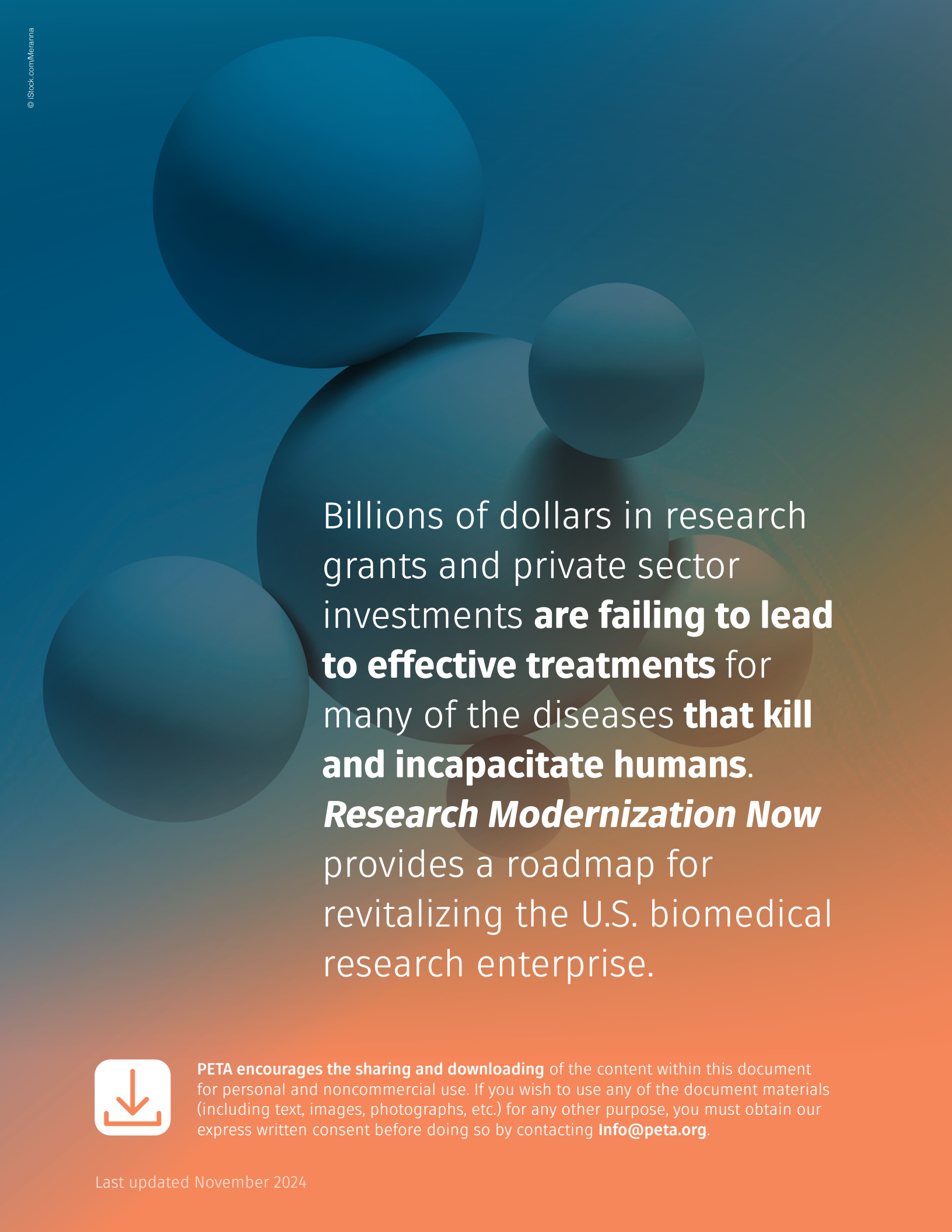
Research Modernization NOW

People for the Ethical Treatment of Animals



Billions of dollars in research grants and private sector investments **are failing to lead to effective treatments** for many of the diseases **that kill and incapacitate humans**. ***Research Modernization Now*** provides a roadmap for revitalizing the U.S. biomedical research enterprise.

Last updated November 2024

Executive Summary

RESEARCH MODERNIZATION NOW

Numerous scientific studies and reviews reveal that experiments on animals fail to lead to effective treatments and cures for human diseases, including the top killers in the U.S. Reliance on animal studies is diverting funds away from more promising areas of research and delaying the development of effective drugs and treatments, limiting our ability to protect human health.

Approximately 47% of the budget of the National Institutes of Health (NIH), which is charged with overseeing the health of Americans, funds experiments on animals. NIH has failed to take effective steps to address the following problems:

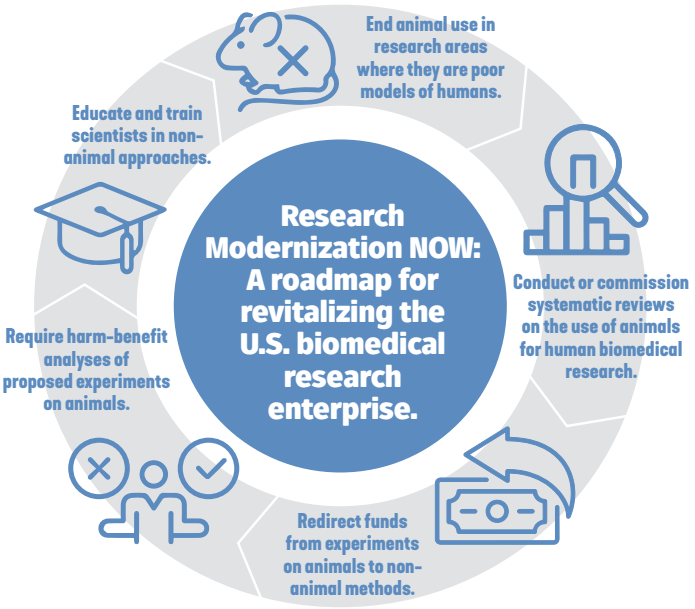
- 95% of all new drugs that test safe and effective in experiments on animals fail in human clinical trials, most because they were not safe or effective in humans.
- The failure rates of new drugs developed using animals in certain disease research areas exceed 95%. Here are a few examples:
  - Alzheimer's disease.....99.6%
  - Cancer 96.6%
  - HIV vaccine.....100%
  - Stroke 100%
  - Sepsis 100%
- 90% of basic research fails to lead to any human therapies within 20 years.
- Up to 89% of experiments cannot be reproduced, even though reproducibility is a critical component of scientific research.

Promising human-relevant research methods, such as organs-on-chips, sophisticated uses of human stem cells, genomics and proteomics, imaging, and computer modeling, can replace animals.

To revitalize U.S. biomedical research and protect human health, PETA proposes the following:

1. End animal use in research areas in which animals have been demonstrated to be poor "models" of humans and their use has impeded scientific and medical progress.
2. Conduct systematic reviews of the efficacy of animal use to identify additional areas in which non-animal methods are available or animal use has failed to protect human health and can, therefore, be ended.
3. Redirect funds from animal studies to reliable, non-animal methods.
4. Implement a harm-benefit analysis system for animal studies that includes an ethical perspective and consideration of lifelong harm inflicted on animals.
5. Educate the scientific community about the benefits of non-animal approaches and train scientists to use them.

This transformation can be initiated today. Without it, the research funded by U.S. taxpayers will fail to provide the discoveries and applications needed to protect human health.



CONTENTS

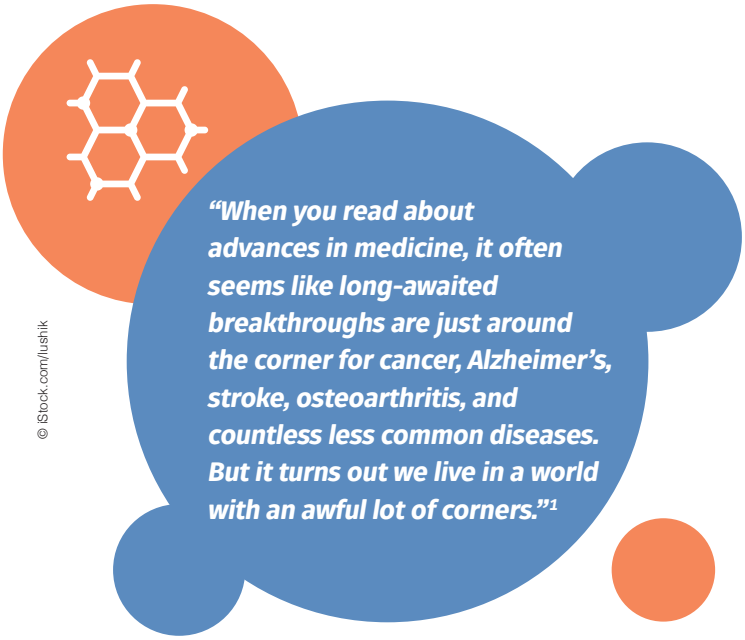
Introduction

The observation (right) by best-selling science journalist Richard Harris resonates with each person who is suffering or who knows someone suffering from an incurable disease—and for good reason: Billions of dollars in research grants and private sector investments are failing to lead to effective treatments for many of the diseases that kill and incapacitate humans.

A primary reason for this failure is a misplaced reliance on animal studies. A great deal of scientific research in the last several decades shows that animal studies are flawed and divert both monetary and intellectual resources from more reliable and relevant methodologies. Critically, intrinsic biological and genetic differences among species contribute significantly to inescapable problems in extrapolating results to humans from other animals, even in the best controlled and best executed study designs.

Along with mounting evidence that experiments on animals do not reliably translate to humans and the increasing development and implementation of technologies that can supplant animal use in laboratories, society's moral acceptance of experiments on animals has decreased.

In this report, we detail the failings of animal experimentation, show how the systems in place are insufficient to correct these failures, offer a plan for replacing animal use in experimentation, identify strategic priorities, and append further information about areas in which there are opportunities for the immediate replacement of animal use.



"When you read about advances in medicine, it often seems like long-awaited breakthroughs are just around the corner for cancer, Alzheimer's, stroke, osteoarthritis, and countless less common diseases. But it turns out we live in a world with an awful lot of corners."[1]

Limited Predictive Value of Research Using Animals

Many in the scientific community are aware of the flaws of experiments on animals. The U.S. National Institutes of Health (NIH) reports that novel drugs fail "in about 95 percent of human studies,"[2] even though they appeared safe and effective in preclinical experiments on animals. A 2014 analysis published in *The BMJ* found that animal studies largely have not furthered knowledge in the field of human health or led to the development of treatments for conditions affecting humans.[3]

Lack of Validity

Problems with internal and external validity contribute to the failure of experiments on animals in the translation of biomedical research from bench to bedside. The internal validity of experiments on animals is undermined by poor study design, including failure to implement processes to prevent bias, such as blinding, in which the individuals conducting the experiments or those analyzing the data do not know whether the animals or samples belong to the treatment or control group. Scientists have found that a lack of measures to reduce bias in experiments on animals likely results in overestimation of the benefits of the treatment studied, noting that this bias affects the trustworthiness of results, wastes resources, and should not be used to inform human clinical trials.[4,5]

Poor internal validity means that many experiments on animals cannot be reproduced, a critical aspect of the scientific process that speaks to the potential validity of a

finding. It is unsurprising, therefore, that a 2015 investigation concluded that between 18% and 89% of all preclinical research, a large part of which involves animal testing, was irreproducible, resulting in billions per year spent on experimentation that is misleading for human health.[6] Former NIH leadership has admitted, "Preclinical research, especially work that uses animal models, seems to be the area that is currently most susceptible to reproducibility issues."[7]

However, the weaknesses of experiments on animals cannot be overcome simply by improving study design, because external validity, or the "extent to which research findings derived in one setting, population or species can be reliably applied to other settings, populations and species,"[8] can never be achieved. Inherent species differences mean that other species cannot serve as analogs for understanding the biological mechanisms of disease and the effects of drugs on humans.

"On average, extrapolated results from studies using tens of millions of animals fail to accurately predict human responses."[9]

Therefore, experiments on animals lack internal and external validity. In other words, they are usually poorly executed, but even if the experimental methods were improved, the results would not translate to humans.

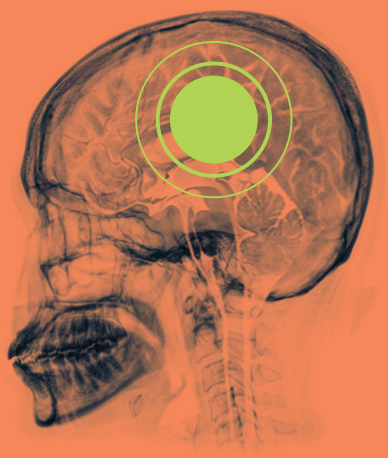
In a 2018 review published in the *Journal of Translational Medicine*, Pandora Pound and Merel Ritskes-Hoitinga discuss species differences as an insurmountable problem of external validity for preclinical animal models.[8] Attempts to control for or correct species differences result in what the authors refer to as the "extrapolator's circle." They write, "[I]f we want to determine whether a mechanism in animals is sufficiently similar to the mechanism in humans to justify extrapolation, we must know how the relevant mechanism in humans operates. But if we already know about the mechanism in humans then the initial animal study is likely to have been redundant."[8]

They also discuss the concerning trend among those involved in experiments on animals to minimize the issue of species differences and the effects on external validity, a problem that is acknowledged by a number of researchers.[10,11] Pound and Ritskes-Hoitinga go on to state that it is unsurprising that the issue of species differences is downplayed, as not doing so would force experimenters to confront the "possibility that the preclinical animal research paradigm no longer has a great deal to offer."[8] There is growing scientific consensus that far more is to be gained from non-animal research methods that are better suited to solving human biomedical research questions.

The difficulties in applying data derived from one species to another are compounded by the confinement and unnatural conditions of laboratory life—including housing,[12,13] diet,[14–16] light cycles,[17–20] noise,[17,21,22] and the temperature and humidity at which animal facilities are kept[23–27]—which thwart animals' ability to engage in natural behavior.[28–30] This deprivation contributes to their stress and alters their physiology and neurobiology, causing them to exhibit various morbidities and psychopathologies unrelated to the experiments at hand.[14,18,29,31–36] Importantly, the fact that animals in laboratories have altered physiology and neurobiology means that they would not even be good "models" for their counterparts in nature. A mouse in a laboratory will not respond to a drug in the same way a mouse in a field would. One then has to ask: How does this biologically distinct mouse reliably represent the biology of humans?

Inherent species differences mean that other animals cannot serve as analogs for understanding the biological effects of drugs and chemicals on humans.

Lack of Clinical Success



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The failure of animal studies in basic and applied research is perhaps most evident in the stark litany of seemingly promising treatments that have not worked in humans. For example, stroke experiments on animals have been an outright failure: 30 years of animal testing have failed to result in any successful translation of drugs that protect against damage or repair the brain after a stroke.[37] Decades of experiments on mice and other animals have generated no new treatment or diagnostic technology for humans with sepsis.[38] Oncology drugs, which undergo extensive animal testing, have a success rate of only 3.4%.[39] This theme pervades many human disease areas.[40] There is an abundance of literature documenting the failure of various animal models of neurodegenerative diseases, neuropsychiatric conditions, women's health issues, and more. (See the appendices for a comprehensive look at disease areas.)

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Misplaced Resources

Despite the growing evidence that experiments on animals are wasteful and can impede medical progress, approximately 47% of all NIH research funding goes toward them.[41] Federal funds available for biomedical research are a finite resource. In the fiscal year 2023, only 21.3% of research project grant applications submitted to NIH were awarded funding.[42] Each decision to approve an application carries with it a refusal to fund other projects, leaving a large opportunity cost in terms of human-relevant research that has the potential to help patients.

"[I]f research conducted on animals continues to be unable to reasonably predict what can be expected in humans, the public's continuing endorsement and funding of preclinical animal research seems misplaced."[4]

Funding for biomedical research is allocated into three categories: basic, translational, and clinical research. NIH defines basic research as that which supports a "greater knowledge or understanding of the fundamental aspects of phenomena and of observable facts without specific applications towards processes or products in mind."[43] A great deal of basic research involves experiments on animals.

NIH perceives basic research, including that which uses animals, as important because its intent is to produce foundational knowledge for a better understanding of the causes and determinants of disease in humans.[44] In other words, the results of animal use in basic research should point the way toward translational and clinical research that should, in turn, benefit humans. However, the evidence shows that this is not the case. To assess whether the promises of basic biomedical research were being fulfilled, researchers identified 101 articles published in the most prestigious medical journals in which the authors explicitly stated that their research would lead to a new application with real potential for a clinical breakthrough. A majority of the articles analyzed (63%) described experiments on animals. The researchers' investigation into the conversion of basic research into clinical applications found that fewer than 10% of these self-proclaimed highly promising discoveries entered routine clinical use within 20 years.[45]

Basic research is a critical step for generating foundational scientific knowledge, but when that knowledge produces no actionable benefits for humans—or the species harmed and killed for it—society's continued investment in and support of it must be reassessed.

In the current system, bringing a new drug to market may cost more than $1 billion and takes an average of 14 years.[2]

The Dangers of Misleading Results

Many novel drugs don't simply fail, representing a huge loss in time and investment—they harm patients. In 2016, a drug intended to help with mood, anxiety, and motor problems related to neurodegenerative disease was administered orally to volunteers as part of a Phase I clinical trial. Six men, ages 28 to 49, experienced such adverse reactions that they had to be hospitalized. One participant was pronounced brain-dead and later died. A report on this incident revealed that the toxicity of the drug in humans "was not observed in animals despite administration of very high doses."[46]

TGN1412 is another tragic example. "After [the] very first infusion of a dose 500 times smaller than that found safe in animal studies, all six human volunteers faced life-threatening conditions involving multiorgan failure for which they were moved to [the] intensive care unit."[47] Five of the six participants were hospitalized for three months after the initial dose, while the other was comatose. Even six months later, participants suffered from headaches and memory loss. One had to have toes and fingers amputated as a result of gangrene.[48]

The opposite is also true: Therapies that have not worked well in animals have sat useless on the shelf while patients have gone without lifesaving treatment. For example, aspirin is widely used in human medicine, but it may have never been approved if it were first tested in animals, for whom it has a wide range of toxic effects that are not observed in humans.[49]

Toxicologist Thomas Hartung noted a number of similar examples in his 2024 article, "The (misleading) role of animal models in drug development," including the following:

> Severe liver injury and multiple deaths forced the termination of a hepatitis B drug trial despite earlier encouraging animal data. Differential species sensitivity to drugs like acetaminophen further highlights the pitfalls of reliance on animal models. Gene therapy vectors that have been safe in animal tests have caused liver failure and brain swelling in children. HIV vaccines, stroke treatments, inflammatory disease agents, and Alzheimer's therapies have all elicited enthusiasm in animal models yet utterly failed in human trials.[50]

Public Opinion and Animal Sentience

Public opposition to the use of animals in experiments has increased steadily, reaching 52% of the population in 2018.[51] In 2024, Gallup reported that 46% of Americans felt that medical testing on animals was "morally wrong," up from 32% in 2004.[52] Another 2024 survey published by the Animal-Human Policy Center at Colorado State University found that approximately 61% of respondents were "very or extremely concerned" about animals used in experimentation and only 22.5% of respondents "somewhat or strongly agreed" that laws in the U.S. aimed at protecting the welfare of animals used in experimentation were "strong."[53] A third 2024 survey by Morning Consult found that 80% of respondents agreed or strongly agreed with the statement "The US government should commit to a plan to phase out experiments on animals."[54] Similar responses were elicited with approximately 85% agreement with both of the following statements: "Government funding should prioritize research methods that do not involve animal testing" and "Animal experimentation should be phased out in favor of more modern research methods."[54]

The public is even less approving of animal use when the experiments are invasive, are viewed as less beneficial or necessary for human health—as in the case of cosmetics testing—or when non-animal methods exist.

Research has revealed that universities and media outlets often exaggerate findings from experiments on animals and "promote research that has uncertain relevance to human health and do not provide key facts or acknowledge important limitations."[55] A study examining media coverage of animal-based preclinical research found that the reports were inflated and often prematurely implied imminent

"breakthroughs" relevant to human medicine. "Of 27 unique published 'breakthroughs', only one had clearly resulted in human benefit. Twenty were classified as failures, three were inconclusive and three were partially successful."[56] A 2021 study found that 69.5% of news articles about Alzheimer's disease research papers omitted any mentions of mice in their headlines and overstated the findings.[57] The use of misleading language in news reporting is not limited to Alzheimer's disease and has also been observed in coverage of other diseases, including cystic fibrosis[58] and multiple sclerosis.[59] Because experimenters rarely publish the results of failed animal studies, other scientists and the public lack access to information about the ineffectiveness of animal experimentation. If the public were fully aware of the extensive evidence that animal use may be hindering the development of effective treatments, opposition to such experiments would likely grow substantially.

The minority of the public that continues to support experiments on animals usually predicates its support on the mistaken belief that oversight bodies would only allow these experiments if they were essential to developing treatments for human disease and if the harm to animals were outweighed by the benefits to humans. Clinician-scientists in Turkey "found that more than 40% of papers based on animal models that were presented at the national orthopaedic congress of their country (population 83 million) over a 9-year span were never published, and of those that were, nearly 40% were never cited or were cited only once. All of this nonimpact cost more than 9400 animals their lives."[60,61] In 2020, researchers who evaluated studies "published in the two clinical journals with the highest Impact Factor in each of 10 surgical specialties found the median number of citations of animal research papers by subsequent human/clinical research over a 10-year span was only one (with the high end of the range being five), suggesting minimal translation of animal studies to research in humans."[60,62]

Recognition of animal sentience has also played a role in the public's growing opposition to experiments on animals. This is particularly true for the species with whom humans share their homes (e.g., dogs and cats) and those perceived as having higher cognitive abilities (e.g., primates). However, public concern for other species has also increased. Philosophers and bioethicists have emphasized that modern views on animal welfare prioritize sentience as a central component of ethical considerations in animal experimentation.[63]

The current state of research on cephalopod, decapod, and insect sentience[64–67] has prompted many countries, including those in the EU as well as Australia, Canada, Norway, Switzerland, and the U.K., to update their animal welfare laws. NIH has solicited feedback from scientists and the public to establish guidelines for the use of cephalopods in experiments,[68] noting that "[a] growing body of evidence demonstrates that cephalopods possess many of the requisite biological mechanisms for the perception of pain."[69]

Recent studies reveal that many animals—in addition to feeling physical and psychological pain and distress—show empathy, self-awareness, and language-like abilities. They also exhibit tool-related intelligence, engage in pleasure-seeking behavior, and have advanced problem-solving skills.[70,71] These realities have prompted academics, intellectuals, philosophers, and ethicists to seek the consideration of animal sentience and consciousness in decision-making about how animals are treated in science and other areas. For example:

- The 2024 New York Declaration on Animal Consciousness, citing empirical evidence of "a realistic possibility of conscious experience in all vertebrates (including reptiles, amphibians, and fishes) and many invertebrates (including, at minimum, cephalopod mollusks, decapod crustaceans, and insects),"[72] called for the consideration of the realistic possibility of conscious experience in other animals as part of the animal welfare decision-making process.
- In 2015, more than 150 academics, intellectuals, and writers backed a report by the Oxford Centre for Animal Ethics that condemned experiments on animals as both morally and scientifically indefensible. "The deliberate and routine abuse of innocent, sentient animals involving harm, pain, suffering, stressful confinement, manipulation, trade, and death should be unthinkable. Yet animal experimentation is just that: the 'normalisation of the unthinkable,'"[73] write the report's authors. They conclude that experimenting on animals contradicts what we now know about animals' ability to experience not only pain but also shock, fear, foreboding, trauma, anxiety, stress, distress, anticipation, and terror.
- In 2012, a prominent international group of neuroscientists issued "The Cambridge Declaration on Consciousness," which definitively stated that "humans are not unique in possessing the neurological substrates that generate consciousness" and that, like humans, "[n]on-human animals have … the capacity to exhibit intentional behaviors."[74]

The statistics on failed translation make it clear that animals are not appropriate human surrogates in biomedical research, but when it comes to their capacity to suffer, how much like humans do they need to be before a critical review of animal-based research is considered mandatory?

"Science is showing how other animals are like us in morally relevant ways, but unlike us in medically relevant ways."[75]

Existing Checks and Balances Are Failing

NIH is the largest funder of biomedical research in the world, and the U.S. has been estimated to be among the world's largest users of animals in experimentation,[76] but the lack of transparent accounting of animals used makes accurate numbers impossible to discern. Despite the existence of laws and committees expected to protect animals in laboratories, no experiments—no matter how harmful—are prohibited. Outdated and incomplete ethical frameworks, insufficient care and welfare standards, lax enforcement, self-serving committees, and the exclusion of 95% to 99% of the animals used in experimentation[77] from enforceable regulations define the reality of animal use in U.S. laboratories.

The federal Animal Welfare Act (AWA) and the Health Research Extension Act of 1985 (HREA) are the only two federal laws that provide minimal standards for the treatment of animals in U.S. laboratories. Both laws are deficient, and critical issues hinder their effectiveness.

The vast majority of animals used in laboratories in the U.S. are not covered by the AWA. This includes approximately 111 million rats and mice[77] and millions of fish, horseshoe crabs, frogs, cephalopods, turtles, purpose-bred birds, and other animals bred for food and fiber who are not recognized as "animals" under the law.[78] Meanwhile, the HREA only applies to institutions receiving taxpayer funding from U.S. federal health agencies, such as NIH,[79] leaving many animals who are used in institutions not funded by NIH without any legal protection. Though some states have laws against cruelty to animals, most have exemptions that exclude animals used in experimentation.[80]

Neither federal law mandates that experimenters not use animals unnecessarily or consider replacing animal use with a non-animal approach, only that they have *considered* alternatives to *specific harmful procedures* they plan to carry out. Even then, the requirement to search for less harmful or distressing procedures is not reliably enforced.

Improving oversight would reduce substantial harm to animals, but it wouldn't solve the problem. A shift away from animal use entirely would eliminate the need for more stringent regulation of animal use and protect the well-being of both humans and other animals.

Rubberstamping: Institutional Animal Care and Use Committees

Established in response to public outcry over cruelty cases involving animals in laboratories, Institutional Animal Care and Use Committees (IACUC) were established with the intent to ensure that institutions using animals in experimentation adhere to the AWA. It was expected "that bodies such as [these] ethical committees will take corporate social responsibility by acting as watchdogs for animal experiments."[81]

In practice, IACUCs lack the essential ethical and scientific diversity to effectively address growing concerns about animal welfare and the ability to avoid animal use.[82] A 2012 study documented that, on average, IACUC membership at top NIH-funded institutions was dominated by animal experimenters.[83] The authors wrote that the "overwhelming presence of animal research and institutional interests may dilute input from the few IACUC members representing animal welfare and the general public, contribute to previously-documented committee bias in favor of approving animal experiments and reduce the overall objectivity and effectiveness of the oversight system."[83]

Ambiguous legislative language and poor oversight by IACUCs have led to inconsistencies in implementation and effectiveness. Multiple Office of the Inspector General (OIG) audits and internal surveys have demonstrated the weaknesses of IACUCs:

- In 1995, the OIG found that IACUCs failed to ensure that experimenters had looked for alternatives to harmful procedures or that the proposed studies were not unnecessarily duplicative of previous experiments.[84]
- A 2000 U.S. Department of Agriculture (USDA) survey of the agency's laboratory inspectors showed that the biggest problem area for IACUCs was the search for alternatives to painful procedures, revealing that "600 to 800 facilities have had trouble with the search for alternatives." USDA inspectors also felt that "undue influence" of principal investigators was a problem for IACUCs.[85]
- A 2005 OIG audit report again highlighted these issues, noting ongoing "problems with the search for alternative research, veterinary care, review of painful procedures, and the researchers' use of animals."[86]
- Problems with IACUCs remained a prominent feature of OIG's 2014 audit report, which warned that IACUCs "are not always adequately monitoring experimental procedures on animals," resulting in "reduced assurance that protocols

are properly completed, approved, and adhered to and that animals are always receiving basic humane care and treatment."[87] The data agreed: Between 2009 and 2011, USDA inspectors cited 531 facilities for 1,379 violations due to IACUCs' failure to adequately review and monitor the use of animals.[87]

But little is changing. The most recent NIH initiative to enhance both rigor and reproducibility in research failed to address the myriad issues with IACUCs and their review processes.[88]

A major failing of U.S. oversight of experiments on animals is that there is no point within the protocol approval process where the harm that will be endured by animals is weighed against the expected benefits of the research. While oversight bodies claim adherence to policies that require the performance of a harm-benefit analysis,[89,90] the bodies that perform the assessment of harm are separate from those assessing benefit, and there is no attempt to balance the results. IACUCs review the harm that will be inflicted on AWA-covered animals or animals involved in NIH-funded protocols, while funding committees are tasked with considering how the experiments might benefit the field. The two committees operate disparately, don't share their opinions with one another, and render binary judgment, resulting in a fragmented and incomplete evaluation system.

The 3Rs Are Insufficient

The 3Rs—the replacement, reduction, and refinement of animal use—have been the longstanding ethical framework guiding the use of animals in biomedical research around the world. Introduced by Russell and Burch in their 1959 book *The Principles of Humane Experimental Technique*,[91] the 3Rs have faced significant criticism in recent years for their failure to prevent unnecessary harm to animals due to their narrow focus on procedural ethics, rather than addressing broader societal and moral questions surrounding animal research. Some scholars argue that the principles do not adequately encompass the complexities of animal welfare and ethical considerations in research.[92–94] Others posit that though the 3Rs may have been fit for their time, science has advanced significantly since their inception, necessitating a modern update.[95–97]

What is clear is that the 3Rs have not been successful. Counter to the principles of reduction and refinement, more animals are used in experimentation now than when the 3Rs concept was published[76,77,98,99] and they continue to be used in procedures that are distressing and harmful. The establishment of 3Rs centers around the globe[100] has not effectively curbed the use of tens of millions of animals in experiments nor has it stopped animals from being used in experiments that have little chance of generating tangible benefits for human health.

Non-Animal Research Methods

A variety of human cell-based and tissue methods, advanced computer models, and other technologies can be used for basic, translational, and preclinical biomedical research. Here are just a few of the exciting examples.

Organs-on-chips include human cells that mimic the structure and function of human organs and replicate human physiology and drug responses more accurately than experiments on animals.

3-dimensional human cell–derived models, such as organoids, replicate self-organized human tissues in a complex structure that recapitulates the function of human organs and tissues.

Sophisticated computer models can simulate human biology and the progression of disease, accurately predicting how new drugs will react in the human body and aiding in biomarker identification and treatment.

"Omics" studies, including genomics, proteomics, and more, give insight into the complex molecular mechanisms that underpin human biology and direct the form and function of human cells and cellular processes.

Non-invasive human imaging with human volunteers allows researchers to better understand the human brain and other organs, even down to the level of a single neuron.

Opportunities for Economic Advancement

The High Cost of Drug Development

By mandating a move away from experiments on animals and toward advanced scientific methods, the U.S. has the opportunity to advance biomedical research, rapidly expand job growth in science and technology, and reduce healthcare costs. In a paper titled "Animal testing and its alternatives—the most important omics Is economics," researchers report that "an economy of alternative approaches has developed that is outperforming traditional animal testing."[101]

In the current system, bringing a new drug to market may cost more than $1 billion and takes an average of 14 years.[2] The high costs of research and development (R&D) may be shifted to patients in the form of increasingly unmanageable price tags for prescription drugs,[102] even though the development of those drugs was likely already subsidized by public funding, meaning patients are essentially "paying twice" for access to lifesaving medications.[103]

During a 2017 conference, then–U.S. Food and Drug Administration (FDA) Commissioner Scott Gottlieb lamented the high cost of drug development and its impact on both patients and the U.S. economy. He discussed the importance of reducing R&D costs "to make sure we're providing an efficient path for the translation of cutting-edge science into practical treatments that are going to benefit patients" and "because the rising cost of drug development is unsustainable."[104] He stated, "Unless we find ways to modernize how we approach our work, and make more efficient use of our resources, then we're going to get fewer medicines, and higher costs," adding, "At a time when people are rightly worried about the rising prices of drugs, and the impact on patient access, we also need to be thinking about these factors that contribute to the high cost of making new medicines."[104]

One factor contributing to the high cost of R&D is the substantial risk associated with developing a product that fails to result in a marketable drug because it does not succeed in human clinical trials. Ninety-five percent of drugs that test safe and effective in animals fail in human trials,[2] most because they are either not safe or not effective.[50,105,106] There are also instances where drugs that make it to market are recalled due to adverse effects or safety concerns that were not detected in animal tests.[50] Failure during the clinical trial phases of drug development is the biggest driver of R&D costs,[107] highlighting the urgent need for better predictive models.[108]

Conversely, drugs that could be effective in humans may never enter clinical trials because they were ineffective or unsafe in animals. Scientists advocating for the use of human-based models during research and drug testing made the following observation:

> [P]otentially effective drug candidates never enter clinical trials owing to negative preclinical tests given that most animal models poorly resemble human conditions and thus have low predictive values. The discrepancies derive from different anatomical layouts and biological barriers, divergent receptor expression and immune responses, host specificities of microorganisms, and distinct pathomechanisms.[106]

With the use of human-relevant technology in place of expensive, time-consuming, and inaccurate experiments on animals, the cost of drug discovery has the potential to decrease dramatically. Experts have estimated that the use of organs-on-chips—just one type of non-animal model—could reduce R&D costs by 10% to 26%, resulting in savings of up to $706 million.[108] By reducing both the expense and time it takes to get effective therapies to market, manufacturers will be able to pass these savings on to patients.[108]

"Drugs showing safety and efficacy in preclinical animal models may show very different pharmacological properties when administered to humans."[47]

Job and Economic Growth in the Technology Sector

The market for human cell–based *in vitro* technology for biomedical research and testing is growing rapidly. According to market research firm DataM Intelligence, "The Global Organ-On Chip Market reached USD 107.5 million in 2022 and is expected to reach USD 796.7 million by 2031 and is expected to grow with a CAGR [compound annual growth rate] of 29.6% during the forecast period 2024–2031."[109] A similar CAGR of 26.5% is predicted for three-dimensional cell cultures, which are expected to reach $14.8 billion by 2028.[110] The markets for induced pluripotent stem cells, 3D bioprinting, and cell-based assays are also expected to continue thriving.[111–113]

Contract research organizations that focus heavily on breeding and supplying animals, on the other hand, are not faring as well. In late 2024, Charles River Laboratories, which was under federal investigation for possible violations of monkey-importation laws, reported a 3.2% decline in revenue in Q2, prompting the company to lay off approximately 600 employees.[114] Inotiv (previously Envigo), another animal supplier that had recently settled a criminal investigation regarding the abuse of dogs it bred for experimentation, reported a 32.8% drop in Q3 2024 revenue, with a consolidated net loss of $26.1 million,[115] and has noted that its financial losses have been due to a decrease in its sales of primates.[116]

Transitioning away from animal experimentation and testing can open new opportunities to retrain laboratory staff, including experimenters, animal technicians and caretakers, animal welfare officers, and breeders in skills that will better equip them for stable and fulfilling careers in growing industries. Building new infrastructure around human-relevant research will fill the gaps left by failing animal breeders and suppliers, creating a wealth of job opportunities that are free from the mental[117–120] and physical[121–123] risks associated with working in facilities with sick, stressed, and captive animals.

New—and more ethical—technology will streamline drug development, making the process safer, cheaper, and more effective. Expanding these techniques allows for the creation of interdisciplinary research teams that will be fundamental in furthering translational science and creating personalized disease models for precision medicine.

Human Biology–Based Methods Outperform Animal Tests

Select cases can demonstrate how research tools based in human biology are better than experiments on animals for predicting outcomes in humans. Here are just a few examples, including several showing how the use of these tools could have prevented morbidity and mortality in humans:

- A human liver–on-a-chip developed by Emulate Inc. in Boston "was able to correctly identify 87% of the tested drugs that caused drug-induced liver injury in patients despite passing animal testing evaluations. These drugs that initially passed animal testing evaluations ultimately caused nearly 250 deaths and 10 liver transplants."[124] In September 2024, the FDA Center for Drug Evaluation and Research accepted this liver chip into its Innovative Science and Technology Approaches for New Drugs Pilot Program, which will allow developers to use the technology to screen new drugs for their potential to cause drug-induced liver injury in humans, one of the leading reasons drugs fail in clinical trials.[125]
- In a 2021 study, researchers at Johns Hopkins University, the Norwegian Institute of Public Health, and U.K. patient safety charity Safer Medicines Trust used human-based *in vitro* methods to reevaluate the diabetes drug troglitazone.[126] Troglitazone had been withdrawn from the market due to severe and fatal liver toxicity that killed at least 63 people. The newer *in vitro* tests predicted this potential hazard, while the preclinical animal studies had not. One author of the study commented, "Patients need safer affordable medicines delivered in their lifetime. The pharmaceutical industry is in crisis, with empty pipelines and skyrocketing costs. Focusing on human biology is the route to developing safer medicines faster and with lower total development costs."[127]
- Working from a large chemical database, a computer algorithm was able to predict the human toxicity of a new chemical better than animal tests.[128] In an interview on the paper, one author noted, "These results are a real eye-opener—they suggest that we can replace many animal tests with computer-based prediction and get more reliable results."[129]
- Emulate and Janssen Pharmaceuticals have demonstrated how a blood vessel–on-a-chip was able to predict a human thrombosis caused by an antibody therapy. This therapy had previously been determined to be safe following preclinical animal tests, but clinical trials had to be stopped after humans given the drug developed blood clots.[130]
- Computational models representing human heart cells predict human cardiotoxicity, which can produce dangerous arrythmias, more accurately than animal tests.[131] Models like these are critical for "improving drug safety, thereby lowering the risk for patients during clinical trials; and speeding up the development of medicines for patients in urgent need of healthcare."[132]

The Need for a Paradigm Shift

If our finite public funds are to be used responsibly, they must fund reliable research and test methods that lead to the effective treatment of diseases and protection of human health. But the evidence that experiments on animals are impeding the development of treatments and cures for human ailments has not prompted sufficient reconsideration of research and funding priorities by NIH or other authorities. Such a paradigm shift is crucial within and beyond the U.S.

The shift in scientific consensus away from the use of animals in experimentation can be observed in several arenas, including publications documenting the limited predictive value of experiments on animals, an increased awareness of animal cognition and sentience, the fast-eroding public support for animal use, and the measures being taken around the world to plan its phase-out.[3,51,52,133] **Research Modernization Now provides a framework by which policy makers, funders, companies, and researchers can plan these necessary interventions**.

Significantly, a move away from experiments on animals will allow for substantial growth in the science and technology sectors, leading to faster returns on investment in drug research and development,[101] as seen after the cosmetics testing ban in the EU. Redirecting research funding priorities toward human-relevant methods—which recapitulate human physiology and biology without using animals or their tissue—will deliver treatments to patients more safely and likely in less time.[50,105,134]

In support of using an evidence-based approach to accelerate the delivery of useful drugs to the patients who need them, a 2017 article called for the elimination of animal use in experiments in which there is clear evidence that animals are not useful or predictive of human disease:

> The literature is replete with examples of contradictions and discordance between animal and human effects, including many cases in which promising animal results have failed to translate to clinically significant efficacy in humans. This is particularly true in some therapeutic areas such as neurodegenerative, psychiatric, and central nervous system diseases, as well as sepsis and inflammatory diseases.
>
> These complexities inherent in translational research present an important opportunity for exploring novel approaches that successfully and efficiently yield outcomes as proximal as possible to eventual human benefit. Supported by several illustrative examples encountered in our drug repurposing program, we propose herein an approach for assessing when it is appropriate to conduct the "last experiment first," that is, progressing directly to human investigations when animal work would likely fail to provide data appropriate for translation into human applications of interest. This represents a significant—and we suggest, avoidable—barrier to drug introduction.[135]

World Leadership

There is an international movement away from using animals in experiments, which reflects the growing consensus in the scientific community that using animals in basic biomedical research or for regulatory assessment requirements is neither ethical nor efficacious. Australia, the EU, Japan, New Zealand, and the U.K. have all banned or limited the use of great apes (chimpanzees, gorillas, and orangutans) in experimentation, and the U.S. no longer awards federal funding for experiments involving chimpanzees.[136]

Major Milestones in the Global Transition to Non-Animal Research

- **2013** The Brazilian Center for Validation of Alternative Methods, which assists the country in validating non-animal methods (NAMs) for research and education, was established.
- **2018** The Netherlands began the Transition Programme for Innovation without the use of animals to accelerate the uptake of animal-free methods.
- **2021** Members of the European Parliament voted almost unanimously in support of a motion for a resolution that would set an EU-wide plan to phase out procedures on live animals in favor of non-animal methods.
- **2022** The U.S. President passed the FDA Modernization Act 2.0, which gave the agency the statutory authority to accept data from non-animal methods in new drug applications.
- **2023** The Government of India passed an amendment to the New Drugs and Clinical Trial Rules that authorizes researchers to use non-animal, human-relevant research methods to test the safety and efficacy of new drugs.
- **2023** The EU responded to the European Citizens' Initiative "Save Cruelty-free Cosmetics - Commit to a Europe without Animal Testing," stating it will "initiate a series of actions to accelerate the reduction of animal testing in research, education and training."
- **2024** The U.K. Department for Science, Innovation and Technology announced several new measures to support the acceleration of non-animal alternatives in research.
- **2024** NIH initiated the Complement Animal Research in Experimentation program to "speed the development, standardization, validation, and use of human-based...NAMs."
- **2024** The New South Wales Government announced that it will establish the Non-Animal Technologies Network to develop NAMs and advise on necessary infrastructure and regulations.

The infographic above highlights some of the major milestones in the global transition away from experiments on animals and toward non-animal research that have taken place since 2013. PETA scientists have played a part in most of these developments, beginning with a 2016 report requested by the Netherlands National Committee for the protection of animals used for scientific purposes, which used information from PETA scientists to publish an advisory report on the country's transition to animal-free innovation. Subsequently, the Transition Programme for Innovation without the use of animals was established, aiming to bring together stakeholders and offer a platform for identifying and developing activities to increase the pace of this transition.[137] PETA's report for the Netherlands committee became the original Research Modernization Deal.

In 2021, after receiving a European version of the Research Modernization Deal from PETA entities, members of the European Parliament almost unanimously supported a motion for a resolution calling on the European Commission to develop an action plan—with a timeline and milestones—to phase out experiments on animals and accelerate the transition to innovation without the use of animals in research, regulatory testing, and education.[138] PETA entities have also played a role in more recent developments in the EU, India, and the U.K.

In the U.S. in late 2022, President Joe Biden signed the PETA-supported FDA Modernization Act 2.0 into law,[139] providing the FDA with the statutory authority to accept data from non-animal testing methods in investigational new drug applications, removing the long-held assumption that tests on animals are required before a drug can proceed to clinical trials.

The following year, the NIH Advisory Committee to the Director Working Group on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research delivered its findings on how NIH can better support non-animal methods.[140] The working group's advice echoed PETA scientists' many recommendations over the years[141] and were promptly accepted by NIH Director Monica Bertagnolli in early 2024.[142] A new NIH program focusing on the development, standardization, and validation of non-animal methods, called Complement-ARIE, was launched by the NIH Common Fund, finally signaling some progress at the long-stagnant agency. But the scale of this new program pales in comparison to what NIH still spends on poorly translatable experiments on animals. The proposed budget for Complement-ARIE was only $35 million for FY25,[143] a mere 0.07% of NIH's total FY25 budget of $50.1 billion[144] and almost 700 times less than what the agency would typically be expected to spend on experiments on animals in that time.[41]

There is still considerable work to be done to move U.S. science policy away from experiments on animals and toward modern, human-relevant methods. Such changes are necessary to improve the quality of biomedical research and for the U.S. to prove itself a world leader in innovative and superior research that will more effectively benefit human health. Research Modernization Now can help stimulate those needed changes.

Plan of Action: Recommendations for Modernizing U.S. Biomedical Research

1. End animal use in research areas in which animals have been demonstrated to be poor "models" of humans and their use has impeded scientific and medical progress.

Multiple reviews have documented the overwhelming failure of animal use to benefit human health in specific areas, including cancer, cardiovascular disease, diabetes, gastrointestinal disorders, inflammation, infectious disease, sepsis, nerve regeneration, neurodegenerative diseases, neuropsychiatric conditions, strokes, and women's health. Since these experiments are generating results that are, at best, useless and, at worst, harmful, experiments on animals in these research areas should be ended as soon as possible and replaced with more effective and efficient non-animal methods. Please find further elaboration on and recommendations for these areas in the appendices.

2. Conduct systematic reviews of the efficacy of animal use to identify additional areas in which non-animal methods are available or animal use has failed to protect human health and can, therefore, be ended.

For research areas in which there is still some question as to whether the use of animals is beneficial, a thorough systematic review should be conducted to determine the efficacy of using animals. Systematic reviews, which critically analyze multiple research studies, are a crucial first step in assessing the effectiveness of animal use. Such systematic reviews should include information about the return on investment received by the public from the results of animal studies, particularly when publicly funded.

Several U.S. funding entities, including NIH, the Department of Veterans Affairs, and the Department of Defense, are members of the Ensuring Value in Research Funders' Forum (EViR), a collection of prominent international funding bodies formed to address waste in clinical and preclinical research. EViR states as its second guiding principle, "Research should only be funded if set in the context of one or more existing systematic reviews of what is already known or an otherwise robust demonstration of a research gap."[145] It explains, "This is important because new research not set in the context of what is already known leads to unnecessary duplication, studies that cannot change decision making (e.g. will not change the meta analysis), or inappropriate design (e.g. inappropriate outcome measures, incorrect prevalence assumptions, failure to learn from past previous studies)."[145] To apply this principle, EViR says that funders

must "[r]outinely assess whether an adequate review has been done and whether the results of that review support the case for further clinical or preclinical research."[146]

The recommendation to conduct systematic reviews of the efficacy of procedures is, therefore, already one that the largest funding bodies in the world agree is a necessary principle for guiding valuable research and reducing waste in research funding, yet there is no concerted effort within the U.S. to put this recommendation into action.

When the National Academy of Medicine, formerly the Institute of Medicine, completed an examination of the scientific necessity of using chimpanzees in behavioral and biomedical research,[147] the effort revealed that harmful studies had been approved, funded, and conducted for years, even though there were alternative methods in virtually every area in which chimpanzees were being used. Institutional oversight bodies and funding agencies had given their stamp of approval to these protocols. However, as we now know, the review processes in place were inadequate. Wherever thorough and objective systematic reviews of animal use for various areas of inquiry have not been conducted, they should be undertaken.

A number of resources exist for facilitating systematic reviews, including software for each step of the review process, tools for assessing study quality, reporting standards, workshops, tutorials, and opportunities to commission systematic reviews from trained researchers.[148–150]

3. Redirect funds from animal studies to reliable, non-animal methods.

The poor predictivity of preclinical experiments on animals has led to high attrition rates in the development of new therapies. As long as 47% of the NIH funding budget goes to experiments on animals, the U.S. will be stalled in the development of effective treatments for human disease. Forward-thinking scientists are developing and implementing methods for studying and treating diseases and testing products that do not entail the use of animals and are relevant to human health. Researchers have created human cell–derived models, "organs-on-chips," *in silico* (computer) models, and other methodologies that can replicate human physiology, diseases, and drug responses more accurately than experiments on animals do. (See the infographic on page 10.)

Studies have repeatedly shown that these new methodologies are better at modeling human diseases than crude experiments on animals are, yet funding for these tools pales in comparison to funding for poorly translatable animal methods.

NIH and other federal agencies must now take the next step and end the funding of experiments on animals that have failed to provide effective treatments and cures. This will free up immense resources that when reinvested in exciting and innovative non-animal methods, career tracks, and institutes—together with bold policy initiatives—will boost the development of far more promising cures and treatments for humans. This will also alleviate the almost unimaginable suffering of millions of animals and help protect human health.

4. Implement a harm-benefit analysis system for animal studies that includes an ethical perspective and consideration of lifelong harm inflicted on animals.

For the benefit of animal welfare and human health, researchers should focus their considerable talent, time, money, and energy on moving away from archaic animal use—prioritizing areas in which the harm inflicted on animals is so great that no benefit could ever justify the experiment. Examples of such studies would include the following: maternal deprivation experiments (tearing infants away from their mothers); psychology experiments that cause fear, anxiety, or depression; drug, alcohol, and food addiction experiments; and painful experiments during which analgesia is withheld. Until all experiments on animals have ended, a system of analysis for a "risk threshold" or "upper limit," similar to that employed in research on humans, should be implemented. Examples of frameworks by which to conduct harm-benefit analyses of animal experimentation can be found in the reports of the U.K. Animals in Science Committee Harm-Benefit Analysis Sub-Group,[151] the report of the Working Group on the Use of Chimpanzees in NIH-Supported Research,[147] and the research of Pandora Pound.[152]

The harm to animals that is considered should not be restricted to that resulting from specific procedures but should also include the inherent harm caused by life in a laboratory, where animals are denied the opportunity to meet their species-specific needs. Currently, the system does not adequately determine the extent to which animals are suffering in these experiments. Until researchers make this critical assessment, they cannot reasonably measure whether the results are worth the pain and suffering.

5. Educate the scientific community about the benefits of using non-animal approaches, and train scientists to use them.

As the fields of animal-free research and testing continue to expand, increased education and hands-on training will accelerate the transition to these methods. In deploying such initiatives, it is important to simultaneously remove the barriers to adopting new technology and build confidence in it. For example, Innovate UK has recognized that overcoming skepticism about the ability of non-animal methods to model biological processes will help remove a major barrier to the use of these methods. Furthermore, conservatism and inertia

obstructing the move away from animal-based methods can be overcome by encouraging scientists "to think beyond their immediate research areas to how their skills, technology and 'know-how' can be leveraged and exploited to accelerate the development and adoption of"[153] advanced non-animal methods. Such educational initiatives must be adopted and given ample financial support across the whole research sector, including academia, scientific and funding communities, and industry, from future scientists to established professionals.

There is a need for additional education and hands-on training in non-animal methods. Students and early-career scientists must be provided with opportunities to develop the skills necessary to contribute to this research field so that the U.S. can compete with international developments. Because many study programs lack sufficient courses about animal-free methods, supplemental training programs have been developed. For example, the European Commission's Joint Research Centre hosts a summer school on non-animal approaches.[154] Similar programs could be replicated in the U.S. at the federal level. Many online resources by experts in the field also exist, including those offered by PETA Science Consortium International e.V.[155] and the Early-Career Researchers Advancing 21st Century Science program by the Physicians Committee for Responsible Medicine.[156] Thus, information about animal-free research and testing is available and should be a component of all biomedical education.

Established researchers using animal-based methods should also be provided with retraining opportunities and encouraged to forge multidisciplinary collaborations to evolve their skills. These collaborations can help them develop new and innovative ways of asking research questions and finding methods for answering them. For example, the Dutch Transition Programme for Innovation without the use of animals created a series of "helpathons," action-oriented workshops centered around a specific question that encourages researchers to think creatively about non-animal approaches through a community forum.[157]

Awareness among scientists of animal-free methods may be increased through the creation of a national center for animal-free research and testing, tenure tracks and professorships based on non-animal methods, and animal-free research leadership positions to advise professors, staff, and students. Universities and other institutions should also be encouraged to develop a departmental body for the transition to animal-free research that can work and advise across different departments. Such bodies could help organize undergraduate, graduate, and postdoctoral programs that use only non-animal methods as well as workshops, seminars, and summer schools on *in vitro* and *in silico* methods.

Funders also need training to identify the most promising advanced animal-free methods with translational potential in order to develop new funding streams. The same applies to grant reviewers to ensure that non-animal methods are not subjected to animal methods bias (the preference for animal-based research methods or the lack of expertise to adequately evaluate non-animal methods).[158] An analysis of the expertise of members on NIH funding panels for basic, translational, and preclinical neuroscience research revealed that the committees were disproportionately biased toward experiments on animals. This bias was correlated with lower funding rates for non-animal research projects. The researchers wrote:

> The implication of these data is that review bodies without sufficient expertise in non-animal methods may not be providing fair review and consideration to research proposals that propose to use non-animal methods. We expect this research to demonstrate the necessity for systemic and cultural change in the biomedical research community and be used to advocate for policies that raise the bar on ethical and effective research.[159]

As the field of animal-free testing methods continues to expand, the scientific and science policy communities must keep pace with these pivotal developments. Increased education and training initiatives are urgently required to build confidence in reliable and relevant non-animal methods that can best protect human health.

Conclusion

The current waste of resources, time, and animals' lives has a direct and disastrous effect on human health. Experiments on animals are not reliably generating the treatments and cures they were promised to produce. Existing oversight of U.S. biomedical research is failing to ensure that animals aren't being used unnecessarily, that their welfare is protected when they are, or that human-relevant methods are being adequately supported. Research Modernization Now provides a roadmap for revitalizing the U.S. biomedical research enterprise. Until this plan is implemented, the research funded by U.S. taxpayers will fail to provide the basic and applied research needed to protect human health.

Detailed information on 23 areas of research and the astonishing failure of animal studies to lead to effective treatments for humans is included in the appendices.

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GLOSSARY

3Rs	Replacement, reduction, and refinement
AD	Alzheimer's disease
AIDS	Acquired immunodeficiency syndrome
ALS	Amyotrophic lateral sclerosis
AWA	Animal Welfare Act
CAGR	Compound annual growth rate
CAR	Chimeric antigen receptor
CDC	Centers for Disease Control and Prevention
CVD	Cardiovascular disease
EViR	Ensuring Value in Research funders' forum
FDA	U.S. Food and Drug Administration
GI	Gastrointestinal
HD	Huntington's disease
hiPSCs	Human induced pluripotent stem cells
HIV	Human immunodeficiency virus
HREA	Health Research Extension Act of 1985
IACUC	Institutional Animal Care and Use Committee
IBD	Irritable bowel disease
IBS	Irritable bowel syndrome
NAGMSC	National Advisory General Medical Sciences Council
NAMs	Non-animal methods; new approach methodologies
NIH	National Institutes of Health
NIGMS	National Institute of General Medical Sciences
NHP	Nonhuman primate
OIG	Office of the Inspector General
OPTN	Organ Procurement and Transplantation Network
PD	Parkinson's disease
PETA	People for the Ethical Treatment of Animals
R&D	Research & development
SCI	Spinal cord injury
SIV	Simian immunodeficiency virus
SUD	Substance use disorder
TBI	Traumatic brain injury
UNOS	United Network for Organ Sharing
USDA	U.S. Department of Agriculture

APPENDICES

Please find in the following pages further details on opportunities to end the use of animals in the following areas of biomedical research. The appendices feature several examples of the implementation of non-animal methods. However, they do not represent an exhaustive account of the scientific literature or developments worldwide.

CONTENTS

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Cancer

Although improvements in screening programs have significantly advanced early cancer detection and reduced mortality rates,[1,2] cancer remains the second leading cause of death in the U.S., with officials estimating over 600,000 Americans deaths from cancer in 2024.[3] Decreased incidence of cancers over the past two decades has been partially attributed to specific lifestyle changes, such as reduced smoking, increased physical activity, and maintenance of stable body weight.[4,5] Though biomedical research has made some strides in understanding carcinogenesis, clinical trials have failed to translate from the laboratory to the clinic effectively. Even after significant investment in research for cancer therapies, the success rate for oncology drugs is lower than 10%.[6]

A recent meta-analysis showed that cancer experiments on animals have smaller effect sizes and are less likely to replicate than non-animal cancer experiments.[7] Oncologists have noted that "crucial genetic, molecular, immunologic and cellular differences between humans and mice prevent animal models from serving as effective means to seek for a cancer cure."[8] Former director of the National Cancer Institute, Dr. Richard Klausner, stated, "The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades—and it simply didn't work in humans."[9] In addition, the enormous pain and suffering experienced by animals raises ethical and welfare concerns.[10,11]

There are several methods by which rodents—predominantly mice—are used in cancer experimentation. These methods are categorized based on the tumor development mechanism: xenografting, genetic engineering, or, less frequently, spontaneous induction through exposure to carcinogenic agents.[12,13]

To create xenografted animals, immortalized or patient-derived human cancer cells are transplanted either under the skin or into an organ of immunocompromised rodents, who may then be subjected to a range of experiments, such as treatment with a drug candidate or a substance of interest. Although xenografting is the most common approach to generate tumors in rodents, an analysis of 1,110 mouse xenograft tumor models concluded that these models face fundamental challenges that hinder their ability to predict therapy outcomes in humans.[14] Transplantation of human cells alters the genetic landscape of mice in ways that are unlikely to happen in humans, and these changes alter responses to drug treatment.

Genetically modified (transgenic) mice are created by inserting or deleting human genes into a mouse's DNA to induce the expression of oncogenes or inactivate tumor-suppressing genes, respectively. Since these modifications happen randomly, researchers cannot control gene expression, and off-target alterations are common.[15] Transgenic mouse cancer models fail to mimic the sporadic nature of tumor development, resulting in unexpected outcomes that would not be present in human patients. Moreover, these models are time-consuming and costly since they require many animals to obtain the desired and stable genotype, and the "surplus animals" are euthanized.[10]

In August 2021, the European Commission's Joint Research Centre published a report on immuno-oncology. It highlighted promising human-based, non-animal methods for developing new therapies, studying cancer biology and immunomodulation, identifying specific molecular biomarkers, and more.[16] Some examples of these human-relevant models for cancer research include three-dimensional platforms, such as bioprinted tumors using patient samples,[17–20] organs–on-a-chip models for precision medicine using different cancer cell lines,[21–25] and patient-derived organoids.[26–28] In addition, cancer genomic datasets[29–33] and machine learning tools[34–37] are available to improve diagnosis and predict responses to therapies in real-time.

Scientists using non-animal methods for cancer research face a smaller translational hurdle since they can use patients' own cancer cells and because these human-relevant methods are grounded in human, not rodent, biology.[38] These new tools and approaches will advance cancer research, produce human-relevant results, and accelerate the field toward precision medicine, but only if funding for them is increased and allocated away from cancer experiments on animals.

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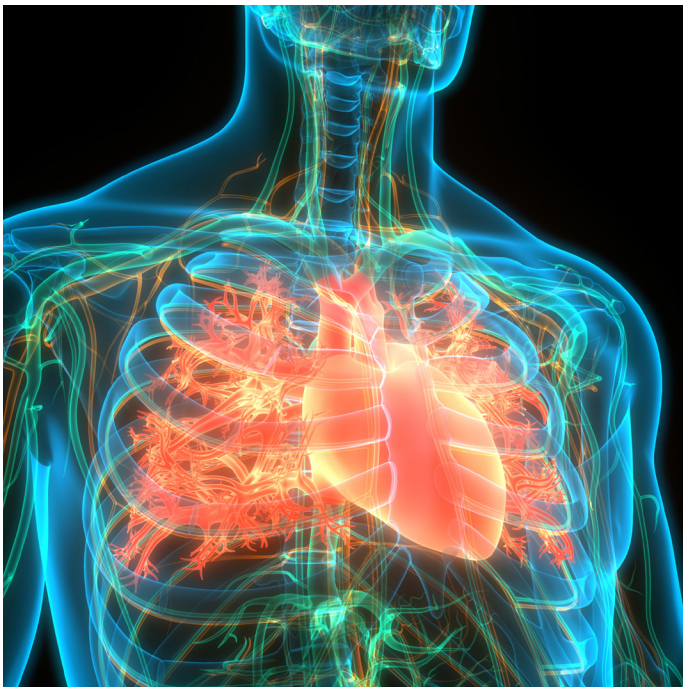

Cardiovascular Disease

Cardiovascular diseases (CVD) are the number one cause of death in the U.S. and worldwide, claiming approximately 17.9 million individuals every year, with mortality rates expected to continue to rise.[1] Despite the availability of therapies for treating CVD, the failure rate of new drugs for CVD treatment was about 75% as of 2022, primarily due to the limitations of animal models in drug discovery and testing.[2] A review of 121 studies using animals for human CVD research found that 79% failed to be replicated in human trials.[3]

Experimenters use a variety of animal species, from frogs to rats to cows, in an effort to study human CVD. Yet, the etiology and pathology of CVD in these animals often differ significantly from those of humans.[2,4] Most species have

distinct cardiovascular functional and structural parameters, including resting heart rate, action potentials, protein isoforms, contraction, and force-frequency response.[5–7] They also exhibit species-specific genetic mechanisms that affect their susceptibility to CVD and responses to drugs intended for human treatment.[4,8,9] For example, rodents are resistant to atherosclerosis,[10] a key component of CVD. Coronary artery disease, which leads to atherosclerosis, rarely occurs in animals and is difficult to induce, often requiring surgical or pharmaceutical interventions that are not relevant to the human context.[11]

Additionally, behavioral and environmental risk factors, such as diet, physical inactivity, smoking, and air pollution[1] are complex and not reliably reproducible in animals. These factors contribute to the limited relevance and poor clinical translation of CVD experiments on animals. A recent study's authors noted that "profound understanding of disease progression is limited. The lack of biologically relevant and robust preclinical disease models that truly grasp the molecular underpinnings of cardiac disease and its pathophysiology attributes to this stagnation."[12]

Human-relevant *in vitro* and *in silico* methods are more suitable for cardiovascular research, as they reflect human biology better than animal models. Researchers have generated heart organoids using human induced pluripotent stem cells (hiPSCs) that mimic the cellular composition of the heart and self-organize to create chamber-like structures. These heart organoids can recapitulate functional impairments seen in conditions such as cardiac fibrosis and hypertrophic cardiomyopathy.[13–15] A team of engineers in Taiwan has developed a microfluidic chip system to rapidly quantify four CVD biomarkers aimed at improving early intervention.[16] A recent study demonstrated that heart-on-a-chip technology can be used to model cardiac arrhythmias.[12,17] Additionally, machine learning techniques, in combination with patient data, can create models to predict CVD risk, enabling earlier identification of diseases and more effective treatment outcomes.[18–20] Scientists and clinicians have collaborated to develop an algorithm that predicts 10-year disease progression in hypertrophic cardiomyopathy using clinical data.[21] Finally, *in silico* modeling and simulation can be employed to assess the mechanistic understanding of cardiac pathophysiology.[22] These methods are valuable platforms for studying the human heart, identifying and screening drugs for CVD treatment, and application in regenerative and personalized medicine.

Considering that "[t]here is no ideal animal model available for cardiac research,"[6] CVD research must evolve toward modern methods that rely on human cells and patient-derived data. These new experimental models are more cost-effective and better recapitulate human physiology.[12] Non-animal research methods provide more accurate biological insights into cardiac function, enhancing the translation of preclinical findings into human benefits compared to animal models.[23–25]

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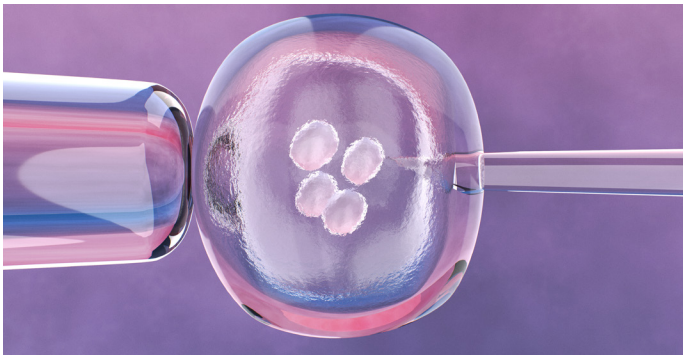

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Cell Therapy

Adoptive cellular therapy (cell therapy) involves transplanting human cells to repair or replace damaged tissue. It uses various cell types, such as hematopoietic stem cells, mesenchymal stem cells, and immune cells, harvested from patients themselves (autologous) or donors (allogeneic), to treat a range of conditions.[1,2] Cell therapy has been explored for treating blood-related diseases, solid cancers, and diabetes, as well as for applications in regenerative medicine.[1,3–6]

Cell therapy research is often conducted using animals, primarily genetically engineered mice, and faces significant limitations. Experiments on animals typically use young, healthy animals who do not reflect the complex etiology of human diseases that are often influenced by age and other co-morbidities. Additionally, experiments on animals lack the long-term analysis and follow-up needed to assess efficacy in humans, posing a challenge in predicting outcomes.[7] Additionally, immune and physiological differences between species lead to poor translation of results.

Though some cell therapies have been approved for use, these treatments still face challenges, especially for solid cancers, due to tumor heterogeneity and the scarcity of tumor-specific antigens.[8] Engineered chimeric antigen receptor (CAR) T-cell therapies have shown antitumor activity in experiments on mice but failed to work in human clinical trials for ovarian and metastatic renal cell cancers.[9,10] One cause for these failures is that preclinical studies are often conducted using immunocompromised mice with xenografted human tumors, whereas, in clinical practice, these cells operate within a patient's complex and intact immune system.[11] For more on the problems with xenograft mouse models, see the section on Cancer (p.23).

Because animals do not accurately replicate human biology, they may also fail to reliably predict adverse effects of cell therapies, such as cytokine release syndrome and immune effector cell-associated neurotoxicity. Additionally, variability in cell preparation and characterization during preclinical experiments on animals can result in inconsistent and irreproducible findings.[7] Non-animal preclinical methods for studying and testing cell therapies include *in vitro* models, such as organoids and those using hiPSCs. These models replicate human physiology more accurately, allowing for high-throughput drug screening, identification of human-specific mechanisms, and personalized medicine approaches.[12,13] Maulana et al. introduced a patient-derived breast cancer-on-chip model that enables real-time monitoring of CAR T-cell activity and prevention of cytokine release syndrome with an FDA-approved drug.[14] In another study, researchers using patient samples and clinical data identified CD22 as a potential marker for CAR T-cell therapy development in triple-negative breast cancer, which, despite ongoing cell therapy clinical trials, is currently without targeted therapy.[15,16]

Interest in adoptive cell therapies has surged in the past decade and continues to expand to various cancers and diseases. Recent advances in engineering technologies, human *in vitro* models, and combination therapies are enhancing cell therapy development, providing robust platforms for studying disease mechanisms and therapeutic interventions, and yielding more applicable results.

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Diabetes

For many years, experimenters have intentionally created symptoms of diabetes mellitus (diabetes) in rodents, pigs, dogs, and primates.[1] However, these models face considerable limitations, such as differing disease progression compared to humans. Experimenters attempt to replicate diabetes pathology in animals by inducing symptoms through poor diet and chemical or viral destruction of pancreatic beta cells, but these efforts consistently fail due to significant limitations, such as tissue necrosis and species-specific differences in susceptibility to diabetes.[2,3]

Beyond technical limitations, using animals to study diabetes also poses significant biological limitations regarding anatomy, physiology, and exposure.[4,5] For instance, mice rely principally on the liver for glucose homeostasis, while, for humans, skeletal muscle is also critical in glucose metabolism.[6] In addition, some transgenic mice models of type 2 diabetes are based on leptin deficiency, which is not an essential contributor to diabetes in humans.[7] Because of a low rate of spontaneous diabetes (only 2%), the LEW-iddm rat model for type 1 diabetes requires compensatory alterations in the rat's immune cell repertoire in order to develop a diabetic profile but still does not entirely mimic the human condition.[1,8] In the same way, the human pancreas differs from that of rodents in its tissue architecture, cellular composition, and insulin regulation.[9]

Many drugs developed to treat diabetes have adverse side effects, such as edema, cardiac risk, and weight gain, with some drugs being withdrawn from the market.[10,11] Recent findings reveal significant human singularities in pathology, environment, ethnicity, and treatment responses among type 2 diabetes patients,[12–15] highlighting why the heterogeneity of diabetes cannot be replicated using animals. As a result, experiments on animals have not led to transferable findings for humans.[2,5]

As interspecies differences continue to emerge, there is a clear need for human-based methodologies to advance diabetes research to bridge the gap between pre-clinical and clinical trials and discover new ways to prevent disease progression.[2,4,16]

Numerous organ-on-a-chip models for studying insulin resistance and glomerular function for diabetic nephropathy have been developed to uncover biological mechanisms and provide insights into effective therapeutic opportunities. For example, a glomerulus-on-a-chip using human cells allows researchers to assess high glucose-induced kidney damage.[17] In another study, the glomerulus-on-a-chip mimicked the human *in vivo* kidney response to injury in patients exposed to serum and toxic agents, providing a valuable tool to investigate renal damage.[18] Another 3D model used cadaveric pancreas islets for continuous insulin measurements, offering a scalable model to study diabetes and perform drug screening.[19] *In silico* modeling using diabetic patient data is also showing promising results.[20–22] For example, a model designed to quantify endogenous and inhaled plasma insulin after a meal was tested in a clinical study with healthy patients and can help estimate the bioavailability and pharmacokinetics of inhaled insulin in humans.[23]

Many other human 3D models are being explored for drug development and considered for future organ transplantation in diabetic patients,[2,24] including stem cells[5,25] and pancreatic islets.[26–28] These innovative approaches, based on patient-derived cells, have the potential to accelerate research on diabetes as they permit investigation into the underlying biological mechanisms of human diabetes-induced complications, which are impossible to replicate in experiments on animals.[3,29]

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Inflammation and Immunology

The use of animals in research to study human inflammation and immunology encompasses a great deal of basic and disease-related research. We will briefly discuss three main areas: the use of animals for HIV/AIDS research, the use of mice for human immune research, and the use of animals to study human sepsis.

HIV/AIDS

The failure to translate experiments on animals into effective human applications of human immunodeficiency virus (HIV) vaccines was acknowledged more than 20 years ago when, in 1995, NIH instituted a moratorium on breeding chimpanzees, the species most commonly used in HIV and acquired immunodeficiency syndrome (AIDS) research at the time, recognizing that studies using this species had failed to produce clinically useful data. Following this, experimenters began to use other nonhuman primate species, notably macaques.

Because humans are the only primates who contract HIV and develop AIDS, experimenters instead infect monkeys with simian immunodeficiency virus (SIV), a virus unique to African primates. The genetic homology between HIV and SIV is only 55%, and SIV is less genetically diverse than HIV.[1,2] Owing to differences in surface proteins and other molecular markers, antibodies that neutralize SIV have no effect on HIV and vice versa.[3] Importantly, the dose of SIV administered to a nonhuman primate in an experiment is often much higher than the typical amount of HIV-1 to which a human is exposed during sexual transmission.[4] Sometimes, experimenters use an engineered SIV/HIV concoction. AIDS researcher Mark Girard has stressed, "One should realize that we still do not know how the SIV or SHIV model compares to HIV infection in humans. Extrapolating from vaccine protection results in nonhuman primate studies to efficacy in man may be misleading."[5]

Even those who use nonhuman primates as models of HIV have admitted that they "do not allow direct testing of HIV vaccines" and that "because of the complexity and limitations of the NHP [nonhuman primate] models, it remains difficult to extrapolate data from these models to inform the development of HIV vaccines."[6] Experimenters have developed dozens of vaccines candidates using monkeys, but all have failed in human trials.[7] At least two clinical trials resulted in an increased likelihood of HIV infection in humans.[8,9] After one of the failed vaccine trials, Anthony Fauci, former director of the U.S. National Institute of Allergy and Infectious Diseases, acknowledged that the original positive results of a macaque study "might be a fluke."[10]

Scientists have noted that "[e]xisting animal models predicting clinical translations are simplistic, highly reductionist and, therefore, not fit for purpose."[11] They reported that clinical attrition data "focusses the attention back on to early target selection/lead generation, but it also questions the suitability of current animal models concerning congruency with and extrapolation of findings for human hosts."[11]

Because of broad failures in nonhuman primate HIV/AIDS research, some experimenters have shifted their focus to mice—a species even more genetically removed from humans.

The "humanized" mouse model for HIV/AIDS research is a mouse who has been partially repopulated with human immune cells, allowing for the animal to be infected with HIV-1. However, humanized mice are limited in their longevity with the disease and retain parts of their murine immune systems, "complicating immune response interpretations."[3] Not surprisingly, the use of humanized mice has also failed to generate valuable results for clinical HIV/AIDS treatment.

Considering the differences between a laboratory environment and human society, experiments on animals will never capture the complexity of this human disease. Mice and rats used in experiments are kept in conditions where the primary pathogens are those found in their feces, and cofactors that may be present in human patients, such as other microbial infections, are absent. This lack of cofactors significantly alters the acquisition and progression of the virus.[1] Nonhuman primates used in HIV research, on the other hand, have been found to harbor confounding infections like Valley fever, which compromises the findings of HIV studies.[12]

Scientists acknowledge that even after costly and unreliable experiments on animals, human data are still needed to determine whether a drug is fit for the clinical setting. Researchers with the U.S. Military HIV Research Program noted that "human clinical trials still appear to be the only reliable way to determine whether an HIV vaccine candidate will have activity or efficacy in humans,"[13] adding to this 2007 comment from the associate editor of *The BMJ*: "When it comes to testing HIV vaccines, only humans will do."[14] Researchers recognize that human *in vitro* models are needed to replicate this human disease and develop treatments.[15]

Recent non-animal HIV research includes interactive molecular dynamics simulations to predict how drug molecules will bind to HIV proteins,[16–19] novel imaging techniques revealing previously unknown aspects of HIV structure that open up the potential for new therapies,[20] and bioinformatics analysis of specimens from individuals with viremia and *in vitro*–infected cells from healthy donors to construct an atlas of HIV-susceptible cell phenotypes.[21] Additionally, single-cell multi-omic analyses of samples from healthy and HIV-infected donors have uncovered differences in T cell populations, protein expression, and glycan expression, which could be instrumental in developing novel immune-targeted therapeutic strategies.[22–24]

Scientists around the world have been studying the immune cells of individuals called "HIV controllers," who can become infected with HIV but can control the spread of the virus without any therapeutic intervention.[25–29] The hope is that immune cells from HIV controllers can be transferred to other HIV-infected patients to help them fight the virus. This promising research is human-specific and requires human-specific testing methods.[30]

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Mouse Immunology

Since the advent of inbred mouse strains in the 1940s and the development of transgenics in the 1980s, mice have been used in alarming numbers for immunology research. Beyond the ethical concerns these numbers raise, most findings generated by these experiments fail to translate to humans and are not replicable.[1,2]

Key physiological and cellular differences between the tissues of mice and humans reveal their inadequacy as human experimental stand-ins and should disqualify the use of mice in experiments.[3,4] Specifically for immunological research, mice have unique dendritic epidermal T cells with sensory functions nonexistent in humans.[5] Similarly, the composition of immune cells in human blood (55-70% neutrophils, 20-40% lymphocytes)[6] is different than that of mice used in experiments (20-30% neutrophils, 70-80% lymphocytes),[7] which affects species-specific immune defense mechanisms.[8,9] Logically, these differences make sense, given that we humans have longer life spans[8] and we "do not live with our heads a half-inch off the ground."[10]

Mice have a unique genetic makeup that contributes to their phenotypic dissimilarities with humans, such as the lack of class II human leukocyte antigen expression on T lymphocytes and differences in the activation of these cells during immune response.[3] These immunological specificities, along with epigenetic modifications unique to mice, hinder the data translation and make comparisons between mice and humans unrealistic and risky.[9,11] For example, a deficiency of CD28 molecules results in severe immune dysfunction in mice, while humans with this deficiency remain healthy.[12] Due, in part, to differences in CD28 expression between species, clinical trials with Fialuridine resulted in organ failure in humans taking only 1/500th of the dose that had been deemed safe in preclinical tests using animals.[13]

A mouse's immunological layout is also altered by the barren, controlled housing conditions in which they are kept in laboratories. Consequently, mice develop a gut microbiome adapted to these conditions,[14] which is distinct from that of wild mice and even more divergent from humans.[15] In a study that analyzed over 1,900 mouse genomes, researchers revealed that humans and mice have only 2% of gut bacteria species in common.[16] The breeding process used to generate specific mouse strains with genetic variations also makes them more susceptible to human pathogens than humans are, adding another point of discrepancy.[11,17] Mice in laboratories fail to represent the genetic variability found among humans or their own species' wild counterparts.[17,18] Despite these many glaring disadvantages, mice continue to be used for immunological research.

Human immunological research is slowly but surely bringing the "human" back into its focus. "Big data" and computational biology – proteomics, metabolomics, and clinical data – integrated with novel 3D models can bridge the gap in translational science and leverage personalized approaches.[19–22] Human samples, such as bone marrow,[23] lymph nodes,[24] tonsils,[22] and liver,[25] are being used to generate patient-derived organoids to address mechanistic and hypothesis-driven immunological studies in different contexts.

A review summarizing the progress of immune-competent human skin disease models recognizes that the failures of experiments on animals to translate into effective treatments for diseases such as fibrosis, psoriasis, cancer, contact allergy,

Mice in laboratories fail to represent the genetic variability found among humans or their own species' wild counterparts.

and autoimmune diseases is due in part, to the immunological nature of these conditions. The authors go on to describe how co-culture, three-dimensional organotype systems, and organ-on-a-chip technology will "enable human models of well-controlled complexity, yielding detailed, reliable data, providing a fitting solution for the drug development process."[26]

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Sepsis

Sepsis is a life-threatening condition caused by the body's response to infection. The most recent global incidence data show that sepsis affected an estimated 48.9 million humans worldwide and resulted in 11 million deaths in 2017.[1] It is a leading cause of death in U.S. hospitals and one of the most expensive conditions to treat.[2,3]

Mice are the animals most commonly used in sepsis research—not because they make good models of human sepsis but because they're cheap, plentiful, small, and docile.[4] The difficulty in reliably translating results from mice to humans is considered a primary cause of the failure of nearly all human trials of sepsis therapies.

In 2013, *Proceedings of the National Academy of Sciences of the United States of America* published a landmark study that took 10 years to complete and involved the collaboration of 39 researchers from institutions across North America, including Stanford University and Harvard Medical School. Dr. Junhee Seok and his colleagues compared data from hundreds of human clinical patients with results from experiments on animals to demonstrate that humans and mice are dissimilar in their genetic responses to severe inflammatory conditions such as sepsis, burns, and trauma.[5]

Former NIH Director Dr. Francis Collins authored an article about these results, lamenting the time and resources spent developing 150 drugs that had successfully treated sepsis in mice but failed in human clinical trials. He called this disaster "a heartbreaking loss of decades of research and billions of dollars."[6] The paper reveals that in humans, many of the same genes are involved in recovery from sepsis, burns, and trauma

but that it was "close to random" which mouse genes might match these profiles. Collins explains it as follows:

> Mice, however, apparently use distinct sets of genes to tackle trauma, burns, and bacterial toxins—when the authors compared the activity of the human sepsis-trauma-burn genes with that of the equivalent mouse genes, there was very little overlap. No wonder drugs designed for the mice failed in humans: they were, in fact, treating different conditions![6]

Even before this landmark study, the criticism of mouse models had been documented in more than 20 peer-reviewed scientific papers. The mice used in sepsis experiments are young, inbred, and of the same age and weight, and they live in primarily germ-free settings. In contrast, it is mostly infant and elderly humans who live in a variety of unsterilized, unpredictable environments who develop sepsis.[7,8] When experimenters induce the condition in mice, the onset of symptoms occurs within hours to days, whereas in humans it takes days to weeks. Mice are not typically provided with the supportive therapy that human patients receive, such as fluids, vasopressors, and ventilators.[9] Unlike humans, mice are rarely given pain relief,[10] another difference that undermines data of already questionable value, as pain affects other physiological processes.

The "gold standard" method of inducing sepsis in mice is through cecal ligation and puncture, a procedure in which experimenters cut open a mouse's abdomen and puncture their intestines with a needle before sewing the animal back up. However, mice's responses to this procedure vary depending on age, sex, strain, laboratory, the size of the needle used, and the size of the incision, which makes results incomparable between laboratories.[11,12] In addition, the procedure causes the formation of an abscess, whose effects may disguise or be disguised by the effects of the sepsis itself.[9] This means that an intervention that appears beneficial for sepsis may only appear beneficial because of its effects on the abscess.

Rats, dogs, cats, pigs, sheep, rabbits, horses, and nonhuman primates, including baboons and macaques, have also been used in sepsis experimentation. None of these species reproduce all the physiologic features of human sepsis. The pulmonary artery pressure responses of pigs and sheep differ from those of humans, so this aspect of sepsis cannot be compared between these species.[13] Furthermore, baboons and mice are less sensitive to a species of bacteria commonly used to induce sepsis in experimental settings.[14] A recent study found that rhesus macaques and baboons differ markedly in their innate immune response to pathogens compared to humans.[15]

A 2019 report from the National Advisory General Medical Sciences Council (NAGMSC) Working Group on Sepsis states, "Despite decades of intensive study of the underlying mechanisms of this condition, no new drug or significantly new diagnostic technology has emerged. Dozens of prospective trials of agents or strategies targeting the inflammatory basis of sepsis have failed."[16] In its report, the NAGMSC Working Group on Sepsis recommended that the National Institute of General Medical Sciences (NIGMS), under NIH, "rebalance" its sepsis research–funding portfolio to "include a more clinical focus."[16] In a "Notice of Information" issued by NIGMS following the NAGMSC report, the institute expressed its intention to support sepsis research that "uses new and emerging approaches, such as clinical informatics, computational analyses, and predictive modeling in patients, and new applications of high-resolution and high-throughput bioanalytical techniques to materials obtained from septic patients" and called the support of "[s]tudies using rodent models of sepsis" a "low priority."[17] More recently, at the 2024 Shock Society Annual Conference, NIGMS announced that they were "unwilling" to fund projects proposing mouse models of human sepsis and encouraged the use of animal-free research methods moving forward.[18] In other words, NIGMS intends to prioritize funding human-relevant sepsis research over sepsis experiments on animals. However, other NIH institutes and funders have yet to follow NIGMS' lead.

In 2015, an expert working group consisting of veterinarians, animal technologists, and scientists issued a report on implementing the 3Rs (the replacement, reduction, and refinement of animal use) in sepsis research.[19] The group identified several methods that could be used instead of animal models, including *in vitro* cell culture models for studying sepsis mechanisms, systems and computation biology for revealing the inflammatory processes occurring during sepsis, three-dimensional cell culture models to explore human disease progression and infectious mechanisms, synthetic human models to recreate disease-related cell types and tissues, and human genomic data to understand how sepsis affects individuals differently and which groups may be more at risk. The authors state that genomic information "will complement or even replace the need for mouse models in disease discovery and drug development."[19]

The following are examples of recent developments in human-relevant sepsis research:

- Scientists in Tokyo used hiPSC-derived liver organoids to model the pathological events of septic-associated liver dysfunction and recovery following infection.[20]
- A team of engineers, doctors, and researchers at Temple University identified an association between neutrophil types and the severity of sepsis using a human lung-on-chip model, which can be used to determine the

appropriate therapeutic intervention based on sepsis severity.[21]

- Researchers in Hefei, China, collaborated with physicians at First Affiliated Hospital to create a six-unit microfluidic device that comprehensively analyzes a sepsis patient's white blood cell activity to monitor disease progression and severity.[22]
- Massachusetts General Hospital scientists and physicians created a microfluidic device to accurately detect a biomarker of sepsis pathophysiology using a drop of blood, aiming to improve disease monitoring.[23]
- Because early detection of sepsis is likely the most critical factor in reducing mortality from this condition,[24] researchers around the globe are exploring various artificial intelligence and machine learning tools to aid in the early prediction and diagnosis of sepsis.[25–33]

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Gastrointestinal Disorders

Gastrointestinal (GI) disorders affect more than a million individuals in the U.S. and account for millions of clinical visits annually, with health expenditures totaling $119.6 billion in 2018.[1] The burden of these diseases is staggering, as they contribute significantly to morbidity, mortality, and healthcare costs, with the prevalence expected to rise.[2] Because of this, tremendous effort has been put into GI disorder drug development, but for many conditions, there has been little success.[3] Treatments are available for GI diseases, but they often entail significant drawbacks, partly because much of the mechanistic knowledge of these diseases has relied on animal models.

Key differences in nonhuman animals render them inappropriate models for studying human GI diseases. The two species most often used in these experiments are rats and pigs.[4] Both have GI tracts that are anatomically dissimilar to those of humans. For example, the jejunum constitutes 90% of the rat's small intestine but only 38% of the human small intestine.[5] Rats lack a sigmoid colon, gallbladder, and cystic ducts, while pig colons are larger than those of humans.[5–7]

Beyond anatomical differences, behavioral disparities impact the relevance of these animal models. Rats typically consume small, frequent meals, whereas humans eat larger, less frequent meals.[8] Pigs, on the other hand, consume more food relative to their body weight than humans do.[4]

Laboratory conditions can further influence the study of GI diseases. In a 2024 study, researchers found that the temperature at which mice are housed within a laboratory can significantly affect their gut motility and microbiota.[9] The source of the animals can also lead to variations in gut microbiomes due to differing environmental factors.[10] Species-specific microbiome differences play another role: Pigs have little Bifidobacterium, a major genus in the human gut.[4] Given the role of gut microbiota in immune response, these differences may significantly impact study outcomes.[11]

Animal models of human GI conditions are criticized for their poor predictive value regarding disease outcomes and clinical efficacy in humans, especially for conditions like irritable bowel syndrome (IBS) and irritable bowel diseases (IBD), the pathogenesis of which remains not fully understood.[12]

IBS is a chronic condition affecting the lower GI tract. Fifteen percent of adults in the U.S. experience IBS symptoms, which include abdominal pain accompanied by diarrhea, constipation, or both.[13] While the exact cause of IBS remains unclear, it is believed to involve a combination of physical and psychological factors, particularly stress and anxiety,[13] which cannot be faithfully simulated in nonhuman models.

Animal models of IBS are typically created by subjecting animals to stress during early development.[14] These models have significant limitations, such as their inability to replicate the constipation or mixed bowel responses of human patients. Additionally, human IBS patients often present with overlapping disorders, such as bladder pain syndrome, chronic pelvic pain, anxiety, and depression—none of which are modeled in experiments on animals. Behavioral changes, such as anxiety or depression, are difficult, if not impossible, to measure in animals (see the appendix on Neuropsychiatric Disorders and Neurodivergence, p. 40). Most experiments use male animals, even though IBS is more commonly diagnosed in females. Additionally, abdominal pain, the primary symptom of IBS, cannot be accurately assessed in animals, as there is no measurable phenotype specific to the visceral pain experienced by humans. These shortcomings make IBS experiments on animals inappropriate for understanding IBS pathophysiology and developing effective treatments.[15]

IBDs, which include ulcerative colitis and Crohn's disease, are chronic inflammatory conditions often affecting the large and small intestines. IBDs impact two to three million people in the U.S.[16,17] IBD patients suffer from rectal bleeding, severe

diarrhea, abdominal pain, fever, and weight loss. The causes of IBDs are believed to involve a combination of genetic, immune, microbial, and environmental factors, although the precise mechanisms are not fully understood.[18]

In IBD research, scientists induce colitis by administering irritating substances or using genetically engineered mice. However, reproducibility remains a significant issue. Different mice strains exhibit varying susceptibilities to chemically induced colitis, and microbiome differences across strains or vendors can also influence the disease development in genetically engineered mice. Given that both genetic and environmental factors contribute to IBD, an animal model that lacks these human-specific characteristics cannot effectively replicate these diseases. For example, genetically engineered mice are often created by mutating a single gene, but human IBDs are polygenic.[19] Furthermore, chemically induced colitis in mice typically results in acute injury over a few days, whereas IBDs in humans develop over years.[20]

A key example of the limitations of animal models is IL-17 inhibition, which effectively treats colitis in mice but has failed in Crohn's disease patients, sometimes even worsening the condition.[21,22] A 2019 review noted that "while there are many *in vivo* models of IBD, none adequately predicts response to therapeutics."[20] The disappointment of IL-17 inhibition in clinical trials illustrates how a treatment that works in animal models can fail in humans. Conversely, some therapeutics that show promise for treating IBDs in patients have failed in mouse models.[23,24]

Given these limitations, it is clear that no animal model can accurately replicate human GI disorders. These conditions are influenced by a complex interplay of environmental, genetic, and microbial factors that cannot be fully captured in artificially induced animal models. Therefore, prioritizing human-relevant research methods, such as organoids, microfluidics, and organ-on-a-chip technologies, is crucial. Recent developments in this area include the following:

- Biological engineers at MIT created a human multi-organ model of ulcerative colitis to study its impact on the gut-liver-immune axis.[25]
- Scientists at the Francis Crick Institute, in collaboration with UCL and Imperial College London, used a multi-omics approach to identify a new biological pathway related to IBDs, finding the gene ETS2, which is linked to higher IBD risk.[26]
- A group of researchers and physicians in Missouri and North Carolina created a neonatal-intestine-on-a-chip to study necrotizing enterocolitis, a deadly GI disease seen in premature infants. They successfully showed that this model can recapitulate disease pathology and plan to use this method for therapeutic testing.[27]
- Physicians and scientists in Boston obtained biopsies, blood, and stool samples from patients at Cincinnati Children's Hospital, Massachusetts General Hospital, Emory University Hospital, and Cedars-Sinai Medical Center to create a longitudinal molecular profile of their microbiomes. Using a multi-omics approach, they were able to identify microbial, biochemical, and host factors involved in IBD-induced dysregulation.[28]
- Researchers and physicians in Houston used patient-derived intestinal organoids to explore the link between telomere dysfunction and IBDs, suggesting that addressing telomeric dysfunction could be a therapeutic strategy.[29]

The anatomical and physiological differences between nonhuman and human GI systems, coupled with the artificial induction of GI diseases in animals, hinder reliable study outcomes. Furthermore, many of these induction methods involve invasive and painful procedures, leaving the animals in distress until they are killed.[14,30–34] Given that animal models of GI diseases do not reliably reflect human pathology and contribute to animal suffering, it is essential to transition toward the numerous non-animal methods using human tissues or consenting patients.

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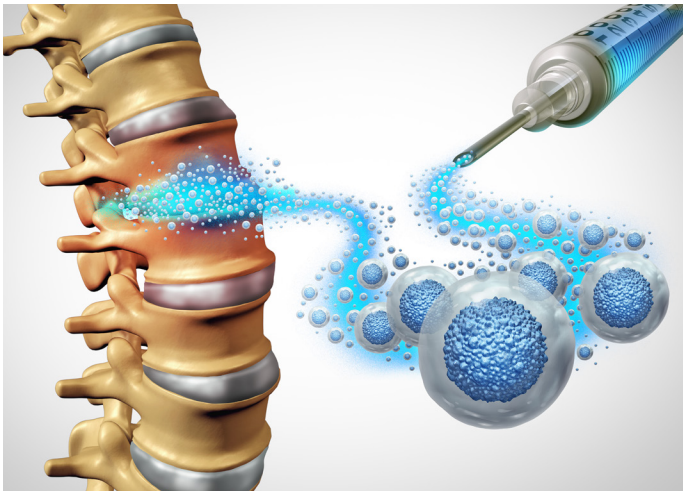

Nerve Regeneration

Many neuroprotective agents have been developed that are successful in treating spinal cord injury (SCI) in animal models, but clinical trials have been disappointing. Neurologist Aysha Akhtar has described three major reasons for this failure: "[D]ifferences in injury type between laboratory-induced SCI and clinical SCI, difficulties in interpreting functional outcome in animals, and inter-species and interstrain differences in pathophysiology of SCI."[1] In a systematic review of the use of animal models to study nerve regeneration in tissue-engineered scaffolds, researchers have said that most "biomaterials used in animal models have not progressed for approval to be tested in clinical trials despite the almost uniform benefit described in the experimental papers."[2] The authors lamented the low quality of described experiments on animals, as necessary detail and rationale had been omitted, making it difficult to compare data.

For example, methylprednisolone, a routinely used treatment for acute SCI, has generated inconsistent results in animal models. A systematic review examining 62 studies of the drug on a wide variety of species, from rodents to monkeys, found that 34% reported beneficial results, 58% reported no effect, and 8% had mixed findings.[3] The results were inconsistent among and within species, even within strains. Furthermore, the variability in results remained even when many of the study design and procedure variables were controlled. The authors pointed out numerous intrinsic differences between, and limitations of, each species/model. They suggested that as a result of these immutable inter- and intra-species differences, no human-relevant animal model can be developed, concluding that the "research emphasis should be on the development and use of validated human-based methods."[3]

Among species, rats are particularly unsuitable for nerve repair or regeneration research. Experts have pointed out three major problems with rat models in this field:

> (1) The majority of nerve regeneration data is now being generated in the rat, which is likely to skew treatment outcomes and lead to inappropriate evaluation of risks and benefits.
> (2) The rat is a particularly poor model for the repair of human critical gap defects due to both its small size and its species-specific neurobiological regenerative profile.
> (3) Translation from rat to human has proven unreliable for nerve regeneration, as for many other applications.[4]

More specifically, the inconsistencies between animal models and the clinical situation are significant[5] and include the following:

(1) healthy animals versus sick patients; (2) short versus long gap lengths (the clinical need for large gap repairs, while 90% of *in vivo* studies are in rats and rabbits where gap lengths are usually ≤3 cm); (3) animal models that almost always employ *mixed sensory-motor* autografts for repairing mixed defects, versus clinical repairs that almost always involve *sensory* autografts (usually sural nerve) for repairing mixed defects; (4) protected anatomical sites in animal models, versus repairs that must often cross articulating joints in humans; and (5) inbred, highly homogeneous animal strains and ages, versus diverse patient populations and ages: It is well recognized that animal models fail to mimic the human condition in terms of the *uniformity* of animal subjects used.[4]

To induce a spinal cord injury in animal models, experimenters use physical force to damage the spinal cord. There are many different methods, such as contusion, which involves displacing the spinal cord by dropping a weight, or distraction, which applies a traction force to stretch the spinal cord. Regardless of the method used, achieving consistency and reproducibility is challenging due to the inability to replicate the same spinal cord injury every time they perform the procedure. For example, in contusion-induced injuries, variability can arise from the rod bouncing after it hits the spinal cord, potentially causing multiple impacts.[6]

In addition to consistency issues, many of these models do not accurately reflect the mechanisms of SCI in humans. A compression model created using forceps does not replicate the acute impact seen in most human SCI, and the devices used for the distraction model often induce injury too slowly to emulate human injury. Chemically induced SCI is employed to study secondary injuries associated with SCI, usually involving the injection or application of a toxic chemical to the area of interest. However, challenges with chemically induced SCI include ensuring accurate delivery of the chemical to the correct region of the spine.[6]

Biomedical engineers have noted that researchers "are incapable of truly mimicking human neural injuries in animal models because of the extensive anatomical, functional, molecular, immunological, and pathological differences between humans and frequently studied animals."[7] Human-relevant methods can bypass these limitations and should be the focus.

Human-relevant methods for studying nerve injury and regeneration have been reviewed by a number of research groups and include human organoids, microfluidics, engineered human tissue scaffold molds, bioprinting, and other *in vitro* uses of human cells. *Ex vivo* models, such as those using three-dimensional engineered scaffolds, bioreactors, neurospheres, and organoids, allow for more controlled studies on specific parameters than animal experiments.[7] Bioprinting can use bioinks containing human cells and materials to construct heterogeneous tissue models in a single step and with remarkable consistency,[8] an aspect of nerve regeneration research that has been notably lacking in animal models.[2]

Engineers and researchers at the University of Pittsburgh Medical Center and Carnegie Mellon University have emulated mild and moderate traumatic brain injury (TBI) using human cerebral organoids. Their study identified important genetic repercussions of TBI on the brain that can be used to diagnose the condition and create personalized treatments for patients.[9] Neuroscientists have engineered human spinal cord organoids that display functional neuronal activity and hold promise for investigating SCI therapies.[10]

Microfluidic devices are "adaptable for modeling a wide range of injuries" and provide advantages over traditional *in vivo* and *in vitro* experiments by "allowing researchers to (1) examine the effect of injury on specific neural components, (2) fluidically isolate neuronal regions to examine specific effects on subcellular components, and (3) reproducibly create a variety of injuries to model TBI and SCI."[11] For example, brain-on-chip platforms offer a promising avenue for personalized medicine, as a patient's own cells can be used to create a custom device to investigate treatment options.[12] Axons-on-a-chip can model diffuse axonal injury, allowing researchers to track the intracellular changes immediately following injury and offering a platform for screening treatments.[13] These systems offer advantages in precision, scalability, and cost-effectiveness when compared to traditional cell culture or experiments on animals and are currently on the market and available for neural regenerative medicine research.[7]

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Neurodegenerative Disease

There is sufficient literature documenting the failings of various animal models of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). While a lengthy appendix could be written for each disease, many of the same limitations of animal models prohibit translation across these conditions, and they will be discussed briefly as a whole.

All these diseases are human-specific, meaning they do not occur naturally in other animals. No animal model has been developed that recapitulates all aspects of a particular neurodegenerative disease.[1] For AD research, the clinical failure rate for new drugs was last estimated to be 99.6%,[2,3] and recent monoclonal drugs approved for AD have been controversial due to adverse effects and questionable efficacy.[4,5]

A bioinformatics analysis comparing the transcriptional signatures of AD, PD, HD, and ALS with mouse models of these diseases produced the following findings:

> [M]ost available mouse models of neurodegenerative disease fail to recapitulate the salient transcriptional alterations of human neurodegeneration and … even the best available models show significant and reproducible differences compared to human neurodegeneration. Although the reasons for the poor transcriptional performance of mouse models varied, the unifying theme was the failure of mouse models to exhibit the variety and severity of diverse defects observed in human neurodegeneration.[6]

These molecular discrepancies underscore the artificial methods used to create such models. Physical and chemical lesioning or systemic administration of toxins are commonly used. These are acute stressors, not long-term degenerative processes, and as such, they initiate events in animal models that are not present in human patients. The acute and immediate nature of disease models, such as the 6-OHDA and MPTP animal models of PD and the 3-NP animal model of HD, fail to capture the progressive nature of the disorders they aim to mimic. In addition, scientists often use young animals to "model" diseases associated with aging,[7] further reducing their relevance. For example, "[c]ommonly used AD mouse models, like the 5xFAD, display amyloid deposits starting at 2–4 months of age…this early accumulation can be translated to Aβ deposits occurring in 4–8 year-old humans, a scenario not found even in the most aggressive cases"[8] of AD.

Genetically modified mouse models exhibit inconsistent pathological and behavioral phenotypes, partly due to variations in transgenes used, inconsistencies in transgene insertion and expression, and differences in mouse background strains.[9] As of 2024, 210 transgenic rodent AD models have been developed.[8] In a review on the relevance and translational validity of these mouse models, researchers described their shortcomings:

> Some transgenic models can present a very aggressive disease phenotype compared to the human form of the disease…while others fail to demonstrate aspects of neuronal loss and dysfunction… Of additional concern is the fact that mouse models often fail to show a substantive neuronal loss even in the presence of amyloid deposits and generate amyloid peptides different from those found in human brain… In some instances, the failures encountered with animal transgene models reflect the fact that they are based on intrinsically flawed hypotheses and the constructs used to interrogate these; in other instances, they reflect a lack of diligence on the part of investigators to ensure best practices in the husbandry and use of these models. Despite their limitations, these flawed models become widely utilized, with their relevance being overstated because of the lack of any viable alternatives, while only lip-service is paid to their validity as they become de rigor and self-perpetuating—driving the field down a blind alley.[3]

Fundamental genetic differences further hinder translation. For example, "knock-in models require the presence of multiple APP [amyloid precursor protein] mutations not found in humans," murine tau differs structurally from human tau, and "key amino acid substitutions make murine Aβ less prone to aggregation when compared to its human counterpart."[8] These differences make animal models of neurodegenerative disease misleading and waste precious time: A genetic target for AD research previously identified as upregulated in mouse models was, unsurprisingly, not found to be upregulated in humans in a recent postmortem study.[10] For PD, nonhuman primate

studies do not "constitute a valid scientific modality for the complete understanding of PD and for the development of future neuromodulation therapeutic strategies."[11]

As in much of biomedical research, animals suffer greatly when used to mimic neurodegenerative diseases. In an analysis of published research on animal models of HD, 51 studies referenced experiments "in which animals were expected to develop motor deficits so severe that they would have difficulty eating and drinking normally."[12] However, only three out of 51 reported making adaptations to the animals' housing to facilitate food and water intake. The authors of this analysis concluded that experimenters are not adhering to the 3Rs principles and compromising not only animal welfare but also the relevance of their studies to HD.[12]

As animal studies fall short, scientists and policymakers are increasingly recognizing the need for human-relevant research strategies. Following a review of AD research, an interdisciplinary panel recommended reallocating funding away from animal studies and toward more promising techniques, such as patient-derived hiPSC models, "omic" technology (genomics, proteomics, etc.), *in silico* models, neuroimaging, and epidemiological studies.[13]

The following are highlights in recent cutting-edge, human-relevant neurodegenerative disease research.

- At Brigham and Women's Hospital, researchers differentiated hiPSCs into neurons that quickly develop protein inclusions mimicking those found in the brains of individuals who died with inclusionopathies. Using this method, the team created more than 60 human cellular models that other laboratories can use to study human neurodegenerative diseases.[14]
- A team of scientists at Washington University in St. Louis used cells from patients with AD to develop a relevant, 3D human cellular model for late-onset AD (which accounts for 95% of cases). This model allows for the study of age-associated neurodegeneration.[15] Another team conducted a proteomic study on the cerebral spinal fluid of patients with AD to identify biomarkers that can be detected decades before symptoms arise.[16]
- Researchers at the Barcelona Institute of Science and Technology developed an organ-on-a-chip to evaluate the brain permeability of nanotherapeutics and facilitate personalized research and therapy for AD.[17]
- At the Vienna BioCenter, scientists created an *in vitro* model of the human dopaminergic system with ventral midbrain–striatum–cortex assembloids to improve the study of PD cell therapies.[18]
- Researchers at the University of Luxembourg used human organoids and assembloids—including those developed with patients' own cells—to understand the early stages of PD and factors influencing susceptibility.[19,20]
- Boston-based Emulate, Inc. engineered a human brain-on-a-chip that represents areas affected by PD, reproduced features of the disease, and can be used to identify and test new therapeutic targets.[21]
- Scientists in Germany used human brain organoids to identify a gene implicated in HD that may damage the brain before symptoms arise and could serve as a focus for drug development. Restoring the function of this gene reversed the HD phenotype.[22]
- University of Central Florida scientists used cells from patients with ALS to develop a disease-specific neuromuscular junction-on-a-chip and tested the effects of a compound on clinically relevant functional measures of ALS.[23]
- In another patient-specific study, a team at Utrecht University used human brain organoids to improve the understanding of synaptic changes in ALS patients before the onset of symptoms.[24]

For decades, experimenters have tormented monkeys, mice, dogs, and other animals in an attempt to model these devastating diseases. However, since other animals don't develop these human neurodegenerative diseases naturally, experimenters have manipulated their genomes to force discrete symptoms. The results, after decades of tests, include more than 100 failed drugs, an untold number of animal deaths, and the continued suffering of humans living with these conditions. For these patients, a shift to human-relevant methods is long overdue.

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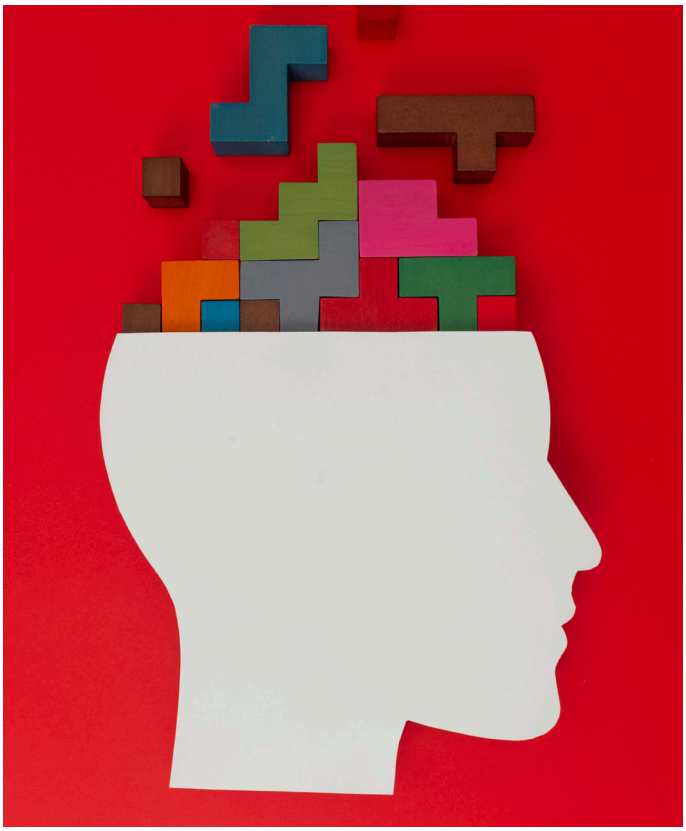

Neuropsychiatric Disorders and Neurodivergence

Like many other animal models of human disease, animal models used in an attempt to study human neuropsychiatric disorders and neurodivergence lack critical aspects of model validity. These deficiencies include (1) construct validity, meaning that the mechanistic underpinnings creating the observed symptoms in animals are different from those that lead to the disorder in humans; (2) face validity, meaning that animals cannot "recapitulate important anatomical, biochemical, neuropathological, or behavioural features of a human disease;"[1] and (3) predictive validity, meaning that results from experiments on animals fail to translate into similar results in humans reliably.

No single animal model replicates all aspects of a human neuropsychiatric condition, and features of human behaviors that represent hallmarks of these disorders cannot be accurately produced or assessed adequately in animals.

For example, human depressive disorders are characterized in part by feelings of sadness, hopelessness, and despair. In an effort to measure "despair" in rodents, the most commonly used behavioral test is the forced swim test, in which an experimenter places a rat or mouse in a container of water with no way to escape or rest. Experimenters falsely interpret the amount of time the animal spends swimming or struggling to escape as a measure of the animal's lack of despair. This misguided notion originated from the observation that swimming and struggling time could be extended by giving the animal some types of human antidepressants (even though this assumption ignores the many false positives and false negatives that the test produces). As has been widely discussed in the scientific literature, an animal's behavior in the forced swim test may represent an evolutionary adaptation to the stressful situation and should not be used to try to determine their mood.[2] The results can be influenced by an animal's strain and many experimental variances, including water depth, container dimensions, and temperature.[3–6]

A PETA neuroscientist and collaborators have published papers discrediting the use of the forced swim test as a valid method for screening antidepressant drugs. Their findings revealed that the use of this test by the world's top 15 pharmaceutical companies did not produce any drugs currently approved for treating depression in humans.[7] They also highlighted actionable steps that regulatory authorities could take to eliminate the use of the forced swim test (and the similar tail suspension test) in the pharmaceutical industry.[8]

Other animal behavioral tests—such as the sucrose preference test (for anhedonia),[9–11] the open field test

and elevated mazes (for anxiety),[12,13] marble burying (for compulsion),[14] chronic unpredictable stress (to induce psychopathologies)[15]—have similar flaws. These concerns have led to the awareness that "some of these assays must be discontinued, and placed in the past; while we seek improved, innovative strategies for outcome measures."[16]

A series of citation analyses demonstrated that researchers studying major depressive disorder in humans rarely cite results from experiments on rats or monkeys, two of the most commonly used species in this field. Instead, they more frequently relied on research results using human cells and human biological data.[17–19] A similar failure of animal studies to contribute to clinical knowledge has been noted in bipolar depression research,[20] and animal studies have been cited as the primary source of attrition (failure of drugs) in neurobehavioral clinical trials.[21] Despite these warnings, thousands of researchers have continued to use flawed assays like the forced swim test to draw erroneous conclusions about an animal's mood[22] or the potential effects of compounds on human depressive disorders.[8]

Significant physiological differences between humans and other animals contribute to the low translation rate. For example, the gene encoding tyrosine hydroxylase, the enzyme involved in dopamine formation, is regulated differently in humans than in mice.[23] Misregulation of tyrosine hydroxylase has been implicated in several psychiatric illnesses, such as bipolar disorder and schizophrenia. In a 2019 study published in *Nature*, 64 researchers analyzed the brains of mice and humans and found substantial species differences in types of brain cells and how they produce proteins critical to neuropsychiatric function. The authors noted numerous "failures in the use of [the] mouse for preclinical studies" because of "so many [species] differences in the cellular patterning of genes."[24] Rodents and humans also diverge in other critical areas for neuropsychiatric research, including the diversity, organization, and volume of neuronal cell types; relevant neural circuitry; volume of neurotransmitters available in specific cell types; and neurotransmitter receptor availability and kinetics.[25]

Beyond the lack of applicability, animal neuropsychiatric models cause immense suffering. To induce "depression," experimenters subject animals to uncontrollable pain through electric shocks or chronic stressors, such as restraining them for extended periods, starving them or denying them water, tilting their cages, forcing them to live in wet bedding, shaking them, or disrupting their circadian rhythms. Animals are often made to live in complete isolation from other members of their species, bullied and physically assaulted by other animals, deprived of parental care, and subjected to genetic or surgical manipulations in an effort to induce a depressed-like or altered mental state. In this field in particular, "animals are likely undergoing experimental procedures that do not provide the epistemic benefit we are sacrificing them for."[26]

Funds should be redirected from the use of animals toward relevant, human-based experimental methods, including the following.

- Human brain organoids: Advanced, 3D *in vitro* cultures of human brain cells that replicate the cellular organization and signaling of human brain tissue. These have been used to study mood disorders, psychoses, and neurodivergence.[25,27–29] Organoids can be combined to form self-organizing assembloids that mimic complex interactions between different parts of the brain,[28,30] such as the cortico-striatal-thalamic-cortical circuit and thalamocortical assembloids recently developed by a team at Stanford University to study human neurodevelopmental conditions like autism, Tourette syndrome, and schizophrenia.[31,32] Researchers at the University of California San Diego and the University of Massachusetts at Amherst are developing disease-specific brain organoids using cells from patients with genetic mutations linked to neuropsychiatric disorders for therapeutic applications.[33–35]
- Omics research: This is being applied to better understand the underpinnings of human neuropsychiatric conditions. The PsychENCODE Consortium, a collaboration of multidisciplinary teams, uses state-of-the-art methods to create large datasets from human postmortem brain samples.[36] Some teams are analyzing existing data to characterize gene variants related to these disorders.[37]
- Brain imaging: Techniques including magnetoencephalography, high-density electroencephalography, magnetic resonance spectroscopy, transport-based morphometry, and functional magnetic resonance imaging—often combined with machine learning and genomics—are being used to study human psychiatric conditions and neurodivergence directly in individuals with lived experience.[38–42]
- Longitudinal studies: Tracking individuals over extended periods provides insights into the effects of environmental stimuli, medical history, and life events on the incidence and progression of neurodevelopmental conditions.[43,44]

- *In silico* clinical trials: Virtual patient models have been used to evaluate the potential of drugs for conditions like attention-deficit/hyperactivity disorder and schizophrenia.[45,46]

Given the psychological distress inflicted on animals and the inapplicability of the results to humans, the use of animals in human neuropsychiatric and neurodivergence experiments should end. Resources must be diverted to human biology-based research like the examples listed above.

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Pandemic Preparedness

To say that the COVID-19 pandemic changed life as we know it is an understatement. However, a silver lining may be its potential to lead to an entirely new era of biomedical research and vaccine development. To accelerate COVID-19 vaccine development, both the FDA and NIH greenlighted landmark human clinical vaccine trials without requiring extensive tests on animals beforehand. Instead, the human and animal testing proceeded in parallel,[1] a change that PETA urged the FDA to extend to all new drugs in development (e-mail communication, May 5, 2020,

https://www.peta.org/wp-content/uploads/2020/05/2020.05.05-FDA-Commissioner COVID-19-letter-FINAL.pdf).

Although time constraint was an obvious factor in this decision, it is essential to note that many species do not respond to SARS-CoV-2 infection in the same way humans do. When *The New York Times* asked about seemingly promising experimental results in rhesus macaques, Dr. Malcolm Martin, a virologist at NIH, "cautioned that monkeys are different from humans in important ways."[2] The interviewer noted that "[t]he unvaccinated monkeys in [the vaccine experiment] didn't develop any of the severe symptoms that some people get following a coronavirus infection" and quoted Martin as saying, "It looks like they got a cold."[2] Even genetically engineered mice, who are made susceptible to the disease, only show mild symptoms. "Humanized" mice (those who are engineered to express human immune factors) do not solve this problem, as "many human factors cross-react with murine cells, which may lead to unexpected phenotypic changes."[3]

Amid the COVID-19 pandemic and outbreaks of other infectious diseases like H5N1, it has become increasingly clear that infectious disease research and pandemic preparedness should be prioritized. Human-relevant research can lead the way.

Many scientists are using innovative non-animal methods to study existing pathogens and those with pandemic potential. These methods include human lung and intestinal organoids, three-dimensional reconstructed human respiratory tissue models, human oral tissue samples from healthy volunteers, advanced computer simulation and supercomputers, human genetic analyses, human challenge studies, human-derived antibodies, and human organs–on-chips modeling human lungs, mouths, eyes, noses, and intestines. Complex *in vitro* human models, such as organoids and organs-on-chips, are expected to be particularly valuable for infectious disease research and developing vaccines and antiviral drugs.[3–7] Here are a few recent examples:

- Human lung and brain organoids are being used to study SARS-CoV-2 infection mechanisms, test potential therapies, and investigate the virus' effects on the brains of healthy individuals and those with comorbidities.[8–12]
- Researchers in Japan created patient-specific livers-on-chips to explore SARS-CoV-2-induced liver dysfunction and to evaluate drugs to treat it.[13]
- Using cells isolated from human lung tissue, researchers engineered human lung organoids to study H5N1 virus replication, host cell survival, and lung immune responses to different viral strains.[14]
- According to a recent review, "microphysiological systems and organoids are already used in the pharmaceutical R&D pipeline because they are prefigured to overcome the translational gap between model systems and clinical studies."[15] The authors explain that complex, human-derived systems like organoids and microphysiological systems will be essential for research on filovirus and bornavirus infection in humans, for which "animal models cannot capture the respective pathogenesis and disease in full."[15]
- Respiratory syncytial virus is being studied using *ex vivo* samples from patients to determine why some have a more severe reaction to the infection[16] and with human airway organoids to develop and test antibody therapies.[17]
- Individuals with post-infectious disease syndromes like long-COVID and myalgic encephalomyelitis/chronic fatigue syndrome have been studied using brain imaging; analyses of skin biopsies, blood, and cerebrospinal fluid; monitoring of diet, sleep, and cardiac measures; and more to phenotype these conditions, understand how they occur, and guide potential therapies.[18]
- *In silico* tools have been used in drug repurposing studies to identify existing therapies that could treat COVID-19.[19]

In addition to adopting non-animal methods to study and develop treatments, it's even more critical to take measures to prevent the spread of emerging pathogens. Ending the importation of wild species into laboratories for experimentation is a key step. Long-tailed and rhesus macaques are the most commonly used nonhuman primates in experimentation, the most commonly traded primate species, and the species that harbors the highest volume of potential zoonotic disease.[20,21] While primate suppliers and buyers claim to support efforts to reduce the use of wild-caught macaques in research, investigations have revealed that international suppliers have falsely labeled wild-caught macaques as captive-bred and sold them to laboratories.[22] This practice risks disease spillover and compromises the results of experiments conducted on these animals, whose health histories are unknown.

Macaques are often captured and imported from regions endemic for melioidosis, a life-threatening illness caused by *Burkholderia pseudomallei*. Though the Centers for Disease

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Control and Prevention (CDC) requires that monkeys imported from these regions undergo a mandatory quarantine, *Burkholderia pseudomallei* can remain dormant for long periods, and animals have been released into laboratories while still infected.[23] Macaques have also been imported while harboring tuberculosis-causing mycobacteria.[24,25] According to the CDC, "In the United States, there is no centralized system for reporting TB in NHP that are not in CDC-mandated quarantine (minimum of 31 days after importation). Therefore, it is unknown how common TB is in NHP in the United States."[26]

Ending the global trade of monkeys for experimentation would eliminate a major risk factor in zoonotic disease spillover, reduce the dissemination of unreliable data collected from animals of unknown origin, and stimulate the move toward human-relevant research methods. This is a critical step in protecting public health and preventing the next pandemic.

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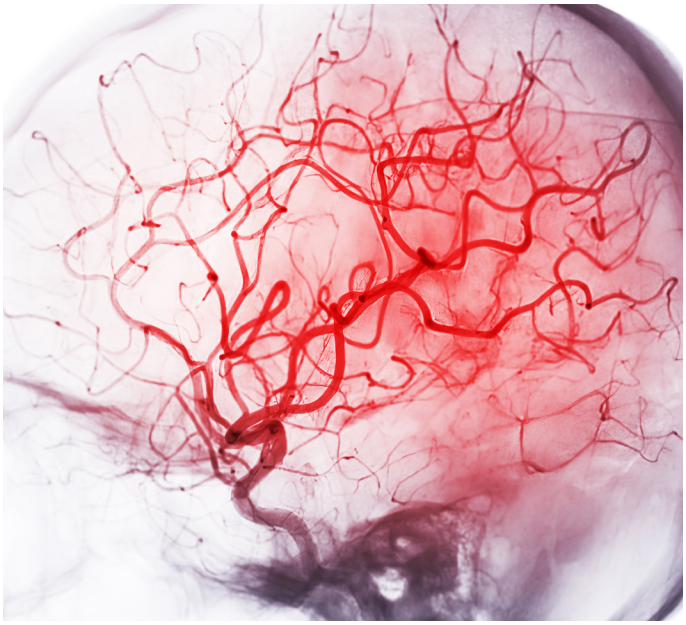

Stroke

A stroke is a serious condition affecting the brain's blood vessels. Strokes are the fifth leading cause of death and a major contributor to disability in the U.S.[1] They occur when blood flow to the brain is interrupted, either by a clot (ischemic stroke) or a burst blood vessel (hemorrhagic stroke), resulting in damage and the death of brain cells due to lack of oxygen. After an ischemic stroke, recanalization (restoration of blood flow to the brain) is the only immediate treatment available in the acute phase.[2] Procedural intervention by endovascular therapy is the standard treatment for ischemic stroke when possible but is only effective in approximately 25% of cases.[3]

Despite over a thousand neuroprotective drugs showing promise in animal models, none have translated into effective human therapies for strokes.[4] Our understanding of the biological processes driving human stroke recovery remains limited,[2] and developing accurate models of the central nervous system is challenging due to the complexity of the human brain. Current animal models, which primarily use rats, lack essential human characteristics, differ in stroke recovery compared to humans, and raise ethical concerns.[4,5] For example, ischemic stroke typically occurs in elderly patients with comorbidities, whereas experiments are predominantly carried out in young, healthy animals who often exhibit spontaneous recovery.[6]

Significant differences in brain composition—such as white matter making up 60% of the human brain but only 10% of the mouse brain[7]—and variations in blood-brain barrier physiology[8,9] play crucial roles in stroke pathology. Additionally, differences in clot composition, neuronal function, and inflammatory processes among species further contribute to the poor translatability of animal models in stroke research.[10–12]

A 2010 analysis of 16 systematic reviews (including 525 different studies) on human stroke interventions tested in animal models revealed that the efficacy of these experiments on animals was overstated by approximately one-third due to publication bias (the propensity of researchers and journals to publish results showing positive outcomes and omit studies with negative or null data).[13] The authors noted that "participants in clinical trials may be put at unnecessary risk if efficacy in animals has been overstated."[13]

In silico modeling shows potential to replace animal experimentation in stroke research. Projects like INSIST (IN-Silico trials for treatment of acute Ischemic STroke) use virtual patients to simulate stroke treatments, replicating clinical characteristics, such as clot properties, vessel geometries, and patient medical records.[14] These models, which allow for virtual drug testing and the detailed study of thrombosis and brain perfusion in humans, "have the potential to lead to a more effective human clinical trial design, reduce animal testing, lower development costs, and shorten time to market for new medical products."[14] A groundbreaking *in silico* trial published in 2021 predicted aneurysm treatment responses using 164 virtual patients with 82 unique anatomies.[15] This model outperformed experiments on animals, identifying new risk factors for treatment failure in days instead of decades. Virtual modeling can also assist patient-tailored clinical decisions for strokes and other neurological conditions. However, regulatory reform for *in silico* trials is urgently needed to advance the field.[16]

Researchers are also exploring new technologies and cell-based methods to enhance recovery by replacing damaged brain tissue with stem cells.[5] Recently, stem cell therapy using patients' bone marrow or allogeneic umbilical cord blood has shown improved neurological outcomes in clinical trials.[2,17–19] In preclinical research, the isolation of human stem cells and hiPSCs has advanced the development of scalable human models in neurobiology.[4,20] Innovative 3D systems, like organs-on-chips and brain organoids,[21,22] may mimic complex cell interactions and *in vivo* physiology better than animal models, while 3D printing[23] enables the creation of detailed nervous system models for preclinical drug testing and clinical applications.

Accurately modeling ischemic responses requires understanding cellular interactions that influence blood-brain barrier permeability, cerebral edema, and neurovascular responses under pathological conditions. Because these interactions ultimately affect stroke outcomes, it is essential to create realistic models. Combining hiPSCs with advanced cell culture technologies has allowed replicating specific human nervous system features. For example, Kook and colleagues developed a vascularized model by coculturing vascular and cerebral spheroids generated by hiPSCs.[24] In

another brain organoid study, Xu et al. observed morphological and synaptic changes in microglia cells after viral exposure.[25] Additionally, microfluidic models enable the use of patient cells and real-time monitoring of human brain dynamics, such as blood-brain barrier permeability and shear stress, which are not feasible in experiments using other species. *Ex vivo* brain slices are another valuable method for studying human brain tissue, as they preserve *in vivo* properties, spatial organization, and complex networks of various cell types.[26]

In recent years, *in vitro* systems for studying strokes and the human nervous system have advanced significantly, becoming sought-after tools for studying human brain function and improving stroke treatment strategies.[9] Now that these tools are available, researchers must adopt them and funders must support their uptake.

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Substance Use Disorder

Fundamental aspects of nonhuman animals make them inappropriate for the study of substance use disorders (SUD). First, the use of and dependence on drugs in humans is a vastly complex experience, one that has been impossible to mimic using animals in a laboratory setting.[1] It has been argued that attempts to model SUD in nonhuman animals, especially rodents, are "overambitious" and that the "'validity' of such models is often limited to superficial similarities, referred to as 'face validity' that reflect quite different underlying phenomena and biological processes from the clinical situation."[2]

> [A]nimal models cannot capture many key aspects of human brain disorders that may be caused by an SUD, which often involve the interplay of genetic, developmental, and environmental factors...In addition, studying the brain in live animals involves invasive techniques that can affect the health and behavior of the subjects, potentially confounding results... Consequently, it's hard to translate research outcomes from animal models into effective clinical treatments for SUDs due to the inter-species differences in neuro systems between human and animal models.[3]

Several diagnostic criteria for SUD are impossible to model in animals since they require an individual to self-report. These include "(i) subjective craving, (ii) taking the substance in larger amounts or for longer than intended and (iii) wanting to cease or reduce substance use but being unable to."[4]

Second, the pharmacokinetic actions of drugs differ among species. For example, "the rate of metabolism of MDMA and its major metabolites is slower in humans than rats or monkeys, potentially allowing endogenous neuroprotective mechanisms to function in a species-specific manner."[5] Pharmacokinetic differences between humans and "model"

animals likely explain why the neurotoxicity seen in rodents after MDMA administration has not been observed in the clinical setting.[5] Since MDMA is being explored not only because of its use as a recreational drug but also for its potential therapeutic use, accurate knowledge regarding its safety in humans is paramount.

Third, serious flaws in the experimental design of substance use experiments on animals skew the interpretation of their results. Unlike humans, whose experience with SUD is primarily shaped by individual choice to consume an addictive substance—often over other rewarding alternatives—animals in laboratories are typically not given this option. When they are, the majority will choose an alternative reward, such as sugar, over the drug.[6] This holds for primates as well as mice and rats. Even among animals with a history of heavy drug use, only about 10% continue to self-administer the drug when presented with another rewarding choice.[7] In a review on the "validation crisis" in animal models of drug addiction, it has been said that the lack of choice offered to animals in these experiments raises "serious doubt" about "the interpretation of drug use in experimental animals."[6]

The nonhuman animal has been called a "most reluctant collaborator" in studying alcohol use disorder and exhibits a "determined sobriety," which the experimenter must fight against to overcome "their consistent failure to replicate the volitional consumption of ethanol to the point of physical dependency."[7] National Institute of Mental Health researchers reason that "it is difficult to argue that [drug self-administration by rodents] truly models compulsion, when the alternative to self-administration is solitude in a shoebox cage."[8]

Despite the epidemic of drug dependence and overdose in the U.S. and the prevalence of SUD research conducted on animals, there are only limited treatment options available for individuals addicted to opioids, nicotine, and alcohol and no approved treatments for marijuana, stimulant, or polysubstance users.[9] Leadership at the National Institute on Drug Abuse has noted that pharmaceutical companies show little interest in investing in treatments for SUD due to the stigma and complexities of the disease.[9,10] While data from animal studies were once hailed as promising in certain drug classes and relapse prevention, most have either failed to be effective in human trials or were not tolerated well by humans.[4,10] Some researchers argue that "these failures illustrate the inability of animal models to capture the complex nature of addiction and its treatment" and that "findings from animal models of addiction have generated a misleading picture of the nature of addictive behavior in humans."[4]

Non-invasive human and human biology-based research methods are now providing answers to questions that the use of other animals is fundamentally unable to solve.

Rutgers University Robert Wood Johnson Medical School researchers authored a review article describing how hiPSCs can provide a "unique opportunity to model neuropsychiatric disorders like [alcohol use disorders] in a manner that … maintains fidelity with complex human genetic contexts. Patient-specific neuronal cells derived from [induced pluripotent stem] cells can then be used for drug discovery and precision medicine."[11]

Forward-thinking scientists around the world are carrying out human-relevant, non-animal research on SUD:

- Researchers are using postmortem human samples to model changes in the brain and brain cells induced by SUD. For example, at the University of Texas Health Science Center and Baylor College of Medicine, researchers engineered a novel hiPSC model of neural progenitor cells and neurons from postmortem human skin cells, directly comparing the new models to brain tissue from the same donors to model opioid-induced brain changes.[12] Heidelberg University scientists conducted an epigenomics study on postmortem brain tissue from individuals with cocaine use disorder to understand how the disorder alters synaptic signaling and neuroplasticity.[13]
- A recent University of Pennsylvania study used 3D genomic datasets to sequence more than 50 diverse human cell types to identify genetic and cell targets that underlie SUD.[14]
- A multi-omics study conducted by a team of researchers across the U.S. as part of the Million Veteran Program used systems biology to reveal key genetic targets for new drugs to treat opioid use disorder.[15]
- University of Central Florida researchers have developed a hiPSC model for studying opioid use disorder and opioid-induced respiratory depression to combat the opioid overdose crisis.[16]
- At North Carolina State University, scientists co-cultured human neurons to form assembloids used to understand single-cell human molecular responses to cocaine and morphine.[17] Human-derived assembloids and organoids "show unique potential in recapitulating the response of a developing human brain to substances"[3] and will also be helpful in studying *in utero* exposure to drugs of abuse.
- Research on better ways to treat human pain is crucial for reducing opioid use disorder incidence and relapse. Researchers at Queen's

University Belfast used *in vitro* and *in vivo* human neuronal models to study a molecular basis for the modulation of nociception in human peripheral nerves.[18] Biotechnology companies like AxoSim, NETRI, and others have developed human neuronal *in vitro* models that can be used for human pain research.

In addition, the funds currently supporting ineffective and wasteful SUD studies in animals could be redirected to support effective drug prevention, rehabilitation, and mental health programs.

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Women's Health

While women face significant health risks independent of sex or gender, many health outcomes are closely linked to the reproductive cycle and can vary throughout a woman's life.[1] Historically underfunded and understudied, women's health issues such as infertility, endometriosis, adenomyosis, and menopausal symptoms require urgent attention.[2]

A significant obstacle to using other species to study women's health is the anatomy of the reproductive tract. For example, mice have a closed reproductive system with tightly coiled oviducts opening into the bursal space. In contrast, the human reproductive system is open to the peritoneal cavity. This allows endometrial cells, shed during menstruation, to flow backward (retrograde menstruation) into the peritoneal cavity. This retrograde menstruation is linked to the development and symptoms of endometriosis. "[F]rom a morphogenetic perspective Müllerian duct development differs considerably in mice and humans,"[3] resulting in the development of fallopian tubes in humans and the Müllerian vagina in mice.

Endometriosis and adenomyosis are closely related gynecological conditions that cause pelvic pain, miscarriage, and infertility and affect around 10% of women.[4–6] Despite being first described centuries ago, significant gaps in the diagnosis and treatment of these conditions are due to the incomplete understanding of underlying mechanisms[5] that have been repeatedly investigated using failed animal models.

Human endometriotic lesions, which are not yet fully characterized, vary significantly in location, size, color, and depth.[7] Additionally, endometriotic lesions have distinct etiologies that are impossible to fully replicate in animal

models, requiring invasive methods such as surgical engraftment, intraperitoneal injection, or direct tissue injection into the endometrium.[7,8] These artificial approaches often result in cellular contamination with non-uterine tissue and local inflammation in animals.[9] Transgenic *de novo* mouse models rarely succeed in replicating endometriosis due to the lethal phenotypes often associated with knocking out essential genes.[8] In addition, the long latency period required for endometriosis to develop—something unachievable with short-lived species like mice—underscores the fundamental limitations of animal models.

The process of menopause and its symptoms vary widely among women, primarily influenced by factors such as the remaining number of eggs in the ovaries, lifestyle, diet, and ethnicity.[10–12] During the menopause transition, fluctuations in estradiol levels in the perimenopausal phase can cause specific, complex, and protracted physiological, behavioral, and neurological changes[10] that experiments on animals fundamentally fail to replicate.

The estrous cycle of other primates and rodents differs considerably from that of humans.[13] The vast majority of nonhuman animals do not experience menopause, and their fertility patterns differ significantly from those of humans. Fertility decline can occur in mice as early as 8 months,[14] or about one-sixth of their potential lifespan. The menstrual cycle of other primates and rodents differs in length, hormone fluctuation, and the ways in which these hormones regulate the hypothalamic-pituitary-gonadal axis compared to humans.[13,15,16]

Given the many biological challenges described above, researchers attempt to replicate menopause and uterine lesions in animals using unnatural methods. Ovariectomy—the surgical removal of ovaries—is considered the "gold standard" for creating these symptoms in animals, but the procedure is an invasive and clinically irrelevant method for inducing menopause. Menopause is a gradual transition—not an abrupt event—and animals do not experience the same symptoms as humans, such as brain fog or the continued release of androgens by the ovaries.[17] Other animal models created by the chemical induction of premature ovarian failure are prone to experimental confounds, such as discrepancies related to the dose and duration of the treatment, the development of unrelated neurological issues,[18] and the inability to model responses to drugs that may reverse premature ovarian failure in humans.[19]

Most experiments use young animals, such as young marmosets, whose physiology drastically differs from the aging humans they aim to mimic. Genetic patterns in the brains of these animals don't align with those of humans in the menopausal transition, meaning cognitive decline caused by estrogen fluctuation and loss during this period cannot be replicated.[20]

To design more effective interventions, it is essential to deepen the understanding of human-specific biological mechanisms that affect women's health and fund the tools necessary for this critical yet often overlooked research.

Collective efforts for phenotypic characterization and biobanking of human endometrial lesions,[21,22] combined with machine learning tools that analyze patient data and wearable devices to identify potential risk factors, can produce data that has been historically difficult to replicate using simpler *in vitro* models. In one study, researchers developed a unified predictive model for the diagnosis of endometriosis using a dataset of over 5,000 women.[23] The model analyzed more than 1,000 variables, including lifestyle, genetic variants, and medical history and identified year of birth and irritable bowel syndrome as significant risk factors.

The limitations of experiments on animals and traditional *in vitro* models have driven the development of advanced microfluidics platforms that accurately recapitulate the human reproductive system.[24] These include the human placenta-on-a-chip, which allows for the study of maternal-fetal interface and pregnancy-related conditions,[25–27] and standardized hiPSC protocols.[28] Another vascularized multicellular model effectively mimics the hormonal fluctuations of the human menstrual cycle,[29] enabling the study of endometrial permeability to contraceptives and serving as a proof-of-concept for studying human embryo implantation, which is impossible to replicate using animal models. Ultrasonographic data has been used to build a 3D bioprinted endometrium for diagnosing congenital uterine anomalies.[30] Recently, the Human Endometrial Cell Atlas was published as a new reference for studying endometrial transcriptomics and guiding the development of human *in vitro* systems.[31]

Shifting resources away from inaccurate animal models and toward improvement in patient care would also profoundly affect outcomes. A recent study highlighted that misinterpreted symptoms are a major contributor to delayed endometriosis diagnoses.[32] To tackle this issue, the authors proposed a comprehensive approach that includes educating physicians, offering specialized courses for medical students, and integrating other healthcare professionals into the diagnostic and care processes.

The human menstrual cycle and endometrium are dynamic and unique to every individual, highlighting the need to prioritize personalized approaches using patient-derived models. Non-animal methods can revolutionize women's health research, offering more accurate models for disease study, drug testing, and precision medicine.

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Xenotransplantation

As the demand for organs grows, the once-experimental idea of using animals for transplants has evolved into a controversial push to breed pigs exclusively for organ harvesting, a practice known as xenotransplantation. There are multiple ways to improve our current system to increase access to viable human organs without xenotransplantation.

According to the United Network for Organ Sharing (UNOS), as of October 2024, over 104,000 people in the U.S. are waiting for organ transplants.[1] Despite this monumental and urgent need, the current system for managing, harvesting, and transporting human organs is highly inefficient. Human organs remain the most compatible and effective option for transplantation, yet inefficiencies in the system lead to the waste of many viable organs. Rather than resorting to genetically engineering, breeding, and killing pigs for organ harvesting, the focus should be on refining the Organ Procurement and Transplantation Network (OPTN), the current U.S. human organ donation system. Creating a separate xenotransplantation network would demand substantial government oversight and funding, adding complexity and potential inefficiency to an already challenging system. Instead, the most responsible and effective solution is to strengthen the current human organ donation process, ensuring that patients receive the best possible transplant options.

Until recently, UNOS was the sole organization managing the OPTN in the U.S., but it has faced decades of criticism for poor management. A 2022 Senate Committee on Finance investigation revealed that organs procured by UNOS were often lost, damaged, delayed, or never collected.[2] A 2022 report by the National Academies of Sciences, Engineering, and Medicine concluded that the U.S. organ transplant system is inefficient, inequitable, and inconsistent and that it needs significant improvement.[3] Human organ transplantation is a critical and, by nature, scarce lifesaving resource. Yet one in five donor kidneys and one in ten donor livers were procured but never transplanted, primarily due to the systemic problems described above.[4]

Moreover, the current system often wastes already available organs. A study of kidney transplants from 2000 to 2015 found that in nearly 8,000 cases, one kidney was used while the donor's other kidney was discarded, often due to minor differences from ideal kidney organ donation criteria.[5] These discarded kidneys would likely function well, especially

compared to long-term dialysis.[6] According to Dr. Dalvin Roth, a Stanford professor and Nobel Prize recipient for his work on kidney exchange programs, transplant centers are pressured to reject kidneys because they are penalized for unsuccessful transplants.[6] However, transplant centers are not penalized for rejecting kidneys.[6] This system perpetuates the organ shortage because *rejected* kidneys may not meet an unrealistic threshold; considering the significant morbidity and mortality of long-term dialysis, transplants offer far greater benefits to patients.[6] Reforming these criteria could significantly increase the number of available kidneys among other organs.

In response, President Joe Biden signed the bipartisan *Securing the U.S. Organ Procurement and Transplantation Network Act* in 2023 to modernize the national transplant system.[7] This legislation aims to ensure that patients receive high-quality human organs,[7] in contrast to animal organs, which harbor risks of rejection and zoonotic infections and raise ethical concerns. In August 2024, the Health Resources and Services Administration announced that the OPTN Board of Directors, which governs national organ allocation policy, would be separately incorporated and independent from UNOS.[8] This is a critical step toward improving efficiency, but additional efforts to expand and improve the OPTN are needed, as human organs remain the best option for transplant patients.

Xenotransplantation introduces additional risks, including transmitting pathogens from animals to humans, a phenomenon known as xenozoonosis. The FDA has recognized this as a significant risk, particularly for transplant patients who are inherently and medically immunosuppressed.[9] These infections could potentially spread to close contacts and the broader community, raising an ethical dilemma by pitting the duty to protect public health against the need to provide organ transplants for patients with end-stage organ failure.[10] Despite genetically engineering animals, raising them in pathogen-free facilities, and undergoing pathogen screening, viruses such as porcine cytomegalovirus or porcine roseolovirus have been reported even after pre-transplant screening.[10] In May 2022, a pig heart transplant recipient died two months after his operation.[11] The autopsy revealed that the pig's heart carried undetected porcine cytomegalovirus and may have contributed to an unforeseen and untimely death in an immunocompromised individual.[11] As of July 2024, all xenotransplant recipients had died,[12] which may highlight the practice's futility but likely also reflects the fact that only high-risk patients have been selected to receive this dangerous, experimental treatment. The risks of xenotransplantation are high compared to human organ transplants, which, when managed efficiently, remain the safest and most effective solution.

Rather than rely on xenotransplantation to solve the organ shortage, the U.S. should make systematic changes to increase the availability of human organs.
For example, experts suggest adopting a "presumed consent" policy, recommended by a 2019 University of Michigan study.[13] In this system, organ donation is the "default" unless individuals opt out, a practice that has already increased donation rates in other countries.[13] Furthermore, the U.S. can implement approaches similar to those of European countries that prioritize broad access to human organs and maximize the efficiency of their organ donation and transplantation systems.[14] Their success is driven by government commitment, an opt-out donation process, fostering a culture of trust and confidence in the system, and establishing dedicated institutions at multiple levels.[14] In addition, proper hospital reimbursement ensures that financial barriers will not impede participation.[14] These measures expand access to human organs and improve the efficiency of the transplantation system. By committing to improving the current U.S. organ donation system, policymakers could increase access to lifesaving human organs without resorting to the ethically fraught, risky, and unnecessary practice of xenotransplantation.

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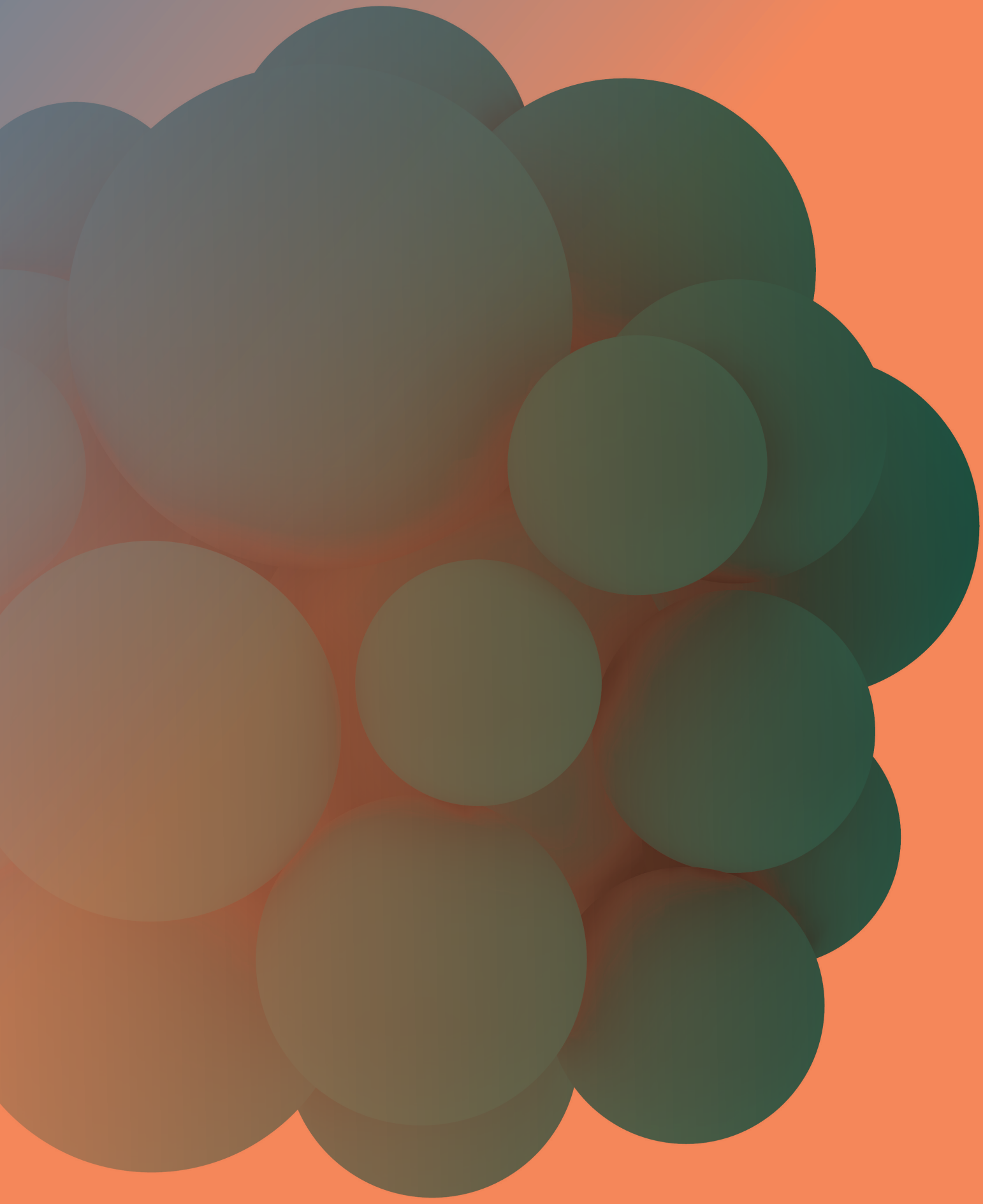

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