Summary: Biomedical developments in the 21st century provide an unprecedented opportunity to gain a dynamic systems-level and human-specific understanding of the causes and pathophysiologies of disease. This understanding is a vital need, in view of continuing failures in health research, drug discovery, and clinical translation. The full potential of advanced approaches may not be achieved within a 20th-century conceptual framework dominated by animal models. Novel technologies are being integrated into environmental health research and are also applicable to disease research, but these advances need a new medical research and drug discovery paradigm to gain maximal benefits. We suggest a new conceptual framework that repurposes the 21st-century transition underway in toxicology. Human disease should be conceived as resulting from integrated extrinsic and intrinsic causes, with research focused on modern human-specific models to understand disease pathways at multiple biological levels that are analogous to adverse outcome pathways in toxicology. Systems biology tools should be used to integrate and interpret data about disease causation and pathophysiology. Such an approach promises progress in overcoming the current roadblocks to understanding human disease and successful drug discovery and translation. A discourse should begin now to identify and consider the many challenges and questions that need to be solved.

Introduction

The genomics era opened a door to understanding genetic changes in susceptibility to diseases, such as single nucleotide polymorphisms, gene copy number variations, and gene deletions and insertions (Zerhouni 2014). The subsequent explosion of related “omics” approaches, including transcriptomics, metabolomics, and proteomics, have provided more details on how gene regulation and protein production are implicated in human disease mechanisms. However, many human illnesses such as cancers, diabetes, immune system and neurodegenerative disorders, and respiratory and cardiovascular diseases are caused by a complicated interplay between multiple genetic and environmental factors (Lango and Weedon 2008). The environmental counterpart to genomics is exposomics, which aims to capture an individual’s lifetime exposure to external factors (e.g., infections, environmental chemicals, drugs, radiation) measured via biomarkers in blood, urine, feces, or breath samples. It provides an opportunity to develop an environmental analog of genome-wide association studies, similarly top down and hypothesis free (Lioy and Rappaport 2011).

Another emerging omics tool is epigenomics—the study of changes in gene activity not attributable to DNA sequence alterations (e.g., DNA methylation and chromatin remodeling). Epigenetic changes including inherited effects and environmentally induced alterations are implicated in disease causation, and epigenomics is being developed in disease research. The U.S. National Institutes of Health (NIH) Roadmap Epigenomics Consortium has provided detailed human epigenomic maps to enhance studies of human disease and development (NIH Roadmap Epigenomics Consortium 2015). Epigenomics is also being explored in environmental health research with many exposures being associated with adverse health effects (Shenderov and Midvedt 2014). These developments provide an unprecedented opportunity to add a new dimension to the study of human diseases.

The 21st century has seen these and many other pivotal advances in science and technology: Together, they offer, for the first time, the possibility of gaining a dynamic systems-level and human-specific understanding of the causes and pathophysiologies of disease (van de Stolpe and Kauffmann 2015). This understanding is a vital need, in view of current failures (Scannell et al. 2012; Kaitin and DiMasi 2011) in health research, drug discovery, and clinical translation (Collins 2011). But these developments in human-specific models and tools require a new research paradigm to unlock their full potential. We suggest it is time for a novel, overarching paradigm for medical research based on adapting and applying the transitional process underway in toxicology that includes reducing reliance on animal models, and instead emphasizing human biology and approaches based on multiscale pathways.

Discussion

In future health research and drug discovery, diseases can be envisaged as the combined outcome of extrinsic causes that include many types of exposures, not just chemical exposures, and intrinsic genetic and epigenetic changes (e.g., Gohlke et al. 2009) that interact at multiple levels (Figure 1). This combined approach would provide a more coherent “big picture” by linking environmental sciences with medical research. Some of the thinking required to develop a more comprehensive framework for understanding disease causation has already begun. Toxicologists and environmental health scientists are already devising new models that explore synergies between toxic exposures and infectious pathogens in complex diseases, exemplified by interactions between the hepatitis B virus and aflatoxin in liver cancer (Birnbaum and Jung 2010).

A new medical research paradigm. To maximize the value of advanced models and technologies, we believe that a new paradigm is needed for fundamental research into human diseases and for drug discovery. The focus should move decisively away from preclinical animal studies and overly simplistic cell models toward a systems biology framework to integrate new types of scientific data, such as from omics, novel human-specific in vitro models, and clinical studies. Such a framework would help enable a comprehensive and dynamic understanding of disease causation and pathophysiology.

A concept that systematically describes links between causes of disease and outcomes could be repurposed from 21st-century toxicology. Since the publication of the U.S. National Research Council (NRC) report calling for a new paradigm (NRC 2007), a transition in toxicology has been underway, actively supported by U.S. regulatory and research agencies both from environmental and medical arenas (Collins 2011), as well as by the European Union [Scientific Committees on Health and Environmental Risks (SCHER) et al. 2013]. The focus in toxicological research turned first to understanding toxicity pathways—the normal cellular processes involving genes, proteins, and small molecules that lead to adverse human health effects when significantly perturbed by chemical toxicants (NRC 2007).

The notion of the cell-level toxicity pathways described in the NRC report (2007) has already been extended to the broader concept of adverse outcome pathways (AOPs), thereby addressing the sequence of changes between the molecular initiating event (e.g., a chemical binds to a cell receptor) and adverse outcomes at the molecular, cellular, organ, organism, and population levels. An AOP is a standardized way to describe concisely the critical mechanisms of toxic effects.
and is enabling the emergence of a new predictive toxicology paradigm [Organisation for Economic Co-operation and Development (OECD) 2012]. This paradigm contrasts with classical toxicology where so-called apical toxicity end points are studied in a series of animal tests for different kinds of toxic effects [e.g., cancer, reproductive and developmental toxicity, or skin allergy (sensitization)]. However, this traditional black box approach sheds little light on the underlying pathways of toxicity. Rather, it merely presents an end result that is not easily accessible to deeper analysis or understanding.

Because of its potential to contribute to deeper knowledge-based human and environmental health assessments, the AOP concept is now established as a comprehensive framework at the OECD to support its international regulatory programs on chemical toxicology (OECD 2013). The OECD has published the first well-characterized AOP, describing chemical potential for causing skin allergy (OECD 2012) (Figure 2), and many others are under development (e.g., Vinken et al. 2013) and review. An essential component of the OECD program is the AOP Knowledge Base (AOP-KB; http://www.aopkb.org) that facilitates scientific collaboration on an international scale to aid both the development and evaluation of AOPs.

**Repurposing the AOP concept for human health research.** We now suggest a novel step in the evolution of pathway concepts—the incorporation of the AOP construct into human health research and drug discovery. Our proposed disease AOPs, like AOPs in toxicology, would describe a chain of causally linked key events causing downstream effects at several biological levels and provide clear mechanistic rationales for diagnostic, preventative, and therapeutic interventions in the era of personalized medicine.

The important commonalities between safety science and health research, drug discovery, and clinical translation argue for the relevance of the AOP concept in all these fields. These common features include a) human biological pathways whose response continuum encompasses efficacy, adaptation, and adversity; b) shared research tools and technologies (e.g., in vitro models, analytical approaches, computational modeling); and c) the benefits of better-structured and transparent weight-of-evidence decision-making frameworks, whether for chemical safety or drug efficacy, that can integrate all the data inputs.

Our proposed AOPs for human diseases are a natural extension of the AOPs developed in toxicology. The central steps will likely be similar, although the molecular initiating events will be more varied. For example, as well as chemical perturbations, infectious and genetic factors may initiate the disease process. Nevertheless, the principles and basic biology will be shared between disease AOPs and toxicity AOPs, and the related information could be integrated into the existing OECD AOP-KB, including information compiled by several programs designed to leverage big data such as the NIH Big Data to Knowledge initiative (https://datascience.nih.gov/bd2k/), as well as information from existing pathways and bioinformatics databases e.g., the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) and the gene–disease database DisGeNET (http://www.disgenet.org/).

In the context of disease research and drug discovery, our disease AOP concept would provide a unified framework for describing relevant pathophysiological pathways and networks across multiple biological levels and for encompassing extrinsic and intrinsic causes. Describing these pathways and networks, along with anchoring molecular initiating events with adverse outcomes, our AOP framework would represent a significant advance over existing concepts, such as disease mechanisms that are often studied in isolation and biological pathways or networks (e.g., for cancers) that are invariably considered only at the molecular or cellular levels.

The disease AOP approach would better exploit advanced experimental and computational platforms for knowledge discovery, since the emergence of AOP networks will identify knowledge gaps and steer investigations accordingly. A commitment to build, curate, and disseminate a pathways framework within the biomedical research field would thus provide considerable impetus to base decisions on mechanistic understanding rather than empirical observation, as has been the case in toxicology.

**Advanced human-specific disease models.** In addition to a strategic and integrated knowledge-based exploitation of omics tools and the introduction of the AOP concept, we further propose a strong focus on human-specific models. Advanced human-specific cell- and tissue-based models (e.g., Singh et al. 2011) and next-generation tools are making possible a fuller, dynamic comprehension of disease pathophysiology and a more reliable and cost-effective drug discovery process (Moorri 2015).

Human-induced pluripotent stem cell technology offers unique access to healthy as well as patient- and disease-specific *in vitro* cell models (Bellin et al. 2012). This could help achieve the holy grail of...
relating disease genotype to phenotype, for example by correlating individual genetic variants with gene expression patterns, disease pathways, and associated outcomes. Models derived from human stem cells have been developed to enhance research into autism spectrum disorders (Marchetto et al. 2010), cardiovascular disease (Zanella et al. 2014), Alzheimer’s disease (Choi et al. 2014), and many other illnesses. In some instances, insights about molecular disease mechanisms and drug effects have emerged from human stem cell systems that were previously missed in nonhuman models (Marchetto et al. 2010; Mitne-Neto et al. 2011).

Human organ-on-a-chip culture devices, combining microfluidics with two- and three-dimensional cell culture, aim to reproduce key architectural, physical, functional, and biochemical features of human organs in vitro. Within miniature cell chambers, highly controlled cell culture allows in vivo–like interactions between multiple cell types (van de Stolpe and Kauffmann 2015). Identifying and independently varying critical cellular and molecular disease contributors is difficult in animal models, but in microfluidic systems, molecular factors and different cell types can be varied independently and simultaneously. Real-time system-level responses become practical (Benam et al. 2015; van de Stolpe and den Toonder 2013). There are already prototype microfluidic models for diseases of the heart, lung, intestine, liver, and kidney and of the vascular, endocrine, musculoskeletal, nervous system, and more (Benam et al. 2015).

Key information is also provided by studies of ex vivo biopsied or postmortem human tissue (Zerhouni 2014; Beach 2013) using powerful analytical tools such as next-generation sequencing (Twine et al. 2011), and novel multiplexed fluorescent in situ cell and tissue visualization technologies for proteins, DNA, and RNA molecules (Weibrecht et al. 2013) using digital pathology platforms that enable quantification of complex staining patterns. In addition, advanced mass spectrometry techniques can provide high-throughput, comprehensive, and quantitative information about proteins in clinical cell (e.g., tumor biopsies) and biofluid samples (e.g., urine, saliva, or plasma) at high sensitivity (Jimenez and Verheul 2014). Interpreting omics data from healthy and diseased tissues using bioinformatics tools has revealed associations with multiple pathways important in (patho)physiology (Andreec et al. 2012), including information on the status and dynamics of regulatory gene networks and pathways (Tonetitsky et al. 2013). Access to biobanks with well-characterized human tissues, and biofluids from phenotyped patients and controls will also be important. Finally, advanced clinical studies to obtain in vivo human information (the true gold standard model) also have much to offer. They may provide new insights into pathology (Rosen et al. 2013; Ledford 2008) and anchor research models of all kinds to real-world illnesses in humans (Koren et al. 2007).

Multidisciplinary data should be integrated and interpreted by means of systems biology tools (van der Stijde et al. 2014). New bioinformatics approaches become even more powerful with the incorporation of cell biology data, and systems biology offers ways to integrate computational and experimental methods at multiple scales from biochemistry through to individual levels (Figure 2). Development and adaptation of integrated software platforms are central to efficient and effective use of data and for predictive computational modeling (Ghosh et al. 2011).

**Figure 2.** Diagram showing different pathways concepts, including the well-characterized adverse outcome pathway (AOP) for chemically induced skin allergy, from chemical structure through molecular initiating event, key events and adverse outcome. DCs, dendritic cells; QSAR, quantitative structure–activity relationships. Reprinted from *Encyclopedia of Toxicology*, Vol. 1, 3rd ed. Adverse outcome pathways: development and use in toxicology, pp. 95–99, 2014, with permission from Elsevier.
budget for a precision medicine initiative involving the omics and a million research volunteers (Collins and Varmus 2015) and, in toxicology, the OECD’s AOP Development Programme (OECD 2013).

Conclusions

Our proposed new research paradigm, adapted from 21st-century toxicology, would involve the following aspects:

• Developing a big picture of human diseases, integrating extrinsic and intrinsic causes, and linking environmental sciences with medical research using systems biology.

• Introducing a disease AOP concept, analogous to toxicity AOPs, with the intention of providing a unified framework for describing relevant pathophysiology pathways and networks across multiple biological levels.

• Creating a strong focus on advanced human-specific research (in vitro, ex vivo, in vivo, and in silico) in place of empirical, animal-based studies.

To accomplish the goals outlined in this article, many questions will need to be considered:

• To what extent can existing and emerging human models and tools be applied to replace animal studies?

• Where are the knowledge and technology gaps?

• How can big data be synthesized into actionable knowledge?

• Can computational models effectively bridge the in vitro–in vivo divide?

• How easy will it be to optimize the derivation of enriched populations of disease-relevant cells from human-induced pluripotent stem cells?

In summary, a new coherent roadmap for medical research promises progress in several areas:

• Revealing common disease pathways.

• Discovering new and multiple human drug targets.

• Improving translation.

• Reducing late-stage drug attrition.

• Facilitating drug repurposing.

• Contributing to the development of personalized medicine.

• Achieving more reliable and valid data in faster time frames and at lower costs.

It will take a formidable effort and redeployment of funds (e.g., away from efforts to improve animal models) to achieve the new paradigm of a multiscale–pathways-based, human-centered concept for disease research. We hope this article will help launch a serious discourse among researchers, policymakers, regulatory agencies, and research-funding organizations around the world and encourage those who have already begun to think along these lines. Unless rethinking of the 20th-century research paradigm starts now, benefits to patients from 21st-century scientific and technological advances will be unduly delayed.

G.L. and T.S. work for Humane Society International, and C.W. works for Humane Society of the United States. Both organizations have as one of their goals the phasing out of animal use in testing and research. J.R.F. is a paid consultant for MB Research Labs and Syngenta and receives reimbursement for travel expenses from the Institute for In Vitro Sciences, Inc., the American Chemical Society, and the Center for Alternatives to Animal Methods at the Johns Hopkins University.

The other authors declare they have no actual or potential competing financial interests.

Gill Langley,1 Christopher P. Austin,2 Anil K. Balapure,3 Linda S. Birnbaum,4 John R. Bucher,5 Julia Fentem,6 Suzanne C. Fitzpatrick,7 John R. Fowlie III,8 Robert J. Kavlock,9 Hiroaki Kitano,10 Brett A. Liddbury,11 Alysson R. Muotri,12 Shuang-Qing Peng,13 Dmitry Sakharov,14 Troy Seidle,15 Thales Trez,16 Alexander Tonevitsky,17 Anja van de Stolpe,18 Maurice Whelan,19 and Catherine Willett20

1Research and Toxicology Department, Humane Society International, London, United Kingdom; 2National Center for Advancing Translational Sciences, National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Bethesda, Maryland, USA; 3Division of Biochemistry, CSIR–Central Drug Research Institute, Lucknow, India; 4National Institute of Environmental Health Sciences (NIEHS) and National Toxicology Program (NTP), NIH, DHHS, Research Triangle Park, North Carolina, USA; 5Division of NTP, NIEHS, NIH, DHHS, Research Triangle Park, North Carolina, USA; 6Unilever R&D, Safety and Environmental Assurance Centre (SEAC), Sharnbrook, Bedfordshire, United Kingdom; 7Office of the Center Director, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Maryland, USA; 8Science to Inform LLC, Pittsboro, North Carolina, USA; 9Office of Research and Development, U.S. Environmental Protection Agency, Washington DC, USA; 10Systems Biology Institute, Tokyo, Japan; 11Genomics and Predictive Medicine, John Curtin School of Medical Research, Australian National University, Canberra, Australia; 12Institute of California, San Diego, School of Medicine, Department of Pediatrics/Rady Children’s Hospital San Diego, Department of Cellular and Molecular Medicine, Stem Cell Program, La Jolla, California, USA; 13Evaluation and Research Center for Toxicology, Institute of Disease Control and Prevention, Academy of Military Medical Sciences, Beijing, China; 14Scientific Research Centre Bioclinicum, Moscow, Russia; 15Research and Toxicology Department, Humane Society International, Montréal, Quebec, Canada; 16Institute of Science and Technology, Federal University of Alfas, Alfas, Brazil; 17National Center of Medical Radiological Research, Obninsk, Russia; 18Philips Research, Eindhoven, the Netherlands; 19Institute for Health and Consumer Protection, European Commission Joint Research Centre, Ispra, Italy; 20Regulatory Toxicology, Risk Assessment and Alternatives, Humane Society of the United States, Washington DC, USA.

Address correspondence to G. Langley, Humane Society International, 5 Underwood St., London N1 7LY, United Kingdom. Telephone: 44 (0) 1422 455300. E-mail: glangley@hsi.org

The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of their organizations.

Acknowledgments

Julia Fentem is employed by Unilever, Sharnbrook, England. Hiroaki Kitano is employed by the Systems Biology Institute, Tokyo, Japan; Dmitry Sakharov is employed by the Scientific Research Centre Bioclinicum, Moscow, Russia; Anja van de Stolpe is employed by Philips Research, Eindhoven, The Netherlands.

REFERENCES

Andreev VP, Petyuk VA, Brewer HM, Karpiwchik YU, Xie F, Clarke J, et al. 2012. Label-free quantitative LC-MS proteomics of Alzheimer’s disease and normally aged human brains. J Proteome Res 11(8):3053–3067. doi: 10.1021/pr3001546.

Beach TG. 2013. Alzheimer’s disease and the “Valley of Death”: not enough guidance from human brain tissue? J Alzheimers Dis 33(Suppl 1):S219–S233.

Bellin M, Marchetto MC, Gage FH, Mummery CL. 2012. Induced pluripotent stem cells: the new patient? Nat Rev Mol Cell Biol 13(11):713–726; doi: 10.1038/nrm3448.

Benam KH, Dautu S, Hassell B, Herland M, Jain A, Jang KJ, et al. 2015. Engineered in vitro disease models. Annu Rev Pathol 10(1):195–262; doi: 10.1146/annurev-pathol-012414-040418.

Birnbaum LS, Jung P. 2010. Evolution in environmental health: incorporating the infectious disease paradigm. Environ Health Perspect 118(A2):A327–A328; doi: 10.1289/ehp.1002691.

Choi SH, Kim YH, Hebisch M, Slivinski C, Lee S, D’Avanzo C, et al. 2014. A three-dimensional human neural cell culture model of Alzheimer’s disease. Nature 515(7526):274–278.

Collins FS. 2011. Reengineering translational science: the time is right. Sci Transl Med 3(99):96cs17; doi: 10.1126/scitranslmed.3002747.

Collins FS, Varmus H. 2015. A new initiative on precision medicine. N Engl J Med 372(3):793–795; doi: 10.1056/NEJMp1500523.

Ghosh S, Matsumiya Y, Asai Y, Hisin KY, Kitano H. 2011. Software for systems biology: from tools to integrated platforms. Nat Rev Genet 12(2):821–832; doi: 10.1038/nrg2936.

Gohike JM, Thomas R, Zhang Y, Rosenstein MC, Davis AP, Murphy C, et al. 2009. Genetic and environmental pathways to complex diseases. BMC Syst Biol 3:60; doi: 10.1186/1752-0509-3-46.

Jimenez CR, Verheul HM. 2014. Mass spectrometry-based proteomics: from cancer biology to protein biomarkers, drug targets, and clinical applications. Am Soc Oncol Educ Book:e504–e501. Available: http://meetinglibrary.asco.org/content/11400504A-144 [accessed 14 May 2015].

Katif KJ, D’Masi JA. 2011. Pharmaceutical innovation in the 21st century: new drug approvals in the first decade, 2000–2009. Clin Pharmacol Ther 89(2):183–188; doi: 10.1038/clpt.2010.286.

Koren E, De Groot AS, Jawa V, Beck KD, Boone T, Rivera D, et al. 2007. Clinical validation of the “in silico” prediction of immunogenicity of a human recombinant therapeutic protein. Clin Immunol 124(1):26–32.

Langley GR. 2014. Considering a new paradigm for Alzheimer’s disease research. Drug Discov Today 19(8):1114–1122.

Lango H, Weedon MN. 2008. What will whole genome searches for susceptibility genes for common complex disease offer to clinical practice? J Intern Med 263(1):116–27.

Lefford H. 2008. Translational research: the full cycle. Nature 453:643–645; doi: 10.1038/453643a.

Lioy PJ, Rappaport SM. 2011. Exposure science and the exposome: an opportunity for cohering in the environmental health sciences. Environ Health Perspect 119:A466–A467; doi: 10.1289/ehp.1104387.

Marchetto MC, Caronec M, Acab A, Yu D, Yee GW, Mu Y, et al. 2010. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell 143(4):527–539.

Metne-Neto M, Machado-Costa M, Marchetto MC, Bengtson MH, Joaazeiro CA, Tsuda H, et al. 2012.
2011. Downregulation of VAPB expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. Hum Mol Genet 20:3642–3652; doi: 10.1093/hmg/ddi284.

Mustri AR. 2015. The human model: changing focus on autism research. Biol Psychiatry; doi: 10.1016/j.biopsych.2015.03.012.

NIH (National Institutes of Health) Roadmap Epigenomics Consortium. 2015. Integrative analysis of 111 reference human epigenomes. Nature 518(7539):317–330; doi: 10.1038/nature14248.

NRC (National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC:National Academies Press. Available: http://dels.nas.edu/Report/Toxicity-Testing-Twenty-first/11970 [accessed 14 May 2015].

OECD (Organisation for Economic Co-operation and Development). 2012. The adverse outcome pathway for skin sensitisation initiated by covalent binding to proteins. OECD Series on Testing and Assessment No. 168. Part 1: Scientific Evidence. ENV/JM/MONO(2012)10/PART1. Paris:OECD. Available: www.oecd.org/oecd/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2012)10/part1&doclanguage=en [accessed 14 May 2015].

OECD. 2013. Guidance Document on Developing and Assessing Adverse Outcome Pathways. OECD Environment, Health and Safety Publications. Series on Testing and Assessment, No. 184. ENV/JM/MONO(2013)6. Paris:OECD. Available: www.oecd.org/officialdocuments/publications/displaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage-en [accessed 14 May 2015].

Pound P, Bracken MB. 2014. Is animal research sufficiently evidence based to be a cornerstone of biomedical research? BMJ 348:g3387; doi: 10.1136/bmj.g3387.

Rosén C, Hansson O, Blennow K, Zetterberg HE. 2013. Fluid biomarkers in Alzheimer’s disease—current concepts. Mol Neurodegener 8:20; doi: 10.1186/1750-1326-8-20.

Scannell JW, Blanckley A, Boldon H, Warrington B. 2012. Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov 11:191–200; doi: 10.1038/nrd3681.

van de Stolpe A, van de Stolpe A, Kauffmann RH. 2015. Innovative human-specific investigational approaches to autoimmune disease. RSC Adv 5:18451-18463; doi: 10.1039/c4ra15794; van der Sijde MR, Ng A, Fu J. 2014. Systems genetics: from GWAS to disease pathways. Biochim Biophys Acta 1842(10):1903–1909.

Vinken M, Landesmann B, Goumenou M, Vinken S, Shah I, Jaeschke H, et al. 2013. Development of an adverse outcome pathway from drug-mediated bile salt export pump inhibition to cholestatic liver injury. Toxicol Sci 136(1):97–106.

Weibrecht I, Lundin E, Killemamia S, Mignardi M, Grundberg I, Larsson C, et al. 2013. In situ detection of individual mRNA molecules and protein complexes or post-translational modifications using padlock probes combined with the in situ proximity ligation assay. Nat Protoc 8(2):355–372.

Zerhouni EA. 2014. Turning the Titanic. Sci Transl Med 6(221):221ed2; doi: 10.1126/scitranslmed.3008924.