

Animal Models of Cancer in Light of Evolutionary Biology and Complexity Science

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1.0 Survival Rates

David Scott, Director of Science Funding at Cancer Research UK, writing about the use animals in cancer research opened his article by stating: “More people are surviving cancer than ever before.”(Scott 2011) He provided a link to the statistics on which he based the statement and went on to declare: “Thanks to decades of research, survival from cancer has doubled in the last 40 years, giving thousands of people more time with their loved ones.” He attributed this to using animals in cancer research stating that this progress “simply wouldn’t have been possible without animal research . . . In some areas there’s simply no other way to get the information needed to make progress against the disease.” As I will show, such statements supporting the importance of animal-based research and testing are not unique. However before addressing the use of animals in cancer research, I will clarify the actual survival rates for cancer and show that the above is very misleading and probably disingenuous.

When the War on Cancer began in 1971, less than 50% of people diagnosed with cancer lived for five years. Currently, the 5-year survival for all cancers is around 67%.[(American Cancer Society 2012) p1] Moreover, the original cancer does not usually kill the patient, rather the metastatic component does. The mortality rate from metastatic disease is essentially unchanged from the 1970s. So why have the 5-year survival rates changed so dramatically? The answer lies in overdiagnosis in general and specifically overdiagnosis secondary to *lead-time bias* and *length bias*.

Figure 1 illustrates lead-time bias, which is the apparent increase in survival rate because the cancer was diagnosed earlier because of screening. The cancer patient actually dies exactly when he would have died without the screening but the time from diagnosis to death is longer because the cancer is detected earlier. This means the therapy had no effect in terms of lengthening life. Furthermore, it means that the mortality rate is unchanged. Because of very aggressive *screening*, many cancers are being diagnosed much earlier than they otherwise would have been. More pre-cancerous lesions are also being diagnosed and counted in statistics even though many would never have proceeded to cancer. This *overdiagnosis* also factors into lead-time bias. Many cancers that are now diagnosed would never have killed the individual but are now counted in survival rate calculations. Along related lines, because of the emphasis on screening, cancers are now being diagnosed in the elderly that would not have been diagnosed a few decades ago. The elderly do not usually succumb to these cancers, which is why they were not screened for them in the past, yet they are now counted as survivors.

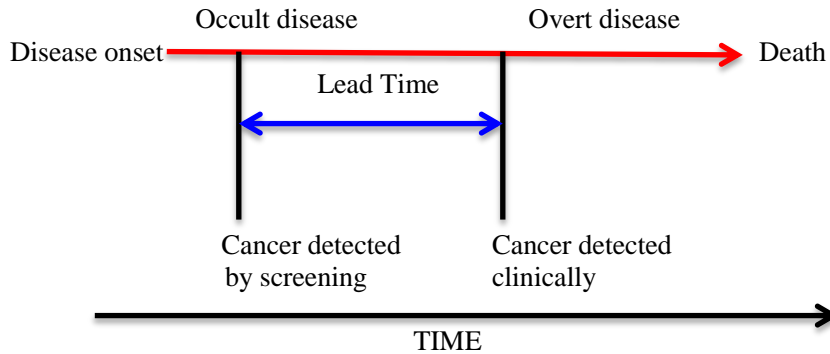


Figure 1. Lead-time bias

Length bias is similar to lead time bias in that it also allows an overestimate of survival because of faulty reasoning. Slowly progressing cancers are more likely to be detected by screens; they grow for a longer period of time hence there is a longer period of time in which to detect them. Rapidly growing cancers are less likely to be detected and more likely to result in death in a shorter period of time

There are other factors complicating the attempt to understand cancer survival rates. Cancer survival statistics are based on death certificates, which are very unreliable. The cause of death is usually given as the most proximate cause, for example pneumonia, when the actual cause, as well as the cause of the pneumonia, was the cancer. In part, the miscalculation of mortality and survival, including the reliance on death certificates, is due to the fact that autopsies are not performed with the same frequency they once were. Furthermore, the people receiving autopsies are disproportionately victims of non-disease, for example trauma patients, poisonings, and murder.(Hendrick 2011) Without an autopsy, the actual cause of death may be missed or the more proximate cause may be listed as the primary cause.

This is not to say there are *no* chemotherapeutic agents that reliably treat cancer and extend life. Some of the leukemias have been treated successfully for decades and are no longer an automatic death sentence for the patient. Regardless of these advances in leukemia, the increase in survival rate referred to by David Scott in the opening paragraph is very misleading. There has been very little progress made in the War on Cancer. Statements such as Scott's are not uncommon among scientists and representatives of scientists who are engaged in research on cancer. I will now examine how animal models have been used during past five decades.

2.0 Prediction in Biomedical Science

Before examining the use of animal models specifically, I need to discuss prediction in biomedical science. Animals can be categorized into essentially nine areas of science and biomedical research (table 1 (Greek & Shanks 2009)). The two areas that concern cancer research and drug development for cancer are numbers 1 and 2, the use of animals as predictive models. Which leads us to the question of what the term *predict* means in science. *Predict* is used in basically two distinct ways in science. First, scientists generate hypotheses, which are then tested. The test usually involves a prediction about an unknown outcome and the hypothesis is strengthened or weakened, perhaps even falsified, by whether the prediction is found to be true. Thousands of predictions are generated by hypotheses and the validity or outcome of the prediction has no immediate meaning outside the specific context of the hypothesis.

This use of *predict* differs immensely from the way the word is used when judging the *predictive value* of a modality such as diagnostic test, research practice, medical intervention, or any practice that can be judged by reality or a gold standard (see table 2). A modality, say a diagnostic test, is not being used to generate a

hypothesis but rather to determine intervention. There are consequences to human health if the test does not do what the physician assumes it does. For example, if a chest x-ray reveals the absence of a pneumothorax (a collapsed lung) there is still a slight probability that the patient does in fact suffer from a pneumothorax. If the physician needs to be 100% certain that the patient does not have a pneumothorax, then a CT scan needs to be performed, as it is the gold standard for diagnosing the condition. All tests that might be useful in diagnosing a pneumothorax can be compared against the CT scan per table 2, because the CT scan is the gold standard.

1. As predictive models for human disease
2. As predictive models to evaluate human exposure safety in the context of pharmacology and toxicology (e.g., in drug testing)
3. As sources of 'spare parts' (e.g., aortic valve replacements for humans)
4. As bioreactors (e.g., as factories for the production of insulin, or monoclonal antibodies, or the fruits of genetic engineering)
5. As sources of tissue in order to study basic physiological principles
6. For dissection and study in education and medical training
7. As heuristic devices to prompt new biological/biomedical hypotheses
8. For the benefit of other nonhuman animals
9. For the pursuit of scientific knowledge in and of itself

Table 1. Categories of animal use in science.(Greek & Shanks 2009)

		Gold Standard	
		GS+	GS-
Test	T+	TP	FP
	T-	FN	TN
Sensitivity = $TP / (TP + FN)$			
Specificity = $TN / (FP + TN)$			
Positive Predictive Value = $TP / (TP + FP)$			
Negative Predictive Value = $TN / (FN + TN)$			
T- = Test negative T+ = Test positive FP = False positive TP = True positive FN = False negative TN = True negative GS- = Gold standard negative GS+ = Gold standard positive			

Table 2. Binary classification test. Allows calculations for determining how well a test or practice compares with reality or the gold standard.

When animal modelers claim that a model is predictive for humans be it for evaluating safety and efficacy or any other property of a drug, they are claiming that the positive predictive value (PPV) and negative predictive value (NPV) are high enough for the modality to be deemed predictive in biomedical science. The same applies when seeking to determine mechanisms of disease. When evaluating modalities in biomedical science and medical practice, PPVs and NPVs greater than 0.9 are needed if the modality is to be considered a predictive test, intervention, treatment, or practice. Ideally, NPVs and PPVs would be very close to 1.0. As we will see, animal models do not approach this standard and hence are not considered predictive for human response to drugs or disease, including cancer.(Collins 2011; Cook et al. 2012; Dixit & Boelsterli 2007; Drake III et al. 2012; FDA 2006; Fletcher 1978; Greek & Greek 2010; Greek, Hansen, et al. 2011; Greek et al. 2012; Greek & Shanks 2009; Greek, Shanks, et al. 2011; Heywood 1990; Horrobin 2003; Kola & Landis 2004; Lumley 1990; M.E. 2010; Markou et al. 2009; O'Collins et al. 2006; Shanks & Greek 2009; Shanks et al. 2009; Sharp & Langer 2011; Sietsema 1989; Suter 1990; Wall & Shani 2008; Weaver et al. 2003; Zielinska 2010)

3.0 Animal Models and Carcinogenesis

Over 400 chemicals had been discovered to cause cancer in animals (Gold et al. 1991; Ames & Gold 1990, 1990; Ames et al. 1996; Ashby & Paton 1993; Fung et al. 1993; McGregor et al. 1994) but only approximately 20-30 were shown to be definite carcinogens in humans as of the 1990s (Ennever et al. 1987; Stoloff 1992; Kleinman 1997). Performing the calculation for PPV, based on this data we find: $30 / (30 + 370) = 0.075$. This is less than what one would expect from random chance or just guessing which chemicals are carcinogens and is certainly unacceptable for a scientific modality. Other studies have been consistent with the 1990s data in that the PPV of animal models is far below what is acceptable in medical science (Coulston 1980; Abbott 2005; Anisimov et al. 2005; Salsburg 1983; Meijers et al. 1997; Dybing & Huitfeldt 1992; Dybing & Sanner 1999). Salsburg compared the predictive value of animal models for carcinogenesis to a coin toss (Salsburg 1983). Granted, every chemical assessed in animal models does not have extensive epidemiological data proving the chemical is definitively *not* a human carcinogen. In light of intra-human differences, some of the chemicals probably could be carcinogens in some people at some time if given in high enough doses. However, this does not negate the fact that a vast majority of these chemicals are clearly not carcinogenic in a clinically significant manner or such would have been noted by physicians as well as individuals affected.

If all the chemicals that tested positive for carcinogenesis in animal models had been prevented from coming to the marketplace, society would have lost many valuable drugs as well as other chemicals. For example, isoniazid an anti-tuberculosis drug that has been used for decades, causes cancer in some animals.(Clayson 1980; Shubick 1980) Phenobarbital, an anti-seizure medication, would not have been approved because it causes cancer in mice and rats.(Clemmensen & Hjalgrim-Jensen 1980) Moreover, studies conducted on mice and rats revealed that 46% of chemicals discovered to be carcinogens in rats were *not* carcinogens in mice, despite mice and rats being phylogenetically more closely related than rodents and humans.(Di Carlo 1984) Di Carlo stated: "It is painfully clear that carcinogenesis in the mouse cannot now be predicted from positive data obtained from the rat and vice versa.(Di Carlo 1984)

Studies like these led David Salsburg then-of Pfizer to state that the "lifetime feeding studies in mice or rats using maximum tolerated doses of the test compound" fail to meet the criteria for predicting human response and suggested that tossing a coin would have yielded better results.(Salsburg 1983) The Centers for Disease Control and Prevention (CDC) stated: "Most of what we know about chemicals and cancer in humans comes from scientists' observation of workers. The most significant exposures to cancer-causing chemicals have occurred in workplaces where large amounts of toxic chemicals have been used regularly."(ATSDR 2002)

Intuitively, one would suspect that mice and rats would share more similarities with each other than either species would share with humans and that nonhuman primates would better predict human response. Beniashvili, writing in the book *Experimental Tumors In Monkeys* stated that monkeys are "highly resistant to

certain blastogenic agents, carcinogenic for other animals” and that, “Spontaneous tumors in monkeys are very rare,” as are lung tumors and tumors of the mediastinum and soft tissues. Beniashvili also noted that skin tumors rarely metastasize in nonhuman primates while they do so in humans.(Beniashvili 1994) There are substantial inter-species differences in the genetics that lead to cancer. Human cancers appear to result from more alterations in genes that do rodent cancers.(Hahn & Weinberg 2002; Rangarajan & Weinberg 2003) Multiple other inter-species differences in carcinogenesis have been described.(Dybing & Sanner 1999; Ennever, Noonan, and Rosenkranz 1987; Habeck 2002; Marsoni et al. 1987; Corry 1952; Rohan et al. 2000; Editorial 2006; Kamb 2005)

The most infamous example of animal models failing to identify a carcinogen is cigarette smoking. Animal models were, and indeed still are, used by tobacco companies to cast doubt on whether their product causes cancer.(Oreskes & Conway 2011) Eric Northrup, a journalist wrote in his 1957 book *Science Looks At Smoking*: “The failure of many investigators . . . to induce experimental cancers [in animals], except in a handful of cases, during fifty years of trying, casts serious doubt on the validity of the cigarette-lung cancer theory.”[(Northrup 1957) p133] The introduction to the book was written by then-chairman of the Yale Department of Pathology, Dr. HSN Greene, who agreed with the statement. Even using current technologies, smoking simply does not cause cancer in a vast majority of lab animals.(Chu et al. 1981) Utidjian states: “Surely, not even the most zealous toxicologist would deny that epidemiology, and epidemiology alone, has indicted and incriminated the cigarette as a potent carcinogenic agent, or would claim that experimental animal toxicology could ever have done the job with the same definition.”(Utidjian 1988) Coulston and Shubick reinforce this stating: “For decades the clinical observation of an association between cigarette smoking and bronchial carcinoma was subject to unfounded doubt, suspicion, and outright opposition, largely because the disease had no counterpart in mice. There seemed no end of statisticians craving for more documentation, all resulting in the fateful delay of needed legislative initiative.”(Clemmensen & Hjalgrim-Jensen 1980)

The use of animal models to cast doubt on the carcinogenicity of smoking continues. William Campbell, president and CEO of Phillip Morris was quoted in the *New York Times* December 6, 1993 as testifying under oath before the US Congress as follows:

Q. Does cigarette smoking cause cancer?

A. To my knowledge, it’s not been proven that cigarette smoking causes cancer.

Q. What do you base that on?

A. I base that on the fact traditionally, there is, you know, in scientific terms, there are hurdles related to causation, and at this time there is no evidence that they have been able to reproduce cancer in animals from cigarette smoking.

Another notable failure for animal models was asbestos. It was not until the 1960s that researchers were able to reproduce *some* of the human effects of the asbestosis in animals. Asbestos had been linked to human cancer much earlier.(Gardner 1938; Wagner 1963; Wagner et al. 1974; Enterline 1978; Smith et al. 1965; Enterline 1988) The *New York Academy of Sciences* assured people in 1965 that: “a large literature on experimental studies has failed to furnish any definitive evidence for induction of malignant tumors in animals exposed to various varieties and preparations of asbestos by inhalation or intratracheal injection.”(Smith et al. 1965)

Casting doubt on the carcinogenicity of smoking and asbestos are not small indiscretions of a model. In terms of the number of deaths due to a failure of a model, these two examples might rank the highest. If a model fails this badly and continues to be used, one must question the motivation of the people using the model. The other side of the coin is the drugs and chemicals that society did not have access to secondary to false positives in animal models. For example, when saccharine first came on the market in the US, many people would not use it because it had caused cancer in rats. Rats have an enzyme in their bladder, which human lack, which interacts with the saccharine and causes cancer.(Cohen & Ellwein 1990). Moreover, even female rats lack the enzyme. Cancer occurred only in male rats. As I will explain, there are simply too many differences between humans and animals for animal models to be used as surrogates for humans in carcinogenicity testing.

4.0 Animal Models and Antineoplastics

The idea behind using animal models in order to ultimately create new treatments for cancer can be summarized as follows: basic research using animal models of cancer will lead to the discovery of mechanisms that can then be targeted in the form of drugs. Targets for new drugs arise from basic research that usually involves animal models and the funding for this type of research has increased dramatically in recent decades. Despite this however, the number of new drugs being approved by the FDA is no greater than 50 years ago. (Munos 2009) The above philosophy also assumes that inter-species extrapolation will be viable. In light of what I just discussed regarding animal models and carcinogenicity, and in light of the fact that evolution holds true for mechanisms related to treatments just as it does for mechanisms of carcinogenesis, we might expect treatments that were safe and efficacious in animals to fail in humans. As we will see, this is indeed the case. Yet, as late as 2011, Harold Varmus, Nobel laureate and then-director of the National Cancer Institute (NCI) confirmed that the NCI would continue to fund animal models and basic research. (Wadman 2011) Varmus is not alone in this position. Nic Jones, the Chief Scientist for Cancer Research UK (CRUK), in 2011 also confirmed support from CRUK for basic research using animal models. When asked if such research might be the reason so little progress has been made in finding new treatments, he replied: "I would argue that we have been using the wrong mouse models." (Editors 2011)

The logic undergirding the above positions is problematic for empirical reasons in addition to evolutionary biology, however. The success rate for new drugs in all areas of development is dismal. Out of 5,000-10,000 chemicals that enter the drug development pipeline only one will enter the market. (European Commission 2008; Hughes et al. 2011) Moreover, the major cost of drug development occurs during the clinical trials and the attrition rate during this stage is equally dreadful. (Unknown 2002; Shaffer 2012; Paul et al. 2010; Schachter 2007) Drugs entering Phase I trials have approximately a 9% chance of coming to market. (FDA 2004; Sarkar 2009; Editorial 2007; Paul et al. 2010) Of the drugs that advance to Phase III, less than 50% are marketed. (Arrowsmith 2011) The failure rate for oncology drugs is even higher. (Editorial 2011; Caponigro & Sellers 2011; Arrowsmith 2011; Begley & Ellis 2012) Only 5% of cancer drugs that have an Investigational New Drug Application (IND) eventually go to market. (Kummar et al. 2007) Lack of safety or efficacy accounts for approximately 90% of drug failures during clinical trials. (Kola & Landis 2004; Arrowsmith 2011). Both safety and efficacy determinations rely on animal models. To complicate matters further, the pipeline in Pharma is drying up and fewer drugs, especially new chemical entities (NCEs) are being marketed. (Editorial 2008; GBI Research 2011)

Why is the attrition rate so high? In large part: animal models. Björquist and Sartipy state: "Furthermore, the compound attrition rate is negatively affected by the inability to predict toxicity and efficacy in humans. These shortcomings are in turn caused by the use of experimental pre-clinical model systems that have a limited human clinical relevance . . ." (Björquist & Sartipy 2007) Then-U.S. Secretary of Health and Human Services Mike Leavitt stated in 2006: "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." (FDA 2006) Johnson et al. found that out of 39 anticancer drugs tested on xenograft mice, only one mimicked the response in humans. (Johnson et al. 2001) Oncology drugs fail more frequently in clinical trials than most other categories. (DiMasi & Grabowski 2007; DiMasi et al. 2010)

There have been many attempts to reproduce human cancers in mice. The nude mouse lacked the *FOX1* gene, the SCID mouse was created with a very deficient immune system, and there have been many more models. All have failed to predict human response and have misled researchers. Zielinska discusses mouse models of cancer stating they: "rarely predict how a human will respond to the same treatment." Zielinska then quotes Marks of the NCI, and who is also head of the Mouse Models of Human Cancers Consortium, as saying: "we had loads of models that were not predictive, that were [in fact] seriously misleading." (Zielinska 2010) The NCI had previously tested mice with 12 anti-cancer drugs being successfully used to treat humans. The mice were growing 48 different kinds of human cancers. The study revealed that 30 out of 48 times (63%) the

drugs that were effective against human cancers were ineffective in the mice that were growing the human cancers. The NCI believes efficacious treatments for human cancers have been lost because of animal testing. (Gura 1997)

The problem of animal models is well known to the drug development community. Cook et al state: “Over many years now there has been a poor correlation between preclinical therapeutic findings and the eventual efficacy of these [anti-cancer] compounds in clinical trials (Johnson et al. 2001; Suggitt & Bibby 2005). . . . The development of antineoplastics is a large investment by the private and public sectors, however, the limited availability of predictive preclinical systems obscures our ability to select the therapeutics that might succeed or fail during clinical investigation.”(Cook, Jodrell, and Tuveson 2012) Singh and Ferrara echo this, stating: “Over 90% of phase 3 clinical trials in oncology fail to meet their primary endpoints despite encouraging preclinical and even early-stage clinical data. This staggering and sobering figure underscores the limitations of existing animal models for the evaluation of potential anticancer agents. The paucity of models is especially apparent with the advent of drugs that target the tumor milieu, or microenvironment, such as anti-angiogenics . . . immunotherapies and compounds directed against tumor-associated fibroblasts.”(Singh & Ferrara 2012)

Wittenburg and Gustafson agree, stating: “The current drug development pathway in oncology research has led to a large attrition rate for new drugs, in part due to a general lack of appropriate preclinical studies that are capable of accurately predicting efficacy and/or toxicity in the target population. . . . One of the most serious challenges currently facing pharmaceutical research of novel anti-cancer therapeutics is the lack of translation of efficacy and safety from preclinical models to human clinical trials, leading to a large attrition rate of investigational compounds. For new oncology drugs, only about 5% of investigational new drug applications submitted progress beyond the investigational phase due to a general lack of preclinical systems that can accurately predict efficacy and toxicity of new agents.”(Wittenburg & Gustafson 2011)

Animal models fail to predict safety as well as efficacy. Reviewers of Phase I trials conducted by the National Cancer Institute (NCI) from 1991-2002 discovered that 15% of participants undergoing single agent chemotherapy agents suffered serious side effects.(Horstmann et al. 2005) Richard Klausner, then-director of the NCI said: “The history of cancer research has been a history of curing cancer in the mouse. . . . We have cured mice of cancer for decades—and it simply didn't work in humans.”(Cimons et al. 1998) In an editorial to two articles, *Nature Medicine* stated: “The complexity of human metastatic cancer is difficult to mimic in mouse models. As a consequence, seemingly successful studies in murine models do not translate into success in late phases of clinical trials, pouring money, time and people’s hope down the drain.”(Ellis & Fidler 2010; Van Dyke 2010) Caponigro and Sellers of the Novartis Institutes For BioMedical Research, Oncology Research and Oncology Translational Medicine stated in 2011: “Despite an improved understanding of the biology of cancer, and an unprecedented volume of new molecules in clinical trials, the number of highly efficacious drugs approved by the regulatory authorities remains disappointingly low. The significant attrition rate of drugs entering clinical trials comes at a high price. This price is paid primarily by the underserved patient and secondarily by the pharmaceutical and biotechnology community, which invests enormous resources perfecting a molecule only to watch it fail in humans . . .”(Caponigro & Sellers 2011)

Cancer researcher Robert Weinberg, of Massachusetts Institute of Technology, was quoted by Leaf in *Fortune* magazine as saying: “And it’s been well known for more than a decade, maybe two decades, that many of these preclinical human cancer models have very little predictive power in terms of how actual human beings—actual human tumors inside patients—will respond . . . preclinical models of human cancer, in large part, stink . . . hundreds of millions of dollars are being wasted every year by drug companies using these [animal] models.”(Leaf 2004) Leaf also quotes Homer Pearce, “who once ran cancer research and clinical investigation at Eli Lilly and is now research fellow at the drug company” as saying: “. . . that mouse models are ‘woefully inadequate’ for determining whether a drug will work in humans. ‘If you look at the millions and millions and millions of mice that have been cured, and you compare that to the relative success, or lack thereof, that we’ve achieved in the treatment of metastatic disease clinically,’ he says, ‘you realize that there just has to be something wrong with those models.’”(Leaf 2004) Others have also pointed out the inadequacy of animal models of cancer, including genetically modified animal models.(Frese & Tuveson 2007; Kerbel 2003; Singh et

al. 2010; Talmadge et al. 2007; Peterson & Houghton 2004; Francia & Kerbel 2010; Johnson et al. 2001; Zielinska 2010; Wade 2009)

Tamoxifen is a good example of the shortcomings of animal models in general. Tamoxifen was originally touted as a birth control pill based on rat studies and was only later found to be an anticancer chemical. Moreover, it was ineffective as an oral contraceptive as it actually increased a woman's likelihood of becoming pregnant.(Jordan & Robinson 1987) Tamoxifen acts by binding to the protein known as tubulin thus inhibiting cell division. After discovered to be effective against cancer, Tamoxifen was shown to causes liver tumors in some strains of rat, but not in mice or hamsters.(Powles 1992) If this had been discovered in preclinical trials, the drug would not have come to market.(Editorial 2003) According to D. N. Richardson of the Imperial Chemistries Industries PLC: "No laboratory tests for anti-tumour activity were carried out for Nolvadex [tamoxifen] until after the activity in human patients had been confirmed."(Richardson 1988) The most common side effect of Tamoxifen is nausea and vomiting, which was not seen in dogs, which are touted as the best species to use when looking for that side effect.(Tucker et al. 1984)

Sadly, even the drugs that do come to market are too frequently not very effective against cancer. In the case of breast cancer, for instance, most women do not benefit from chemotherapy. As a general rule, one-third of women diagnosed with breast cancer would have improved without the chemotherapy and one-third would have died with or without it. Only one-third actually benefit from the treatment. Along the same lines, chemotherapies for cancer have decreased the size of the tumors but at the expense of an increase in frequency of secondary tumors and a very adversely affected lifestyle. Furthermore, most chemotherapy does not prolong life or result in a longer, high quality life.(Bear 2003; Savage 2008; Mittra 2007)

5.0 Evolved Complex Systems

Medical science was very different in the 19th century when animals were first used as models for humans. The structure of DNA had not been elucidated, scientists thought the poliovirus entered via the nose (it enters through the gut) (Paul 1971), the notion of a *magic bullet* (that for every disease, or at least every infectious disease, a chemical existed that could interact with the single site causing the malady and thus cure the disease without harming the rest of the body) via Ehrlich and Salvarsan (Ehrlich & Hata 1910) was foremost in the minds of drug developers, the modern synthesis in evolution was brand new (Mayr 2002), and the creationist-based position of the influential animal modelers was that animals and humans seemed to be more or less the same except for humans having a soul.(LaFollette & Shanks 1994; Bernard 1957; Elliot 1987) There were no organ transplants, infectious diseases were still a major killer in the developed world, the fields of cognitive ethology and animal cognition were unheard of, and differences between ethnic groups (Cheung et al. 1997; Couzin 2007; Gregor & Joffe 1978; Haiman et al. 2006; Spielman et al. 2007; Stamer & Stuber 2007; Wilke & Dolan 2011) and sexes (Holden 2005; Kaiser 2005; Simon 2005; Wald & Wu 2010; Willyard 2009) in terms of disease and drug reactions had not yet been discovered. Physics was just beginning to cast off the shackles of determinism and reductionism but chaos and complexity theory was still on the horizon. It was a different world. People in the 1800s are to be excused for thinking that animals and humans would react more or less the same to drugs and disease. I will now bring the reader into the current scientific environment as it relates to our topic.(LaFollette & Shanks 1993; LaFollette & Shanks 1994, 1996; Shanks & Greek 2009; Shanks, Greek, and Greek 2009; Greek & Greek 2010; Greek, Shanks, and Rice 2011)

Two major advances in science, that are relevant to our topic, have occurred in the past three decades. First, the field of evolutionary biology has continued to develop. The new division of evolutionary biology known as evolutionary developmental biology, or evo devo, is one example of the important advances in the field of evolution. Evo devo arguably began in 1978, when Lewis (Lewis 1978) published his findings on the anterior-posterior layout of the fruit fly, *Drosophila*. In 1984, the homeobox genes were discovered by McGinnis et al.(McGinnis et al. 1984) The homeobox genes are responsible for the body plan of "bilaterian"

organisms. Bilaterians, of which humans are an example, are symmetrical around two axes. The homeobox genes are responsible for the way the body is configured: the arms here, the thorax there and so on. (Gellon & McGinnis 1998) The homeobox are active in early embryogenesis, organizing the cell and anterior–posterior body layout. (Slack et al. 1993) While there are differences among species—for example, there are nine homeobox genes in flies contrasted with thirty-nine in mammals—the overall use of the homeobox is the same. Discoveries such as the homeobox allowed scientists to appreciate the fact that mammals, and animals in general, have much in common in terms of their genetic composition. The differences among species were not to be completely explained by different species having different genes.

The concepts learned from evo devo and evolutionary biology in general tie in closely with discoveries from the Human Genome Project (HGP) (McPherson et al. 2001; Venter et al. 2001) and other spin-off projects. Prior to the HGP, scientists thought the number of genes was proportional to the complexity of the organism. The number of genes in some organisms was known or approximated; therefore, the scientists involved in the HGP were looking for an estimated 100,000+ genes in humans. As the project advanced, it became clear that humans had nowhere near this many genes. This was perplexing.

Because of evo devo, the HGP and its spinoffs, and speculation by King and Wilson (King & Wilson 1975) in the 1970s, scientists now know the following. All mammals have more or less the same genes. Some species have a few genes that other species do not have, but one could more or less build any mammal using the genes from another. The differences among species lie, in large part, in the *regulation* and *expression* of the same genes. The genes that build the body are known as structural genes, while the genes that turn the structural gene on and off are called regulatory genes. Think of your genetic composition as the keys on a piano. Every piano has the same keys (structural genes). But each piano can be played so as to produce a variety of tunes. The reason for this is that the structure of the piano allows for keys to be pressed at various intervals and in various combinations. The sheet music dictates when and how to press the keys. Likewise, the regulatory genes (the sheet music) tell the structural genes (the keys) when to be active (expressed) and for how long. For example, humans and mice both have the gene that allows mice to grow a tail. In humans, this gene is not activated during embryogenesis, hence humans have no tail. (Evidence for this is found in the fact that, very rarely, this gene will be turned on in humans and the baby will be born with a tail.) Traits can be determined or modified based on how long a gene or set of genes is activated, for example allowing the thumb position to migrate down the hand or for the fingers to lengthen.

There are other differences among species and almost all are related to evolution. Different enzymes metabolize different drugs, metabolize the same drugs at different rates, and form different metabolites, all of which influence toxicity and dosing. There are also differences in how many copies of a drug-metabolizing gene various animals have. If species A has 10 copies and species B has one copy, then species A might metabolize a drug 10 times faster than species B. This also has significance for dosing and for toxicity. For example, trastuzumab (Herceptin), an anti-breast cancer drug, is prescribed for women who carry multiple copies of, or overexpress, the gene *HER-2/neu*. (Gonzalez-Angulo et al. 2006)

Species, and even individual humans, can differ in genetic composition. For example, there may be differences in:

- The presence (or absence) of certain genes.
- The presence (or absence) of certain alleles.
- The background genes and modifier genes that influence the genes being perturbed by drugs or disease.
- The regulation and expression of genes.
- Gene networks.
- Alternative splicing, which allows one gene to form or be part of forming many different proteins.
- Proteins and protein–protein interactions.
- Gene–protein interactions.
- Old genes evolving to perform new functions.

- Horizontal gene transfer (HGT). HGT occurs when genes from one organism are incorporated into another organism without the recipient organisms being the offspring of the donor. For example, resistance to anti-bacterial drugs can occur through HGT.
- Epigenetics. Epigenetics is the relatively new field that studies changes in gene expression that can be inherited and that occur without changing the underlying DNA sequence. For example, because of environmental influences, a regulatory gene may be changed such that it is turned on or off thus allowing a disease to manifest.
- The presence of gene and chromosomal mutations such as single nucleotide polymorphisms (SNPs), copy number variants (CNVs), duplications, inversions, deletions, and insertions.

In response to a perturbation to the system, such as a drug or disease, even one of the above differences can result in life or death consequences. Furthermore, *convergent evolution* can result in the same trait being present but being mediated by very different pathways in different species. Different molecules can also perform the same function. *All* of these types of differences are present in every species.

There are, of course, similarities among species. Some of these similarities are referred to as *conserved processes*, which are basic functions of a cell that have been present since early evolutionary times. The homeobox, described above, is an example of a conserved process. Conserved processes occur in living complex systems that have differences like those outlined above. These differences result in the conserved process being influenced by various factors that are unique to each species and even each individual within each species. Importantly, we understand how modifications in the genome, like those mentioned above, have resulted in the evolution of different body types and indeed different species. (Gellon & McGinnis 1998; Wagner et al. 2003; Amores et al. 1998; Garcia-Fernandez 2005) Therefore, even when animals and humans share genes and traits, they will most likely still react differently to diseases and drugs.

The second major change in science that is relevant to our discussion is the development of chaos and complexity science, replacing, in part, the deterministic paradigms. For centuries, physics, and science in general, saw the world through the eyes of Descartes and Newton. Newtonian physics is closely connected to reductionism and determinism. Reductionism maintains that everything can be reduced to its component parts, those parts examined and understood, and then the whole explained based on it being the sum of the parts. Determinism means that once for certain systems, once the initial conditions are known only one outcome is possible. Reductionism and determinism lead to a very linear process with A leading to B leading to C and so on. The Newtonian physics of inclined planes, velocities, forces, a point representing an object, and so forth explores simple systems amenable to reductionism and determinism. The early 20th century saw advances in science that challenged reductionism and determinism. For instance, relativity and quantum mechanics revolutions in physics could not be explained by reductionism. Later in the 20th century chaos and complexity science would be developed, thus changing the way reductionism and determinism were viewed by all of science.

Reductionism worked very well for science and still has a role to play. But some systems are not the simple systems that conform so well to study by reductionism. Some systems are *complex systems* and have rules and characteristics of their own. Complex systems are more than merely a sum of their component parts. Complexity is related to chaos theory. Chaos is perhaps best understood by examining the original experiments performed by Lorenz in 1961. While running weather simulations on a computer, Lorenz shortened a number in an equation from six decimal places to three. When he re-ran the program he found the results were very different from the original. Translating from computer-speak, what he found was that where it had been sunny on day 15, it now rained. Because of the extremely small change in the initial conditions of the program, the outcome was essentially the opposite from the original. This very small change in initial conditions is what phrases like “a butterfly flaps its wings in China and causes a tornado in Kansas” are referring to. Seemingly unimportant differences between two situations or systems can translate into major differences in outcomes. For example, you may eat chocolate but it can kill your dog. The reason for this is the fact that dogs lack the enzyme, or have the enzyme but only in very small quantities, that metabolizes a potentially toxic ingredient in

chocolate known as theobromine. Something as simple as the presence or absence of an enzyme can have fatal consequences.

Lorenz's computer experiment, along with work done by other scientists including Poincaré, gave rise to chaos theory and complexity theory. A major difference between chaotic systems and complex systems is that chaotic systems are deterministic. Given enough computer power and knowledge of the system, outcomes could be predicted. This is not the case with complex systems because they exhibit, among other things, *emergent* properties. Emergence is the presence, in a system, of new properties that could not have been predicted even with total knowledge of the component parts from which the emergent property arose. Financial markets, the behavior of ant colonies, cells, and living organisms are examples of complex systems whereas the weather and the red spot on Jupiter are examples of chaotic systems.

Complex systems, including humans and other animals, have the following characteristics.(Ahn et al. 2006; Alm & Arkin 2003; Cairns-Smith 1986; Csete & Doyle 2002; Goodwin 2001; Jura et al. 2006; Kauffman 1993; Kitano 2002, 2002; Monte et al. 2005; Morowitz 2002; Novikoff 1945; Ottino 2004; Sole & Goodwin 2002; Van Regenmortel 2004, 2004; Van Regenmortel & Hull 2002; Vicsek 2002; Woodger 1967)

1. The whole is greater than the sum of the parts. This is, in part, because of emergent properties. Because complex systems exhibit the characteristics of emergence and the whole being greater than the sum of its parts, they cannot be completely described via reductionism.
2. Different levels of organization exist and a perturbation to the system as a whole may affect each level differently.
3. There are a large number of components or parts and these can combine to form modules that interact with each other and the environment. Feedback loops also exist among the parts and modules.
4. The system displays robustness, meaning it is resistant to change, and redundancy, meaning that the loss of one part may be compensated for by another part.
5. Complex systems are best described by differential equations and are examples of nonlinearity. Nonlinearity means that a small perturbation may have no effect on the system or a very large effect. Cause does not give rise to effect in the linear way it does in a simple system.
6. The particular manifestations of complex systems and chaotic systems are both determined in part or in whole by initial conditions. For example, changing or deleting just one gene in a living complex system might result in death or in no noticeable change whatsoever. This has important implications as studies have demonstrated that deleting a gene in a mouse may result in the death of one strain but not another. Similarly, a gene may be required for human development but not the development of mice or other animals.

Other factors leading to complications for trans-species extrapolation, and even intra-species extrapolation, are the stochastic nature of mutations and the intrinsic fluctuations and environmental disturbances (noise) of the system (Chen & Lin 2011; Chen & Wang 2006). While addressing these concepts is beyond the scope of this chapter, they are nonetheless important factors and I refer the reader to Chen et al for more information.

Humans and animals are living complex systems that have different evolutionary trajectories. Therefore, animals and humans have very different initial conditions in the form of the genetic differences listed above. It follows that one species may respond to perturbations such as drugs and disease in a manner that cannot be predicted based on the response of a different species. Moreover, all of the characteristics of a complex system, and the differences between complex systems that have occurred because of evolution, have a major impact on inter-species extrapolation. This was not appreciated during the era of the Nuremberg trials. Predicting outcomes within a complex system is problematic; predicting an outcome for one complex system based on the outcome from another is virtually impossible. Nevertheless, this is exactly what scientists are attempting to do when they test a drug on a mouse or monkey in an attempt to ascertain what it will do to a human.

6.0 Personalized Medicine

Because humans are evolved complex systems it should not be surprising that even individual humans react differently to drugs and disease. Gabor Miklos writes: “There is enormous phenotypic variation in the extent of human cancer phenotypes, even among family members inheriting the same mutation in the adenomatous polyposis coli (APC) gene believed to be causal for colon cancer. In the experimental mouse knockout of the catalytic gamma subunit of the phosphatidyl-3-OH kinase, there can be a high incidence of colorectal carcinomas or no cancers at all, depending on the mouse strain in which the knockout is created, or into which the knockout is crossed . . . Thus, although a mutation-cataloging research megaproject may be a diverting occupation for sequencing centers and gene hunters, leading scientists should think carefully before they tout its therapeutic promise to patients and politicians. The simple truth is that the money would be much better spent if research priorities were reevaluated. A good place to start would be to dismiss the fallacious notion that single mutations in primary tumors are the optimal starting point for research that would lead to the discovery of new, more effective cancer drugs. The clinical reality is that it is not single genes, but rather the properties of aneuploid-based methylated networks that allow metastatic cancer cells to explore novel niches in different genetic backgrounds and to rapidly become resistant to drug-based therapies.”(Miklos 2005)

Serrano et al. discussed the role of the gene *SULT1A1* and 33 alleles of the enzyme CYP2D6 and 3 of CYP2C19 in the metabolism of tamoxifen. They discovered that out of 182 patients, 8 were poor metabolizers of tamoxifen, 151 were extensive metabolizers, 17 were intermediate metabolizers and 3 were ultra metabolizers.(Serrano et al. 2011) Such intra-species differences alter treatment strategies.

Powell et al examined circulating tumor cells (CTCs), cells that are circulating in the bloodstream that are derived from the original cancer.(Powell et al. 2012) They discovered that the CTCs were genetically diverse. This is important but perhaps even more important some of the cells have genes turned on such that they can implant into other tissues easier. This also explains why patients respond so differently to treatments and why different treatments may be needed in the same patient. It also, again, supports the notion that animal models are never going to be predictive modalities for human response to drugs and disease.

Heng, writing in *JAMA*, discussed the use of reductionism in biomedical research. Heng noted that approaching living systems like they were clockwork systems was rewarding in the early days of science. However, science is now studying human disease at the level where complexity science becomes important hence reductionism can be misleading. Heng: “Likewise, chemotherapy often initially reduces tumor size but also produces severe adverse effects leading to other complications, including the promotion of secondary tumors. Most important, little evidence exists that more aggressive chemotherapies prolong life for many patients. In fact, chemotherapies may have overall negative effects for some patients.(Heng 2008) One reason for this is what are classified as the same cancer can have different genetic profiles in addition to being caused by different genetic aberrations.(Wood et al. 2007; Heng 2007; Heng et al. 2006) Complicating matters further, epigenetic factors that result in mutations in regulatory genes, can account for a high number of differences in response to chemotherapy.(Brower 2011) (For more on personalized medicine see (Greek, Menache, and Rice 2012).)

7.0 Productive Areas of Research

There are many research modalities with a history of providing results that are applicable to humans and society should demand that biomedical researchers that use animal models switch to these methods. All of these methods are either human-based or come from the physical sciences. Nobel laureate Sydney Brenner, who was awarded the Prize for research on *Caenorhabditis elegans*, advocated for more research using *Homo sapiens* even referring to *Homo sapiens* as “the model organism.”(Ledford 2008) Human-based research includes epidemiology, *in vitro* research with human tissues, clinical observation, post-mortem examinations, computer

modeling based on human findings, genome-wide association studies, *enforced* post-marketing drug surveillance, microdosing, and research with human stem cells among other methods. The first chemotherapies were largely based on human observation, not mechanism-based. Lord and Ashworth state: “Clinicians will attest that cytotoxic chemotherapy regimens, developed with the limited biological information available at the time of their development, remain the mainstay of treatment for most cancers. . . . Following the discovery of chemotherapeutics, the next significant advance came in the 1960s with the straightforward notion of combining drugs. The rationale for this came from the treatment of tuberculosis, for which antibiotics, each with a different mechanism of action, were more effective when used in combination. For cancer, it was considered that the development of resistance to a battery of agents used concurrently, rather than a single drug, was less likely.”(Lord & Ashworth 2010)

Brennan et al. also emphasize human-based research noting: “. . . the cumulative adult death rate from cancer adjusted for the size and age of the population has improved by less than 5%.” They contrast the mortality rate from adult cancers with mortality in the pediatric population, pointing out that the cure rate for pediatric cancer is approximately 80%, up from 30% in 1971. Why is this the case? Brennan et al. state: “Most of the progress in improving outcome for pediatric cancer has come from clinical research. Indeed, the majority (>90%) of pediatric cancer patients are enrolled on treatment protocols and there is now abundant evidence that research protocols have helped optimize treatment intensification, drug dosing and timing, chemotherapeutic drug combination, and the identification of prognostic features of disease in relation to treatment plans. In sharp contrast, only 3% of adult cancer patients are enrolled on research protocols. These numbers suggest that the advances in patient outcome for pediatric cancer since the beginning of the war on cancer can be attributed in part to the coordinated participation in clinical research protocols. . . .”(Brennan et al. 2010) Brennan et al. also opine state that inter-species extrapolation is “an incredible challenge.”(Brennan, Federico, and Dyer 2010)

Human-based research also includes prevention and environmental factors. Former president of the American Cancer Society, Dileep G. Bal et al state that two-thirds of cancer deaths in the US can be prevented by proper diet, proper weight, and avoiding known risk factors like smoking. They note that diet equals smoking in terms of prevention. Pan et al support this conclusion with their twenty-nine year study finding that: “Red meat consumption is associated with an increased risk of total, CVD, and cancer mortality.”(Pan et al. 2012) The role of diet should be emphasized in light of the fact that ““Western cancers’ [are] spreading to developing world.”(Coghlan 2012; Bray et al. 2012) This is mainly secondary to the increasing standard of living with the concomitant increases in consumption of the Western diet and smoking.

8.0 Societal Concerns

The nonpredictive and misleading nature of animal models must be placed into the context of societal concerns regarding the use of animals in research and testing. Giles writing in *Nature* states: “In the contentious world of animal research, one question surfaces time and again: how useful are animal experiments as a way to prepare for trials of medical treatments in humans? *The issue is crucial, as public opinion is behind animal research only if it helps develop better drugs.* Consequently, scientists defending animal experiments insist they are essential for safe clinical trials, whereas animal-rights activists vehemently maintain that they are useless.” (Giles 2006) (Emphasis added.)

The Institute for Laboratory Animal Research (ILAR 2004) and other proponents of using animals in research (Frey 1983) have views similar to Giles. An editorial in *Nature* in 2009 reinforced the above stating: “Animal-research policies need to be guided by a moral compass—a consensus of what people find acceptable and unacceptable.”(Editorial 2009) What does society find acceptable?

A survey conducted by YouGov in the UK, France, Germany, Italy, Sweden and the Czech Republic asked under what conditions should the use of dogs, cats, and primates in research be allowed.

- 81% of people surveyed agree or strongly agree the new law should prohibit all experiments causing pain or suffering to primates.
- 79% of people agree or strongly agree the new law should prohibit all experiments on animals which do not relate to serious or life-threatening human conditions.
- 84% of people surveyed agree or strongly agree the new law should prohibit all experiments causing severe pain or suffering to any animal.
- 73% of people disagree or strongly disagree that the new law should permit experiments causing pain or suffering to cats.
- 77% of people disagree or strongly disagree that the new law should permit experiments causing pain or suffering to dogs.(ECEAE 2012)

The Pew Research Center and the American Association for the Advancement of Science (AAAS) revealed, in 2009, that only 52% of nonscientists supported the use of animals in general in scientific research.(Pew/AAAS 2009) In 1999, MORI and *New Scientist* (Aldhous et al. May 22, 1999) asked people whether they favored using animals with 24% answering yes 64% answering no. The questions were then divided into several categories. Respondents were questioned about experiments in which mice would be subject to pain, illness or surgery, and 61% stated that they disapproved using mice in order to study how the sense of hearing works. That percentage dropped to 32% when the question concerned the use of mice to ensure a new drug to cure childhood leukemia was safe and effective. When monkeys were substituted for mice the disapproval went from 64% to 75% and 32% to 44%, respectively. As the previous sections reveal, animals cannot in fact be used to predict safety and efficacy and are in reality used for basic research, which The Organisation for Economic Cooperation and Development defined as: “Experimental or theoretical work undertaken primarily to acquire new knowledge of phenomena and observable facts without any particular application or use in view. It is usually undertaken by scientists who may set their own agenda and to a large extent organise their own work.”(Organisation for Economic Cooperation and Development 1963)

While the above polls reveal variation in response the general message is clearly that society is uncomfortable with using animals in basic research. Since animal models are not predictive for human response to drugs and disease, including cancer, it would appear that, if society understood this, it would not approve of using animals in such a fashion.(Greek & Greek 2010)

Contrast this entire chapter with the following statements.

From the American Medical Association’s White paper: “Animal research holds the key for solutions to AIDS, cancer, heart disease, aging, and congenital defects.”(American Medical Association 1992) Michael F. Jacobson, executive director of the *Center for Science in the Public Interest* stated in 2008: “We must test animals to determine whether a substance causes cancer.”(CSPI 2008) Similarly, Huff et al. observe: “Chemical carcinogenesis bioassays in animals have long been recognized and accepted as valid predictors of potential cancer hazards to humans.”(Huff et al. 2008)

The Foundation for Biomedical Research (FBR) states: “Animal research has played a vital role in virtually every major medical advance of the last century, for both human and animal health. From the discovery of antibiotics, analgesics, anti-depressants, and anesthetics, to the successful development of organ transplants, bypass surgery, heart catheterization, and joint replacement, practically every present-day protocol for the prevention, control, and cure of disease is based on knowledge attained through research with laboratory animals. More than half of the Nobel Prizes in Physiology or Medicine have been given for research involving animals. Since 1900, modern medicine and public health have boosted the average lifespan in United States by almost 30 years. Much of this progress came from knowledge gained through animal research. Many diseases that once killed millions of people every year are now either preventable, treatable, or have been eradicated altogether. The survival rates for many other major diseases are at an all-time high thanks to the discovery of powerful new drugs, the development of new surgical procedures, and the design of sophisticated medical devices. Research with animals has played a critical role in nearly all of these advances.”(FBR 2010)

Understanding Animal Research of the UK states: “As this [development of new treatments] is a relatively large area of research and testing, accounting for 18% of animal procedures in 2010, it is useful to split it into three main areas. Animals are vital in all three.

- research to find new vaccines and treatments
- developing new medicines and vaccines and improving existing ones
- testing potential new medicines to make sure they are effective and safe”
(Understanding Animal Research 2011)

Botting and Morrison write: “In truth there are no basic differences between the physiology of laboratory animals and humans. . . . we can not think of an area of medical research that does not owe many of its most important advances to animal experiments.”(Botting & Morrison 1997) Fomchenko and Holland state: “GEMs [genetically engineered mice] closely recapitulate the human disease and are used to predict human response to a therapy, treatment or radiation schedule . . . GEMs that faithfully recapitulate human brain tumors and will likely result in high-quality clinical trials with satisfactory treatment outcomes and reduced drug toxicities. Additional use of GEMs to establish causal links between the presence of various genetic alterations and brain tumor initiation . . .”(Fomchenko & Holland 2006) There appear to be reasons why animal models continue to be used that have nothing to do with the efficacy of the practice.

9.0 Summary

Animal models of cancer have failed both for carcinogenesis and chemotherapy. There has been empirical evidence for the failure of animal models for decades but only recently has a Theory been developed to place the evidence in context. Based on the fact that animals and humans are evolved complex systems, one species should not be expected to be predictive for another for responses to disease and drugs. A modality, be it a research modality or treatment modality, that fails to perform as needed should be abandoned just as bloodletting and trephination have been abandoned by physicians. The concern society has expressed for using sentient animals in research in general is cause for further concern regarding the continuation of a failed modality.

Despite claims that research using animals to model human cancer has increased the life span of people with cancer, the use of animal models for developing new treatments for cancer has not been rewarding. In reality, it has been very misleading. In this chapter, I discussed the use of animal models for determining carcinogenesis and discovering chemotherapies. I also examine the empirical evidence pertaining to the predictive value of animal models for these purposes and placed this evidence into the context of scientific theory in the form of the Theory of Evolution and Complexity Theory. I then presented the position of society in general, as indicated by surveys as well as opinions from the scientific literature, regarding the use of animals in research and contrasted this stance with the actual benefits that result from animal models of cancer. I conclude that from a scientific perspective, animal models of cancer have failed and will continue to fail to predict the response of humans to carcinogens and antineoplastic therapies regardless of genetic modifications to animals or the use of chimeras. When this failure is placed in the context of what society demands in return for allowing scientists to use sentient animals, such as mammals, in such research, the continued use of animal models of cancer is difficult to justify.

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