity testing of medical devices (Sarode & Roy, 2019), an important step forward in mitigating blood clots in patients with blood-contacting medical devices. Common features that allowed these models to proceed through the development

pipeline include sufficient data and testing, clear endpoints, rigorous benchmarking studies, and evaluation metrics.

To maximize scientific utility and achieve regulatory acceptance, continuous collection and evaluation of NAMs data through pilot programs and comparative assessments are essential. Ideally, FASEB recommends this process coincide with regular stakeholder meetings and public comment opportunities to exchange information and assess potential regulatory implications for end-users. Despite the tremendous promise of NAMs, federal agencies recognize that current non-animal systems cannot yet replace all animal studies. As a result, federal agencies like the EPA and FDA frequently launch pilot programs and/or case studies to study the predictivity of certain models before publishing draft policies and risk assessments. Because NAMs are largely in the development phase, this type of flexible and iterative approach is necessary to ensure the best available data informs agency decision-making. Therefore, in formulating next steps for NAMs research, FASEB urges NIH to leverage data collection opportunities (pilot programs, case studies, working groups, etc.) and public comment periods. This level of engagement could facilitate the agency's ability to redirect resources according to the latest science while ensuring that subsequent policy implementation reflects multiple stakeholder perspectives.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

2a-b. One of the primary challenges in building robust, reproducible, and reliable NAMs is the lack of validation data. This problem largely stems from the fact that there is no clear consensus on effective strategies for evaluating NAMs, resulting in the overall absence of uniform guidelines or criteria to conduct validation studies. As NIH outlines future areas of investment, FASEB strongly advises prioritizing the development of validation guidelines before allocating funding toward new technology development. Furthermore, to ensure model development and validation are considered concurrently, we recommend providing equal and parallel funding for validation studies alongside future NAM grants, initiatives, or projects. One potential approach to achieve this goal and reduce experimental bias is collaborating with external organizations to perform independent validation studies. FASEB recognizes that model validation—and scientific confidence more broadly—is a complex, time-consuming, and expensive process that requires standardized metrics, meaningful endpoints, benchmarking studies, as well as consultation with regulatory and public stakeholders. However, without consistent validation guidelines and confirmatory data from human and animal studies alike, the development of NAMs will continue to outpace scientific standards and applications, resulting in inefficient use of research time and federal dollars.

Additionally, data reporting requirements and transparency of results associated with NAMs research remain very limited, creating an additional challenge in establishing robust and reproducible alternatives. To develop safe and efficacious NAMs, the scientific community must have reliable, accessible information demonstrating that alternatives perform as well as or better than traditional animal studies. FASEB supports the recent recommendations from the previous ACD Working Group regarding ways to improve animal research rigor and reproducibility and applauds NIH's recent Guide notice (NOT-OD-23-057) encouraging the use of the ARRIVE Essential 10 in all publications resulting from vertebrate animal and cephalopod research. However, standardized reporting requirements for NAMs have not received equal attention or uptake. General frameworks

and databases such as the Materials Design Analysis Reporting (MDAR) and the European Commission's Tracking System for alternative methods towards Regulatory acceptance (TSAR) represent useful steps forward, but are not broadly used and do not resolve the rigor and reproducibility shortcomings of NAMs.

Therefore, to ensure NAMs are evaluated with the same level of rigor and reproducibility as animal studies, FASEB recommends that NIH partner with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) "Validation Workgroup" to develop validation and reporting strategies for NAMs. The workgroup's charge could include conducting comparative studies with human and animal data (e.g., closest clinical data available) and establishing a set of minimum reporting requirements for publications. Because the current workgroup's scope is limited to toxicology, we recommend working with ICCVAM to expand this effort or create parallel groups focused on NAMs validation and reporting in biomedical research more broadly. Considering NIH's current participation in ICCVAM, FASEB considers this to be a suitable and streamlined approach to push the field forward while acknowledging the current regulatory, validation, and reporting constraints. Recognizing that the current ACD Working Group is developing a landscape analysis of the advantages and disadvantages of various NAMs, we suggest providing this information to the potential new group to inform next steps and ensure harmonization across NAMs-related entities. Finally, this new workgroup may benefit from meeting regularly with industry groups, scientific societies, and other stakeholders to share data, best practices, and conduct independent validation studies.

2c. In addition to the absence of reporting requirements, a major challenge in successfully integrating novel technologies across research approaches is the lack of funding mechanisms available to study model characterization, particularly for novel technologies. To effectively validate NAMs, a thorough understanding of how a model functions is essential. This includes identifying mechanisms of action, scientific context of use, and risk of bias. However, characterization studies are often not considered "fundable" activities, resulting in delays in their widespread use and relevance. As the Working Group articulates high-priority areas for NIH investment, FASEB advises exploring ways to modify current funding opportunities to accommodate this gap in the field or create new funding mechanisms specifically dedicated to NAM characterization.

A second challenge is the considerable time and costs to conduct nonanimal studies. This often varies in different sectors depending on discipline-specific needs and the complexity of the research question. To inform NIH's future investment strategy in NAMs, FASEB recommends conducting cost-effective analyses of proposed technologies with existing methods, including animal studies. Specific aspects to consider through these analyses include time, scalability, and resource efficiency. Not only will the costs associated with scaling NAMs for broad deployment be significant, but in many cases, combinations of multiple NAMs may not necessarily outperform single tests involving animals, further increasing costs. Although such analyses will require substantial time and effort to complete, this information ensures proper stewardship of future federal investments and can facilitate the public's understanding of the current NAMs landscape.

Another factor restricting the integration of NAMs across the biomedical research enterprise is insufficient engagement between regulators, NAM developers, and end-users. While current federal agency efforts are laudable—including the EPA NAMs Work Plan, the FDA Alternative Methods Working Group, ICCVAM, and the National Center for Advancing Translational Sciences (NCATS)—this piecemeal approach prohibits the formation of a cohesive and shared knowledge base.

Therefore, FASEB strongly recommends strengthening interagency partnerships to develop a coordinated NAM approach that enables science to advance efficiently while minimizing administrative and regulatory burden. One early goal to work towards through these partnerships is developing a strategic plan with milestones to align NAM research priorities across the various federal entities. Additionally, we encourage interagency collaborations to leverage the expertise of end-users and professional societies through workshops and comment periods. This is an essential step towards advancing the scientific community's commitment to the 3Rs and promoting an open dialogue about complex research topics.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

3b. To ensure equitable distribution of resources across labs, it is essential to have access to scientific information and adequate support for research staff and infrastructure. As noted earlier, developing minimum reporting standards for NAMs represents a crucial step in improving rigor, reproducibility, and transparency in this field. However, another benefit of an accepted set of reporting standards is improved access and sharing of cross-disciplinary knowledge and expertise. This will enable research labs of all sizes and capacities to prioritize future research topics, acquire appropriate resources, and identify potential collaborators.

Secondly, FASEB recommends NIH provide additional support for research infrastructure, shared resources, and technical staff to promote fair allocation and access to animal and non-animal methodologies. In many cases, particularly for resource-limited institutions, researchers lack the necessary tools and expertise to utilize novel technologies essential to their field and career development. Suggested strategies for addressing this gap include increasing infrastructure grants (G20, C06) to enable institutions to build state-of-the-art facilities and expanding the number of shared technology hubs—such as those funded through the National Institute of General Medical Sciences (NIGMS) National and Regional Resources R24 program—to areas with historically less NIH funding. Specific funding and career development opportunities are needed for core facility and technical staff to ensure scientific expertise keeps pace with rapid technology development, including NAMs. One way to achieve this is expanding opportunities for trainees on F-, K-, and T-grants to facilitate their exposure and training with novel methods. To achieve efficient and equitable technology sharing, FASEB urges NIH to harness the full potential of core facilities, shared resources, and staff scientists. Because non-animal model development and validation are critically dependent on animal studies for the foreseeable future, FASEB considers these recommendations as opportunities to advance animal and non-animal research simultaneously.

3c. Maximizing the translatability of NAMs and reducing inherent biases requires implementing standards for rigor and reproducibility, as well as fostering collaboration across different disciplines. As noted in question 2a-b, FASEB recommends prioritizing the development of uniform guidelines that NAM developers and users can use to support validation studies and evaluate scientific confidence of these models. This effort serves as an opportunity to strengthen collaborations with other federal agencies and appropriately complements our previous recommendations to fund parallel validation studies and create minimum reporting standards. If possible, guidelines should be consistent across federal agencies to streamline NAM development and validation while minimizing end-user administrative burden.

Recent work from ICCVAM and the National Academies of Sciences, Engineering, and Medicine (NASEM) can provide valuable insights to achieve this goal. For example, ICCVAM published a recent framework (van der Zalm, 2022) for establishing scientific confidence in NAMs that outlines five essential elements for determining their adequacy: fitness for purpose, human biological relevance, technical characterization, data integrity and transparency, and independent review. While the authors note that the focus is primarily on pesticides and industrial chemicals, the framework is intended to be adaptable to other fields. As another example, NASEM recently published a report, Building Confidence in New Evidence Streams for Human Health Risk Assessment: Lessons Learned from Laboratory Mammalian Toxicity Tests, that outlines the various barriers to broad deployment of NAMs in EPA-related decision-making. Similar to the ICCVAM framework, the report highlights five components for building scientific confidence in NAMs: intended purpose and context of use, internal validity, external validity, biological and experimental variability, and transparency. The discrepancies between the two documents—as well as other resources— demonstrate the need for interagency partnerships to build unified guidelines with a shared vocabulary, definitions, and objectives that can propel innovation forward.

Finally, to optimize translatability in an evidence-based manner, FASEB recommends NIH develop new mechanisms to foster collaboration between animal researchers and NAM developers. Another recent NASEM report, Nonhuman Primate Models in Biomedical Research, underscores how nonhuman primates (NHPs) remain essential for NIH-supported biomedical research given the lack of qualified and validated NAMs to answer complex research questions. To address this, the report emphasizes the importance of enhanced collaboration between NHP researchers and NAMs developers to expand the applicability of non-animal systems. FASEB concurs with the report's suggested strategies for accomplishing this and encourages NIH to establish multi-laboratory funding opportunities, cross-disciplinary challenge programs, and annual conferences or symposia that mobilizes varying perspectives and expertise. For the latter, we strongly advise partnering with scientific societies to leverage their knowledge and networking capabilities for maximal impact and effective policy implementation.

Conclusion

FASEB appreciates the opportunity to offer comments on strategies for maximizing the development and use of NAMs. Stakeholder feedback is central to sound policymaking. As scientists that strongly support the use of various resources to advance biomedical research, including humane animal studies and non-animal models, we look forward to future engagement opportunities on this topic and the Working Group's final recommendations.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/FASEB-Response_ACD-WG-RFI-NOT-OD-23-140_August-2023.pdf

Description: PDF Copy - FASEB Response (NOT-OD-23-140)

Email: ncharalambakis@faseb.org

Submit date: 8/2/2023

I am responding to this RFI: On behalf of myself

Name: Nigel Yarlett

Name of Organization: Pace University

Type of Organization: University

Type of Organization-Other:

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Hollow fiber technology provides a useful and accessible model to replace animals particularly in the early (phase I) stage of drug development. Cells grown on hollow fibers are biochemically and physiologically representative of 3D tissue because they are polarized possesing a basal attachment surface and an apical surface. A single cell type (eg. intestinal epithelial) or a mixture of cell types (intestinal epithelial, goblet, Paneth, etc) can be cultured on the surface of the fibers (extracapillary space); which in combination with endothelial cells lining the inside of the hollow fiber (intracapillary space) provides a 3D tissue model with tight junctions, that has been used to replace animal models for the culture of intestinal parasites. The improvement in tight junctions using an endothelial cell lining is supported by data obtained using (a) lactulose/mannitol uptake and/or (b) trans-epithelial electrical resistance (TEER). Because these cells are fed by a continuous flow of growth medium that can be sampled or have additions made to it via side ports. This NAM permits the acquisition of preliminary pK/pD and dose-response data for experimental drugs. Since the majority of test compounds do not proceed beyond this analysis, the in vitro HFB model provides a method for streamlining the drug development process limiting animal models to the final stages at which point most drugs do proceed to clinical trials. The utility of this method was recently described by Love and McNamara (Expert Opinion on Drug Discovery 2021, 16(1):59-74) as “The bioreactor system may be a much more physiologically relevant environment in which to study co-cultures of human pathogens such as Cryptosporidium”.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The robustness and reproducibility of the method is maintained by employing a robust set of standards. Briefly these include (but are not limited to) the inoculum of a defined cell number; a defined period post inoculum (based upon the doubling time of the selected cell-line) before the HFB is sampled allowing the host cells to reach a sufficient density prior to use. These parameters can be established for any selected cell line by monitoring changes in glucose metabolism, glucose permeability assay, and TEER measurements. There is a plethora of published pK/pD data in the literature, which can be used as a reference bank for comparison of pK/pD data for established compounds that can be compared to data for the same compound obtained using the HFB.
   A limitation to the HFB is the inability to visualize the cells during disease progression due to the thickness of the cartridge housing the fibers and the opacity of the cartridge case. This can be

overcome by modifying existing cartridges to include a port on one side through which several fibers leave and pass through a flat glass plate that is served with a branch from the intracapillary medium. The flow pressures can be equilibrated with the main cartridge by optimizing the dimeter of the tubing. This would provide a section that can be visualized under the microscope without invasion of the main bioreactor. This addition would expand the capability of the system and permit the use of the bioreactor to study intestinal pathology during disease progression. Such a modification would also benefit use of the HFB to study changes in the interactions that occur in the microbiome during disease progression.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   The HFB has potential for replacing animals in multiple studies. It also provides a unique model to replace current animal invasive studies, facilitating the study of cell-cell interaction during disease progression which is challenging using conventional animal models. Limiting factors are discussed in section 2, as are potential solutions. In summary, the HFB provides a versatile method to produce 3D cultures for the replacement of animal models. Potential uses include: the in vitro study of cancer and other physiological disease states such as obesity and diabetes using immortalized cells, primary cells or stem cells. Some publications demonstrating the potential of this method to replace animals models are attached below.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/1.-Publications-using-HFB-to-replace-Animal-Models.pdf

Description: publications using HFB to replace animal models

Email: nyarlett@pace.edu

Submit date: 8/2/2023

I am responding to this RFI: On behalf of an organization

Name: Xuejun H Parsons

Name of Organization: San Diego Regenerative Medicine Institute

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   The difficulty of crossing "the valley of death" in drug development is the pounding consequence of a vast amount of Federal and private investments only go to maintain the status quo of mainstream biomedical research in the non-human model organisms or systems that do not reflect the complexity of humans, thus have little implications for the prevention and treatment of human diseases. Without a readily accessible and effective human model system to unlock the mysteries of human development and disorders, the road of desperately seeking cures has become all but a dead end to real world remedy. Due to the restriction on human embryonic and fetal materials available for study, there is a fundamental gap in our knowledge regarding the molecular networks and pathways underlying human embryogenesis. As a result, the normal human developmental pathways that generate the enormous diversity of CNS neurons as well as the molecular controls in human embryonic cardiogenesis remain poorly understood. Derivation of pluripotent human embryonic stem cells (hESC) provides a powerful in vitro model system to investigate the molecular controls in human embryonic development as well as an unlimited source to generate the diversity of human cell types across the spectrum of developmental stages for repair. However, the conventional, chaotic, multi-lineage differentiation approaches of pluripotent cells not only do not reflect the well-controlled human embryogenesis in vivo, but also pose a big challenge for characterizing, identifying, and validating developmental-stage- and cell-type-specific functional elements in human embryonic development in a comprehensive manner. Development and utilization of novel hESC models that emulate human tissue and organ formation in vivo will not only provide missing knowledge regarding organogenesis in human embryonic development, but also facilitate rapid progress in identification of molecular and genetic therapeutic targets for the prevention and treatment of human diseases.
   Our technology breakthrough enables neuronal or cardiac lineage-specific differentiation direct from the pluripotent state of hESC with small molecule induction, providing much-needed in vitro model systems for investigating molecular neurogenesis and cardiogenesis in human embryonic development [1-4]. It opens the door for further unveiling genetic and epigenetic programs embedded in the human CNS and heart formation using genome-wide high-throughput high resolution profiling approaches. These studies will not only contribute tremendously to our knowledge regarding molecular embryogenesis in human development, but also allow direct control and modulation of the pluripotent fate of hESC when deriving an unlimited supply of clinically-relevant lineages for therapies.
   The limit capacity of cardiomyocytes of the heart as well as neuron circuitries of the brain/spinal-cord for self-repair constitutes a significant challenge to traditional medicine for tissue and function

restoration in seeking cures for those serious diseases and conditions. To date, the need to restore vital tissue and function for a wide range of incurable or hitherto untreatable neurological and heart diseases remains a daunting challenge to the conventional mode of drug development. Although stem cell therapy represents a promising regenerative medicine approach closest to provide a cure for those diseases, demonstrating stem cell production at the scale and product purity adequate to heal the damaged or lost tissues that have naturally limited capacity for repair, such as the human heart and brain, has been a big challenge for traditional adult stem cell sources or products, including so-called induced pluripotent adult/stem cells (iPSC) that are in fact adult cells reprogrammed with oncogenes or cancer cells harboring oncogenes, and another adult stem cell Ponzi scheme or scam.

In regenerative medicine, human embryonic stem cell (hESC) research holds huge promise for treating major human diseases that have been challenging to traditional medicine. As neurological and cardiovascular diseases incur exorbitant costs on the healthcare system in US and worldwide, there is a strong focus on translating hESC technology innovations to provide potentially life-saving treatments or cures for these major health problems.

The emerging areas of hESC-based regenerative medicine present innovative, more effective therapeutic solutions for many major health problems that have been challenging to traditional drug development, not only having tremendous economy and health impact, but also provide unique opportunities to transform current research, regulatory, and clinical practices. Traditional drug development usually starts with drug leads discovered in non-human simple model organisms, thus requires lengthy and costly both demonstration in animal model testing and establishment of proof-of-concept and safety in human trials. As a result, millions of drug leads have vanished before even reach clinical trials, and for few lucky ones, have encountered the very high drug failure rate in human trials. Among those very few drugs that eventually obtained their market approvals, there were not any cures, or even meaningfully effective treatments, for Alzheimer disease, Parkinson's disease, stroke, spinal cord and brain injuries, heart disease and failure, or a host of other disorders that destroy lives. Unlike traditional R&D, hESC-based therapy products have been developed directly with human cells with proof-of-concept already established in humans, which simplifies the development process, lowers the costs, shortens the time consumption, and increases the probability of clinical success dramatically.

Emerging hESC research breakthrough innovations provide much-needed hESC model systems to bridge the knowledge gap in human embryonic neurogenesis and cardiogenesis, and present hESC as a novel, advanced therapeutic strategy for a wide range of incurable or hitherto untreatable neurological and heart diseases that affect millions and cost billions, thus overcoming the major bottlenecks in the regenerative medicine market, potentially shifting current research and clinical practices and creating new scientific paradigms for CNS and cardiac repair.

Ref: (1) Parsons XH. PluriXcel: Emerging Technologies of Regenerative Medicine. 2016. Book ISBN-13: 978-3-659-95970-7; ISBN-10: 3659959707; EAN: 9783659959707. (2) Parsons XH. Directing pluripotent hESC towards lineage-specific cell therapy derivatives for regenerative medicine. Gene and Cell Therapy: Therapeutic Mechanisms and Strategies, 2015; Chapter 31:795-818, Print ISBN: 978-1-4665-7199-0, eBook ISBN: 978-1-4665-7200-3. (3) Parsons XH. Direct conversion of pluripotent hESC under defined culture conditions into human neuronal or cardiomyocytes cell therapy derivatives. Methods Mol. Biol. 2016;1307:299-318. DOI: 10.1007/7651_2014_69. PMID: 24500898. (4) Parsons XH. Direct conversion of non-functional pluripotent hESC into functional

somatic elements creates scientific paradigms to address key challenges to traditional medicine and biofabrication. Regen. Med. Ther. 2017;1:1-15. DOI: 10.36959/654/389.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

On December 29, 2022, President Biden signed into law the FDA Modernization Act 2.0 that legitimizes alternatives to animal testing for advancing a drug or product to human trials. Enacting such a law for a drastic change in FDA regulation, passed by Congress and welcomed by animal welfare groups, was only made possible by the advancements in hESC research that have begun to offer increasingly viable alternatives to animal testing. Human embryo-originated hESC and their cell/tissue/organ products/models offer viable, adequate, timely, the most cost-effective, and superior alternatives with higher quality and safety standards to animal testing.

In order for a drug to be approved in the US, FDA typically requires toxicity tests on small and large animals. Companies use tens of thousands of animals for such tests each year. Yet more than nine in 10 drugs that enter human clinical trials fail because they are unsafe or ineffective. Organ chips and organoids based on adult stem/cancer cells (e.g., induced pluripotent adult/stem cells [iPSC] that are in fact adult cells reprogrammed with oncogenes or actually cancer cells harboring oncogenes) have also proven to be inadequate as alternatives to animal toxicity testing because the tolerance threshold of adult/cancer cells/iPSC to toxic chemicals/drugs is much higher than the tolerance threshold of normal human tissues or organs. For example, the drugs screened by the liver chips of Emulate based on iPSC/cancer-cells either failed in clinical trials because they were toxic to the liver or were approved for market but then withdrawn or scaled back because of liver damage (see the New York Times report “Could the Next Blockbuster Drug Be Lab-Rat Free?”).

hESC are derived from human embryos, negating the species-species differences observed in animal testing. hESC and their derivatives have very low tolerance threshold and are very sensitive to any toxic chemicals/drugs, providing more timely, predictive, reliable alternative methods to animal testing, ensuring higher quality and safety standards for drugs, reducing the time and cost of drug/product development and accelerating product development.

Our hESC-based novel platforms also provide scale-up capability enabling the creation of human replacement tissue/organ products/models. Future progress will lead to the development and commercialization of multi-cellular 3D human CNS/heart-related models or products [e.g., micro-hearts/brains/spinal-cords], which can be used for rapid and high fidelity safety and efficacy evaluation of human therapeutic candidates, thus leading to advances in technologies used in the regulatory review of medical products; and will be readily adaptable in drug efficacy and toxicity testing; and for commercialization and therapeutic development of replacement tissue and organ products [1-4]. These studies will provide powerful tools to increase the biological complexity of human-based in vitro models and assays to mimic the in vivo structure, behavior, and function of the human CNS/heart, which are controllable, reproducible, and scalable, and can be monitored and validated against responses on multiple hierarchical levels. It will pave the way for further development of cutting-edge automated high-content systems for systematic functional assembly of the in vitro replacement tissues and organs from pluripotent hESC in a 3D setting that reflect the biological complexity, microenvironment niche, and function of the in vivo human organ system, enabling automated high content and high-throughput analysis of CNS or heart circuitry and

dynamics, and systems developmental biology models of the complex human embryonic development.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Emerging hESC research breakthrough innovations provide much-needed hESC model systems to bridge the knowledge gap in human embryonic neurogenesis and cardiogenesis, and present hESC as Innovative Solutions of future medicine, including

Health Solutions:

Human Embryonic Stem Cell (hESC)-based innovative platforms focus on developing Regenerative Medicine Advanced Therapy (RMAT) targeting serious or life-threatening diseases and conditions in unmet medical needs that have been big challenges for traditional medicine, presenting hESC as a novel, advanced therapeutic strategy for a wide range of incurable or hitherto untreatable neurological and heart diseases. hESC-based innovative platforms offer game-changing enabling technologies to provide RMAT products in large quantity and high quality with adequate cellular capacity to regenerate the neuron circuitry and the contractile heart muscle, ensuring high degrees of efficacy and safety of the hESC-derived therapeutic products, thus robust clinical benefit leading to therapies. It not only constitutes clinically representative progresses in both human neuronal and cardiac therapeutic products for treating a wide range of incurable or hitherto untreatable neurological and cardiovascular diseases, but also offers manufacturing innovation for production scale-up and creation of replacement tissue or organ products.

Scalable Solutions:

Mending the Broken Heart: Lack of a scalable human cardiac stem cell source with adequate heart muscle regeneration potential remains a major setback for heart replacement, and fabricating a human heart is still beyond reach. Our innovative hESC Platform enables direct conversion of pluripotent hESC uniformly into a large supply of human cardiac stem or precursor cells for heart replacement or bio-fabrication [patent: USPTO# 9,428,731], providing a practical scalable solution for heart regeneration.

Neuron Circuitry Repair: Due to lack of a scalable human neuron source, the need to restore vital tissue and function for a wide range of neurological diseases remains a daunting challenge to conventional drug development. Our innovative hESC Platform enables direct conversion of pluripotent hESC into a large supply of human neurons for neuron circuitry repair and nerve tissue bio-fabrication [patent: USPTO# 8,716,017], providing a practical scalable solution for CNS regeneration.

Please note hESC research provides a unique opportunity to transform medicine and life sciences. There is no alternative to hESC research, and hESC research breakthroughs do not apply to, do not work with, or cannot be replaced by induced pluripotent adult/stem cells (iPSC) that are in fact adult cells reprogrammed with oncogenes or cancer cells harboring oncogenes, a scientific Ponzi scheme or adult stem cell lie by the Bush administration.

Our hESC breakthrough innovations using small molecule induction at the pluripotent stage of hESC are unique to embryo-derived hESC that are highly acetylated and unmethylated, maintained under the defined culture conditions, sustain epiblast pluripotency, and respond to developmental signals at embryonic/epiblast stage; our hESC technologies do not work for hESC maintained on feeder cells; our hESC technologies do not work for cancer cells or induced pluripotent adult/stem cells

(iPSC) that are in fact reprogrammed from adult cells/different tissues with oncogenes, deacetylated and highly-methylated, and not responding to developmental signals at embryonic/epiblast stage [1-4].

iPSC are in fact cancer cells or adult cells reprogrammed with oncogenes, commonly-known as flawed reprogramming or oncogenesis. It is common knowledge for anyone with a doctor degree in science or medicine (PhD or MD) that iPSC contain oncogenes, have oncogenic potential, are in fact cancer cells, but not stem cells. hESC are called pluripotent stem cells (PSC) because hESC have unlimited differentiation potential. Growing evidence indicates that iPSC do not even have unlimited differentiation potential, the definition of pluripotency, and it is completely false to call such cells PSC. hESC have unlimited differentiation potential, genomic/epigenomic/cell-line homogeneity, highly-acetylated and unmethylated, across all hESC lines. iPSC have different differentiation potential, genomic heterogenicity, cell line variations, because the different tissues they used for reprogramming have different genomic imprints and are highly-methylated that cannot be reversed by genes, causing their cell line variations, genomic heterogenicity, different differentiation potential, and also, most importantly, genomic instability and oncogenic potential. iPSC are not at embryonic stage and do not respond to developmental signals at embryonic stage as hESC do. One essential aspect of stem cells is their long-term genetic stability. Stem cells can maintain long-term, stable growth in culture, while cancer cells grow abnormally crazy and mutate fast. The initial cluster of iPSC papers was published in top scientific journals, such as Nature, Cell, and Science, in lightning speed, or only a few weeks, without any scientific evidence or data to show the long-term genetic stability of iPSC or iPSC could maintain long-term stable growth. And over a decade later, there is still absolutely no scientific data to show the long-term genetic stability of iPSC or iPSC could maintain long-term stable growth. Without the data of long-term genetic stability, the line between stem cells and cancer cells is bleared. In fact, iPSC have been reportedly associated with abnormal gene expression, accelerated aging, and immune-rejection following transplantation owing to introducing foreign oncogenes and instability/abnormality to the adult genome, and serious spontaneous mutations, the sign of cancer cells, have been identified in human iPSC clinical trials.

Email: parsons@sdrmi.org

Submit date: 8/2/2023

I am responding to this RFI: On behalf of myself

Name: Matthew Rand

Name of Organization: University of Rochester

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   I am very supportive of the concept of novel alternative methods to advance biomedical research. I am a little unclear what constitutes "novel" and why there's an emphasis on "novel" when there is in fact much room to optimize conventional alternative methods to serve this purpose. Perhaps "optimize" can equate with "novel" in this regard.
   I advocate the use of the Drosophila model for studies to advance understanding of human biology, circuits, systems and disease. The fly has already proven itself in advancing progress into understanding specific biological processes or human states, with several Nobel laureates able to emphasize this point. Flies hold an exceptional niche for characterizing Gene X Environment interaction, using highly controlled experimental paradigms and executing experiments in relatively short time frame. Where I see this model needing more development is in characterizing responses to environmental exposures and identifying conserved pathways in resistance and susceptibility traits. The fly can be easily "humanized" with introduction of human gene homologs to the fly genome. In short, Drosophila stand ready for more exploitation of NAMs.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The ability to conduct whole animal experiments in a highly controlled experimental system is already in place with Drosophila. This reduces the challenge for establishing robustness and reproducibility. The divergence of some fundamental physiological processes (e.g. flies use glutamate for neurotransmission where acetylcholine is used in vertebrates) bring the need to validate truly homologous molecular events. These limitations are nonetheless, identifiable and should not dissuade use of the model.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Maximizing the research value of Drosophila for NAMs will require a concerted and coordinated effort, by several investigators, to carry out characterization of numerous fundamental G X E experimental conditions. This will lead to a database to define core pathways that underlie conserved susceptibility or resistance profile in flies and people.

Email: matthew_rand@urmc.rochester.edu

Submit date: 8/4/2023

I am responding to this RFI: On behalf of an organization

Name: Diana Navon

Name of Organization: Better Science Campaign

Type of Organization: Other

Type of Organization-Other: Non Profit

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Currently Developed and Used Successfully:
   Organ-on-a-chip Technology: Organ-on-a-chip platforms are miniaturized devices that mimic the structure and function of human organs, allowing researchers to study biological processes in a more physiologically relevant environment. These systems have shown success in modeling diseases and drug responses, offering a more accurate representation of human biology compared to traditional cell culture methods.
   Single-Cell Sequencing: Single-cell sequencing enables the analysis of individual cells within complex tissues, providing insights into cellular heterogeneity and gene expression patterns. This method has been instrumental in understanding cell types, developmental processes, and disease mechanisms at the single-cell level.
   Machine Learning and AI: Machine learning and artificial intelligence are revolutionizing biomedical research by analyzing large datasets, identifying patterns, and making predictions. These methods have been used successfully in medical imaging analysis, drug discovery, and personalized medicine.
   Multi-Omics Integration: Integrating data from multiple omics levels (genomics, transcriptomics, proteomics, etc.) allows for a more comprehensive understanding of biological processes. It has been instrumental in identifying biomarkers, understanding disease mechanisms, and developing targeted therapies.
   Maximizing Scientific Utility:
   Data Sharing and Open Science: To maximize the utility of novel alternative methods, researchers should embrace open science principles and share their data, methodologies, and findings with the scientific community. This fosters collaboration, reproducibility, and accelerates progress.
   Benchmarking and Standardization: Establishing benchmarks and standardized protocols for novel alternative methods is crucial to ensure consistency and reliability across studies. This facilitates comparisons between different research groups and increases the overall scientific utility of these approaches.
   Collaborative Networks: Building collaborative networks that involve researchers from different disciplines and institutions can accelerate the development and application of novel alternative methods. Such networks foster knowledge exchange and provide access to diverse expertise and resources.
   Advancing Progress into Understanding Biological Processes and Human States:

Unraveling Cellular Heterogeneity: Novel alternative methods like single-cell sequencing have provided valuable insights into the heterogeneity of cell populations within tissues, leading to a deeper understanding of complex biological processes and disease states.
Personalized Medicine: Integrating multi-omics data and machine learning approaches have paved the way for personalized medicine. By considering individual variability, researchers can develop targeted therapies and treatment plans tailored to patients' specific needs.
Potential Limitations to Addressing Human Variability:
Limited Diversity in Datasets: Biases in data collection can lead to limited representation of certain populations, potentially overlooking critical aspects of human variability. Ensuring diverse and inclusive datasets is crucial to address this limitation.
Ethical and Regulatory Challenges: Developing novel alternative methods that account for the ethical considerations and regulatory requirements of human research can be challenging. Balancing scientific advancement with patient safety and privacy is essential.
Revolutionary Impact on Understanding/Treating Human Health:
Rare and Underserved Diseases: Novel alternative methods have the potential to shed light on rare diseases and conditions that have been historically understudied. By uncovering disease mechanisms, these methods can lead to the development of targeted therapies for currently underserved patient populations.
Neurological Disorders: Advancements in understanding brain circuits and neural systems through alternative methods can revolutionize the treatment of neurological disorders, potentially leading to more effective interventions for conditions like Alzheimer's, Parkinson's, and autism.
Precision Medicine: Integrating multi-omics data and novel alternative methods can propel the field of precision medicine forward. Tailoring treatments to individual patients based on their unique genetic makeup and biological characteristics can lead to more effective and personalized healthcare.
In conclusion, novel alternative methods in studying human biology, circuits, systems, and disease states have already demonstrated success and potential in advancing scientific understanding and medical research. Maximizing their scientific utility requires open science, standardization, and collaboration. While these methods have the potential to address human variability and revolutionize human health research, ethical considerations and diverse data representation must be carefully considered to fully realize their impact. Their application in underserved areas of biomedical research can lead to groundbreaking discoveries and the development of innovative treatments for various diseases.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
Catalyzing the development and validation of novel alternative method technologies is critical for their successful integration into research approaches. Addressing challenges related to robustness, replicability, reproducibility, and reliability of these technologies, as well as bolstering technology readiness, requires strategic approaches. Additionally, overcoming factors limiting their integration across research approaches involves careful planning and collaboration. Here are some thoughts on each aspect:
Challenges for Building in Robustness, Replicability, Reproducibility, and Reliability:

Standardization and Validation Protocols: Establishing standardized protocols for experimental design, data collection, and analysis is essential to ensure the robustness and reproducibility of novel alternative methods. Rigorous validation against known benchmarks or gold standards can bolster the reliability of the technologies.
Data Sharing and Open Science: Encouraging data sharing and open science practices fosters collaboration and allows independent replication of results, ensuring the reliability of the technologies and the datasets they produce.
Quality Control and Quality Assurance: Implementing rigorous quality control and assurance measures throughout the research process helps identify and mitigate potential sources of variability and bias, leading to more reliable results.
Cross-Validation with Existing Methods: Comparing the results obtained from novel alternative methods with those obtained using established techniques can help identify areas of agreement and discrepancies, strengthening the validity of the new technologies.
Strategies for Bolstering Technology Readiness and Reliability:
Interdisciplinary Collaboration: Bringing together experts from diverse fields can lead to a more comprehensive understanding of the technologies and their applications, enhancing their readiness and reliability.
Pilot Studies and Iterative Development: Conducting pilot studies and iterative development can help refine and optimize novel alternative methods before full-scale implementation, ensuring their readiness and reliability.
External Evaluation and Peer Review: Seeking external evaluation and peer review of the technologies and their validation processes can provide valuable feedback and strengthen their credibility.
Investment in Research Infrastructure: Adequate funding and resources dedicated to research infrastructure, such as advanced equipment and computational resources, can enhance the readiness and reliability of novel alternative methods.
Factors Limiting Successful Integration and Potential Solutions:
Resistance to Change: Researchers may be hesitant to adopt novel alternative methods due to the inertia associated with established techniques. Educating the scientific community about the benefits and potential of these technologies can overcome this resistance.
Lack of Resources and Expertise: Limited access to specialized equipment, computational resources, or expertise can impede the integration of novel alternative methods. Collaborative initiatives and shared resources can help overcome these limitations.
Regulatory and Ethical Concerns: Meeting regulatory requirements and ethical considerations when using novel technologies can be challenging. Engaging with regulatory agencies and ethics committees early in the development process can help address these concerns proactively.
Data Complexity and Analysis Challenges: The complexity of data generated by novel alternative methods can be daunting. Investing in data analysis training and developing user-friendly analysis tools can aid researchers in extracting meaningful insights from the data.
Validation Bottlenecks: The validation process for novel alternative methods can be time-consuming and resource-intensive. Streamlining validation procedures and establishing collaboration between researchers and regulatory bodies can accelerate technology readiness.
In conclusion, catalyzing the development and validation of novel alternative method technologies requires a concerted effort from the scientific community, funders, and regulatory bodies.

Robustness, replicability, reproducibility, and reliability can be enhanced through standardization, data sharing, and quality assurance. Strategies like interdisciplinary collaboration, pilot studies, and peer review can bolster technology readiness. Overcoming resistance to change, resource constraints, regulatory challenges, and data complexity requires proactive planning, collaboration, and investment in research infrastructure. By addressing these challenges and implementing strategic solutions, novel alternative methods can be seamlessly integrated into research approaches, advancing scientific knowledge and contributing to improved understanding and treatment of human health and disease.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Use of Novel Alternative Methods to Study Human Biology, Circuits, Systems, and Disease States: Novel alternative methods have opened up exciting avenues in studying human biology and disease states. These approaches often complement traditional techniques and offer unique advantages. Examples of novel alternative methods include organ-on-a-chip technology, single-cell sequencing, machine learning and AI, and multi-omics integration.

Current Development and Successful Use:

Organ-on-a-chip technology: These microfluidic devices replicate organ structures and functions, enabling researchers to study complex biological processes in a more physiologically relevant environment. They have been successfully used to model diseases, drug responses, and toxicological effects, reducing the need for animal testing.

Single-cell sequencing: This method allows researchers to analyze individual cells within tissues, uncovering cellular heterogeneity and gene expression patterns. It has led to significant advancements in understanding cell types, developmental processes, and disease mechanisms.

Machine learning and AI: These approaches analyze large datasets, identify patterns, and make predictions, revolutionizing biomedical research. They have been used successfully in medical imaging analysis, drug discovery, and personalized medicine.

Multi-omics integration: Integrating data from various omics levels (genomics, transcriptomics, proteomics) provides a more comprehensive understanding of biological processes. It has been instrumental in identifying biomarkers, understanding disease mechanisms, and developing targeted therapies.

Advancing Progress into Understanding Specific Biological Processes or Human States: Novel alternative methods have advanced our understanding of various biological processes and human health states, such as:

Cellular Heterogeneity: Single-cell sequencing has revealed the presence of distinct cell populations within tissues, leading to a deeper understanding of complex biological processes and disease heterogeneity.

Precision Medicine: Multi-omics integration, combined with machine learning, has paved the way for precision medicine. This approach considers individual genetic makeup and biological characteristics to tailor treatments, resulting in more effective healthcare.

Potential Limitations in Addressing Human Variability: Despite their promise, novel alternative methods may face limitations in addressing human variability:

Data Representation: Biases in data collection can result in limited representation of diverse populations, potentially overlooking crucial aspects of human variability. Efforts to ensure diverse and inclusive datasets are essential to address this limitation.

Ethical Considerations: Some novel alternative methods might involve ethical and privacy concerns when used in human research. Proper ethical oversight and adherence to regulatory guidelines are necessary to address these issues.

Revolutionary Impact on Understanding/Treating Human Health: Novel alternative methods have the potential to be truly revolutionary in understanding and treating human health, especially in underserved areas of biomedical research:

Rare Diseases: By providing new insights into rare and understudied diseases, these methods can accelerate the development of targeted therapies and improve patient outcomes.

Neurological Disorders: Advancements in understanding brain circuits and neural systems can lead to breakthroughs in treating neurological disorders, such as Alzheimer's, Parkinson's, and autism.

Personalized Medicine: Precision medicine based on multi-omics and AI approaches has the potential to transform healthcare, offering tailored treatments and prevention strategies for individuals.

Drug Discovery: Novel alternative methods can enhance drug discovery by identifying new drug targets, predicting drug responses, and reducing the reliance on animal models.

In conclusion, the use of novel alternative methods to study human biology, circuits, systems, and disease states has already shown great promise and potential. Their successful integration into research approaches relies on addressing challenges related to data robustness, replicability, and reproducibility. Emphasizing interdisciplinary collaboration, standardization, and data sharing can maximize their scientific utility. By overcoming limitations in addressing human variability and focusing on underserved areas of biomedical research, these novel methods have the potential to be truly revolutionary in understanding and treating human health.

Email: betterscienceccampaign@gmail.com

Submit date: 8/6/2023

I am responding to this RFI: On behalf of myself

Name: Dr Thea Sesardic

Name of Organization: None

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   - Most promising and already accepted NAMs have been those addressing relatively simple biology i.e where mode of action and targets are well understood and in vitro systems are available.

   - Since human biology, systems and disease act on many organs with very complex interaction, simple in vitro systems cannot replace information obtained with whole animal models. Construction of synthetic whole animals have been in development and may improve with better models targeting specific application.

   - Data mining and machine learning are already being used to develop NAM and may be more extensively explored with other approaches.

   - Future research should also focus on better understanding of mode of action and biology of drugs and biologicals which are currently tested in animal model (as a priority). Particular focus on infection models.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Develop and validate new methods prior to licensing and at pre-clinical/clinical studies so to avoid use of animals once the products are licensed.
   Develop understanding how changes ton clinically relevant parameters can be determined in vitro.
   More difficult to validate NAMs if animal model is already approved and included in the licensing documents
   Develop new mathematical models to allow validation of several in vitro methods against a single in vivo model.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Formulate research funding by including industrial and regulatory partners
   Increase funding for interlaboratory training / on lime training.
   Increase funding for co-ordination of proficiency and collaborative studies and distribution of reference reagents or standards

Submit date: 8/6/2023

I am responding to this RFI: On behalf of myself

Name: Antonia Laskaris Moore

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Organ-on-chip systems are being used to study the embryonic development of human organs, drug interactions, and the effects of specific substances on a human organ. Advantages include: results that are human-specific, obtained more rapidly than with animal testing, more reproducible than with animals, and don't inflict pain on or result in the death of animals.
   Advanced computational techniques are enabling the development of virtual models that simulate biological processes, drug interactions, and the toxic effects of substances on human bodies.
   Micro-dosing is being used in human studies to study the effects of substances.
   Micro-sampling of human tissues is being used for similar purposes.
   Advanced statistical learning tools to analyze vast quantities of healthcare data that already exist and are being underutilized by researchers. This potentially prevents animal studies that have already been done as well as animal studies for disease states that are specific to humans or certain human populations and which therefore can't produce human-relevant results.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   NIH should create a flexible framework for evaluating new alternative methods by forming a committee of scientists familiar with the process.
   Generous funding should be offered to researchers and institutions to support novel technologies.
   Animal methods should be used only when researchers can clearly show that doing so will provide relevant human health information and that the intended animal method has advantages over any available non-animal method.
   Enhancing transparency in the ethical review process will allow public input on funded studies, reflecting changing attitudes towards animal use in research.
   NIH should also address bias in medical journals that favor studies using animals. Researchers with valid results from non-animal studies should have more outlets for publishing their findings.
   NIH should educate researchers and the public on the failures of animal methods and should promote the idea that valid research can be conducted without animal use.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Offer research grants and incentives specifically targeted at projects utilizing alternative methods. This financial support will encourage researchers to explore and invest in these novel technologies.

In modern scientific research, collaboration among different institutions is crucial. To overcome wasteful duplication of efforts, NIH should promote national and international research collectives. These networks will enable scientists to share findings, learn from each other, and accelerate progress. NIH should provide frameworks for project management and data sharing to facilitate these partnerships. Collaboration, as seen during the COVID-19 crisis, enhances the development of innovations, maximizes the value of products, and fosters a collective effort to analyze new methods and address their flaws.

Email: toniamoore618@gmail.com

Submit date: 8/6/2023

I am responding to this RFI: On behalf of myself

Name: Hyun Jung Kim

Name of Organization: Cleveland Clinic

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   It is crucial to develop new and innovative methods that can bridge the gaps between in vivo animal models, in vitro cell culture models, and human clinical studies. The human microbiome plays a significant role in human health and diseases, making its contribution extremely important. However, the current experimental models fail to represent the right populations of the human microbiome (e,g, the mouse gut microbiome only harbors 15% of the human microbiome). Additionally, current tissue-engineered models, including organoid cultures, cannot maintain a stable host-microbiome ecosystem format for longitudinal studies. To overcome these limitations, a new alternative method called Organ-on-a-chip can be used. This method enables the manipulation of minimal determinants in the system, allowing for the modulation of various biological, physiological, and mechanical factors. This can lead to the discovery of disease mechanisms and testing of therapeutic interventions in the preclinical stage.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   New methods like the Organ-on-a-chip are still developing and face challenges like consistency, scalability, reproducibility, and affordability for research. However, many researchers, including commercial entities and government agencies like NCATS, are working to improve these methods. Collaborations between academic research institutions and hospitals have already led to significant advancements, but more funding is needed to accelerate drug development and interdisciplinary innovations.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   We must establish a dedicated budget to support high-risk, high-reward research that involves multiple collaborative consortia. This is a crucial factor in fostering a collaborative environment. Furthermore, we fully acknowledge the importance of translational research that enables the practical dissemination of the developed technology, which aligns with the “Bedside to bench to bedside” concept. This funding approach is essential for researchers who face challenges in securing funding through the traditional NIH mechanism.

Email: biomelab.2022@gmail.com

Submit date: 8/14/2023

I am responding to this RFI: On behalf of an organization

Name: Mark Eichelberg

Name of Organization: American Physiological Society

Type of Organization: Professional org association

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   "The American Physiological Society (APS) appreciates the opportunity to comment on the Request for Information on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research (NOT-OD-23-140). APS represents a multidisciplinary community of nearly 10,000 scientists and educators focused on understanding the function of living organisms. APS strongly agrees with recent statements from NIH recognizing that animal models are critically important to biomedical research (1). APS also supports the principles of the "3Rs" (replacement, reduction, and refinement) and the development and use of alternative approaches, including novel alternative methods (NAMs), where they are scientifically justified and appropriate. With recent rapid advancement of microfluidic devices, researchers have access to novel in vitro tools including organ-on-a-chip and body-on-a-chip systems which allow more complex modeling of biological systems. However, despite the sophistication of these technologies, they do not recapitulate the total complexity of a living animal and should be viewed as supplemental to animal models as tools to understand life, health, and disease.
   Microphysiological systems such as organ-on-a-chip are of particular interest to the pharmaceutical industry. As preclinical models they offer potential as high-throughput drug screening platforms and for accurate prediction of pharmacokinetic parameters, promoting a "fail early fail fast" approach that could significantly lower the cost of drug development (2). The technology offers several advantages over traditional cell culture models. Microfluidic devices enable the study of cell and tissue function in the presence of physiologically relevant factors such as mechanical stretching and perfusion, which are difficult to model in other in vitro systems. By using fluidic coupling of multiple chambers lined by different organ cell types, a body-on-a-chip system was demonstrated to predict the pharmacokinetics and pharmacodynamics of cisplatin and nicotine (3). A number of other case studies have been summarized in a report from a workshop with the FDA and representatives of the pharmaceutical industry (4).
   Although these successes underscore the promise of NAMs, there are several important limitations to these technologies as currently implemented. While they can be much more complex than traditional cell culture, microphysiological systems are reductive models, and are unable to replicate the entire physiology of an organ. Additionally, data derived from NAMs are generally limited to parameters that can be easily observed and measured, such as the concentration of a metabolite or the rate of cell death. It is difficult to design a system capable of measuring a range of physiological responses, including tissue and organ morphology and function, and impossible to measure neuropsychological responses such as behavioral changes or pain. This limits the utility of NAMs as

tools for drug discovery. Because of these drawbacks, researchers will continue to rely on the currently available tools for drug development, including animal models.

Ultimately, the clinical value of these technologies depends on their ability to predict toxicological or therapeutic results in human trials. As the technologies are still in development, there is a lack of validating data to verify their predictive power. NIH investment should support the validation and assessment of these technologies alongside their development. NIH should also clarify for investigators that translational and preclinical studies should not rely on data from unvalidated models when established models are available. Finally, NIH should continue to invest in research using established models while the appropriate role of NAMs in the drug development process remains unclear.

1. Jorgenson L. 8 Dec. 2022. Catalyzing Research with Novel Alternative Methods [Online]. National Institutes of Health. https://osp.od.nih.gov/catalyzing-research-with-novel-alternative-methods/. [22 July 2023].

2. Ewart L, et al. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. Commun. Med. 2, 154, 2022. https://doi.org/10.1038/s43856-022-00209-1

3. Herland A, et al. Quantitative prediction of human pharmacokinetic responses to drugs via fluidically coupled vascularized organ chips. Nat. Biomed. Eng. 4(4):421-436, 2020. https://doi.org/10.1038/s41551-019-0498-9

4. Baran SW, et al. Perspectives on the evaluation and adoption of complex in vitro models in drug development: Workshop with the FDA and the pharmaceutical industry (IQ MPS Affiliate). ALTEX-Altern. Anim. Ex. 39(2):297-314, 2022. https://doi.org/10.14573/altex.2112203"

2. Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:

Translatability of preclinical models is a persistent challenge in biomedical research and one of the leading causes for the high cost of drug and therapy development. NAMs such as organ-on-a-chip and multi-organ chips have the potential to become powerful tools for drug efficacy and toxicology testing, but they face several unique challenges that must be addressed to maximize their predictive power. To account for these challenges and make the best use of available resources for fundamental and translational research, APS makes the following recommendations:

- NIH should support the characterization of the cells used in NAMs, including the development of new immortalized organotypic cell lines and cell culture methodologies that improve the consistency and replicability of cell behavior.
- NIH should prioritize independent validation of NAMs before they can be considered as physiologically relevant models of function or disease.
- NIH should emphasize the supplementary role of NAMs to established methods used in academic research, including the use of animal models.

As more microphysiological model systems reach the market, reproducibility will be a significant concern. Run-to-run reproducibility is highly dependent on the source of cells used. Systems which rely on the use of primary cells may face challenges with consistent sourcing of cells, as well as the susceptibility of primary cells to differentiation. Induced pluripotent stem cells (iPS cells) may be used to provide improved consistency between experiments, but they can be limited in their ability to exhibit the fully mature differentiated phenotypes of cells found in a living organism. Neither iPS

cells nor primary cells are easy to scale for high-throughput applications, so while they are not considered ideal as physiologically relevant models, established cell lines are sometimes used. However, many of the most widely used cell lines poorly represent organotypic cell function in a human, and improper stewardship of many cell lines has resulted in contamination with viruses, mycoplasma, and other cell lines. The most appropriate types of cells will need to be determined for each use case, and validation standards will need to account for any potential variability due to the types of cells used. NIH should direct effort toward the characterization of cells used in microphysiological models and improved reproducibility in cell behavior.

Before NAMs are adopted as reliable tools in the drug development pipeline, they will require rigorous demonstration of their performance. Because most academic studies only use a small number of chips, often with unique device configurations, validation will need to involve large-scale studies using standardized protocols. NIH should work with manufacturers to ensure that validation studies are carried out by independent third-party entities to avoid conflicts of interest and minimize experimental bias. NIH should also consider NAMs derived from animal cells as a tool for technology development and validation. Animal chips can support validation through assessing concordance of results based on parallel in vivo studies or extensive existing pharmacology data. Showing strong agreement of results between animal and human NAMs can help to demonstrate clinical relevance.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Because of the tremendous potential economic benefit of integrating NAMs into drug development workflows, the pharmaceutical industry will almost certainly be a leader in exploring potential applications. This will likely occur in a stepwise manner as researchers identify applications that provide superior results to existing models. To maximize the research value of NIH investments in NAMs, APS provides the following recommendations:

- NIH should prioritize the development and validation of NAMs for use in fields that lack models that are relevant to human health, such as liver toxicity studies and certain infectious diseases.

- NIH should improve the reproducibility of results from NAMs by supporting training of personnel, standardization of experimental procedures, and facilitating sharing of data, methods, and reagents.

- NIH should support fundamental research at academic institutions to characterize cellular and molecular physiology and to support the development and validation of NAMs.

There is particular value in the development of NAMs for use in fields of study that do not already have access to physiologically relevant in vitro or animal models, or where such models are poor representatives of human function. Examples include modeling of liver toxicity, infectious diseases with narrow host ranges, and genetic diseases that are not recapitulated by animal models. Each context of use may have unique chip design criteria depending on the disease or condition studied and the number of measurable outputs desired. However, it remains critical to validate these models for each use case. NIH should continue to engage with the research community to identify gaps in the availability of models and focus investments to address those gaps.

Reproducibility will be a significant challenge as NAMs become more widely utilized, and NIH should take steps to minimize undesired variance across labs. Important steps include coordination of personnel training and standardization of methods and procedures. Collaborative initiatives such as postdoc exchanges can help personnel understand best practices for performing experiments. Additionally, efforts to stimulate sharing of data, materials and reagents, and detailed experimental protocols will be essential to controlling the variability that can occur due to small differences in techniques.

NIH should also consider the role of academic researchers in the development and improvement of these new platforms. The efforts of academic researchers to characterize human cellular and molecular physiology have been indispensable to the development of NAMs. Fundamental research to identify the mechanisms of function and disease in biological systems can enable better, more physiologically relevant models to be developed, and improve our ability to validate these models. NIH should provide funding opportunities to enable researchers to contribute to the development and characterization of NAMs. However, continued funding of research using established methods remains crucial to further our understanding of biological systems. The importance of fundamental research to the development of new models underscores the value in continued investment in a variety of research tools and methods, including animal models, cell culture, and organoids, and emphasizes the role of NAMs as supplemental to existing methods.

APS appreciates the efforts of the Working Group to engage with stakeholders to understand the needs of the research community. As these technologies continue to mature, they will likely enable significant progress in basic and translational biomedical research and may provide a humane alternative to animal models in some contexts. Coordination and collaboration across federal agencies, particularly FDA, will help to maximize their potential. We look forward to continued engagement with the Working Group as they move toward final recommendations.

Submit date: 8/14/2023

I am responding to this RFI: On behalf of myself

Name: Sumita T. Jonak

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   1. This is the way 21st century science needs to be moving! Coming from an IT background, I found the life sciences world woefully behind in the dark ages of incremental progress masquerading as "novel" discoveries. This is particularly unchecked in the basic research arena where NIH is often the sole funder, sometimes for academic curiosities of dubious value with no human-relevant end-goal.
   2. It's time we call out some "basic research" for its downright pedestrian thinking and lack of innovation. As a taxpayer, I'm disappointed that our money is being doled out in fruitless pursuits like parabiosis...what is the scientific merit of parabiosis, a 150-year old surgical technique, today? How does that translate to helping advance human health? How many times do we have to re-prove that high-fat diets are bad for humans by feeding mice high-fat diets?
   3. Career PIs with an NIH grant history need to be challenged to produce novel discoveries merit-worthy of being an NIH awardee. There are insufficient opportunities for investigators who pursue NAMs, or non-animal research.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   1. Human-relevant technologies require a concerted effort akin to what we did for the Human Genome Project, where the full might of the US government and its splendid resources were marshaled towards a shared goal. We must do the same for human-relevant technologies to succeed and actually deliver on the promise of precision medicine.
   2. Over half of animal studies fail robustness, replicability, reproducibility and reliability, yet we still, albeit incorrectly, consider models like humanized mice to be a gold standard.
   3. The "whole body" or "whole organism" retort used to be a solid end-of-discussion, but it's the wrong whole system, the wrong whole body. So whatever glimpse of knowledge is gleaned is specious as it has a paltry 5% successful translation rate to humans.
   Let me repeat that ---> 5% hit rate.
   Cost ---> $2.6 billion, with a B.
   Time ---> 10-15 years.
   No other industry has those kinds of numbers and still exists with a business as usual bravado.

   This data is coming from NIH, FDA, and the pharma industry itself.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

1. Life sciences, particularly pharmaceuticals, has failed to progress proportionally to other industries. What business spends $2.6 billion dollars per successful product, with over a decade in R&D, only to have a 95% failure rate? Imagine if 95% of your Google searches failed. Imagine if Apple didn't update the iPhone for a decade. Imagine if The Walt Disney Company spent $2.6B on a theme park and 95% of the rides failed.
So why do we accept failure in Big Pharma as the cost of doing business? Maybe it's time to change the “business as usual” mindset.
2. The begins with changing how basic research is funded, and prioritizing areas that do not rely on animal models. Fifty years later, the 3Rs needs to focus on “replace” by supporting human-relevant technologies. Funding NAMs is the key to achieving this mindset shift and actually achieving innovative discoveries so we can solve for the remaining 93% of human diseases! (of the 7000+ human diseases, only about 500 have treatments/cures)
3. Animal models are expensive, forcing only a few, limited novel compounds to be tested. With computational modeling, researchers can explore the entire drug discovery space of 10^60 molecules, well beyond just the 10 million (10^7) we’ve worked with over the past 100 years.
BUSINESS ECONOMICS:
Preclinical testing methods based in human biology:
+ Avoid opportunity cost of delayed drug discovery.
+ Avoid compounding errors by relying on animal models, including humanized mice, that fail to mimic human disease and treatment response.
+ Quickly and cost-effectively assess accurate clinical response against disease models on promising drug candidates.
+ Validate drug safety and efficacy using human-relevant techniques.

All of the above begins with NIH setting the tone by funding truly novel, innovative research that gets us closer to solving human diseases. Let's put NIH money where it matters most!

Email: stjonak@gmail.com

Submit date: 8/14/2023

I am responding to this RFI: On behalf of an organization

Name: Alan R Jacobson

Name of Organization: Wake Forest Institute for Regenerative Medicine

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Wake Forest Institute for Regenerative Medicine (WFIRM) is a pioneer in the development of organ-on-a-chip, or microphysiological systems (MPS). Over the past 15 years, the use of these 3D tissue and cell culture models have become more widely accepted, thanks in large part to institutions like WFIRM advancing the technology by standardizing protocols for producing these organ tissue equivalents (OTEs) and performing reproducibility and validation studies.
   WFIRM has successfully created models for nearly all human organs and tissues using the OTE platform and have shown these OTEs more faithfully recapitulate their respective human biology. Furthermore, based upon our expertise in regenerative medicine, the approach used to develop the OTEs takes into account how these organs and tissues would be used clinically. This insight has allowed WFIRM to improve upon typical 3D culture systems by including components of the extracellular matrix (ECM), a major driving force of cellular communication, which has been shown to be vital for these miniature organs to reproducibly behave like natural organs. This knowledge base has been shared with the greater science community through both leading scientific publications and a national and international network of collaborators who have continually pushed the platform towards greater experimental studies and improvements. While hurdles within the field of 3D culture remain (see below), it is safe to say that widespread adoption of the technology has been accomplished, with almost all life science laboratories incorporating some level of 3D cell culture to compliment 20th century 2D cell cultures.
   WFIRM continues its leadership role in this field through ongoing efforts in studying the various ways in which the OTE platform can be utilized across complex biological questions. Examples include (key references provided in attachment);
   - Personalized Medicine: Our researchers have had significant impact in the oncology field by using patient derived tumor cells to create personalized cancer OTEs, and rapidly testing chemotherapeutic agents. These efforts have resulted in more specific drug regimens and, ultimately, better clinical outcomes
   - Development of disease specific OTEs, allowing for more detailed studies of the etiology and biochemistry underlying the pathology. A non-exhaustive list includes;
     - Cystic fibrosis (lung OTE)
     - NASH (liver OTE)
     - Cardiac diseases (heart OTE)
     - Necrotizing Enterocolitis (NEC, gut OTE)
     - Fibrosis (multiple tissue and organ types)
   - Development of a testing platform for gene therapies

- Use of liver OTE to study gene therapy delivery
- Development of OTE platform to study medical threats from space travel
  - Study cellular and tissue effects of solar energetic particles and galactic cosmic ray radiation
  - Study human cellular pathways to enable long space flights
  - Minimize energy consumption
  - Initiate a torpor-like state or other potential hibernation inducers

More recently, WFIRM has been utilizing the OTE platform to study threats of importance to the Nation's security. Work funded through BARDA has focused on understanding the pulmonary effects of chlorine intoxication. This work, using the lung OTE, is providing a greater understanding of the biochemical pathways involved in chlorine exposure as a function of time and dose, providing new opportunities to identify both biomarkers and potential therapeutic targets. The platform is also amenable to assessing medical countermeasures (MCMs), and we are currently evaluating a library of FDA-approved compounds to determine if there are existing drugs that can be repurposed for responding to a chlorine attack or accidental release.

A second program, funded by the DoD, is using our OTE platform to study viral infections. The overall objective of this work is to provide host response data to train an AI/ML algorithm for predictive toxicological and pathological applications. Though only one year into this program, our team has identified both common as well as unique host responses as a function of the type of virus as well as the lethality of the virus. This level of detail, obtained on individual organs, provides distinctive insights to the way in which a virus infects and spreads, without the often confounding "noise" associated with trying to study these early-stage phenomena in an in vivo system.

While much work using 3D cultures is limited to a single organ or tissue, the WFIRM OTE platform is able to be connected to form a circuit of organs, allowing the study of more complicated, human-like system. These types of circuits have been used to identify the mechanism of toxicity for previously FDA approved compounds that were later pulled from the market because of severe adverse events that arose post-approval. This type of circuitry is laying the foundation for a full-fledged human-on-a-chip system, which would fulfill the FDA's Modernization Act, to name but one aspect of the use of the technology.

WFIRM is also conducting another DoD funded study to understand species differences for various known drugs. As is well known to those in the field, success rates for transitioning promising basic research into clinically useful drugs is abysmal. Much of the attrition is believed to be due to poor animal models, which are not predictive of human physiology. As a consequence, promising drugs studied in animals often fail to show the same response in humans. Using OTEs representing different animal species to study drug response can lead to better indicators as to the most appropriate animal models. In a similar vein, these studies can provide guidance when invoking the Animal Rule, where it is deemed unethical to expose humans to certain pathogens (eg., Ebola virus). In these cases, the FDA's Animal Rule allows you to use an animal proxy in order to get the drug approved. However, there is no simple way to know which animal would be the best surrogate, especially when no human data is available

One of the biggest strengths of the OTE platform is that it is an agnostic technology in the sense that the OTEs can be constructed from essentially any cell source, including patient-derived and iPS cells. This versatility means that what has historically been drug discovery for white males can be expanded to include the true genotypic diversity that represents humanity. In fact, we would say

that the OTE platform has the potential to revolutionize human health. Arguably the most significant aspect of the 3D system is the ability to study mechanistic biochemistry. Isolating an organ to understand the etiology of an organ-specific disease can only be accomplished using these human 3D tissue models. The study of how the disease begins, how it changes the cells and surrounding tissue, and how it propagates, provides a level of detail that has hitherto been unknown. The data obtained will undoubtedly include biomarkers useful in diagnostics and therapeutics, as well as new therapeutic targets for combating these diseases. As SARS-CoV-2 showed the world, there are differences in how viruses attack based on sex and phenotype. The OTE platform allows for modeling this diversity. What were once marginalized populations can easily be incorporated into any disease studies or drug development effort using OTE technology. Likewise, for specific diseases, OTEs can be constructed to represent patient populations. For those diseases that are considered neglected or orphaned, or not sufficiently lucrative for industry to work on, the OTE platform can be used to more critically study the disease, identify potential targets, and screen existing libraries for interventions. As these 3D systems are essentially sophisticated cell cultures, they are amenable to modern molecular biology such as gene editing. This would allow for the development of disease-specific OTEs using, for example, CRISPR/Cas technologies.

In short, WFIRM's OTE platform represents a system that has the potential to completely change the way in which diseases are studied, how biomarkers and new targets are discovered, and how drug and toxicity testing take place. What's more, the technology includes race and sex differences, representing one of the most diverse biological platforms available to study pathologies, not just for the general population, but for those neglected and orphan diseases, and those that effect only certain subsets of humanity.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

   As a technology, the OTE or MPS platform has been rapidly adopted by the greater life sciences community. Basically, these systems represent a more sophisticated cell culture and the methods of making them are not technically difficult. However, the relative infancy of the technology means that there are no universal standards that define verification and validation across all available systems.

   One of the greatest challenges currently limiting this technology is the availability of a well characterized and diverse set of cells that are representative of the human population. The primary reason we need large Phase 3 clinical studies is to capture the potential adverse events across a hugely diverse population, not simply sex and race, but a host of other pheno- and genotypic characteristics, as well. Having a reliable cell bank from which we could consistently and reproducibly create the OTEs is imperative.

   While multiple commercial choices exist for tissue chips or the components needed to create an organoid, comparing the results from one manufacturer with those of another is tenuous at best, and may be more akin to comparing apples to oranges. This is not to say that these multiple platforms are "right" or "wrong". As with any platform, the choice of which one to use is most often dependent on what question one is trying to answer. Still, there needs to be a standard way by which one can determine that data from a liver OTE, for example, made by one manufacturer or lab, can be compared to another liver OTE made through a different process.

NCATS has taken the initiative and has formed an Innovation and Quality consortium to address some of these questions, ultimately however, it may take a regulatory agency, such as the FDA, to make standardization a priority. While individual companies or labs can use the OTE platform as they see fit, the objective of WFIRM, and many groups in the field, is to develop a product that will be used for diagnosing or testing treatments for humans, and therefore will require the FDA's approval. Reaching the point where the OTE platform can replace animal models will only be achieved when the FDA becomes comfortable with the data being generated. Defining benchmark bioassays or biomarkers that can be used for the purposes of validating the system would allow for the cross-referencing of data and provide more assurances to all those interested in utilizing the technology that the data is reliable and reproducible.

There is also one other "elephant in the room" issue that remains largely ignored by many in the field. Most of the cells used in these platforms, unless patient derived, represent a minor component of the diversity of the human population. As we all are aware, the recent SARS-CoV-2 pandemic made it clear that there are measurable response differences dependent upon age, race, and sex. As with many new technologies, one does not want to add too many variables at any one point in an experiment. However, the field itself needs to address the diversity issues head-on and an understanding both from the science perspective as well as the regulatory perspective is required. How we define diversity in experiments that are based on these OTEs will help facilitate a more encompassing acceptance of the technology.

While the use of the 3D cell culture platform has provided the research community with an expanded tool for studying biochemistry and pathophysiology, WFIRM is most interested in utilizing our OTE platform to better serve the patient. For this to become a greater part of medical care, there remains several hurdles to overcome. First, how to design a true human-on-a-chip. Although we, and others have shown you can create a circuit of OTEs encompassing as many as 9 different organs, this requires both engineering and microfluidics to maintain the correct distribution of any small molecule or biologic tested. There is also the need for a universal blood surrogate, that would allow for long-term longitudinal studies. Second, how to build and incorporate a physiologically-based pharmacokinetic (PBPK) model into the OTE platform that would accurately represent what is observed in vivo. Lastly, how can we use this platform to eliminate the use and need of any animal studies across the whole of science. None of these are fatal flaws in the platform, rather they are where WFIRM believes effort and funding should be made available in order to solve these shortcomings.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

The truth of the matter is that WFIRM has been concerned about the types of issues raised by Focus Area 3 since our groundbreaking work in the field. As a result, we have begun to address this through what we are calling the Human Sensor Consortium. This consortium represents a group of both academic and industry partners that are tackling the problems highlighted above; standardization, verification and validation, sex, age, race, phenotype, and genotype, to name a few. The need to recruit a multidisciplinary team to solve these problems was evident from the start. For biologists, cell sourcing, cell expansion, and a universal blood surrogate are important. For the pharmacologist, modeling adsorption, distribution, metabolism, excretion (ADME) are paramount, while for the bioengineer, the circuitry and microfluidics necessary to mimic how a human body

absorbs an active pharmaceutical ingredient is a priority. Enlisting the expertise of bioinformatics and computational scientists allows the design of algorithms that have the capability to discern nuances within the data that human eyes may miss.

The importance of creating such a group of scientists is that these different views, experiences, and scientific directions provide a more holistic approach to the design and use of the OTE platform. Based on capabilities to understand toxicology and predicting drug efficacy, the OTE system is on the cusp of offering an incredible tool across all of the life sciences. One of the potential applications is in the design of an Human Organoid Atlas, where one can begin to map out the various pathways that are perturbed by insults as a function of sex, race, age, and phenotype. This can include geographical points so that we can begin to understand the impact of living in an area where industries are active, big cites, or farming communities, for example.

Having such a consortium will allow the MPS and OTE platforms to standardize a variety of parameters across different chip constructs and cell sources. As the database of validation and verification continues to expand, more and more industries will grow confident in the value of these systems. Companies like cosmetics, consumer products, and chemical manufacturing, will more readily adapt the platform to their needs, recognizing that experimental readouts represent human response. Furthermore, the tedious conversions from animal data to human data will no longer be necessary.

As discussed above, one of the most significant hurdles that need to be overcome for more inclusive adoption of the technology is for a regulatory body to provide their seal of approval. For WFIRM, with a focus on the patient, this regulatory agency is the FDA. While the Agency has been directed under the recent legislative Modernization Act to minimize the use of animals, the pathway forward is ill defined. WFIRM and the Human Sensor Consortium are designed to help provide a roadmap towards the acceptance of these miniature organs as legitimate surrogates for human response. For example, a major question that remains in the field is how does one compare all the available animal in vivo data with the OTEs? While we are now amassing human OTE data, and there is a wealth of data on human clinical results, the piece of information that is missing is how the animal OTE corresponds to the animal in vivo data. Understanding this will allow the FDA to understand the relationship between the human OTE and the clinical trial results. Steps like this are vital to providing the hard scientific evidence that can show the benefits, as well as any potential risks, the microphysiological systems provide.

An important part of the Human Sensor Consortium is the recognition that machine learning and artificial intelligence has reached a point where this technology can provide additional insights into the life sciences. As data related to specific diseases are compared across species and both in vivo and with OTEs, the amount of data that can be captured provides the ideal type of training sets for these algorithms. It is anticipated that eventually, these algorithms will be able to provide better ways to understand pathophysiology of diseases, discover new biomarkers, new targets, and make predictions as to what are the best ways to develop actual cures. Furthermore, through the Consortium, we will be able to include the entire phenotypic diversity of humanity, so that any potential biases within the algorithm will be minimized. This not only provides a more inclusive study of historically underserved populations, but also allows us a greater scientific understanding of the potential genetic drivers of these differences, be they positive or negative.

WFIRM whole heartedly believes that as our platform and the Human Sensor Consortium define the ways by which these systems of systems can be validated, the risks minimized, and the benefits

maximized, agencies such as the FDA will have greater confidence of the data and will be more likely to adapt the OTE platform as a more predictive model than any animal model. As the USG shows confidence in the platform, the greater industrial community will take note, and be reassured that the OTEs are in fact reproducibly providing more sophisticated and human-derived data than what has been historically relied upon.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/WFIRM-References-Alternative-Methods-RFI-August-2023.docx

Description: WFIRM key references

Email: ajacobso@wakehealth.edu

Submit date: 8/14/2023

I am responding to this RFI: On behalf of an organization

Name: Erin A Huber

Name of Organization: RTI International

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   In the broad sense NAMs have been at the core of biomedical research for decades. They have contributed to our understanding of foundational aspects of human health and fundamental biology. In more recent years, rapid progress in the field has led to advancements in both the development and deployment of novel NAMs, especially in the fields of toxicology, pharmacology, and public health. The responses provided here will focus on these fields; however, the concepts, challenges, and solutions discussed apply across NAMs applications and sectors (i.e., academia, government, and industry).
   The ever-greater integration of human-derived primary cells into in vitro studies has improved their in vivo human relevance. While often allowing the validation and expansion of cell line-based studies, the use of primary cells has also highlighted the limitations of an over-reliance on cell lines for extrapolation to human biology. For example, two studies demonstrated that the molecular mechanisms involved in the health effects of exposure to the ubiquitous ambient air pollutant ozone differed significantly between primary cell and cell line–based models (Bowers et al., 2018; McCullough et al., 2014). Unfortunately, the greater in vivo relevance of primary cell–based in vitro models also raises new challenges with their use and extrapolation to human- and population-level outcomes. The increase in the use of primary human cell–based models has begun to allow the field to appreciate the existence of inter-donor/inter-individual variability at the cellular level and examine its influence on biology and health in ways that cannot be addressed by isogenic cell lines or inbred animal strains (Bowers et al., 2018, 2021; Burnett et al., 2019, 2021; McCullough et al., 2016). Additionally, given the expanding use of in vitro NAMs in toxicology, the field needs to develop a more thorough understanding of the impact of inter-individual variability to provide context for the interpretation and extrapolation of in vitro data to in vivo biology and health outcomes. Studies such as these have highlighted how much remains to be learned regarding our understanding of the influence of inter-individual variability on human health and the underlying cellular and molecular mechanisms. Unfortunately, researchers are often apprehensive to use primary cells in their studies because of the higher cost compared to cell lines and required investment of time into establishing methods within a research group. This challenge is further compounded by the frequent usage of small numbers of primary cell donors (i.e., $n \leq 3$), which is typically accompanied by limited biological and technical replication. Unfortunately, results from these studies may be neither representative of the range of effect/response to experimental treatment in the population nor sufficient to accurately reflect the presence or absence of statistical significance if conducted in a larger donor population. Ensuring the rigor of these studies, and their ability to represent the diverse biology of the human population, requires greater levels of project

funding, longer project durations, and the development of sufficient data to establish guidance on a data-driven approach to the design of studies that involve primary cells. Additionally, advancing the integration of primary cells into NIH research would benefit greatly from efforts to increase access to primary cells from a broad range of donors.

Whether primary cell–- or cell line–based, in vitro studies have traditionally relied on monoculture (i.e., composed of a single cell type) models, these models lack the biological complexity that exists within the tissues that they are used to represent in vivo. Cells exist within a microenvironment that is shaped by various cell types, extracellular matrix, and other intrinsic factors. Building in vivo-relevant biological complexity in in vitro models is critical to understanding the contributions of different cell types and other biological factors to tissue structure and function under healthy and disease states. It is also necessary to maximize the scientific utility of in vitro models themselves, and the use of in vitro data for in silico modeling. Although efforts to develop multicellular in vitro models are still in their relative infancy, it is important to emphasize that their impact on understanding human health is dependent on their ability to reflect the composition, architecture, and function of their comparable in vivo tissue. Although challenging, emphasis should also be placed on developing multicellular systems that allow for the interrogation of the role of individual cell types as potential targets or mediators of experimental treatments.

In addition to in vitro and in vivo, NAMs can be utilized to predict chemical properties and interactions through the use of quantitative structure-activity relationship (QSAR) models. QSAR models are generated on algorithms to perform either regression or classification predictions based on the structural analysis and property comparison of a library of chemicals (Neves et al 2018.). These algorithms are able to utilize experimental results such as bioactivity to then predict the activity of chemicals with similar structural features. In silico NAMs are successfully being used in product development through the assessment of potential chemical impact with the analysis of chemical structural features following the ICH M7 guidelines (EMA, 2018). These guidelines are used to determine whether chemicals are potentially mutagenic or carcinogenic through QSAR models and alert researchers to structural features of concern. Thus, research/development efforts can be informed on likely challenges facing the development of a therapeutic agent rather than relying on in vitro screening alone.

Although not NAMs themselves, Adverse Outcome Pathways (AOPs) have been and are being developed to understand the biological mechanisms underlying a broad range of exposure-related adverse health endpoints/outcomes (see https://aopwiki.org for examples). AOPs leverage existing data to develop an understanding of key events that occur between a molecular initiating event (i.e., the initial interaction of a test substance with a cell) and an apical outcome at the individual/population level. They also provide a means to integrate data of different types (e.g., in silico, in vitro, in vivo, and epidemiological). Despite being described as “adverse” outcome pathways, the mechanistic information contained within AOPs can also be useful in understanding the cellular and molecular mechanisms involved in disease and the identification of materials with therapeutic potential. For example, the in vitro assay(s) associated with a key event necessary for the progression toward a disease state could be used to help identify chemicals capable of modulating the key event and thus mitigating a disease state.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

Although in vivo data are often regarded to be a "gold standard" against which NAMs should be evaluated, challenges these models have been widely criticized for their variability, with high failure rates that require repeat testing (Lewis et al., 2012). For example, recent evaluations of data from acute oral toxicity and skin sensitization testing have shown that animal data are often accompanied by large error terms and poor repeatability (Karmaus et al., 2022; Rooney et al., 2021). NAMs have the potential to provide more consistent data; however, achieving robustness, reproducibility, and reliability will require significant investments of funding, time, and coordinated effort. One key step toward this goal is harmonizing NAMs models and methods to bolster technology readiness, reliability, and the interpretation of resulting data. Developing consensus best practices in both the execution and reporting of NAMs-based studies will enhance reproducibility, reliability, and facilitate inter-lab evaluation of system and model robustness. Accomplishing these things in a transparent and accessible manner will be critical to building confidence in their value in biomedical research within both the scientific community and the general public. Achieving these goals will also benefit from conducting NAMs research in structured environments that emphasize technical and conceptual training, the formulation of defined study plans/protocols, clear reporting of data and methods, and oversight at the institutional and funding agency levels. Further, developing streamlined quality and performance metrics and best practices will provide researchers with the ability to efficiently complete experimentation and the sharing of methodology and data to the biomedical research community and end users of the resulting data. I The impact, transparency, and value of these data would be enhanced by reporting raw and processed data, as well as negative data and associated controls (positive and negative), as well-annotated and publicly accessible datasets. These efforts would be aided by the establishment of an NIH-funded and supported publicly accessible repository for data generated in NIH-funded studies. The successful implementation and acceptance of NAMs would benefit greatly from additional funding for training and workshops for researchers in methods and usage of advanced technologies.

Benchmarking systems-level outcomes using extant in vivo animal and human data and defined responses is critical to the development of NAMs. In addition, NAMs developers need to consider the comparability of in vitro and in silico-derived data with in vivo outcomes. Further studies are needed for multi-comparability research and trials and support for similar work with models with a range of experimental treatments to build context of use. Reliability of these technologies would benefit from funded projects for toxicological and pharmacological testing including a larger spread of chemical classes and species. Currently, in silico models mostly analyze 2D structural features because of the "lack of beneficial results" and "high computational computing cost" when calculating 3D structural features, but chemicals are not only 2D. Factors such as chirality and movement have led to instances where one conformer interacts in ways another does not (e.g., thalidomide). If researchers can leverage computational models (in silico analyses) to establish qualitative and quantitative comparability between in vitro and in vivo results and biological outcomes, then there is a possibility to predict some of the conformer-specific interactions. Emphasis should be placed on developers and researchers providing the rationale for these methods with additional information on how these models and methods work within the specific context. To further this effort, it is essential to increase the thoroughness with which NIH-funded research methods and data are reported. Overall additional funding and support are needed to not only

develop methods but also provide validation of models and methods, to understand the strengths and limitations of the NAMs and grow community confidence. This will benefit from the development and required use of reporting standards and peer-reviewed protocols.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Historically, NIH-funded research has led to both revolutionary and incremental advances in technologies for biomedical sciences. Unfortunately, the broad adoption of these advances is significantly limited by a lack of technical reporting standards for methods and data and minimal incentives and funding for investigators to ensure that their discoveries are transferable to other users. Supporting transparency through detailed and rigorous reporting of data and methods is also fundamental to the independent reproduction and validation of study findings. Although ensuring the dissemination of the technology is valuable for the advancement of the field, it is absolutely critical for building the context of use that is required to support the integration of NIH-funded advances into real-world public health applications (e.g., chemical/materials safety assessments, pharmaceutical development). Methodological transparency and reproducibility would be aided by the requirement for methods used in published NIH-funded research to be: (1) made available on one of a list of appropriate open-access methods repositories (e.g., Protocol Exchange) and (2) described in sufficient detail to allow complete reproduction by an unaffiliated lab. Submissions to such protocol repositories are typically given a DOI number, which can then be referenced in manuscripts or other presentations of NIH-funded research. Results of NIH-funded research have led to the award of numerous patents in the novel approach methodologies field. By definition, the focus on "novel" approaches and methodologies implies that the field will have great opportunities for the development/discovery of profitable intellectual property. Although the purpose of protecting intellectual property by patent is not lost on this group, it does, by definition, limit the sharing and deployment of new technology equitably. Similar to the requirement that the published results of NIH-funded research must be publicly available as per the NIH Public Access Policy, the field would benefit greatly from practical limitations on the sequestration of taxpayer-funded research as personal or institutional/ organizational intellectual property.

The integration of reliable, reproducible, and accessible NAMs can be enhanced by providing funding opportunities that are specifically directed to support cross-sector collaborative/consortium efforts across multiple labs for the harmonized implementation of new methods. Crucially, these efforts should require, or at least incentivize, the inclusion of investigators/participants that are representative of the end users of both the method and the resulting data. For example, an effort to integrate a new method into chemical safety assessment should include risk assessors and study directors/principal investigators from commercial entities that would utilize the method as study team members. Additionally, funding opportunities could be made available to support training sessions or workshops in which interested users could be directly trained on the use of a novel method/technology. The impact of such NIH-supported efforts would be enhanced by requiring the publication of a detailed accounting of the method, the integration effort, successes, or failures/challenges.

Importantly, the thorough reporting of methods and accessibility of new technology goes hand in hand with data reporting and accessibility. To ensure transparency and equitable accessibility, taxpayer-funded study data need to adhere to Findable, Accessible, Interoperable, and Reusable

(FAIR) reporting guidelines, which can be facilitated by the provision of appropriate metadata (Boeckhout et al., 2018). The combination of accessible methods and data will facilitate independent reproduction and validation of NIH studies and associated new methodologies.

Understanding the relationship between experimental to an experimental treatment and observed outcomes is crucial for interpreting study results and extrapolating to human health outcomes. The current standard of practice in most toxicology and pharmacology studies is to use and report nominal treatment doses/concentrations (i.e., the expected amount of the test substance that is applied to the experimental system) without quantifying or characterizing the actual amount of the test substance that interacts with, or is absorbed by, exposed cells/tissues. Given that in silico models are developed using in vitro and in vivo data, they are also frequently impacted by the lack of exposure characterization. Unfortunately, nominal and actual concentrations in an experimental system can vary because of a range of processes including solubility, adsorption, evaporation, and degradation. Further, the impact of differences between experimental exposure models and in vivo human exposures may further limit the translation of NAMs-based study results to "real-world" human exposures. Determination and reporting of nominal and actual exposure concentrations and absorption/metabolism in NAMs-based studies incurs significant study cost and time; however, these data add valuable context for the interpretation, utilization, and translation of study results. Thus, the translation of NIH-funded NAMs studies would benefit from strong encouragement, in the form of guidance and additional funding, to collect and report these data.

To maximize translatability and minimize potential biases in the NAMs space, proposed research needs to account for variability and be inclusive of different demographic, disease, and susceptibility groups within the population to adequately extrapolate in vitro generated data to public health. Computationally based NAMs (in silico methods) can complement the use of primary cell-based in vitro systems (discussed above) to represent the diversity and variability of human biology by simulating human difference based on known or theorized data (RTI SynthPop™). The use of such in silico models can also inform the design of laboratory studies to increase the rate and breadth of scientific advancement. Further, human variability models can be used to identify vulnerable population groups that may be at greater risk. With the increased availability of electronic health records–based data, databases such as RTI SynthPop™ are able to cross-compare numerous variables by linking datasets to better predict human behavior and risk factors in infectious disease spread through existing trends. To address potential bias in NAMs that would inadvertently limit the diversity or extendibility of the results, we encourage suggestions mentioned above, specifically reporting frameworks that make clear assumptions, parameters, and domain of applicability. Not only would this greatly improve the quality and the interpretation of NAMs data but would also give transparency that is not currently the norm. Several reporting frameworks have been presented to different communities (OECD, 2004, 2021; Piir et al., 2018) and there has been active discussion on furthering them for NAMs (Hartung et al., 2019).

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/RFI_Response_RTI-International_NIH_Catalyzing_Development_Use_of_NAMs_Final.pdf

Description: The document is the formal response to NOT-OD-23-140.

Email: ehuber@rti.org

Submit date: 8/14/2023

I am responding to this RFI: On behalf of myself

Name: Sparsha Saha

Name of Organization: Harvard University

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Growing human organs and personalizing treatment -- get rid of the animal! Let's test on "ourselves" through growing mini versions of our own organs.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The main issue or barrier is the 'publish or perish' reality of academia that breeds inertia instead of innovation. We'll need regulations that are binding to bring about positive changes.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   na

Submit date: 8/15/2023

I am responding to this RFI: On behalf of myself

Name: Merel Ritskes-Hoitinga

Name of Organization: Utrecht University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   please see attachment

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   please see attachment

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/Submission-NIH-Request-Ritskes-Hoitinga.docx

Description: Input to NIH request by Merel Ritskes-Hoitinga, prof. in Evidence-Based Transition to Animal-Free Innovations

Email: j.ritskes-hoitinga@uu.nl

Submit date: 8/15/2023

I am responding to this RFI: On behalf of an organization

Name: Mary McElroy PhD ERT MBA

Name of Organization: Charles River Laboratories

Type of Organization: Other

Type of Organization-Other: CRO

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Are currently being developed and/or used successfully, including features that maximize scientific utility:
   CRL perspective: Currently perform multiple routine regulatory safety assessment in vitro studies according to OCED guidelines (examples below).
   CRL is developing complex in vitro models for discovery and safety assessment supported with peer-reviewed publications from CRL scientists (examples below).
   Regulated safety assessment tests (examples)
   Routine regulatory tests to predict skin irritation & corrosion (OECD TG 431, 439), ocular irritation & corrosion (OECD 437, 492, 492B), acute toxicity (OECD 129), skin absorption (OECD 428), phototoxicity (OECD 432, 498), skin sensitization (OECD 442C, 442D, 442D, 497)
   Complex 3D/organoid in vitro systems in development for safety and/or discovery (examples from CRL)
   The development and validation of a full thickness human skin model to determine the skin irritating properties of (antimicrobial) formulations (paper submitted to in Vitro Toxicology)
   Complex human lung 3D model (MucilAir™) –replaced 90-day inhalation rat tox study for directly toxic pesticide called Chlorothalonil (detail provided in this OECD case study published in 2022, pdf (oecd.org))
   Lung in vitro model EpiAirway™ prediction model developed to predict GHS hazard classifications https://pubmed.ncbi.nlm.nih.gov/29904643/
   Evaluation of 3D Human Intestinal Organoids as a Platform for EV-A71 Antiviral Drug Discovery https://pubmed.ncbi.nlm.nih.gov/37190047/
   Are advancing progress into understanding specific biological processes or human states, including potential limitations to addressing human variability:
   CRL RESPONSE
   Multi-donor cell-models including models generated from patients with specific disease condition e.g. Epithelix™ with complex 3D models from cystic fibrosis, COPD patients.
   Could be truly revolutionary for understanding/treating human health, including currently underserved areas of biomedical research.
   CRL RESPONSE
   Multi-organ chip with matured tissue niches, endothelial layer, and vascular flow (with immune cells (e.g. Bourchare et al., 2022)

In vitro testing battery to determine Developmental Neurotox (guidance-evaluation-of-data-developmental-neurotoxicity-in-vitro-testing.pdf (oecd.org))
NAMs which account for differences in gender, age, and disease state.
Accurate blood brain barrier (BBB) modeling including vascular cells, pericytes and astrocytes.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

Challenges for building in robustness, replicability, reproducibility and reliability of the technologies and the ensuing datasets;

CRL RESPONSE

1. Agreement on panels of reference items with known in vivo toxicity/mechanism of action in humans and availability to use these panels to validate.
2. Human cell supply, consistency, characterization, and availability
3. Adoption of standardized quality system for study conduct.
4. In silico modeling and understanding computational models which predict in vivo exposure from in vitro data.
5. Machine learning models.
6. Lab to lab variation, biological variation (individual differences, etc.).

strategies for bolstering technology readiness and reliability these technologies; and

CRL RESPONSE

A common theme from multiple stakeholders is the lack of funding to validate models. More support and funding in the pre-competitive space for co-development/co-validation of common platforms will be important.
Gaining confidence in NAMS by performing case studies with involvement of regulators in projects like Risk-Hunt3R (Home - RISK-HUNT3R)

factors potentially limiting the successful integration of these technologies across research approaches and potential solutions

CRL RESPONSE

For model systems that need to be validated with test chemicals – an agreed chemical panel should align with regulatory expectations to avoid delays/discussions after validation.
Recommend that regulators are included in the process of the development and validation of NAMs at an early stage of the process i.e. draft protocols shared for comment ahead of validation studies.
Required laboratory equipment to perform studies should not be cost prohibitive and not be specific to one manufacturer.
Recommend that regulators are included in the process of the development and validation of NAMs at an early stage of the process i.e. draft protocols shared for comment ahead of validation studies.
Required laboratory equipment to perform studies should not be cost prohibitive and not be specific to one manufacturer.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

PLEASE NOTE THAT THE TEXT ABOVE THS BOX DOES NOT MATCH THE DESCRIPTOR AT THE TOP OF THE PAGE (FYI).

areas in which coordinated approaches across research disciplines or research sectors would dramatically advance the development and or use of these technologies.

CRL RESPONSE

Currently the process of regulatory acceptance/confidence will benefit from coordination across multiple stakeholders e.g. regulator advice, in vitro and in vivo scientists, computational modelling, advantaged statical analysis and subject matter experts for endpoint analysis, toxicologists etc.

Example of a systematic approach to gaining regulatory confidence in NAMs has been published by van der Zalm, A.J., Barroso, J., Browne, P. et al. A framework for establishing scientific confidence in new approach methodologies. Arch Toxicol 96, 2865–2879 (2022).
https://doi.org/10.1007/s00204-022-03365-4

Alignment between different regulatory authorities is also important e.g. between FDA, EMA. ECHA and EFSA (and EPA).

Approaches for sharing technology deployment equitably across labs, including incentives for reliable and reproducible methods integration.

CRL RESPONSE

Consortia with multiple stakeholders to ensure that research is focused on models with potential for most impact and adoption occurs as quickly as possible (e.g. Landscape New Approach Methodologies (NAMs) | RIVM

Practical guides to multi-stakeholder partnerships (communication, consultation, collaboration, and coproduction), managing stakeholder conflicts of interest.

Inclusive nature of multi-stakeholder partnerships to promote transparency and give credibility to development initiative.

Training is also important and a source where protocols / SOPs are accessible, and version controlled.

Ensuring selected in vitro models are widely available to CROs/Biotech. Little value in validating a good/robust model is cells/tissues which are difficult to acquire regularly.

Factors for consideration when maximizing translatability and minimizing bias regarding human variability.

CRL RESPONSE

Ensure multi-donor cell sources (sex, age, ethnicity, history).

Minimum acceptable criteria for cell sources.

Ethical permission for use of in vitro assays.

Pooled samples where available and/or makes sense (i.e., PBMCs).

Email: mary.mcelroy@crl.com

Submit date: 8/15/2023

I am responding to this RFI: On behalf of an organization

Name: Mary C McElroy PhD MBA ERT

Name of Organization: Charles River Laboratories

Type of Organization: Other

Type of Organization-Other: CRO

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   are currently being developed and/or used successfully, including features that maximize scientific utility:
   CRL RESPONSE
   CRL perspective: Currently perform multiple routine regulatory safety assessment in vitro studies according to OCED guidelines (examples below).
   CRL is developing complex in vitro models for discovery and safety assessment supported with peer-reviewed publications from CRL scientists (examples below).
   Regulated safety assessment tests (examples)
   Routine regulatory tests to predict skin irritation & corrosion (OECD TG 431, 439), ocular irritation & corrosion (OECD 437, 492, 492B), acute toxicity (OECD 129), skin absorption (OECD 428), phototoxicity (OECD 432, 498), skin sensitization (OECD 442C, 442D, 442D, 497)
   Complex 3D/organoid in vitro systems in development for safety and/or discovery (examples from CRL)
   The development and validation of a full thickness human skin model to determine the skin irritating properties of (antimicrobial) formulations (paper submitted to in Vitro Toxicology)
   Complex human 3D tumor model recapitulating the TME including stroma and immune cell components. https://pubmed.ncbi.nlm.nih.gov/37190054/
   Complex human lung 3D model (MucilAir™) –replaced 90-day inhalation rat tox study for directly toxic pesticide called Chlorothalonil (detail provided in this OECD case study published in 2022, pdf (oecd.org))
   Lung in vitro model EpiAirway prediction model developed to predict GHS hazard classifications https://pubmed.ncbi.nlm.nih.gov/29904643/
   Evaluation of 3D Human Intestinal Organoids as a Platform for EV-A71 Antiviral Drug Discovery https://pubmed.ncbi.nlm.nih.gov/37190047/
   are advancing progress into understanding specific biological processes or human states, including potential limitations to addressing human variability; and
   CRL RESPONSE
   Multi-donor cell-models including models generated from patients with specific disease condition e.g. Epithelix with complex 3D models from cystic fibrosis, COPD patients.
   3D oncology platform using patient matched tumor, immune and stromal cells.

Broaden the current o3D oncology panel by enabling the culture of >500 PDX models in 3D co-culture

could be truly revolutionary for understanding/treating human health, including currently underserved areas of biomedical research:

CRL RESPONSE

Multi-organ chip with matured tissue niches, endothelial layer and vascular flow (with immune cells (eg. Bourchare et al., 2022)

In vitro testing battery to determine Developmental Neurotox (guidance-evaluation-of-data-developmental-neurotoxicity-in-vitro-testing.pdf (oecd.org))

NAMs taking into account differences in gender, age, disease state.

Accurate blood brain barrier (BBB) modeling including vascular cells, pericytes, astrocytes.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

challenges for building in robustness, replicability, reproducibility and reliability of the technologies and the ensuing datasets;

CRL RESPONSE

1. Agreement on panels of reference items with known in vivo toxicity/mechanism of action in humans and availability to use these panels to validate.
2. Human cell supply, consistency, characterization, and availability
3. Adoption of standardized quality system for study conduct.
4. In silico modeling and understanding computational models which predict in vivo exposure from in vitro data
5. Lab to lab variation, biological variation (individual differences, etc.).

strategies for bolstering technology readiness and reliability these technologies; and

CRL RESPONSE

A common theme from multiple stakeholders is the lack of funding to validate models. More support and funding in the pre-competitive space for co-development/co-validation of common platforms would also be welcomed.

Gaining confidence in NAMS by performing case studies with involvement of regulators in projects like Risk-Hunt3R (Home - RISK-HUNT3R)

factors potentially limiting the successful integration of these technologies across research approaches and potential solutions

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Required laboratory equipment to perform studies should not be cost prohibitive and not be specific to one manufacturer.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

areas in which coordinated approaches across research disciplines or research sectors would dramatically advance the development and or use of these technologies.
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Currently the process of regulatory acceptance/confidence will benefit from coordination across multiple stakeholders e.g. regulator advice, in vitro and in vivo scientists, computational modelling, advantaged statical analysis and subject matter experts for endpoint analysis, toxicologists etc.
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https://doi.org/10.1007/s00204-022-03365-4
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Ensuring selected in vitro models are widely available to CROs/Biotech. Little value in validating a good/robust model if cells/tissues which are difficult to acquire regularly.
factors for consideration when maximizing translatability and minimizing bias regarding human variability.
CRL RESPONSE
Ensure multi-donor cell sources (sex, age, ethnicity, history).
Minimum acceptable criteria for cell sources.
Ethical permission for use of in vitro assays.
Pooled samples where available and/or makes sense (i.e., PBMCs).

Email: mary.mcelroy@crl.com

Submit date: 8/15/2023

I am responding to this RFI: On behalf of an organization

Name: Stephen Heinig

Name of Organization: Association of American Medical Colleges

Type of Organization: Professional org association

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Please see our attached comment letter, PDF format.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/AAMC-Comments-to-NIH-on-Novel-Alternative-Methods-Aug-2023.pdf

Description: Comment letter of the AAMC in response to the RFI NOT-OD-23-140

Email: sheinig@aamc.org

Submit date: 8/15/2023

I am responding to this RFI: On behalf of myself

Name: Caroline Hoemann

Name of Organization: George Mason University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Coagulopathy is a major cause of morbidity in patients with severe lung infection, but difficult to manage due to the lack of specific tests that explain why platelet levels are dangerously low. C reactive protein is a biomarker that reveals the presence of inflammation, and D-dimer can reveal the presence of coagulation but cannot discriminate between bleeding or thrombosis.
   The cultured blood clot system is a novel thrombus model and Novel Alternative Method (NAM) being developed in my lab. In this device, a small sample of blood is collected in sterile tube with clot activator, then held at body temperature for 4 hours. Transcriptomics of the cultured clot relative to fresh blood of healthy donors led us to identify a pattern of novel biomarkers of thromboinflammation that are released by neutrophils exposed to pro-coagulant conditions. In healthy donors, selected biomarkers released by cultured clots are induced to the same donor-specific amplitude over time, suggesting that the device could be developed and used as a personalized medical device to monitor health and disease states. We also determined that the device is capable of detecting innate immune responses (or lack thereof) to certain drugs. Lipidomics additionally showed that certain drugs enhance the release of lipid mediators tied to pain. We believe that our cultured clot device has promise as a NAM to explore personalized drug responses by systemic primary human white blood cells. Identification of a panel of novel cultured clot biomarkers in human plasma could help discriminate thrombosis from bleeding, for example.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The cultured clot device has the potential to reveal human innate immune mixed cellular responses that mouse models are unable to detect, because mice have a different innate immune system compared to humans. For example, mice are lacking IL-8/CXCL8, a key inflammation mediator. Arginase-1, a key enzyme involved in alternative inflammatory responses, is expressed in neutrophils in humans, and monocytes/macrophages in mice. Mice also lack the gene encoding FUT3, the enzyme responsible for producing the Lewis blood antigen. Lewis blood groups are associated with cardiovascular disease and susceptibility to infection by certain microbial pathogens. The human cultured blood clot is a novel tissue engineered device that could be used to develop a panel of biomarkers to profile thrombotic disease states and provide ideas for new therapeutic approaches. The device could also be used to screen drugs or bioactive factors for anti-inflammatory or pro-inflammatory activity. One challenge will be in collecting a large number of samples to determine the potential effect of demographics on response profiles. An on-line database could be built to generate large data for pattern profiling.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Challenges of the cultured clot device as a NAM technology include that it is a closed system and lacks vasculature or endothelial cell responses. One strategy to overcome this limitation would be to develop co-culture systems, however the endothelial cells would be either cell lines or obtained from limited primary cell sources. Alternatively, bioactivity of cultured clot soluble factors could be tested in endothelial barrier models. Another challenge is reproducibility and sensitivity of biomarker detection. Use of Mass Spectrometry and enrichment strategies for targeted biomarkers is one strategy that will improve the reliability of the technology. Statistical methods will also be important for identifying response patterns in cultured clots generated with patient blood instead of blood from healthy human donors. Another challenge in deploying this NAM technology is the regulatory hurdles that need to be overcome to implement the cultured clot system in the clinic or hospital setting.

Email: choemann@gmu.edu

Submit date: 8/15/2023

Name: Eric Jonak

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Human diseases require technologies rooted in human biology for us to effectively help patients. Animal models fail to recapitulate human physiology and are not human-relevant in drug discovery.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   To catalyze the development and validation of NAMs, we must have a concerted government funding effort like we did for the Human Genome Project. We must shift money and resources from flawed animal models to human-relevant technologies.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   There are 2 complementary approaches: (1) NIH funding methodologies must prioritize NAMs, which means boosting NCATS's budget; and (2) Some of NAM technologies should fall in the public domain to enable open-source R&D.

Email: ejonak@gmail.com

Submit date: 8/15/2023

Name: Marian Casey

Type of Organization: Not applicable

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Human diseases require technologies rooted in human biology for us to effectively help patients. Animal models fail to recapitulate human physiology and not human-relevant in drug discovery.

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3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   There are 2 complementary approaches: (1) NIH funding methodologies must prioritize NAMs, which means boosting NCATS's budget; and (2) Some of NAM technologies should fall in the public domain to enable open-source R&D.
   We also need to amend the Animal Welfare Act to add rats, mice, fish as protected “animals” and mice so they are not used in research.

Submit date: 8/15/2023

I am responding to this RFI: On behalf of myself

Name: Stephen Ferguson

Name of Organization: National Institute of Environmental Health Sciences

Type of Organization: Government agency

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   NAMs are being developed across the biomedical research community with great aspirations to understand and cure disease. These efforts have largely been driven by biomedical engineering teams that lack the transdisciplinary resources to advance the sophisticated dynamics of pathophysiological action. This is evident from the widespread use of immortalized cancer cells in these complex systems that are presumed to reflect healthy cells from human simply because they were derived from a human tissue, but in fact often poorly reflect differentiated tissue-like functionality. To realize the potential of advanced cell systems computational models that represent the next generation of biomedical tools, we must raise the bar on the scope, quality and differentiability of human cells available to scientists and clinicians from the more than 40 tissues collected in guideline animal toxicology studies. NIH has a unique opportunity to foster formation of centers of excellence that address this critical need and better realize the hope of all organ donors that their gift make a meaningful contribution to human health. Establishing best practices to transition patient-derived cells from healthy, diseased, and pre-disease donor tissues to researchers in critical need of greater physiological relevance in therapeutic development and disease etiology modeling is essential to realize the potential of NAMs. NIH has stated clear goals towards protection of our most sensitive/susceptible populations. These elevated biomedical resources would enable expanded opportunities for cell therapy, geospatial focused testing of epidemiological hypotheses, and enhance modeling of pathophysiological action for drug therapeutic development, environmental chemicals risk assessments (e.g., mixtures) and green chemistry screening. Access to these cells enables both complex and simpler microphysiological systems to be developed for all human cell types, which can be combined with 3D cell culture tools to enable lego-like assembly of more complex organ system axes. Moreover, these investments would enable broad coverage of lifestyle and genetic drivers of disease that better capture the ranges of interindividual variability. Sadly, with existing cell sourcing option, the grand hopes of tissue engineering are extremely hampered by the lack of access to high quality human cells to establish benchmarks and display recognizable aspects of tissue function and disease that enable mechanistic understanding and advances to human health. Ultimately, computational models in tandem with cell systems can integrate information from patients (e.g., haplotypes of susceptibility, lifestyle, drug therapeutics) to reveal a more complete understanding of xenobiotic interactions and human translation.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   NAMs must be right-sized in complexity to the question at hand. For many scenarios, simple systems can be advanced with exiting technologies and approaches into validation efforts in the near term. However, careful consideration into the investment of these efforts should be made if the translational application of these NAMs is unclear. Can a simpler system give the same answer? If so, those systems should be validated with highest priority while more complex systems sort out their respective contexts of use. NIH can serve to coordinate these types of efforts to accelerate fit-for-purpose use of NAMs to a wide range of human health effects. Prioritizing these systems should consider both near-term feasibility and unmet needs (multi-tissue/organ axes).

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   To realize the greatest value with NAMs, we must establish a higher bar for our cell sourcing with NAMs. Cancer cells do not generally function analogous to normal, healthy cells, yet we rely on them to somehow do so in many NAMs. We must establish tissue benchmarks of tissue-like functionality for all human tissues that set the bar for functionality. This is particularly true with liver and kidney where we know the dynamics of metabolism and transport of chemicals play essential roles in disease progression in response to xenobiotic exposures. How can we account for scaling of organs/tissues in an intentional manner that tune NAMs to physiological relevance. In an ideal scenario, NAMs can serve as building blocks for cell therapy to help us develop approaches not only to understanding human biology and disease, but to translate those learnings into remediated tissues that can cure or block the progression of disease. For this, we must begin with highly differentiated cell sources from donor tissues that allow us to establish the boundaries of predictive biology.
   One concept to consider is creation of drug safety. NAMs are envisioned to replace animal testing needs in biomedical research. However, the vast array of possible pathological findings in an animal greatly exceeds the present ability of NAMs to cover anything that might go wrong in drug/chemical safety. Imagine a future world where all human tissues for a given patient could be modeled in a single cell system (e.g., 3D spheroid models) to more comprehensively cover their relative safety in context with health and susceptible donor cells. Induced pluripotent stem cells have been envisioned to fulfill these types of lofty goals, but our ability to differentiated iPS cells to mature functionality is extremely limited for many tissues. To maximize the potential of NAMs, first we must establish benchmarks of cellular/tissue function for NAMs. Next, mapping of requisite cells into appropriate models holds the promise of addressing idiosyncratic toxicity and unraveling the complexities of human disease.

Email: stephen.ferguson@nih.gov

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Emily R. Trunnell, Ph.D.

Name of Organization: People for the Ethical Treatment of Animals

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

   Translating basic science and pre-clinical research into meaningful, affordable outcomes for patients is a critical challenge in biomedical research. Despite decades of research and billions of dollars invested in animal-based models of human biology, circuits, systems, and disease states, effective treatments for many debilitating and deadly human diseases remain elusive. The "translation gap" between data emerging from biomedical research and understanding/treating human health is due, in part, to the limitations of animal models.

   Species differences in anatomy, physiology, and gene expression—affecting developmental trajectories, metabolism, immune responses, disease susceptibility, and more—make translating data from an animal experiment into a human-relevant preventative measure, treatment, or cure extremely difficult. Animal models are often oversimplified and artificial versions of a complex human behavior, trait, or pathology, with targets that may be meaningful in an animal laboratory but are ultimately inadequate for humans. Poor study design combined with the confinement and unnatural conditions of laboratory life further undermine the internal validity of animal research. Depending on the disease area of interest, novel drugs for humans fail in clinical trials between 90 and 100% of the time. The vast majority (90%) of "highly promising" basic science discoveries (most of them from experiments on animals) make no difference at all for human patients (Contopoulos-Ioannidis 2003).

   The failure of animal-based research models and assays is contributing to the increased costs of drug development and the public's declining trust in science. If our finite public funds are to be used responsibly, they must fund reliable research and test methods that lead to effective treatment of diseases and protection of human health.

   Motivated by both the ethical concerns surrounding animal-based experimentation and testing as well as the limited translatability of animal-based data, advances in novel, non-animal methods (a.k.a. novel alternative methods or NAMs) like complex, 3-D cellular models, such as microphysiological systems, organoids, spheroids, and 3-D bioprinted structures derived from human cell lines and based in human biology have expanded in the past decade. Many of these models simulate human physiology and disease more accurately than traditional in vivo animal models do because they do not have to overcome the translational species hurdle. Currently, these tools are accessible to researchers working directly on their application and development. However, given their potential to improve preclinical and basic research as well as ongoing advances in their design, it is essential that investigators with knowledge or access gaps have the opportunity to take advantage of these cutting-edge in vitro methods. We cannot know how much progress might have been made if funding agencies had already made novel, non-animal methods a priority, but there is

now a chance for them to catch up. It is both scientifically and ethically imperative that the NIH make the shifting of funding priorities toward non-animal methods and away from animal-based methods its agency-wide priority.

There are many examples that demonstrate the scientific utility of non-animal methods over animal-based research for advancing progress into understanding specific biological processes or human states, including currently underserved areas of biomedical research. Here are just a few of the papers that demonstrate or describe their potential to be truly revolutionary for understanding/treating human health:

Adegbola A, Bury LA, Fu C, Zhang M, Wynshaw-Boris A. Concise review: Induced pluripotent stem cell models for neuropsychiatric diseases. Stem Cells Transl Med. 2017;6(12):2062-2070.

Al-Hilal TA, Keshavarz A, Kadry H, et al. Pulmonary-arterial-hypertension (PAH)–on-a-chip: Fabrication, validation and application. Lab Chip. 2020;20(18):3334-3345.

Allen A, Deshmukh H. All on “CHIP”: Using microfluidics to study neutrophil ontogeny. Transl Res. 2017;190:1-3.

Arzua T, Yan Y, Jiang C, et al. Modeling alcohol-induced neurotoxicity using human induced pluripotent stem cell–derived three-dimensional cerebral organoids. Transl Psychiatry. 2020;10(1):347

Barrile R, van der Meer AD, Park H, et al. Organ-on-Chip Recapitulates Thrombosis Induced by an anti-CD154 Monoclonal Antibody: Translational Potential of Advanced Microengineered Systems. Clin Pharmacol Ther. 2018;104(6):1240-1248.

Bergers LIJC, Reijnders CMA, van den Broek LJ, et al. Immune-competent human skin disease models. Drug Discov Today. 2016;21(9):1479-1488.

Beydag-Tasöz BS, Yennek S, Grapin-Botton A. Towards a better understanding of diabetes mellitus using organoid models. Nat Rev Endocrinol. 2023;19(4):232-248.

Blaurock-Möller N, Gröger M, Siwczak F, et al. CAAP48, a new sepsis biomarker, induces hepatic dysfunction in an in vitro liver-on-chip model. Front Immunol. 2019;10:273.

Brown D, Namas RA, Almahmoud K, et al. Trauma in silico: Individual-specific mathematical models and virtual clinical populations. Sci Transl Med. 2015;7(285):285ra61.

Brown JA, Codreanu SG, Shi M, et al. Metabolic consequences of inflammatory disruption of the blood-brain barrier in an organ-on-chip model of the human neurovascular unit. J Neuroinflammation. 2016;13(1):306.

Cerchia C, Lavecchia A. New avenues in artificial-intelligence-assisted drug discovery. Drug Discov Today. 2023;28(4):103516.

Cerneckis J, Bu G, Shi Y. Pushing the boundaries of brain organoids to study Alzheimer's disease. Trends Mol Med. 2023;29(8):659-672.

Cohen A, Ioannidis K, Ehrlich A, et al. Mechanism and reversal of drug-induced nephrotoxicity on a chip. Sci Transl Med. 2021;13(582):eabd6299.

Cuní-López C, Stewart R, White AR, Quek H. 3D in vitro modelling of human patient microglia: A focus on clinical translation and drug development in neurodegenerative diseases. J Neuroimmunol. 2023;375:578017.

Dauth S, Maoz BM, Sheehy SP, et al. Neurons derived from different brain regions are inherently different in vitro: A novel multiregional brain-on-a-chip. J Neurophysiol. 2017;117(3):1320-1341.

De Filippis L, Halikere A, McGowan H, et al. Ethanol-mediated activation of the NLRP3 inflammasome in iPS cells and iPS cells-derived neural progenitor cells. Mol Brain. 2016;9(1):51.

Diebel LN, Wheaton M, Liberati DM. The protective role of estrogen on endothelial and glycocalyx barriers after shock conditions: A microfluidic study. Surgery. 2021;169(3):678-685.
Dirven H, Vist GE, Bandhakavi S, et al. Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review. Sci Rep. 2021;11(1):6403.
Ehling P, Meuth P, Eichinger P, et al. Human T cells in silico: Modelling their electrophysiological behaviour in health and disease. J Theor Biol. 2016;404:236-250
Ethier SP, Guest ST, Garrett-Mayer E, et al. Development and implementation of the SUM breast cancer cell line functional genomics knowledge base. NPJ Breast Cancer. 2020;6:30.
Ewart L, Apostolou A, Briggs SA, et al. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. Commun Med (Lond). 2022;2(1):154.
Fernández-Costa JM, Tejedera-Vilafranca A, Fernández-Garibay X, Ramón-Azcón J. Muscle-on-a-chip devices: a new era for in vitro modelling of muscular dystrophies. Dis Model Mech. 2023;16(6):dmm050107.
Fosse V, Oldoni E, Bietrix F, et al. Recommendations for robust and reproducible preclinical research in personalised medicine. BMC Med. 2023;21(1):14.
Haggarty SJ, Silva MC, Cross A, Brandon NJ, Perlis RH. Advancing drug discovery for neuropsychiatric disorders using patient-specific stem cell models. Mol Cell Neurosci. 2016;73:104-115.
Hartung T. A call for a Human Exposome Project. ALTEX. 2023;40(1):4-33.
Hoang P, Wang J, Conklin BR, Healy KE, Ma Z. Generation of spatial-patterned early-developing cardiac organoids using human pluripotent stem cells. Nat Protoc. 2018;13(4):723-737.
Hockney S, Parker J, Turner JE, et al. Next generation organoid engineering to replace animals in cancer drug testing. Biochem Pharmacol. 2023;213:115586.
Landhuis E. Deep learning takes on tumours. Nature. 2020;580(7804):551-553.
Lee CT, Chen J, Kindberg AA, et al. CYP3A5 mediates effects of cocaine on human neocorticogenesis: Studies using an in vitro 3D self-organized hPSC model with a single cortex-like unit. Neuropsychopharmacology. 2017;42(3):774-784.
Levy RJ, Paşca SP. What Have Organoids and Assembloids Taught Us About the Pathophysiology of Neuropsychiatric Disorders?. Biol Psychiatry. 2023;93(7):632-641.
Lieberman R, Kranzler HR, Levine ES, Covault J. Examining the effects of alcohol on GABAA receptor mRNA expression and function in neural cultures generated from control and alcohol dependent donor induced pluripotent stem cells. Alcohol. 2018;66:45-53.
Lim K, Donovan APA, Tang W, et al. Organoid modeling of human fetal lung alveolar development reveals mechanisms of cell fate patterning and neonatal respiratory disease. Cell Stem Cell. 2023;30(1):20-37.e9.
Lyu Z, Park J, Kim KM, et al. A neurovascular-unit-on-a-chip for the evaluation of the restorative potential of stem cell therapies for ischaemic stroke. Nat Biomed Eng. 2021;5(8):847-863.
Kim H, Park HJ, Choi H, et al. Modeling G2019S-LRRK2 Sporadic Parkinson's Disease in 3D Midbrain Organoids. Stem Cell Reports. 2019;12(3):518-531.
Meigs L, Smirnova L, Rovida C, Leist M, Hartung T. Animal testing and its alternatives—the most important omics is economics. ALTEX. 2018;35(3):275-305.
Meng F, Meyer CM, Joung D, Vallera DA, McAlpine MC, Panoskaltsis-Mortari A. 3D bioprinted in vitro metastatic models via reconstruction of tumor microenvironments. Adv Mater. 2019;31(10):1806899.

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Additional Supporting Resources:

Contopoulos-Ioannidis DG, Ntzani E, Ioannidis JP. Translation of highly promising basic science research into clinical applications. Am J Med. 2003;114(6):477-484.
Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. J Transl Med. 2018;16:304.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

If non-animal methods (a.k.a. novel alternative methods or NAMs) are to live up to their potential to transform biomedical research and catalyze discovery, their adoption must be commensurate with intense rigor. Otherwise, we risk abandoning critical methodologies and experiments not because they are fundamentally incorrect, but because they were improperly used. This would be a tragedy. Good laboratory and good cell culture practices are imperative. To aid in ensuring the robustness, replicability, reproducibility, and reliability of the technologies and the ensuing datasets, the NIH can provide dedicated funding for researchers in different laboratories to repeat experiments and fund accessible, public data repositories to promote transparency and data sharing. The NIH should also mandate that grantees adhere to high quality reporting standards, several of which have been recommended in the literature (see Supporting Resources). The UK's National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) is currently undertaking a user testing study of its Reporting In Vitro Experiments Responsibly (RIVER) guidelines and have recently made a preprint available on these recommendations (The RIVER Working Group). These recommendations should ideally be in place for all research funded or undertaken by the NIH, but are increasingly important for non-animal methods so that their value is fully appreciated, validated, and trusted.

Supporting Resources:

Emmerich CH, Harris CM. Minimum Information and Quality Standards for Conducting, Reporting, and Organizing In Vitro Research. Handb Exp Pharmacol. 2020;257:177-196.
Hartung T, De Vries R, Hoffmann S, et al. Toward Good In Vitro Reporting Standards. ALTEX. 2019;36(1):3-17.
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The River Working Group. Reporting in vitro experiments responsibly – The RIVER recommendations. MetaArXiv preprints. Updated June 21, 2023. Accessed August 15, 2023. https://osf.io/preprints/metaarxiv/x6aut/.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

While there are research methods that can be used to study living humans (such as imaging), most methods are necessarily reductive. It will likely be the case that researchers or research groups need to use several non-animal methods (a.k.a. novel alternative methods or NAMs) in order understand a biological system or disease state. The benefit of non-animal, human biology-based methods is that, unlike animal-based methods, the system or state of interest is no longer in an entirely different species. Many of these platforms can even be used to study systems and states in the individual patient of interest, using tissue and cell samples or genetic data, for example.

A key strategy for bolstering technology readiness and the reliability of these technologies and ensuring their successful integration across research approaches and potential solutions is to increase funding for, access to, and training in these methodologies. This could be done by 1) making funding for non-animal research more readily available, 2) prioritizing non-animal research methods in training opportunities, and 3) establishing and expanding animal-free biomedical research resources.

1) Make funding for non-animal research more readily available: Decisions about grant funding must prioritize applicants who currently use non-animal methods, are making the transition from animal to non-animal methods, or are developing and/or validating non-animal methods. The NIH should offer Program Project Grants or Center Grants (P01/P30/P50) to investigators interested in establishing centers for non-animal methods at their institutions. The NIH should offer grant supplements to investigators who want to switch to non-animal methods mid-funding.

2) Training opportunities must prioritize non-animal research methods. The NIH should offer Institutional Training Grants to trainees at the undergraduate, graduate, and postdoctoral levels to receive training that would allow them to make the transition from animal to non-animal research methods. It should place particular emphasis on post-doctoral training fellowships that allow young scientists to receive training in non-animal methods. The NIH should offer Continuing Education Training Grants with the explicit purpose of establishing educational programs to train researchers on available non-animal methodologies. The NIH should offer awards to early stage investigators who are looking to switch from using animal models to conducting non-animal research. The NIH Director's Early Independence Award should prioritize applicants who currently use non-animal, clinically-applicable methods; are making the transition from animal to non-animal methods; or are developing and/or validating non-animal methods. The NIH Bench-to-Bedside and Back Program should prioritize pairing basic science researchers using animal models with Intramural Research Program (IRP) clinical researchers. The goal should be to assist those researchers interested in permanently switching from animal-based research to clinical work. The NIH Graduate Partnership Program should prioritize those students who are hoping to use non-animal methods in their research but do not have access to those tools at their home institution. These are just a few ideas.

3) Establish/Expand Animal-Free Biomedical Research Resources: The Office of Strategic Coordination—within the Office of the Director—should use the NIH Common Fund to establish multiple centers for non-animal methods across the U.S., as we suggested in a recent submission to an NIH Common Fund RFI. The NIH should establish Core Facilities at the NIH IRP that will provide investigators with access to resources and experts in the use of non-animal methods. Suggestions for such core facilities include a microphysiological systems core, an animal-free antibodies core, and a three-dimensional tissue printing core. The NIH should expand the current Human Tissue and Organ Research Resource. The NIH should require grant recipients to share their human bio samples with the "All of Us Research Program" biobank.

As mentioned above, it is imperative that with increased funding for non-animal methods comes a mandate of rigorous practices, reporting, and data sharing.

Email: emilyt@peta.org

Submit date: 8/16/2023

I am responding to this RFI: On behalf of myself

Name: Vito Mennella

Name of Organization: University of Cambridge/MRC Toxicology unit

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   We are building airway cellular models using CRISPR Cas9 KO which recapitulate disease states, in particular rare diseases of the lung such as Primary Ciliary Dyskinesia caused by single gene mutations. These cellular models will be used as a tool to understand toxicities caused by Particulate Matter in the environment and to test RNA and read through therapies.
   We are building immune-airway co-culture models, including from cells (airway, endothelial, macrophages etc.) obtained from same patient to mimic human specific biology and for personalised medicine.
   We have developed a deep phenotyping pipeline in airway cellular models combining several spatial biology tools to understand how well the models replicate in situ organization of the tissue (Volume EM and Super-resolution microscopy) and how the tissue respond in healthy, disease and toxic states (spatial transcriptomics, Bio-ID)
   We are performing a comparative analysis of airway immortalised lines and primary cells (bulk and ScRNAseq, proteomics) to understand the potential differences in response caused by sex, age, ethnicity and immortalization procedures.
   NAMs can have a transformative power to model rare diseases for RNA therapies and analysis of toxicity and for rapid response to airway diseases as shown already in the case of Covid-19 pandemic and Cystic Fibrosis research.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   It is important to perform a comparative analysis of immortalised lines and primary cells (bulk and ScRNAseq, proteomics) to understand the potential differences in response caused by sex, age, ethnicity and immortalization procedures.
   It should be a key strategy decision the creation of a repository with characterised cell lines available to researchers worldwide, which also provide access to primary cells from patients with matching characteristics.
   There is a need for standardization of cellular material, models and platforms used for analysis and access as some of them are commercial available and too expensive for individual laboratories and institutes.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Building a NAM taskforce divided by tissue/organ modelling that establishes guidelines. Mandatory requirements to share methodologies for publication with NIH funding and when accessing cell/tissue banks

Studies need to include analysis in primary cells taking into account sex, age and ethnicities. As this might be difficult to obtain for basic research laboratories, a centralised source should be made available, which will also improve standardization.

Email: vm430@cam.ac.uk

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Vicky Robinson

Name of Organization: NC3Rs

Type of Organization: Other

Type of Organization-Other: Science organisation, including research funder

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Novel alternative methods (NAMs) are increasingly being used in biomedical research to address a range of important scientific questions relevant to human health and the development of safe and efficacious treatments. In the UK the development of NAMs is primarily led by the NC3Rs (https://nc3rs.org.uk/), a government-backed organisation with core funding from the Medical Research Council and Biotechnology and Biological Sciences Research Council, which are part of UK Research and Innovation. The NC3Rs welcomes the opportunity to respond to this Request for Information and recognises the leadership that the NIH could bring to the area of NAMs.
   The NC3Rs invests in the development of NAMs through its research and innovation funding schemes with over £100M committed to date – over two-thirds of this has been for NAMs that avoid the use of animals. The primary focus of our investment is to develop models, tools and technologies that reduce reliance on in vivo models or improve animal welfare, however, we have demonstrated that NAMs provide considerable additional scientific value in terms of their predictivity, reliability and translational relevance as well as commercial opportunities. Examples of the impacts of the NC3Rs investments can be found in the reviews of our research funding portfolio and CRACK IT Challenges innovation programme that were published in 2019.
   NC3Rs Research review: https://nc3rs.org.uk/sites/default/files/2021-09/NC3Rs%20Research%20Review%202019.pdf
   CRACK IT review: https://nc3rs.org.uk/sites/default/files/2021-09/CRACK%20IT%20Review%202019.pdf
   Note: There are various definitions of NAMs across geographical locations and technical disciplines, with the term being used to encompass new approach methodologies, non-animal methods/models and novel alternative methods. While the fundamental aims of these approaches are improved science through the development of non-animal approaches, their use across multiple sectors has resulted in different definitions. We use NAMs specifically to refer to the use of approaches that replace the use of animals for assessing the toxicity of drugs and chemicals. However, for the purpose of responding to this consultation we have used NAMs to mean novel alternative methods in line with the NIH definition. Further information on the NC3Rs definitions can be found at https://nc3rs.org.uk/who-we-are/3rs#replacement.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

The availability of complex in vitro models such as microphysiological systems and new advances in artificial intelligence technologies are providing for the first time the opportunity to make substantive and wholesale changes to the way animals are used. These are exciting times but there is much to be done to exploit and capitalise on this momentum in NAMs. Significant barriers remain despite the large investments in NAMs development worldwide. Barriers include poor awareness about the availability of NAMs and a lack of confidence in their utility. These are compounded by a reluctance in the research community to change practice that is in part based on the long-term polarisation of the animal research issue, and a frequent bias in the system that favours in vivo models over non-animal approaches. For the academic sector the latter includes the "publish or perish" culture which reduces the incentive to shift to new approaches (animal or NAMs) even when the model has a poor record of translation. Careers are often built on the use of specific in vivo models and shifting from these can be challenging because of the difficulty in securing funding to use new models and tools without an existing track record in them. Often the level of validation required for NAMs is high and beyond that required for most gold standard animal models – as a result there is no incentive to develop or use better models because of the hurdles faced and the perceived disadvantage researchers may experience for example with delays to publications or additional requests for studies from reviewers.

All of these factors mean that there is often a long lag between the development of NAMs and their deployment into routine practice. A range of approaches are required to address this. We have focused on building confidence in the use of NAMs, raising awareness about their availability and utility, building capacity and skills, and de-risking their adoption into common research practice. Our programmes are summarised below. A central theme underpinning these is the importance of engaging the research community in developing solutions, facilitating partnerships between academic and industrial stakeholders and working internationally.

NAMs funding pipeline from concept to commercialisation:

We have established a comprehensive funding pipeline that supports initial concept development through to comparative, validation, feasibility and reproducibility studies and where appropriate commercialisation. The pipeline is intended to ensure that NAMs developed with NC3Rs support are well-characterised and ready for deployment. We have introduced a dedicated funding scheme to address issues relating to the validation and the transferability of NAMs. Our Skills and Knowledge Transfer awards allow researchers to gain experience and expertise in the use of alternative approaches by facilitating the dissemination of models and tools between the developer's lab and that of end-users helping to build confidence by allowing new and existing/standard approaches to be used in parallel. Examples of the NAMs specific awards we have made can be found at https://www.nc3rs.org.uk/our-portfolio/search?activity_type%5b%5d=7&r%5b%5d=19 .

The investment in NAMs needs to extend to their commercialisation so that they are widely available for use as "off the shelf" products and services. This includes support for technologies that underpin model development and the use of NAMs. For example, we have supported research into fully-synthetic, self-assembling peptide hydrogels to replace the use of animal-derived matrices and the associated issues with batch-to-batch variation that compromise the reliability and reproducibility of in vitro models. The research has recently been commercialised – the market

value for such innovations to support NAMs is significant yet the commercial benefits have on the whole yet to be exploited.

We have dedicated funding through our Business Growth Scheme to support the business and commercial opportunities that arise from the development of NAMs emerging through our CRACK IT Challenges innovation programme. This scheme has supported the formation of several companies including Vivaltes – a Netherlands based SME that has developed in silico models for toxicity testing.

Publishing detailed methodologies and validation studies:

We have launched a dedicated publishing platform for NC3Rs-funded researchers. There is a lack of information in the public domain on NAMs to allow researchers to critique them, assess their suitability for use in their own labs or reproduce findings from them. We have addressed this for NC3Rs-funded research by launching the NC3Rs gateway (https://f1000research.com/nc3rs) in partnership with the publisher F1000Research.

The gateway provides a dedicated platform for NC3Rs grant holders to publish methodologies and validation studies. Although relatively early days, the metrics are very positive. For example, there are 35 articles published on the gateway – based on data from the gateway and PubMed these have been viewed 78,000 times and downloaded 11,000 times. The gateway papers have also been cited 167 times with almost a quarter of these being in papers that have used the methods described.

More broadly, however, there is still little motivation and few incentives for researchers to publish methodologies because of the lack of recognition and reward for this, despite the increased focus across the sector on research integrity and the importance of transparent methodologies to this. This needs to be addressed at a national and international level and we would welcome the opportunity to collaborate on this.

Enhancing the utility of in vitro models:

Evidence suggests that issues related to internal validity and bias are commonplace in in vitro studies. We have set out to address this as it undermines confidence in their use by others. We have recently published as a pre-print the RIVER recommendations for reporting in vitro studies (https://nc3rs.org.uk/our-portfolio/river-recommendations). The recommendations are focused on six areas that all documents reporting in vitro research should include for a reader to assess the methodological rigour and reliability of the study. The recommendations have been developed by an international expert group, convened by the NC3Rs, which includes a representative from the NIH. The recommendations are undergoing user testing prior to publication in the peer-reviewed literature. The goal is for the RIVER recommendations to be endorsed and actively used by journals (e.g. in editorial checks and/or guidance to authors) and funders (e.g. in the description of in vitro experiments in grant applications) so that the reliability of the studies can be fully evaluated. The NIH has been proactive in encouraging the use of the ARRIVE 2.0 guidelines for reporting animal research and we would welcome similar leadership on the RIVER recommendations.

Much of the investment in NAMs to date has been in ensuring their biological relevance. It is essential that there is work in parallel to ensure that NAMs are easy to use, reproducible and robust – these remain key barriers to adoption for disease modelling, efficacy and safety studies. We are tackling this for organ-on-chip technology with our recently launched SensOoChip CRACK IT Challenge which focuses on incorporating longitudinal, multiparametric monitoring capabilities through advanced engineering approaches to improve the utility and reproducibility of connected organ-on-a-chip devices (https://nc3rs.org.uk/crackit/sensoochip). We aim to demonstrate that the multiparametric datasets generated can be interrogated and modelled to improve understanding of

the local physiological environment, reproducibility of the devices and the effect of drug administration. We have committed almost £3M to the Challenge which is also sponsored by five global pharmaceutical companies, illustrating the importance of efforts focused in this area.

Supporting the NAMs skills base:

We have recently evolved our PhD Studentship Scheme so that it only supports training in the development and application of NAMs (previously it supported projects on reducing and refining animal use). This evolution is an important step for ensuring the UK has a scientific workforce able to respond to future demands from the sector for expertise and skills in NAMs.

Championing policy and regulatory changes:

The adoption of NAMs and the addressing of barriers that prevent their rapid use requires a change in the policy and regulatory environment. The UK is widely regarded as having comprehensive legislation on the use of animals in scientific procedures that only permits the use of in vivo models where there are no alternatives.

We recently commissioned an independent report on the review and oversight processes for animal research in the academic sector, from funder to ethics committee (equivalent to the IACUC) and regulator. The report highlighted significant gaps in the consideration of NAMs in all parts of the system (https://nc3rs.org.uk/sites/default/files/2023-02/Rawle%20project%20report.pdf). We are currently working with UK funders to address the recommendations in the report and in particular how NAMs can be better considered as part of the peer review process. It is unlikely the findings of the report with regards to NAMs are unique to the UK.

The role of journals in facilitating the use of NAMs has not been explored to any extent, although anecdotal evidence suggests that it is not uncommon for referees and editors to ask for additional animal studies when data from NAMs is submitted, particularly for demonstrating physiological relevance, even if the in vivo models are known to have limitations. This reflects the lack of confidence in alternatives and the inertia around animal models. There is a significant piece of work to be undertaken to look at the role journals could play to expedite the use of NAMs and we would be pleased to work with the NIH and other international partners to deliver this.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

   We have addressed this in our response to question 2.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/NC3Rs-responseRFI.08.23.pdf

Description: The NC3Rs response with hyperlinks.

Email: vicky.robinson@nc3rs.org.uk

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Carter Alleman

Name of Organization: American Society for Pharmacology and Experimental Therapeutics

Type of Organization: Professional org association

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Many studies in pharmacology and toxicology have been utilizing NAMs to advance our understanding of toxic substances, risk assessments, and drug development, especially with the most recent rapid developments in machine learning and AI technologies. Many groups are adopting the reduce, refine and replace animal use in research studies. Using NAMs with sufficient data, testing, and evaluation metrics are the hallmarks of what allowed these models to proceed through the development pipeline.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   ASPET believes that in order to build more confidence in the performance, replicability and reproducibility of NAMs in order to reliably use them, there needs to be clear consensus on effective strategies for evaluating and standardizing them and their use. A current challenge is the absence of guidelines or criteria to conduct validation studies on different types of NAMs. NIH should prioritize the development of validation and standardization guidelines before allocating funding toward new technology development. This should include emphasis on NAMs data confirmatory with human and animal studies and developing new mechanisms to foster collaboration between animal researchers and NAMs developers to perform validation studies. Moreover, strengthening interagency partnerships to develop a coordinated NAMs approach that enables science to advance efficiently while minimizing administrative and regulatory burden.
   Other challenges to building in robustness, replicability, reproducibility and reliability of NAM include data reporting requirements for NAM studies, as well as the time and costs to conduct non-animal studies, although the latter can vary by discipline and sector. ASPET recommends conducting cost-effective analyses of proposed NAMs with existing methods like animal studies. Specific aspects to consider through these analyses include time, scalability, and resource efficiency. Not only will the costs associated with scaling NAMs for broad deployment be significant, but in many cases, combinations of multiple NAMs may not necessarily outperform single tests where animals are used.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   As mentioned before, to qualify NAMs for regulatory use, more robust data collection, evaluation, and standardization of NAMs is needed. One way to achieve that is through pilot programs and comparative assessments across disciplines and sectors. Most of the current non-animal models are still in the development stage and cannot yet replace all established animal models. Launching pilot and/or case studies to study the predictivity of certain models before publishing draft policies and

risk assessments, as currently done by some agencies like FDA and EPA, is a great flexible approach that can help ensure that the best available data informs agency decision-making. Therefore, as NIH formulates the next steps for NAMs research, ASPET recommends employing data collection opportunities like the mentioned pilot and case studies to help redirect resources according to the latest science while ensuring that subsequent policy implementation reflects multiple stakeholder perspectives.

ASPET also recommends that NIH provides support for research infrastructure, shared resources, and technical staff to promote fair allocation and access to animal and non-animal methods for conducting research. This is because using novel technologies requires resources and training use such methods that resource-limited institutions may not have. To address this, NIH may consider increasing infrastructure grants (G20, C06) to enable institutions to build facilities to keep up the emerging technologies. Moreover, providing targeted funding and career development staff scientists to ensure scientific expertise keeps pace with rapid technology development, including NAMs. One way to achieve this is expanding opportunities for trainees on F-, K-, and T- grants to facilitate their exposure and training with novel methods.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/ASPET-Response-to-RFI-on-NAMs.pdf

Description: ASPET Comment Letter to RFI on NAMs

Email: calleman@aspet.org

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Merel Ritskes-Hoitinga, Remco Westerink, Anne Kienhuis

Name of Organization: Institute for Risk Assessment Sciences (IRAS) Toxicology, Faculty of Veterinary Medicine, Utrecht University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   please see the attached file

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   please see the attached file

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   please see the attached file

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/IRAS-TOX-document-submission-NIH-Request-for-Information-rw_ak_mrh.docx

Description: Input to the NIH request by the Institute for Risk Assessment Sciences Toxicology

Email: j.ritskes-hoitinga@uu.nl

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Katherine McGuire

Name of Organization: American Psychological Association

Type of Organization: Professional org association

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

   APA recognizes that the development of new methods and tools will propel biomedical and behavioral research forward, including both animal and non-animal models. We support the Advisory Committee to the NIH Director's decision to investigate how NAMs may enhance NIH research studies. However, APA agrees with the comments of the Federation of American Societies for Experimental Biology (FASEB) that animal studies are at present and in the foreseeable future the most valid and reliable method for numerous areas of research. Clarifying the contexts in which NAMs may be appropriate and highlighting their use to supplement research with animals is an important step in adjusting end-user and public expectations until validation strategies, metrics, and regulatory pathways become more defined.

   The development of new technologies to extend the boundaries of understanding is a touchstone of scientific progress. New and emerging technologies such as in chemico methods, computer simulations, organs-on-chips and 3D digital imaging are making exciting contributions to our understanding of biology and behavior. Each of these technologies must proceed through the development pipeline with common steps: undergoing testing, providing convincing data and clear endpoints, rigorous benchmarking studies, and evaluation metrics. To maximize scientific utility and achieve clinical and regulatory acceptance, continuous collection and evaluation of NAMs data through pilot programs and comparative assessments are essential. Scientists who are acting within the high standards of NIH's peer review guidelines, rather than pressure from outside advocacy organizations, must determine when and how novel alternative technologies should be employed to ensure the health and safety of the public.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

   New models must be validated to receive acceptance. As NIH outlines future areas of investment, APA agrees that model development and validation should be considered concurrently and that providing parallel funding for validation studies alongside future NAM grants, initiatives, or projects seems reasonable. Consistent validation guidelines and confirmatory data from human and animal studies alike are needed. Otherwise, the development of NAMs will continue to outpace scientific standards and applications, resulting in inefficient use of research time and federal dollars (and increased pressure for NIH to apply technologies before they can be considered safe and ready).

   To inform NIH's future investment strategy in NAMs, APA supports FASEB's recommendation to conduct cost-effective analyses of proposed technologies with existing methods, including animal studies, looking at time, scalability, and resource efficiency. It is possible that combinations of

multiple NAMs may not outperform single tests involving animals, further increasing costs. While this type of analysis may be resource-intensive, this information would facilitate proper investment of federal dollars and may support the public's understanding of the state of the science involving NAMs.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   "APA agrees with a recent National Academy of Sciences, Engineering and Medicine (NASEM) recommendation that NIH develop new mechanisms to foster collaboration between animal researchers and NAM developers. The NASEM report, "Nonhuman Primate Models in Biomedical Research underscores how nonhuman primates (NHPs) remain essential for NIH-supported biomedical research given the lack of qualified and validated NAMs to answer complex research questions, such as those pertaining to brain function and behavior (Homberg, et al., 2021). To address this, the report emphasizes the importance of enhanced collaboration between NHP researchers and NAMs developers to expand the applicability of non-animal systems. APA encourages NIH to establish multi-laboratory funding opportunities, cross-disciplinary challenge programs, and annual conferences or symposia that mobilize varying perspectives and expertise. For the latter, we strongly advise partnering with scientific societies to leverage their knowledge and networking capabilities for maximal impact and effective policy implementation.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/APA-comment-to-NIH-re-NAMs-8.16.23.pdf

Description: Comment in PDF letter format with references

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Colin Duckett, Vice Dean for Basic Science, School of Medicine

Name of Organization: Duke University

Type of Organization: University

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Please see amended file attached.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Please see our amended file attached.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Please find our amended file attached.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/NIH-RFI-Response_nonanimal-models_2023-08-16_DUSOM-1.pdf

Description: Please find our institutional letter attached. We'd be grateful if you could replace the document that we uploaded previously; it contained some typos and referencing errors.

Email: colin.duckett@duke.edu

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Mikalah Singer

Name of Organization: Center for Contemporary Sciences

Type of Organization: Nonprofit research organization

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   See attachment.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   See attachment.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   See attachment.

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Description: Full comment attached as file.

Email: mikalah@contemporarysciences.org

Submit date: 8/16/2023

I am responding to this RFI: On behalf of myself

Name: John Wikswo

Name of Organization: Vanderbilt University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   There is an ever-growing emphasis on the use of in vitro and in silico models, either alone or coordinated, to address biomedical questions that cannot be readily studied in humans. In silico models have the benefit of not requiring humans, animals, or living cells, but their specification and validation clearly require experimental data recorded from the real system that is being modeled numerically. In vitro cellular models, such as microphysiological systems (MPS), e.g., organoids or single or coupled organ chips, require hardware to maintain these living models and acquire the desired data. Hence, novel alternative methods (NAMs), either in vitro or in silico, will benefit from advanced, novel microfluidic controls and sensors that would simplify, accelerate, extend, and parallelize the required experiments. Similarly, the commercial production of biopharmaceuticals, such as antibodies or functional proteins, could be accelerated by technologies that automate and parallelize the steps following creation of cells genetically engineered to produce the desired biomolecules: selected clonal populations must be expanded, studied, optimized, and fed. Novel microfluidic hardware, sensors, and software might increase the throughput of cell-line and media optimization by an order of magnitude or more, which would be significant given the realization that a single saved day in the development of a pharmaceutical could save a million dollars, and millions of lives might be saved by the accelerated development of a vaccine that addresses an emerging pandemic. While much emphasis has been placed on the refinement of the biological aspects of these approaches, very little attention has been paid to the underfunded area of the development of the hardware infrastructure that is required to support and control the biology. Substantial funding of technology will be required to maximize the scientific utility of NAMs. These technologies will also advance our understanding of specific biological processes or human states, and could be truly revolutionary for understanding and treating human health.
   John Wikswo, Vanderbilt University

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Unfortunately, much of our knowledge of diseases and their pharmaceutical treatment has been derived from animal experiments and simple in vitro cell culture, neither of which may adequately recapitulate the human situation or its response to treatment. For example, although rats can demonstrate addictive behaviors and, for a variety of reasons, are the preferred animal model for opioid addiction research, neurologically and pharmacologically, of course, rats are not humans. This problem is being addressed in part with microphysiological systems (MPS), e.g., organoids or single or coupled organ chips, which represent an entire class of NAMs to study health and disease. There

is a growing realization that organs-on-chips or organoids populated with human cells may serve as in vitro models of human physiology and pathology that are of particular value when it is impossible to obtain in vivo data from humans. However, the national emphasis on human MPS has not addressed the need to validate these models in terms of the vast prior experience with animal experiments. The question of "Does rat model human?" suggests an alternative question "Does human chip model human?" that will be challenging to answer quantitatively. A question that could readily be answered is "Does rat chip model rat?" For organ chips or organoids to make a substantial scientific contribution, there must be a detailed comparison of the chip or organoid to the organism it recapitulates, and such comparisons are most readily made by starting with very well characterized animal models. By comparing in vitro studies in human and rat or other animal chips with in vivo studies in animals, we will not only determine how well the animal chip approximates the animal, but also improve our understanding of how well any of the chips approximate animal and human physiology, pharmacology, and toxicology. The development and acceptance of novel alternative methods would be accelerated by the creation of an innovative platform for the systematic comparison of these methods across species using MPS. Additional funding is needed for advancing organ-on-chip perfusing and sensing technologies to provide enhanced flexibility and dynamic range for understanding many key biological questions and for facilitating drug screening and development, including the in vitro recapitulation of pharmacokinetics and circadian and ultradian hormonal rhythms.

John Wikswo, Vanderbilt University

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

   The scaling of novel alternative methods presents a variety of challenges, particularly with the integration of multiple cutting-edge technologies. Massively parallel bioreactors for organ chips, organoids, or the pilot production of biopharmaceuticals require careful coordination of microfluidic pumps, valves, sensors, microprocessors, and control algorithms, and possibly the introduction of digital twins for process optimization and control. In turn, the arrays of sensors that will be needed for NAMs may span the breadth of analytical chemistry and quantitative biology, including mass spectrometry proteomics and metabolomics, electrochemical and optical measurements of the concentration of multiple chemical species, single-cell and population gene expression profiling with RNAseq, and optical determination of cellular phenotypes, each of which has different requirements for sample collection, preparation, and measurement. In the past, a technician or scientist provided both the coordination and connection of these different technologies, but this approach fails to scale, particularly in today's economy. Robot scientists and self-driving laboratories should help address the demands for increased throughput, lower assay cost, optimal design of experiments, and faster results. However, there appear to be very few NIH funding opportunities to support the requisite technological integration. Several National Laboratories funded by the Department of Energy have major programs wherein their staff develop, for example, advanced mass spectrometers and modeling and experimental tools for bioreactors, and there a number of academic groups that are pursuing these or related topics. What is missing from the NIH funding portfolio are programs that support the development and integration of advanced technologies in support of NAMs.

   John Wikswo, Vanderbilt University

Email: john.wikswo@vanderbilt.edu

Submit date: 8/16/2023

I am responding to this RFI: On behalf of an organization

Name: Link Parikh

Name of Organization: Rocket Technology, Inc.

Type of Organization: Other

Type of Organization-Other: Industry

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   This white paper outlines a comprehensive approach to utilizing IBM's Watson to address the National Institutes of Health's (NIH) interest in innovative methodologies to advance biomedical research. By integrating a. Program and Engineering Lifecycle Management, artificial intelligence governance, neural network, and data science capabilities, and integrated open source software bill of materials cybersecurity, we aim to foster collaboration, accelerate discovery, and reduce reliance on traditional models, aligning with the NIH's goals specified in the Request for Information (RFI). We call this solution the Ecosystem Management and Modeling Solution (EMMS), recognized as a Rocket Technology solution in the IBM Global Solutions Library. The platform suite, data scientists, and support regime is available directly through Rocket Technology and consists of an integration of
   - IBM watsonx and CloudPak for Data (includes COTS NLP, neural network, and its security and governance environment)
   - IBM Engineering Lifecycle Management (our version for Scientific Discovery Management)
   - Sonatype NEXUS for Scanning Open Source and custom software bill of materials (to prevent the now widespread software vulnerability).

   Industrial programs have achieved speeds of 2-3X in complex R&D-based federal and commercial programs with unique innovations and insights that generate leaps in scientific discovery, capability, and capacity.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   2. THE ADVANTAGE OF NEURAL NETWORKS IN DRUG DISCOVERY

   Neural networks, especially deep learning architectures, are revolutionizing therapy development. Here's an expanded view of how these advanced computational models can be employed to support various stages of therapy development:

   2.1. Understanding Disease Mechanisms
   - Complex Pattern Recognition: Neural networks can analyze complex biological data to identify underlying patterns and relationships, providing insights into disease mechanisms.
   - Genomic Analysis: They can process large-scale genomic data to uncover genetic markers and mutations that contribute to disease, paving the way for targeted therapies.

   2.2. Drug Target Identification

- Protein Structure Prediction: By predicting the 3D structure of proteins, neural networks help researchers understand how drugs can interact with targets in the body.
- Drug-Target Interaction Modeling: Neural networks model how potential drugs interact with their targets, shortening the time needed to identify promising compounds.

2.3. Drug Design and Optimization

- De Novo Drug Design: Neural networks can design new drug-like molecules that match specific criteria for binding to a target, providing a starting point for medicinal chemistry.
- Optimization of Lead Compounds: These models can optimize the properties of lead compounds to improve their efficacy, reduce toxicity, and enhance other pharmacokinetic characteristics.

2.4. Predicting Drug Safety and Efficacy

- Toxicity Prediction: Neural networks analyze chemical structures to predict potential toxicity, helping to prioritize compounds early in the development process.
- Efficacy Modeling: By integrating clinical and pre-clinical data, neural networks can create models that predict a drug's efficacy in treating specific conditions.

2.5. Personalized Therapy Development

- Patient Segmentation: Neural networks analyze individual patient data, allowing for the development of personalized treatment protocols.
- Therapy Response Prediction: By understanding individual genetics and disease profiles, neural networks can predict how patients will respond to specific therapies.

2.6. Enhancing Clinical Trials

- Trial Design Optimization: Neural networks can create simulations to determine the optimal design for clinical trials, including sample size, duration, and methods.
- Real-time Monitoring: These models can continually analyze trial data to detect anomalies, provide insights, and ensure the integrity of the trial.

2.7. Post-Market Surveillance

- Adverse Event Monitoring: Neural networks can continuously analyze post-market data to detect unexpected side effects, allowing for rapid response and ensuring ongoing patient safety.
- Treatment Optimization: By tracking long-term outcomes, neural networks can help in refining treatment guidelines and optimizing therapy protocols over time.

The application of neural networks in therapy development represents a transformative approach that leverages complex data analysis, predictive modeling, personalized medicine, and real-time monitoring to accelerate and enhance the process. From understanding disease at the molecular level to optimizing clinical trials and monitoring post-market effects, neural networks are driving a new era of innovation in therapy development.

This comprehensive approach can lead to more targeted, effective, and safer therapeutic interventions. Moreover, it significantly reduces the time and cost associated with traditional drug development processes.

3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:

3. IMPLEMENT A. IBM “WATSONX” – A NEW ERA IN INTELLIGENT SOLUTIONS COUPLED WITH, B. PROGRAM/ENGINEERING LIFECYCLE MANAGEMENT SOLUTIONS WILL TOGETHER DRIVE VALUE FOR NAMS SOLVING CHALLENGES WITH MATURE COTS AND TOOLS THAT ARE OPEN AND FIT INTO EXISTING ARCHITECTURES OR DELIVERED AS A SERVICE.

3.1 watsonx is the latest and leading capability that NIH must adopt now using a small-team, focused approach by a small business

Neural networks, particularly deep learning models, are playing a significant role in transforming the pharmaceutical landscape by aiding in drug discovery.

3.1.1. Predictive Modeling

- Drug Interaction Analysis: Neural networks can predict how drugs will interact with targets in the body, helping researchers identify promising compounds.
- Toxicity Prediction: By analyzing chemical structures, these models can forecast potential toxicity, thereby reducing risks and costs in clinical trials.

3.1.2. Biomarker Identification

- Genomic Data Analysis: Neural networks analyze vast genomic data to identify specific markers for diseases, aiding in targeted therapy development.
- Personalized Treatment Plans: By recognizing unique genetic markers, neural networks can help in creating patient-specific treatment protocols.

3.1.3. Accelerating Clinical Trials

- Patient Recruitment: Neural networks can analyze patient data to identify ideal candidates for trials, speeding up enrollment.
- Real-time Monitoring: These models enable continuous monitoring of trial participants, ensuring safety and efficacy.

3.1.4. Enhancing Drug Repurposing

- Existing Drug Analysis: Neural networks can identify new uses for existing drugs, reducing development time and costs.

3.1.5 Leveraging Decision Optimization Tools in Therapy Development

Decision optimization tools add another layer of efficiency in the development of new medicines and therapies. Here's how:

3.1.5.1. Resource Optimization

- Laboratory Management: By optimizing resource allocation, labs can run more efficiently, speeding up the research process.
- Supply Chain Optimization: Tools can ensure the timely and cost-effective sourcing of necessary research materials.

3.1..5.2. Clinical Trial Optimization

- Trial Design: Optimization tools can design the most efficient trial structures, considering factors like sample size, trial length, and geographical location.
- Risk Mitigation: They help in creating strategies that minimize risks and uncertainties in the trial process.

3.1.5.3. Regulatory Compliance

- Automated Compliance Checks: Optimization tools can automate adherence to regulations, ensuring that the development process remains within legal boundaries.

3.1.5.4. Strategic Decision Making

- Market Analysis: They assist in analyzing market trends, potential competitors, and help in shaping strategic business decisions.
- Investment Optimization: Tools can optimize investment across various research domains, ensuring that funds are utilized where they can be most impactful.

By integrating neural networks with decision optimization tools, the pharmaceutical industry can create a powerful synergy that speeds up the discovery of new medicines and therapies. This combination enhances predictive modeling, optimizes resources, personalizes treatment strategies, and ensures more efficient clinical trials. Such a collaboration represents a substantial leap forward in medical science, promising faster, more targeted, and more effective therapeutic solutions. If required, this information can be transformed into a white paper or a detailed research document tailored to specific needs or audiences.

3.2. PROGRAM/PORTFOLIO LIFECYCLE MANAGEMENT FOR SPEED

3.2. 1 We can now manage Grant and Research Programs more Efficiently and in an Integrated Portfolio to Drive Insights and Overall Speed and Cost Efficiency.

IBM Engineering Lifecycle Management (ELM) platform can provide substantial support in speeding up drug discovery programs by offering an integrated and streamlined approach to managing the entire lifecycle of a drug discovery project.

3.2.2 Collaborative Environment:

- Interdisciplinary Collaboration: ELM facilitates collaboration across various departments, including researchers, data scientists, clinicians, and regulatory experts. It allows real-time sharing of data and insights, enabling a cohesive and efficient workflow.
- Secure Communication: With security features, it ensures that sensitive data related to drug discovery remains protected while still allowing for effective collaboration.

3.2.3 Requirements Management:

- Capture and Trace Requirements: It allows teams to clearly define, capture, and trace requirements throughout the discovery process. This ensures alignment with the project objectives, compliance with regulations, and helps in avoiding costly errors or oversights.
- Impact Analysis: Helps in understanding the impact of changes in requirements, allowing for more informed decisions and reducing delays in the project timeline.
- Advanced Data Analytics and Integration:
- Data Integration: ELM can integrate data from various sources, such as research labs, clinical trials, and third-party databases, creating a comprehensive data environment.
- Real-Time Analytics: Offers analytics tools to interpret complex data, which can lead to quicker insights into the effectiveness of compounds, patient responses, etc.

3.2.4. Project Management:

- Planning and Scheduling: Provides tools for robust planning, scheduling, and resource allocation, ensuring that the project stays on track and within budget.
- Monitoring and Reporting: Real-time monitoring of project progress and automatic generation of status reports keep stakeholders informed and facilitate timely decision-making.

3.2.5. Simulation and Modeling:

- Integration with Modeling Tools: Allows integration with simulation and modeling tools that can be vital in predicting the behavior of biological systems, reducing the need for extensive laboratory testing.

3.2.6. Compliance and Regulatory Support:

- Automated Compliance Checks: Offers automated checks to ensure that the drug discovery process complies with various regulatory standards, reducing the risk of non-compliance.

- Document Management: Helps in managing essential documents and maintaining proper version control, which is vital for regulatory submissions and audits.

3.2.7. Traceability and Transparency:

- Traceability Matrix: ELM creates a traceability matrix that links all aspects of the project, from requirements to tests to deliverables. This ensures complete transparency and facilitates troubleshooting and audits.

3.2.8. Customization and Scalability:

- Customizable Workflows: Tailors workflows to suit the specific needs of the drug discovery project, improving efficiency.
- Scalable Solutions: Can scale according to the size and complexity of the project, ensuring that it remains agile and responsive.

IBM Engineering Lifecycle Management platform offers a cohesive, transparent, and efficient framework that can significantly speed up drug discovery programs. By integrating various aspects like collaboration, data analytics, project management, compliance, and more, it ensures that the entire process is streamlined and aligned with the goals. Its adaptability to the specific requirements of the drug discovery domain makes it a valuable tool in accelerating the time to market for new therapeutics.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/NIH-RFI-Final.-Rocket-Technology-8-16-23pdf.pdf

Description: White Paper: Leveraging IBM's Watson to Catalyze the Development and Use of Novel Alternative Methods in Biomedical Research

Email: link.parikh@rocket-technology.com

Submit date: 8/17/2023

I am responding to this RFI: On behalf of an organization

Name: Lindsay Marshall

Name of Organization: The Humane Society of the United States and the Humane Society Legislative Fund

Type of Organization: Other

Type of Organization-Other: Not for profit; Animal Protection

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   In terms of the success of the novel alternative methods (NAMs) referred to in the RFI, we would like to raise a point of clarity. This is that the definition of success may vary according to the respondent or the intended outcome. As an animal protection organization with a firm grounding in science, we believe that the success of NAMs lies in their ability to fully replace live animals across the spectrum of NIH-funded research programs. To evaluate the degree of this success requires a clearer understanding of how and where NAMs are being currently used and indeed, where animals are being used. These data are not always publicly available. Therefore, in order to effectively gauge the success of NAMs we suggest that NIH carry out a landscape analysis of intramural and extramural NIH research programs to map the use of non-animal NAMs across the NIH centers and beyond, for all NIH-funded research. We can only evaluate whether the application of potentially disruptive technology (ies) has (have) had an impact if there is a baseline measurement against which to compare. The US Environmental Protection Agency is leading the way with its workplan which contains comprehensive efforts to evaluate animal use both within the agency and by the industry it regulates [1]. This should facilitate monitoring reduction in animal numbers concomitant with successful development and use of NAMs and we suggest that NIH consider adopting a similar process for NAMs. Additionally, as stated in the Government Accountability Office (GAO) report of 2019 [2] – " The National Institutes of Health Revitalization Act of 1993 directs the Director of NIH to prepare a plan to conduct or support research into methods of biomedical research and experimentation that do not require the use of animals, that reduce the number of animals used in such research, and that produce less pain and distress in such animals. The act also directs NIH to prepare a plan for establishing the validity and reliability of the new methods it develops, encouraging the scientific community's acceptance of these methods, and training scientists in using such methods. The act further directs NIH to periodically review this plan and, as appropriate, make revisions and include those revisions in a biennial report. In response to the act, in September 1994 NIH established ICCVAM [Interagency Coordinating Committee on the Validation of Alternative Methods] as an ad hoc committee."
   We are waiting for NIH to "prepare a plan to conduct or support research into methods of biomedical research that do not require the use of animals, that reduce the number of animals used, or that reduce pain and distress in animals; establish the validity and reliability of those methods; encourage the scientific community to accept such methods; and train scientists in their use", as

directed by the NIH Revitalization Act. We propose that the success of animal replacement requires moving from advancing progress with NAMs to revolutionising research through the substitution of animals with NAMs. To achieve this, NIH must do more than require that researchers consider alternatives and show familiarity with searching databases for NAMs (as is currently required in proposal applications) and should expand the recommendation from GAO that metrics are developed "to assess the progress [the agencies] have individually or collectively made toward reducing, refining, or replacing animal use in testing and (2) incorporate those metrics into the committee's biennial progress reports" to encompass animal use across NIH-funded research and testing [2].

Successful NAMs application should also be evaluated based on discipline. For fundamental research, success may be (for example) recapitulation of the specific physiological feature, determination of the mechanism of action, dissection of a molecular process – NAMs are already capable of all of these and being used successfully for these purposes. For regulatory testing, where reliability across many laboratories and operators is required to ensure confidence in the data, it seems that more understanding of variability is required before NAMs can be applied successfully. This is ongoing, with the Interagency Coordinating Committee on the Validation of Alternative Methods releasing its draft report on the "Validation, Qualification and Regulatory Acceptance of New Approach Methodologies" that aims to "establish confidence in new approaches that replace, reduce, or refine the use of animals in testing" earlier this month [3]. We are also encouraged that NIH has been active in supporting this with its Tissue Chip Testing Center funding. We hope that NIH can continue to fund these critical efforts until such time that NAMs are embedded in regulatory testing strategies globally. For applied and translational research, perhaps where NIH is most invested given its mission to apply fundamental knowledge "to enhance health, lengthen life, and reduce illness and disability," we see exciting advancements in the application of NAMs already and these will continue to improve as the technology(ies) develops and evolves. For example, human induced pluripotent stem cells have been used to create a neuro-muscular junction on a chip and revealed the auto-antibody dependent role of complement fixation in the impairment of neuronal conduction typical of demyelinating neuropathies [4]. These data formed part of an Investigational New Drug submission to the Food and Drug Administration for a clinical trial with an anti-complement monoclonal antibody [5]. NIH funding will be key to these advances in the future.

For safety testing more applicable to regulatory decision making, liver chips have been used to reveal species-specific toxicities – indicating the potential of the organ-chips for replacement of animals in elements of the preclinical testing pipeline [6] and human liver chips have proven more effective than animals for screening potential liver toxicants (demonstrating 87% specificity and 100% sensitivity for twenty-seven known compounds) [7].

Considering the advancements in progress offered by the NAMs, referred to briefly above, it seems apparent that this is a fast paced and rapidly evolving field and is attracting researchers across the globe. As one example, the second annual Microphysiological Systems (MPS) World Summit held in Berlin in June 2023 [8] attracted over 1,200 participants. In fact, registration had to be closed early and people placed on waiting lists due to the overwhelming demand to attend this event.

The revolutionary nature of the NAMs in enabling better understanding of human health and disease is evidenced in their ability to tackle issues not previously possible or underserved. In many ways, regulatory science and toxicity testing are leading the way in terms of applying NAMs to understand human health risks. The seminal reports from the National Research Council (NRC) [9]

and the National Academies of Sciences, Engineering, and Medicine [10] provided recommendations for developing and validating NAMs, outlining where these tools may be best integrated for use in evaluating chemical risk. A recent National Academies study further examined the variability of mammalian laboratory tests and recognized that "sole reliance on animals would limit the ability to screen large numbers of chemicals" [11]. However, currently, the limitation of using live animals for screening large numbers of compounds is not restricted to chemical safety testing, it has resonance and merit in consideration of the value and success of the human health research more akin to what NIH funds, where reliance on animal models restricts our ability to fully understand human biology, in healthy and disease states.

For biomedical research, there are many examples where NAMs are offering insight into conditions where animal models do not exist, fail to translate or where we simply do not have the cellular or molecular understanding of the human condition to know where to begin to create an animal model. Patient-derived cells and induced pluripotent stem cells (iPSCs) can be used to model the intricate microenvironment of rare diseases and enable understanding of the molecular and cellular reactions. This approach requires comparatively fewer resources and less development time than the creation of in vivo models and is increasingly adopted by the pharmaceutical industry [12]. Application of MPS for rare diseases could help to mitigate the risks linked with substantial research and development expenses, and the potential for diminished profit margins, and may incentivize investment in the field of rare diseases. Collectively, the recent advancements in MPS models for rare diseases provide opportunities to elucidate complex mechanisms of rare disease development, as well as to pinpoint novel disease biomarkers that would not be possible using animals.

1. USEPA, 2021. New Approach Methods Work Plan (v2). U.S. Environmental Protection Agency, Washington, DC. EPA/600/X-21/209.
2. Government Accountability Office, 2019. GAO 19-629. Animal Use in Research: Federal Agencies Should Assess and Report on Their Efforts to Develop and Promote Alternatives
3. The Interagency Coordinating Committee on the Validation of Alternative Methods, 2023. Draft guidance document "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies."
4. Rumsey, J.W., et al., Classical Complement Pathway Inhibition in a "Human-On-A-Chip" Model of Autoimmune Demyelinating Neuropathies. Advanced Therapeutics, 2022.
5. https://clinicaltrials.gov/study/NCT04658472?term=SAR445088&rank=2
6. Jang, K.J., et al., Reproducing human and cross-species drug toxicities using a Liver-Chip. Science Translational Medicine, 2019. 11: p. eaax5516.
7. Ewart, L., et al., Qualifying a human Liver-Chip for predictive toxicology: Performance assessment and economic implications. 2021.
8. https://mpsworldsummit.com/mps-world-summit-2023/
9. National Research Council, Toxicity Testing in the 21st Century: A Vision and a Strategy. 2007, Washington DC: The National Academies Press. 113.
10. National Academies of Sciences, E., and Medicine; Division on Earth and Life Studies; Board on Environmental Studies and Toxicology; Committee on Incorporating 21st Century Science into Risk-Based Evaluations., Using 21st Century Science to Improve Risk-Related Evaluations. 2017, Washington DC (US): National Academies Press (US).

11. National Academies of Sciences, Engineering, and Medicine. 2022. New Approach Methods (NAMs) for Human Health Risk Assessment: Proceedings of a Workshop–in Brief. Washington, DC: The National Academies Press. https://doi.org/10.17226/26496
12. Irrechukwu, O., et al., Applications of microphysiological systems to disease models in the biopharmaceutical industry: Opportunities and challenges. ALTEX, 2023.

2. Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:

In order to catalyze the development and validation of novel alternative method technologies, researchers must be encouraged and supported to develop these technologies and, at the same time, be made to feel confident that a shift away from animals towards NAMs will not damage their career or future prospects. One simple, straightforward way to help achieve this is to ensure that all NIH funding calls prioritize NAMs. NAMs should be mentioned before animal models and requests for application should make it clear that NAMs are the preferred tools and that development of new NAMs or utilization of available NAMs are acceptable and will be prioritized above the use or development of animal models.

It is very encouraging to see where NIH is specifying NAMs in funding calls- for example in RFA-TR-22-032, where the use of tissue chips is specified in the RFI title, however this is a narrow funding call relating to regulatory use of chips, and we would like to see this language adopted more widely across more of the NIH RFI to encourage researchers to make the move away from animals. We appreciate that NIH does make reference to NAMs in some of its requests for application, but frequently these are situated far below mention of animal models. This could (even unintentionally) create the assumption that use of animals would be the preferred option and potentially lead researchers to back away from submitting proposals where NAMs are the research tool if they think that animals are needed or that proposals reliant on animal models are more likely to receive grant funding. We appreciate that there is a need to ensure equity and accommodate all applicants, but also we see that animal research has enjoyed effectively unlimited funding since research began, whereas the same cannot be said for NAMs-based projects. This can result in very steep competition for researchers dedicated to using NAMs in place of animals - submitting applications reliant on potentially high-risk, novel, non-animal tools alongside proposals describing well-established and often well understood animal models can result in lower ranking of the more innovative NAMs proposal. In this case, it is not enough to simply allow proposals to be submitted to the same funding call, there must be a firm steer in the text of the call that proposals using or developing NAMs will be prioritized above projects solely reliant on animal models.

As one favorable example, in RFA-ES-22-006, the objectives describe the potential to use human cell lines as the first objective, with other organisms listed under that. We commend the NIH on this, and on a more recent RFA, RFA-ES-23-008, which states that "[c]urrent methods to evaluate the thousands of chemical compounds with unknown DNT potential remain largely ineffective due to the complexity of neurodevelopment, which involves multiple key processes, one or more of which may be perturbed by a given environmental agent. Also, there are concerns about the current framework of DNT assessment, which is largely based on rodent guideline studies and are often time- and resource-intensive. As a result, environmental compounds with unknown potential to cause DNT remain largely untested. There is therefore a critical need to develop additional resources, new methods, and approaches that can be directly applied in the integrated testing

strategies to evaluate environmental compounds for DNT effects" (emphasis added). In this call, NAMs are described as the first five objectives with animal-based tools appearing underneath, at the end of the list. Until such time that animals are no longer required for any research and testing, we suggest that NIH adopts this format for all funding calls, such that the objectives clearly describe the NAMs as the very first item before any description of possible other, animal-based methodologies. Additionally, we have found other funding calls where animal data are requested alongside NAMs and we think that this could be refined to request that applicants use human data (wherever these are available) or historical animal data before resorting to new animal experiments. We are gratified to see that NIH is already funding many innovative projects that are using or developing NAMs but unfortunately these are still in the minority, compared to the use of animals in NIH-funded research. Our analysis of the NIH RePORTER database has shown that, so far in 2023 under the R01 scheme, new projects using microphysiological systems have received less than 0.5% of the total funding awarded to all new projects. Calculating how much NIH funding supports animal use is very difficult, but NIH itself has stated that "almost 50% of the NIH-funded grants and contracts that we support involve animals" [1]. Ensuring that NAMs are mentioned in the title of funding calls, are described in the first line of the explanatory text and making NAMs the first objective(s) of the call will help to raise awareness that the NAMs are valued, creditable tools that can be adopted without damaging a researcher's prospects for funding. It is sadly not enough to simply allow proposals employing the NAMs to be submitted and evaluated alongside animal-based research projects; there is a need to level the playing field for NAMs developers and raise awareness amongst potential applicants that NAMs are the preferred method, if we are to catalyze the creation and uptake of these tools.

There is an urgent need for more funding to be dedicated to the creation, development, validation, and application of NAMs. The "challenges for building in robustness, replicability, reproducibility and reliability of the technologies and the ensuing datasets; strategies for bolstering technology readiness and reliability these technologies; and factors potentially limiting the successful integration of these technologies across research approaches and potential solutions" articulated in this question all require an injection of considerable funding that is sustained, and ideally increased, over time. We cannot build rigor or understand reliability without investing in these technologies; the understanding of variability needed to bolster readiness requires the support of multiple laboratories and these will all help to lead to increased confidence in the tools and therefore facilitate their integration across research processes. We therefore urge NIH to consider setting aside an increasing percentage of its budget each year to be awarded to projects addressing the challenges raised in this request for information, to ultimately realize the potential of NAMs and accelerate animal replacement across the breadth of NIH-funded programs. In order to do this, it will be necessary to adopt the metrics for tracking the development of NAMs that we suggest in point 1 of this RFI, and then we suggest that NIH calculates the annual spend on projects dedicated to NAMs development and increases the total annual funding by at least 5-10% every subsequent year. This does not require additional funding, it is merely a judicious redirection of the existing budget, and along with funding calls that clarify the priority of NAMs we feel that this strategy will be successful in addressing all the queries in this RFI. To evidence this concept, we urge NIH to carry out, or support the execution of, retrospective reviews of those priority areas of NIH-funded biomedical research that are using, or have used, animals. These reviews should focus on the translational value of the research in order to demonstrate where the animal models are most likely

to fail and identify those research subjects where no further funding should be awarded to projects reliant on animals. To allow the shift in NIH funding to keep pace with NAMs progress, the reviews should be ongoing such that as NAMs develop further, the funding can match this and enable more projects to move away from live animal models and employ the NAMs instead.
Furthermore, there is a need to ensure that proposals focused on the use of novel, innovative NAMs are not being reviewed less favorably due to reviewers' lack of familiarity with these new tools. We would like to propose two ways to address this. The first is that NIH develops a panel of NAMs experts and ensures that they are included in proposal review. We have anecdotal evidence that proposals using novel NAMs are likely to score less highly than projects using "traditional", more well-known models of animals or even those projects using new iterations of animal-based tools. These decisions seem to reflect the level of comfort or familiarity in reviewers assessing the novel nonanimal tools rather than due to a lack of scientific credibility of the NAMs. Ensuring that relevant expertise is available to review project proposals could help, not only with improving awareness (among the wider grant review panel) of what the NAMs are capable of, but also the NAMs expert could play a role in indicating where more investment or development is needed. Either way, this would be a fairer assessment of these new technologies and of their capacity to address research questions of interest to NIH. The second is that NIH grant review criteria are adapted to include consideration of the replacement potential of the technology described. We encourage NIH to require that a dedicated section on Replacement, Refinement and Reduction (the 3Rs) is included, either within the section on vertebrate animals or, ideally, as a stand-alone section that also considers NAMs. Here, applicants would provide details of how the project would contribute to the 3Rs and indicate the scale of impact for the work in terms of reduction, refinement, and replacement of animals. For training grants and fellowships, this section could also include details of 3Rs specific training courses that the applicant will attend and use this as a metric for progress toward replacement of animals in NIH funded research. We submitted comments along these lines to the review of NIH grant criteria that was open to public comment earlier this year and we anticipate seeing the revised criteria published in due course, and hope that our comments were taken on board.

1. Sally Rockey, Use of Animals in NIH-Supported Biomedical Research, ILAR Journal, Volume 52, Issue Suppl_1, 2011, Pages 482–484, https://doi.org/10.1093/ilar.52.Suppl_1.482b

3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:

One issue that we feel it important to raise here is that of confidence. This has been recognized across biomedical research, irrespective of the technology involved. The crisis in confidence in the use of animals [1] and the well-known failures with translational potential of animals (nine out of every ten drugs that are tested on animals will fail in patients [2]) and also the reproducibility crisis [3] has reduced public trust in science and has had the undesirable knock-on effect that now NAMs are held to higher standards than animal models ever were. It is important that, in this regard, biomedical research is not siloed and that there are opportunities to collaborate across other disciplines, including, but not limited to, toxicology and regulatory sciences, since these fields are also grappling with the issues of scale, variability, and uncertainty.

It seems apparent that improving confidence in the NAMs is necessary for their application in advancing scientific enquiry. In the report from the National Academies workshop on Variability and Relevance of Current Laboratory Mammalian Toxicity Tests and Expectations for New Approach Methods (NAMs) for use in Human Health Risk Assessment, participants noted that "strong confidence that the [NAMs] methodology is not highly variable and is reproducible, and that the cellular-level outcomes are relevant to humans" could help enable the shift away from animals, and that "integrating the results from different approaches (shorter-term animal studies, human studies, and one or more NAMs) would enhance the representation and understanding of variability and increase the confidence and policy application of NAMs [4]. We suggest that one of the many ways to improve confidence is to ensure open and unbiased reporting. We would urge NIH to adopt the recommendations articulated in, for example, ProMap [5] and PRIVAT [6], to enhance clarity of reporting for NAMs under development and thus help to improve confidence in the reliability and use of such tools.

The National Academies report on Nonhuman Primate Model Systems: State of the Science and Future Needs [7] examined "[w]ays to increase coordination and collaboration between researchers who use nonhuman primates and those who use new approach methodologies to enhance the value of all methods and tools" as one element of the statement of task. The committee found that "[c]ontinued development and validation of new approach methodologies (in vitro and in silico model systems) is critically important to support further advances in biomedical research. This may reduce the need for nonhuman primate (NHP) models in the future, and/or enhance their utility. Additionally, this may help to mitigate shortages in NHP supply and the high cost of NHP research" and reached the conclusion that "efforts to reduce reliance on nonhuman primates (NHPs) in biomedical research will require investment in opportunities to facilitate direct interaction and collaborative research among investigators using NHP models and those developing in vitro and in silico approaches to expand the applicability of new approach methodologies to research questions for which NHPs are currently needed. At present, however, few mechanisms for fostering such interaction and collaborative research are available" (emphasis added). It is crucial that the opportunity for stakeholders to interact and collaborate is built into any future strategy prioritizing the use and development of NAMs. NIH could provide dedicated funding opportunities to foster collaborations devoted to the application of NAMs to replace NHP (initially, and then consider how to achieve this for all animals used in NIH-funded research). There are ongoing opportunities that could be exploited more effectively to bring together the animal users and NAMs developers, where these groups are distinct from each other (although we recognize that often these may be the same and frequently NAMs are used alongside animals in the same research lab). For example, the NIH Advisory Committee to the Director NAMS Working Group, is hosting a webinar on August 21, 2023 [8]. This has a stellar list of speakers who already have great familiarity within the NAMs fields, including Dr Nicole Kleinstreuer of the National Toxicology Program's Interagency Center for the Evaluation of Alternative Toxicological Methods and Prof Thomas Hartung of Johns Hopkins Center for Alternatives to Animal Testing. We hope that this event has been promoted to NIH grant holders who are using animals as one way to bring the disparate groups together and promote the interactions and conversations that will be required to scale the technologies, share technology

deployment and coordinate approaches across research disciplines or research sectors to dramatically advance the development and/or use of these technologies.

One overarching strategy to achieve this coordination and advance deployment could be to adopt the Common Fund as a mechanism for supporting the transition away from animals that underpins the development and use of NAMs. The Common Fund already supports high risk, high reward research, supporting "highly innovative research with the potential for broad impact in biomedical, behavioral, or social sciences within the NIH mission" [9]. An infusion of money from the Common Fund could allow for the coordinated development, scaling, and integration of NAMs into biomedical research and provide a strong signal of the direction of NIH research- away from poorly performing animal models towards these more innovative and translational NAMs.

We fully appreciate the support that NIH has put into NAMs so far, and, considering the impact that this small amount of funding has had is a sure indicator of the potential to transform biomedical research if NAMs were given significantly increased support. Over the last 10 years, (since 2012) NIH has invested heavily in tissue chips, including the Tissue Chips in Safety Testing for disease modelling in space and for the Tissue Chip Testing Centers to address issues of reproducibility. We applaud this intention of NIH to support the development and application of these tools. However, the total sum of 361.5 million over 13 years, averaging around 33 million annually, represents a mere one thousandth of the total budget of NIH. Shifting a percentage of total NIH funding (as articulated in our response to question 2) along with dedicating money from the Common Fund to support proposals dedicated to NAMs sends a strong, inspirational signal to researchers and the public that NIH is firmly behind these new technologies, and to facilitating the replacement of animals in NIH-funded programs.

1. Kilkenny C, Parsons N, Kadyszewski E, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLoS One. 2009;4(11):e7824. doi:10.1371/journal.pone.0007824
2.https://ncats.nih.gov/ntu/about#:~:text=The%20average%20length%20of%20time%20from%20target%20discovery,successful%20drug%20can%20be%20%241%20billion%20or%20more.
3. Fitzpatrick BG, Koustova E, Wang Y. Getting personal with the 'reproducibility crisis': interviews in the animal research community. Lab Anim. 2018;47:175–177.
4. National Academies of Sciences, Engineering, and Medicine. 2022. New Approach Methods (NAMs) for Human Health Risk Assessment: Proceedings of a Workshop–in Brief. Washington, DC: The National Academies Press. https://doi.org/10.17226/26496
5. https://osf.io/x85gh/
6. https://osf.io/bwypt/
7. National Academies of Sciences, Engineering, and Medicine. 2023. Nonhuman Primate Models in Biomedical Research: State of the Science and Future Needs. Washington, DC: The National Academies Press.
8. https://acd.od.nih.gov/working-groups/novel-alternatives.html
9.https://doi.org/10.17226/26857.https://commonfund.nih.gov/highrisk#:~:text=The%20Common%20Fund%27s%20High%2DRisk,scientists%20conducting%20highly%20innovative%20research.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/HSUS_HSLF_Response-to-NIH_NAMs-RFI_August-16.pdf

Description: The pdf is a copy of the comments submitted on the online form in case there are any issues with that. Thank you for the opportunity to comment.

Email: lmarshall@hsi.org

Submit date: 8/17/2023

I am responding to this RFI: On behalf of myself

Name: Tao Zhang

Name of Organization: SUNY-Binghamton University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Currently being developed in our lab is an in vitro and in silico modeling combined approach to simulate the drug secretion into breast milk during lactation. We are developing a human mammary cell based system to estimate key parameters related to drug transfer to breast milk and using these parameters as inputs into physiologically based pharmacokinetic models to predict drug secretion into breastmilk. Our intention is to build a well characterized system that has the robustness, replicability, reproducibility and reliability to give reliable predictions.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The major challenge for building in robustness, replicability, reproducibility and reliability of the technologies is that the development of such technologies with those qualities require a lot of time and efforts to optimize and simplify the process. Very often those work is not considered as novel and rarely being supported by funding agencies.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   The NIH may consider supporting research from middle and small research institutions that develop robust, applied and useful technologies which doesn't require very expensive equipment. It is so common that research proposal for technology development is not deemed as valuable or novel if there is no large core facility or expensive equipment being used.

Email: zhangt@binghamton.edu

Submit date: 8/17/2023

I am responding to this RFI: On behalf of an organization

Name: Ramkumar Menon

Name of Organization: The University of Texas Medical Branch at Galveston

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   My reproductive biology laboratory has developed multiple alternative methods to test human reproductive organs, specifically pregnancy-related organs. We have developed various microphysiologic system-based organs on a-chip (OOC) models. These include amnion membrane, chorio-decidual interface, amniochorion/decidua, 2nd and 3rd-trimester placenta, and vagina-cervix-decidua. These OOCs have been used to develop both 'healthy' and 'disease' models of the organs. To confirm the physiological validation of our model system, we have recreated the healthy and disease state in animal models and determined that the OOC models are suitable alternatives to existing animal models and provide reproducible data.
   The models developed include (1) Ascending model of infection associated with lower uterine infection, (2) ascending model of infection associated with preterm birth, (3) exposure of cervicovaginal and placental organs to environmental toxicants, (4) testing of drugs for during pregnancy. These models have provided an alternate approach to testing single-cell systems, tissue explants, or animal models that do not reliably represent the human system.
   in addition, we have now developed a 3D bioprinted feto-maternal interface that will be used for drug screening at NCATS intramural drug screening labs, and this will be used for repurposing drugs during pregnancy.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   RFAs to support current and ongoing projects and to identify new collaborative opportunities in research associated with NAMs.
   Sustained funding of already initiated projects is essential to continue the mission of NIH and other entities.
   Working in alliance with intramural programs and bolstering intramural and extramural collaborations (especially in NICHD) and accessibility of intramural biobanks will be immensely helpful.
   Accessibility to samples by studies conducted by networks (e.g. MFMU)
   Factors limiting advancement.
   Funding in this area is still very restricted, specifically in reproductive medicine.
   Availability of reliable cells and cell lines
   Restrictions in getting tissues to develop first and 2nd-trimester human pregnancy-associated cell lines where investigators are modeling them using term pregnancy (delivered) tissues.
   Interdisciplinary collaborative opportunities need to be encouraged

Monopoly by certain microphysiologic companies in promoting the “one fits all” model of OOCs has substantially hampered progress (e.g., Emulate chips).

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Industry/Academia/FDA partnership is one essential approach
   Intramural and extramural investigator collaborations where skills and technologies developed by both entities can be utilized
   Accessibility to biobanked samples and subject databanks will be good for translational research
   I am attaching a PDF of NAM based study for drug PK testing funded by NIH.
   These are examples of how a NAM can benefit and adavance the field .
   These models are developed using cell lines developed from human tissues and immortalized and characterized. Resources are available and we are happy to collaborate and support NAM research using the technology and tools that we have developed.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/Frontiers-Pharmacology-2023-Microfluidic-technology-and-simulation-models-in-studying-pharmacokinetics-during-pregnancy-1.pdf

Description: Manuscript

Email: ra2menon@utmb.edu-

Submit date: 8/19/2023

I am responding to this RFI: On behalf of an organization

Name: Rachel E. Levinson

Name of Organization: Arizona State University

Type of Organization: University

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/NIH-RFI-NAMs-Signed-Response-w-Carlo-KED-letterhead.pdf

Description: This is an UPDATED version of response submitted on 08/18/2023

Email: rachel.levinson@asu.edu

Submit date: 8/30/2023

I am responding to this RFI: On behalf of an organization

Name: Alexandra Shifflett

Name of Organization: Transnetyx

Type of Organization: Biotech pharmaceutical company

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

   CAPABILITIES

   Transnetyx is a 25-year-old biotechnology company focusing on the rigor and reproducibility of animal research. Our work ensures that every researcher has the most efficient path to discovery, and that the path is paved with reproducible methods and ethical animal welfare practices. While additional Novel Alternative Method (NAM) Technologies are being developed to provide relevant data for human biology, there is still—and will always be—a need to address the biological variables that exist in animal models.

   Standard measures have been documented through the PREPARE1 and ARRIVE 2.02 Guidelines but are not enforced in the research community in a meaningful way. Thousands of publications exist documenting the importance of the implementation of these guidelines3,4; however, even as a global scientific partner at leading research institutions, we find that only 13.4% of our customers are utilizing diagnostic services that allow laboratories to easily achieve standards clearly defined in the guidelines. Two such services include Genetic Monitoring (background strain testing) and Microbiome testing of novel rodent models. For reference, Transnetyx has served over 10,000 unique researchers thus far in 2023 across 65 countries globally.

   Transnetyx is traditionally recognized as an automated genotyping provider and will process over 1M genotyping samples in 2023; however, only 534 researchers (of our over 10K unique researchers) will have utilized Genetic Monitoring and/or Microbiome thus far in 2023. Further comparison shows that we have processed nearly one million genotyping samples in FY 2023 whereas we have only processed a total of 4,391 samples for microbiome analysis and 4,391 genetic monitoring samples. These services directly address Section 8 in the ARRIVE 2.0 Guidelines, which NIH endorsed in 2020.

   CURRENT SUCCESSFUL IMPLEMETATION AT NIH:

   Transnetyx has been a trusted scientific partner to NIH for over a decade, and has contracts established within IC Director Offices that subsidize the cost of Transnetyx diagnostic services for their laboratories, including NIAID, NIEHS, NIA, NIDA, NIEHS, NEI, NIAMS, and NICHD. Transnetyx has global data proving the increase in researcher adoption of services when mandated or simply supported by executive leadership.

   Example

   Under the leadership of Dr. Steven Holland, NIAID invested in both genotyping and genetic monitoring on behalf of their researchers, directly supporting two of the biological variables defined in the ARRIVE 2.0 guidelines.

There has been an 88% increase in the adoption of Genetic Monitoring in year two of the contract. If IC leadership invests in reproducibility measures, adoption rather than opposition occurs within laboratories. We are very encouraged by NEI's contract investment in Genetic Monitoring for FY 2024. This was a decision made following their first full year under a Genotyping contract with Transnetyx.

The question of why these reproducibility measures are lacking in animal research is not only to be put squarely on the shoulders of each Scientific Director though. Additional challenges are outlined below that must be addressed to ensure the successful integration of these technologies across animal research at NIH.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

KEY CHALLENGES:

1. Lack of standard of practice across NIH for genotyping, background testing, microbiome, colony management, or animal research facility software. The lack of consistency and support negatively impacts reproducibility measures in animal studies.
2. Fiscal responsibility for standardized laboratory practices has not been adopted at the leadership level across all ICs; this is firmly recognized as a deterrent to adoption for laboratories.

STRATEGIES FOR BOLSTERING TECHNOLOGY READINESS:

1. Implementation of campus-wide animal research facility software: Transnetyx offers a robust facility software that can help NIH effectively manage animal care, operations, and compliance processes. This provides NIH ICs with centralized management, full data transparency, operational and project completion, and accelerated cost recovery. Transnetyx is deeply encouraged by the expressed interest of multiple ICs interest in tick@lab and are glad to provide references upon request.
2. Empower leadership to make reproducibility measures “non-negotiable” for their labs. NIH should establish a standard of science and expect their researchers to adopt technologies that meet the expectations set in the ARRIVE 2.0 guidelines. This immediately reduces the reproducibility crises and provides visibility to a public that is increasingly aware of where research dollars are spent.
3. Reinstate events like the annual Research Festival to ensure researchers are aware of firmly vetted scientific partners such as Transnetyx. Transnetyx has secured six separate DIR contracts5 and has an entire suite of services6 developed to align animal researchers with the ARRIVE 2.0 Guidelines easily and affordably.
4. Financially incentivize labs who integrate reproducibility measures in their animal research through more robust funding and/or larger cage-space allotments.

FACTORS LIMITING ADOPTION OF DIAGNOSTIC SERVICES ESTABLISHING REPRODUCIBILITY:

1. OD decision making. Throughout our tenured partnership with NIH, we have observed a lack of authority in investing in and mandating practices that serve reproducibility in research. The financial burden of change at the laboratory level unfortunately outweighs many lab’s decisions to do the “right thing.” This leads to lack of oversight, inconsistencies, wasted research dollars, and wasted animal lives.

2. Resistance to change is a human condition that must be considered. When there is no direct consequence to continuing old methods, there is a perceived notion that the newly proposed methods are not necessary.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Please see attached appendices, numbers three and four.

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/RFI-NAMs-submission.pdf

Description: Transnetyx: NAMs RFI Submission

Email: ashifflett@transnetyx.com

Submit date: 8/31/2023

I am responding to this RFI: On behalf of myself

Name: James P Sluka

Name of Organization: Indina University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Multiscale computational models that can map from the in vitro scale to the human and population scales.
   Toxicity Testing in the 21st Century (National Research Council. Toxicity Testing in the 21st Century: A vision and a strategy. Washington, D.C.: The National Academies Press; 2007.) and the 3 Rs advocate for the development of in vitro assays to reduce the use of animals in medical research. One approach to this challenge is the development and use of novel alternative methods (NAMs) in biomedical research. These alternatives include more extensive use of in vitro models to predict in vivo responses in humans. However, this requires building computational models that can carry out the prediction. This IVIVE translation requires new methods of mapping the spatiality of in vitro models to the spatiality of in vivo system to make reliable in vivo predictions.
   In recent years it has become clear that spatiality is a key component of both the normal and abnormal function of tissues and organs. The spatial arrangement of the cells is an emergent property of tissues and is critical for the proper functioning of the tissue or organ. In the pharmacological and toxicological domains, the exposure of individual cells to drugs/toxicants depends on the layout and perfusion of spatially organized tissue. The modeling modality that can capture this spatially resolved complexity is multiscale modeling known as “Virtual-Tissues” (VTs). VTs provide mechanism-based (hence defensible and understandable) insights into the spatially dependent mechanisms underlying biological processes.
   VTs can include multiple mathematical, biological, and spatial treatments of the system under study. For example, combining sets of ordinary differential equations (ODEs), commonly used for modeling reaction kinetics, signaling and for Physiologically Based Pharmacokinetic Modeling (PBPK), with spatial representations of tissues including vasculature, cells, extracellular matrix, sub-cellular reactions and signaling (for example PMID:27636091). This range of biological concepts and mathematical and computational representations allows VTs to couple across spatial and temporal physiological scales, e.g., to explore how drugs or toxicant exposure at the whole body level translate to tissue and cellular effects within the body.
   A key modeling issue that is often neglected is the need to spatially model both the in vitro system and the in vivo system. Spatiality effect both systems and there is a requirement that the difference in spatiality are explicitly treated in any attempt to map from an in vitro model (like a sheet of cells in a petri dish or cells in a hanging drop) to the spatial layout in vivo. The spatial layout of the two systems is different, as is the perfusion and migration of diffusible species (drugs, toxicants, hormones, oxygen, etc). Therefore, there is a critical need in modern IVIVE modeling to develop

reliable, mechanism-based mappings from spatially defined in vitro models to spatially defined in vivo predictions.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   There needs to be significant effort expended to develop USER FRIENDLY technologies for properly annotating biological experiments and data. Ontologies and data formats exist but are rarely used, largely because of their complicity and lack of a usable, intelligent interface.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   The key route to MAXIMIZE VALUE is to maximize the chances that a research result will be REUSED in research not directly related to the project that originally generated the data. REUSE requires robust methods of documenting, annotating and sharing. The approach of FAIR is applicable but to date there are few tools to help realize FAIR documentation and no robust method for sharing the FAIR annotations.
   Data should be easily findable, once FAIR annotated, by any web search engine. Data consumers should not need to know where a particular piece of data resides.
   1. Online tools should be developed that allow a user to EASILY generate an ontology-based description of the basic components in their experiment. What are the CORRECT names for genes, proteins, tissues, cells, drugs, disease, ...
   2. These structured annotations should be included with ALL publications including papers, deposited data sets, computational models and so forth.
   3. Publishers should be REQUIRED to include this information. Yes, the NIH/NSF/EPA etc. CAN require this type of compliance by simply requiring grantees to only publish in journals that follow the standard. (Note that a comprehensive set of annotations would NOT increase the printed length of a publican since the annotations are not meant to be human read and therefore could be set in 0.01 pt type. Automated processes don't care what the point size of text is and can extract the information as needed. Digital formats, like PDF, can include the information without increasing the document's length.)

Email: jsluka@indiana.edu

Submit date: 9/3/2023

I am responding to this RFI: On behalf of myself

Name: Robyn Tanguay

Name of Organization: Oregon State University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   There is a wide diversity of research platforms and models being developed for specific applications to understand the cause and underlying mechanisms of human disease. Much of our current understanding of biological processes and disease have arisen from animal research, and certainly we have much more to discover.
   First, the NAM abbreviation is problematic, as I would hope we will continually evolve our approaches using the best available science to make human health discoveries and protections. Also, sometimes the "old methodology" may be the best available science. Finally, the NIH should strongly delineate that NAM is not a synonym for "non-animal models", as it would seem we could throw out the best available science if that is how this term is interpreted.
   Although I agree that some of the in vitro new approach methodologies show tremendous promise, I am concerned that the biological complexity could be sacrificed if we move to the simple systems too soon. For example, in assays developed to screen for chemical or chemical mixture toxicity, using simple systems could miss important biological activity of these chemicals. Environmental chemicals are not drugs, so they may interact with gene products in unexpected way to cause disease. We are not yet able to screen for or predict these effects.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Certainly validation of any assay is essential, and this is no different for new approach methodologies, but we need to be clear what we are validating these new approaches against. With any experimental system, reproducibility must first be demonstrated and this is where some new approach methodologies offer substantial advantages over traditional laboratory rodent models because more data could be collected at lower cost which should increase our ability to assess and confirm data quality.
   Strategies to bolster technology readiness could be highly coordinated case studies that span model systems. Ideally this would not be through traditional NIH funding mechanisms that are too cumbersome and restrictive.
   Major factor would be sociological acceptance of these new approaches which is completely understandable.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Much of the challenges for building robustness replicability and reproducibility and reliability are related to funding limitations. Most of the innovative and forward thinking research occurs in academia, yet funding aimed at transitioning into new approach methodologies is largely unfunded, and not fundable through the current funding mechanisms.
Perhaps substantially more SBIR funding could address some of these challenges where academic and the private sector could work more closely to implement, optimize, and evaluate these new technology.

Submit date: 9/3/2023

I am responding to this RFI: On behalf of an organization

Name: Ellen Berg

Name of Organization: Alto Predict, LLC

Type of Organization: Biotech pharmaceutical company

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/Berg-Revised-Response-to-NOT-OD-23-140-3Sept2023.pdf

Description: Berg Revised Response to NOT-OD-23-140 3Sept2023

Email: eberg@altopredict.com

Submit date: 9/4/2023

I am responding to this RFI: On behalf of myself

Name: Yoichi Watanabe

Name of Organization: University of Minnesota

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   We are developing mathematical models of biological processes involved in the radiation response of tumor and normal cells using kinetic models and stochastic cellular models in multi-dimensions. Multi-scale models are a key component in this approach.
   I believe that the mechanistic processes in biological systems can be mathematically represented. If not, that indicates the need for more knowledge and understanding. Therefore, modeling activities are valuable to assess the current state of knowledge and plan for future areas of experimental studies.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   The model requires not only mechanistic understanding but also the numerical values of the parameters in the model. To overcome the shortage of data, well-designed animal experiments are needed.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Assembling a team of experts in mathematics, computation, biology, and medicine is important to undertake the required project. NIH can provide funds necessary to pull all necessary expertise for successful undertaking.

Email: watan016@umn.edu

Submit date: 9/4/2023

I am responding to this RFI: On behalf of an organization

Name: Catharine E. Krebs

Name of Organization: Physicians Committee for Responsible Medicine

Type of Organization: Nonprofit research organization

Role: Select one

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Uploaded File: https://osp.od.nih.gov/wp-content/uploads/ninja-forms/7/PCRM-RFI-NOT-OD-23-140-NAMs-2023-09-04.pdf

Description: All comments are included in the attached document.

Email: ckrebs@pcrm.org

Submit date: 9/4/2023

I am responding to this RFI: On behalf of myself

Name: James A. Glazier

Name of Organization: Indiana University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   While in vitro NAM studies of the metabolic, signaling and regulatory networks within cells are essential to developing an understanding of how cells work as individuals, they lack the higher level context which is critical to the identification of more subtle collective sources of robustness and disease. While organoid culture and multi-organoid approaches provide additional context, these models still are quite far from reproducing in vivo complexity. Virtual Tissues, Multiscale-agent-based mechanistic models of tissue organization, homeostasis, function and dysfunction are essential to understanding how the patterns of control active within individuals lead to emergent function and dysfunction at larger scales. Virtual Tissues have the advantage that all aspects of a simulation can be seen in a longitudinal context and thus can serve as a critical way to bring experimental endpoint assays like spatial 'Omics methods to life. The detailed information available in NAM experiments in turn can allow the creation and validation of vastly more detailed and quantitatively predictive Virtual Tissue models than were previously available. Thus VTs and NAMs can form a productive virtuous cycle, where VT models of the NAMs themselves allow more stringent validation of the models than would be possible in vivo, the NAMs provide detailed intracellular pathway data for VT creation, VTs allow the design of more informative organotypic NAMs and the extrapolation of NAM results to whole organ or organisms contexts.
   Virtual Tissue models have been used in developmental toxicological contexts to predict tissue-and organ-level effects from single cell responses in the context of vascular damage, palate formation and urogenital disruption by estrogen mimics. They have also been used to explore the factors leading to systemic liver recovery or failure after injury--specifically why perturbations with apparently similar effects at the level of individual cells can lead to dramatically different outcomes at the whole organ level (complete recovery vs liver necrosis and death). In an immunological context such models are essential to understanding immune response and spatial organization of infection, e.g., in developing improved therapies for tuberculosis or leishmania infection. These approaches are potentially revolutionary for scientific understanding because the enormous advances in experimental technology and understanding at the molecular level have not been complemented by equivalent advances in experimental techniques and understanding at higher scales (e.g. we still don't understand effectively how extracellular matrix is synthesized or remodeled or how it contributes to homeostasis or pathology, or how local and lymphatic immune components interact to lead to rapid or chronic infection, chronic inflammation or autoimmune diseases).

Virtual Tissues, in principle, allow the generation of unlimited heterogeneous patient populations. In practice, our understanding of the actual range of variability and covariance of key parameters controlling such models in human populations is somewhat limited and needs further exploration. Medicine is fundamentally unscientific since control and repetition are, in their nature impossible. It is impossible to treat and not treat the same individual or give them two different treatments to explore which is more effective. Virtual tissues enable the creation of virtual controls to evaluate the effectiveness of treatment much more rapidly than with current methods and to create an unlimited number of virtual test subjects to explore millions of possible treatment approaches. Such approaches are especially important in treating individuals with unusual or rare health profiles or diseases.

Developed Virtual Tissue models could enable the transition of biomedicine from an exploratory science to engineered control, enabling the DESIGN of treatment strategies to take individuals from a current undesirable health state as close as possible to a desired final outcome given the range of treatment options available.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

Effective software and standards infrastructure requires many layer--there is no single layer of "computational infrastructure." Modern systems like internet protocols or Cell-phone based navigation systems like Google Maps, integrate dozens of different layers of software and data components of standards. Each one of these needs to be independently built and verified, but is maximally useful in a confederation. OMICs workflows and data standards illustrate some of these issues, but the representation of the many spatial and temporal scales of biological organization and of experiments and clinical interventions will require much more sophisticated approaches--climate models and GIS systems may be the best prototypes for what is needed here. At the most basic level, description of biology (conceptual models) needs to be fully separated from the computational methods used to instantiate that model (such approaches exist for network models but not for most scales of modeling or for the interconnections between scales).

Critical gaps in the integration of Virtual Tissues with NAMs include a great deal of superficially simple but actually quite challenging infrastructure--including 1) Software tools to develop multiscale conceptual models of cell and higher-level biological behaviors and organization, 2) Standards for this description. 3) Standards for the method-independent description of VT models, 4) Approaches to the modularization of VT model components to enable the reuse and extension of their components (including the standardized description of model component inputs and outputs, agreed upon approaches to parameterizing complex biological processes and descriptions of the domains of component validity and defaults). The lack of this critical infrastructure for shareability and reuse means that most VTs are limited to single research groups and the knowledge embodied in them is stranded or lost. Where such standards exist, as with SBML and network modeling, it has led to much more rapid acceptance of these approaches by regulators and experimental biologists. Even here, limitations like the limited modularity and interconnectability of SBML models, and the lack of meta standards for such interconnection, impede the interconnection and assembly of components describing subnetworks, preventing the rapid progress that has occurred, e.g. through software and data interoperability in bioinformatics/Omics.

Beyond the modularization and standardization of model component, API and data descriptions at all scales, a critical concept for the development of complex reconfigurable models is white-box testing, the incorporation of functional tests into the design and distribution of model components. Design of such tests is challenging and difficult to retrofit to existing models.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   There are still open fundamental questions about HOW to compare the outputs of VTs which predict spatial structure with experiments and how to handle the intrinsic stochasticity of both simulations and experiment. NAMs and VTs both allow the creation of many replicates of the same situation, but we lack systematic ways to integrate these replicates.
   Some critical needs are formalisms for the specification of the purpose and limitations of wet-lab and in silico experiments, the description of wet-lab and computational experiments processes and the set of possible experimental manipulations (not all conceivable experiments are possible). The latter are essential for the design of optimal control systems (e.g., to design informative new experiments, or to personalize therapies to achieve desired health-state outcomes).
   Integration also requires new approaches to the formal description of the outputs of complex spatio-temporal experiments and mechanistic models and their variability. An additional need is formalism to describe the manipulation of output data during analysis, so, e.g., a published figure in a paper would include the raw data that contributed to it as well as the complete reproducible set of transformations used to generated the processed figure.
   Maximizing research value of models and data requires a significant amount of effort in internal annotation, presentation, documentation beyond that required for data generation. New and more effective approaches for the organization, interfacing, annotation and distribution of VTs are all needed to bootstrap the integration of VTs with their experimental partners.
   Method-independent model description is also a key to the long-term value of models, since it enables the biological understanding and data embedded in a model to persist past the lifetime of the initial platform on which the model was developed.
   In addition to a focused effort to develop the multiple layers of infrastructure needed to make VTs and NAMs maximally productive, More support for the infrastructure for relatively loose collaborative networks is key to the bootstrapping of these technologies. These are technologies that develop rapidly and in unexpected directions. SIngle PI support does not promote the extensive effort needed for optimal sharing and reuse and national centers are too exclusive and unwieldy for the flexible collaborations these very large-scale projects will require.

Description: The Role of Virtual Tissue Computer Simulations in Maximizing the Utility of NAMs

Email: jaglazier@gmail.com

Submit date: 9/5/2023

I am responding to this RFI: On behalf of an organization

Name: James A. Glazier and Tomas Helikar on behalf of the Steering Committee

Name of Organization: GLIMPRINT (Global Alliance for Immune Prediction and Intervention)

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Mechanistic biological computational models are instrumental in representing both in vitro and in vivo biological systems, from organoid cultures to humans. As reliance on animal testing decreases, the need to use computational models to extrapolate knowledge from in vitro experiments to entire animals becomes paramount. In Vitro to In Vivo Extrapolation (IVIVE) necessitates models that are transferable, clear, and defensible. As the intricacy of in vitro experiments increases, we need models to interpret, design, and control this evolving complexity to help make the information from in vitro methods more predictive of whole-organism responses.
   While in vitro studies of cellular metabolic, signaling, and regulatory networks lay the foundation for understanding individual cell function, they often miss the broader context, which can radically affect systemic outcomes. This context becomes vital to identifying collective sources of robustness and disease. Although organoid cultures provide more insights, they still lack many aspects of in vivo organization and function. Multi-scale mechanistic models, which offer a comprehensive simulation perspective, can bridge this gap. Such models can paint a holistic picture of tissue function and dysfunction, explaining how patterns of behavior of individual cells lead to larger-scale phenomena. Models can also enable the creation of dynamic predictions from experimental measurements that require the use of fixed samples and the inference of important but experimentally unmeasurable quantities. Multiscale mechanistic models have been used successfully to predict tissue effects from cellular responses in areas including vascular damage, liver recovery, or immune response.
   Drug and therapy development can be a billion-dollar, decade-long endeavor. Multiscale mechanistic computer simulations can assist in all stages of drug discovery, from target identification to understanding compound effects, to personalizing treatments. Beyond reducing the need for animal testing, multi-scale mechanistic models could save upwards of $100M per clinical indication in drug development by providing earlier prediction of drug failure and improved approaches to treatment delivery and scheduling. Models also have a critical role in improving the design of clinical trials and their interpretation. The drug and therapy development pipeline requires many different types of models, from Molecular Dynamics studies of protein-drug docking to network models of intracellular molecular pathways, multicellular and tissue models of systemic responses, Pharmacokinetic and pharmacodynamic models of drug availability, metabolism and elimination, models of biomechanics and radiation exposure and response and population models of clinical trial protocols and outcomes. We must establish criteria for when to employ each model type, improve their individual verifiability, and develop effective and reliable transitions and interconnections between them to ensure that they are acceptable in life-critical regulatory contexts.

Pairing experimental methods like organoids with computational modeling could significantly improve our understanding of complex conditions, from neurodegenerative disorders to cancers. Such models would more precisely emulate human diseases, offering a platform to test treatments and study disease progression. Computational models can also democratize research. A repository of validated, extensible, and interconnectable mechanistic models would allow smaller teams globally to undertake significant studies in all areas of basic and applied biomedicine.

However, the level of interoperability and reuse of mechanistic models and tools significantly lags that in fields like 'Omics or ML/AI. Delivering the potential of mechanistic models requires much more effort in the development of computational and social infrastructure for standardized model description and annotation, model interconnectability, and model evaluation.

Human biology's complexity requires multi-scale, multi-approach modeling tools. However, a significant roadblock is the lack of public datasets due to privacy concerns. Solutions could lie in generating synthetic health data, maintaining patient anonymity, and legal compliance. Technologies like Generative Adversarial Networks (GANs) and Recurrent Neural Networks (RNNs) can produce such synthetic data. Imagine the potential if all clinical datasets were accessible in a synthetic format for unrestricted R&D use.

The ever-evolving landscape of computational biology holds immense promise, and multiscale mechanistic models stand at the forefront. However, to truly harness their potential, we need to invest much more in developing enabling standards, tools, and infrastructure in addition to more traditional biomedical research topics. We also need to establish mechanisms to enable collaborative, progressive scientific and technological development and to support loosely coupled community development. This new mode of collaborative transdisciplinary research and development offers the promise to reshape and improve the efficiency of research while offering more accurate, humane, and efficient solutions to the challenges of modern biology.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

Mechanistic multiscale models have emerged as a promising approach to maximizing the ability to design and analyze experimental NAMs to obtain results normally requiring whole animal methods. However, their adoption is hindered by challenges in data and model sharing, model reusability and extensibility, standardization, software interoperability and model validation.

Challenges Hindering Progress:

1) Ambiguities in Naming Conventions: Publications often lack consistent naming. A PUBMED search with "TGFB" retrieves thousands of articles, despite the gene's actual nomenclature being TGFB1, TGFB2, etc.

2) Risks Associated with Complex Models: Current mechanistic models have expanded in complexity and range of application. While more complex models potentially offer more detailed predictions, they also have a higher risk of failure or inconsistency and are harder to validate.

3) Limited Integration Across Platforms: At the experimental level, in vitro models are still unable to be combined to reliably replicate results in whole animals. Computationally, mechanistic models lack a standard architecture that would allow them to be interconnected or to be moved from one platform to another. Thus different groups cannot create libraries of mechanistic models, e.g., of cell types or organ systems, which can then be integrated by others to create novel models. There is also a lack of standard approaches to integrate in vitro assays with their mechanistic simulation

counterparts, to facilitate model development and validation. Without this integration, the development of holistic computational models of entire organisms remains infeasible.
4) Data Sharing Dilemmas: Virtual Tissues (VTs) suffer from a lack of universal tools and standards for sharable development and dissemination. Consequently, the insights in individual models remain trapped within individual research groups.
Propelling the Use of Mechanistic Models:
1) Smart Annotation Tools: An immediate need is tools that automate the comprehensive, structured cataloging of experimental details. A tool prompting researchers to use standard annotations, especially gene and protein names, would be a step in the right direction. These machine-readable annotations can be subtly incorporated into publications for easy access by search engines.
2) Bridging the Gap Between Modelers and Experimentalists: Collaboration is key. Often, experimental data doesn't serve modelers' needs. Early-stage dialogues can tailor experiments for more relevant data outcomes.
3) Evolving Modeling Standards: Current standards cater to specific biological scales or mathematical methodologies. The next generation of standards should address the intricate nuances of multi-scale, multicellular models.
4) Standards bridging experiment and computation. Many of the key tasks in both wet-lab and in-silico biology do not have standardized sharable descriptions, impeding the replication and cross validation of results and methodologies.
5) Some critical needs are formalisms for the specification of the purpose and limitations of wet-lab and in silico experiments, the description of wet-lab and computational experiments processes and the set of possible experimental manipulations (not all conceivable experiments are possible). The latter are essential for the design of optimal control systems (e.g., to design informative new experiments, or to personalize therapies to achieve desired health-state outcomes)
6) Integration also requires new approaches to the formal description of the outputs of complex spatio-temporal experiments and mechanistic models and their variability. An additional need is formalism to describe the manipulation of output data during analysis, so, e.g., a published figure in a paper would include the raw data that contributed to it as well as the complete reproducible set of transformations used to generated the processed figure.
7) Open-Access Data Platforms: For mechanistic models to advance, accessible repositories for model validation data are crucial. Facilitating collaborations and transparent data sharing can foster the growth of these models.
8) Embracing Collaborative Networks: European-style networks showcase the power of collective effort. Such cooperative structures can streamline resources and expertise, promoting rapid advancements.
9) Comparative Analysis for VT Outputs: Methods for contrasting VT outcomes with traditional experimental results need development, considering the inherent stochasticity in simulations and laboratory tests.
10) Systematic efforts to integrate Machine-Learning/Artificial Intelligence approaches with mechanistic models have the potential to accelerate discovery more effectively than either approach on its own. The range of possible synergies is very broad.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

To truly maximize the VALUE of research, it's pivotal to enhance the reusability of technology, data, and biological understanding beyond the confines of the original research project. This demands an open, transparent sharing of both laboratory and computational models, along with their associated data, in ways that align with best practices like FAIR (Findable, Accessible, Interoperable, and Reusable) principles.

Promoting open access to these technologies is key. They should not only be easily accessible but also have robust support mechanisms, potentially via foundations or similar entities. Additionally, ensuring that these technologies are continually updatable, reusable, interoperable and extendable – much like the open-source nature of Python – is of importance.

Open-source licensing of software infrastructure, model components, APIs and standards can be valuable, allowing for commercial exploitation while safeguarding the interests of original developers. A case in point is the Llama 2 (large language model, like ChatGPT) license by Meta AI, which incorporates an acceptable use policy. This policy ensures that larger commercial entities contribute to the originating organization when leveraging open-source technology.

Infrastructure advancement should facilitate real-time, interactive collaborations on models and their abstraction into reusable components, empowering the community to refine and expand existing models iteratively. As we progress, it's crucial that open-source licensing terms support commercial ventures. This approach will ensure the translation of tax-payer-supported technologies in clinical and other settings and contribute to their sustainability. Fostering relationships between modelers and clinicians and the private sector is essential.

While data integration poses challenges, given the varied techniques and toolkits across labs, the qualitative essence of these datasets remains invaluable. Harnessing computational techniques that amalgamate data from diverse labs can facilitate data reuse, either qualitatively or as a hybrid with quantitative data. Such strategies could pave the way for models demanding fewer experiments, reducing animal usage.

There is a pressing need for a dedicated funding to support infrastructure and data sharing endeavors, particularly in areas of data annotation, presentation, and documentation. Streamlining these processes is fundamental to bridging the gap between mechanistic computational models and their experimental counterparts. The longevity of models hinges on creation of effective method-independent modular model descriptions. This ensures that the biological understanding and data encapsulated within a model transcend the lifespan of the initial development platform and that components of a model (especially validated components) are available for extension, reuse and repurposing in other contexts.

As we strive to diminish reliance on animal research, tapping the vast reservoir of previously collected animal data becomes a prudent strategy. Integrating data from diverse sources, especially when stored across diverse databases or when presented using inconsistent data standards, is extremely time-consuming and difficult. A concentrated effort to collate and standardize these data is imperative. This labor-intensive task demands expertise spanning statistics, bioinformatics, and domain-specific knowledge. An integral aspect of this collaboration is the meticulous annotation of data with metadata, detailing aspects like experimental conditions, species, and variables. The sheer diversity in animal models, species, and research objectives means datasets are highly heterogeneous. Analyzing and drawing meaningful conclusions from such diverse data will be

challenging. Understanding the context and limitations of the data, including the specific research questions for which it was originally collected, is essential for accurate interpretation and appropriate use.

Lastly, syncing with large-scale European initiatives, such as EDITH, is also critical. Collaborative efforts on such scales can amplify the impact and reach of our research endeavors.

Email: jaglazier@gmail.com

Submit date: 9/5/2023

I am responding to this RFI: On behalf of myself

Name: Gregor Neuert

Name of Organization: Vanderbilt University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Current traditional approaches and current Novel Alternative Methods (NAMs) often measure cellular responses to instantaneous changes in the extracellular environment, which are simpler to control, but lack physiological relevance. What is missing is an innovative technology that focuses on experimentally modeling the dynamic and gradual changes that cells experience within the human body. These dynamic changes are crucial as cells in our bodies are constantly exposed to fluctuating concentrations of hormones, growth factors, nutrients, and therapeutic drugs. These variations occur in conjunction with changes in blood flow through tissues, creating complex and ever-changing cellular environments in-vivo.
   Future novel alternative methods, called dynamic Novel Alternative Methods or dNAMs, should replicate in vivo fluctuations in environmental conditions, providing a more realistic environment for studying cellular responses in vitro. A notable advancement of dNAMs should be its compatibility with a wide range of laboratory assays for both cell populations and single cells. This compatibility would allow researchers to explore cellular behavior under dynamic conditions without sacrificing the ability to conduct standard laboratory analyses, as is often the case with other technologies such as microphysiological or organs-on-chips systems.
   The impact of dNAMs is significant in advancing our understanding of human biology, circuits, systems, and disease states. By focusing on gradual and physiological changes, the dNAM approach will provide insights into how cells respond in contexts that more closely resemble real-life scenarios. The dNAM technology has the potential to revolutionize our understanding and treatment of human health and therefore addresses an underserved area of research as it stands in contrast to traditional methods and existing novel alternative methods that use escalating acute concentration changes and that often overlook the effects of gradual changes on cellular behavior.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Validating dynamic Novel Alternative Methods (dNAMs) presents multifaceted challenges that demand comprehensive solutions. dNAMs that model in-vitro the in-vivo physiology dynamics should be implemented by including multiple replica, controls, and experimental conditions within a single experiment. Furthermore, the technology should be benchmarked using acute conditions against standard laboratory techniques, ensuring the introduction of minimal differences.
   Post-sample collection, the dNAMs technology should include semi or fully automated data analysis to mitigate human bias and errors. This combination of meticulous experimental design and automated analysis will contribute to the generation of high-quality data and robust computational

insights. While these measures are effective initially, further development is necessary to automate the dynamic profile generation process, experiment execution, and data analysis, enhancing the efficiency and reliability of the dNAMs technology.

To bolster dNAMs technology readiness and reliability, the proposed roadmap should include commercialization efforts. The transition to a commercial platform will foster rigorous testing, validation, and optimization, strengthening the technology's robustness and applicability. The infusion of resources and expertise from the commercial sector will contribute significantly to technology maturity.

However, we recognize that the paradigm shift we propose could face resistance in integration of dNAMs into existing laboratory workflows. A potential factor limiting seamless integration is the need for researchers to adapt their methodologies to accommodate dNAMs novel technology which could be overcome by providing funding opportunities to develop dNAMs through grant supplements, technology development grants for individual investigators (R21 / R01 grant mechanisms), consortium grants (U grant mechanisms), collaborative grants between universities and startup companies (SBIR, STTB grant mechanisms), and collaborative funding opportunities between NIH and NSF. Additionally, addressing the above listed limitation requires proactive outreach, training, and demonstrations to illustrate the benefits of our technology in augmenting research outcomes.

Catalyzing the development and validation of dNAMs involves the strategic navigation of these challenges. To build robustness, replicability, reproducibility, and reliability into the dNAM technologies and datasets, rigorous experimental design, quality controls, and automation are key. Bolstering dNAMs technology readiness and reliability needs a comprehensive commercialization strategy that promotes thorough testing and optimization. To overcome the challenge of integrating dNAMs across research approaches, fostering a culture of adaptability and investing in training and education initiatives will be instrumental. Additionally, supplying adequate funding that encompasses both basic research, hardware and software technology development, and technology commercialization is pivotal for the successful progression of the transformative dNAM approaches.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Maximizing the research value of dynamic Novel Alternative Methods (dNAMs) involves strategic considerations that span accessibility, compatibility, and holistic experimental design. To ensure broad accessibility, dNAMs should be designed to cater to a diverse spectrum of researchers. A concurrent strategy is to render dNAMs compatible with standard laboratory assays, easing their seamless integration into established workflows.

Despite these proactive strategies, potential challenges persist. Building robustness, replicability, reproducibility, and reliability into dNAMs and the resulting datasets demands meticulous quality control mechanisms, standardized protocols, and automated data processing. To bolster technology readiness and reliability, concerted efforts are necessary, including rigorous testing, optimization, and validation. This journey is further accelerated through collaborations with industry partners who can infuse expertise and resources into the refinement process.

A factor constraining the integration of these dNAMs technologies across research approaches is the requirement for a skilled and quantitatively adept workforce. The effective use of dNAMs necessitates proficiency not only in conducting experiments but also in harnessing computational

tools for data analysis. Addressing this limitation mandates investments in training programs (T31, T32, F31, F32, K00, K99, etc.) and educational initiatives that empower researchers with the competencies needed to navigate these novel methodologies.
Strategies to maximize research value, therefore, encompass diverse dimensions. To enhance accessibility, dNAMs should be crafted with a broad user base in mind. Compatibility with existing laboratory assays enables integration, bolstering adoption rates. Concurrently, emphasis on cohesive experimental design and computational analysis workflows ensures that each experiment yields comprehensive insights. Overcoming challenges requires a multi-pronged approach, from instituting stringent quality control mechanisms to engaging in collaborative partnerships for validation. Moreover, investing in training and education initiatives is pivotal for overcoming the workforce challenge and enabling researchers to fully harness the potential of these transformative dNAMs.

Description: Please feel free to reach out to me with additional questions.

Email: gregor.neuert@vanderbilt.edu

Submit date: 9/5/2023

I am responding to this RFI: On behalf of an organization

Name: Gwynne Grasberger

Name of Organization: Drexel University

Type of Organization: University

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   The value of any modeling approach is based on the assertion that known similarities between the model and the subject matter permit conclusions that additional features observed in the model will also be observed in the domain to which the model is applied. An optimal future state is one in which our understanding of human biology is sufficient to design modeling systems that accurately reflect the complexity of that biology.
   Depending on the biological system or disease state, different combinations of methods may be required to provide the strongest body of evidence.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Many of the issues with rigor and translatability in animal models must also be addressed for non-animal models, such as considerations of human biological relevance, study design, statistical analysis, data sharing, and reporting. However, there are additional considerations for rigor and translatability that are unique to the development of NAMs, where development of new technologies and methodologies can outpace scientific consensus on standards.
   Not all code is open source and not all methods have complete documentation. The context of use is not always made clear. State space explosion is an issue for model checking. Disadvantages of modeling and simulation include the cost of running several different simulations. Simulation-based approaches require appropriate levels of fidelity.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Accuracy measures the correctness of predictions made by the model while fidelity measures the general agreement between the models on any input. Simulation fidelity can and should be decomposed into its constituent components of resolution, error/accuracy, sensitivity, precision, and capability. Limit the fidelity required and implemented to that which is actually needed.
   Differing levels of fidelity, missing functionality are factors potentially limiting successful integration.

Email: researchdevelopment@drexel.edu

Submit date: 9/5/2023

I am responding to this RFI: On behalf of myself

Name: Joshua F. Robinson

Name of Organization: University of California, San Francisco (UCSF)

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Our laboratory leverages in silico and in vitro models to study embryogenesis and placentation in the context of human disease, toxicology and environmental health. In particular, human primary and stem cell models have greatly increased our ability to study interactions that underlie biological processes necessary for aspects of early human development (e.g., neural tube development, placental development) and diseases that otherwise cannot be studied in vivo or may not be properly represented in traditional animal models. We have demonstrated that these model systems (e.g., primary human trophoblasts, human embryonic stem cell neural differentiation model) on cellular and molecular levels align with their in vivo counterpart. Using these model systems, we are able to interrogate the molecular bases of development and apply innovative tools (omics, functional genomics) to define mechanisms that lead to impairment of biological processes and disease. Examples of this work from our lab include the identification of molecular pathways affected in the context of impaired early human neural cell function, providing insight into pathways that underlie defects in central nervous system development. On a larger scale, collectively, the use of alternatives, including cell-free systems (reporter assays), human cell lines (e.g., hESCs), non-mammalian models (zebrafish) and animal-reduction models (whole embryo culture), have been critical in defining the role of major signaling pathways (e.g., retinoic acid signaling) in development and disease.
   More complex model systems e.g., co-culture models, organoids, bioengineered systems are emerging, and will provide novel ways to investigate interactions among different cell-types that more closely mimic the human condition. I envision that these systems will be widely used as they mature in their applicability and reproducibility. Several roadblocks exist due to 1) the cost of these systems; 2) the necessary expertise and workforce; and 3) the lack of financial/technical support to implement these systems. And while NAMs have greatly improved the ability to investigate interactions underlying fundamental processes in developmental biology, there is a lack of understanding when it comes to human variability and how genetics or other factors may increase or decrease risk of developmental disease. Alternative biological models may capture some of this variability; similar to rodent studies; however, long-term, computational models seem to provide the best way to address human variability.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   In my specific research domain, securing funding for translational projects that seamlessly integrate computational, in vitro experiments at different levels, in vivo studies, and human investigations can

be challenging. Nevertheless, it is these very projects that play a pivotal role in advancing the development and validation of alternative methodologies while providing a substantial dataset for a comprehensive assessment of both achievements and significant limitations.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   I anticipate that integrated methodologies encompassing computational techniques such as machine learning, diverse biological and experimental models, and in vivo/human studies will facilitate a comprehensive exploration of the applicability domain of New Approach Methodologies (NAMS), thereby enhancing their research utility significantly. It is imperative that these strategies are tailored to specific domains of development or disease. Although many of these systems exhibit wide-ranging applicability, it is crucial to ascertain their effectiveness within specific developmental or disease contexts.
   For instance, in the field of toxicology, we employ Adverse Outcome Pathways to establish connections between molecular initiating events and subsequent alterations at molecular, cellular, morphological, and organismal levels. It would be of great interest to observe how a diverse array of NAMS can, in a consistent and dependable manner, forecast interactions leading to specific adverse outcomes or diseases.

Email: joshua.robinson@ucsf.edu

Submit date: 9/5/2023

I am responding to this RFI: On behalf of an organization

Name: Megan R LaFollette

Name of Organization: The 3Rs Collaborative

Type of Organization: Nonprofit research organization

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**

Dear Working Group Members,

The 3Rs Collaborative (3RsC) appreciates the opportunity to provide comments on the Request for Information (RFI) (NOT-OD-23-140) regarding the development and use of Novel Alternative Methods (NAMs). We are a U.S.-based non-profit whose mission is to advance better science – for both people and animals – through facilitating collaborative 3Rs efforts on refinement, reduction, and replacement. Catalyzing the use of NAMs is therefore a central part of our organization. We currently have an initiative devoted to advancing microphysiological systems (MPS) that includes 96 members from 44 organizations (primary commercial developers). Furthermore, we are launching an initiative to advance the use of artificial intelligence in biomedical research. We appreciate the National Institutes of Health (NIH) Advisory Committee to the Director's (ACD) approach to promoting and investing in the use of NAMs in biomedical research.

The 3Rs Collaborative has identified three central themes to aid the Working Group and NIH in outlining future areas of investment:

- Enhance collaborations with non-profits and scientific societies especially those that encourage collaboration between various stakeholders (e.g., researchers utilizing animal models and/or NAMs, NAMs developers, etc.) and sectors (e.g., academics, industries, contract research organizations, regulators, developers, and IACUCs).
- Endorse and invest in implementing scientifically justified NAMs using evidence-based techniques to create change by promoting positive attitudes, providing training on implementation, and assisting in the normalization and socialization of 3Rs progress.
- Provide investments for the independent characterization and validation of currently developed and future developed NAMs (especially those that have been commercialized) to ensure that these technologies are well-characterized and validated for contexts of use.

Please find the 3Rs Collaborative's comments on each RFI topic below.

Topic 1: The use of NAMs to study human biology, circuits, systems, and disease states.

a. How NAMs are currently being developed and/or used successfully, including features that maximize scientific utility:

b. How NAMs are advancing progress into understanding specific biological processes or human states, including potential limitations to addressing human variability; and

c. How NAMs could be truly revolutionary for understanding/treating human health, including currently underserved areas of biomedical research:

3RsC Response:

High-level summary:

- In vitro methods, such as MPS, are currently being used successfully in many circumstances to predict chemical/drug toxicity, PK/PD, and drug efficacy (especially in areas without translational animal models or for personalized medicine) as well as for quality control testing of vaccines.
- Key features that maximize utility include clear contexts of use, clear methodology/cell source/cell culture practices, and reliability efforts.
- Current potential limitations include limited recapitulation of portions of an organ or single organ and potentially limited external stimuli.
- NAMs could be truly revolutionary especially in addressing underserved areas of biomedical research without translational animal models such as rare diseases as well as accelerating the drug development process

NAMs are currently being developed and used successfully to understand and treat human health. This includes using in vitro methods such as developing MPS. The 3Rs Collaborative's MPS initiative has quarterly organ-specific workshops with the IQ MPS Affiliate that demonstrate some of these applications. For example, MPS can be used to predict toxicity of drug candidates early in drug discovery to determine which candidates to proceed into animal tests (Wang e al. 2022). MPS can be used to predict pharmacokinetics and pharmacodynamics (Baran et al. 2022) as well as predict permeability of drugs into organs and study organ development (Pellegrini et al. 2020). They may be particularly useful for conditions that currently do not have translational animal models. For example, MPS can be used to identify complex diseases such as DILI (Ewart et al. 2022) which are not predicted in animals. In addition, MPS have taken diseased tissues from human patients to directly examine the efficacy of treatments. This includes a wide variety of models including lung models for human diseases such as allergic rhinitis, cystic fibrosis, and COPD. Additionally, MPS can incorporate many aspects of the human immune system. In vitro assays have successfully been used to replace animal models in quality control batch testing of vaccines and certain toxicological tests such as for skin sensitization. Features that maximize their scientific utility include development of MPS for a clear context of use, processes for good data and cell sourcing, and a focus on reliability and reproducibility.

Along with this critical advance of science, as with all model systems, MPS have limitations. Current MPS systems are largely homogeneous with efforts to introduce heterogeneity underway. They typically only recapitulate some features of a single organ or a limited number of organs. Therefore, they may not always predict whole-body responses. They also are housed in incubators without variable external conditions which limits their ability to predict relevant variability that results from external stimuli. Finally, there are certain conditions that MPS cannot recapitulate such as whole body behavior responses, large bone fractures, or mental health conditions.

Beyond in vitro methods, in silico methods are currently being developed and used to predict toxicity or efficacy as independent predictive tools and to generate synthetic data which simulate experimental studies (e.g., virtual control animals or digital twin models). Although current applications may be related more to the assessment of hazardous chemicals (Borba et al 2022), they could be expanded to include study of human biology, circuits, systems, and disease states. Digital twins may also be used to assist in the design of clinical trials. These applications can enhance knowledge gained from current human and animal studies and play an important role in the implementation of the 3Rs (refinement, reduction, and replacement).

NAMs are advancing progress into understanding specific biological processes and human conditions and can be truly revolutionary for understanding and treating human health. Data on

translation between animals and humans are expanding and we may find them similar (e.g.,) or different (e.g.,). The differences are increasingly being understood to be caused by species sensitivity and the complex physiological differences between humans and animals. How to incorporate NAMs into regulatory nonclinical pharmaceutical safety assessment is discussed in . As stated above, NAMs have the potential to address human variability – and ultimately provide translational results, by purposely building relevant biological variability into these models from their initial development in ways that could be like calls to introduce systematic heterogenization into animal studies (Voekl et al. 2020). For example, in vitro methods are increasingly being developed from primary cell lines with genetically diversity replacing cell lines. NAMs may be useful to address underserved areas of biomedical research if targeted efforts are made towards these aims. Support for these applications and consensus on purposeful relevant variability must be made to ensure their application.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

High-level Summary:

- Funding a national U.S.-based, balanced 3Rs (replacement, reduction, refinement) center whose focus is on harnessing the power of collaboration to increase implementation of all 3Rs (including NAMs) using evidence-based techniques is recommended. NAMs forwards all 3Rs by replacing some animal models, reducing required animal numbers by guiding study design, and refining necessary tests thereby reducing pain/distress.
- Key challenges for NAMs include (1) a lack of funding/work on the independent characterization, validation, and qualification of these technologies, and (2) lack of engagement with best practices such as good cell culture practice and RIVER.
- Strategies for bolstering technological readiness and reliability of these technologies and widespread integration of these technologies includes (1) funding to independent validation and data sharing (especially of widely available commercial products or open access methods), (2) incentives to include NAMs data in regulatory submissions, and (3) support for non-profits that provide educational materials and advocate for responsible inclusion and analysis of NAMs in partnership with an understanding of the in vivo human and animal models.
- Key factors limiting the integration of NAMs across research is a lack of funding directed at efforts to move NAMs from primary research, through validation, and into widespread implementation. Current efforts such as NICEATM will be instrumental in the socialization and normalization of the use of NAMs and their acceptability.
- Solutions to integrate technologies include funding for independent characterization/validation and funding focused on increasing implementation of NAMs. Of note, we recommend balanced, accurate messaging on NAMs that neither over-promises nor holistically condemns animal research – as this can alienate and activate cognitive dissonance in scientists currently utilizing animal models. Progress will be facilitated by all researchers working together to develop the best model possible to support the scientific objective with ethical considerations for both animals and humans.

2a-b. One of the primary challenges in building robust, reproducible, and reliable NAMs is a lack of adequate funding for characterization and validation efforts specifically and lack of consensus on validation strategies. Therefore, as NIH outlines future investment areas, the 3RsC advises a strong prioritization on funding characterization and validation efforts and consensus efforts related to

validation before allocating funds towards the development of additional new NAMs.
Characterization is particularly critical when considering cell source and hardware as variability and supporting efforts and consensus on cell sourcing is essential. Ideally, validation efforts should be performed by independent organizations to those who are developing the NAMs – especially as this ensures the transferability of these technologies to additional laboratories.
Building confidence in NAMs is a complex, but critical pursuit to ensure their widespread use. One strategy to bolster technology readiness and reliability is to encourage good publication and data sharing practices that are reproducible and demonstrate when a NAM has been shown to be equal or superior to another assay that relies on an animal model. In particular, the 3RsC encourages the use of the RIVER (Reporting In Vitro Experiments Responsibly) which was developed by the NC3Rs, which was closely involved in developing the widely encouraged ARRIVE guidelines. Furthermore, as stated above, it is important for end-users to understand what amount of variability is tolerable and perhaps representative of the larger population and what variability is the result of poor reproducibility and consistency in application. The 3RsC encourages NIH to partner with commercial technology providers of MPS in particular as these technologies are often built for higher scalability and reproducibility compared to initial model development.
The 3RsC furthermore encourages that NIH continue to partner with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Validation Workgroup and Consideration of Alternative Methods Working Group to help develop validation, reporting, and best practice strategies for NAMs. These working groups benefit from meeting regularly with stakeholders outside of the federal government including academia, industry groups, non-profits, and other stakeholders.
The 3RsC advises that a wider availability of failed and successful clinical drug tests and their reasons for failure would be helpful for development and validation of NAMs. Additionally, it is our perspective that human data (when available) should be considered the gold standard of comparison. Therefore, model development that relates directly to human information has the highest potential for success. Some examples have been sought in the chemical space evaluating historical data from occupational hazard observations (e.g., Ponder et al. 2022)
Overall, the 3RsC recommends a multi-pronged approach to bolster technology readiness and reliability of these technologies. This includes funding to facilitate connections between commercial developers with future end-users, funding for independent validation and data sharing, and incentives to include relevant, reliable NAMs data in regulatory submission. A core part of bolstering readiness and reliability of these technologies is increasing general confidence and use of them. To achieve this aim, we recommend providing funding for US-based non-profits (such as the 3RsC) that provide educational materials and advocate for NAMs widespread usage. This may include efforts to provide widespread, standardized training and methods for use of NAMs.
2c. The 3RsC finds there are several factors that are potentially limiting the successful integration of these technologies across research approaches and potential solutions. There is a sometimes termed “valley of death” that occurs between the development of new 3Rs technologies and their widespread implementation. Unfortunately, widespread implementation does not occur solely from funding basic research or even create policies or recommendations. These difficulties in moving from basic research/concepts to implementation can be seen in efforts to implement blinding, fixing sex bias, implementing environmental health monitoring, and refined rodent handling methods (Karp et al. 2022, Karp et al. 2018, Luchins et al. 2023, LaFollette et al. 2019). Research professionals

are unlikely to implement new techniques unless they believe the techniques are beneficial, there is professional pressure to use them, and they are confident in their ability to use them well. For NAMs, there are often entrenched beliefs that animal data is more robust than NAMs, there is a lack of professional pressure to use them (or even examples of successful use), and a lack of confidence in the performance of NAMs.

Another challenge that limits the integration of NAMs across research approaches and potential solutions is the lack of a primary, unbiased independent scientific center in the United States that addresses all 3Rs through a combination of approaches including funding streams, training, meta-research projects, dissemination, and implementation efforts. It is important to promote NAMs not only for safety/toxicity testing but also efficacy and basic research. Without a central informational center, scientists focused on animal research and scientists focused on NAMs may engage with disparate information sources. This segregation of knowledge may prevent the true integration of NAMs across research.

For an excellent example of an effective central 3Rs organization, we recommend the NIH look to the UK-NC3Rs. The NC3Rs was established in 2004 and now receives a 10 million pound budget per year. The early establishment and adequate funding of such a center has accelerated the implementation of 3Rs approaches within the UK. Since the adoption of the European Union Directive 2010/63/EU, nearly every country has created a government funded 3Rs center to not only develop novel methods, but also promote and accelerate the adoption of the 3Rs within their country (Neuhaus 2022a). An overview of their status, financial/organizational structures, and activities can be found in Neuhaus 2022b.

To address these challenges, a multi-pronged approach is needed. To build confidence in NAMs by the wider scientific field, there must be funding for the necessary characterization and validation tests as described above as well as concerted effort to disseminate these findings to the wider scientific community. Furthermore, there must be concerted efforts to provide widely accessible engagement with and training in NAMs. To build confidence in NAMs by regulators, we recommend incentives to include validated data from NAMs in addition to animal tests. Funding for groups that highlight or champion successful use of NAMs (while also addressing a realistic view of their challenges and solutions) by early adopters to encourage more widespread usage will be essential going forward to mainstream the integration of NAMs across the community.

To encourage current animal model researchers to engage with and utilize appropriate NAMs, there must be carefully constructed messaging on the topic. Unfortunately, there is not enough balanced messaging on the status quo and realistic near future for NAMs. When groups focus primarily on condemning animal studies to encourage greater use of NAMs, they often alienate the very groups that they need to work with. Balanced messaging about NAMs that highlight their ability to complement or supplant animal tests in specific validated contexts of use (without overpromising on NAMs) is more likely to help successfully integrate these technologies across fields. Therefore, the 3RsC encourages funding for such balanced, targeted dissemination and implementation efforts.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

High-Level Summary

- Coordinated approaches across research sectors are needed to both understand challenges and successes of animal research models, as well as assist in the widespread use of these technologies.

- Equitable deployment across labs will require support for making higher-throughput, lower-touchpoint and less costly NAMs.
- To maximize translatability and minimize bias, efforts should be made to implement standards for rigor and reporting.

3a. To maximize the research value of NAMs, it is critical that clear efforts are made to increase their widespread use. These new techniques are only valuable if they are used. There are several 'novel' approaches that have been developed and validated but lack widespread implementation. Therefore, it is essential that as NAMs are developed to replace animal studies that coordinated efforts are made to increase their implementation. This requires not only formally funded validation efforts, but also formally funded dissemination efforts by experts in communication and widespread behavior change. The 3RsC recommends funding to implement a coordinated approach across research disciplines and sectors to encourage widespread implementation using proven science communication and behavior change efforts.

3b. The dissemination efforts described above will also help ensure equitable deployment of NAMs across labs. Not only must these labs learn about and be convinced of the utility of NAMs, but they must also have adequate support for any staff, infrastructure, or external contracts to employ these NAMs. Therefore, the 3RsC recommends NIH provide support for research infrastructure, resources, and technical staff to allow access to NAMs. It is essential that training is provided throughout the scientific research careers, and especially for new scientists.

3c. To ensure translation (and widespread use) of NAMs, the 3RsC once again encourages NIH to foster collaborations between current animal researchers and NAM developers/users. It is critical for NAMs developers to understand the current challenges and successes with animal research to address key gaps directly. Additionally, developing positive relationships between these stakeholders will encourage more widespread use. These collaborations may include efforts such as diverse working groups, conferences/symposiums, and training efforts. We advise partnering with scientific societies and non-profits like the 3RsC to leverage their positive relationships and networks for the best impact.

To maximize the translation of NAMs and minimize bias related to human variability, it is important to implement standards for rigor and reporting such as the RIVER guidelines as stated above. It would also be beneficial to establish guidelines like the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE), but for in vitro and in silico studies. When possible, any guidelines or recommendations that are developed should be streamlined across federal agencies to assist the development and validation of NAMS.

The 3RsC recommends the strategies cited in recent work by ICCVAM and the National Academies of Science, Engineering, and Medicine (NASEM). ICCVAM recently published a framework for establishing scientific confidence in new approach methodologies (van der Zalm, 2022). Five essential elements are recommended: fitness for purpose, human biological relevance, technical characterization, data integrity and transparency, and independent review. Although this framework was developed to evaluate NAMs for pesticides and industrial chemicals, it is easily adaptable to biomedical research.

Conclusion

The 3Rs Collaborative appreciates the opportunity to comment on strategies for maximizing the development and use of NAMs. Stakeholder feedback is crucial to policymaking. There is a clear need for funding to focus on 3Rs research, reproducibility, translation, and dissemination. As

scientists that strongly support the development and implementation of the 3Rs of biomedical research, including replacement technologies such as NAMs, we look forward to future engagement on this topic and the Working Group's final recommendations.

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Description: The 3Rs Collaborative's response

Email: meglafollette@na3rsc.org

Submit date: 9/5/2023

I am responding to this RFI: On behalf of an organization

Name: Sue Leary

Name of Organization: Alternatives Research & Development Foundation

Type of Organization: Other

Type of Organization-Other: non-profit foundation

Role: Member of the public

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   "The Alternatives Research & Development Foundation (ARDF) was established in 1993 to provide funding for researchers developing non-animal research methods that hold the potential to reduce or replace the use of animals in testing, biomedical research, and education. With 30 years of experience funding non-animal research methods, now often called "new approach methods" or "novel alternative methods" (NAMs), we have watched with great enthusiasm as the interest in using NAMs for biomedical research has rapidly expanded in recent years. Our funding portfolio has shifted dramatically over the past decade, from primarily applications aimed at toxicology to the majority being focused on biomedical research models aimed at understanding fundamental aspects of human biology and biological processes. As a funding organization, our primary focus is supporting the researchers who are advancing NAMs science, and we hope that this request for information receives a robust and informative technical response from the diverse community of NAMs researchers. We appreciate this opportunity to provide feedback on NIH's approach and potential timeline.
   We applaud the NIH for its current initiative exploring opportunities to increase its investment in NAMs research and encourage it to move forward with these efforts expeditiously. Increasing NIH investment in the development of NAMs is an essential factor in addressing NIH's mission to "enhance health lengthen life and reduce illness and disability." It is also an ethical imperative in two respects, by reducing the use of animals in research—an undisputed aim of the 3Rs framework, which NIH endorses—and by creating more human-relevant models with greater predictive ability for human health applications. The limitations of animal models in addressing many of our most pressing medical challenges are well documented and overcoming these challenges will require new and innovative approaches.
   A frequent response to concerns about the limitations of animal models is that NAMs simply "aren't there" yet and that their limitations exceed those of most animal models. While this is currently the case for many (though not all) research domains, it is also a self-fulfilling prophecy. Developing any model, animal or non-animal, requires substantial investment. Continuing to treat NAMs as a marginal research area will ensure that these breakthroughs take much longer than necessary to emerge. The only way to realize the full potential of NAMs for understanding human biology, circuits, systems, and disease states is to substantially increase NIH's investment in this area, and to treat the development of NAMs as a high-priority research area, on par with other major initiatives

NIH has undertaken in recent years with the aim of revolutionizing a specific disease or scientific domain.
We acknowledge that any significant shift of resources and scaling up of new investments is a major undertaking for any organization, however, we hope that NIH will address this initiative with the urgency it deserves. Counterpart research agencies in Europe, and even other agencies in the U.S., have recognized the importance of investing in NAMs research for several years; NIH has a lot of lost time to make up for on this front. However, NIH's position as the premier health research agency in the world means it could play an important leadership role in influencing the trajectory of some of the most exciting and promising research discoveries in the twenty-first century.

In its recent workshop on "Catalyzing Development and Use of Novel Alternative Methods the ACD Working Group addressing this issue laid out several key themes, including the promise of NAMs for addressing scientific challenges important to patient communities, particularly those related to population variability and health disparities. These aspects have proven especially resistant to progress using animal models, but they are incredibly important to creating more effective and equitable interventions in pursuit of NIH's mission. We hope that the current and ongoing conversations about the potential power of NAMs prompt greater recognition that failing to fully exploit these technologies fails the patient communities that are waiting for these breakthroughs.

We encourage NIH to pursue its effort in supporting NAMs on several fronts and to avoid waiting on lengthy deliberations by various committees. NIH should assume a leadership role in NAMs research and development and there are several approaches for prioritizing research investments. It is not necessary to have an NIH-wide consensus before moving forward with different initiatives. Indeed, we applaud NIH's recent initiative to establish Translational Research Centers for Microphysiological Systems (RFA-TR-23-001). This FOA is an outstanding example of the NIH's ability to make a significant impact in a specific, targeted research area, and we hope it is just the start of much more substantial investments.

Although funding is crucial, we would also like to underscore the importance of policy and cultural leadership, which NIH is also well positioned to leverage. Encouraging collaboration between researchers, through thoughtfully developed policies and requirements, as well as data-sharing and standardization, will be essential for creating productive, rigorous, and reliable NAMs technology. NIH should also take a greater role in encouraging the uptake of NAMs in areas they have proven successful. The biomedical research community, like any other professional culture, is subject to many cultural pressures that make groups and individuals resistant to change. Acceptance of NAMs science will require attitude shifts across all levels of the biomedical research enterprise, from NIH leadership to study section members. We encourage NIH to take these cultural factors seriously and to make explicit, evidence-based efforts to address them.

ARDF's own funding strategy is to prioritize the potential impact for replacing animals in the near- to mid-term in specific research contexts of use, which reflects our mission as an organization. Within that approach, we also prioritize non-animal models to replace the use of animals in research that generally causes severe pain, such as wound healing and osteoarthritis. We realize that NIH has a broader mission to improve human health, but we hope that its efforts in the NAMs space are not limited only to research areas with few or low-quality animal models. NIH's past timidity in supporting NAMs for the purpose of replacing animals has been disappointing, and we hope that its

current interest signals a shift in its approach. Developing biologically accurate, scientifically useful, and reliable NAMs for use in biomedical research is a tremendous undertaking that will require substantial investment. We hope NIH will commit to becoming a leader in supporting NAMs development and will serve as a catalyst to usher in the next era of biomedical research advances.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   (see question 1)

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   (see question 1)

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Description: PDF version of ARDF response

Email: grants@ardf-online.org

Submit date: 9/5/2023

I am responding to this RFI: On behalf of an organization

Name: Gina Lento

Name of Organization: vivoVerse, Inc.

Type of Organization: Biotech pharmaceutical company

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   The notice for this workshop (NOT-OD-23-140) mentions "These "novel alternative methods" (NAMs) include in chemico strategies (e.g., experiments on biological molecules like DNA and proteins in test tubes); in vitro methods (e.g., exploring the nature of cells and tissues by culturing them in sterile chambers); and in silico computational models that simulate how these biological systems work and predict outcomes to refine hypotheses (e.g., to define how potential drugs interact with their biological targets and to refine clinical intervention and procedures that increase patient safety and treatment efficacy)."

   An important omission from this list of NAM approaches is that of alternative in vivo small model organisms (e.g., the microscopic nematode, Caenorhabditis elegans (or C. elegans); zebrafish; planarian flatworms and others). Among these, C. elegans stands out for reasons that are explicitly relevant to the areas of information sought here. Below we summarize a small selection of the vast body of research on this small model organism that addresses this call for information.

   In the area of The use of novel alternative methods to study human biology, circuits, systems, and disease states:
   - Neurodegeneration. Caldwell et al. (2020; doi:10.1242/dmm.046110) notes that while C. elegans has served as a model organism for "multiple transformative discoveries that have refined our understanding of biology for ~60 years", its considerable attributes have been powerful for modeling neurodegeneration. Their 2020 paper highlights how C. elegans has been used as a model for Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Huntington's disease (HD) and Parkinson's disease (PD).
   - Human reproductive system. Athar and Templeman (2022; doi:10.1016/j.cbpa.2022.111152) demonstrate how C. elegans have been pivotal in uncovering mechanistic insights into reproduction. It has helped us to understand female reproductive health—reproductive aging, chemical-induced reproductive dysfunction, and reproductive cancers. Reproductive spans and age-related deterioration of reproductive tissue in human females and C. elegans hermaphrodites are comparable. Similar patterns of fertility and hallmarks of reproductive function decline with age.
   - Metabolism, cellular transport and environmental toxicology. Hartman et al. (2021; doi:10.1080/10937404.2021.1884921) discuss the emergence of C. elegans "as an important model in biomedical and environmental toxicology." The routes of chemical uptake by C. elegans (absorption through the cuticle (or 'skin') and by ingestion) are relevant to modes of human exposure to environmental toxins. "Emerging evidence suggests that the worm's microbiome

exhibits the potential to alter chemicals prior to intracellular uptake" making them useful in understanding xenobiotic and drug metabolism. Further, "the biochemical toxicological processes classically referred to as 'Phase I, II, and III'… are present in worms." C. elegans has been used to study the toxicology of environmental pollutants that are a threat to humans including heavy metals, pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls.

- Genetic variation in toxicology. The Hartman et al. authors, one of whom is a scientific advisor to vivoVerse, (ECA) and another a vivoVerse customer (JNM), point out that "using a model system, one can address both weaknesses in xenobiotic assessments ethically and robustly. However, a gap exists in the translation of xenobiotic toxicity mechanisms in most model organisms because most assessments of xenobiotic responses use only one genetic background. This situation is akin to making xenobiotic-induced disease risk assessments from a single subject. Therefore, in order to define population-level risk factors, one requires a discovery platform that determines whether xenobiotic response pathways vary across genetically diverse individuals, and C. elegans is ideally suited for this purpose. The C. elegans species has genetic diversity similar to humans and genetically distinct individuals are found worldwide."
- Genetic homology to humans. Kim et al. (2018; doi:10.1152/physiolgenomics.00063.2018.) note that "Comparative proteomic analysis of 18,452 C. elegans protein sequences revealed that human gene homologs exist for ~83% of the C. elegans proteome. Moreover, C. elegans homologs were found to exist for ~60 – 80% of human protein-coding genes."

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   In the area of Approaches for catalyzing the development and validation of novel alternative method technologies:

   - In vivo NAMs using small model organisms play a key role in early research to deliver fast answers to a discovery problem, such as the function of a gene, indicators of toxicity pathways, or define novel therapeutic entry points. Of the whole organism models,"… C. elegans is certainly the fastest and most amenable to cost-effective medium/high-throughput technologies." (Kaletta and Hengartner, 2006; doi:10.1038/nrd2031)

     - challenges for building in robustness, replicability, reproducibility and reliability of the technologies and the ensuing datasets;
     - We, at vivoVerse, agree that these are critical quality standards that all NAMs should meet, and we can show how the C. elegans model is amenable to meeting these quality standards. We have taken steps to ensure these quality standards are met by multiple assays we have developed for developmental and reproductive toxicology (DART) and developmental neurotoxicity (DNT) assessments. We are preparing a manuscript demonstrating our results with these assays, and if any reader is interested in a pre-print of the manuscript, we welcome their contact at support@vivoverse.com.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

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Description: Cover letter

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Submit date: 9/5/2023

I am responding to this RFI: On behalf of myself

Name: Brian Johnson

Name of Organization: Michigan State University

Type of Organization: University

Role: Scientific researcher

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   New approach methodolgies including throughput compatible cellular coculture and multiculture technologies are vital for birth defects research and prevention. These technologies allow researchers to study the interactions between different cell types and tissues in a controlled environment, mimicking the conditions of the developing embryo. By using coculture and multiculture systems, researchers can identify the factors that influence normal and abnormal development, as well as test potential therapies genetic modifiers in susceptible populations or interventions to prevent or treat birth defects. Cellular coculture and multiculture technologies are therefore essential tools for advancing our understanding of the causes and mechanisms of congenital anomalies, and for developing novel strategies to improve the health and quality of life of affected individuals.
   In animal models, developmental and reproductive toxicity is often driven by chemical insults to intercellular signaling pathways where multiple molecular initiating events can be affected across cell types involved in secreteing or sensing a given signal. The effectiveness of the drug discovery approach for toxicity testing as recommended by the National Academy in 2007 is problematic in this scenario since monoculture based reporter assays do not include all the sensitive molecular initiating events. Engineering multicellular assays with functional intercellular communications with the throughput capability to expose them to chemical libraries to discover unknown chemical modulators and test for interactions between commonly targeted toxicity pathways is vital.
   Our work in this area involves the development of a novel microphysiological culture model that recapitulates the molecular and cellular sequence of lip and palate development (Johnson et. al. 2021). We demonstrated a 3D microphysiological model (3D MPM) platform which was micromilled into a commercial microtiter plate. We use simple open microfluidic principles to engineer direct 3D epithelial-mesenchymal interactions (E-Mi) by creating vertically pinned cell embedded hydrogels, that when overlaid with epithelium are used to create an E-Mi perpendicular to the imaging plane at the plate bottom making them ideal for high content imaging, enabling multi-parameter quantitative analyses. Demonstrating the practical utility and biological fidelity of this approach, we showed that this platform recapitulates a paracrine Sonic Hedgehog (SHH) signaling response, whereby SHH ligand produced from the epithelium generates a gradient of pathway activity in the adjacent mesenchyme. The 3D MPM generated robust SHH pathway activation which was chemically antagonized in a concentration:response fashion at multiple molecular targets in the SHH pathway (both SHH secretion and sensing) supporting the utility of this approach for high-content and throughput compatible chemical screening as well as to understand genetic predispositions to

orofacial clefts. Together, these findings demonstrates a novel and practical microphysiological model with broad utility for investigating epithelial-mesenchymal interactions in development.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**

Researchers in toxicity testing and drug discovery encounter several difficulties when applying new advanced cell culture models to their research. These include overcomplicated microfluidic pumps and valves, unfamiliar materials such as PDMS, low throughput, limited adaptability and especially limited time to figure things out. Yet, there remains great potential for these systems to enable important scientific discoveries through use of stem-cell derived human cell lines, cocultures of different cell types, biomimetic extracellular matrices, microfluidic perfusion platforms, 3D culture, tissue-chip technologies, tissue architecture, and organ functionality . To address these issues, platforms that integrate with familiar formats including standard commercially available polystyrene cell culture plates are critical for successful integration of these technologies.

Devices constucted using PDMS and adhesives used to fasten glass bottoms onto microplates sequester lipophilic small molecules making them unsuitable for drug and chemical screening . In addition to user familiarity, the integration with well plates allows for combination of microfluidic operation with traditional well plate functionality, enabling use in either modality – as a microfluidic device or a well plate – thus, opening the door to new assay combinations. During prototype development these plates are CNC milled for rapid iteration and prototyping during assay development. Microchannels (80um to 1000um wide) are milled out of the existing structure and ports are drilled into the bottom of the wells to interface the wells with each other. The bottom of the plate can be covalently bonded with a thin sheet of polystyrene or left open for suspended applications and integrated vertically with other well plates or sample formats. Subsequently, completed designs can be injection molded in high volume. This innovation in design facilitates the adoption of these devices into HTS laboratories as well as aid in the adoption of microfluidic technologies in standard biological laboratories.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**

Adverse outcome pathway and computational modeling are two complementary approaches to anchor microphysiological models.

Adverse outcome pathways (AOPs) are conceptual frameworks that describe the causal links between molecular-level perturbations and adverse effects at higher levels of biological organization. AOPs can help design toxicity testing models that are more relevant, efficient and predictive of human and environmental health risks. By using AOPs, toxicologists can identify key events and biomarkers that are indicative of adverse outcomes, and use them to design in vitro assays that can measure the effects of chemical exposures.

Computational models are essential tools for predicting the human health outcomes of exposure to environmental chemicals. However, these models often rely on extrapolating data from animal studies or in vitro assays, which may not accurately reflect the complex interactions and dynamics of human physiology. New approach methodologies including microphysiological systems (MPS), also known as organ-on-a-chip devices, offer a promising alternative for generating human-relevant data in a controlled and reproducible manner. MPS can mimic the structure and function of human

tissues and organs, and can be integrated with sensors and microfluidics to monitor and manipulate the cellular microenvironment. By combining computational and MPS approaches, we can establish a quantitative understanding of how the results obtained from MPS translate to effects in human populations. This will enable us to improve the accuracy and reliability of risk assessment, and to identify safer and more effective interventions for disease prevention and treatment.

Submit date: 9/5/2023

I am responding to this RFI: On behalf of an organization

Name: Aubrey Schoenleben

Name of Organization: University of Washington

Type of Organization: University

Role: Institutional official

1. **Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:**
   Please see the attached letter.

2. **Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:**
   Please see the attached letter.

3. **Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:**
   Please see the attached letter.

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Description: Response letter

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