New Translational Medicine

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New Paths Forward in Translational Medicine: Innovative Biological Models

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Introduction

Translational research lies at the heart of drug development. "Translational" (or "bench-to-bedside") research is the process whereby basic science discoveries are harnessed to develop new drugs, devices, and therapeutic approaches for use in human patients.[1] A classic success story in translational research was the development of insulin therapy for diabetes, which began in 1869 with the discovery of the pancreatic islets of Langerhans, continued with Nobel Prize-winning experiments on the digestive physiology of dogs in the 1920s, and culminated in the large-scale commercial production of genetically engineered insulin in 1982.[2,3] In a more recent example, the 1995 discovery that spinal muscular atrophy (SMA) is caused by mutations in the Survival Motor Neuron 1 (*SMN1*) gene led directly to the development of the first therapy for SMA, an antisense drug that was approved by the FDA in December 2016.[4,5]

Lately, there has been a sense of crisis in the drug discovery field. Despite huge advances over the last 30 years in biomedical technology and basic science insights into disease mechanisms, there has been an increasingly high failure rate of new candidate drugs developed during the same period.[6] Currently, it takes longer than a decade and USD 2.6 billion on average to develop a new drug from target discovery to market entry, and only one out of ten drug candidates entering clinical trials receives market approval.[7] The 90% of drug candidates of that fail in clinical trials have been found to do so because of low efficacy and safety issues.[8] Failure rates are highest for cancer, mental health disorders, cardiovascular disease, and neurological disease, four of the leading causes of morbidity and mortality worldwide.[9]

This situation has prompted extensive reappraisals of current approaches to translational research.[10–17] Major issues that have been identified as

likely contributing to failure of new drug candidates in clinical trials include:

- Suboptimal trial design, poor choices of patient populations (for example, studying patients whose disease has already progressed too far for successful treatment), lack of validated disease and target engagement biomarkers, failure of drugs to engage their intended targets at the doses used, and insufficiently sensitive outcome measures.
- Lack of rigor in preclinical animal studies, including inadequate sample sizes, poor study design, inappropriate statistical methods and the failure to seek replication of positive results or report negative results.
- Poor predictive value of many currently used preclinical *in vitro* and *in vivo* model systems.

Of these, perhaps the most pervasive worry on the preclinical side concerns the inherent predictive validity of commonly used animal models of disease. Drug discovery relies heavily on genetically engineered animal models of disease, especially mice. The cancer field also makes heavy use of mouse xenograft models: immunodeficient mice into which patient-derived tumors or tumor cell lines have been transplanted. However, it is has become increasingly clear that many mouse models fail to accurately recapitulate the human disease and/or to predict the efficacy and clinical side effects of candidate drugs.

Fortunately, there is a seismic shift happening in the field of translational research, as emerging technologies offer new possibilities for creating more accurate, informative, less expensive, and higher-throughput biological models for drug discovery. Genome editing techniques like TALENS and CRISPR have revolutionized the precision, scale, and speed with which we can generate new disease models, in large animals as well as rodents.[18–20] Burgeoning databases of human "omics" data are enabling the reverse translation of clinical findings to inform preclinical studies, generate new animal models, and test the validity of existing models. Furthermore, developments in the field of bioengineering are spawning new *in vitro* systems for studying disease biology and the effects of novel drugs on human cells.

Building better animal models of disease

In theory, the ideal animal model of disease would have the following features (reviewed in references[21, 22]):

1. Replicate the human disease phenotype (at all levels from the molecular to the behavioral);
2. Share underlying biological mechanisms with the human disease;
3. Have predictive validity with respect to drug efficacy and safety in humans.

In practice, these criteria are rarely, if ever, met. For example, the Alzforum website now lists 127 genetically engineered mouse models of Alzheimer's disease (AD), and not one of them has yet been shown to *completely* fulfill any of these criteria.[23–25] Key pathological features of AD in humans include not only amyloid plaques but also tau tangles and neuronal cell death. Most AD model mice do develop amyloid deposits, but most don't develop tau tangles or show neuronal cell death. In addition, it's still unclear how well any of these mouse models reproduce the underlying mechanisms of AD, especially for sporadic AD. And, to date, none of the many drug candidates developed in mouse models of AD has proven effective in halting or even slowing disease progression in human clinical trials.

Issues that likely contribute to the imperfect accuracy of mouse models of disease include:

- Major differences in the basic biology of rodents and humans.
- Lack of homology of molecular targets: e.g., mice and humans express different isoforms of beta-amyloid.[26]
- Lack of homology in molecular pathways: transcription factors bind to overlapping but different sets of genes in mouse vs. humans, and in some cases transcriptome changes in mouse models of a particular disease barely resemble those seen in humans.[25, 27–30]
- Lack of genetic diversity in inbred mice versus humans.
- Comorbid conditions associated with age-related diseases in humans that are not reproduced in mouse models.
- Environmental risk factors that contribute to most common human diseases but not reproduced in mouse models of those diseases.

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Despite these limitations, mouse models nonetheless have been invaluable for establishing causative roles of specific genes and gene variants in disease, for understanding biological pathways of disease, and identifying new drug targets. They have also been critical in the development of the 10% of drug candidates that *do* make it through clinical trials. To give just a few examples:

- Despite the fact that mouse models of rheumatoid arthritis (RA) don't perfectly model human RA, these models were pivotal in the development of anti-tumor necrosis factor (anti-TNF; a translational success that helped launch the biopharmaceutical industry);[31]
- The recent discovery of the first therapy for SMA would likely have been impossible without *SMN2* transgenic mice;[4,5]
- The development of most successful anti-cancer drugs has relied on mouse xenograft models.[32]

It is also possible that many cases of "failure to translate" are due to poor design and interpretation of preclinical studies rather than to inadequacies in the animal models studied.[13] Nonetheless, given the expense and ethical issues of working with vertebrate animals, we should not only improve the design of preclinical studies, but also develop the best models we can. Environmental as well as genetic risk factors contribute to most for the specific purpose exploring gene/environment interactions.[33–35] In reverse translational approaches (see below), researchers can use human clinical data to inform the creation of animal models that more accurately mimic human disease states and compare disease phenotypes in currently existing animal models to those of human patients. Meanwhile, new genome editing technologies like CRISPR are enabling the production of more accurate genetically engineered animal models.[18–20]

Reverse translation

In "reverse translational" (or "bedside-to-bench") research, data from human subjects is used to develop new hypotheses for testing in the laboratory and to develop new animal models and therapeutics. Although reverse translation is a recently coined term, this kind of research has been done for centuries. For example, Edward Jenner's 1796 discovery of the first smallpox vaccine was based on the observation that milkmaids who had previously caught cowpox developed resistance to smallpox, and the vaccine's success helped lay the foundation for modern immunology.[36]

Over the past few years, molecular profiling of human patients has yielded vast quantities of "omics" data that can be harnessed for reverse translational research. For example, genome-wide association studies (GWAS) and next-generation sequencing (NGS) have identified hundreds of novel genes and gene variants associated with risk of or protection against human diseases, including common sporadic disorders. These data can be used to create new genetically engineered iPSC (induced

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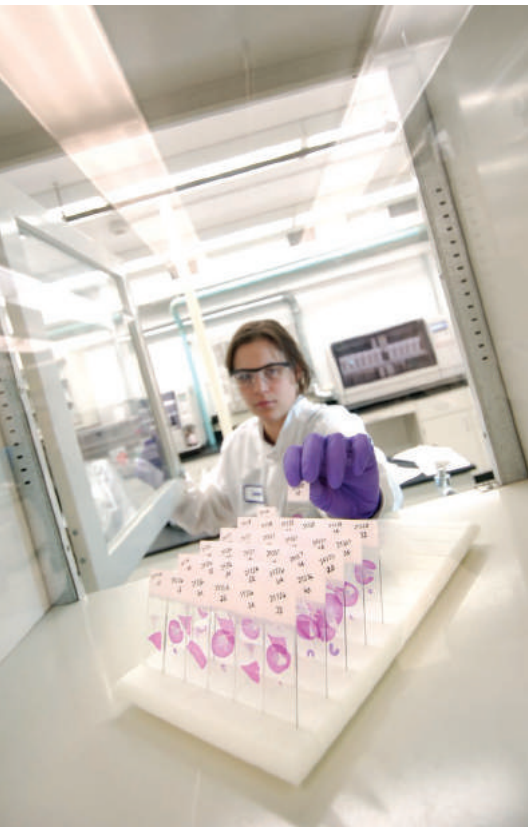
pluripotent stem cell) models as well as animal models of disease, which then can be used both to explore the functions of the newly identified genes, and to discover new disease pathways and candidate drug targets.[37–39] This kind of reverse translational approach to generating animal models offers a special boon for the study of rare inherited diseases, for many of which no animal models have previously been available.[40,41]

Molecular profiling of patient tissue samples can be used to identify patterns of RNA and protein expression that correlate with disease resistance and/or responsiveness to therapeutics. In the cancer field, for example, gene and protein expression profiling of tumors has begun to define molecular signatures associated with better responses to immunotherapy and higher patient survival rates; these signatures can also suggest new targets for drug development.[42–45] Molecular profiling can also be used to screen and optimize cell-based therapeutics. In one recent study, molecular profiling of over 100 different preparations of dendritic cell (DC) vaccines targeting prostate cancer identified a signature of DC gene and protein expression that correlated with the induction of strong anti-tumor responses in patients.[46] In a modern spin on the development of the smallpox vaccine, immune profiling of humans who show resistance to certain diseases (Alzheimer's disease, progressive multifocal leukoencephalopathy) has been used to develop antibody therapies for these diseases.[47,48]



Another important application of the reverse translational approach is in the analysis of results of failed clinical trials.[49,50] In one example, the anti-IL-12B p40 antibody, which showed promise as a therapeutic for multiple sclerosis (MS) based on results in mice and marmoset models of experimental autoimmune encephalomyelitis (EAE), failed in human trials. Subsequent analysis of disease progression in the mouse and marmoset EAE models vs. human MS showed that (1) the initiation and progression phases of the disease are driven by different mechanisms in primates, (2) the mouse replicates only the initiation mechanism, and (3) the drug blocks only the initiation mechanism.[50]

Targeting environment-gene interactions: the microbiome and metabolome

Most common human diseases result from interactions between environmental factors such as diet and exercise, with genetic risk factors. However, outside the areas of cancer and metabolic diseases, environmental risk factors are typically not built into animal models. This situation is changing now, in part as a result of increased awareness of the roles of the *microbiome* and the *metabolome* in health and disease.

The microbiome is the array of micro-organisms (bacteria, fungi, and other single celled organisms) that populate the gut, skin, respiratory tract, and other parts of the body that are directly exposed to the environment. The gut microbiome (the most thoroughly studied) has now been shown to play essential roles in nutrient digestion, drug metabolism, and the development and function of the immune and nervous systems.[51,52]

The human microbiome contains around 1,000 species of bacteria, whose exact numbers and proportions vary from person to person.[53] The composition of an individual's microbiome can also change over time in response to environmental factors, including diet and sex hormones.[54] Alterations in the gut microbiome have been linked to a growing list of diseases, including obesity, diabetes, irritable bowel syndrome, cardiovascular disease, cancer, and autism.[55,56,57] The gut microbiome also helps determine drug efficacy and side effects.[58,59] Transfer of gut microbiota from one animal to another allows direct testing of suspected roles of the microbiome in disease, and may also enable the creation of new animal models of disease.[60,61]

The metabolome is the full set of small molecule chemicals (sugars, amino acids, lipids, etc.) found in a given biological sample, including metabolites generated by the microbiome as well as those produced by an individual's own cells. The metabolome lies at the direct interface of the environment with the genome and microbiome, and provides a dynamic readout of the current state of an individual's health.[62,63]

High-throughput profiling of the microbiome and metabolome, made possible by recent developments in genome sequencing and chemical analytic technologies (e.g., automated, quantitative NMR and liquid or gas chromatography coupled with mass spectrometry) can identify new disease

signatures and translatable biomarkers, and generate hypotheses for reverse translational research.[57,62,64] These approaches now have been used to discover the first pre-clinically successful microbiome-targeting drugs in the areas of cancer and cardiovascular disease.[55] In the latter case, an untargeted metabolomics screen in human patients suggested that a microbe-derived metabolite trimethylamine *N*-oxide (TMAO) was associated with greater disease risk.[65] Subsequent animal studies confirmed a causal link between elevated TMAO and atherosclerosis, and identified a small molecule inhibitor of the microbial TMAO pathway that attenuates disease progression in mouse models.[55]

The microbiome and the metabolome each offer huge, virtually untapped sources of potential drug targets. Whereas the human genome provided 20,000 gene targets, the human microbiome offers several *million*, and the metabolome offers not only targets but also natural product drug leads.[56]

"Disease-in-a-dish" models

Cell culture systems are advantageous for preclinical studies because they offer simplified biological models in which environmental factors can be tightly controlled. Compared to *in vivo* studies, *in vitro* studies are generally much faster, can be done at much higher throughput and lower cost, and are largely free of the ethical issues.

To date, 2D cell culture models have been the norm for early-stage drug screening. However, some traditional 2D cell culture models are inherently non-physiological because they (1) lack the 3D architecture under which cells normally function and communicate with one another, and (2) employ non-human cells and/or immortalized cell lines that have been selected based on their ability to grow under non-physiological conditions. In addition, most 2D culture systems used in drug screening include only one cell type. However, we now know that many human diseases involve dysfunctional interactions between two or more cell types. For example, defects in glial, immune, and/or vascular cell function initiate or contribute to neuronal degeneration in many neurological

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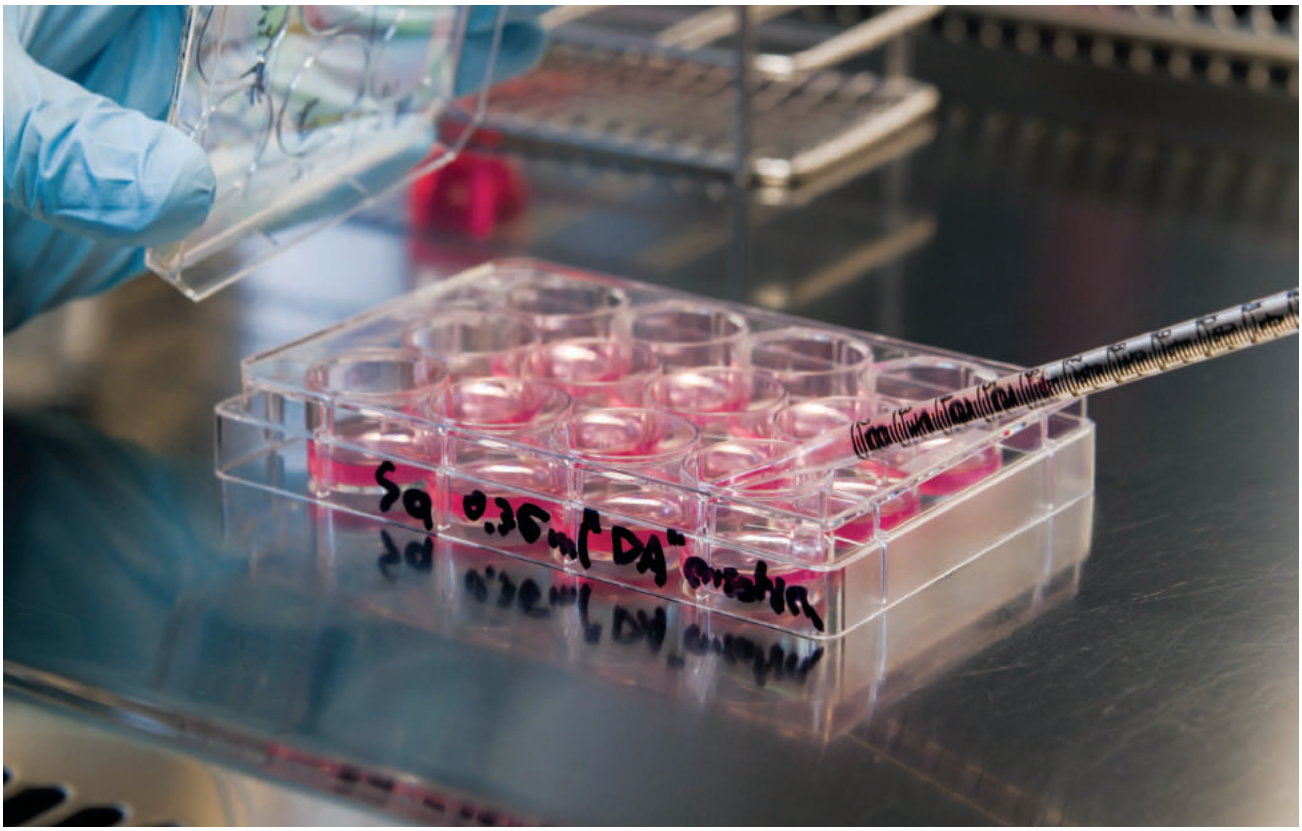
diseases, including Huntington's disease, ALS, and Alzheimer's disease. Similarly, the growth of tumor cells is strongly affected by their interactions with stromal, immune, and vascular cells.

Over the past decade, advances in stem cell biology, tissue engineering, and microfluidics have spawned a plethora of exciting new *in vitro* model systems that mimic *in vivo* 3D cellular architecture and can include multiple cell types. These include the following:

Induced pluripotent stem cell (iPSC) cultures: iPSCs have caused a revolution in translational research because they provide unlimited supplies of human cells for *in vitro* studies.[66] iPSCs can be cultured in 2D, or in 3D to create "organoids" with complex architectures.[67–70] For example, human iPSC-derived neural precursor cells can self-organize into "mini-brains" that contain many classes of neurons (as well as astrocytes), develop functional synapses and circuits, and show region-specific patterning (e.g., cortical layers).[69,71] A key feature of iPSC technology is that it can be used to generate cultures of specific cell types carrying a patient's own, individual genetic makeup. In many cases, patient-derived iPSCs recapitulate cellular phenotypes similar to those seen in human patients *in vivo*.[67,70] For example, iPSC-derived neurons generated from fibroblasts of patients with genetic forms of Parkinson's disease show many disease-appropriate abnormalities, including reduced neurite outgrowth, increased sensitivity to oxidative stress, and elevated α-synuclein levels.[70] Thus, iPSCs offer powerful tools for precision medicine, including *ex vivo* testing of patient-specific disease mechanisms and drug responses. Moreover, using gene-editing technologies like CRISPR or TALENS, it is possible to introduce precise mutations or combinations of mutations into iPSCs for the purpose of analyzing genotype-phenotype relationships—a boon for the study of both single-gene and complex genetic disorders.[70,72]

Bioprinting: In bioprinting, 3D tissue- or organ-like structures are constructed layer-by-layer by 3D printing machines that deposit precisely patterned sheets of living cells, extracellular matrix, and other bioreagents.[73,74] Bioprinted organs can incorporate multiple cell types and can be created from either primary cells (including tumor cells) or iPSCs. One challenge has been to provide these artificial organs with a blood supply, as tissue development and function *in vivo* requires functional, hierarchically organized vasculature. Recently, methods have been



> Up to five different organ types have been functionally coupled on a single hardware platform."

developed to incorporate preformed vascular beds into bioprinted tissues, which can then form functional connections with the vascular system of a living host animal.[75,76]

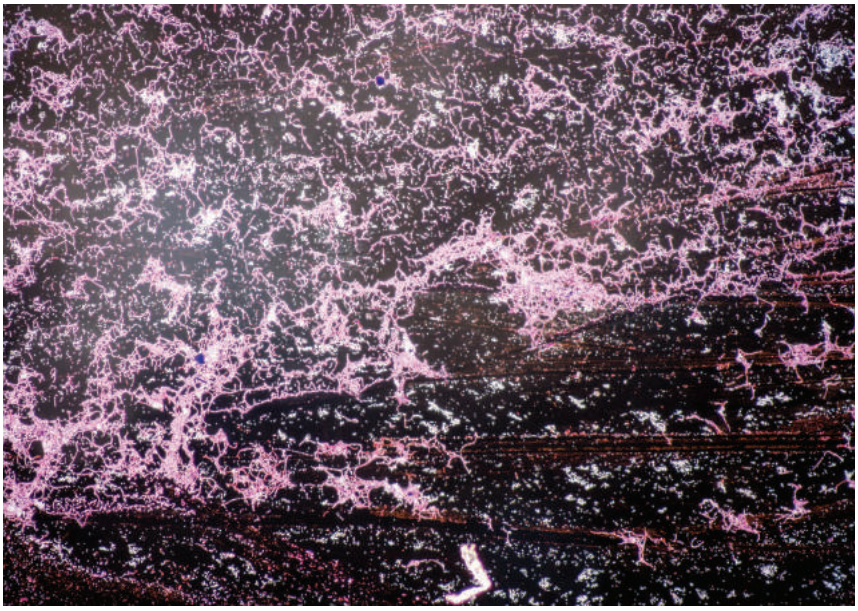
Organ-on-a-chip, patient-on-a-chip: Bioprinted tissues can be combined with microfluidic, "lab-on-a-chip" platforms that provide tissue perfusion, delivery of compounds, and continuous measurements of tissue responses. This "organ-on-a-chip" approach has now been used to create *in vitro* models of several diseases, including cancers, lung diseases and nonalcoholic steatohepatitis (NASH).[47,48,77,78] In the latest generation of this technology, called "body-on-a-chip" or "patient-on-a-chip," up to five different organ types have been functionally coupled on a single hardware platform, allowing the study of disease and drug effects on complex organ system interactions.[48,78]

In addition to the advantages previously cited, these new "disease-in-a-dish" models can all be used for high-throughput drug screens and cell- and organ-specific toxicology screens. They can be also used for high-throughput phenotype-based screening. Phenotype-based screening used to be basis of all drug development, but in recent decades had been largely replaced by target-based approaches. Phenotype-based screens are now having a renaissance, because they may be better for discovering "first in class" drugs, and can identify drugs that exert beneficial effects by acting on multiple biochemical pathways simultaneously.[79, 80]

Non-rodent models

Non-traditional small animal models

Non-traditional small animal models, such as the nematode worm *C. elegans*, the fruit fly (*Drosophila melanogaster*) and zebrafish, can provide a bridge between *in vitro* and rodent models. They are cheap, fast, and easy to breed,



and are readily amenable to genetic manipulations that enable the study of basic mechanisms of biology and disease.[81] Compared to *in vitro* systems, intact animals can be used to assess a much broader range of phenotypes, including behavior, gut motility, and cardiovascular function and zebrafish even possess a blood brain barrier.[82] *C. elegans* and zebrafish have the additional advantage of being transparent, so their cells can be fluorescently labeled and visualized over time in living animals. Many genetic and biological pathways are conserved from lower organisms to humans. For example, over 80% of human disease genes are conserved in zebrafish, and 60% in the fruit fly, and mutation of human disease genes in lower organisms often produces cellular phenotypes comparable to those seen in humans.[83,84] In addition, invertebrates and lower vertebrates often have unique regenerative abilities (zebrafish, for example, can regenerate heart tissue), and so are of particular interest in regenerative medicine.[81,85]

Additional advantages of these non-traditional animal models are that they can be used for:

- High-throughput phenotype-based screening (see section above).
- Reverse genetic screens to identify new molecular partners of disease genes and new drug targets.
- Enhancer/suppressor screens for drug discovery.
- Combined screening and counter-screening (for therapeutic and adverse effects of drugs) in the same assay.

Automated phenotypic assays have been developed for several of these organisms, as have species-specific mechanism of action discovery tools.[79,81,86,87]

High-throughput drug screens have now been conducted in worm, fruit fly, and/or zebrafish for drugs to combat infectious diseases, cancer, neurodegeneration, aging, cardiomyopathies, and many other diseases.[79,81,87–89] A number of approved cancer drugs, including crizotinib, gefitinib, and vandetanib, were developed or validated in the fruit fly[88] and a drug discovered in zebrafish is now in clinical trials for treatment of hematological malignancies.[79]

Large animal models

The anatomy and physiology of rodents differs from that of humans in many crucial ways, including the following (reviewed in reference 90):

- Immune mechanisms. For example, lymphocytes are the predominant population of white blood cells in mouse blood, but neutrophils are the main type in humans.
- Composition of the microbiome.
- Cardio- and cerebrovascular architecture.
- Complexity of the CNS and behavioral repertoires.
- Proportions of glial cells. In the cerebral cortex, the glial: neuron ratio is 10:1 in humans, compared to 1:1 in mice,[91] a difference that may be critical when modeling neurodegenerative diseases, where glia are now believed to play major roles in most diseases.

Large mammals often reproduce key features of human biology and disease more closely than rodents do and are closer to humans genetically: rodents have only 48-66% genetic homology with humans, whereas swine and New World monkeys have 80% and Old World monkeys (e.g. baboons) up to 99%.[92] Animals that are closer to humans in size are necessary for testing surgical devices and procedures, and also are more suitable for dosage, delivery and safety studies. Large animals that have proven useful in studying human diseases include the pig (cardiovascular disease, obesity, cystic fibrosis)[90,93–95] and non-human primates (neurological and psychiatric disease).[92,96]

Apart from the issues of cost and ethics, the major limitation to using large animal models in translational research has been the difficulty of creating genetically engineered animals. This situation is changing now with the advent of gene editing technologies, which have now been successfully applied in pigs (over 25 lines of gene-edited mini-pigs have now been generated) and non-human primates.[94,95]

Conclusion

During the past few years, researchers involved in translational science and drug discovery have been harnessing exciting new technologies to enable the reappraisal and fine-tuning of traditional models, and to create new models. As detailed above, there is increasing emphasis on overcoming limitations of currently available *in vitro* and *in vivo* models by improving ease and cost of use, and predictive validity. These innovative model systems include new animal models that more closely recapitulate human disease syndromes, and new *in vitro* models that support easier, deeper study of human disease pathways and "personalized" drug testing.

The mission of the inaugural Charles River World Congress on Animal Models in Drug Discovery & Development is to describe and discuss some of these cutting-edge innovations and breakthroughs in translational tools and methods in drug discovery research and development. The conference has been designed to create a platform that fosters the sharing of knowledge, views and collaborations through a mix of presentations and interactive networking sessions. We anticipate that conference attendees will be inspired to be a part of breakthroughs in translational tools and methods in drug discovery research and development.

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