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

I am responding to this RFI:	on-behalf-of-an-organization
Name	Emily R. Trunnell, Ph.D.
Name of Organization	People for the Ethical Treatment of Animals
Type of Organization	nonprofit-research-organization
Type of Organization - Other	
Role	scientific-researcher
1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	Translating basic science and pre-clinical research into meaningful, affordable outcomes for patients is a critical challenge in biomedical research. Despite decades of research and billions of dollars invested in animal-based models of human biology, circuits, systems, and disease states, effective treatments for many debilitating and deadly human diseases remain elusive. The “translation gap” between data emerging from biomedical research and understanding/treating human health is due, in part, to the limitations of animal models. Species differences in anatomy, physiology, and gene expression—affecting developmental trajectories, metabolism, immune responses, disease susceptibility, and more—make translating data from an animal experiment into a human-relevant preventative measure, treatment, or cure extremely difficult. Animal models are often oversimplified and

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	pathology, with targets that may be meaningful in an animal laboratory but are ultimately inadequate for humans. Poor study design combined with the confinement and unnatural conditions of laboratory life further undermine the internal validity of animal research. Depending on the disease area of interest, novel drugs for humans fail in clinical trials between 90 and 100% of the time. The vast majority (90%) of “highly promising” basic science discoveries (most of them from experiments on animals) make no difference at all for human patients (Contopoulos-Ioannidis 2003). The failure of animal-based research models and assays is contributing to the increased costs of drug development and the public’s declining trust in science. If our finite public funds are to be used responsibly, they must fund reliable research and test methods that lead to effective treatment of diseases and protection of human health. Motivated by both the ethical concerns surrounding animal-based experimentation and testing as well as the limited translatability of animal-based data, advances in novel, non-animal methods (a.k.a. novel alternative methods or NAMs) like complex, 3-D cellular models, such as microphysiological systems, organoids, spheroids, and 3-D bioprinted structures derived from human cell lines and based in human biology have expanded in the past decade. Many of these models simulate human physiology and disease more accurately than traditional in vivo animal models do because they do not have to overcome the translational species hurdle. Currently, these tools are accessible to researchers working directly on their application and development. However, given their potential to improve preclinical and basic research as well as ongoing advances in their design, it is essential that investigators with knowledge or access gaps have the opportunity to take advantage of these cutting-edge in vitro methods. We cannot know how much progress might have been made if funding agencies had already made novel, non-animal methods a priority, but there is now a chance for them to catch up. It is both scientifically and ethically imperative that the NIH make the shifting of funding priorities toward non-animal methods and away from animal-based methods its agency-wide priority. There are many examples that demonstrate the scientific utility of non-animal methods over animal-based research for advancing progress into understanding specific biological processes or human states, including currently underserved areas of biomedical research. Here are just a few of the papers that demonstrate or describe their potential to

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	health: Adegbola A, Bury LA, Fu C, Zhang M, Wynshaw-Boris A. Concise review: Induced pluripotent stem cell models for neuropsychiatric diseases. Stem Cells Transl Med. 2017;6(12):2062-2070. Al-Hilal TA, Keshavarz A, Kadry H, et al. Pulmonary-arterial-hypertension (PAH)–on-a-chip: Fabrication, validation and application. Lab Chip. 2020;20(18):3334-3345. Allen A, Deshmukh H. All on “CHIP”: Using microfluidics to study neutrophil ontogeny. Transl Res. 2017;190:1-3. Arzua T, Yan Y, Jiang C, et al. Modeling alcohol-induced neurotoxicity using human induced pluripotent stem cell–derived three-dimensional cerebral organoids. Transl Psychiatry. 2020;10(1):347 Barrile R, van der Meer AD, Park H, et al. Organ-on-Chip Recapitulates Thrombosis Induced by an anti-CD154 Monoclonal Antibody: Translational Potential of Advanced Microengineered Systems. Clin Pharmacol Ther. 2018;104(6):1240-1248. Bergers LIJC, Reijnders CMA, van den Broek LJ, et al. Immune-competent human skin disease models. Drug Discov Today. 2016;21(9):1479-1488. Beydag-Tasöz BS, Yennek S, Grapin-Botton A. Towards a better understanding of diabetes mellitus using organoid models. Nat Rev Endocrinol. 2023;19(4):232-248. Blaurock-Möller N, Gröger M, Siwczak F, et al. CAAP48, a new sepsis biomarker, induces hepatic dysfunction in an in vitro liver-on-chip model. Front Immunol. 2019;10:273. Brown D, Namas RA, Almahmoud K, et al. Trauma in silico: Individual-specific mathematical models and virtual clinical populations. Sci Transl Med. 2015;7(285):285ra61. Brown JA, Codreanu SG, Shi M, et al. Metabolic consequences of inflammatory disruption of the blood-brain barrier in an organ-on-chip model of the human neurovascular unit. J Neuroinflammation. 2016;13(1):306. Cerchia C, Lavecchia A. New avenues in artificial-intelligence-assisted drug discovery. Drug Discov Today. 2023;28(4):103516. Cerneckis J, Bu G, Shi Y. Pushing the boundaries of brain organoids to study Alzheimer's disease. Trends Mol Med. 2023;29(8):659-672. Cohen A, Ioannidis K, Ehrlich A, et al. Mechanism and reversal of

drug-induced nephrotoxicity on a chip. Sci Transl Med. 2021;13(582):eabd6299.

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

Dauth S, Maoz BM, Sheehy SP, et al. Neurons derived from different brain regions are inherently different in vitro: A novel multiregional brain-on-a-chip. J

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	A, McGowan H, et al. Ethanol-mediated activation of the NLRP3 inflammasome in iPS cells and iPS cells-derived neural progenitor cells. Mol Brain. 2016;9(1):51. Diebel LN, Wheaton M, Liberati DM. The protective role of estrogen on endothelial and glycocalyx barriers after shock conditions: A microfluidic study. Surgery. 2021;169(3):678-685. Dirven H, Vist GE, Bandhakavi S, et al. Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review. Sci Rep. 2021;11(1):6403. Ehling P, Meuth P, Eichinger P, et al. Human T cells in silico: Modelling their electrophysiological behaviour in health and disease. J Theor Biol. 2016;404:236-250 Ethier SP, Guest ST, Garrett-Mayer E, et al. Development and implementation of the SUM breast cancer cell line functional genomics knowledge base. NPJ Breast Cancer. 2020;6:30. Ewart L, Apostolou A, Briggs SA, et al. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. Commun Med (Lond). 2022;2(1):154. Fernández-Costa JM, Tejedera-Vilafranca A, Fernández-Garibay X, Ramón-Azcón J. Muscle-on-a-chip devices: a new era for in vitro modelling of muscular dystrophies. Dis Model Mech. 2023;16(6):dmm050107. Fosse V, Oldoni E, Bietrix F, et al. Recommendations for robust and reproducible preclinical research in personalised medicine. BMC Med. 2023;21(1):14. Haggarty SJ, Silva MC, Cross A, Brandon NJ, Perlis RH. Advancing drug discovery for neuropsychiatric disorders using patient-specific stem cell models. Mol Cell Neurosci. 2016;73:104-115. Hartung T. A call for a Human Exposome Project. ALTEX. 2023;40(1):4-33. Hoang P, Wang J, Conklin BR, Healy KE, Ma Z. Generation of spatial-patterned early-developing cardiac organoids using human pluripotent stem cells. Nat Protoc. 2018;13(4):723-737. Hockney S, Parker J, Turner JE, et al. Next generation organoid engineering to replace animals in cancer drug testing. Biochem Pharmacol. 2023;213:115586. Landhuis E. Deep learning takes on tumours. Nature. 2020;580(7804):551-553.

Lee CT, Chen J, Kindberg AA, et al. CYP3A5 mediates effects of cocaine on human neocorticogenesis: Studies using an in vitro 3D self-organized hPSC model with a single cortex-like unit. Neuropsychopharmacology. 2017;42(3):774-784.

Levy RJ, Paşca SP. What Have Organoids and Assembloids Taught Us About the Pathophysiology of Neuropsychiatric Disorders?. Biol Psychiatry. 2023;93(7):632-641.

Lieberman R, Kranzler HR, Levine ES, Covault J. Examining the effects of alcohol on GABAA receptor mRNA expression and function in neural cultures

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	pluripotent stem cells. Alcohol. 2018;66:45-53. Lim K, Donovan APA, Tang W, et al. Organoid modeling of human fetal lung alveolar development reveals mechanisms of cell fate patterning and neonatal respiratory disease. Cell Stem Cell. 2023;30(1):20-37.e9. Lyu Z, Park J, Kim KM, et al. A neurovascular-unit-on-a-chip for the evaluation of the restorative potential of stem cell therapies for ischaemic stroke. Nat Biomed Eng. 2021;5(8):847-863. Kim H, Park HJ, Choi H, et al. Modeling G2019S-LRRK2 Sporadic Parkinson's Disease in 3D Midbrain Organoids. Stem Cell Reports. 2019;12(3):518-531. Meigs L, Smirnova L, Rovida C, Leist M, Hartung T. Animal testing and its alternatives—the most important omics is economics. ALTEX. 2018;35(3):275-305. Meng F, Meyer CM, Joung D, Vallera DA, McAlpine MC, Panoskaltsis-Mortari A. 3D bioprinted in vitro metastatic models via reconstruction of tumor microenvironments. Adv Mater. 2019;31(10):1806899. Mobini S, Song YH, McCrary MW, Schmidt CE. Advances in ex vivo models and lab-on-a-chip devices for neural tissue engineering. Biomaterials. 2019;198:146-166. Mullen S, Movia D. The role of extracellular vesicles in non-small-cell lung cancer, the unknowns, and how new approach methodologies can support new knowledge generation in the field. Eur J Pharm Sci. 2023;188:106516. Muñiz AJ, Topal T, Brooks MD, et al. Engineered extracellular matrices facilitate brain organoids from human pluripotent stem cells. Ann Clin Transl Neurol. 2023;10(7):1239-1253. Neufeld L, Yeini E, Pozzi S, Satchi-Fainaro R. 3D bioprinted cancer models: from basic biology to drug development. Nat Rev Cancer. 2022;22(12):679-692. Nguyen VVT, Gkouzioti V, Maass C, Verhaar MC, Vernooij RWM, van Balkom BWM. A systematic review of kidney-on-a-chip-based models to study human renal (patho-)physiology. Dis Model Mech. 2023;16(6):dmm050113. Nzou G, Wicks RT, VanOstrand NR, et al. Author Correction: Multicellular 3D neurovascular unit model for assessing hypoxia and neuroinflammation induced blood-brain barrier dysfunction. Sci Rep. 2020;10(1):20384 Ochalek A, Mihalik B, Avci HX, et al. Neurons derived from

sporadic Alzheimer’s disease iPSCs reveal elevated TAU hyperphosphorylation, increased amyloid levels, and GSK3B activation. Alzheimers Res Ther. 2017;9(1):90.

Otero M, Canals J, Belio-Mairal P, et al. Advanced non-animal models in biomedical research: Autoimmune diseases. Publications Office of the European Union; 2022.

Park J, Wu Z, Steiner PR, Zhu B, Zhang JXJ. Heart-on-chip for combined cellular dynamics measurements and

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	Biomed Eng. 2022;50(2):111-137. Patel VS, Amin K, Wahab A, et al. Cryopreserved human precision-cut lung slices provide an immune competent pulmonary test system for "on-demand" use and long-term cultures. Toxicol Sci. 2023;191(2):253-265. Pičulin M, Smole T, Žunkovič B, et al. Disease progression of hypertrophic cardiomyopathy: Modeling using machine learning. JMIR Med Inform. 2022;10(2):e30483. Ramirez S, Mukherjee A, Sepulveda S, et al. Modeling traumatic brain injury in human cerebral organoids. Cells. 2021;10(10):2683. Richards DJ, Li Y, Kerr CM, et al. Human cardiac organoids for the modelling of myocardial infarction and drug cardiotoxicity. Nat Biomed Eng. 2020;4(4):446-462. Romania P, Folgiero V, Nic M, et al. Advanced Non-Animal Models in Biomedical Research: Immuno-Oncology. Publications Office of the European Union; 2021. Ronaldson-Bouchard K, Vunjak-Novakovic G. Organs-on-a-chip: A fast track for engineered human tissues in drug development. Cell Stem Cell. 2018;22(3):310-324. Rosenbluth JM, Schackmann RCJ, Gray GK, et al. Organoid cultures from normal and cancer-prone human breast tissues preserve complex epithelial lineages. Nat Commun. 2020;11(1):1711. Santhanam N, Kumanchik L, Guo X, et al. Stem cell derived phenotypic human neuromuscular junction model for dose response evaluation of therapeutics. Biomaterials. 2018;166:64-78 Sebastian R, Jin K, Pavon N, et al. Schizophrenia-associated NRXN1 deletions induce developmental-timing- and cell-type-specific vulnerabilities in human brain organoids. Nat Commun. 2023;14(1):3770. Scarnati MS, Halikere A, Pang ZP. Using human stem cells as a model system to understand the neural mechanisms of alcohol use disorders: Current status and outlook. Alcohol. 2019;74:83-93. Schiller AM, Howard JT, Convertino VA. The physiology of blood loss and shock: New insights from a human laboratory model of hemorrhage. Exp Biol Med (Maywood). 2017;242(8):874-883. Shrirao AB, Kung FH, Omelchenko A, et al. Microfluidic platforms for the study of neuronal injury in vitro. Biotechnol Bioeng. 2018;115(4):815-830. Siekmeier PJ. Computational modeling of psychiatric illnesses via

well-defined neurophysiological and neurocognitive biomarkers. Neurosci Biobehav Rev. 2015;57:365-380.

Sokolowska P, Zukowski K, Janikiewicz J, Jastrzebska E, Dobrzyn A, Brzozka Z. Islet-on-a-chip: Biomimetic micropillar-based microfluidic system for three-dimensional pancreatic islet cell culture. Biosens Bioelectron. 2021;183:113215.

Soscia D, Belle A, Fischer N, et al. Controlled placement of multiple CNS cell

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	2017;12(11):e0188146. Spijkers XM, Pasteuning-Vuhman S, Dorleijn JC, Vulto P, Wevers NR, Pasterkamp RJ. A directional 3D neurite outgrowth model for studying motor axon biology and disease. Sci Rep. 2021;11(1):2080. Strelez C, Jiang HY, Mumenthaler SM. Organs-on-chips: a decade of innovation. Trends Biotechnol. 2023;41(3):278-280. Tao T, Wang Y, Chen W, et al. Engineering human islet organoids from iPSCs using an organ-on-chip platform. Lab Chip. 2019;19(6):948-958. Tian L, Prasad N, Jang YY. In vitro modeling of alcohol-induced liver injury using human-induced pluripotent stem cells. Methods Mol Biol. 2016;1353:271-283. Urresti J, Zhang P, Moran-Losada P, et al. Correction: Cortical organoids model early brain development disrupted by 16p11.2 copy number variants in autism. Mol Psychiatry. 2021;26(12)7581 Venkat V, Abdelhalim H, DeGroat W, Zeeshan S, Ahmed Z. Investigating genes associated with heart failure, atrial fibrillation, and other cardiovascular diseases, and predicting disease using machine learning techniques for translational research and precision medicine. Genomics. 2023;115(2):110584. Vuorenpää H, Björninen M, Välimäki H, et al. Building blocks of microphysiological system to model physiology and pathophysiology of human heart. Front Physiol. 2023;14:1213959. Wei W, Cardes F, Hierlemann A, Modena MM. 3D In Vitro Blood-Brain-Barrier Model for Investigating Barrier Insults. Adv Sci (Weinh). 2023;10(11):e2205752. Wevers NR, Nair AL, Fowke TM, et al. Modeling ischemic stroke in a triculture neurovascular unit on-a-chip. Fluids Barriers CNS. 2021;18(1):59. Zamprogno P, Wüthrich S, Achenback S, et al. Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. Commun Biol. 2021;4(1):168. Zhong X, Harris G, Smirnova L, et al. Antidepressant paroxetine exerts developmental neurotoxicity in an iPSCderived 3D human brain model. Front Cell Neurosci. 2020;14:25. Zhuang P, Sun AX, An J, Chua CK, Chew SY. 3D neural tissue models: From spheroids to bioprinting. Biomaterials. 2018;154:113-133.

Ziraldo C, Solovyev A, Allegretti A, et al. A computational, tissue-realistic model of pressure ulcer formation in individuals with spinal cord injury. PLoS Comput Biol. 2015;11(6):e1004309.

Additional Supporting Resources:

Contopoulos-Ioannidis DG, Ntzani E, Ioannidis JP. Translation of highly promising basic science research into clinical applications. Am J Med. 2003;114(6):477-484.

Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity in preclinical

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	Transl Med. 2018;16:304.
2. Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:	If non-animal methods (a.k.a. novel alternative methods or NAMs) are to live up to their potential to transform biomedical research and catalyze discovery, their adoption must be commensurate with intense rigor. Otherwise, we risk abandoning critical methodologies and experiments not because they are fundamentally incorrect, but because they were improperly used. This would be a tragedy. Good laboratory and good cell culture practices are imperative. To aid in ensuring the robustness, replicability, reproducibility, and reliability of the technologies and the ensuing datasets, the NIH can provide dedicated funding for researchers in different laboratories to repeat experiments and fund accessible, public data repositories to promote transparency and data sharing. The NIH should also mandate that grantees adhere to high quality reporting standards, several of which have been recommended in the literature (see Supporting Resources). The UK's National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) is currently undertaking a user testing study of its Reporting In Vitro Experiments Responsibly (RIVER) guidelines and have recently made a preprint available on these recommendations (The RIVER Working Group). These recommendations should ideally be in place for all research funded or undertaken by the NIH, but are increasingly important for non-animal methods so that their value is fully

2. Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:	Resources: Emmerich CH, Harris CM. Minimum Information and Quality Standards for Conducting, Reporting, and Organizing In Vitro Research. Handb Exp Pharmacol. 2020;257:177-196. Hartung T, De Vries R, Hoffmann S, et al. Toward Good In Vitro Reporting Standards. ALTEX. 2019;36(1):3-17. OECD. Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, No. 286, OECD Publishing, Paris. Published December 10, 2018. The River Working Group. Reporting in vitro experiments responsibly – The RIVER recommendations. MetaArXiv preprints. Updated June 21, 2023. Accessed August 15, 2023. https://osf.io/preprints/metaarxiv/x6aut/.
3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:	While there are research methods that can be used to study living humans (such as imaging), most methods are necessarily reductive. It will likely be the case that researchers or research groups need to use several non-animal methods (a.k.a. novel alternative methods or NAMs) in order understand a biological system or disease state. The benefit of non-animal, human biology-based methods is that, unlike animal-based

3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:	entirely different species. Many of these platforms can even be used to study systems and states in the individual patient of interest, using tissue and cell samples or genetic data, for example. A key strategy for bolstering technology readiness and the reliability of these technologies and ensuring their successful integration across research approaches and potential solutions is to increase funding for, access to, and training in these methodologies. This could be done by 1) making funding for non-animal research more readily available, 2) prioritizing non-animal research methods in training opportunities, and 3) establishing and expanding animal-free biomedical research resources. 1) Make funding for non-animal research more readily available: Decisions about grant funding must prioritize applicants who currently use non-animal methods, are making the transition from animal to non-animal methods, or are developing and/or validating non-animal methods. The NIH should offer Program Project Grants or Center Grants (P01/P30/P50) to investigators interested in establishing centers for non-animal methods at their institutions. The NIH should offer grant supplements to investigators who want to switch to non-animal methods mid-funding. 2) Training opportunities must prioritize non-animal research methods. The NIH should offer Institutional Training Grants to trainees at the undergraduate, graduate, and postdoctoral levels to receive training that would allow them to make the transition from animal to non-animal research methods. It should place particular emphasis on post-doctoral training fellowships that allow young scientists to receive training in non-animal methods. The NIH should offer Continuing Education Training Grants with the explicit purpose of establishing educational programs to train researchers on available non-animal methodologies. The NIH should offer awards to early stage investigators who are looking to switch from using animal models to conducting non-animal research. The NIH Director’s Early Independence Award should prioritize applicants who currently use non-animal, clinically-applicable methods; are making the transition from animal to non-animal methods; or are developing and/or validating non-animal methods. The NIH Bench-to-Bedside and Back Program should prioritize pairing basic science researchers using animal models with Intramural Research Program (IRP) clinical researchers. The goal should be to assist those researchers interested in permanently switching from animal-based research to clinical work. The NIH Graduate Partnership Program should prioritize those students who are hoping to use non-animal

3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:	at their home institution. These are just a few ideas. 3) Establish/Expand Animal-Free Biomedical Research Resources: The Office of Strategic Coordination—within the Office of the Director—should use the NIH Common Fund to establish multiple centers for non-animal methods across the U.S., as we suggested in a recent submission to an NIH Common Fund RFI. The NIH should establish Core Facilities at the NIH IRP that will provide investigators with access to resources and experts in the use of non-animal methods. Suggestions for such core facilities include a microphysiological systems core, an animal-free antibodies core, and a three-dimensional tissue printing core. The NIH should expand the current Human Tissue and Organ Research Resource. The NIH should require grant recipients to share their human bio samples with the "All of Us Research Program" biobank. As mentioned above, it is imperative that with increased funding for non-animal methods comes a mandate of rigorous practices, reporting, and data sharing.
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