Animal models in an age of personalized medicine

Personalized medicine is based on intraspecies differences. It is axiomatic that small differences in genetic make-up can result in dramatic differences in response to drugs or disease. To express this in more general terms: in any given complex system, small changes in initial conditions can result in dramatically different outcomes. Despite human variability and intraspecies variation in other species, nonhuman species are still the primary model for ascertaining data for humans. We call this practice into question and conclude that human-based research should be the primary means for obtaining data about human diseases and responses to drugs.

KEYWORDS: complexity ■ evolution ■ personalized medicine ■ prediction ■ species ■ variation

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The observation that humans responded differently to drugs formed the basis for the field of personalized medicine [1,2]. These are cases of intraspecies differences and can be traced back to differences in genetic make-up. Some examples include copy number variants, SNPs, gene regulation and epigenetics.

The age of personalized medicine has come on the heels of changes in physics that include the study of chaotic and complex systems. These changes took on their current form in the 1960s, with Edward Lorenz conducting computer-based weather simulations. He rounded off a variable in the simulation from six decimal points to three and ran his computer program again only to find that he obtained completely different results (typified by Figure 1). What Lorenz discovered was that very small and seemingly insignificant changes in initial conditions resulted in major changes in the long-term outcome. Today, this is part of a discipline known as chaos theory. Complexity and the study of complex systems are closely related to the chaos theory.

In any given complex system, small changes in initial condition can also result in dramatically different outcomes. An example of this would be a common population splitting into two populations, undergoing different evolutionary trajectories, and eventually becoming separate species. The resulting two different species would be expected to demonstrate different traits including different responses to chemicals and other environmental perturbations. These interspecies differences have been empirically confirmed. For example, humans share the receptors for odor detection with other primates but, because the genes for the receptors have evolved differently since splitting from our common ancestor, each species uses the receptors in different ways [3,301].

We examine the practice of using models, specifically animal models used in drug development but also including animal models of disease, that have far more differences between the model and the complex system being modeled than the intraspecies differences exploited by personalized medicine and pharmacogenomics.

Prediction

Pharmacogenomics and personalized medicine are concepts, practices or modalities with the express purpose of predicting human response to drugs and disease. While this point may appear self-evident and even simplistic, it deserves a brief consideration. Medical practice based on science involves understanding the fundamentals of human anatomy, biochemistry and physiology. One must also appreciate the mechanisms of medications and the pathophysiology of disease. Medicine began the transition from being superstition and anecdote based and, to a large degree, based on the notion of a special creation, to being science based, a little more than a century ago. It is only within the last two decades that we have witnessed medical science attempt to leave the one-size-fits-all approach and embrace genome-based personalized medicine.

What has gone largely unsaid in today's transition, as well as yesteryear's casting off of superstition, is that all of the advances of medical science have as their aim the ability to predict human response. This started in large part by understanding at least some aspects of

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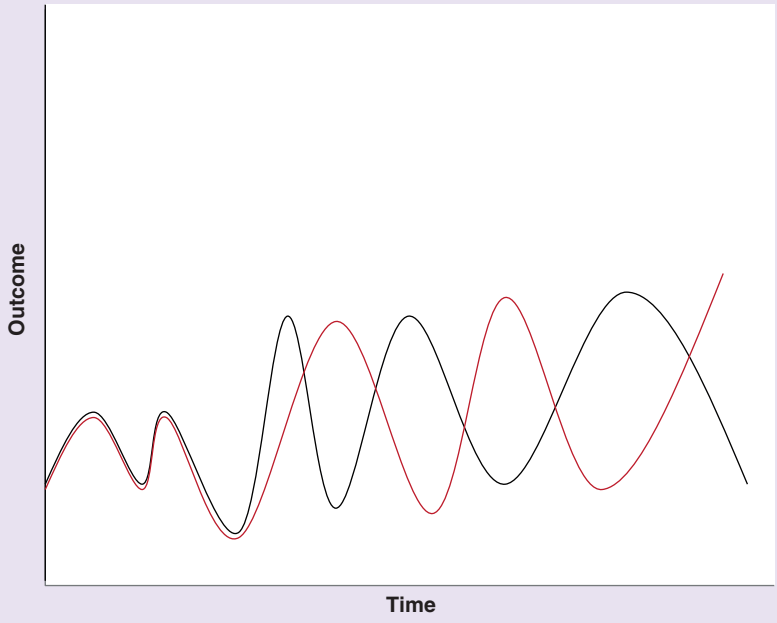

Figure 1. Dependence upon initial conditions. Two almost identical systems (or equations) are represented by the black and red lines. Rounding off a variable from six significant digits to three (or similar very small changes in initial conditions) can lead to trajectories that eventually result in opposite outcomes or effects.

the system one was treating – the pancreas, the liver, the heart and so forth – using reductionism. Next came statistics and the ability to predict, not individual response, but the responses of most members of a group. Refinements have changed the size of the group from all members of *Homo sapiens* to: white males over the age of 50 who have all the major risk factors for coronary artery disease; black females under the age of 30 diagnosed with a certain type of breast cancer and so forth. Statistics has enabled the medical practitioner to increase the probability of success but not to the extent that personalized medicine offers.

Now would be a good time to refresh our memories on what predict means in medical science and how prediction is evaluated and/or calculated in specific instances. Put simply, prediction means knowing with what probability the effect a disease or drug will have on a specific patient. This means the practitioner can assign the right medical intervention (including none at all) to the specific patient. Personalized medicine aims for a correct probability of 1.0 or, getting it right 100% of the time. How is this calculated?

The ability of a practice, modality or test (and we are using the term 'test' in a very broad sense of the word) to correctly predict outcomes can be calculated by using values from a 2 × 2 table (see Table 1 for values and Box 1 for how to perform the calculations). As this is standard fare for all readers of *Personalized Medicine* we will not go into more detail. Suffice it to say, the goal of personalized medicine is to assign intervention or prognosis based on tests that give a sensitivity, specificity, positive predictive value and negative predictive value of 1.0. Currently, such values are not realistic; therefore, values as close as possible to 1.0 are sought.

Historically, the problem with reaching this goal has been that individual humans, even monozygotic twins, did not always respond identically to diseases and drugs. Personalized medicine has as its objective the discovery and implementation of knowledge that enables the practitioner account for these discrepancies.

Intraspecies differences

Scientists, especially in pharma (the pharmaceutical industry in general), know that there is tremendous variation among humans in response to drugs, disease and genetic mutations. Allen Roses, then-vice-president of genetics at GlaxoSmithKline, said that fewer than a half of the patients prescribed some of the most expensive drugs derived any benefit from them: "The vast majority of drugs – more than 90% – only work in 30 or 50% of the people." Most drugs had an efficacy rate of 50% or lower [4]. Diseases also affect members of the same species differently. There is variation between sexes, among ethnic backgrounds, among age groups and so forth [5–18,302]. All of this is well known to readers of *Personalized Medicine*.

The reasons for this variation are also well known. As Shanks and Greek point out in *Animal Models in Light of Evolution* [19], there is more to the differences between and within species than merely different genes. Regulatory genes can make the exact same genome result in very different phenotypes, as can other factors and interactions. Similarly, Snyder recently opined that the important differences among people are not to be found in the genes but rather in the sections of DNA that affect gene expression and protein binding [20,303]. Differences in transcription factors, which control the rate of binding of DNA to mRNA, play a large role in this. Kasowski *et al.* performed research that revealed that large differences exist between individual humans in two transcription factors. These differences were associated with SNPs, gene duplications, deletions and inversions of DNA, and with differences in gene expression. Snyder used the Kasowski *et al.* [304] study to point out that we now have evidence that SNPs and other variations have a major effect on

Table 1. Binary classification test.

		Gold standard	
		GS+	GS-
Test	T+	TP	FP
	T-	FN	TN

FN: False negative; FP: False positive; GS+: Gold standard positive; GS-: Gold standard negative; TN: True negative; TP: True positive; T+: Test positive; T-: Test negative.

"large numbers of regulatory elements that control gene expression." Kasowski hypothesized that this variation might explain variation in disease susceptibility [304]. The Kasowski *et al.* study also revealed large differences between humans and chimpanzees in that 32% of RNA polymerase II binding regions differed. This difference must be considered because 99.29% of coding sequences are the same in humans and chimpanzees.

Strains of mice vary in their response to drugs, diseases and even gene disruption. Deleting one gene in strain A may prove lethal while deleting the same gene in strain B may result in no discernable effect or the effects may vary considerably [21–26]. One strain cannot predict the outcome for another [27]. Rohan *et al.* demonstrated that different strains of mice have a tenfold variation in range of response to angiogenesis. They suggest that genetic factors may be responsible for this variation [28].

Hunter *et al.* studied transgenic mice and discovered that metastatic efficiency varied up to tenfold between the originals and their offspring [29,30]. They then performed gene analysis on the 17 genes that were associated with metastatic efficiency [31]. Hunter *et al.* concluded: "Because all tumors were initiated by the same oncogenic event, differences in the metastasis microarray signature and metastatic potential are probably due to genetic background effects rather than different combinations of oncogenic mutations [32]. Consistent with our observations in metastasis, several laboratories have shown similar strain differences with regard to oncogenesis, aging and fertility in transgenic mouse models [33–35]. Data on both primary tumors and metastases reinforce the notion that tumorigenesis and metastasis are complex phenotypes involving both inherent genetic components and cellular responses to extrinsic stimuli" [31].

This is also true in the human model of disease. Miklos stated: "There is enormous phenotypic variation in the extent of human cancer phenotypes, even among family members inheriting the same mutation in the *APC* gene believed to be causal for colon cancer" [36]. He goes on to describe how in mouse models, knocking out "the catalytic γ subunit of the phosphatidyl-3-OH kinase" can result in a high incidence of cancers or no cancers. The results vary with the strain used [36]. Dixit and Boelsterli point out that while tolcapone causes hepatotoxicity, in some humans, rodent studies did not reveal this [37,38]. However, after humans were noted to have hepatotoxicity tests were performed on different strains from the original rodents and hepatotoxicity was seen [38].

Even monozygotic twins do not always respond in the same way to diseases and drugs [39–42].

The implications of these differences are profound. Because of differences in genes, for example SNPs, the same vaccine may not protect all children [43,44]. In the future, such children may be able to receive a personalized vaccine. It is estimated that "between 5 and 20% of people vaccinated against hepatitis B, and between 2 and 10% of those vaccinated against measles, will not be protected if they ever encounter these viruses" [44,45]. Currently, tests can be performed prior to prescribing codeine, tamoxifen [46], chemotherapies for various cancers [47], for determining the dose of warfarin and 6-mercaptopurine [48–51] and others are being developed [52,53]. Pharma is reacting to these discoveries. Milne stated: "There's a trend toward the death of the blockbuster, so people are moving toward the niche buster" [54].

Genetic differences in the form of epigenetics also raise the possibility that a genetic variant will not be expressed or will be expressed differently in one individual than in another. The presence of modifier genes may also influence the activity of a biomarker. One must also question whether the biomarker under consideration is causally and directly related to the effect being studied. Humans are complex systems (see below). While biomarkers are currently being intensively studied, they will probably not be a panacea because of these concerns. This further enforces our position that small differences between individuals may be the difference between a treatment being efficacious or toxic [55–60].

Box 1. Calculating values for a binary classification test.

- Sensitivity = TP/TP + FN
- Specificity = TN/FP + TN
- Positive predictive value = TP/TP + FP
- Negative predictive value = TN/FN + TN

FN: False negative; FP: False positive; TN: True negative; TP: True positive.

Of course, all of the above revolves around evolution. Changes in allele frequencies result in different responses to the same perturbation. Futuyma famously stated: "Evolution… is the central unifying concept of biology. By extension, it affects almost all other fields of knowledge and must be considered one of the most influential concepts in western thought" [61]. This is really the basis for personalized medicine.

Complex systems

Medical research as a science can perhaps rightly trace its roots back to the French physiologist, Claude Bernard. Bernard sought to bring medicine onto the same intellectual plane as the physical sciences. In doing so, Bernard was a strict causal determinist, meaning that if X caused Y in a monkey, X would also cause Y in a human. Bernard stated: "Physiologists . . . deal with just one thing, the properties of living matter and the mechanism of life, in whatever form it shows itself. For them genus, species and class no longer exist. There are only living beings; and if they choose one of them for study, that is usually for convenience in experimentation" [62].

He was also offended by the statistical nature of medicine "This false idea leads certain physicians to believe that medicine cannot but be conjectural; and from this, they infer that physicians are artists who must make up for the indeterminism of particular cases by medical tact. Against these antiscientific ideas we must protest with all our power, because they help to hold medicine back in the lowly state in which it has been so long … if based on statistics, medicine can never be anything but a conjectural science; only by basing itself on experimental determinism can it become a true science … I think of this idea as the pivot of experimental medicine, and in this respect experimental physicians take a wholly different point of view from so-called observing physicians" [62].

Despite Bernard's importance in the overall development of medicine as a science, history has proven Bernard wrong regarding the lack of importance of statistics while the fields of evolutionary biology and complexity theory have proven his deterministic view naive. To make things even worse for Bernard's legacy, personalized medicine is replacing statistics in determining which treatment to use for which patient and how a specific disease interacts with the **internal milieu, but that further undermines** his "genus, species and class no longer exist" position.

Bernard was also a creationist and this is not unimportant. Bernard was not a creationist in the current sense of the word, rather he rejected evolution for other reasons, but reject it he did. LaFolette and Shanks stated: "Moreover, certain types of creationism may involve a commitment to the interchangeability of species. Those who think that all creatures are products of a designer would likely assume – on grounds of ontological simplicity – that the designer took the same basic stock of parts and rearranged them to produce different species. Certainly this was one response to the discovery of homologous structures by 19th century comparative anatomists: what Darwin would see as evidence of descent with modification, creationists were apt to see as evidence of a designer's variations on a basic common blueprint. According to creationists, the main difference between men and animals was merely that the designer added an extra ingredient – a soul. But the basic body parts remained constant. Under these assumptions, if we knew how a rat's liver functioned, we would likewise know how a human liver functioned (once we had adjusted for differences in size and weight)" [63].

Human parts and animals parts were thought to be interchangeable. Humans had souls and were sentient, otherwise animals and humans were more or less identical. Bernard stated: "Now the vital units, being of like nature in all living beings, are subject to the same organic laws. They develop, live, become diseased and die under influences necessarily of like nature, though manifested by infinitely varying mechanisms. A poison or a morbid condition, acting on a definite histological unit, should attack it in like circumstances in all animals furnished with it; otherwise these units would cease to be of like nature; and if we went on considering as of like nature units reacting in different or opposite ways under the influence of normal or pathological vital reagents, we should not only deny science in general, but also bring into zoology confusion and darkness…" [62].

This view accounts for the importance placed on reductionism in the early days of biomedical research. This view of creationism also explains, at least in part, why the creationist surgeon Leonard Bailey transplanted the heart of baboon into Baby Fae [63].

Our discussion of complex systems must be set in the background of reductionism. Complexity is in some way opposite to the concept of reductionism. The great biologist Ernst Mayr defined reductionism as: "The belief that the higher levels of integration of a complex system can be fully

explained through a knowledge of the smallest components" [64]. Another way of viewing reductionism is divide and conquer. Learn what all the parts of an engine do and one can deduce the function of the entire engine.

Pistons, for example, are capable of description on their own, without reference to the system from which they are removed. The parts of a complex living system on the other hand must be described based on the interaction of the parts. Merely describing individual parts in isolation is insufficient to fully describe the system as a whole. The whole is greater than the sum of the parts. A complex system can only be described in light of the organization of the individual parts [23].

Complex systems should also be contrasted with simple systems. One can consider pistons and engines to be examples of simple systems, as are inclined planes and objects represented by a point in Newtonian physics. Simple should not be confused with easy to understand or manipulate although some simple systems are both. Rather, a simple system can usually be described solely using Newtonian physics and or reductionism and determinism. While Newtonian physics certainly acts on complex systems, the complex system is best described by partial differential equations that do not have solutions. Cells are complex systems and anything composed of cells is a complex system. However, even a complex system can be treated as a simple system, for example, a rodent falling in a vacuum. The level of organization under consideration is important in a complex system whereas it is not in a simple system. Complexity as a science can be thought of as organized chaos. Complexity lies between the conditions that lead to chaos and those that result in determinism. Complexity tends to refer to systems. Living systems, such as animals, are examples of complex systems. Complex systems have many properties that are relevant to personalized medicine in general and our discussion of animal models in particular [65–68]. See **Box 2 & Figure 2** for characteristics of a complex system [21,22,65–76,305].

To put the above in the context of using animals to model humans in research and testing, very small differences in the genetic make-up of two otherwise very similar species can result in very different responses to drugs and disease. Similarly in the arena of personalized medicine, very small differences in genetic make-up of two otherwise very similar humans can result in very different responses to drugs and disease.

Mayr also pointed out that the current concepts of complex systems and complexity theory, although considered unique to the last quarter of the 20th century, have actually been around since Alex Novikoff described it in 1947 [64]. Novikoff spelled out in considerable detail why an explanation of living organisms has to be holistic: "What are wholes on one level become parts on a higher one … both parts and wholes are material entities, and integration results from the interaction of parts as a consequence of their properties … [holism] does not regard living organisms as machines made of a multitude of discrete parts (physicochemical units), removable like pistons of an engine and capable of description without regard to the system from which they are removed" [77]. On a similar note, the biologist Cairns-Smith pointed out: "Subsystems are highly interlocked … [P]roteins are needed to make catalysts, yet catalysts are needed to make proteins. Nucleic acids are needed to make proteins, yet proteins are needed to make nucleic acids. Proteins and lipids are needed to make membranes, yet membranes are needed to provide protection for all the chemical processes going on in a cell. The whole is presupposed by all the parts. The interlocking is tight and critical. At the center everything depends on everything" [78].

Box 2. Characteristics of a complex system.

- Complex systems are robust, meaning they have the capacity to resist change [21,22,69–74,305]. This can be illustrated by the fact that knocking out a gene in one strain of mouse may produce no noticeable effects
- Redundancy tends be a part of complex systems and may explain some aspects of robustness. For example, many members of the kingdom Animalia exhibit gene redundancy [21,22,69–74,305]
- Different parts of a complex system are linked to and affect one another in a synergistic manner. In other words, there is positive and negative feedback in a complex system [75]. This is why overloading one part of a complex system with say vitamins, may not result in a healthier individual. The feedback system results in the rest of the system acting to simply excrete the unneeded vitamins
- Complex systems are also modular. But failure in one module does not necessarily spread to the system as a whole as redundancy and robustness also exist [65–68]
- The modules do communicate though. For example, genes tend to be part of networks, genes interact with proteins, proteins interact with other proteins and so on
- Complex systems communicate with their environment; they are classified as dynamic [65–68]
- Complex systems are very dependent upon initial conditions [67]. For example, very small changes in genetic make-up can result in dramatic differences in response to perturbations of the living system
- The causes and effects of the events that a complex system experiences are not proportional to each other. Perturbations to the system have effects that are nonlinear, in other words large perturbations may result in no change while small perturbations may cause havoc [65–68]
- The whole is greater than the sum of the parts [67,70–72,76]
- Complex systems have emergent properties. An emergent property cannot be predicted by full knowledge of the component parts. For example, the formation of a flock of birds and hurricanes are examples of emergent phenomenon as is perhaps consciousness [67]

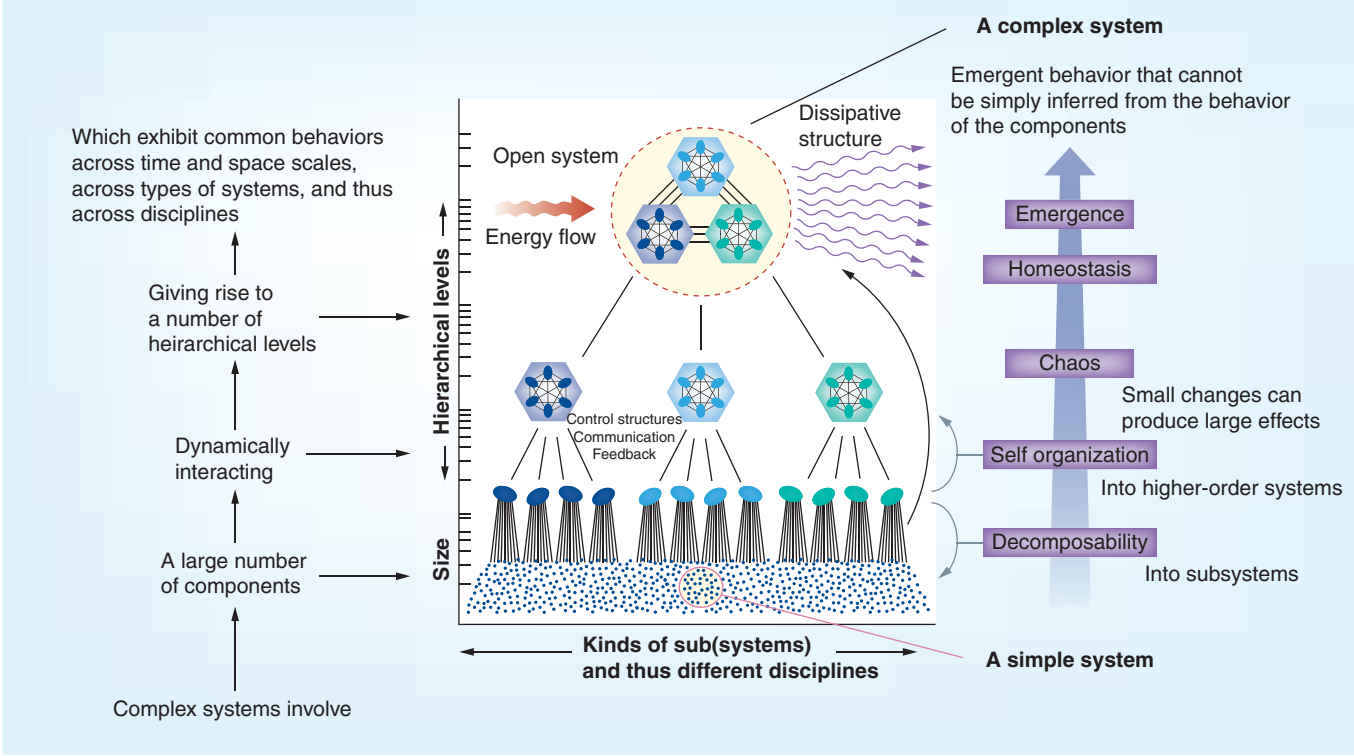

Figure 2. A complex system.
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Many scientists have written about complex systems [79,80]. In particular, they have addressed what this means for studying human disease and drug response [72]. Van Regenmortel said in 2004 that while the reductionist method has been effective in explaining the basis of numerous living processes, it has reached its limit. In support of this, he states that complex systems have emergent properties that cannot be explained or predicted, by studying their individual parts. He acknowledged that the reductionist approach was successful in the early days of molecular biology but today is having a detrimental influence on drug discovery and vaccine development [23]. Jura *et al.* stated: "Linear models based on proportionality between variables have been commonly applied in biology and medicine but in many cases they do not describe correctly the complex relationships of living organisms and now are being replaced by nonlinear theories of deterministic chaos" [75].

Living systems such as chimpanzees, mice and humans are obviously examples of complex systems. It should be equally obvious, therefore, why extrapolation between species will be problematic: small changes on the genetic level of a living complex system can have ramifications through the system that result in very large differences between species. Indeed, that is what evolution is all about. The claim that humans and rats are the same animal dressed up differently at the biochemical level [78], just isn't true. Moreover, it is irrelevant to point to observed similarities in genetic make-up between species, since the details of the differences are in the interactions between conserved genes, not in the genes themselves. It is as though humans and rats have a common genetic keyboard on which different phenotypic tunes are being played. What matters is not similarity with respect to the keyboard, but differences with respect to the order and timing of the pressing of the keys [19,81].

For more on complexity and biomedical research see the following references [23,67,75,76,79,82–85].

Interspecies differences

Animals are frequently used to predict human response to drugs and disease. Knowing that all animals are examples of complex systems and that even individual humans respond differently, it is not surprising that animal models fail to fulfill this function [19,86]. Scientists in and out of pharma recognize this and are expressing concern, but perhaps the most vocal have come from pharma and the regulatory agencies [19,21,86–120,306]. Alan Oliff, former executive director for cancer research at Merck Research Laboratories in West Point, Pennsylvania, USA, stated in 1997: "The fundamental problem in

drug discovery for cancer is that the [animal] model systems are not predictive at all" [99].

Along similar lines, in 2006, then-US Secretary of Health and Human Services Mike Leavitt stated: "Currently, nine out of ten experimental drugs fail in clinical studies because we cannot accurately predict how they will behave in people based on laboratory and animal studies" [306].

Many analysts today believe the pharmaceutical industry to be in a state of crisis. The pharmaceutical industry has long relied on the blockbuster, and new blockbuster drugs simply are not on the horizon. The search for new drugs is a risky and expensive business. Estimates about the cost of developing a new pharmaceutical compound vary from a low of US$800 million to nearly $2 billion, based on an optimum timeline of 10–12 years [88]. All of this is occurring at a time when the pipeline for new molecular entities is drying up and patents are expiring. Pharma cannot afford to lose what would otherwise be good medications for humans because of lack of efficacy or toxicity in animal models [307]. The search for a new drug candidate begins typically with high throughput screening of 5000–10,000 promising chemical entities, subsequently narrowed down to a pool of a few hundred compounds that will enter into preclinical laboratory and animal testing. Of those unique compounds, fewer than 10, on average, will show enough potential to qualify for Phase I human testing to establish basic safety [308]. Even so, many new drug candidates that show promise in the laboratory and even early human trials go on to fail in late-stage clinical trials.

The clinical phase of drug development is by far the most expensive part of the entire process, accounting for 80% of the total cost. However, apart from the significant financial cost to the drug manufacturer there is also a major public health burden in terms of adverse drug reactions, which are now a significant cause of morbidity and death in developed countries [121]. Safety testing of all new drugs relies heavily on animal toxicity studies, which has led to a growing scientific concern about the predictive power of animal models [19,86,122–124]. Many efficacious drugs are being weeded out by animal tests. Approximately 11% of all new chemical entities in clinical development are eliminated because of toxicity seen in animals [125,126]. Despite all the costly preclinical testing, a compound entering Phase I human clinical trials has only an 8% chance of reaching the market [127]. When a drug candidate has completed the lead optimization process, there is still a 99.8% chance it will not make it to market [128]. The failure to eliminate ineffective or toxic drugs costs money but perhaps not as much as the failure to recognize effective ones. The NCI has stated that society may have lost cures for cancer because of animal models [99]. McGee stated: "Dixit uses a variation on a famous real estate phrase to explain what scientists are looking for: Prediction, prediction, prediction is everybody's call, everybody's desire. We all want to predict safety and efficacy early so that we have fewer and fewer drugs failing so we can reduce the cost of drug development. It's not the cost of developing a successful drug; it's the cost of having unsuccessful drugs" [109].

The following two comments, made by government and regulatory officials, suggest that these disturbing figures are in fact an acknowledgement of the *status quo*. A US FDA toxicology reviewer stated in 1998 that "Most of the animal tests we accept have never been validated. They evolved over the past 20 years and the FDA is comfortable with them" [Menache A, Pers. Comm.]. Across the Atlantic, similar words were echoed by a UK Home Office minister in 2004, who acknowledged an acceptance of the *status quo* when she stated that the UK Government "has not commissioned or evaluated any formal research on the efficacy of animal experiments … and has no plans to do so" [309].

Profound interspecies, as well as intraspecies differences have been revealed for absorption [129–134], distribution [107,135], metabolism [136–146], elimination [147,148], and toxicity [38,88,127,134,149–157]. Collectively, absorption, distribution, metabolism, elimination and

Table 2. Sensitivity and positive predictive values for toxicity studies from rats and dogs.

Human	Toxic effects found in humans	53
	Toxic effects found in humans only	23
Rat	Toxic effects also found in humans	18
	Toxic effects not found in humans	19
	19 FP	
	35 FN	
	Sn = 18/(18 + 35) = 34%	
	PPV = 18/(18 + 19) = 49%	
Dog	Toxic effects also found in humans	29
	Toxic effects not found in humans	24
	24 FP	
	24 FN	
	Sn = 29/(29 + 24) = 55%	
	PPV = 29/(29 + 24) = 55%	

FN: False negative; FP: False positive; PPV: Positive predictive value; Sn: Sensitivity.

toxicity are referred to as ADMET. Some specific examples follow.

Litchfield conducted a study in 1962 (see TABLE 2) comparing toxicity in humans with toxicity in rats and dogs. He studied six drugs for 39 physical signs in the three species. He reported that toxicities that occurred in rats only were rarely observed in humans and that toxicities observed in dogs occurred only slightly more frequently in humans, while those that occurred in both rats and dogs showed approximately a 70% concordance (meaning sensitivity) with humans [158]. The positive predictive values are listed in TABLE 2.

Suter compared testing the drugs ergoloid mesylates, bromocriptine, ketotifen, cyclosporine, FK 33–824 and clozapine for toxicity in animals and humans. The sensitivity for toxicity for the animal tests was 0.52 and the predictive value positive was 0.31 [159]. Similar results have been obtained from other studies [152,160–165]. Numerous scientists have also commented on the lack of the predictive ability of animal models in drug development [166,167], including carcinogenic studies [168,169]. For example, Salsburg remarked: "Thus the lifetime feeding study in mice and rats appears to have less than a 50% probability of finding known human carcinogens. On the basis of probability theory, we would have been better off to toss a coin…" [168].

Fourches *et al.* analyzed MEDLINE abstracts for 1061 compounds known to cause hepatotoxicity in humans and found that the concordance among species was around 39–44% [170]. A study of 23 chemicals revealed only four were metabolized the same in humans and rats [139]. Sietsema, studied 400 drugs and compared the oral bioavailability in humans with three other species (FIGURES 3–5) [171]. Sietsema concluded: "Each of the plots is really nothing more than a scattergram."

Predicting efficacy is also a problem for animal models. Littman and Williams of Pfizer [89], writing about using humans as models for other humans, stated in 2005: "There are many examples of drugs that were effective in standard animal disease models but lacked efficacy in human disease. Xenograft models of cancer are a prime example." Johnson *et al.* reported in 2001 that out of 39 anticancer drugs tested on xenograft mice, only one mimicked the response in humans [172].

Browne and Taylor summarize the problem succinctly enough: "The premarket failure rate of drug candidates has been measured and remeasured from varying perspectives but always leads to the unavoidable conclusion that the process is inefficient. More than 50% of failures are due to lack of efficacy or unexpected animal toxicity … The failure rate is so high – about 1

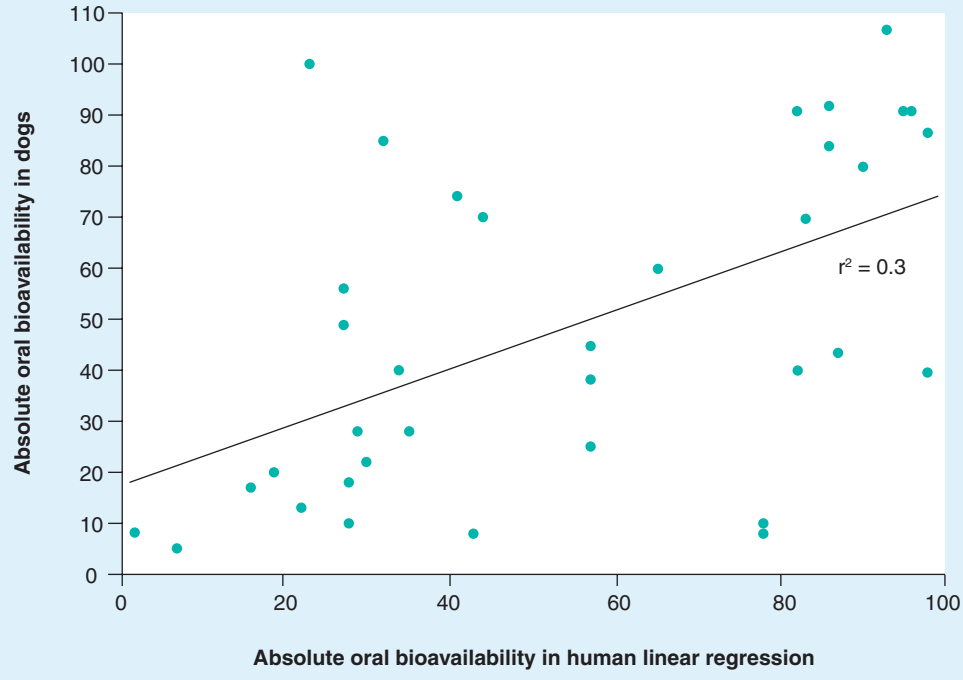

Figure 3. A comparison of the absolute bioavailability of selected drugs in dogs and humans.
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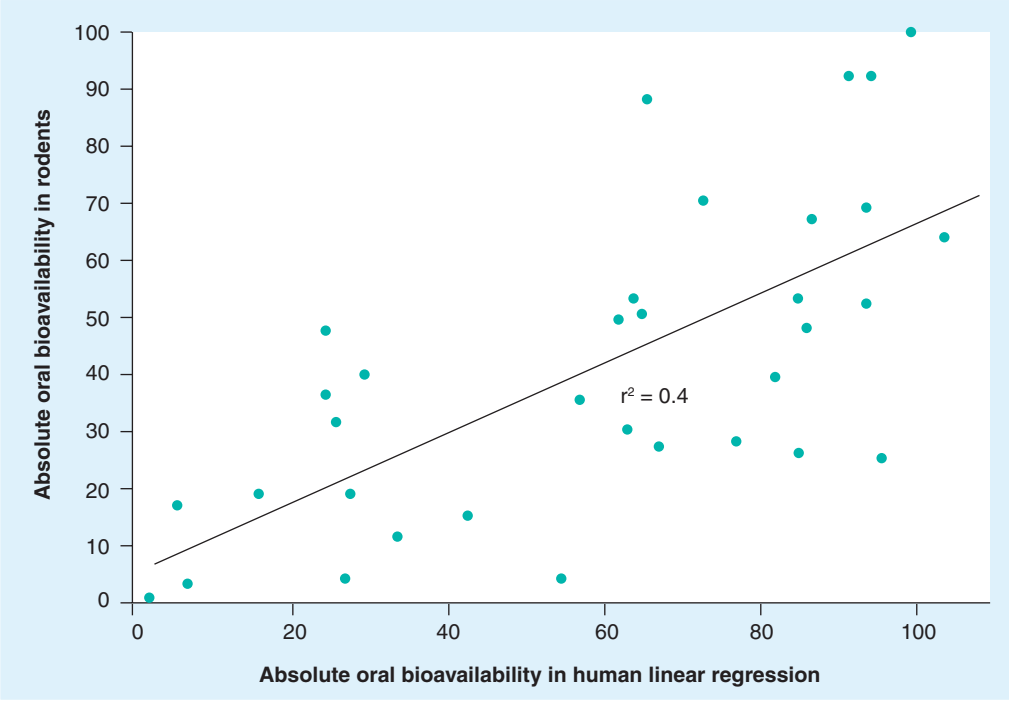

Figure 4. A comparison of the absolute bioavailability of selected drugs in rodents and humans.
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in 10 drug candidates survives from initiation of clinical evaluation to market launch, despite the expense and time committed to drug development, approved drugs have frequently been withdrawn from the market due to severe adverse reactions. Between October 1997 and September 1998, a number of FDA-approved drugs were withdrawn but not before being prescribed to 20 million patients in the US alone" [92].

The consequences of interspecies differences are not confined to pharma and or the study of ADMET and efficacy. Researchers in academia studying drug properties or disease mechanisms also encounter the problems of interspecies differences when using animal models [19]. Alleles that cause phenylketonuria and Sanfilippo syndrome in humans do not cause mental retardation in rhesus macaques [173,174]. Studying strokes and cerebral hemorrhage in animals has led to multiple medical treatments that resulted in harm to humans [175–178]. Transgenic animals are not producing better results [115,179,180]. The xenograft mice, which have cancers from human tumors, have not worked out for predicting the human effects of anticancer drugs. Sausville, then-associate director of the division of cancer treatment and diagnosis for the developmental therapeutics program at the NCI stated: "We had basically discovered compounds that were good mouse drugs rather then good human drugs" [99].

Evolution

Complexity aside, there are reasons from evolution to expect the aforementioned differences. Species evolved with different trajectories resulting in different responses to their environments. Selective pressures have resulted in different genes, the same genes but with different SNPs and copy number variants, and also in different regulation and expression of the same genes. For example, humans have lost 510 sections of regulatory DNA that are present, indeed vital, in other animals including chimps [181]. Zhang *et al.* identified the *Pax6* gene as a very important regulator in the development of the human brain. It represses some genes while activating others. Interestingly mice do not require *Pax6* for brain development nor do zebrafish [182].

Numerous studies have revealed gene-expression differences among nonhuman primates [183–185]. Pai *et al.* studied the epigenetic mechanism of methylation to modify DNA in order to assess changes in gene regulation between humans and chimpanzees. They state: "In particular, we estimate that, in the tissues we studied, interspecies differences in promoter methylation might underlie as much as 12–18% of differences in gene-expression levels between humans and chimpanzees" [186]. Gene-expression varies greatly intra- and inter-species, in humans [187–190] and in animals [191–194].

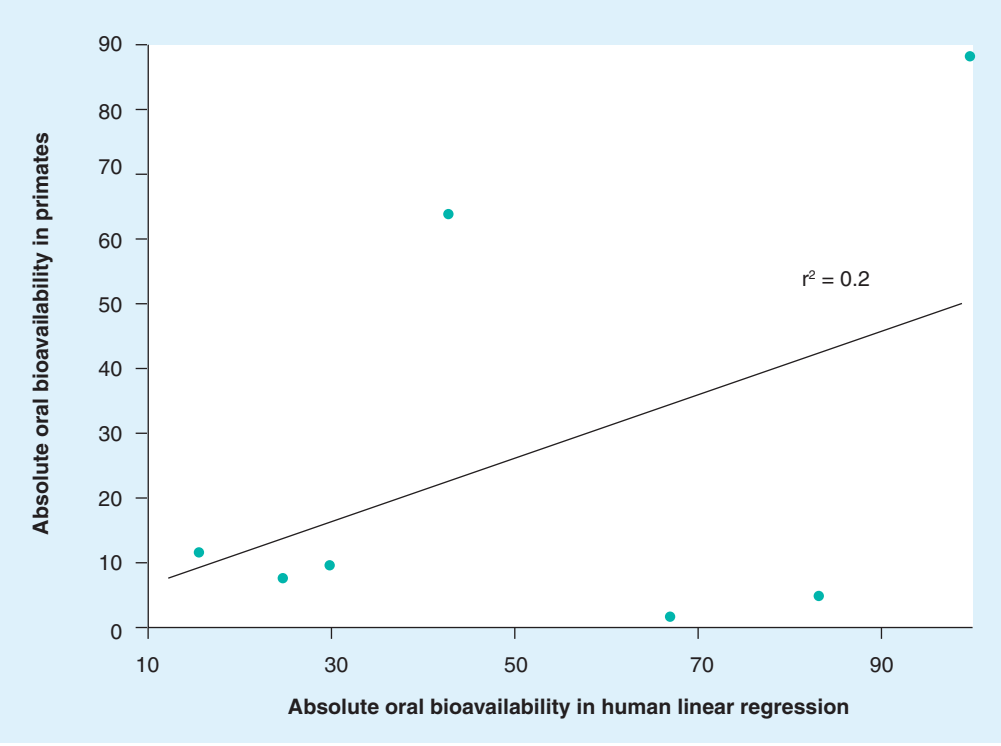

Figure 5. A comparison of the absolute oral bioavailability of selected drugs in primates and humans.
Reproduced with permission from Dustri-Verlag, Inc. © [171].

Coleman compared gene-expression profiles for the *CFTR* in tissues from humans and mice (FIGURE 6) [195]. Wide variation was noted. Nowick *et al.* compared the expression of genes for transcription factors in human and chimpanzee tissues and found significant differences in brain tissue [196]. Enard *et al.* compared the transcriptomes and protein-expression patterns of humans, orangutans and chimpanzees for brain tissues and tissues from the livers. They also examined tissues from three closely related rodent species. Enard *et al.* comment: "Our results show that that large numbers of quantitative changes in gene expression can be detected between closely related mammals. They furthermore suggest that such changes have been particularly pronounced during recent evolution of the human brain. The underlying reasons for such expression differences are likely to be manifold, for example, duplications and deletions of genes, promotor changes, changes in levels of transcription factors and changes in cellular composition of tissues" [197].

Moser *et al.* discovered that genes involved in the synthesis of plasmalogens were expressed differently in various tissues in humans and chimpanzees [198]. Sun *et al.* discovered that miRNA gene-expression profiles for embryonic stem cells were generally conserved between humans, mice and rhesus macaques but they also discovered gene-expression profile differences in three miRNA clusters [199]. Dere *et al.* studied gene-expression profiles from human *HepG2*, mouse *Hepa1c1c7* and rat *H4IIE* hepatoma cells after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and found significant differences [200]. Even different strains of mice have been revealed to have different gene-expression profiles [201].

With regard to all of the above, it must be stated that genes work in networks that are themselves complex systems and that form parts of wholes that are also complex systems. Per Goodwin, if we assume a 100-gene network with each gene switched off or on, then the number of states the network can be is 2^{100}. If the network proceeded through every state in some sequence and stayed in that state for one microsecond, it would take billions of billions of years to get through all the states. Humans have around 20,000 genes and can be expressed between 0 and 1.0 giving a number of states of at least $2^{20,000}$ [85]. This should give some idea of the complexity of the system and why small changes in it can have enormous implications.

Even when an animal model produces the same response to perturbation of the system that a human does, this does not ensure predictability. The mechanisms producing the outcome may vary. This is illustrated by convergent evolution,

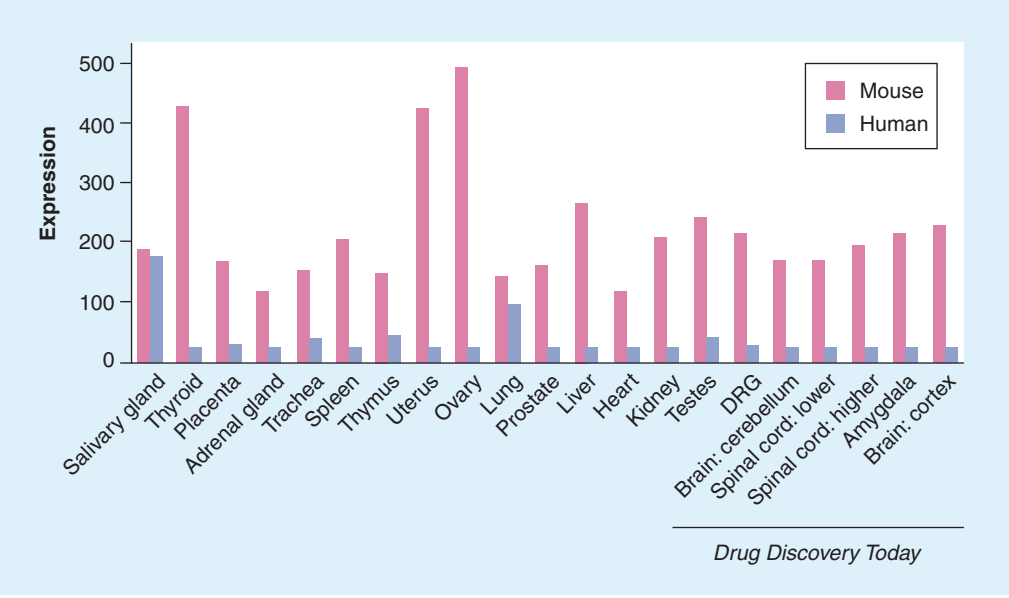

Figure 6. Gene-expression profile based on gene-chip technology for the relative expression of the *CFTR* in 21 tissue types from human (blue) and mouse (pink).
DRG: Dorsal root ganglion.
Reproduced with permission from Elsevier © [195].

the fact that the same outcome can be achieved via different evolutionary paths. For example, the eye of the octopus and human evolved along separate paths, as did the wings of birds and bats. The antifreeze proteins in two cold-water species of fish, the Arctic cod and the Antarctic notothenioids, are similar but evolved separately [202]. Independent evolution has also resulted in various frogs, newts and fish that utilize tetrodotoxin from bacteria to block sodium channels in their prey or enemies. However, these animals have evolved mutations in the genes coding for sodium channels such that the channels are resistant to the toxin [203,204,310].

Numerous studies have been published outlining the many differences between species that impact on predicting drug and disease response [98,130,205–211,311]. Reasons historically given for the differences between animals and humans can be seen in Box 3. A more current accounting for the differences between species can be seen in Box 4. Box 3 communicates empirically derived differences

Box 3. Historical reasons given for why animal and humans differ in response to drugs.

- Differences in absorption
- Differences in distribution
- Differences in elimination
- Differences in toxicity
- Differences in metabolism. Biotransformation results in different chemicals in different species These chemicals may also have different levels of biological activity in different species
- Differing efficacy and/or toxicity due to varying sensitivities of cells, organs and proteins such as receptors
- The dose used in animals was dramatically different from the dose used in humans
- Differences in living conditions such as food, air, water and other environmental factors
- Differences between strains
- Differences in underlying pathology
- Presence of genetic defects
- Differences in previous chemical exposures
- The use of young animals whereas the population of humans involved is much older
- The population of humans has comorbidities whereas the animals are otherwise healthy
- The animals are more homogeneous than the human population
- The animals studied were all male whereas the humans are of both sexes
- The time to treatment or diagnosis in the animals differed from the time to treatment or diagnosis in humans

Data taken from [130,141,205].

Box 4. Based on our current understanding of science, reasons why species, strains and even individuals differ in response to perturbations such as drugs and disease.

- Living organisms such as humans and other animals are examples of complex systems and hence have the properties listed in **Box 2**
- Living organisms such as humans and other animals are also examples of different species, which are themselves the result of the operation of evolutionary mechanisms over long periods of time. These differences in evolutionary history can be manifest by:
  - Differences with respect to specific genes present. Genes can be lost or gained over evolutionary time
  - Differences with respect to chromosomal or gene alteration such as:
    - Deletions (chromosome, gene or nucleotide)
    - Insertions (chromosome, gene or nucleotide)
    - Inversions
    - Duplications (chromosome, gene or nucleotide)
    - Copy number variants
    - SNPs
  - Differences with respect to mutations in the same gene (where one species has an ortholog of a gene found in another)
  - Fossilization of genes. Genes no longer in use will be discarded or fossilized meaning they will undergo degeneration contributing to the 'junk' DNA in our genome
  - Different molecules (hence derived from different genes) can accomplish the same function
  - Horizontal gene transfer also known as lateral gene transfer
  - Alternative splicing
  - Old genes can acquire new functions and the same function can be accomplished by different genes
  - Genes can be regulated and expressed differently because of the environment and miRNA
  - Genes operate in the presence of background genes and modifier genes
  - Changes in gene-expression profiles
  - Differences with respect to proteins and protein activity and protein–protein interactions
  - Differences in genetic networks
  - Differences with respect to organismal organization (humans and rats may be intact systems, but may be differently intact)
  - Differences in environmental exposures
  - Other differences with respect to evolutionary histories

All of the above facts are subject to the properties of complex systems referred to in **Box 2**.
This applies to every level of organization and has implications for what can be learned using reductionism (which has served science well) in order to predict behaviors of the system at higher levels of organization. Given the number of variables illustrated and given the fact that humans and other animals are complex systems, there is every reason to suspect that interspecies extrapolation, on the level of organization where prediction about drug and disease response occurs will be impossible. Immense empirical evidence supports this [19,81,86].

among species whereas **Box 4** explains those differences in a more encompassing way. Many of the differences outlined in **Box 3** could be overcome given the proper motivation. The reason animal models will fail to predict human response is not for the reasons outlined in **Box 3**, but for the evolution-based reasons as applied to complex systems that are outlined in **Box 4**. Coordinating drug-dosing schedules between animal models and humans will not overcome the fact that animals and human are complex systems with different evolutionary trajectories. Merely substituting a gene into an animal model will not negate the other factors, outlined in **Boxes 2 & 4**, which influence drug and disease response. The differences between complex systems are far greater than the presence or absence of a gene or genes, or differences in feeding or dosing schedules, or other differences that might best be described as superficial.

An appropriate example might be found in a simple system, namely the quest to build a machine that discharges more useful energy than it consumes. Every such attempt can be criticized based on the component parts, the way they interact, and the fact that mistakes of a mathematical nature were made. But the real criticism of such a quest lies in the second law of thermodynamics. Such a machine is simply not possible and while all attempts to construct one can be criticized on various and sundry levels and for various and sundry reasons, in the final analysis even if all the reasons were addressed, the quest would still fail.

Because of the differences between evolved complex systems described above, it should come as no surprise that moving discoveries from animal-based research to the clinic (translational medicine) has been very challenging [212–218]. This has been addressed by current NIH director Francis Collins, in his proposal for the National Center for Advancing Translational Sciences (NCATS) [219]. It has also been addressed in the combined NIH and FDA *Critical Path Initiative* [220]. While both of these initiatives emphasizes the failures of animal models and the need for human-based research, we maintain that they do not go far enough in abandoning animal models when used to predict human response to drugs and disease. Rather than attempting to improve animal models, we encourage the FDA, NIH and the scientific community in general to embrace the science that undergirds personalized

medicine and acknowledge the implications of this science for animal models.

Conclusion

The use of animals as human models for drug and disease research ignores the very principles upon which personalized medicine is based. Science has gone from thinking all mammals were variations on the same theme (creation based) to acknowledging that even individuals that started out as the same zygote may have important genetic differences. We conclude that the concepts that undergird personalized medicine should be extended to medical research in general and that human-based research should be the primary means for obtaining data about human diseases and response to drugs.

Future perspective

If scientists are sincere about conducting medical research with the aim of benefiting humans, then they should immediately stop using animals as predictive models for human responses to drugs and disease. The NIH and other funding organizations should consider transferring the current funding that is going to animals as predictive models to human-based research that is consistent with the concept of personalized medicine. This would include developing predictive human-based ADMET and efficacy testing, studying diseases both in intact humans and in tissues obtained from humans, developing software to aid in these pursuits, and generally following the scientific principles that have been set forth for advancing the field of personalized medicine.

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Executive summary

Prediction

- In medical science, in order for a practice, test or modality to be considered predictive it must be found to have a very high probability of obtaining the correct answer. Occasional correlation is not synonymous with prediction.

Intraspecies differences

- Personalized medicine is based on differences in drug and disease responses between individual humans, despite the genetic similarity of these humans.

Complex systems

- Humans and animals are examples of complex systems, thus extrapolating results of drug testing and disease response among species is expected to be problematic.

Interspecies differences

- Empirically, we find that extrapolating results of drug testing and disease response among species is problematic, and in fact, does not reach the level of probability necessary to be considered predictive.

Evolution

- Differences among species in genes, gene regulation and expression, and the networks the genes and proteins operate in explain the empirically observed low probability that any two species will respond the same to drugs and disease.

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