

Pre-ACD Vote

# **CATALYZING THE DEVELOPMENT AND USE OF NOVEL ALTERNATIVE METHODS**

*December 2023  
Report to the Advisory  
Committee to the Director*

Pre-ACD Vote

**Catalyzing the Development and Use of Novel Alternative Methods**

## Preamble

*The 21<sup>st</sup> century is a time of expeditious technological acceleration. Increasing use of new and improved biomedical technologies, such as gene editing, artificial intelligence (AI), and induced pluripotent stem cells, are fundamentally changing the way science is done. When successfully combined with traditional research methodologies, these novel alternative methods (NAMs) enable research to be done more quickly, by more researchers, at a more affordable cost. Importantly, these advances can also drive innovation in scientific methodologies themselves, opening doors to new scientific approaches, including complementary and more effective non-animal-based approaches. Taken together, harnessing the power of emerging technologies to advance novel scientific approaches holds tremendous promise for helping us better understand fundamental biology to advance human health.*

*Successful deployment of NAMs, whether for conducting basic research, uncovering disease mechanisms, or translating knowledge into products or practice, relies on bringing together many disciplines, technologies, data, and areas of expertise. Interdisciplinary collaboration drives creativity and innovation, and teams of people coming from different backgrounds can create out-of-the-box solutions that would otherwise never be imagined. Each sector within the biomedical research enterprise has a role to play in catalyzing the development and use of NAMs. By integrating these perspectives and needs early in technology conception, researchers can develop NAMs that provide high-quality, reproducible findings with the highest relevance to human biology.*

## ACKNOWLEDGEMENTS

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We also would also like to thank all of those that have been at the forefront of developing and using NAMs for the past several decades. We have learned tremendously from these efforts as this work has been truly catalytic in terms of spurring both tremendous scientific opportunity and inspiration for generations to come regarding the promise of innovation.

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## EXECUTIVE SUMMARY

Scientific breakthroughs are often propelled by the introduction of new technologies that can transform how scientists study health and disease. While some technologies are truly novel and disruptive, the majority are discovered through a continuous and iterative cycle of development, standardization, validation, and uptake. As technological capabilities have improved over the last decade, they equip researchers with a new set of methods that offer unique precision and can potentially reduce reliance on animal model systems for specific types of studies. These “Novel Alternative Methods” (NAMs, sometimes defined as New Approach Methodologies, Non-Animal Methods, or New Alternative Methods) provide a complementary approach to traditional models while offering tremendous promise for enhancing understanding of the human system and for more effectively treating human conditions.

The burgeoning field of NAMs is quite diverse and each has its own unique strengths and weaknesses depending on the context of use. Accordingly, in December 2022, Acting NIH Director Dr. Lawrence Tabak convened the Working Group, comprising experts across disciplines and sectors to assist NIH in prioritizing the development and use of NAMs with the highest potential for catalyzing biomedical research. The Working Group were asked to identify the landscape of NAMs currently being deployed; assess their strengths and limitations; and characterize the types of research in which they hold the most promise of complementing and/or replacing animal studies. Ultimately these findings are intended to inform the Advisory Committee to the Director (ACD) in providing recommendations to the NIH for priority settings and future investments.

### **NAMs DEFINED** (see Appendix A for additional definitions)

***In silico***: Experiments performed by computing platforms or custom hardware, encompassing mathematical modeling and simulation, machine learning, and other computational techniques.

***In chemico***: Experiments performed on biological molecules, such as proteins and DNA, outside of cells, which may be used to study how these molecules interact with each other and with drugs.

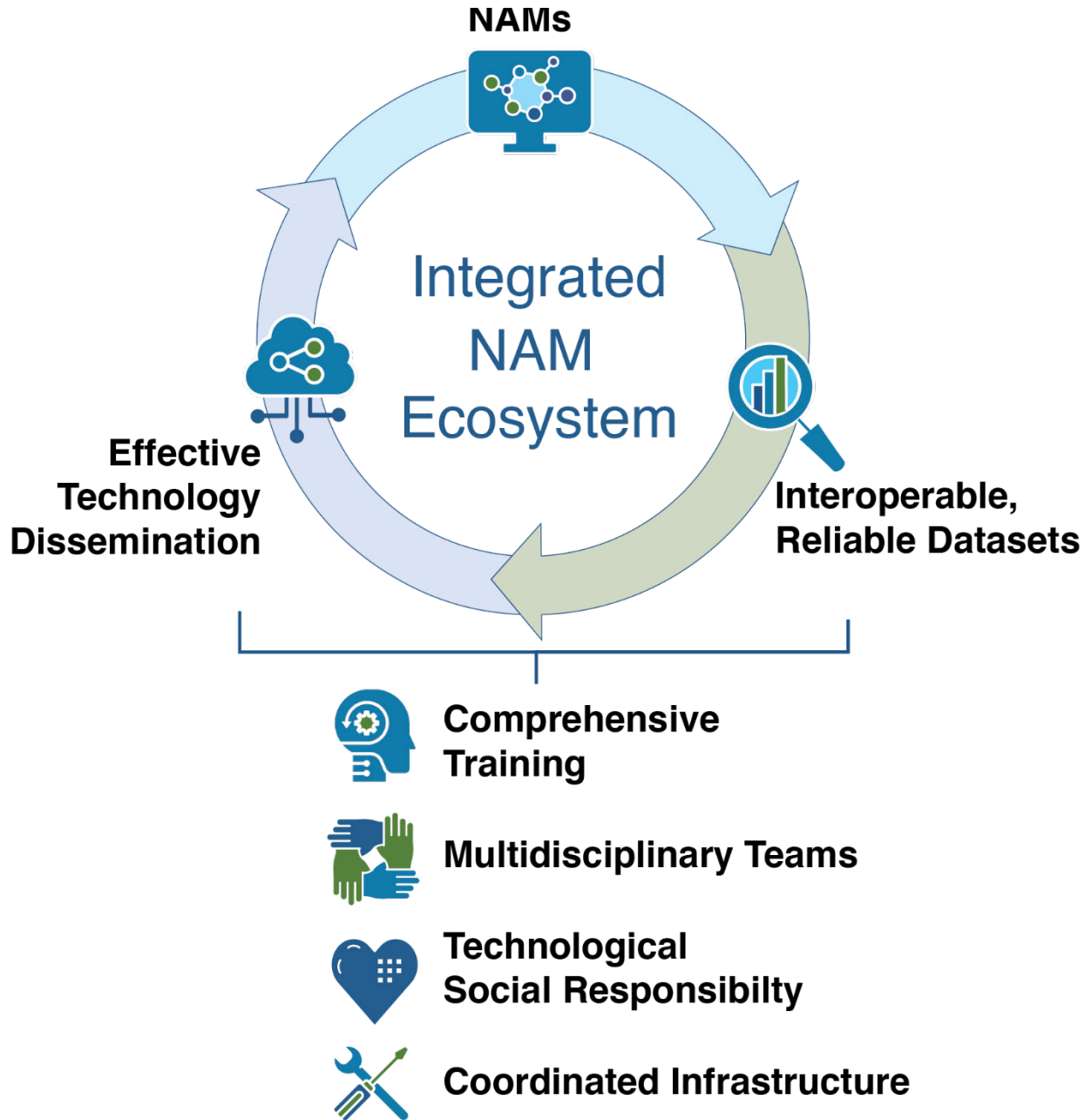
***In vitro***: Experiments performed on cells outside of the body, including various types of cell, organoid, and tissue culture techniques.

The Working Group worked intensively throughout 2023, meeting with developers and users of NAMs to explore not only the current context of use, but also opportunities for future development and deployment. For the purposes of the WG’s discussions and findings, NAMs were classified into three general categories of technologies: computational modeling and predictive technologies (*in silico*), cell-free methods and assays (*in chemico*), and cell-tissue-organoid culture models (*in vitro*). Each model offers unique strengths that, when utilized individually or in combinations, expands researchers’ toolboxes to improve upon and answer previously unanswerable biomedical research questions, and to ask new questions. For NAMs to be effective and reliable, these strengths and weaknesses should be assessed prior to incorporation into a study, and context of use should be considered in NAMs development.

It was clear throughout the WG’s deliberations that NAMs are already incredibly valuable for conducting basic research, uncovering pathophysiological mechanisms, and translating discovery and knowledge into products or practice. Numerous areas of biomedical research are already benefitting from NAMs, including cancer, diabetes, cardiovascular disease, Alzheimer’s disease, mental illness, infectious disease, rare diseases, and more. However, no one individual NAM can currently fully recapitulate human physiology. For NAMs to reach their full potential, much more needs to be done to unite and interconnect the underlying disciplines, technologies, data, and areas of expertise. By integrating these perspectives and needs early in technology

conception, researchers can develop NAMs that provide high-quality, reproducible findings with the highest relevance to human pathophysiology.

Recognizing that integration is the key for delivering on the transformative promise of NAMs, the Working Group identified seven thematic clusters of high priority needs that should be addressed in moving forward. Ideally, these needs would be addressed in tandem and are depicted in the figure below.





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In support of these needs, the Working Group identified key recommendations and activities that NIH should pursue in each of these areas:

<b>RECOMMENDATIONS TO CATALYZE THE DEVELOPMENT AND USE OF NAMs</b>	
<b>Recommendation 1.</b>	<b>Prioritize the development and use of combinatorial NAMs.</b>
<b>Recommendation 2.</b>	<b>Establish resources, infrastructure, and collaborations to promote the use of interoperable, reliable, and well curated/high quality datasets produced from research using NAMs.</b>
<b>Recommendation 3.</b>	<b>Promote effective dissemination and interconnection of NAMs technologies.</b>
<b>Recommendation 4.</b>	<b>Invest in comprehensive training to bolster continuous advances in NAMs development and use.</b>
<b>Recommendation 5.</b>	<b>Facilitate multidisciplinary teams with expertise across technologies and the lifecycle of NAMs development and use.</b>
<b>Recommendation 6.</b>	<b>Promote social responsibility in both the creation and deployment of NAMs across the research lifecycle.</b>
<b>Recommendation 7.</b>	<b>Support and maintain coordinated infrastructure to catalyze effective and responsible NAM development and use.</b>

The recommendations above are not mutually exclusive and hinge upon the importance of putting together diverse, multi-disciplinary teams with the right complementary knowledge. Breaking down silos includes setting up collaborations between groups (e.g. disciplines, sectors), training scientists in a multi-disciplinary fashion, creating standardized language to communicate across specialties and sectors, and building and maintaining an infrastructure to foster data interoperability and integrated models. As all new scientific advances build upon prior scientific advances, it is essential to keep researchers who work with traditional models engaged throughout this endeavor. Similarly, as the ultimate goal is the advancement of medicine, it is important to consult with the clinicians who will put this new knowledge into use, to make sure that the results produced are translationally relevant, heed regulatory guidelines, and can be integrated into existing workflows in clinical settings.

While this report is focused on NIH, the vast and expansive applicability of the recommendations necessitates participation across sectors. Each sector has a role to play in supporting an integrated ecosystem for catalyzing the development and use of NAMs. In addition to NIH, the Working Group expect this report to serve as a basis for other stakeholders to take in consideration in the development and myriad uses of NAMs. The large-scale uptake of any new technology by researchers, practitioners, and patients will require a cultural shift, while deploying new technologies will require communication, training, and building confidence in NAMs among the research and technology development community and the public.

## SECTION I. CHARTING THE COURSE FOR THE DEVELOPMENT AND USE OF NAMs

From its foundation to the present day, NIH has funded research into the development and application of novel technologies and approaches. While not traditionally categorized as “novel” or “alternative”, the NAMs field itself has seen tremendous growth over the past 15 years alongside ever-expanding technological capabilities. Often, NIH-funded researchers use these methods to help guide, or in tandem with animal studies, bolster evidence for their conclusions in going from simpler to more complex models. In many cases, NAMs allow scientists to control variables and establish clearer roles for the building blocks of biological systems, while research in animal models is critical to understanding just how these fundamental pieces interact in a living organism as it behaves over time in its environment. These NAMs are applied and continue being developed in a wide range of areas of basic and clinical research, including cancer, diabetes, cardiovascular disease, Alzheimer’s disease, mental illness, infectious disease, and rare and genetic diseases.

**Historical Context.** NIH-funded researchers have used NAMs extensively for scientific and medical discovery, and they continue to hold great promise for the future. As NAMs become increasingly sophisticated, the prospect of refining, reducing, and replacing the use of animal models in research (also referred to as the 3Rs)<sup>1</sup> becomes more feasible. Accordingly, NIH continues to invest into identifying and developing appropriate biological systems, including NAMs, to maximize research translation. However, each model has strengths and limitations, which may vary depending on the specific research questions being addressed. By strategically increasing NIH’s portfolio by investment into NAMs, NIH can provide researchers with tools that are complementary to, and potentially replacements of, traditional models that hold great promise in establishing more accurate, relevant, and reliable research into human health and disease.

It is important to acknowledge the many long-standing efforts within the biomedical research community along with a recent convergence of factors that highlight this as an ideal time to invest strategically in the development and use of NAMs. Recent reports have demonstrated the need for such strategic prioritization, including the May 2023 release of a congressionally requested and NIH funded, National Academies of Sciences, Engineering, and Medicine (NASEM) analysis on the state of the science for nonhuman primate (NHP) model systems, including the current role of NHPs in biomedical research, future needs for NHPs, and opportunities for new approach methodologies to complement or reduce NHPs in biomedical research.<sup>2</sup> The analysis concluded that while there are currently no alternative approaches that can replace NHP models to answer research questions that require complete multiorgan interactions and integrated biology, there is value in working towards that goal. The NASEM analysis noted that efforts to reduce reliance on NHPs in biomedical research will require direct interaction and collaborative research among investigators using NHP models and those developing *in vitro* and *in silico* approaches to expand the applicability of NAMs to research questions for which NHPs are currently needed. The current Working Group report and recommendations also build on recommendations by the NIH Advisory Committee to the Director (ACD) Working Group on Enhancing Rigor, Transparency, and Translatability in Animal Research, which were adopted by the ACD, to explore alternative approaches and to improve selection, design, and relevance of animal models.<sup>3</sup> NIH recognizes that to support rigorous and impactful science, the choice of model or integrated models and methodologies used in biomedical research studies must be selected based on scientific appropriateness and relevance to human health and disease.

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<sup>1</sup> [caat.jhsph.edu/russell-and-burchs-principles-of-humane-experimental-techniques/](http://caat.jhsph.edu/russell-and-burchs-principles-of-humane-experimental-techniques/)

<sup>2</sup> [nationalacademies.org/our-work/nonhuman-primate-model-systems-state-of-the-science-and-future-needs](https://nationalacademies.org/our-work/nonhuman-primate-model-systems-state-of-the-science-and-future-needs)

<sup>3</sup> [acd.od.nih.gov/documents/presentations/06112021\\_ACD\\_WorkingGroup\\_FinalReport.pdf](https://acd.od.nih.gov/documents/presentations/06112021_ACD_WorkingGroup_FinalReport.pdf)

**Working Group Approach.** Acting NIH Director Dr. Lawrence Tabak proposed the initial NAMs Working Group charge to the ACD in November 2022<sup>4</sup> and formally launched the Working Group in December 2022.<sup>5</sup> Led by Drs. Howard Chang and Lyric Jorgenson, the Working Group met monthly from January to November 2023, reviewing the state-of-the-science, assessing current and planned initiatives, and meeting with experts across disciplines and sectors.

Early on, the WG's deliberations clearly showed that the breadth and diversity of NAMs warranted additional mechanisms for feedback, especially to determine which areas of research are currently underserved or may benefit from more sophisticated or easier to use NAM technologies. Accordingly, NIH held a public workshop in August 2022 to discuss current uses, challenges, and opportunities of NAMs, and included representatives from academic, non-profit, private, and government partners.<sup>6</sup> The workshop participants brought expertise across scientific disciplines and experience in both developing and using NAMs. To capture input from the public more broadly, including non-traditional partners, the NIH issued a public Request for Information (RFI) (NOT-OD-23-140) to hear more from these stakeholders.<sup>7</sup> NIH shared the 85 responses with the Working Group and the public, which they reviewed and used to inform their deliberations and recommendations (See APPENDIX B).

#### CHARGE TO THE ACD NAMs WORKING GROUP

- Identify the types of alternative methods being developed for use in biomedical research and assess their general strengths and weaknesses for studying human biology, circuits, systems, and disease states;
- Characterize the types of research, condition, or disease for which alternative methods are most applicable or beneficial; and
- Articulate high-priority areas for NIH investment in the use and development of novel alternative methods with human applicability to:
  - Advance progress into understanding specific biological processes or states; and
  - Augment the tools and capabilities for biomedical research to complement and/or potentially replace traditional models.

**Scoping the Task.** It became increasingly clear that challenges facing progress in developing and using NAMs begin with a fragmented system for how to conceptualize, define, and standardize these technologies. Clear and interoperable definitions are needed to catalyze the field but also promote use of NAMs more broadly across the biomedical research ecosystem. In the WG's discussions, they focused on the following categories of NAMs, defined as follows: **in silico methods**, experiments performed by computing platforms or custom hardware, encompassing mathematical modeling and simulation, machine learning, and other computational techniques; **in chemico methods**, experiments performed on biological molecules, such as proteins and DNA, outside of cells, which may be used to study how these molecules interact with each other and with drugs; and **in vitro methods**, experiments performed on cells outside of the body, including various types of cell, organoid and tissue culture techniques.

While the Working Group acknowledged that there is a broad and diverse set of tools and techniques that could fall under the rubric of "alternatives", they agreed about an initial focus on areas in which emerging technological advances can provide new or improved models for interrogating biological processes or states. For example, NIH-funded research in single cell eukaryotes (e.g., yeast) and invertebrate animals (e.g., fruit flies, *C. elegans*) has been transformative and have reduced reliance on traditional vertebrate animal models.

<sup>4</sup> [acd.od.nih.gov/documents/presentations/11032022\\_Biomedical\\_Research.pdf](https://acd.od.nih.gov/documents/presentations/11032022_Biomedical_Research.pdf)

<sup>5</sup> [acd.od.nih.gov/documents/presentations/12082022\\_Proposed\\_ACD\\_Novel\\_Alternative.pdf](https://acd.od.nih.gov/documents/presentations/12082022_Proposed_ACD_Novel_Alternative.pdf)

<sup>6</sup> [osp.od.nih.gov/events/nih-workshop-on-catalyzing-the-development-of-novel-alternatives-methods/](https://osp.od.nih.gov/events/nih-workshop-on-catalyzing-the-development-of-novel-alternatives-methods/)

<sup>7</sup> [grants.nih.gov/grants/guide/notice-files/NOT-OD-23-140.html](https://grants.nih.gov/grants/guide/notice-files/NOT-OD-23-140.html)

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Studies in these models have resulted in nine Nobel prizes in Physiology and Medicine because some of the fundamental biological mechanisms are conserved from these animals all the way to humans, revealing detailed insights into cancer,<sup>8</sup> the mechanisms of sleep,<sup>9</sup> memory and learning,<sup>10</sup> and the brain functions,<sup>11</sup> amongst others. Additionally, research studies can be increasingly conducted with human participants and NIH remains committed to direct epidemiological and non-invasive studies involving humans. While these approaches play an important role in reducing the number of animals necessary for research, the focus of this assessment is on a subset of NAMs that can take advantage of new technologies, more faithfully represent human pathophysiology and serve as models of tissue/organ injury, regeneration, disease and treatment.

**Finally, the WG scoped its task on NAMs to focus on those with the greatest potential for human relevance.**

The NIH mission is to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability. Thus, clinically relevant questions should serve as the driver for the science, and the development of NAMs and their use, whether alone or in concert with other NAMs and more traditional models, occur in response to those questions.<sup>12</sup> With *in vitro* methods, the use of human cell lines and human tissue surrogates can increase human relevance and simultaneously provide potential to reduce the use of traditional animal models. Further, *in silico* methods can guide *in vitro* and *in vivo* studies and ones that use data from patients, or model and predict conditions and treatments can increase clinical relevance and translatability of findings. With this said, and given the complexity of human biology and disease, it is challenging to envision a near-term future where animal studies are not necessary for improving public health and protecting the public and patients from unintended harm. 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.<sup>13</sup> Some NAMs, such as computational models, may reveal unexpected and complex causal relationships among variables that are unlikely to emerge exclusively in animal studies.<sup>14</sup> In this context, animal models may be applied in a complementary fashion to confirm novel hypotheses. The continued development of NAMs will improve the ability to progressively decrease that dependence even more and improve the ability to translate fundamental biomedical research to patients.

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<sup>8</sup> [nobelprize.org/prizes/medicine/1995/summary/](https://nobelprize.org/prizes/medicine/1995/summary/)

<sup>9</sup> [nobelprize.org/prizes/medicine/2017/press-release/](https://nobelprize.org/prizes/medicine/2017/press-release/)

<sup>10</sup> [nobelprize.org/prizes/medicine/2000/kandel/facts/](https://nobelprize.org/prizes/medicine/2000/kandel/facts/)

<sup>11</sup> [nobelprize.org/prizes/medicine/2002/summary/](https://nobelprize.org/prizes/medicine/2002/summary/)

<sup>12</sup> [nationalacademies.org/our-work/variability-and-relevance-of-current-laboratory-mammalian-toxicity-tests-and-expectations-for-new-approach-methods--nams--for-use-in-human-health-risk-assessment](https://nationalacademies.org/our-work/variability-and-relevance-of-current-laboratory-mammalian-toxicity-tests-and-expectations-for-new-approach-methods--nams--for-use-in-human-health-risk-assessment)

<sup>13</sup> Bartha PFA (2010) *By parallel reasoning: the construction and evaluation of analogical arguments.* Oxford University Press, New York

<sup>14</sup> M. Ferrante et al. *Molecular Psychiatry* (2019) 24:479–483

## SECTION II. CAPITALIZING ON THE OPPORTUNITY OF NAMs

NIH funds research using animal and non-animal approaches to investigate biology from molecules, to systems, to whole organisms and how they interact with their environments. Depending on the biological system or disease state, different combinations of methods may be required to provide the strongest body of evidence, so when and how to replace animal models with NAMs needs to be determined by the appropriateness of the model for the research hypothesis. While NAMs have grown increasingly sophisticated in mimicking human biology, it is important that the community fully understand their strengths and limitations, as well as areas of opportunity given the current state of science.

The Working Group would be remiss if they did not acknowledge that significant efforts have been dedicated to improving and reducing use of animals by enhancing their translational relevance, supporting their greater reproducibility, and relieving pain and discomfort. NIH is committed to replacing, reducing, and refining the use of animals in biomedical research. Significant effort and investment have been dedicated to developing capabilities that advance the ability to model human biology without the use of animals. Currently, these capabilities are focused on investigating basic biological and disease mechanisms, while some are beginning to support toxicology testing. The rapidly evolving understanding of disease biology complemented by the development of increasingly complex non-animal models will converge to support a future that is more effective at supporting research that improves public health while equipping researchers with capabilities to choose a model most suitable for study.

The promise of NAMs is recognized by both researchers and the public, with numerous federal and private sector initiatives underway both domestically and abroad. There are many existing reviews detailing the strengths and weaknesses of various NAMs that the Working Group will not recapitulate as part of this report. However, to augment the report findings, the Working Group reference a recent NASEM non-human primates and NAMs consensus report<sup>15</sup> to which several of the Working Group members contributed, which describes examples of NAMs, recent changes in the regulatory landscape that are shaping the use of alternatives for studies involving human safety, and needs and opportunities for facilitating collaboration between non-human primate researchers and those who develop and use NAMs. Importantly, in 2022, the U.S. Congress directed the NIH to assess its current NAMs portfolio which was shared with the WG group to inform their understanding of potential gaps and needs. These analyses demonstrate increasing use of NAMs in NIH funded projects, and that NAMs are being developed and applied in a wide range of areas, including cancer, diabetes, cardiovascular disease, Alzheimer's disease, mental illness, infectious disease, rare diseases, and more. Rather than focus on specific diseases or conditions, the Working Group decided that their recommendations would be most informative if they could be applied to the entirety of the NIH portfolio. Thus, the WG's findings and recommendations are disease agnostic and focus more broadly across various stages of the research lifecycle, as described in further detail below.

**Conducting Basic Research.** NAMs have shown immense promise for basic research studies in which controlling variables and delineating precise building blocks of biological systems are needed. NAMs such as *in vitro* human cell cultures or single cell organisms are often utilized by researchers to elucidate fundamental functions of cells and the basic biological mechanisms of cell functions. Complex *in vitro* models like 3-D tissue cultures can also provide information on how cells interact with each other in a localized environment, such as how different cell types in the same tissue interact or how cells like immune cells can move through 3-D structures. *In silico* models can identify novel molecules, simulate target engagement and drug-drug interactions (DDI), predict combinatorial therapy efficacy and toxicity, and guide preclinical studies and patient

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<sup>15</sup> [nationalacademies.org/our-work/nonhuman-primate-model-systems-state-of-the-science-and-future-needs](https://www.nationalacademies.org/our-work/nonhuman-primate-model-systems-state-of-the-science-and-future-needs)

stratification strategies. *In silico* models and *in vitro* NAMs typically recreate isolated components of the body (i.e., individual cells, tissues, or organs) and multi-tissue units recapitulating important aspects of specific component interactions (such as organ-organ communication, inflammation, infection, or cancer). As such, while researchers have made tremendous strides in more sophisticated, multi-physiological system NAM models, they currently cannot substitute for the whole organism. The Working Group emphasizes that this is not a unique challenge for NAMs, as most animal models also fail to replicate the complexity of the human body as an integrated system. That said, this is an area of intense opportunity moving forward.

**Uncovering Human Patho/Physiological Mechanisms.** As alluded to in the previous section, an area of immense opportunity is use of suitable NAMs for studying the complexities of human biology and disease, especially for multi-organ interactions; the intersection of brain, cognition, and behavior; the body's response to infectious disease; intricacies of human development across the lifespan; and characterizing long-term, systemic, and developmental health effects of environmental and drug exposures. Traditional animal models remain the gold standard for studying many aspects of human health and disease but have similar limitations in terms of replicating the complexity of human physiology (and even more so for predicting the course of human disease). Integrated NAMs could provide researchers with a host of new approaches to study human physiology, which could be used in tandem with human disease datasets as an integrated and predictive model. NAMs are also uniquely suited to make accessible entirely new areas of research that are difficult to study in living people and animals (such as pediatric conditions and rare and complex diseases). Development of more physiologically relevant NAMs could be deployed for better prediction of disease outcomes for these conditions as well as assist in the development of therapeutic agents. For example, current animal models often lack sufficient predictive value which has slowed drug development for the treatment of nervous system disorders,<sup>16</sup> and utilization of suitable NAMs derived from human data and cells could enhance and expedite predictive studies. In areas that are unable to move forward due to lack of appropriate model accessibility, such as with NHPs in the face of the current NHP supply shortage, the biomedical research community is looking for ways to use NAMs to supplement and reduce reliance on these extremely limited and invaluable resources.

**Translating Knowledge into Products or Practice.** The use of NAMs currently is perhaps most evident in translational disciplines, especially for drug development and discovery, as they can provide testing at scale. Sometimes, researchers need to explore hundreds, thousands, or even millions of possible targets, such as when searching for new drug compounds and exploring their efficacies and toxicities. The number of assays that can be performed is generally inverse to the complexity of the system, meaning that it is much more effective, rapid, and cost-effective to use NAMs such as *in vitro* cell and tissue models to run these screens than to test each drug using animal models. NAMs may also be transformative for studying rare conditions in which disease incidence is low or inadequate, no traditional models exist, or large clinical trials are not feasible. However, like traditional models, NAMs must also be subject to robust qualification and testing measures to ensure relevance in the context of their proposed use, and to ensure their predictive value in regard to safety and efficacy in the case of therapeutics in humans. Improvements in these capabilities are clear as there is evolving regulatory guidance regarding these approaches. When breadth or cost-effective exploration is the goal, high throughput NAMs may be a more practical model.

Toxicology research and testing studies have been of increasing focus. This is commensurate with a renewed investment in the development and adoption of NAMs for these types of studies. Toxicology testing aims to

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<sup>16</sup> [nationalacademies.org/our-work/therapeutic-development-for-nervous-system-disorders-in-the-absence-of-animal-models-a-workshop](https://www.nationalacademies.org/our-work/therapeutic-development-for-nervous-system-disorders-in-the-absence-of-animal-models-a-workshop)



identify and characterize human health hazards associated with environmental/drug exposures and develop approaches for mitigating or eliminating risk. NIH already has several investments in this domain, such as:

- Toxicology in the 21st Century (Tox21)<sup>17</sup>, a U.S. federal collaboration whose partners include the National Toxicology Program (NTP)<sup>18</sup>, National Institute of Environmental Health Sciences (NIEHS), National Center for Advancing Translational Sciences (NCATS), U.S. Environmental Protection Agency (EPA), and U.S. Food and Drug Administration (FDA). Tox21 enables new high-throughput and alternative methods to evaluate chemicals efficiently.
- The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM),<sup>19</sup> an office within NIH/NTP, evaluates alternatives to animal use for chemical safety testing with a focus on scientific publishing. Additionally, NICEATM runs the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM).<sup>20</sup>

Developing NAMs for standardized testing methods is uniquely tractable due to the long history in characterizing toxicological health effects, the consistency of the aims of those methods, the availability of historical data from many years of testing from which to develop computational models, and the ability to compare new approaches to traditional animal-based approaches.

**Prioritizing Rigor and Reproducibility: Unique Considerations for NAMs.** Rigorous and reproducible study design, as well as promulgation of study findings and technology use, remain a challenge across the research enterprise. It is important to acknowledge that any type of research study must prioritize integration of robust research practices to maximize scientific integrity. The development of NAMs alone will not solve the problem and animal models must also improve (as discussed in greater detail by the ACD Working Group on Enhancing Rigor, Transparency, and Translatability in Animal Research).<sup>21</sup> However, the various NAMs, like all technologies and models, have their own unique challenges that, if left unaddressed, can limit their utility and ability to advance understanding of human health. The lack of consensus in terms of definitions and standards, as well as appropriate technological benchmarks, remain a challenge for emerging technologies and for integration and deployment of various NAMs. These ambiguities should be considered upfront in NAMs development with appropriate caveats, and described in technology use. For example, it is important to account for biological variability from donor source, especially for *in vitro* methods that can provide patient-specific studies and investigate effects of sex, ethnic background, age and state of health or disease. Likewise, it is key to mitigate selection bias and perpetuation of biases., particularly from *in silico* methods). There are also specific equity considerations that should be accounted for when using NAMs, such as understudied diseases, heterogenous populations, and lifespan. When paying special attention to human applicability, development and use of the models must take into consideration the complexity of interacting biological and environmental factors. However, these challenges also represent immense opportunity: the ability to incorporate human variability into study design offers researchers the potential to represent human diversity in a way that may not be readily accessible otherwise.

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<sup>17</sup> [ntp.niehs.nih.gov/whatwestudy/tox21/index.html](http://ntp.niehs.nih.gov/whatwestudy/tox21/index.html)

<sup>18</sup> [ntp.niehs.nih.gov/](http://ntp.niehs.nih.gov/)

<sup>19</sup> [ntp.niehs.nih.gov/whatwestudy/niceatm/index.html](http://ntp.niehs.nih.gov/whatwestudy/niceatm/index.html)

<sup>20</sup> [ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam/index.html](http://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam/index.html)

<sup>21</sup> [acd.od.nih.gov/working-groups/eprar.html](http://acd.od.nih.gov/working-groups/eprar.html)

## SECTION III. HIGH PRIORITY NEEDS & RECOMMENDATIONS

It is clear that NAMs are already incredibly valuable for conducting basic research, uncovering patho/physiological mechanisms, and translating knowledge into products or practice. However, no one individual NAM can fully recapitulate or simulate human physiology, and the goal should be an optimized, clinically relevant predictive model. Better comparisons between animal models, NAMs, and humans are needed to understand the full potential of different NAM systems, identify the best combinations in which to use the different systems and technologies to answer complex biological questions, and ultimately increase translatability. Research support is needed to compare relevant animal, NAMs and human models to validate translational potential, reduce reliance on singular methods, and identify strengths and weaknesses in hybrid-model approaches.

For NAMs to reach their full potential, **more must be done to unite and interconnect disciplines, technologies, data, and areas of expertise.** By integrating these perspectives and needs early in technology conception, researchers can develop NAMs that provide high-quality, reproducible findings with the highest relevance to human biology. Recognizing that integration is the key for delivering on the transformative promise of NAMs, the Working Group identified seven thematic clusters of high priority needs that should be addressed moving forward (see figure). While the scope of this report was focused on NIH, the Working Group emphasizes that each sector has a role to play in supporting an integrated ecosystem and should address these needs in tandem.

### HIGH PRIORITY NEED 1: COMBINATORIAL NAMs

**Opportunity/Need.** More sophisticated NAMs will be achieved by catalyzing approaches that integrate and combine different methods. Technology combinatorial effect is a phenomenon where the integration or combination of different technologies or their components results in a more significant impact than the sum of their individual effects. In the context of NAMs, the strategic combination of NAMs can lead to breakthroughs that would not have been possible with any single NAM in isolation. This type of interaction and integration of technologies can lead to new opportunities, increased efficiency, improved performance, or the development of entirely new tools.

Hybrid or integrated NAMs can be used for closed-loop systems (i.e., either data-driven or model driven) throughout a research cycle and address the “black box” of *in silico* methods. For example, data from patients can be used to generate hypotheses by training large scale models, which can be validated through *in vivo* and *in vitro* models, which can then inform and generate new hypotheses for *in silico* models and for human health. Combining modeling and machine learning, a hybrid AI approach, increases the ability to explain data resulting from these NAMs. Successful combinatorial approaches may include any of the following:

- Only newly developed NAMs
- A mix of previously developed NAMs
- A mix of previously developed NAMs and newly developed NAMs
- NAMs and animal model systems

The desired outputs of the technologies and their successful outcomes need to be determined ahead of model development. There is a need to bring stakeholders together to identify what makes the most sense to fund by identifying the research questions based on what is not yet well-served by current methodologies, including animal research. It is important to include experts in different technologies so the decision on what



to invest in is rooted in what is possible. When NIH reviews proposals to evaluate what to further develop, reviewers need to be cognizant of and if needed trained to appreciate the goal and technology behind the NAMs along with their usability for addressing specific research questions.

For successful integration across approaches, there is a need for multidisciplinary teams (see High Priority Need 5) with access to interoperable high-quality datasets (see High Priority Need 2). Part of integration is engagement with end users to make sure the approach is “fit for purpose”, easily adoptable, and scalable (see High Priority Needs 3 & 6). There must be avenues to pass on knowledge and methods about how to use these different technologies – workshops, “visiting preceptorships”, “hubs” where people can go to learn (see High Priority Needs 4 & 7).

**RECOMMENDATION 1. Prioritize the development and use of combinatorial NAMs. Specific NIH activities to pursue include:**

- 1.1. Establish benchmarks and standards for individual NAMs and combinatorial NAMs to foster technology integration efforts and demonstrate impact of combinatorial effect (see High Priority Need 2: Interoperable, Reliable Datasets).
- 1.2. Support research comparing and benchmarking relevant animal, NAMs, and human models to validate translational potential, reduce reliance on singular methods, reduce costs, and identify integration frameworks and strengths and weaknesses in model approaches.
- 1.3. Initiate a combinatorial technology pilot for developing process/feasibility of complementing or reducing reliance on a current animal model (i.e., nonhuman primate) for an experimental area/condition.
- 1.4. Track NIH’s investment in NAMs, including combinatorial NAMs, to identify gaps, support new initiatives, identify promising areas for continued investment, and bolster scientific/proposal review for tools and resources.

## HIGH PRIORITY NEED 2: INTEROPERABLE, RELIABLE DATASETS

**Opportunity/Need:** Researchers seeking to appropriately validate the performance of NAMs need access to reliable and interoperable *high-quality* datasets (including accompanying metadata), generated using both traditional and NAM approaches. Such datasets enable qualification and validation of new methods against benchmarks and established methods to inform the degree to which NAMs are complementary or advantageous to traditionally employed models. For example, once an *in silico* method has been validated, it can be deployed to test hypotheses generated using other datasets. Access to reliable, high-quality datasets increases the overall efficiency, reproducibility, and validity of comparisons critical for method development. Interoperable datasets facilitate collaboration and generation of meta-analyses from multiple studies to provide a more comprehensive understanding of the effectiveness and limitations of the methodologies. Additionally, validated NAMs can generate datasets that include measurements, simulations of drug response and biomarker expressions and can generate predictions that can be further analyzed.

Due to the breadth of methodologies that fall under the category of NAMs and the wide swath of fields and sectors that develop and use them, strategies are needed for promoting FAIR (findable, accessible, interoperable, and reusable) data to foster linkage. There is a need for standards for data/metadata collection to leverage heterogeneous NAM types and maximize data sharing and reuse across the biomedical research enterprise. Increased dataset interoperability and reliability can only catalyze the use and development of NAMs if the data is accessible and easy to find. To achieve this, there is a need for improved structuring and curation for standards-based harmonization across studies.

Importantly, researchers and institutions often lack the infrastructure for establishing robust data sharing practices to promote reuse of experimental data; this is further complicated by limited funding and resources for data sharing, career pressures to retain exclusive access, or the lack of expertise needed to make datasets reusable or interoperable. As a leader in data science and the world's largest public funder of biomedical research, NIH can serve as a forerunner in addressing these challenges, as it has done with its recent NIH Data Management and Sharing Policy<sup>22</sup> and data sharing resources created through the National Library of Medicine (NLM) and the Office of Data Science Strategy (ODSS). Additionally, the NIH Institutes and Centers hold the requisite expertise needed to identify data, confirm their quality, and facilitate sharing of data sets and tools needed to qualify and benchmark NAMs. There are unprecedented collections of data and large cohort databases containing data from animal and clinical studies, *in vivo* studies, environmental exposure, genetic and -omics data, and more. Many global organizations and federal agencies have ongoing efforts in this space, so connecting NIH's efforts with those of these partners will be key.

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<sup>22</sup> <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-21-013.html>

**RECOMMENDATION 2. Establish resources, infrastructure, and collaborations to promote the use of interoperable, reliable, and well curated/high quality datasets produced from research using NAMs. Specific NIH activities to pursue include:**

- 2.1. Define and address barriers to creating shared, reliable, and interoperable datasets, including heterogeneous data/metadata terminology, formats, and standards; inconsistent governance practices and quality curation; and lack of qualified personnel for effective communication, translation, and adoption. Develop and disseminate methods for assessing the quality of NAM data, leveraging existing data quality metrics.
- 2.2. Establish and maintain NAM data management policies and infrastructure to facilitate heterogeneous NAM data sharing and integration, including:
  - creation of registries, harmonization of nomenclature, development of ontologies, etc.
  - development of policies to promote timely and accessible publication of studies with concurrent deposition of related datasets, assigned codes, and algorithms.
- 2.3. Identify or establish a designated repository for NAM data sharing, consistent with FAIR principles, privacy protections, and security practices, with sufficient metadata requirements to promote equitable reuse of high-quality NAMs data.
- 2.4. Create alliances and collaborations for collecting, managing, sharing, and publishing high-quality NAMs data, including increasing access to hard-to-access data such as:
  - Industry data, focusing on the pre-competitive space and regulatory approval submissions.
  - Unpublished data, particularly from failed studies (in an effort to address survival bias).
- 2.5. Crowdsourcing new methodologies that enable access to quality data to use for qualification or generated by NAM research, to improve characterization of data and increase confidence in NAM-generated data.

### HIGH PRIORITY NEED 3: EFFECTIVE TECHNOLOGY DISSEMINATION AND INTERCONNECTION

For effective technology dissemination, developers of NAMs need to consider the users of these models starting from ideation, including the academic and industry partners who will be using them, the regulators who will be evaluating them, and the communities who will both be informing and will be informed by them. NAMs developers must have a deep understanding of the end user and what they need, whether it be a physician, a patient, a researcher, or a pharmaceutical developer.

Rapid dissemination of reliable technology across the research community requires clarity regarding technology “maturity” for use and dissemination. Clear descriptions of the methods are needed to ensure the reliability and availability of resources (e.g., tools, reagents, cells, algorithms, and natural language processing and machine learning code) to create a standard (i.e., major source of variability with *in vitro* methods is with the cell sources). There is also a need for a process for verifying and validating the integrated technologies as a whole rather than individually. In these efforts, the community must be cognizant that a failure does not mean that every component is invalid, but rather that the components may need to be integrated in a different way or that certain components may need to be eliminated, supplemented, or substituted out for others.

#### **RECOMMENDATION 3. Promote effective dissemination and interconnection of NAMs technologies. Specific NIH activities to pursue include:**

- 3.1. Establish mechanisms to support testing, validation, qualification, and benchmarking of integrated systems to maximize uptake of these systems by the community, including frameworks for describing which stakeholder should advance which component.
- 3.2. Create accessible and reliable sources and repositories for disseminating validated NAMs.
  - Integrate strategies for deploying technologies broadly and equitably, including to under-resourced organizations and research areas.
  - Create and expand access to donated human tissue repositories including both typical and atypical/disease samples of tissues and cells.
- 3.3. For integrated NAMs, incentivize research focused on making the technology simpler, faster, and cheaper (e.g, automation, miniaturization) and promote accessibility through easily navigable licensure procedures to manage intellectual property, commercial applications, and use issues.
- 3.4. Define expectations for NAM studies to follow established reporting guidelines for funders and publishers regarding NAMs development and use (e.g., RIVER (Reporting *In Vitro* Experiments)).

To move these technologies into widespread use, there must be targeted efforts to broaden technology reach beyond the experimental; in other words, the emphasis should be on development of tools for the sake of understanding human physiology rather than on the development of tools for the sake of developing new tools. The challenge that the tools will address needs to be well defined. Platforms and technologies should be designed to be “fit for purpose”, i.e, sufficiently complex only to the point of what is needed to answer the biological question. All of this will require strategies for long-term investment to move technology from the bench to broader use and will benefit from collaborations with experts in user-centered design.

## HIGH PRIORITY NEED 4: COMPREHENSIVE TRAINING

New and evolving tools and technological capabilities should be disseminated to a wide scientific user base, along with the knowledge required to wield them. The dissemination of skills to use these technologies across the research community is essential for unleashing the catalytic possibilities with the use of NAMs. To enable the broadest possible impact of newly developed methods and their rigorous application, support should be provided for comprehensive training. This approach requires targeted strategies across career stage and role in the biomedical research ecosystem. Any proposed training efforts must center around equity and accessibility to technological approaches and training from early on in the development process, including considerations for lower resourced areas.

Well-designed training courses have the potential to promote interdisciplinary and collaborative actions as well as to provide the backbone for the cultural change required to actuate the use of novel tools and technologies. Distributed workforce training programs can utilize and bolster industry and academic partnerships, while fellowship programs can boost early-career cross-sector training. Opportunities to develop “train the trainer” programs can further aid in the development and expansion of use of these methods and increase the confidence in the results of these methods. Integrative cross -technologies and -scientific experts, who have a deep understanding and proficiency in multiple distinct technology or scientific fields and possess the ability to integrate knowledge and principles from these domains to solve complex problems or create innovative solutions, are needed to bridge the gaps between various disciplines and leverage their interdisciplinary expertise to develop holistic and synergistic approaches that require the integration of technology from diverse fields. Similarly, technology translators, who possess the ability to facilitate effective communication and collaboration among diverse stakeholders in a multidisciplinary environment, also play a crucial role in enhancing interdisciplinary collaboration, promoting innovation, and ultimately improving health outcomes by facilitating effective communication and mutual understanding among experts from diverse backgrounds. Both integrative cross -technologies and -scientific experts and technology translators are crucial for enhancing interdisciplinarity of training sessions while facilitating effective communication and mutual understanding of NAM technologies among trainers and trainees from diverse backgrounds.

Different training will be necessary for the development and use of different types of NAMs. For example, training in cyberoperations is necessary to support the use of NAMs for research and deployment utilizing patient data. Considerations of patient privacy, cybersecurity, and respect for participant autonomy must be considered in regard to data handling, data analysis, and safety and security of hardware devices, especially those that become part of a closed-loop system. Importantly, for NAMs to be successfully used and deployed, training is needed across the NAMs pipeline from development and deployment to scientific review. This means that training is also needed for reviewers in terms of understanding proposals and the unique value of NAMs.

**RECOMMENDATION 4. Invest in comprehensive training to bolster continuous advances in NAMs development and use. Specific NIH activities to pursue include:**

- 4.1. Incentivize cross-training opportunities across scientific disciplines, animal to human approaches, and technologies, including across sectors.
  - Initiate mechanisms to support multiple aspects of NAMs-based research, especially the frontier of merging abiotic and biotic NAMs and combinatorial expertise across traditional models.
  - Establish trainings in responsible data management and sharing unique to NAMs data types to foster integration.
  - Promote training for grant reviewers to better understand how to evaluate the use of NAMs in fundamental and applied research grants.
- 4.2. Create funding mechanisms for technology developers to both receive and advance training in different methods and strategies for reliable technology deployment.
- 4.3. Invest in training across the research to implementation pipeline, including addressing hurdles in bringing technologies to fruition, such as regulatory and policy requirements, patient care, etc. For example:
  - Embed academic researchers in industry, regulatory agencies (e.g. FDA or EPA), national laboratories, and policy not-for-profits (e.g. AAAS, PCRM).
  - Create collaborations between researchers and clinicians to incorporate patient perspectives in NAMs development.
  - Foster entrepreneurship training.
- 4.4. Promote awareness and understanding of NAMs through publicly available educational course modules and workshops covering the lifecycle of NAMs, from conceptualization to dissemination, use, and commercialization.

**HIGH PRIORITY NEED 5: MULTIDISCIPLINARY TEAMS**

The full potential of NAMs can be realized through collaboration across multiple sectors and disciplines and by integrating cross-disciplinary research with its implementation. This is an endeavor where biologists, biomedical engineers, data scientists, integrative/cross technologies experts and many other experts should engage in a team-science approach to create innovative solutions in catalyzing NAMs with the highest relevance to human biology. Moreover, the progression from traditional to newly evolving methodologies is not only about technological advancements, but it also reflects a broader shift in the biomedical landscape influenced by economic, regulatory, and public sentiments. The scientific relevance and cost-effectiveness of new methods, combined with regulatory needs and incentives, have accelerated the momentum toward developing innovative research approaches. Additionally, the fusion of diverse fields from epidemiology, genetics, cell biology, and immunology with engineering (i.e., “biological engineering”) and machine learning with biomedical research is enabling a more holistic approach, redefining the boundaries of what is possible. This interdisciplinary synergy is central to the promise of NAMs, underscoring the importance of collaboration in pioneering solutions that could increase understanding of human biology and how best to model or mimic the human condition. Amidst this transformation, the scientific community stands on the brink of a new era,

driven not just by the quest for knowledge but by the aspiration to reshape the very fabric of how biomedical research is conducted.

To achieve the full potential of collaborative interdisciplinary efforts, there is a need to engage along the lifecycle of NAMs development with teams that have specialty expertise, in addition to those who work with more traditional models and who would ultimately apply the research findings to human patient cohorts. This includes regulatory expertise and connections regarding future evaluation of technologies as well as expertise in community and patient engagement (see High Priority Need 6: Socially Responsible Technologies). There must be incentives to bring together academics, technology providers, and industry partners in a pre-competitive, consortium-type environment. Each sector comes with its own needs and different levels and types of resources. The success of multidisciplinary teams relies upon the communication between disciplines and will require 1) standardized language to speak across specialties and sectors; 2) professionals who possess extensive knowledge and expertise in multiple scientific and technological disciplines; 3) individuals who possess the unique ability to facilitate effective communication and collaboration among diverse stakeholders in a multidisciplinary environment; and 4) increased data sharing across sectors.

**RECOMMENDATION 5. Facilitate multidisciplinary teams with expertise across technologies and the lifecycle of NAMs development and use. Specific NIH activities to pursue include:**

- 5.1. Develop funding opportunities to support multi-disciplinary teams, considering potential:
  - Scientific, technological, and engineering needs
  - Regulatory or policy requirements
  - Ethical considerations
  - Patient/public adoption
- 5.2. Support incentives for multi-laboratory coordination, especially mechanisms for supporting expertise across the lifecycle of development and use.
- 5.3. Create novel funding opportunities such as cross-disciplinary challenge programs or prize competitions.
- 5.4. Promote annual conferences or symposia that mobilize varying perspectives and expertise and establish resources and mechanisms to assist researchers in connecting with experts across disciplines, sectors, and research stages.
- 5.5. Support pilot studies incorporating multidisciplinary expertise focused on studying the predictivity of certain models before publishing draft policies and risk assessments.

**HIGH PRIORITY NEED 6: SOCIALLY RESPONSIBLE TECHNOLOGIES**

Integrating societal norms into the development and use of emerging technology is a mainstay of building trust and promoting uptake of technological advancement. This is a long-studied field, and there is immense value in learning from these experts to ensure new technologies do not fall into the traps of the past. Additionally, those developing and using NAMs should work in tandem with bioethicists, patients, and policymakers to create and sustain a responsible culture of use. Again, not unique to NAMs, but there are considerations that researchers should build into their studies from conception through deployment.



In creating models that recapitulate human biology, researchers should prioritize the autonomy, privacy, and needs of the individual(s) sharing the source data or biospecimens. For example, cells used for *in vitro* methods originate from donor tissues; thus issues of consent, identification, return of value, etc., need to be discussed and addressed early in the conceptualization of the model. Additionally, while use of human biospecimens can capture unique aspects of human biology, they still fail to represent the diversity of humans in general. *In silico* methods offer tremendous promise for complementing *in vitro* and *in chemico* methods in this domain. However, considerations around algorithmic biases in collection, analysis, and interpretation, as well as the cultural considerations and potential for stigmatization, need to be considered. Recommendations made throughout this report, including standards and documentation in the development of these models, are a first step in addressing these issues.

In the development and use of any emerging or evolving technology, there will be required tradeoffs between access, cost, and consistency/reproducibility. How these factors are individually weighed will greatly influence the best course for moving forward, especially in terms of equity and accessibility. There are also tradeoffs regarding commercialization incentives and the use of incentive structures or subsidies may be required especially early on in development to promote access. Critical consideration must be given to striking a balance where actions around intellectual property can incentivize commercialization instead of creating unwarranted barriers to entry. Deciding whether and when intellectual property is warranted to advance broader goals is often a fact-dependent question, and there are not likely to be bright-line rules around using intellectual property rights to promote socially responsible creation and deployment of NAMs.

**RECOMMENDATION 6. Promote social responsibility in both the creation and deployment of NAMs across the research lifecycle. Specific NIH activities to pursue include:**

- 6.1. Foster equitable development and use of NAMs for research and public benefit. For example:
  - Support research to characterize unique attributes of NAMs to understanding individual differences, method biases, etc., and to recognize, minimize, and correct for variability and biases.
  - Promulgate guidance for considering sources of tissues, cells, and data/metadata used to develop NAMs and whether they are sourced ethically or represent population diversity.
  - Promote open sharing of technology and data when possible.
- 6.2. Strengthen interagency partnerships to develop a coordinated federal approach to NAMs that enables science to advance efficiently, safely, and ethically while minimizing administrative and regulatory burden.
- 6.3. Support cost effective analyses of proposed technologies with existing methods, including animal studies, looking at time, scalability, and resource efficiency.
- 6.4. Support bioethical research on ethical, legal, and social issues unique to NAMs, including research to maximize responsible deployment, promote equity, and provide “return of value” to research participants and communities.
- 6.5. Partner research initiatives with robust public engagement to incorporate social norms and promote awareness of emerging technologies.

Importantly, maintaining scientific integrity and public trust requires honest, transparent, and balanced discussion regarding the opportunities and limitations of NAMs. To be clear, there will be the Working Group expect positive return on the public’s investment in NAMs, but there is risk in overpromising technological



capabilities that damage confidence in the biomedical research enterprise more broadly. The research community has the responsibility to the public to be open and honest in terms of communicating the NAMs agenda.

## HIGH PRIORITY NEED 7: COORDINATED INFRASTRUCTURE

Coordinated infrastructure is required for addressing virtually all the high priority needs above. In order to meet the full potential of NAMs to help researchers uncover new discoveries about human health and disease, there must be dedicated resources and venues to support these activities.

These investments should prioritize venues for strengthening dialogue across and even within sectors and geographical boundaries. With rapid development of any technology, infrastructure is needed that can minimize duplication of effort and maximize taxpayer funding with equity as a priority.

### **RECOMMENDATION 7. Support and maintain coordinated infrastructure to catalyze effective and responsible NAM development and use. Specific NIH activities to pursue include:**

- 7.1. Create mechanisms for disseminating NAMs resources, technologies, and expertise efficiently, equitably, and reliably across researchers and institutions. For example:
  - Protocols for technology development and use, qualification of reagents and equipment, tracking of materials and experimental details, and standard operating procedures for teams.
  - Clearing houses and repositories for easy, reliable, and inexpensive access to specialty reagents and custom syntheses.
  - Knowledge bases for tracking NAMs, how they are used, for what purposes, and how in combination with other models.
- 7.2. Promote or establish consortia and venues for sharing established best practices, standards, definitions, frameworks, and harmonized approaches for NAMs.
- 7.3. Invest in infrastructure to support institutions in keeping pace with the rapid evolution of NAMs, including:
  - Establishing "recruitment and placement" platforms and collectives so that researchers can identify colleagues with specialized expertise.
  - Supporting small
  - to mid-scale physical laboratory infrastructure.
- 7.4. Identify opportunities to build upon existing efforts both nationally and internationally to link resources and identify a clear source of coordination for NAMs resources.
- 7.5. Establish dedicated and centralized core facilities as national or regional resources to develop and run NAM assays to reduce costs, leverage scale, and provide training.

## SECTION IV. SUCCESSFUL INTEGRATION: TRANSFORMATIVE PROJECTS

### TRANSFORMATIVE PROJECT EXEMPLAR 1: LEVERAGING *IN VITRO* AND *IN SILICO* TECHNIQUES FOR ACTIONABLE INSIGHTS IN COMPLEX BIOLOGICAL MODELS

**Human Applicability.** The process of cell culture is intricate, particularly with the advent of new cell lines and the sourcing of specific lines from patients. Optimizing cell culture is vital for ensuring reproducibility and reliability. However, traditional methods are often time-consuming and resource-intensive, especially when dealing with a limited number of cells, such as those from individual patients. Assessing cell morphology, a crucial aspect of this process, is influenced by numerous factors, making it both time-consuming and subjective. Furthermore, as *in vitro* assays grow in complexity, there is a notable absence of standard tools or metrics for evaluating performance or biological function within these models. It is important to note that commonly used morphological metrics may not accurately reflect the biological function of the network, such as oxygen transport.<sup>23</sup>

**Challenges for Traditional Models.** The challenge extends to assessing drug responses within organoids or microphysiological systems, especially when handling high-throughput image datasets, which is both difficult and labor-intensive. Omics-based approaches are increasingly employed to identify disease mechanisms and drug responses, as well as to detect adverse effects. However, the insights derived from omics-based data in *in vitro* assays do not always yield valuable inferences.<sup>24</sup>

**Combinatorial Approach.** Combining *in vitro* and *in silico* techniques offers a scalable approach that aligns with the objectives and constraints of cell culture processes. For instance, machine learning algorithms have been effectively used to optimize cell culture feeding strategies. This optimization enhances cell growth and metabolite production, leveraging *in vitro* cell culture process data to create and evaluate both linear and nonlinear models through real-time experiments.<sup>25,26</sup>

Additionally, machine learning has been employed to quantify cell morphology in a scalable and cell-agnostic manner, extending its applicability across various cell types.<sup>27,28,29</sup> This includes the application of tools such as random forest regression models for assessing the biological functions of microphysiological systems.<sup>30</sup> Additionally, the fusion of simulation and predictive models with omics data from *in vitro* assays can help to enhance therapeutic performance.<sup>31</sup>

The use of computer vision tools for analyzing high-throughput image datasets from *in vitro* assays enables detailed and automated analyses. An example of this is the automated single-organoid morphology analysis in

<sup>23</sup> Tronolone JJ, Mathur T, Chaftari CP, Jain A. Evaluation of the morphological and biological functions of vascularized microphysiological systems with supervised machine learning. bioRxiv [Preprint]. 2023 Jan 15. Update in: Ann Biomed Eng. 2023 Mar 13.

<sup>24</sup> Yue R, Dutta A. Computational systems biology in disease modeling and control, review and perspectives. NPJ Syst Biol Appl. 2022 Oct 3;8(1):37.

<sup>25</sup> Rashedi M, Rafiei M, Demers M, Khodabandehlou H, Wang T, Tulsyan A, Undey C, Garvin C. Machine learning-based model predictive controller design for cell culture processes. Biotechnol Bioeng. 2023 Aug;120(8):2144-2159.

<sup>26</sup> Rashedi M, Rafiei M, Demers M, Khodabandehlou H, Wang T, Tulsyan A, Undey C, Garvin C. Machine learning-based model predictive controller design for cell culture processes. Biotechnol Bioeng. 2023 Aug;120(8):2144-2159.

<sup>27</sup> Welter EM, Kosyk O, Zannas AS. An open access, machine learning pipeline for high-throughput quantification of cell morphology. STAR Protoc. 2023 Mar 17;4(1):101947.

<sup>28</sup> Sherman J, Verstandig G, Brumer Y. Application of machine learning to large in-vitro databases to identify cancer cell characteristics: telomerase reverse transcriptase (TERT) expression. Oncogene. 2021 Aug;40(31):5038-5041.

<sup>29</sup> Mergenthaler P, Hariharan S, Pemberton JM, Lourenco C, Penn LZ, Andrews DW (2021) Rapid 3D phenotypic analysis of neurons and organoids using data-driven cell segmentation-free machine learning. PLoS Comput Biol 17(2): e1008630.

<sup>30</sup> Tronolone JJ, Mathur T, Chaftari CP, Jain A. Evaluation of the morphological and biological functions of vascularized microphysiological systems with supervised machine learning. bioRxiv [Preprint]. 2023 Jan 15. Update in: Ann Biomed Eng. 2023 Mar 13.

<sup>31</sup> Yue R, Dutta A. Computational systems biology in disease modeling and control, review and perspectives. NPJ Syst Biol Appl. 2022 Oct 3;8(1):37.

chemotherapy dose-response experiments, which has identified significant dose-related effects on organoid features like circularity, solidity, and eccentricity.<sup>32</sup>

These technologies are mutually beneficial: *in silico* methods depend on data from *in vitro* systems, and conversely, *in silico* techniques maximize the utility of *in vitro* assay data. This interdependence facilitates the design of *in vivo* studies and potentially reduces the reliance on preclinical *in vivo* models. By streamlining this process, these combined technologies not only optimize research efficiency but also contribute to more precise and effective biological research outcomes.

## TRANSFORMATIVE PROJECT EXEMPLAR 2: UNCOVERING NEW UNDERSTANDING OF NEUROPSYCHIATRIC DISORDERS

**Human Applicability.** Developmental and degenerative disorders of the human nervous system collectively represent one of the largest causes of disability and disease burden worldwide, yet there is a limited understanding of the mechanisms leading to disease. Moreover, currently there are relatively limited therapeutic approaches.

**Challenges for Traditional Models.** Animal models have yet to prove their fidelity and utility for studying psychiatric disorders, which are behaviorally defined. Primate models represent a promising avenue, but access to genetically engineered non-human primates is often limited and developing these models often takes many years. Human cellular models, such as organoids or assembloids, maintain the genetic background, but the inherent variability of patient-derived cell lines and inconsistencies between organoids have been problematic and they do not recapitulate circuit-level features or display behavioral outputs.

**Combinatorial Approach.** Concepts concerning psychiatric disorder pathophysiology and therapeutic targets have shifted over the years. There have been strong advances in identifying the genetic architecture and susceptibility for many of these conditions, including autism spectrum disorders, schizophrenia, epilepsy, intellectual disability, addictions, mood disorders, Alzheimer disease, and others. There is a need for an integrated model of the human disease to complement, reduce reliance or replace animal models, but the pathophysiology of psychiatric disorders may impact multiple cell types including neurons, microglia, astrocytes and possibly vascular cells, and in some cases neuro-immune interactions. Current stem cell technologies can recreate *in vitro* 3D organoid models using donor cells that can recapitulate some of the cellular diversity and complex cellular architecture of the central and peripheral nervous system and model cell migration and circuit assembly in a genetic background of disease vulnerability. Some of these methods are being developed at scale, in a way that may enable drug screening. Moreover, xenograft transplantation of organoids could allow for advancing cell maturation in an *in vivo* environment and enable therapeutic testing on human cells in an *in vivo* context. There are also significant efforts to develop multi-omics assays to capture and perturb the genome, transcriptome or chromatin landscape, as well as functional assays that capture the activity of neurons. Coupled with machine learning-aided taxonomy, neural organoids and assembloids may fill the need for integrated systems via high throughput production techniques.

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<sup>32</sup> Matthews JM, Schuster B, Kashaf SS, Liu P, Ben-Yishay R, Ishay-Ronen D, et al. (2022) OrganoID: A versatile deep learning platform for tracking and analysis of single-organoid dynamics. *PLoS Comput Biol* 18(11): e1010584.

## TRANSFORMATIVE PROJECT EXEMPLAR 3: COMBINING *IN VITRO*, *IN VIVO* (CLINICAL AND PRECLINICAL), AND *IN SILICO* APPROACHES TO MINIMIZE DEPENDENCE ON PRECLINICAL *IN VIVO* MODELS

**Human Applicability.** Transdermal drug delivery, as an evolving field, presents a significant shift from traditional methods of drug administration. This method is increasingly favored for its potential to enhance patient compliance, particularly in those who find oral or injectable routes challenging. A key advantage of transdermal delivery is its ability to circumvent pre-systemic metabolic effects and reducing the likelihood of adverse effects through decreased systemic exposure.

**Challenges for Traditional Models.** Despite these advantages, there are substantial challenges in the development and assessment of transdermal therapeutics. Currently, the evaluation of these drugs' toxicity and efficacy relies heavily on traditional *in vitro* and *in vivo* assessments.

*In vivo* studies, particularly those utilizing animal models like rats, are a staple in assessing the safety and efficacy of transdermal drugs. These models offer a more comprehensive understanding of how a drug behaves in a living system, including its metabolism, distribution, and excretion. However, reliance on these models presents several challenges. Firstly, *in vivo* testing is time-consuming, often requiring extended periods to observe long-term effects of the drug. Secondly, it is costly.

Moreover, translating findings from animal models to humans is not always straightforward due to physiological differences between species. This can lead to uncertainties in predicting how a drug will perform in humans based on animal data alone.

In summary, while transdermal drug delivery offers several advantages over traditional routes, it also presents unique challenges in terms of development and safety assessment.

**Combinatorial Approach.** In the realm of dermal exposure, *in silico* physiological modeling plays a crucial role. It is instrumental in predicting the effects of therapeutic agents and in assessing the impact of different formulations on transdermal disposition. This approach is becoming increasingly important as it offers a more efficient and potentially more accurate method for evaluating drug efficacy and safety.

The integration of *in silico* predictions, rooted in *in vivo* clinical data, stands as a pivotal advancement in pharmacological research, particularly in the realm of transdermal therapeutics. This approach leverages scalable and accurate computational models, harnessing extensive datasets derived from *in vivo* clinical trials. Such data encompasses a vast array of parameters and coefficients, which are instrumental in training sophisticated Quantitative Structure-Activity Relationship (QSAR) models. These QSAR models are adept at predicting the potential utility of compounds when administered transdermally.<sup>33</sup> By doing so, they have the potential to significantly reduce the reliance on traditional *in vivo* preclinical models.

In a similar vein, the application of machine learning techniques in pharmacological studies offers a transformative approach to predicting drug toxicity. By analyzing and interpreting *in vitro* human data, particularly transcriptome profiles, machine learning algorithms can make informed predictions about a compound's toxicity.<sup>34</sup> This approach provides valuable insights that can guide and inform subsequent *in vivo* studies, thereby optimizing the research process and potentially reducing the need for extensive *in vivo* testing.

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<sup>33</sup> Maharao N, Antontsev V, Hou H, Walsh J, Varshney J. Scalable in silico Simulation of Transdermal Drug Permeability: Application of BIOiSIM Platform. Drug Des Devel Ther. 2020 Jun 11;14:2307-2317.

<sup>34</sup> Gardiner LJ, Carrieri AP, Wilshaw J, Checkley S, Pyzer-Knapp EO, Krishna R. Using human in vitro transcriptome analysis to build trustworthy machine learning models for prediction of animal drug toxicity. Sci Rep. 2020 Jun 12;10(1):9522.

## TRANSFORMATIVE PROJECT EXEMPLAR 4: COMBINATORIAL APPROACHES TO IMPROVE TREATMENT OF CHRONIC INFLAMMATORY CONDITIONS

**Human Applicability.** There is increasing appreciation that chronic inflammatory conditions as exemplified by endometriosis, ME/CFS, chronic Lyme disease, and Crohns, are not “one disease” – i.e., that patients can likely be classified into subgroups on a molecular basis, in a manner similar to cancer patients but using different kinds of molecular markers as somatic mutations are not yet established as causative. Many of these diseases skew strongly female, are difficult to diagnose, and lack effective, safe therapies for large fractions of the afflicted. For example, an estimated 200 million women worldwide suffer from endometriosis, which is so lacking in adequate therapies that many patients resort to repeated major surgical procedures to alleviate debilitating symptoms.

**Challenges for Traditional Models.** A compendium of factors makes these conditions extremely difficult to model in animals or with current computational models. First, for many chronic inflammatory diseases, genetic linkage and GWAS studies have identified multiple loci associated with disease, yet individuals with genetic signatures may be free of disease. This conundrum suggests that there may be mitigating factors, such as undiscovered protective loci, or exposure to infection, environmental chemicals, or stress in ways that cause epigenetic modifications across multiple loci; emerging data supports these hypotheses. Second, animal models fail to capture much of the human patient symptoms adequately, especially pain, fatigue, and uterine-specific symptoms such as heavy menstrual bleeding. Pain mechanisms, especially the kinds of chronic pain associated with inflammatory diseases, are notoriously sexually dimorphic in humans. Finally, human patients often have multiple co-morbid conditions, and chronic inflammatory diseases often afflict multiple organ systems. They are thus very challenging to study in the context of the traditional NIH Institute/Center funding models, which tend to isolate organ systems (e.g. NIDDK, NHLBI) and disease triggers (e.g. NIAID), precluding easy construction of multi-disciplinary clinical teams.

These factors together motivate NAMS that can capture the diversity of patient sub-populations and point to precision approaches based on a compendium of knowledge that cannot be gleaned from traditional models.

**Combinatorial Approach.** Endometriosis, which afflicts about 10% of U.S. women, provides a useful example to illustrate integration of multiple types of NAMs in chronic inflammatory diseases. New approaches are starting to chip away at the significant individual bottlenecks that still exist in diagnostics, including in patient symptom phenotyping, imaging, and molecular markers, though approaches thus far have focused almost exclusively on binning patients into “disease or no disease” categories, without providing clinically actionable solutions for definitive therapies. Several heralded molecular markers described in blood have not reached clinical replicability or acceptance. Enhanced understanding and deployment of pain phenotyping at the research (e.g. fMRI) and clinical levels is starting to yield some path forward to potential targeted therapies from among those that already exist. More advanced bioinformatics analysis of genetic loci, by using multi-variate analysis of genotypes to cluster patients into subgroups according to correlated changes in multiple SNPs, are starting to yield mechanistic insights into precision medicine approaches for patient subgroups, and insights into overlap with other diseases that are co-morbid such as inflammatory bowel disease. Translating sets of correlated SNPs into testable hypotheses involving potential therapeutic targets requires new approaches in human systems modeling, as the animal correlates are lacking. There is growing appreciation of the dire need for sexually dimorphic systems models for how inflammation changes across the lifespan, from pre-puberty, through puberty, and through later life stages, especially as endometriosis is often erroneously believed to be a disease of women in their twenties through late 40s, when it actually afflicts a significant fraction of women in their early teens and postmenopausally. Beyond genomics, a growing compendium of multi-omic data sets on lesion properties and on dysregulation of other affected organs is yielding insights into

immunological factors operative at the sites of local inflammation. However, unknown is how more systemic immunological factors, including potential immune system rewiring from prior infection, contribute to overall immunological response, as has been demonstrated by the causative role of EBV infection in triggering multiple sclerosis. These types of analyses, that promise to yield critical insights into the critical pathophysiological factors distinguishing different patient subgroups from each other, are crucial for the design of MPS models to capture disease phenotype in precision fashion.

The design and use of MPS for chronic inflammatory diseases is still in its infancy, in part because the aforementioned design principles are still scant, and in part many of the essential *in vitro* tools for sexually dimorphic immunologically-competent MPS are yet to be developed or are far from being deployed at scale. For example, control of steroid hormone microenvironment requires careful attention to the equilibrium between hormones and plasma proteins, along with metabolism; this can be especially challenging in the most commonly-used MPS formats, which absorb steroids. Most inflammation processes involve complex interactions between immune cells and multiple cell types in tissues. Scaling MPS models to accurately represent these processes requires integration with computational models of cell-cell crosstalk in a dynamic microenvironment, and design of MPS hardware that enables appropriate acquisition of information noninvasively (eg imaging, sampling media) or at endpoints, recognizing that each MPS replicate may be expensive to produce. Finally, computational modeling of systems pathophysiology can point to design of interacting MPS modules to represent organ-organ crosstalk. For example, in endometriosis, some patients may have a gut-liver-uterus-neuroimmune axis. While modeling neuroimmune – tissue interactions in MPS is still a nascent field, it is integral to understanding complex inflammatory diseases where pain is a prominent phenotype.

## TRANSFORMATIVE PROJECT EXEMPLAR 5: DATA INTEGRATION ACROSS NAMs, TRADITIONAL MODELS, AND THE CLINIC TO EMULATE PATIENT-SPECIFIC TUMOR-IMMUNE ENVIRONMENTS

**Human Applicability.** Current projections show that 40% of population will be diagnosed with cancer within their lifetime. Despite major advances in cancer diagnosis and therapy, many patients do not respond or relapse after treatment. Metastatic progression of circulating cancer cells to distant organs remains the major determinant of poor outcome, being the cause of 90% of all cancer deaths. While decades of cancer biology research have focused on oncogenic transformations leading to the emergence of primary tumors, much less attention has been directed to studying how tumor cells alter their microenvironment and colonize a distant organ, and what determines their dormancy and activation. This gap is largely due to the lack of adequate experimental models.

Metastatic progression is particularly difficult to study, both in patients and in the existing models. Cell culture and animal models remain poor predictors of human pathophysiology of cancer, its metastatic dissemination and response to treatment. Two additional aspects are contributing to the incredible complexity of cancer: (i) biological heterogeneity of cancer cells and their microenvironments among the cancer patients and even within the same patient, and (ii) cancer cell interactions with the immune system. Both the animal models and cultures of cancer cell lines largely fail to recapitulate these critical aspects. The development of predictive human tissue/organ models of cancer metastasis that can recapitulate key aspects of cancer pathophysiology would be transformative to cancer research and pre-clinical validation of new therapeutic modalities, representing a real opportunity for integrated NAMs to address the major scientific and clinical challenges of cancer research and therapy.



**Challenges for Traditional Models.** Metastatic dissemination involves a complex series of events including extravasation from vasculature, homing to target tissue, and environmental adaptation fostering interactions with resident cells and the target-specific extracellular matrix with distinct biophysical and biochemical foundations that are different of those at the site of origin. Metastatic cells from primary tumors tend to assume new and rapidly evolving molecular programs as they colonize the new organ environments. The complexity of these processes that is further compounded by the spatiotemporal heterogeneity of cancer cells and their interactions with immune cells. These aspects of metastatic progression are particularly difficult to model in cultures of cancer cell lines and experimental animals, requiring human tissue contexts and individualized, patient-specific contexts. Appropriate models of cancer metastasis would maintain the tumor cells in a low-proliferative state with the display the metastatic phenotype associated with drug resistance. The models of interest would provide biologically meaningful and tightly controllable environments designed to elucidate mechanistic drivers of metastasis and therapeutic predictions using NAMs validated against matched patient outcomes. Integrated NAMs designed to recapitulate the key steps of metastatic dissemination will be critical for advancing understanding of biological mechanisms governing metastatic progression and paving the way for development of drugs for late-stage disease.

**Combinatorial Approach.** The studies of cancer progression, metastasis and responses (or resistance) to drugs or immunotherapy are an area that would critically benefit from the patient-specific NAMs and the integrated NAMs approaches. A recent example of an *in vitro* NAM of this kind is the multi-organ chip with matured tissue niches linked by vascular flow. Each tissue is cultured in its own optimized environment and is separated from the common vascular flow by a selectively permeable endothelial barrier. This design allows the interlinked tissues to maintain their molecular, structural and functional phenotypes over long culture times while enabling tissue cross talk by molecular species, extracellular vesicles and circulating cells. The model recapitulated the pharmacokinetic and pharmacodynamic profiles of doxorubicin observed clinically, and allowed for the identification of early miRNA biomarkers of cardiotoxicity. One can envision that NAMs of this kind, configured by linking to each other the human tissues representing cognate metastatic sites (e.g., bone, brain, liver, lung) engineered from iPSCs could be used to study metastasis of unprocessed tumor cells obtained from a patient along with the matching immune cells and introduced into the vascular circulation. The experimental studies in such organs-on-chip platforms can be further supported by advanced imaging and gene editing (to track cells and their functions), spatial genomics and computational modeling. Integration of these studies with the *in silico* and *in chemico* NAMs and benchmarking against clinical data and relevant animal studies offer tremendous potential for bringing us closer to emulating the patient-specific architectural, cellular, and phenotypic features of tumor-immune environments and cancer cell extravasation into diverse tissues.

## SECTION V. CONCLUDING REMARKS

The synthesis of understanding and innovation across models and fields is no small feat. Ideally, researchers seek to design an innovation-implementation cycle whereby researchers iterate on work done across models to inform human health, generating data from which the cycle repeats. For NAMs, the real opportunity to integrate AI, computational modeling, and 3-D organoids, human genomics, and more, into an increasingly sophisticated model provides a powerful opportunity to maximize the public's investment in biomedical research. Ultimately, this fuels the NIH in achieving its mission by providing researchers with the diversity of tools needed to answer complex questions about human health and disease.

Solving the complexities of disease will most certainly require creative and effective mechanisms for catalyzing partnerships across the biological and biomedical research enterprise. The recommendations described throughout this report stress the importance of uniting diverse, multi-disciplinary teams that include not only the researchers, but a vast number of those treating, caring for, and afflicted by human disease. Breaking down silos shall not only require setting up collaborations between groups, but also training scientists in a multi-disciplinary fashion, creating standardized language to speak across specialties and sectors, and building and maintaining an infrastructure so as to be able to foster data interoperability and integrated models. Additionally, the large-scale uptake of any new technology will need to be supported by culture change, in which needs of the groups are addressed. Enhancing communication through all stages of NAM development and application will be essential to building trust and incorporation of the work we do.

Finally, at the end of the day, the biomedical research enterprise is working to advance the human condition. Here is where our Working Group found the most hope and promise of spurring advances in NAMs. Whether it be to diversify our research toolkit or enable new inquiries into underserved areas of disease, we firmly believe NAMs will transform our collective capabilities to promote health. We thank the NIH for the willingness to work with the community to chart a bold, ambitious, and equitable path forward to achieve this important goal.



## APPENDICES

### APPENDIX A – Definitions and Terms

#### NAM APPROACHES

***In chemico methods:*** experiments performed on biological molecules, such as proteins and DNA, outside of cells, which may be used to study how these molecules interact with each other and with drugs. Specific *in chemico* terms:

- **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR):** a biotechnology that allows precise editing of the genome through use of guide RNA and an endonuclease that cleaves DNA.<sup>35</sup>
- **Direct Peptide Reactivity Assay:** a common *in chemico* model used in toxicology in which the interaction of chemicals with proteins is used to predict whether they will react with skin.<sup>36</sup>

***In silico methods:*** experiments performed by computing platform or custom hardware, encompassing mathematical modeling and simulation, machine learning, and other computational techniques. Specific *in silico* terms:

- **Computational model:** a general term describing the use of computers to simulate complex systems<sup>37</sup>
- **Machine learning:** often used synonymously with artificial intelligence (AI/ML), an approach in which computers analyze and make predictions on data<sup>38</sup>
- **Artificial intelligence:** often used synonymously with machine learning (AI/ML), a computer system with the ability to learn how to perform tasks rather than carry out programmed instructions<sup>39</sup>
- **Natural language processing:** a term for computer algorithms that automate the processing of unstructured language, both extracting meaning from it and generating it<sup>40</sup>

***In vitro methods:*** Experiments performed on cells outside of the body, including various types of cell, organoid and tissue culture techniques. Specific *in vitro* terms:

- **Microphysiological systems (MPS):** an *in vitro* platform composed of cells; explants derived from tissues/organs; and/or organoid cell formations of human or animal origin in a micro-environment that provides and supports biochemical/electrical/mechanical responses to model a set of specific properties that define organ or tissue function.<sup>41</sup>
- **Tissue chip:** also called organ chip or organ-on-chip, a form of MPS in which cells are grown as a three-dimensional structure within a microfluidic chip.<sup>42</sup>

<sup>35</sup> Redman M, King A, Watson C, et al. What is CRISPR/Cas9? *Archives of Disease in Childhood - Education and Practice* 2016;**101**:213-215.

<sup>36</sup> Jon F. Lalko, Ian Kimber, G. Frank Gerberick, Leslie M. Foertsch, Anne Marie Api, Rebecca J. Dearman, The Direct Peptide Reactivity Assay: Selectivity of Chemical Respiratory Allergens, *Toxicological Sciences*, Volume 129, Issue 2, October 2012, Pages 421–431,

<sup>37</sup> [nibib.nih.gov/science-education/science-topics/computational-modeling](http://nibib.nih.gov/science-education/science-topics/computational-modeling)

<sup>38</sup> [commonfund.nih.gov/bridge2ai](http://commonfund.nih.gov/bridge2ai)

<sup>39</sup> [nibib.nih.gov/science-education/science-topics/artificial-intelligence-ai](http://nibib.nih.gov/science-education/science-topics/artificial-intelligence-ai)

<sup>40</sup> [nlm.nih.gov/research/focus/Natural.html](http://nlm.nih.gov/research/focus/Natural.html)

<sup>41</sup> [ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/mps](http://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/mps)

<sup>42</sup> [ncats.nih.gov/tissuechip/about](http://ncats.nih.gov/tissuechip/about)

## Pre-ACD Vote

- **Organoids:** *in vitro*-generated cellular systems that emerge by self-organization, include multiple cell types, and exhibit some cytoarchitectural and functional features reminiscent of an organ or organ region. Organoids can be generated as 3D cultures or by a combination of 3D and 2D approaches (also known as 2.5D) that can develop and mature over long periods of time (months to years). For the nervous system, they are generally constructed from pluripotent stem cells but can also be derived from donor tissues with growth potential (such as glioblastoma organoids).<sup>43</sup>
- **Assembloids:** self-organizing cellular systems resulting from the combination of a type of organoids with another type of organoids (for example, dorsal forebrain with ventral forebrain) or with different specialized cell types (for example, cortical organoid with endothelial cells) that result in integration.

## TECHNOLOGY VALIDATION, QUALIFICATION, AND BENCHMARKING

- **Benchmarking:** rigorous comparison of the performance of different technologies or approaches to determine the strengths of each or to provide recommendations regarding their suitability for the purpose at hand.<sup>44</sup> Benchmarking has several benefits, including increasing awareness of emerging technologies and approaches (NIH, 2023) and understanding how the performance of a new approach methodology compares with that of *in vivo* approaches. Benchmarking is also appropriate for comparing new approach methodologies relative to the same or different intended uses for a model, allowing for identification of the most appropriate technologies and approaches for specific COUs.<sup>45,46</sup>
- **Validation:**
  - **Analytical validation:** evaluation of data processing algorithms that convert technology-collected measurements into outputted metrics.<sup>47</sup>
  - **Clinical validation:** demonstrating that technology adequately identifies, measures, or predicts a meaningful clinical, biological, physical, functional state or experience in the specified (1) animal cohort and (2) context of use.<sup>48</sup>
  - **Technology validation:** demonstrating that a particular method is reliable and relevant in a specific research application.<sup>49</sup>
- **Verification:** ensuring, through demonstration of precision, reliability and reproducibility, that a device is measuring and storing data accurately.<sup>50</sup>
- **Qualification:** process used by the FDA, by which an alternative method is demonstrated to have sufficient reliability and rigor in a specific context of use to be applied in drug development.<sup>51</sup>

<sup>43</sup> Paşca, S.P., Arlotta, P., Bateup, H.S. *et al.* A nomenclature consensus for nervous system organoids and assembloids. *Nature* **609**, 907–910 (2022).

<sup>44</sup> Weber, L.M., Saelens, W., Cannoodt, R. *et al.* Essential guidelines for computational method benchmarking. *Genome Biol* **20**, 125 (2019).

<sup>45</sup> Mangul, S., Martin, L.S., Hill, B.L. *et al.* Systematic benchmarking of omics computational tools. *Nat Commun* **10**, 1393 (2019). 4

<sup>46</sup> Wu, Yi MD; Li, Shizhen MD; Yuan, Jingxiong MD; Zhang, Hang MD; Wang, Min MD; Zhang, Zhenxiong MD; Qin, Renyi MD, PhD. Benchmarking: a novel measuring tool for outcome comparisons in surgery. *International Journal of Surgery* **109**(3):p 419-428, March 2023.

<sup>47</sup> Goldsack, J.C., Coravos, A., Bakker, J.P. *et al.* Verification, analytical validation, and clinical validation (V3): the foundation of determining fit-for-purpose for Biometric Monitoring Technologies (BioMeTs). *npj Digit. Med.* **3**, 55 (2020).

<sup>48</sup> Goldsack, J.C., Coravos, A., Bakker, J.P. *et al.* Verification, analytical validation, and clinical validation (V3): the foundation of determining fit-for-purpose for Biometric Monitoring Technologies (BioMeTs). *npj Digit. Med.* **3**, 55 (2020).

<sup>49</sup> [ntp.niehs.nih.gov/whatwestudy/niceatm/iccvm/international-partnerships/icatm](https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvm/international-partnerships/icatm)

<sup>50</sup> Baran SW, Bratcher N, Dennis J, Gaburro S, Karlsson EM, Maguire S, Makidon P, Noldus LPJJ, Potier Y, Rosati G, Ruiter M, Schaevitz L, Sweeney P, LaFollette MR. Emerging Role of Translational Digital Biomarkers Within Home Cage Monitoring Technologies in Preclinical Drug Discovery and Development. *Front Behav Neurosci.* 2022 Feb 14;15:758274..

<sup>51</sup> [fda.gov/drugs/biomarker-qualification-program/biomarker-qualification-program-submission-frequently-asked-questions](https://www.fda.gov/drugs/biomarker-qualification-program/biomarker-qualification-program-submission-frequently-asked-questions)

## Pre-ACD Vote

- **Fit for purpose:** intended use of a technology or approach is supported by validation/qualification information<sup>52,53</sup>
- **Context of use (COU):** defines the manner and purpose of use for a technology or approach (how and when it will be used).<sup>54</sup> This term can generally be applied for any intended use of a methodology. COU elements include what is measured and in what form, and the purpose of the technology or approach in the testing of hypotheses or decision making/action.<sup>55</sup>

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<sup>52</sup> 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.

<sup>53</sup> FDA and NIH. 2016. BEST (Biomarkers, Endpoints, and other Tools) Resource.

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.

<sup>54</sup> Baran SW, Brown PC, Baudy AR, Fitzpatrick SC, Frantz C, Fullerton A, Gan J, Hardwick RN, Hillgren KM, Kopec AK, Liras JL, Mendrick DL, Nagao R, Proctor WR, Ramsden D, Ribeiro AJS, Stresser D, Sung KE, Sura R, Tetsuka K, Tomlinson L, Van Vleet T, Wagoner MP, Wang Q, Arslan SY, Yoder G, Ekert JE. 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*. 2022;39(2):297–314. doi: 10.14573/altex.2112203. Epub 2022 Jan 21..

<sup>55</sup> 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.

## **APPENDIX B – NIH Request for Information (RFI)**

The NIH posted a Request for Information (RFI) for the community to share insights on the challenges and opportunities for the further development and use of novel alternative methods (NAMs) in research (NOT-OD-23-140), with the NIH Guide Notice copied below.<sup>56</sup> Input received from the RFI was shared with the ACD Working Group on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research to inform their recommendations and report. The full submissions to the RFI are posted on [osp.od.nih.gov/get-involved/previously-released-compiled-public-comments](https://osp.od.nih.gov/get-involved/previously-released-compiled-public-comments).

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<sup>56</sup> [grants.nih.gov/grants/guide/notice-files/NOT-OD-23-140.html](https://grants.nih.gov/grants/guide/notice-files/NOT-OD-23-140.html)

# Request for Information on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research

**Notice Number:**  
NOT-OD-23-140

## Key Dates

**Release Date:**  
June 12, 2023

**Response Date:**  
September 5, 2023

## Related Announcements

[NOT-OD-23-164](#): Notice to Extend the Response Date of NOT-OD-23-140: Request for Information on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research

## Issued by

Office of The Director, National Institutes of Health ([OD](#))

## Purpose

The National Institutes of Health (NIH) seeks public input on challenges and opportunities for the further development and use of novel alternative methods (NAMs) in biomedical research. NIH investment in these methods have proven beneficial tools across basic and clinical research studies, being developed and applied to interrogate cancer, diabetes, cardiovascular disease, Alzheimer's disease, infectious disease, rare diseases, and more. Each NAM approach has unique strengths and limitations that vary depending on the specific research question being addressed.

To identify areas in which the development and use of NAMs provide the most value to biomedical research, NIH sought the assistance of the Advisory Committee to the Director ([ACD](#)), an advisory group that provides advice on matters pertinent to NIH mission responsibilities in the conduct and support of biomedical research, medical science, and biomedical communications. The purpose of this request is to inform the NIH and the development of the ACD's recommendations on high-priority areas for future investment.

## Background

Biomedical researchers rely on a combination of innovative methods, models, and technologies to answer complex questions about human health and disease. The use of any given approach is based on its ability to answer the research question under study. While animal models remain an invaluable resource for researchers' addressing the complexity of human biology, rapid advances in technology are catalyzing the development and use of complementary, nonanimal based approaches. 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). The development of these NAMs holds tremendous promise for increasing the tools available to achieve the NIH mission and potentially reduce and refine the future use of animals in some areas of research in the future.

To identify areas in which the development and use of NAMs provide the most value to biomedical research, in January 2023, the NIH Director charged an Advisory Committee to the Director (ACD) Working Group with articulating high-priority areas for NIH investment (see [ACD Working Group on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research - NIH Advisory Committee to the Director](#)). In pursuit of its charge, the ACD Working Group has been assessing the value and limitation of NAMs and needs for the development of new and/or more effective NAMs. The ACD Working Group has also been meeting with experts across research sectors to understand both public and private sector investment and identify gaps/areas of opportunity to maximize the value of NAMs to advance biomedical research. Collectively, these activities are being summarized in the ACD Working Group's initial landscape

## Pre-ACD Vote

assessment on the challenges and opportunities in the development and use of NAMs that will be used to inform its final recommendations regarding high-priority areas for NIH investment.

### Request for Information

To support the activities of the ACD Working Group, NIH is requesting information from the interested individuals and communities on challenges and opportunities for the development and use of NAMs in biomedical research. Input sought includes, but is not limited to, feedback on the following:

- **The use of novel alternative methods to study human biology, circuits, systems, and disease states.** 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. NIH is particularly interested in hearing how NAMs:
  - are currently being developed and/or used successfully, including features that maximize scientific utility;
  - are advancing progress into understanding specific biological processes or human states, including potential limitations to addressing human variability; and
  - could be truly revolutionary for understanding/treating human health, including currently underserved areas of biomedical research.
- **Approaches for catalyzing the development and validation of novel alternative method technologies.** 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. NIH is particularly interested in hearing from the public on:
  - 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.
- **Strategies for maximizing the research value of novel alternative method technologies.** Depending on the biological system or disease state, different combinations of methods may be required to provide the strongest body of evidence. NIH is particularly interested in hearing from the public on how to scale these technologies to more effectively advance scientific inquiry or improve translation, including:
  - areas in which coordinated approaches across research disciplines or research sectors would dramatically advance the development and or use of these technologies.
  - approaches for sharing technology deployment equitably across labs, including incentives for reliable and reproducible methods integration.
  - factors for consideration when maximizing translatability and minimizing bias regarding human variability.

### How to submit a response

All comments must be submitted electronically at <https://osp.od.nih.gov/request-for-information-rfi-catalyzing-the-development-and-use-of-novel-alternative-methods-to-advance-biomedical-research>. It is not necessary to address each question/item.

Responses must be received by 11:59:59 pm (ET) on September 5, 2023.<sup>57</sup>

Responses to this RFI are voluntary and may be submitted anonymously. You may also voluntarily include your name and contact information with your response. Other than your name and contact information, please do not include in the response any personally identifiable information or any information that you do not wish to make public. Proprietary, classified, confidential, or sensitive information should not be included in your response. After OSP has finished reviewing the responses, the responses may be posted to the OSP website without redaction.

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<sup>57</sup> Submission deadline was originally August 16, 2023 prior to extension.

## Inquiries

Please direct all inquiries to:

NIH Office of Science Policy

Telephone: 301-496-9838

Email: [SciencePolicy@od.nih.gov](mailto:SciencePolicy@od.nih.gov)

## **APPENDIX C – Agenda and Participants, August 2023 Public Meeting**

On August 21, the NIH held a public workshop on approaches, challenges, and opportunities relating to the development of Novel Alternative Methods (NAMs). The workshop also featured discussion on identifying incentives and barriers to successful implementation of NAMs technologies. The agenda of the meeting is copied below. A recording of the event can be found at: [videocast.nih.gov/watch=49776](https://videocast.nih.gov/watch=49776). A meeting summary will be posted at: [www.acd.od.nih.gov/working-groups/novel-alternatives.html](https://www.acd.od.nih.gov/working-groups/novel-alternatives.html).





**NOVEL ALTERNATIVE METHODS (NAMs) WORKING GROUP**  
**CATALYZING DEVELOPMENT AND USE OF NOVEL ALTERNATIVE METHODS**

August 21, 2023 (all times ET)  
(<https://videocast.nih.gov/watch=49776>)

**AGENDA**

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**9:00 AM Welcome**

[Howard Chang, MD, PhD](#) & [Lyric Jorgenson, PhD](#) – ACD NAMs Working Group Co-Chairs

**9:15 AM The Opportunities and Challenges for NAMs in Biomedical Research**

*Successful deployment of NAMs, whether for conducting basic research, uncovering disease mechanisms, or translating knowledge into products or practice, relies on bringing together disciplines, technologies, and data. This session focuses on research areas for which NAMs have been impactful to identify best practices for leveraging these approaches.*

Moderator: [Nancy Lane, MD](#) – ACD NAMs Working Group

Presenters:

- [Nathan Price, PhD](#) – Thorne HealthTech (*novel mechanisms*)
- [Thomas Hartung, MD](#) – John Hopkins University (*translation/product development*)
- [Nicole Kleinstreuer, PhD](#) – U.S. National Institutes of Health (*regulatory processes*)
- [Chirag Patel, PhD](#) – Harvard Medical School (*inter-individual differences*)

**10:30 AM BREAK**

**10:45 AM Cross Sector Approaches for Driving NAMs Use and Development**

*Each sector within the biomedical research enterprise has a role to play in catalyzing the development and use of NAMs. This session focuses on the unique and complementary efforts underway to identify synergies and potential gaps in needed collaboration.*

Moderator: [Danilo Tagle, PhD](#) – ACD NAMs Working Group

Presenters:

- [Alex Carlisle, PhD](#) – National Alliance Against Disparities in Patient Health (*nonprofit*)
- [Elijah Peterson, PhD](#) – U.S. National Institute of Standards and Technology (*government*)
- [Yvette Seger, PhD](#) – Federation of American Societies for Experimental Biology (*scientific society*)

**12:00 PM BREAK**

## DISCUSSION OF HIGH PRIORITY NEEDS

*The following sessions focus on identifying potential high priority needs for catalyzing NAMs use and development with human applicability to (1) advance progress into understanding specific biological processes or states or (2) augment the tools and capabilities for biomedical research to complement and/or potentially replace traditional models. Specific goals will include identifying incentives for integrating efforts and barriers to success.*

### 1:00 PM Developing Integrated and Multi-System Models

Moderator: [Szczepan Baran, VMD](#) – ACD NAMs Working Group

Discussants:

- [Graca Almeida-Porada, MD, PhD](#) – Wake Forest University
- [Blanca Rodriguez, PhD](#) – University of Oxford
- [Roser Vento-Tormo, PhD](#) – Wellcome Sanger Institute
- [Terry Van Vleet, PhD](#) – AbbVie

### 2:00 PM BREAK

### 2:15 PM Leveraging Diverse Datasets for Maximally Useful NAMs

Moderator: [Gordana Vunjak-Novakovic, PhD](#) – ACD NAMs Working Group

Discussants:

- [John Burke, PhD](#) – Applied Biomath
- [Anna Gourmelon](#) – Organisation for Economic Co-operation and Development
- [Donna Mendrick, PhD](#) – U.S. Food and Drug Administration
- [Ivan Rusyn, MD, PhD](#) – Texas A&M University
- [James Zou, PhD](#) – Stanford University

### 3:15 PM BREAK

### 3:30 PM Equitably Deploying Robust and Reliable NAMs into Practice

Moderator: [Antonio Baines, PhD](#) – ACD NAMs Working Group

Discussants:

- [Jessie Carder](#) – U.S. Department of Agriculture
- [Megan LaFollette, PhD](#) – The North American 3Rs Collaborative
- [Michael Moore, PhD](#) – Tulane University; AxoSim
- [Manu Platt, PhD](#) – U.S. National Institutes of Health
- [Nicholas Tatonetti, PhD](#) – Columbia University

### 4:30 PM DISCUSSION AND NEXT STEPS

[Howard Chang, MD, PhD](#) & [Lyric Jorgenson, PhD](#) – ACD NAMs Working Group Co-Chairs

### 5:00 PM ADJOURN