CANINE CANCER

Why it is rampant, how it might be prevented, and an exciting new treatment strategy for dogs with cancer

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This work is dedicated to my children, Alex, Trevor and Samantha. You are the light in my life, always showing me the way forward. And to my mentors, Sidney Weinhouse, Peter Magee, and George Hitchings, who set me down this path so many years ago.

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The Canine Cancer Epidemic

"The Good Lord, in his infinite wisdom, gave us three things to make life bearable: Hope, jokes, and dogs; but the greatest of these was dogs."

Robyn Davidson

The word cancer comes from the Greek word *Karkinos* , meaning crab-like or claw-like. It was coined by Hippocrates, the father of medicine, about 2400 years ago, and describes the physical appearance of tumors— hard, like a crab's shell— and heavily vascularized, the large, truncating blood vessels feeding it seeming to early physicians like a crab's appendages. And of course, cancer is tenacious, never letting go, just as a crab will not let go once it has gripped you in its pincers. If you have had

a dog gripped by the pincers of cancer, then you know how tenacious that grip is. You know how relentless cancer is. You know that resorting to chemo is a stopgap measure at best, and too often, an excruciating one. Well, the exciting news in this book is that something new and very different has just been discovered— something that gives every appearance of being able to release both our dogs and ourselves from the pincer-like grip that cancer has held us in for so long. This discovery is so fundamental, so transformative that it may *eliminate* cancer as a major disease of both humans and dogs. I do not say this lightly, or without the foundation of a long, productive career to back it up. I have been a professional cancer research scientist for more than 40 years now, [1] and so, through experience, can recognize that what has unfolded right in front of me is so fundamental, so enabling, that it can literally change everything.

And it was discovered in dogs.

There are more dogs with cancer in the United States than there are people with cancer!

There are about 90 million dogs alive at any one time in the United States, and the National Cancer Institute (NCI) estimates that 6 million of them will be diagnosed with cancer

every year. That equates to a cancer incidence in our dogs of at least 6,600 per 100,000 per year, which very well may be the highest in all of the animal kingdom. [2] The cancer incidence in our own species, which is already considered to have reached epidemic proportions, is 442 per 100,000 per year. [3] Thus, cancer incidence in our dogs is *almost 15 times higher than it is in us!* This leads to the remarkable statistic that there are almost 3 1/2 times more dogs with cancer in the United States than there are people with cancer— even though there are 3 1/2 times more people than dogs living here! Looked at in a different way that considers lifespan, we long-lived humans have a lifetime cancer risk of a little over 40% (risk for men and women combined), while that of our much shorter-lived dogs has now reached an astronomical 50%. Compare this to the lifetime cancer risk of virtually all other large, long-lived species, which is about 4% [4] — including wolves in the wild, the species from which our dogs originated, and even primitive members of our own species, who lived much shorter lifespans than we do today. Cancer has accelerated dramatically in both humans and dogs until now it is the number one cause of death in each. This is far, far outside the norm of the animal kingdom. Outside of humans and dogs, cancer is generally a rare cause of death in the animal world. Clearly, something very unnatural is

happening to us, and especially to our dogs. And it is only getting worse with every passing year. But what is it? What is causing this epidemic of cancer? A contaminated environment? Carcinogens in our food and the food that we give to our dogs? Chemicals that we put into our dogs' bodies to kill fleas and ticks and other pests? Chemicals that we spray on our lawns that pass into a dog's body when it runs and plays on that lawn? You might be relieved to learn that these things probably play only a small role in the abnormal rates of cancer plaguing our two species— they're not innocuous, but they are not the primary drivers of cancer in our dogs; or in us. On the surface, the actual answer as to the cause of the aberrant levels of cancer plaguing our two species is much more complex— explaining why it took so long to be discovered. But once understood, it is almost incomprehensible how we could have remained blind to it for all these decades.

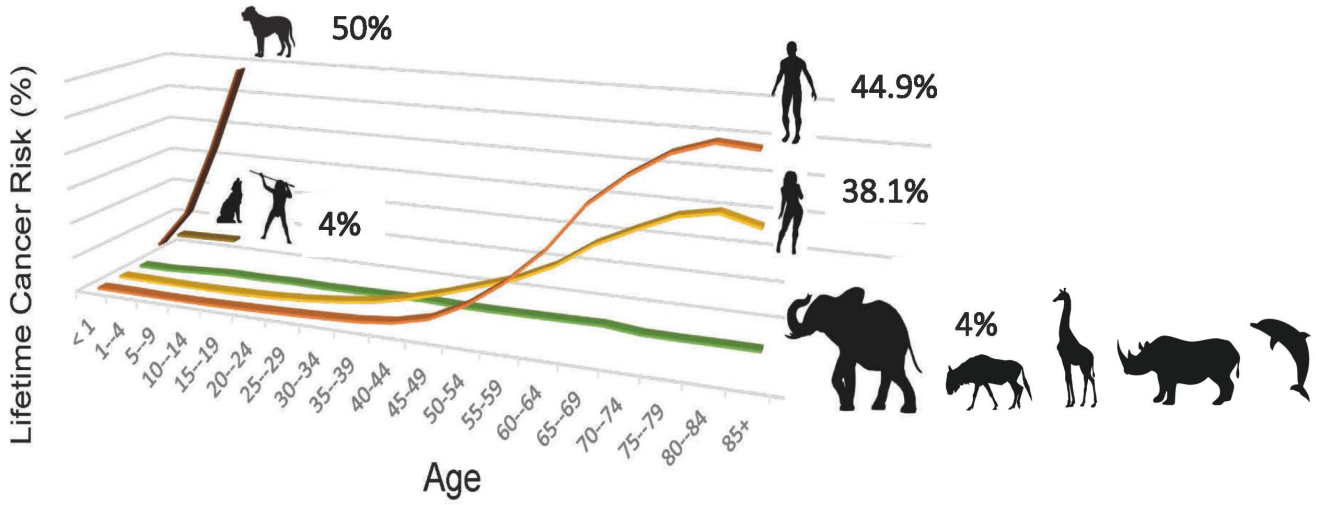

Figure 1.1 Lifetime cancer risk is dramatically higher in modern humans than it was in our primitive ancestors, and it is also dramatically higher in our dogs, especially our larger breeds of dogs, compared to their ancestors, a species of wolf. The high cancer risk of modern humans and dogs represents an aberration in the animal kingdom. Other large, long-lived animals such as the elephant maintain a constant, low cancer risk throughout their lifetimes. Why?

One of the interesting things that comes out of the figure above is the fact that large, long-lived species can have a very low cancer risk, despite having such massive bodies. Doesn't that strike you as strange? That such large bodied animals have the same cancer risk as much smaller species doesn't really make sense, at least not on the surface of it. Since animal cells from one species to another are all roughly the same size,

irrespective of how big the animal is, the bodies of massive creatures can have *orders of magnitude* more cells than much smaller ones. If all cells have a roughly equal chance of becoming cancerous, which also seems logical, then the more cells an animal has, the greater should be the risk in that animal for one or more of those cells to undergo malignant transformation and become a cancer cell. Comparing a blue whale that weighs 400,000 pounds, and a bottle-nose dolphin that weighs 400 pounds, the whale has a thousand times more cells at risk for cancer. Logically, it should have a thousand times higher risk of getting cancer than the dolphin has, just from the number of cells at risk in each animal. Yet this is not what we see in Nature. Cancer risk does not scale with body size, the way it logically should, if all things were equal. Clearly, all things are *not* equal. A gigantic animal like an elephant or a whale, has the same 4% lifetime risk of developing cancer as a dolphin, a manatee, or a wolf. This bit of illogic occurring in the natural world was first pointed out by a trio of British scientists, and became known as “Peto’s Paradox,” after the most alliteratively named member of the group. Although there have been many attempts to explain Peto’s Paradox over the years, none has succeeded. Until now. That is the exciting surprise awaiting you in this book. My

laboratory has solved the riddle of Peto's paradox by uncovering a heretofore unknown, fundamental equation of vertebrate speciation. It is so fundamental that it must be considered a natural law; hence its name, the *lex naturalis* . Please forgive my exuberance, but it is not overstating to say that the *lex naturalis* is the $E = mc^2$ of biology. A big claim, I know. But one that you are likely to agree with once you see its power to explain past evolutionary outcomes, and even to predict future ones.

Uncovering the *lex naturalis* revealed that cancer is a fundamental force opposing vertebrate speciation, and that unique, species-specific mechanisms of tumor suppression evolved as a counterforce to neutralize this opposing force. Species-specific mechanisms of tumor suppression thus enable increased size to evolve without increased cancer risk. This means that cancer is very different from one vertebrate species to the next, because every species has evolved a unique, species-specific mechanism of tumor suppression, and therefore deals with cancer in its own unique way. I cannot stress enough how different this is from the paradigm that has dominated, and continues to dominate, cancer research for the past 50 years. This dominant paradigm holds that cancer is pretty much the same thing from one species to the next, and this dogma was

used to support the idea that drugs for humans with cancer can be discovered using mice with cancer to test those drugs. The *lex naturalis* demolishes this paradigm, holding virtually the opposite: Because unique, species-specific mechanisms of tumor suppression exist for every vertebrate species, one vertebrate species, such as a mouse, *cannot* be used to create a valid model system with which to study cancer in another vertebrate species, such as a human. The troubling offshoot of this paradigm-demolishing discovery is that *all* of the drugs—chemo and otherwise— that exist in the oncologists' pharmacopeia have been discovered in mice! They are therefore mouse cancer drugs, not human cancer drugs. This undoubtedly explains the dismal 7% improvement in two-year survival for cancer patients that has occurred over the past 27 years. [5] As oncologist Azra Raza has pointed out, [6] the same 32% of cancer patients that could not be effectively treated 40 years ago still cannot be effectively treated today. Death is the relentless result of their illness. And with new cancer drugs costing *$1000 per day* to produce only a few more months of life, not only patients' families, but our entire healthcare system has been put on the verge of financial collapse. What about drugs to treat cancer in dogs? Almost all of the drugs used to treat canine cancer are doubly removed from the reality

imposed by the *lex naturalis* because they were discovered in *mice* , and developed as drugs in *humans* . Their use in dogs is nothing but an afterthought.

But the news is not all bad. In fact, uncovering the *lex naturalis* appears to have finally put into our hands the means to *win* the war on cancer that President Nixon declared five long decades ago. It provides a prescription, writ for both ourselves and our dogs, that holds by far the most promise of any discovery to date, to release both of our species from the pincer grip of cancer. By showing us how to rejuvenate our natural, species-specific mechanisms of tumor suppression, the uncovering of the *lex naturalis* is poised to make cancer a rare disease again, just as it was in pre-modern humans, and just as it was— and still is— in wolves.

This discovery comes just in the nick of time for our own species, as World Health Organization (WHO) projections show that a *quarter of a billion people* will be newly diagnosed with cancer during the coming decade— a catastrophe of biblical proportions that is far beyond the capacity of any imaginable healthcare system. The *lex naturalis* shows how we can avoid this catastrophe, both the one in *our* near future, and the one already occurring in our dogs. These are big, bold claims, I know. But the evidence supporting the l*ex naturalis,*

and what it means for our two species, is overwhelming—fitting together everything that we know about cancer, like a solved Rubik's cube. You will see this as you read this book.

Hang on. This is going to be an exciting ride.

But first things first. To understand how the *lex naturalis* was discovered in dogs, we first have to unravel the origins of the most profound of inter-species relationships ever to occur on this planet. It certainly was not one that was preordained in the stars.

2

The origin of our dogs

When the Man awoke he said, "What is Wild Dog doing here?" And the Woman said, "his name is not Wild Dog anymore, but the First Friend, because he will be our friend for always and always and always."

Rudyard Kipling

The story of man and dog is one of the richest in the animal kingdom. No other species has made such an impact on humankind. No other species has so firmly rooted itself into our families, to the point that we consider them to be equal members. No other species— not horse, nor cat, nor bird, nor

fish— makes us feel the way our dogs make us feel. No other species makes us reach out a hand, or stoop to pet an animal that we have never met before. We adopt no other species into our lives the way we do our dogs. And no other species adopts us the way our dogs do. A dog immediately knows when he or she has been adopted by a human, and immediately responds by loving and protecting that human, working hard to earn a place in that family. The bond between dogs and humans is very, very deep. This is because, for the past 35,000 years or so, humans and dogs have been evolving *together* on this planet, almost as if we and they were one biological unit. Our lives, our work, and, recent evidence suggests, *even the DNA* of we and our best friends have all been evolving in parallel. Several recent studies have thus demonstrated that genes associated with adaptation to severe environments, [7] with digestion and metabolism, [8] and even cancer, [9] have undergone parallel evolution in our two species. But with respect to the life histories of *wolves* and humans, this kinship between canines and humans is a recent phenomenon, preceded by millennia of hostility. This makes the story of man and dog even more remarkable.

An unlikely alliance

The initial, natural relationship between wolf and man was strictly as blood, tooth and claw enemies— competitors for the same game, the same water resources, and the same living spaces. Humans would not tolerate wolves. Nor would wolves tolerate humans. When wolf and man met, whether it be by accident, or in contest over a carcass, attack by one or the other was all but certain— an attack which had a very good chance to be fatal to one or the other, because in relative terms, wolf and man were roughly equivalent in their ability to harm each other. At the time that man and wolf had come together, *Homo sapiens* had invented new weapons, most notably the projectile spear that could be thrown to kill at a distance. But on their side, wolves had strength, speed, sharp claws and teeth, and the fact that they traveled in packs. It did not help the relationship that wolf furs were coveted for their warmth by humans; nor that human flesh, particularly that of human young, was an acceptable substitute for bison or deer for the wolf. There was nothing in this initial relationship that gave any hint that anything other than violence would ever come of it. Even today, while we love our dogs, we are generally unmerciful to wolves. They are hunted with almost instinctive fervor. Even when they were on the endangered species list, exceptions were permitted such that a wolf that posed a “possible threat” to

livestock could be hunted and killed. As an example, consider the demise of ‘06, a radio-collared female alpha wolf known as 832F to researchers, that was shot to death from a helicopter in Wyoming in 2012, a chilling reminder of the decimation of the American wolf population from an estimated 380,000 in the early 1900’s, to about 2,200 today. We are not now and have never been kind to wolves. In the entire history of the *Endangered Species Act* , wolves are the only species to go from protected to hunted in a single day. And although most modern wolves have learned to keep their distance from humans, that has not always been the case. Consider the wolf of Gysinge , which killed and devoured 11 children and one adult in central Sweden during a three-month period in 1820-1821. The relationship between wolf and humans has always been strained, so much so that there are only bad wolves in the fairy tales that we tell to our children. I don’t know if we will ever learn enough about animal communication to decipher what wolf mothers tell their cubs about we humans, but I doubt that it is complimentary. So how did these natural enemies, man and wolf, come to eventually produce the most profound inter-species alliance on the planet? To answer this question, let’s start at the beginning. Where did the evolutionary trajectories

of these two species, so bitterly at odds with each other, first intersect?

Evolutionary origins of the wolf

You may be surprised to learn that the canid story begins in North America. Here, in the late Eocene, about 39 million years ago, a creature known as *Hesperocyon* , the first canid genus, arose. *Hesperocyon* had long hind-limbs and a long back and tale. At this time in North America, as well as virtually all of the planet, the weather was beginning to cool down. The Antarctic polar ice cap began to expand, producing cold water that currents pushed around the globe, initiating dramatic decreases in global temperature. Many of the animals of the early and middle Eocene epoch that depended on the tropical climate that had prevailed for millions of years were driven into extinction by the drop in temperature that occurred during the late Eocene and early Oligocene— the Eocene Oligocene Extinction Event (EOEE). Two relatively large impacts have been suggested to have precipitated the EOEE, sending debris into the atmosphere that may have blocked solar radiation, initiating what may have been as much as an 8 degree C temperature change. These impacts caused the 40 km (25 mile)

wide Chesapeake Bay impact crater , and the 100 km (62 mile) wide Popiga impact crater in central Siberia. A new climate marked by the advent of changing seasons began, giving homeothermic (warm-blooded) animals such as mammals a clear advantage over those that could not control their body temperature. In North America, northern latitudes were covered with forests, while mid latitudes were covered with grasslands that produced the first large herbivores. Armadillos at this time were ten feet long, and rhinoceroses were 18 feet tall and 27 feet long.

Hesperocyon appears to have been a canid adapted for forest life. It probably focused on small rodents such as voles and hamsters as its major food source, two species that also arose at this time. By the early Miocene, about 23 million years ago, *Hesperocyon* and several hyena-like and coyote-sized canids that had evolved from it, all became extinct, with the exception of two lineages known as *Notocyon* and *Leptocyon* . The *Notocyon* lineage gave rise to a series of short-faced, heavy-jawed, massive canids all of which appear to have died out in the middle Miocene, about 20 million years ago. It is the small fox *Leptocyon* that survived the rigors of the Miocene, giving rise, in North America, to three genera: *Canis, Urocyon,* and *Vulpes* . *Urocyon* and *Vulpes* are two species of fox that

survive today— the gray fox, and the true, or red fox. The canines from which wolves, and eventually dogs would evolve, appear to have arisen in what is now the southwestern United States, about 9 million years ago. Each of these genera adopted different survival strategies, exploiting different prey species, different methods of hunting, different body size, different life spans, and as we shall discuss in detail, improved mechanisms to suppress cancer that were required to increase body size and lifespan during speciation.

One of the features that apparently helped the *Canis* genera thrive and populate the rest of the world was the development of paired upper and lower teeth capable of both shearing and chewing. And indeed, populate the rest of the world canids did. From their birthplace in the American southwest, canids spread to Eurasia 8 million years ago; to western Europe 6 million years ago; and to Africa 4 million years ago, during the age of our early ancestor, the *Australopithecines* , and 3.8 million years before the first members of our species, *Homo sapiens,* would arise there.

The evolution of these dispersed canids continued in response to the different environments they colonized. The gray wolf, *Canis lupus* , became fully developed as a species in northern Eurasia about one million years ago. [10] This particular

wolf species became spectacularly successful, by the end of the Pleistocene reaching virtually every accessible habitat on the planet. By three hundred-thousand years ago, *Canis lupus* had spread all over Europe and Asia. And when the Bering Strait between Eurasia and North America dried up, creating a land bridge (Berengia), *Canis lupus* crossed back into North America, the origin of her ancestral species.

Upon her arrival in North America, the gray wolf had to compete for a time with the dire wolf (*Canis dirus* , "fearsome dog"), a much larger, extremely muscular canid species (re-imagined in the popular series, *Game of Thrones*), but one that may have lacked the brain power of *Canis lupus* . (For why else would so many dire wolves and so few gray wolves be at the bottom of the LaBrea tar pits? https://tarpits.org/topics/dire-wolves). Equipped by evolution to be the master of virtually any environment, the gray wolf thrived while the dire wolf succumbed to extinction. The ability to adapt to any environment was the tool kit that Nature had provided to *Canis lupus* — a tool kit that some enterprising members of this species would eventually offer in barter to the upstart, oddly bipedal, relatively hairless creatures now moving out of Africa, spear in hand, competing with them for resources.

Evolution of Canis lupus familiaris, the modern dog

In this book, I refer to the wolves that initiated first contact with humans as epi wolves. I do so because I believe that epigenetic processes played a critical role in the establishment of the collaboration between wolves and humans. Epigenetics refers to modifications to the genome that regulate the way it is expressed— which genes are transcribed, and translated into protein, and how actively that transcription and translation occurs. One epigenetic process that regulates transcription is called DNA methylation, a process in which specific cytosines in DNA— usually cytosines next to guanine, so-called CpG dinucleotides— are methylated on their 5-carbon atom, creating 5-methylcytosine. Researchers have shown that epigenetic processes such as DNA methylation are capable of recording early life experiences as semi-indelible marks upon the brain, epigenetic marks that can alter behavior. [11] Other researchers have found that differences in the methylation status of a variety of genes known to be related to behavior distinguish wolves from dogs. [12] Applying this work to first contact, I hypothesize that the first successful, lasting collaboration between wolf and man relied not only on the wolf— probably female— that initiated first contact with humans, but even more

so on her offspring who would have been epigenetically imprinted with the knowledge that these particular humans need not be feared— knowledge obtained by observing their mother. In turn, the offspring of these epi wolves would have reinforced this epigenetic imprinting upon *their* pups, and so on, down through the generations. In my writings published in the scientific literature I have proposed that such epigenetic changes, which are reversible, eventually undergo transition to genetic changes, which are much less easily reversible. In this way, over time, learned behaviors can transition into instinct.

Most people believe that humans created the dog by domesticating the wolf; that we took wolves from the wild and gradually taught them how to help and protect us. Nothing could be further from the truth. We did not create dogs by domesticating wolves. Rather, an ancestral wolf with a mix of neurobehavioral elements probably far removed from most of her kin approached our hunter gatherer forebears and proposed, so to speak, a collaboration. In return for being allowed to feed off the remains of the carcasses of animals that humans had killed, such epi wolves would extend the sensory perception of man many orders of magnitude. They would do this by using their profoundly more sensitive olfactory and auditory senses to detect the approach of potential enemies— man or beast— and

warn the encampments of the humans who had agreed to the collaboration. The establishment of such a collaboration between wolf and man thus brought immediate benefits to both species.

But contrary to what most people believe, we did not initiate the relationship that led to the evolution of dogs. They initiated the relationship with us. Or rather, their ancestor, a species of grey wolf that is now extinct, did. More precisely, one member of this now extinct species, an individual that was almost certainly exceptional in certain aspects of its neurobiology, made the deliberate decision to explore the possibility of establishing a relationship with humans.

A recent large scale project led by a team from Oxford University, but which involved many of the world's experts on canine evolution, has come to the tentative conclusion that dogs were "domesticated" not once, but twice in human history—once in East Asia, and once in Western Europe, and by way of two apparently different sub-species of now extinct wolves, *Canis lupus variabilis* , and the other an unidentified subspecies of extinct *Canis lupus* . [13] These researchers began by sequencing the entire genome of a 4,800-year old dog from a bone that had been excavated from a neolithic tomb site known as Newgrange , in Ireland. The Newgrange tomb site is large,

and was built around 3,200 B.C.E. by Stone Age farmers. It is thus older than both Stonehenge and the Egyptian pyramids. The bone that was found there was part of the ancient dog's tympanum , part of the inner ear. The way this bone is constructed, the DNA inside had been completely protected for millennia, and the scientists were able to isolate pristine samples for their analysis. (*Many times, the analysis of such ancient DNA is complicated by the presence of DNA from other animals and from bacteria. But inside the tympanum, it is completely isolated from the possibility of such contamination* .) This international research group also obtained mitochondrial DNA from bones and hair of 59 ancient dogs living 3,000 to 14,000 years ago, and compared them to the genomes of 2,500 modern dogs that had been previously reported. (*Mitochondrial DNA is often used in such studies because it is passed down through the female lineage only, making it less complicated to analyze, and because in any ancient cell under investigation, there are several thousand copies of a target mitochondrial gene, compared to just two copies of most genes from nuclear DNA; this large number of copies of genes makes isolation and analysis of mitochondrial DNA easier* .) As noted above, the end result of this complex set of analyses indicated that dogs were "domesticated" twice— arising independently from two

now extinct wolf lineages on opposite sides of the Eurasian continent. This also roughly fits the available archeological data, which is replete with dog fossils in the East and West that are 12,000 or more years old, but in which there are no fossils from central Asia that are older than 8,000 years. Another twist to the story is that the dogs from the East seem to have replaced the dogs from the West upon their arrival in Europe. Apparently, the eastern Eurasian canines had some quality that ancient Europeans found lacking in their Western-origin dogs. Perhaps dogs from the east had a more sensitive trigger to set off their barking, making them better watch dogs; barking is not something that wolves commonly do, as we will discuss below.

It is important to note that the field of canine origins as delineated by genome studies is still in a state of flux. Thus, another group of experts studying DNA from bone fragments of Neolithic dogs dispute a dual origin, and showed that the dogs that they studied had not yet evolved the requisite enzymes to digest starch, thought to be an important adaptation occurring in dogs that resided in farming communities. [14]

Yet another group of researchers on an expedition to the northernmost part of Eurasia, Russia's remote Taymyr Peninsula, used the effects of global warming to uncover ancient bones. [15] They searched river banks in Taymyr at spots

where the permafrost had melted to reveal the remains of Wooly Mammoths and other long dead animals. Among these bones they found a rib from which it was possible to isolate DNA and sequence it. This analysis showed that it was an extinct sub-species of wolf that was ancestral to both modern dogs and modern wolves. Carbon dating showed that the wolf from which the rib bone had come had died 35,000 years ago. A jaw bone, from a different wolf, was found to be even older. When compared to the genome of modern dogs, the genome sequenced from the Taymyr wolf's rib bone suggested that the ancestors of today's dogs split from the ancestors of modern wolves as far back as 40,000 years ago— much earlier than the dates proposed in most other genetic studies utilizing modern wolves and dogs. [16] , [17] , [18]

So, putting all of the evidence together that is available at this moment, *Canis lupus* and *Homo sapiens* appear to have initiated long-lasting alliances possibly twice, at roughly the same time, but at opposite ends of the huge continent of Eurasia, between 30 and 40 thousand years ago. This story of the origin of dogs will almost certainly have further twists and turns as more ancient canine remains are discovered, and further canine DNA is isolated and sequenced.

The Last Glacial Maximum

The wolf's evolution into the dog was in full swing during what is known as the Last Glacial Maximum, a period in Earth's history when ice sheets were at their greatest extension from the poles. Imagine a landscape in which mobile glaciers from the Arctic were slowly expanding southward, scraping the earth like great, frigid chisels and pushing everything in their path southward. The world at this time was inhospitable, cold and windy, with frequent storms and a dust-laden atmosphere. It was a harsh landscape for humans to endure; harsh, too, for the wolf; even one so well-equipped by Nature as *Canis Lupus* . This extension of growing ice sheets reached its maximum about 22,000 years ago, after which the ice began to slowly retreat as a result of a natural global warming, leaving a boulder strewn landscape marking its edges. [19]

The most extensive biome on the Earth's surface during the Last Glacial Maximum, known as the mammoth steppe , was characterized by an arid, cold, savannah-like climate covering most of the northern hemisphere from Spain to North America and from the arctic islands to China. It has recently been demonstrated by analysis of ancient seed DNA that the vegetation of the mammoth steppe was dominated by forbs, a kind of herbaceous, broad-leafed flowering plant with a very

high nutritional value. The animals that consumed these forbs and were able to thrive in this landscape included the wooly mammoth, the bison, the woolly rhinoceros, the giant sloth and the ancestral horse. The mammoth steppe was the environment in which an ancestral wolf consolidated its relationship with man, and began its long evolution into the dog. It was an environment dominated by mega fauna for which puny humans should have been no match, and which also presented a significant challenge for even a pack of wolves accustomed to hunting together. Such mega fauna not only included the mammoths, giant sloths, rhinoceros and bison mentioned above, but also a preternatural hunter whose prey certainly included our ancestors. This hunter we now call *Smilodon,* the saber-toothed lion. One can only imagine by what feared name our ancestors knew this giant cat— imagination surely amplified by recently discovered fossilized footprints of a giant *Smilodon* tracking her probably human prey 50,000 years ago along an ancient Argentinian beach. [20] The enormity of these footprints shows what our ancestors were up against if this huge, killer cat caught their scent.

Were epi wolves dumpster divers?

It used to be a common idea that some wolves began to evolve toward the modern dog by frequenting human trash heaps, where they could scrounge for food scraps. That idea was discarded by most anthropologists and paleontologists when it was discovered by genetic analysis that dogs separated from their wolf ancestors when humans were still hunter gatherers, and long before the advent of human agriculture, which occurred only recently, about 10,000 years ago. It is assumed that hunter gatherer societies did not stay in any one place long enough to produce trash heaps, which would have been a signature of later agricultural societies. But one might consider that trash heaps come in all types and sizes. It is believed that human hunters scouring the mammoth steppe routinely killed large mammals, even the largest, the wooly mammoth. In fact, there is credible evidence to suggest that the disappearance of the wooly mammoth, and the other large mammals that roamed North America during the Last Glacial Maximum, was caused by human predation upon them. [21] Certainly, humans that had dispatched a wooly mammoth, a giant sloth, a wooly rhinoceros or a bison would consume as much of the carcass as they could, collecting the pelt for clothing, the meat for consumption, and so on. But even the most frugal hunter gatherers would have left behind considerable remnants from the carcass of such a

large animal. These carcass remnants were themselves “trash heaps” of a sort— the hunter-gatherer sort. It is possible that the association between wolves and humans may have begun by wolves scavenging at the remains of such human predation. Is that why dogs have such a fondness for bones? It may have been the least dominant of the wolves— those that fit into the hunting nature of their packs least well— that would be most attracted to scavenging at such carcass refuse sites as an alternative survival strategy. Let’s consider this idea in a little more detail.

Canis lupus evolved to be a pack hunter. Unlike bears, or even the more closely related jackals and hyenas, *Canis lupus* alive today almost never resort to scavenging human garbage. They will eat carrion left by human hunters at kill sites, but no trash scavenging problem exists with wolves, as it does with bears raiding human refuse sites. The *Canis lupus* alive today prefer to hunt, just as they did 200,000 years ago when *Homo sapiens* first arrived on the scene. It is not in their nature to approach human habitation with the purpose of scavenging a meal, the way hyenas are known to do. [22] When pressed upon by encroaching humans, gray wolves just move further north, and keep hunting. They are an ancient species, much older than ours, and set in their ways, a logical strategy in as much as

those ways have been so successful. How we see them behave today is how they behaved millennia ago. Our mistrust of them, and theirs of us, remains as it was millennia ago. Unfortunately, that will never change.

But we know that it did change once, perhaps even twice, for at least some wolves, and some humans. What enabled the collaboration to commence between two such profoundly opposed species? The answer to this question, I believe, can be found in the science of outliers. The large-scale studies noted above that show two long-lasting canine-human alliance events, one in East Eurasia and one in West, really identify the advent of a dog industry; two separate dog industries, in fact, separated by the breadth of a continent. Certainly, prehistoric humans wanted whatever new invention their neighbors (or enemies) had acquired just as much as we do today. When was the last time you saw someone without a cell phone at the ready? The "invention" of the dog must have swept through early human culture like iPhones did in modern times. Dogs enhanced the sensory perception of humans by many orders of magnitude. Humans, for example, have a limited, one-dimensional sense of smell. We can detect that a smell exists, but not much more. Canines have a *four-dimensional* sense of smell. They can detect not only the presence of a smell, but also the exact

direction from which it is emanating, and when it was placed there. [23] It appears that canines even have the ability to smell the time of day from the scent of the earth, which changes as the position of the sun warms it differently with each passing hour, increasing or decreasing the concentration of volatiles in the air. The way that we see the world— with our eyes— dogs smell it. Even though the canid brain is one tenth as large as the human brain, that part of the brain that is devoted to olfaction is 40 times larger in the dog than in humans; and, depending upon breed, dogs have between 125 million to 300 million scent glands compared to about 5 million in humans. This translates to a canid sense of smell that is 10 million times more sensitive than that of humans. A dog's auditory system is also superior to that of the human— they can hear things four times as far away as humans can, and the range of their hearing— how high or how low in frequency— is about twice as broad as that of man. Dogs thus truly do sense the world differently than we do, and adding their senses to ours must certainly have helped our ancestors.

Canines may have given our hunter gatherer ancestors an evolutionary edge over similar, competing groups of humans, such as the Neanderthals. Thus, while there is abundant fossil evidence that *Homo sapiens* had canine companions, there is no

evidence whatsoever that Neanderthal tribes did. Pat Shipman, a retired Adjunct Professor of Paleontology at Penn State University, has theorized that this may be one reason that the Neanderthals went extinct. [24] In ancient times, a family without a dog was at a disadvantage compared to a family with a dog. Dogs could protect a family at night, providing warning of any approaching enemy, man or beast, and with their incredible olfactory senses they could help in the hunt during the day.

It has recently been demonstrated that dogs process the faces of human beings in a different part of their brain than they do the faces of other dogs. [25] Thus, the special relationship that dogs have created with humans appears to be hard-wired into them. Like the dogs of today, epi wolves and the early dogs that they gave rise to, clearly must also have had the remarkable capacity to process human faces in fine detail, to identify their human collaborator, and a sort of hard-wired emotional attachment and loyalty to that person, or to that family. Just as epi wolf pups may have learned from their epi wolf mothers that particular humans need not be feared, subsequent generations of epi wolves raised by particular humans could receive the same information from their human “mothers” (male and female.) Their deference, embedded epigenetically, was reserved for their human “mother,” and for other humans

designated as friendly by their human “mother.” With regard to strangers, epi wolves reverted to their ancestral behavior. This neurobehavioral quality of being able to recognize individuals and develop allegiance to them, along with their incredible olfactory and hearing abilities, made alliance with an epi wolf of tremendous survival value for our species.

The industrialization of the dog

From the start, the cooperation between man and epi wolves was a two-way street. Each gained from association with the other. With their keen olfactory sense, epi wolves that had been adopted into a human tribe could track and corner game too large for them to kill by themselves. With the invention of throwing spears, early *Homo sapiens* could follow behind, following the barks and howls of their hunting partners. When they arrived on scene to find a mammoth or other mega fauna creature cornered by their epi wolves, they could stay back, out of harm’s way, throwing spears from a distance until the beast was dead.

Such increases in sensory perception gained by our hunter gatherer forebears by their association with epi wolves had many uses. Inter-tribal conflict was probably rampant as our

primitive ancestors competed for the limited resources that the harsh glacial environment provided. Attacks by rival tribes for food and females were probably commonplace, and the epi wolves that had adopted a particular human tribe could warn that tribe of the approach of strangers. Human attackers were of course not the only dangers that epi wolf-adopting tribes faced. As noted above, the mega fauna inhabiting the mammoth steppe included human kinds' most deadly predator, the saber-toothed cat, a massive beast that probably found our species to be quite edible, and easy to catch. That large cats can exist on a diet exclusively of humans was demonstrated by the Tsavo lions, two sibling felines that killed and devoured 75 of the workers attempting to build the Kenya-Uganda railway in 1898 — a time when firearms were present on scene (but dogs were not.) Once a big cat has developed a taste for human flesh, or becomes attracted to the ease of catching them as compared to other wildlife, they can specialize in the catching, killing and eating of humans as their preferred food source. This, the Tsavo lions clearly showed to us.

The largest of the saber-toothed cats was *Smilodon populator* , which reached a weight of 1000 pounds (a modern tiger reaches a weight of about 600 pounds). This gigantic cat had enormous claws and canine sabers that extended from their

jaws fully twelve inches. *Smilodon populator* has the distinction of being the only prehistoric cat known to have driven an entire species into extinction. The victim of this annihilation was itself a formidable predator, the saber-toothed cat-like marsupial *Thylacosmilus* — which probably also had our hunter gatherer ancestors on *its* menu. *Thylacosmilus* disappeared in the fossil record almost immediately after *Smilodon* entered the marsupial's home range of South America. Perhaps it was man's association with epi wolves that were evolving into dogs that prevented *Homo sapiens* from joining this list of species made extinct by *Smilodon* . But at some point after dogs became commonplace in human cultures, the tables were turned on the saber-toothed cats, and *Smilodon* was driven into extinction about 12,000 years ago. Would humans have been able to drive *Smilodon* into extinction, instead of the other way around, without the dog by our side? Most people have witnessed how dogs protect their human masters from threats such as bears. One can imagine early dogs behaving the same way toward *Smilodon* . In a face to face encounter with a thousand-pound saber-toothed cat our early ancestors would clearly not have fared well, throwable spear or not. But with his dogs feinting and charging, feinting and charging, distracting such a giant cat, the human may have been

able to position himself to the cat's unprotected side, from which a lethal spear throw to the heart could be possible. The barking of such dogs would also have alerted other members of the tribe to come with their spears and lend assistance in the slaying of the distracted saber tooth. Certainly, the timing is correct for this scenario, as our current understanding of the origin of the dog places that origin well before the extinction of *Smilodon* and his cousins. It was not the loss of prey which drove *Smilodon* into extinction, because wooly mammoths and the other mega fauna did not become generally extinct until 10,000 years ago, with populations surviving on St. Paul Island until 5,600 years ago, and on Wrangel Island until 4,000 years ago.

Although the exact cause of the extinction of *Smilodon* remains controversial, I will put my money on the evolution of the man/dog interspecies collaboration as the most probable cause. If dogs had not evolved when they did, perhaps *Homo sapiens* would have gone the way of *Thylacosmilus* . Perhaps some genetic remnant of this memory is why dogs still generally have an adversarial relationship with cats to this day.

But all of this represents the *industrialization* of the dog. It does not say much about the *creation* of the dog. This industrialization of the dog would be somewhat analogous to

the business person today who spots a new invention and says, “now that’s something I could sell.” An enterprising hunter-gatherer entrepreneur who got his hands on a few dogs could see that he had a bright future ahead of him. Everyone would want one of these. But again, this is about how the invention of the dog moved from East to West and then throughout the world. It tells us the how of the gene flows of dogs that geneticists are able to detect by careful sequence analysis of modern and ancient dogs and wolves. It does not tell us very much at all about the creation of the dog from the wolf.

The earliest alliance of man and wolf

As noted above, dogs show attachment and loyalty to humans, qualities that are not associated with the gray wolf. Not at all. So where did they come from? How is it that dogs retain the sensory enhancements of their ancestral wolves, but have acquired new traits that not only allow them to tolerate *Homo sapiens* , but which create an urge in *Canis lupus familiaris* to attach themselves to human beings; in fact, to devote themselves to particular human beings? What turned some members of *Canis lupus* into *Canis lupus familiaris* ? Somewhere in the East, and also somewhere in the West, one

particularly receptive wolf, our epi wolf, approached one particularly receptive human, and a collaboration was born. But what made these individuals, from these otherwise warring species, different? What made them receptive to each other? What allowed their collaboration to get off the ground?

DHEAS: The secret ingredient that enabled the bond between humans and dogs

Dehydroepiandrosterone sulfate (DHEAS) is an androgenic steroid that serves as a precursor for the synthesis of testosterone and estradiol in vertebrate animals. It also acts as a neurosteroid, influencing the growth, sprouting and survival of neurons, [26] and participates in brain development during adolescence. [27] Such broad neurological activity may enhance the ability of long-lived animals to acquire and store memories, enabling them to access those memories to assist in solving future problems.

Virtually all vertebrates synthesize and secrete DHEAS in their gonads— the exceptions being dogs and primates, which synthesize and secrete DHEAS primarily from their adrenal glands. [28] It is a fascinating aspect of comparative biology that

very high levels of *circulating* DHEAS appear to occur exclusively in primates, with the highest levels by far in humans. In our species, DHEAS begins to be synthesized and secreted into the circulation at adrenarche , a developmental period characterized by the appearance of the *zona reticularis* in the adrenal gland (adrena l = adrena rche) at about eight years of age. Adrenarche is a distinct developmental stage in humans, occurring prior to gonadarche. Whereas the purpose of gonadarche is clearly the synthesis of the sex steroids that effect sexual maturation in male and female vertebrates, the purpose of adrenarche has been unclear.

My laboratory has recently identified an additional function for circulating DHEAS—and in the process has uncovered the purpose of adrenarche. It turns out that *circulating* DHEAS is the central element of a tumor suppression mechanism that evolved in primates— *and in parallel in dogs, although to a more limited extent* . The evolution of this DHEAS-based tumor suppression mechanism *created* the primate lineage, enabling primates to evolve, over time, from mouse-sized creatures to gorilla-sized (and even larger) species over the course of some 66 million years. [29] Such increases in size— which were of a magnitude even greater than the evolution of the Blue whale from the wolf-sized land mammal *Pakicetus* — would not have

been possible without the simultaneous evolution of the DHEAS-based tumor suppression system, and a variety of additional “improvements” that occurred in the primate lineage at every point characterized by new species with increased body size. Each one of these “improvements” contributed to a “kill switch” that is triggered in cells that sustain a potentially cancer-causing mutation. The p53 tumor suppressor is the most frequently mutated gene in human cancer, inactivated by mutation in more than half of all tumors, and inactivated by other means in virtually all of the rest.

DHEAS and DHEA are the Dr. Jekyll and Mr. Hyde of androgen biology

When p53 is inactivated, the “kill switch” that my lab uncovered is triggered, and large amounts of DHEAS from the bloodstream are transported into the affected cell. Once inside the p53 mutant cell, DHEAS is de-sulfated to DHEA, which is the equivalent of turning Dr. Jekyll into Mister Hyde. Whereas DHEAS is innocuous even when circulating at very high concentrations in the blood, DHEA is an extremely potent inhibitor of one of the most important enzymes in the cell—Glucose-6-phosphate dehydrogenase (G6PD). Astonishingly, DHEA is an *uncompetitive* enzyme inhibitor— a class of

enzyme inhibition unique for its ability to rapidly become *irreversible, with potentially deadly results for the cell in which it occurs*. In the words of Athelton Cornish-Bowden, a famous British enzyme kinetics expert:

> *"Any metabolic pathway in which uncompetitive inhibition can occur can respond catastrophically to the presence of inhibitor."* [30]

Let's first examine what happens in the normal cell in which p53 is intact and active.

G6PD is the cellular "factory" where the co-factor NADPH is produced. NADPH has many important functions in the cell, but one of the most important is that it is required for the synthesis of selenoproteins, many of which act as the "firemen" of the cell. What "fire" is there in the cell that would require selenoprotein "firemen?" Metabolic processes in the cell produce a byproduct referred to as reactive oxygen species (ROS), one example of which is hydrogen peroxide. ROS oxidize— *burn* —cellular macromolecules, and if they are not kept in check, they will burn down the cell. The selenoprotein "firemen" keep ROS in check, preventing them from burning down the cell. And how they do this is completely dependent upon NADPH. Let me explain in a little more detail.

HMG CoA reductase is a unique enzyme in intracellular metabolism because it requires *two* NADPH molecules to make just one molecule of its product, mevalonate. This makes HMG CoA reductase, and the mevalonate pathway, very sensitive to the intracellular concentration of NADPH. Mevalonate is a critical intermediate in the synthesis of selenoprotein "firemen," because all selenoproteins require isopentenyl pyrophosphate in order to come into existence. Selenoprotein "firemen" also need to be continuously "re-charged" by NADPH to remain active, and NADPH can therefore be envisioned as the flame-retardant material in the fire hydrants that the firemen use to keep ROS from burning down the cell. In cells with functional p53, DHEAS remains in the circulation, and therefore no DHEA forms inside the cell that could initiate potentially irreversible, uncompetitive inhibition of G6PD. In cells with active p53, then, selenoprotein "firemen" abound, their fire hydrants are filled with NADPH, and ROS are maintained at levels that are survivable by the cell.

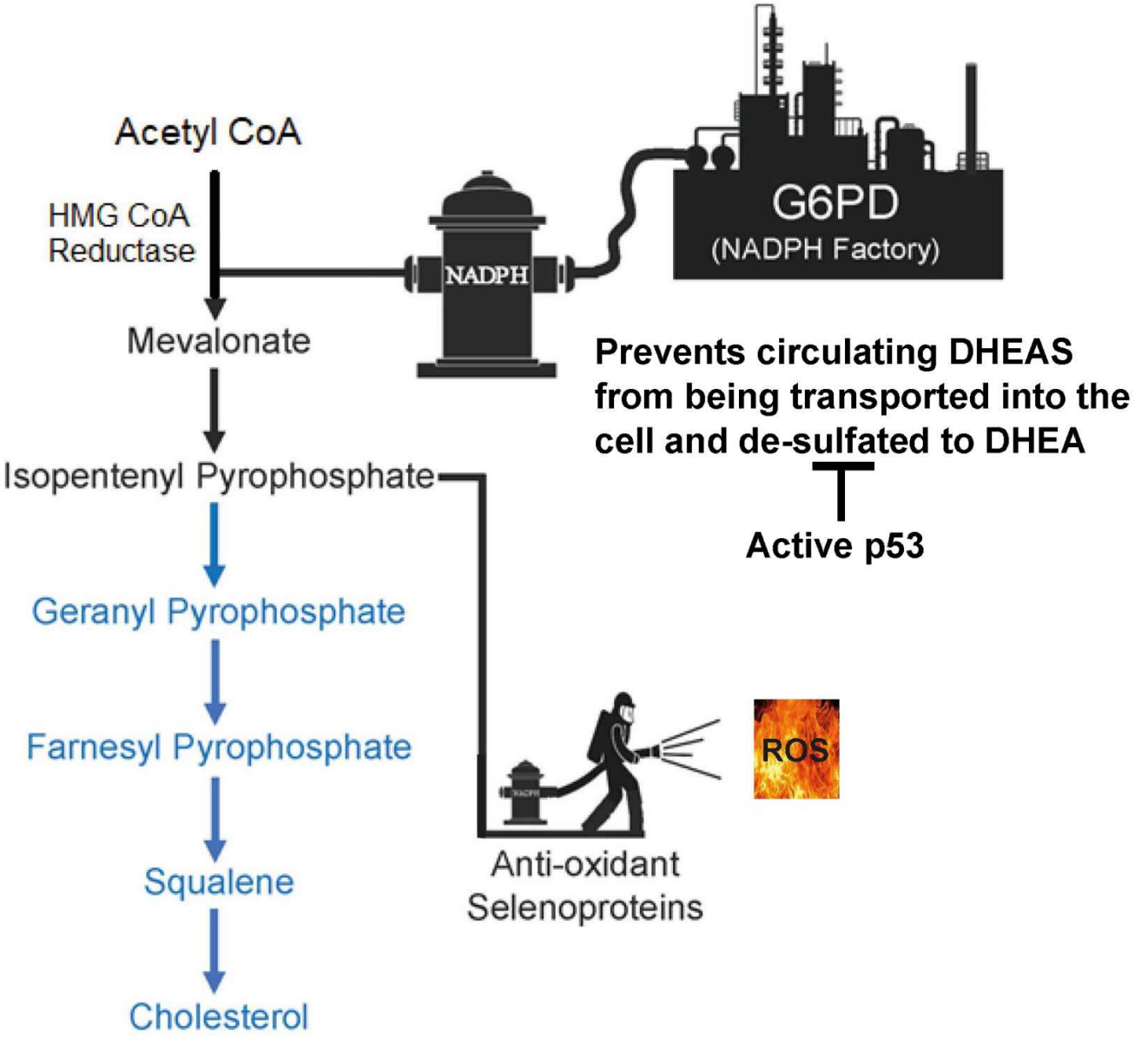

Figure 2.1 In normal cells with functional p53, G6PD factories produce the NADPH required to both bring selenoprotein "firemen" into existence, and to keep their fire hydrants full.

Now let's examine what happens when p53 is *inactivated* in a cell, causing that cell to become pre-malignant.

It had always been very puzzling why Nature would evolve primates to have levels of circulating DHEAS that are hundreds

of times higher than in other vertebrates— thousands of times higher when the primate we are considering is human— when its proximate metabolite, DHEA, is a deadly inhibitor of so critical an enzyme as G6PD. My lab's discoveries, first, that species-specific mechanisms of tumor suppression are a fundamental element of vertebrate speciation, and then, that our species-specific mechanism of tumor suppression is based upon DHEAS, explains this conundrum.

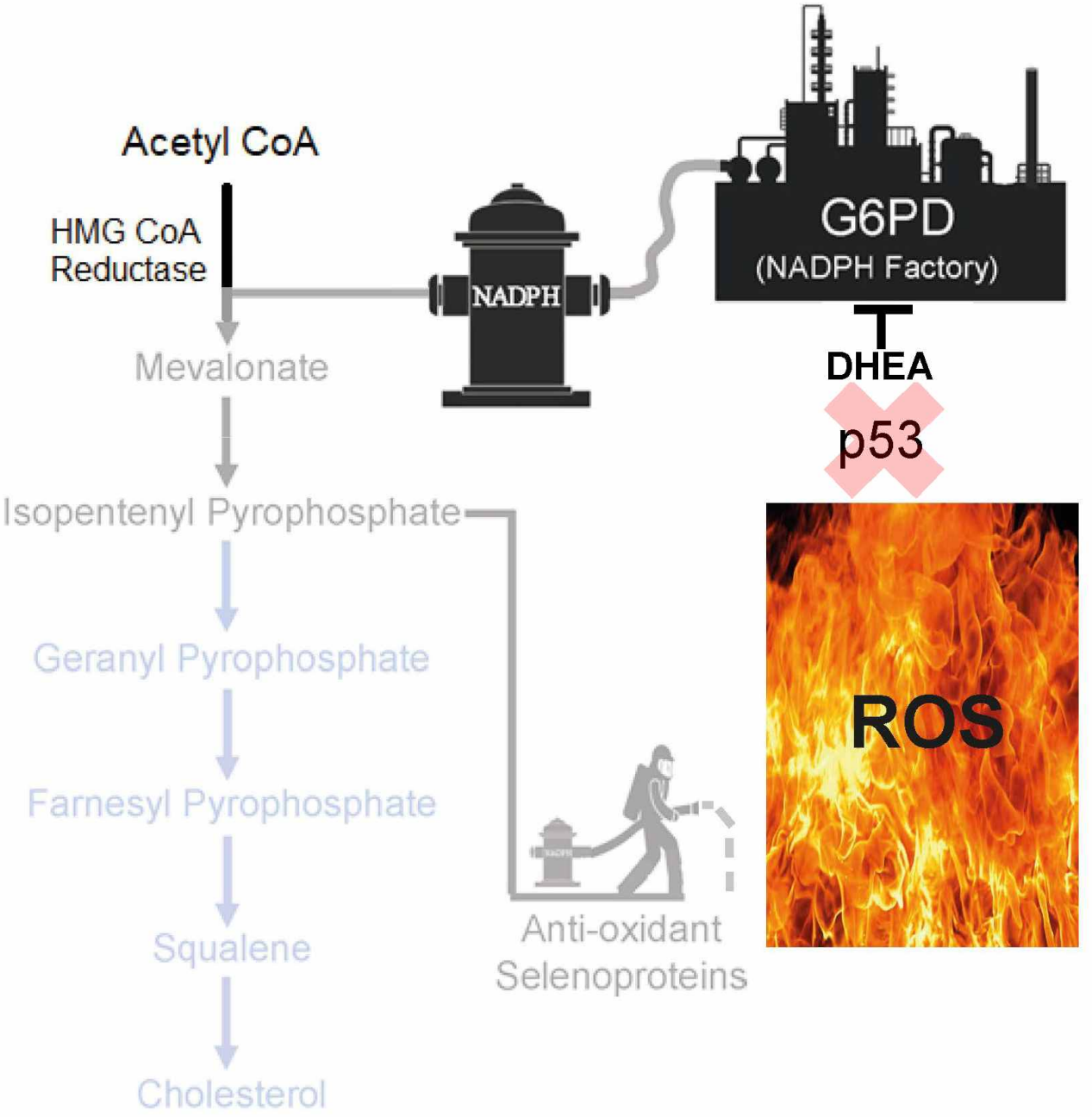

Figure 2.2 Inactivation of the p53 tumor suppressor in a human cell triggers the DHEA-mediated "kill switch" tumor suppression mechanism, killing the cell by runaway ROS.

DHEAS (Dr. Jekyll) is kept at very high levels in the bloodstream—i.e., literally right outside every cell—so that if a p53 mutation occurs in any cell anywhere in the body, large

quantities of DHEAS can rapidly be pumped into that cell and converted to DHEA (Mr. Hyde). DHEA shuts off the flow of NADPH. With no NADPH to fuel HMG CoA reductase, the selenoprotein "firemen" disappear, their fire hydrants run dry, ROS flare out of control, and the p53-affected cell burns down. This ensures that pre-malignant cells that would have gone on to become tumors are killed off while they are still at the single cell stage. My lab has proposed that such a "kill switch" tumor suppression mechanism fundamentally controls lifetime cancer risk in both humans and dogs.

The purpose of adrenarche

So what is the purpose of adrenarche? Adrenarche ensures that the "kill switch" tumor suppression mechanism becomes operational just prior to the onset of adult body size. Why? The whole point of species-specific mechanisms of tumor suppression is that they enable a larger species to evolve from a smaller one. *The advantages of having a larger body size— to revamp predator/prey relationships, to enable the traverse of greater distances in search of food, etc.—is the driving force.* To evolve a bigger body size than its immediate ancestor, a new species of vertebrate animal must simultaneously evolve an improved tumor suppression mechanism— a species-specific

tumor suppression mechanism— that is so superior to that of the smaller ancestor that it maintains the smaller ancestor's cancer risk even with the bigger body that has more cells at risk. But it is *adult body size* that is the driving force for tumor suppression improvement. During their early development, humans and other primates— indeed, all other vertebrates— have a reduced cancer risk by virtue of their small body size. This is because of the fact that such a small body contains a much smaller number of cells at risk for malignant transformation. But a big change in body size is about to begin with the passing of adolescence, at which time cancer risk is going to dramatically increase due to the much greater number of cells that the much larger adult body is made of— if nothing happens to offset that risk, that is. But something does happen. In humans, adrenarche pours vast quantities of DHEAS into the bloodstream just prior to the onset of adult body size, ensuring that the "kill switch" tumor suppression mechanism comes on line exactly when it is needed: when gonadarche starts building the adult male and the adult female body. Peto's paradox— noted in chapter one— is thus resolved. Cancer risk does not increase with increasing body size in different species because new, larger species are not permitted to evolve from smaller

ones unless they simultaneously evolve an improved, species-specific tumor suppression mechanism.

But bigger body size is not the only way to improve the chance of survival, especially in primates.

Bigger body size vs. increased exposure to cancer-causing substances. The back and forth trade-off that characterized primate evolution, especially for our species

I have presented evidence elsewhere (see reference 29) that exposure to carcinogenic polycyclic aromatic hydrocarbons (PAH) that were kicked up into the biosphere by the Chicxulub asteroid impact not only killed off the dinosaurs, but also created the unique DHEAS-secreting adrenal gland that brought the primate lineage into existence. I have further argued that a subsequent impact, 10 million years later— the one that initiated the so-called Paleocene Eocene Thermal Maximum (PETM)— rendered the Earth re-contaminated with PAH, and that these PAH carved changes into the primate genome; changes that consisted of still further improvements to the constantly evolving primate tumor suppression system. As more and more such “improvements” evolved, they presented primate speciation with three distinctly different opportunities.

“Improvements” in their tumor suppression mechanism could be “spent” creating new species with larger adult body size; or new species with longer lifespans; or new species with the capacity to tolerate increased carcinogen exposure— or, of course, new species with some combination of these three elements. The first primates, created by the PAH kicked up by the Chicxulub impact, were tiny, exemplified by *Archicebus achilles* , a diminutive basal Haplorrhine primate that is thought to have weighed perhaps 38 grams as an adult. These diminutive first primates had all they could handle acclimating to the extreme PAH contamination caused by the Chicxulub impact. So they “spent” all the capital derived from their new, DHEAS-secreting adrenal gland tolerating such extreme PAH exposure, remaining tiny.

One of the first improvements to the primate kill switch tumor suppression mechanism, an improvement that occurred during the PAH-contaminated PETM, was the deletion of a portion of the Gulonolactone Oxidase gene (GLO), creating the Haplorrhine lineage. Inactivation of GLO rendered the Haplorrhine lineage unable to synthesize vitamin C, but this was a necessary trade-off in order to enable G6PD inhibition to become more toxic in p53-affected pre-malignant cells.[29] A little later in the PETM, GLO deletion was followed by an

additional improvement mutation in the Glucose-6-phosphatase gene (G6PC), creating the anthropoid lineage (Figure 2.3, below). This G6PC improvement combined with GLO deletion to drive DHEA inhibition of G6PD toward irreversibility.[29] And exactly how this kill switch improvement was generated in the primate genome is telling. Demonstrating how PAH exposure shaped primate speciation during the PETM, the two improvement mutations that occurred in the G6PC gene both consisted of G to T mutations. Such G to T mutations are the "signature" mutations of PAH, and are rare in the absence of PAH exposure. [31]

Anthropoid primates could "spend" the combined GLO/G6PC improvement capital on increased body size, and began to do so in their speciation strategies. But the improvements to the primate kill switch tumor suppression mechanism that would eventually produce our species were nowhere near completed. Some 25 million years after the PETM, a dramatic improvement in adrenarche created the Catarrhine lineage, enabling primate speciation to further deploy increased body size as a mechanism of niche exploitation. This resulted in such species as *Aegyptopithecus zeuxis* , whose body mass of about 6.7 kg represented a more than 150-fold increase over that of *Archicebus achilles* (38

gram) and other basal primates (Figure 2.3, below). But the primate linege would have never gotten much larger than *Aegyptopithecus zeuxis* if additional, major improvements to the kill switch had not occurred. To see how this still diminutive lineage produced gorillas, and orangutans, and other large primates, we have to re-trace our steps to hunt for a missing ingredient.

Long time no C

The loss of the ability to synthesize vitamin C was an "improvement" in tumor suppression that was necessary for primates to advance through the PAH-contaminated PETM, and to become "poised" to increase body size (which would occur after the G6PC mutations noted above). However, loss of the ability to synthesize vitamin C deprived primates of one of their most powerful antioxidants. This was not without negative effect. Aging is thought to be the result of oxidation damage to DNA and other macromolecules, and rate of aging is a primary determinant of lifespan. Loss of the capacity to synthesize antioxidant vitamin C thus almost certainly meant that primates could not evolve the longer lifespans that make large bodies efficient. Loss of the ability to synthesize vitamin C antioxidant

thus acted as a barrier, preventing further increases in body size in the primate lineage.

This barrier was overcome by two separate and distinct mutations in the uric acid oxidase gene (UOX), one occurring in gibbons, and another occurring in the hominid primates (see Figure 2.3, below). UOX is the enzyme that in other species degrades uric acid— another very powerful antioxidant. These other species that degrade uric acid have active GLO, and can therefore depend on vitamin C for their antioxidant needs. By disabling UOX, the gibbons and the hominid primates overcame their loss of vitamin C antioxidant, by replacing it with uric acid antioxidant. Inactivation of UOX caused remarkable increases in circulating uric acid— in humans such levels can reach 400 micromolar— putting us and other hominids at risk for the potentially debilitiating malady of gout, a form of painful arthritis in which uric acid crystalizes in joints. [32] But the advantages inherent in replacing vitamin C with uric acid were apparently so great that it was worth the risk of developing gout.

The hominids evolved even further manipulations of uric acid physiology, in which uric acid transport was placed directly under the control of p53. [33] SLC2A9 is the cell surface protein that transports uric acid from the circulation into the

interior of the cell. In hominid primates, cells with active p53 can use SLC2A9 to pump uric acid into their interiors, which will then assist the selenoprotein firemen in controlling ROS inside the cell. But in hominid cells in which p53 has been inactivated, such inactivation switches SLC2A9 activity completely off, depriving affected cells of uric acid antioxidant. It is easy to see how this dramatically improved the primate kill switch tumor suppression mechanism: In cells that experience inactivation of p53— and are therefore in a pre-malignant state — DHEAS is imported into the cell, de-sulfated to DHEA, which inhibits G6PD, inhibiting the synthesis of selenoprotein "firemen" and emptying their fire hydrants of NADPH. This allows ROS levels to flare up out of control. Because p53 is inactivated in the cell, SLC2A9 is switched off, preventing the influx of uric acid that would otherwise dampen the ROS flare up. In this way, the kill switch tumor suppression was greatly improved, and the antioxidant barrier that had been preventing significant increases in body size was removed. This resulted in the evolution of such large primates as gorillas and orangutans, and the extinct *Gigantopithecus* , which, at three times the body mass of a gorilla, is believed to have been the largest primate ever to have evolved.

For a detailed analysis of the evolution of the kill switch tumor suppression mechanism of primates, and in particular that of our species, the reader is directed to the citations in reference 29.

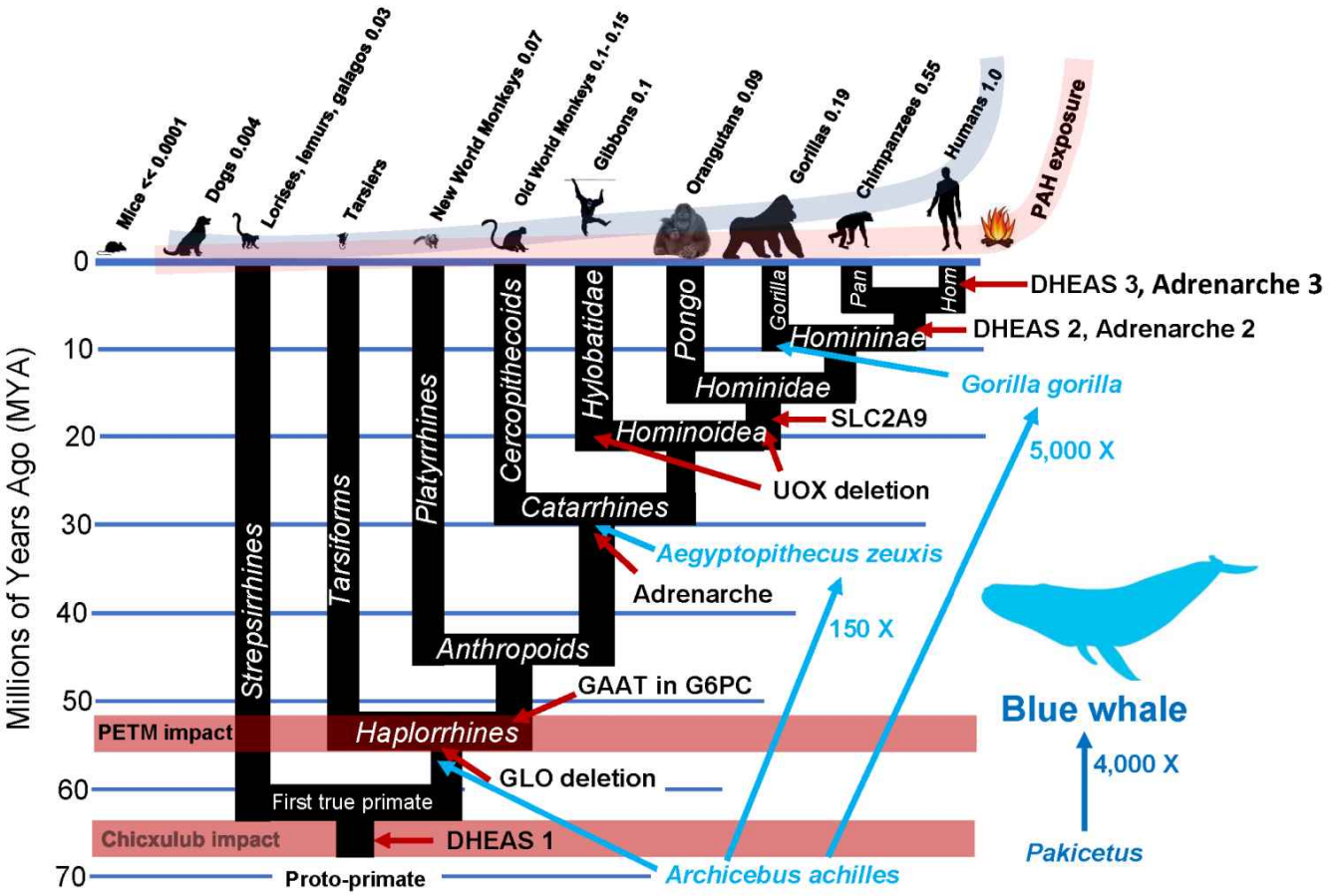

Figure 2.3 Primates evolved a lineage-specific "kill switch" tumor suppression mechanism based on very high levels of circulating DHEAS, and the irreversible uncompetitive kinetics of DHEA's inhibition of G6PD. Many "improvements" to this kill switch evolved over time, and the value of these "improvements" could be "spent" to increase body size,

increase lifespan, increase tolerance to carcinogen exposure, or some combination of these speciation events. The numbers above each species show circulating DHEAS levels, relative to human, which is set at 1.0.

Emergence of the killer primate

The last common ancestor of chimpanzees and humans— here we will call this creature Pretor—distinguished itself from the other hominidae by including red meat in its diet; i.e., it was the first predator primate. This characteristic carried over to Pretor's descendants, such that chimpanzees conduct raiding parties to capture, kill and eat smaller primates, and humans took this to a whole new level, learning to kill the megafauna that co-habited their landscape, and subsequently domesticating cattle so that meat became a primary food source for our species. Red meat is carcinogenic, [34] and red meat cooked over a fire becomes enriched in PAH, making it particularly carcinogenic. [35] This addition of red meat to the primate diet could not have happened without species-specific improvements in the kill switch tumor suppression mechanism to accommodate this increase in carcinogen exposure. As shown in figure 2.3, above, chimpanzees (and Pretor) increased

their levels of circulating DHEAS, and better focused adrenarche on the period of time preceding onset of adult body size. But their consumption of red meat did not approach that of humans, as neither did their consumption of cooked meat—although chimpanzees are known to preferentially scavenge at recent sites of natural fire. [36] Humans thus evolved much higher levels of circulating DHEAS as an important improvement in their species-specific tumor suppression mechanism. Also, adrenarche became completely separated from gonadarche only in humans, enabling the kill switch to be activated more completely before the onset of adult body size. The "capital" obtained by virtue of these kill switch improvements was spent primarily enabling the consumption of cooked red meat. Although there were substantial increases in body size during *Hominini* evolution— inter-tribal warfare and interaction with large predators were strong selection pressures favoring increased body size—the consumption of cooked red meat significantly constrained increased body size. Consequently, humans could never attain the body size of some of their fellow hominids, which remained vegetarians.

Gorillas and orangutans followed such a vegetarian evolutionary trajectory. This kept their carcinogen exposure extremely low, enabling the gorilla to spend its kill switch

improvement capital on a dramatic increase in body size; and the orangutan to spend much of its kill switch improvement capital on an expanded lifespan— the word “orangutan” is Malay for “old man of the forest.”

The company of dogs

Epi wolves exposed themselves to PAH-mediated genomic stress when they began their collaboration with we pyrophilic humans, joining us in our smoke-filled habitats. It may be that this relationship never could have gotten off the ground were it not for the fact that wolves, like primates, were made unique by the presence of circulating DHEAS coursing through their veins. And again like primates, female wolves uniquely produce most of their circulating DHEAS from their adrenal gland. While wolves/dogs have low circulating DHEAS compared to primates, it is worth considering that, nevertheless, those levels are 40 times higher than are found in mice, rats and most other species (Figure 2.3, above). This level of circulating DHEAS, combined with the smaller size of epi wolves and the first dogs, compared to “wild-type” wolves, may have been all that was necessary to tolerate the increased PAH exposure that was part of their new life with humans. There is also the fact

that smoke builds up from the ceiling down, and being four-legged and lower to the ground, epi wolves/dogs had less smoke exposure. They could also modulate such exposure by remaining outside the human habitat when they needed to. The parallel evolution of species-specific mechanisms of tumor suppression based upon circulating DHEAS thus enabled our two species to initiate and maintain this most remarkable of all inter-species collaborations.

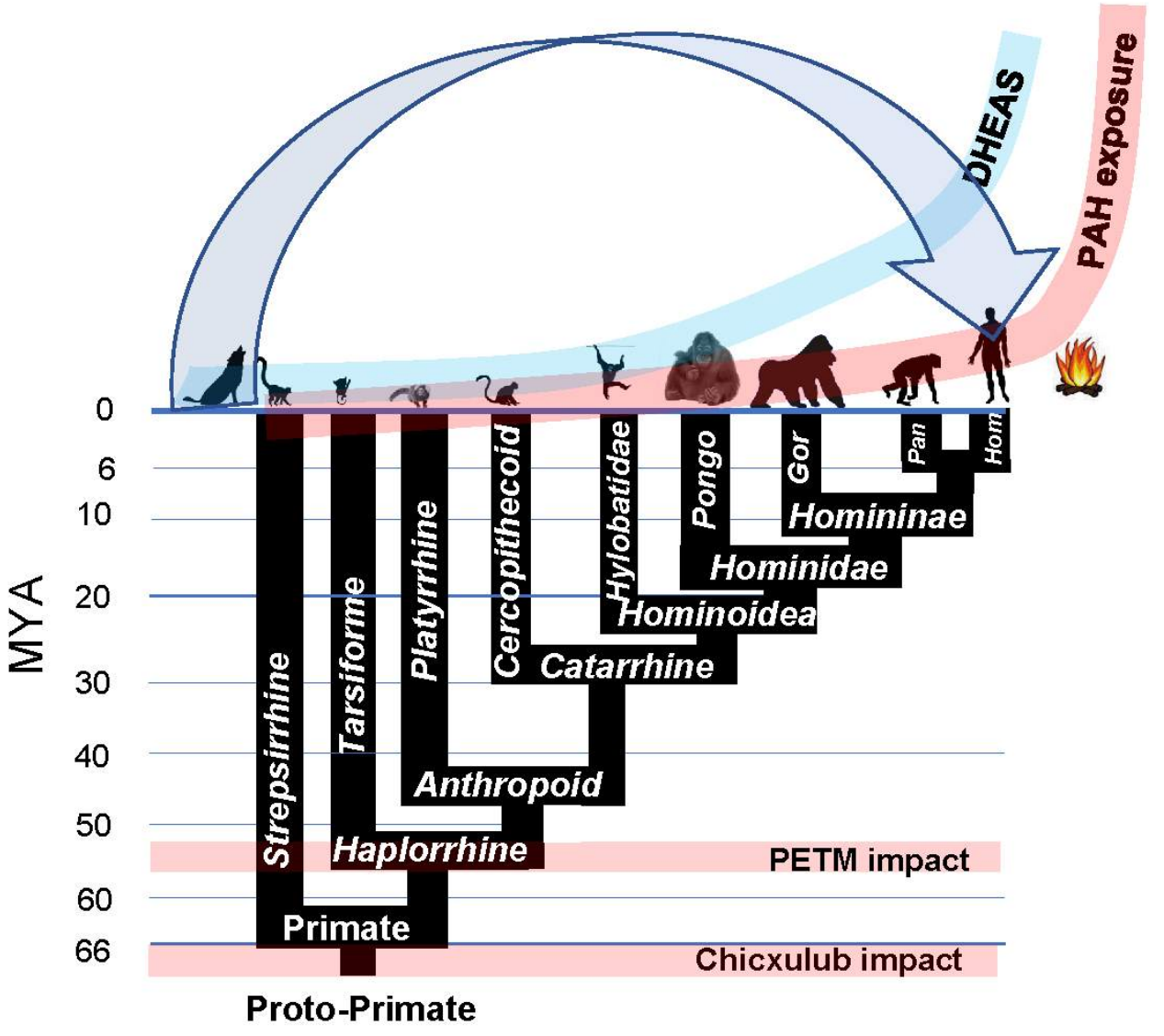

Figure 2.4 Compared to other mammals, the evolution of canine biochemistry and physiology has paralleled that of humans. Epi wolves may have been able to form relationships with pyrophillic humans because they, like primates, were unique among vertebrate animals in having DHEAS-secreting adrenal glands, and an analog of adrenarche. [37]

Other ways that DHEAS helped establish the relationship between epi wolves and humans

Like most biological features, such as the distribution of human height in males and females of our species, biochemical and behavioral features can be plotted as bell-shaped curves about a mean, the mean representing the average expression of the feature in the population. How far away a feature is from the mean is generally measured in terms of standard deviation, as shown in the figure below. As you can see, about 38.2% of the values that comprise this normal curve lie within an area stretching one-half standard deviation to the right of the mean, and one -half standard deviation to the left. These represent those values that are most closely related to the mean, the average. Sixty-eight percent of all the values represented by this normal curve fall within one standard deviation to the left

and right of the mean, and so on, until we see that only about 0.1% of the total values lie three standard deviations to the left , and 0.1% to the right of this normal curve— representing extremely rare values indeed. If we could draw our normal curve to greater scale—, that is, if the size of the population was very large— we would see that there might exist values five, or six, or even more standard deviations from the mean— exceedingly rare values indeed.

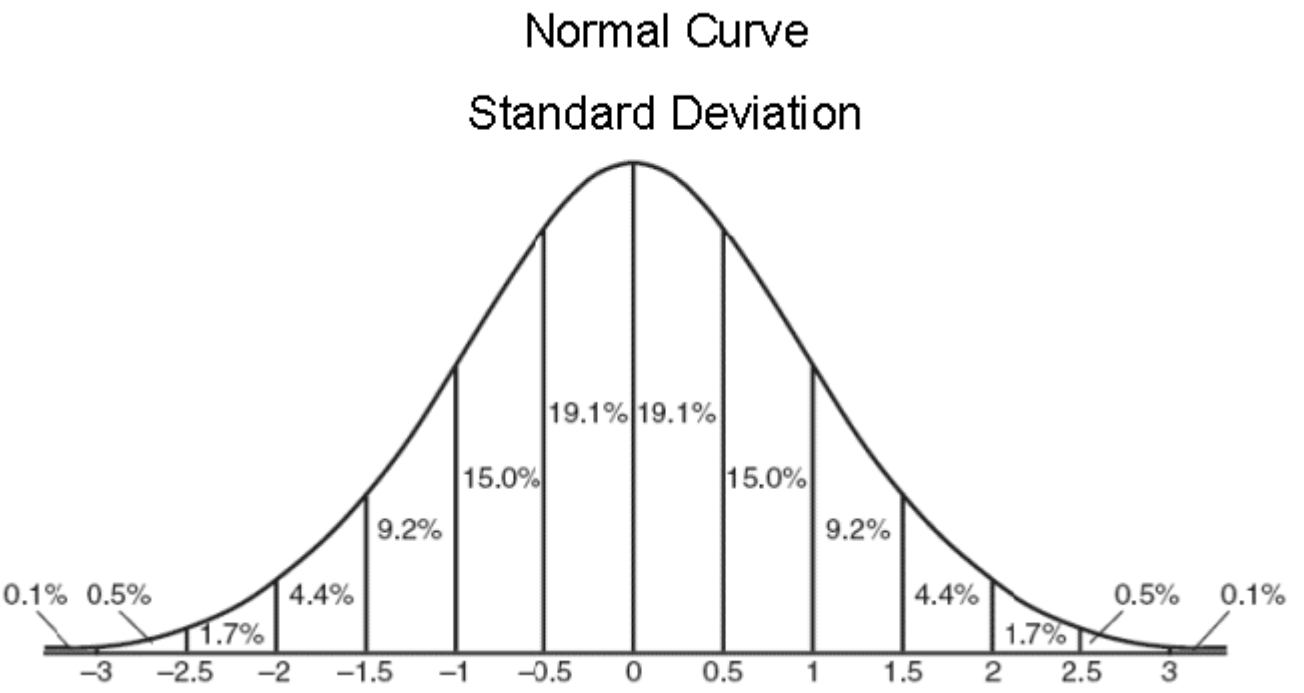

Figure 2.5 Standard deviation from the mean shows how far away from average a feature is

It is very likely that our epi wolf occupied an outlier position in the bell-shaped curve for a complex mix of wolf behaviors, and

that being so far from the mean of normal wolf behavior enabled it to initiate a new relationship with human beings. The various biochemistries underlying epi wolf behavior would have plotted out as a bell-shaped curve, with variability extending in both directions away from the mean; those plots would very likely have produced bell-shaped curves with different means for male and female ancestral wolves, similar to how human height produces different bell-shaped curves for men and women. There would in fact be bell-shaped curves for every imaginable body attribute, behavior, and underlying biochemistry, making every wolf a complex mix of such curves, and some wolves virtually unique.

DHEAS and the fight or flight response

All vertebrate species have built into them what is called the "fight or flight " response, a response that becomes activated by the perception of a potentially life-threatening danger. The amygdalae , two small almond-shaped clusters of nuclei located bilaterally, deep within the brain, initiate the fight or flight response, sending a signal to the hypothalamus that potential danger has been detected. The hypothalamus incorporates all sensory data and makes the decision as to whether the threat is

real. If the threat is deemed real, the adrenal gland is stimulated *via* the sympathetic nervous system and releases adrenaline (epinephrine), and cortisol, which raises blood pressure and increases blood sugar and the release of fatty acids into the blood stream, preparing muscles throughout the body for action. But at this point in the response to life-threatening events, the canine and human species appear to diverge, *together* , away from virtually all other vertebrate species.

In primates, the "fight-or-flight" response appears to exist on a behavioral continuum, with the "fight or flight" response sequestered toward one end, and an opposing, "tend and befriend" response on the other. "Tending" refers to nurturing activities that tend to reduce stress in both the giver and the stressed individuals that the giver tends. "Befriending" has been defined as the creation and maintenance of social networks that assist in this process. In humans, testosterone seems to push toward "fight or flight," and so "tend and befriend" appears to be a female response to stress more so than in males. [38] While knowing when to run or fight certainly has survival value, so, too, is it important for primates to be able to reduce stress by the "tend and befriend" strategy.

As we noted above, cortisol is a primary mediator of the "fight or flight" response, and therefore may oppose or even

block the “tend and befriend” response. DHEA is well known to oppose cortisol, blocking its effects on many levels. It has therefore been suggested that the high levels of circulating DHEAS may have evolved to permit our species to live together in large groups (“tend and befriend” instead of “fight or flight.”) [39] Co-habitation in large groups would offer a common defense against prehistoric carnivores and competing tribes, and would also have expanded the range of potential mates, important for the maintenance of diversity in the primitive human gene pool.

Are some of the aspects of DHEA-associated brain development (for example, enhanced memory and attention) that have been demonstrated to be operative in adolescent humans, also emerging in the species with which we have developed the closest relationship? Certainly, dogs, like primates, show the ability to remember events and to use such memories to later advantage. For example, border collies have been demonstrated to be capable of learning the names of more than 1,000 different objects; to be capable of executing a variety of instructions given in reference to such a large number of objects (for example to retrieve or otherwise manipulate a specific object from a very large group); to be capable of rapidly understanding the name of a new object by the process

of exclusion (i.e, capable of identifying a never-before-seen object by realizing that every other object already has a given name); and to understand the concept of categories. [40] Clearly, if DHEA contributes to memory and attention in dogs (the way it has been shown to do in humans), this contribution alone would be sufficient to explain the unexpected presence of circulating DHEAS in canines. However, for our story, DHEA's opposition to cortisol, and therefore its potential to inhibit the "fight or flight" response, and to promote instead the "tend and befriend" response, may bear directly on the initiation of the alliance that has occurred between our two species. As we have noted, behaviors have complex biochemical underpinnings, with the center of the bell-shaped-curve for a behavior representing the most common expression of that behavior, and the left and right ends of the bell-shaped-curve representing rare variations of that behavior. I propose that epi wolves that formed successful alliances with humans were those drawn not from the mean of the bell-shaped-curve for "fight-or-flight," but rather from the other extreme in which "tend-and-befriend" was the dominant behavior. Thus, successful epi wolves were likely to be female, not male wolves, and small, even for their sex. But they were also likely to be *outlier* females, with a

variety of additional biochemical, physical, and behavioral elements that were far from the mean.

The “cuddling” variable

A recent study has demonstrated that dogs, as part of their socialization with humans, have acquired human-like methods to bond with their owners. For example, human mothers and their infants engage in something called “mutual gaze”— a prolonged staring into each other’s eyes— which has been shown to elevate oxytocin levels in both the mother and the infant, creating a strong bond between the two. Oxytocin is part of the brain’s reward system, and is well-known to increase feelings of love and trust, important aspects of bonding. A recent study by Miho Nagasawa and colleagues [41] showed that dogs are able to elicit oxytocin secretion in their owners by engaging in mutual gazing, similar to the way human infants elicit oxytocin secretion in their mothers. Furthermore, in these experiments mutual prolonged gaze by the dog elicited closer interaction by the owner, such as petting behavior, which in turn elicited oxytocin secretion by the dog. No such effect was observed in wolves raised by hand from birth. To prove the association of mutual gaze with oxytocin secretion, Nagasawa

and colleagues administered oxytocin by nasal spray to a new group of dogs before presenting them to their owners. Such oxytocin treatment significantly increased the duration of mutual gaze initiated by the dogs, which again increased oxytocin secretion in their owners. These authors propose that the evolution of such a self-perpetuating oxytocin-mediated feedback loop participated in by dogs and their humans has played an important role in the bonding that dogs have established with us, and we with our dogs. I agree with them. But I also believe that DHEAS played an equally important, perhaps even more fundamental role in the establishment and deepening of the relationship between humans and canids. Since current wolves do not engage in mutual gazing with humans, this behavior must have been extremely rare among potential epi wolves, further stratifying them from the other wolves alive at the time.

Another recent discovery may bear upon the hyper-sociability of dogs toward humans that they have bonded with. In a study led by Dr. Bridgett von Holdt of Princeton, gene variants in canines were observed within the same genetic region in which deletion in humans causes Williams-Beuren Syndrome (WBS). [42] Patients with WBS are slow to fully develop, retain childlike features into adulthood, and possess a

hyper-sociable personality. [43] One of the sociability genes that resides in the deleted region of WBS patients is known as GTF2I, which codes for a protein that binds to and controls the expression of many other genes. One of the main functions of GTF2I appears to be to regulate oxytocin's effects. Thus, when GTF2I is deleted, as in WBS patients (or it is modified by less drastic changes, as in dogs), a hyper-sociable personality is the result- apparently GTF2I deletion releases oxytocin from all constraint to work its socializing magic. But in other humans, who have the GTF2I gene *duplicated* , creating *extra copies* of the protein, oxytocin is prevented from working; such duplications are observed in patients with certain forms of autism spectrum disorder, and result in anti-social, aloof behavior. [44]

There is a direct connection between DHEAS and oxytocin. Both serum oxytocin and DHEAS are reduced in young patients with Attention Deficit Hyperactivity Disorder (HDAD) complicated by aggressive behavior, [45] and administration of DHEAS to near term pregnant women has been shown to elevate serum oxytocin levels. [46] Clearly, DHEAS and oxytocin are part of a biochemical pathway that remains to be fully described. In my studies I have observed, as I have noted, a profound response in dogs administered DHEA. The response

is near universal, even in very sick dogs. It is a remarkable thing to see. I have proposed that both the epi wolves who initiated interaction with humans, and the humans who accepted that interaction, may have had unusually high levels of circulating DHEAS, enabling that interaction.

The “Woof! Woof! Woof!” variable

Even with their extremely acute olfactory and auditory senses, epi wolves would not have proved useful to early humans unless they actually used those acute senses for the benefit of the human tribe to which they became attached. This eliminated the vast majority of wolves, even those with a dominant “tend-and-befriend” behavior pattern, because *wolves do not bark* . Barking is a very rare phenomenon in wolves, accounting for only about 2% of their vocalizations. [47] Barking is a behavior much like any other in that its occurrence will plot as a bell-shaped-curve. But in this instance, it is not barking, but rather the absence of barking (or barking only 2% of the time when vocalizing), that represents the mean of the bell-shaped-curve. Moving four or five or six standard deviations to the left of the mean, there will be wolves that bark incessantly (probably driving their pack mates crazy.) It was from this pool of rare

wolves that successful epi wolves were drawn, a pool made even smaller by the fact that they also had to have “tend and befriend” as their dominant interactive behavior.

“Detect-and-Protect”

There were clearly additional behaviors that were important for epi wolves to have, behaviors that were equally as rare. For example, dogs are well known for their bravery in defense of their humans. In fact, just this evening on the news there was a story of a woman who had been attacked and nearly killed by a large, predatory bear in Western Pennsylvania, the woman having been drug by the bear by her leg more than 90 yards before her chihuahua successfully got the bear to loose its grip, and then stayed between the woman and the bear while she crawled to her escape. [48] This is an example of a fact of which we are all aware: our dogs are completely fearless in our defense. While the biochemical underpinnings of this fearlessness in defense of their human is at present unknown, it may be closely related to the bonding solidified by oxytocin secretion. Thus, with respect to the trait of barking upon detection of a threat, and fearlessness in defense of their human, I refer to these behaviors together as “detect and

protect." Clearly, a simple two-dimensional bell-shaped curve is inadequate to describe the behavioral variables that enabled an epi wolf to establish a long-lasting relationship with a human. In the three-dimensional bell-shaped curve below, the red cube barely visible at the intersection of the "tend-and-befriend" and "detect-and-protect" X and Z abscissas, and near the base of the Y ordinate, is meant to convey the rarity of all the requisite behaviors occurring in the same epi wolf.

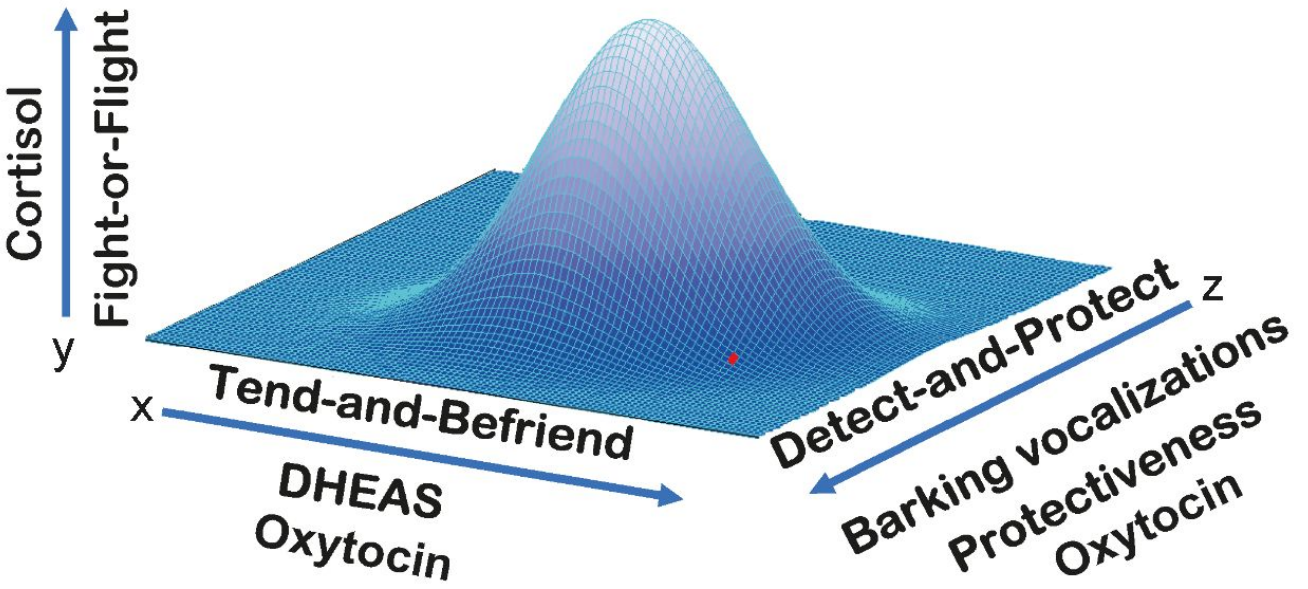

Figure 2.6 Multi-variable representation of the biochemical and behavioral variables that may have uniquely qualified epi-wolves to initiate contact with humans. The tiny red rectangle represents the rare combination of behavioral, neurobiological, and neurochemical features that distinguished an epi wolf from her peers, and made her seek out human contact.

To summarize the qualities that I believe our epi wolf must have had:

1. First and foremost, our epi wolf was almost certainly female.

2. She had much higher than average circulating levels of DHEAS, which blocked the cortisol-mediated “fight-or-flight” response, favoring the “tend-and-befriend” response instead.

3. Her higher than average circulating DHEAS (and its enhancement of memory and attention) may have made her a “thinker” among her peers, much less quick to act on instinct, much more open to new possibilities.

4. She was probably on the slow end of the growth curve, smaller than most of her peers, with a tendency to resolve conflict by “tend or befriend,” cowering and submissiveness, or some other mechanism that did not involve aggression.

5. She was probably a poor hunter, and did not fit well into the organized hunting parties of her pack.

6. She had a strong tendency to bark, which may have contributed to her failure as a hunter.

7. She was the most submissive member of her pack, her high DHEAS levels causing her to almost always choose “tend-and-befriend” over “fight-or-flight.”

8. She was probably constantly hungry, as her less aggressive attitude often made her last to feed at a carcass.

9. Being of higher intelligence for a wolf, she could realize that hers' was an untenable situation.

10. She may have been expelled from her pack by an alpha female.

The Artemis hypothesis of epi wolf "domestication"

Was the unique behavioral, neurobiological and biochemical makeup of our female epi wolf enough to initiate the relationship with humans? Almost certainly not. In the harsh reality of the paleolithic, most humans would have killed any wolf that they could get close enough to hurl a spear towards. This would certainly have been true for the male hunters of virtually all paleolithic tribes, alpha males all, perhaps led by a super alpha male. Any stray wolf unlucky enough to come into contact with such a band of primitive humans would have met with a swift and ignominious fate, her teeth quickly adorning a necklace, her fur turned into a fine, if small blanket.

And our epi wolf would have almost certainly been a stray, forced out of her pack.

But imagine a different kind of first contact; one in which the members of each participating species were both outliers—individuals who were both quite some distance from the mean of their respective bell-shaped curves, one wolf, one human. Thus, the epi human was probably also an outlier with respect to average human behavior. It is possible that the epi human was a male, but I don't think so. Male bonding in hunter gatherer societies must certainly have been extreme, and rigorously enforced, with little chance for any tribesman to live very long as an outlier. In my opinion, the epi human that enabled the relationship with the epi wolf was an outlier female; an independent woman who rejected aspects of her hunter gatherer society, and thereby prevented herself from getting pregnant and heading down the normal path of a gatherer female. Perhaps she was an accomplished huntress. She may have been the Artemis of her tribe, deeply communing with nature and rejecting the attention of men. In Greek mythology, Artemis was the goddess of wild nature, the mistress of animals. Artemis not only hunted animals, she also protected them. Like Artemis, perhaps this epi woman had a powerful father who could protect her from tribal aggression; one who would listen to her ideas and her different ways of looking at the world and not just dismiss them as the alpha

males in her tribe would. Perhaps she had a father who had enough control over the tribe that he could enforce her idea to let the nearby female wolf and her cubs alone…

The epi wolf had watched Artemis at her secret place for several days, keeping quiet and hidden, and always downwind. Hungry, cold and alone, she had finally run out of options. One morning, soon after Artemis had arrived, the epi wolf came out of hiding and approached Artemis submissively, crawling on all fours, eyes down except for furtive glances for weapons. Artemis was startled at first— this was a wolf approaching her, after all— but instead of reflexively reaching for her spear, something inside her staid her hand, and compelled her to watch, and wait. You see, Artemis, like the epi wolf approaching her, had higher than normal levels of circulating DHEAS, so she was right-shifted, away from the "fight-or-flight" reflex, and toward the "tend-and-befriend" reflex. Artemis and the epi wolf stared at each other for a very long time without moving, each one wondering if this was really happening; if this member of the enemy species was different; if this one might, just might, be trusted. Artemis may have made the first move, offering the hungry epi wolf a piece of dried

jerky. Once the epi wolf accepted and devoured it hungrily, more was offered. Then Artemis noticed something that she had never seen in a wolf before. This one was gazing straight into her eyes; holding that gaze. A gaze that appeared to be communicating something. But what? Appreciation? Submission? Friendship? Then Artemis understood. The epi wolf's gaze was communicating trust. "I am going to trust you," the epi wolf was saying. Silently, the epi wolf might be adding in her thoughts, "I have no other choice. You have not attacked me yet, so perhaps you are different; as I am different." But the epi wolf's uninterrupted gaze made Artemis feel something special for this young wolf laying submissively just a few feet away. Artemis handed the epi wolf more jerky, and more, until her day's provisions were exhausted. When the submissive wolf inched closer, Artemis laughed and tried to show her that the bag was empty; there was no more food. But then she realized, as the exhausted young wolf laid its head upon Artemis's thigh, that it was not food that the wolf was after. It was trust; it was safety; it was companionship. With some hesitation, but hesitation overcome, Artemis laid her hand upon the

wolf's head. "Yes, you can trust me," she said softly, hoping the wolf would understand her meaning by her tone. "Yes, we can be friends. You are safe with me. And I won't let anyone else hurt you, either." After a moment, she stroked the wolf's head with a downward movement of her hand, in what was the first petting of a canine by a human. Oxytocin was secreted in each of their brains, and coursed through their veins, forming a bond between them.

Artemis kept her epi wolf hidden in her secret place, which she knew was safe; it was a place that no one else ever went to. Every day she came to the site with more food, and the wolf would quickly reappear. They would play together, and the wolf would end each play session by nudging, her way to ask for more petting, which she always received. The bond between them grew stronger and stronger.

After a few weeks, Artemis noticed that the epi wolf was pregnant. This is when she went to her father, Zeuxis, chief of the clan, and told him about the wolf. Zeuxis, being protective of his daughter, as any father would, insisted upon seeing this wolf that Artemis claimed was not really a wolf— at least not like any

wolf the clan had ever known before. When they approached together, the epi wolf reverted to her aggressive heritage, growling and snarling at this human hunter drawing near to her, spear in hand. But Artemis calmed her, and Zeuxis was amazed beyond words to see his daughter put her arm around the wolf, and the wolf respond by licking her face. A wise man, he lowered his spear. He knew enough to trust his daughter. But for an hour he stood at the ready, in case this strange wolf's behavior reverted to that with which he had become accustomed over a lifetime of competition and conflict with this predator species so parallel to his own. After a while, with Artemis coaxing both sides, her father finally moved closer, kneeling, and the epi wolf bowed her head in submission. Artemis showed him how to stroke the wolf's head, something that felt to Zeuxis like a strange thing to do to a living wolf. It would take time for wolf and hunter to trust each other, but each trusted Artemis, and so they would put their mistrust aside.

As Zeuxis prepared to leave, the epi wolf began to bark, loudly and repetitively, at something off in the

distance. "What is she doing?" asked Zeuxis. "Wolves don't do that."

"I know," said Artemis. "She's different, I keep telling you." Then Artemis looked off into the distance, far down the valley. "She hears something; or she smells something."

Zeuxis looked carefully in the direction that the wolf was looking as she barked.

"There's nothing there, child."

"She's never wrong," Artemis replied, muzzling the epi wolf with her hand as they watched and waited.

Twenty minutes later, one of the young tribesmen from their clan moved out of the trees, a thousand meters away, and started up the hillside, every few meters stopping to look intently at the ground, searching for signs, and bending to test the soil. He was tracking Zeuxis.

"It's Solto," said Zeuxis, which made Artemis frown. Solto was Zeuxis's chief lieutenant, and he had been actively courting Artemis for months, which she had even more ardently discouraged. Zeuxis hoped she would eventually come around, for Solto was a fine man. But he knew his daughter was headstrong, and

different, and he would not force her into anything that she did not choose for herself.

But unlike Artemis, Zeuxis wasn't frowning. Not at all. He was realizing just how much warning this strange wolf had given them at the approach of someone unknown to her. After a while of staring off into the distance, deep in thought, he turned, knelt down upon the soft ground, and petted this strange wolf's head.

In my opinion, the legend of Artemis is more than a legend. It may very well describe the epi human who enabled the approach of the first epi wolf. And it may not have been a one-time event. As noted above, the most recent evidence is that the domestication of the dog happened twice, once in Eastern Eurasia, and once in Western Eurasia, from distinct wolf populations in each locale. [49] I would bet a lot that the Artemis legend repeated itself in both locations, and that both the epi wolves and the epi humans that were involved in each origin event similarly resided far to the left of their respective neurobehavioral means. If this is indeed what happened, then we owe the evolution of our beloved dogs to the repeatable science of outliers. But is there any precedent outside of Greek

mythology to support even a single real-life Artemis, let alone our repetitive outlier scenario?

A few months after I penned my Artemis hypothesis I was surprised to hear of a present-day huntress who provides splendid support for it. In Mongolia there today lives a 13-year-old girl who has learned to hunt with a Golden Eagle. Golden eagles are magnificent creatures that can stand over three feet tall and can have a wingspan of seven and a half feet. In the barren landscapes of the mountainous regions of Mongolia, they can be seen soaring high above, searching the tundra thousands of feet below. When they spot prey, they swoop down in an astonishing arc that ends with a shocked victim being impaled by sharp talons, and lifted into the sky by giant, muscular wings. Fearless hunters, golden eagles have been known to attack animals as large as full grown deer. A raptor of this size and ferocity would be difficult for a full-grown man to tame and handle, but this young girl from Mongolia, whose name is Aisholpan Nurgaiv, has done just that. Her story is now the subject of a National Geographic documentary, *The Eagle Huntress* . Like Artemis, Aisholpan had a father who supported her outlier life style. I was also pleasantly surprised to learn that Aisholpan was not the only eagle huntress in history. In 1932, National Geographic photographed another such huntress,

Mongolian Princess Nirgidma, with her hunting eagle. *So there is precedent for the Artemis hypothesis of wolf domestication—and even for it happening more than once* . Even today, in modern times, outliers rule. And frequently, these extraordinary beings who push our species forward, are outlier women.

Figure 2.7 Aisholpan Nurgaiv, the Eagle Huntress. National Geographic

3

Rex the Wonder Dog

"Unforeseen surprises are the rule in science, not the exception"

Physicist Leonard Suskind

I received my doctorate in cancer biology at the Fels Cancer Research Institute in Philadelphia, part of the Temple University School of Medicine. My thesis research focused on two different areas, one related to the anti-cancer effect of dehydroepiandrosterone (DHEA), which I found could inhibit the growth of colon tumors in mice— not cure, or make disappear, but simply delay the progression of. This point— that DHEA never produces complete tumor regression in mice

— is critical for what will come later in this chapter, so I ask you to underscore it in your memory. The second half of my thesis research project involved a new idea that I had been working on, linking enzymatic methylation of DNA to cancer — the idea that would subsequently play a major role in the birth of an entirely new specialty which would come to be called the epigenetics of cancer. It turned out that the National Cancer Institute (NCI) found this new idea to be quite interesting, and they provided me with a grant to continue researching it, which I did at the Children's Hospital of Los Angeles (CHLA, part of the University of Southern California School of Medicine.) One of my projects at CHLA was to figure out why about half of the kids receiving a drug called cytosine arabinoside (araC) for acute lymphocytic leukemia (ALL) responded and went into remission, while half did not, rapidly declining, and dying. It was during this project that I first developed the concept of drug-induced DNA hypermethylation (DIDH) as a cause of drug resistance, showing, first *in vitro* ,[50] and then *in vivo* ,[51] in kids with drug-resistant ALL, that DIDH could account for at least some of the cases in which children with ALL failed to respond to araC. In the *in vivo* work in children with ALL, the protocol blocking DIDH induced remissions in children who had been very close

to death. I still remember how wonderful it felt to see these children sitting up in their beds, feeling the embrace of their parents, and the faces of those parents, filled with tears of joy. This was the first time that I had been able to bring an idea that I had created in my own brain into existence in the real world, and it had enabled another human being to stay alive. If I hadn't been hooked already on spending my life doing more of this, I was hooked then. This small clinical trial at Children's Hospital was the first ever demonstration that DIDH occurred in actual patients, as I had proposed it would, and that blocking this epigenetic phenomenon could have a profound effect on treatment outcome.

Based upon the fact that this research was likely to receive further funding from the National Cancer Institute, I was offered a professorship at the Brody School of Medical Sciences, part of the University of North Carolina system.

It was on the cross-country drive to this new post, in my jeep, that I fleshed out drug-induced DNA hypermethylation in the form of a grant proposal to the NCI. I have to say that the transition from Los Angeles to the east coast of North Carolina was an interesting one for me— and probably also for the North Carolinians who had to integrate this west coast barbarian into their genteel culture. At the North Carolina border I came upon

a huge sign which read, “Welcome to North Carolina. Compliments of the North Carolina Coon Hunters Association,” with a picture of a racoon on it, apparently cornered up a tree. I could not resist, and so I untied my surfboard from the roof of my jeep and posed with it in front of this sign, scratching my head, with a puzzled look on my face. My friends back in Southern California got a kick out of the photograph when I sent it to them.

Once I was unpacked and had gotten situated, a helpful secretary in my new department, the Department of Pharmacology, helped me get my grant off to NCI, and I set about equipping my new laboratory using the start-up money that the department had provided.

Greenville was a very conservative city, not accustomed to a new professor arriving with his surfboard strapped to the roof of his Jeep, refusing to wear socks, let alone a suit and tie. The culture shock went in both directions. Eastern Carolinians at the time were not really accustomed to the requirements of a molecular biologist, as I was one of the very first to take up residence in this part of the state. Back then, we used a procedure called “Southern blotting” (named after the inventor, not the direction) combined with intensely radioactive nucleotide-substituted DNA probes to determine the

methylation status of a section of a gene. In this procedure, the DNA of interest is cut into small fragments with enzymes called restriction endonucleases, and then separated according to size using a procedure called polyacrylamide gel electrophoresis. The single-stranded DNA fragments in the gel are then transferred to a nylon membrane, and the membrane is placed in a Tupperware container [52] containing a solution that has the radioactive probe in it. The idea is that your radioactive probe, which is a single-stranded DNA corresponding to a region of your gene of interest, will hybridize to the complementary DNA fragment on the nylon membrane. The final step is taking that nylon membrane, which has columns of DNA fragments on it, one or more of which may have hybridized to your probe, and putting it in contact, in the dark, against x-ray film, and placing the nylon membrane and film inside a light-tight x-ray cassette, similar to the ones physicians use to x-ray broken bones. The information that we seek— the DNA sequence, the methylation status of a section of a gene; whatever— burns an image onto the film, and it is the developed film that we read this information from. You may have seen such X-ray films placed on a light box, whereupon an actor playing a doctor on one of your favorite hospital shows proceeds to make a diagnosis based upon what he sees on the

film. This is the same principle. Molecular biologists, at least back then, went through this tedious procedure to get our end result— an X-ray film into which bands representing our data had been etched by radioactivity.

I was ready to perform my first such experiment in my new lab, but realized that I still did not have the X-ray cassettes that I needed to develop the film in. I called up the department of surgery, and asked where they got theirs. I was given the number of a hospital supply company in the neighboring town of Farmville. When I dialed the number, the voice of a young woman with a very heavy southern drawl answered, and I proceeded to tell her the reason for my call. Before I had even finished, she began to berate me, and it took a full thirty seconds before I understood exactly what she was saying in her beautiful Southern drawl.

“Sir, this is a legitimate hospital supply company. We do NOT, sir, NOT sell x-rated cassettes. You will have to go somewhere else for that sort of thing!”

It took another sixty seconds before I could calm her down and get her to understand what I was saying, in my apparently impenetrable Yankee-speak. But finally she did, and then she became apologetic in the extreme way that only well-bred Southern women can be. Apparently, in her company what I

was looking for was referred to as a “metal” cassette, not an “X-ray” cassette, in order to distinguish it from the cardboard type of cassettes that were also available. When she was finally through with her explanations and apologies, she got back to the business at hand and asked me what kind of X-ray cassette I was looking for, because they had a large selection. By this time, I just could not resist, and so I said, “Well, do you have *The Devil in Miss Jones* ?”

Click. The phone went dead.

True story.

In any case, I eventually got my x-ray cassettes, and was able to get to work, generating the data that I would need in order to submit my grant application to the National Cancer Institute. I received a very good, fundable score on my NCI grant application , and subsequently published a series of results in top tier cancer journals, ranging from Cancer Research to the Proceedings of the National Academy of Sciences . It was during my work in North Carolina that George Hitchings took an interest in what I was doing, and became an additional mentor to me. George was the inventor of mercaptopurine, an important drug for the treatment of childhood leukemia, for which he won the Nobel Prize.

Rex the wonder dog

Grants from the NCI require that you re-apply for renewal after their initial period is over, showing what you have accomplished, and how you plan to move the study forward. When it came time for my renewal application, I had done well, and so I received a very good score from the study section that would go on to renew funding for my research program. When the medical school found out about my renewal, they made a big deal out of it— Greenville not yet being known as a hotbed of cancer research. At that time, my wife and I had two young sons, seven and five years old, and we lived on the third floor of an elevator-less apartment building just off campus. As we were walking down the stairs one Sunday morning, my eldest, Alex, pointed to the newspaper on a neighbor's stoop, and said, "Daddy, you're in the newspaper!" To my surprise, the article about my cancer research project, and the renewal of my grant, along with a picture of me in my well-worn lab coat, filled the front page. The only reason that I mention this now is because something happened as a result of this article that would eventually change everything, something that would eventually lead me to rebel against the paradigm that cancer was the same disease in all species. A local veterinarian saw the article, and the following day he gave me a call. One of his long-time

clients owned a champion Rottweiler, named Rex, and he wanted to show me an X-ray that he thought I would be interested in. When he arrived at my office, the X-ray he pulled out of an over-sized manila envelope astonished me. It showed the outline of a large dog, his body literally filled with golf-ball sized masses. I had only seen one other case in my life in which so much tumor occupied so much internal volume; that was at an autopsy of a little girl at Children's Hospital, a little girl with a gigantic form of Wilms tumor. The poor girl herself weighed only 60 pounds, and the tumor that bulged from her abdomen like the monster that it was, when weighed during her autopsy tipped the scales at 29 pounds. [53]

The vet told me that he had biopsied one of the dog's masses, and he showed me the pathology report that he had subsequently gotten back. Soft tissue sarcoma was the diagnosis, a tumor type with a dismal prognosis. He told me that the owners of the dog had seen the newspaper article about my work, and had brought it to him to inquire if there was anything that I could do for their beloved Rex. The owners had declined to start the dog on chemotherapy, after being told that it was unlikely to help and would probably make the animal's last days miserable. The vet told me that he had gone online to the National Library of Medicine (PubMed Medline) and had

read some of my published papers on DHEA. Since I had written that DHEA was natural and that it inhibited tumorigenesis in mice, did I think it might help this dog? I told him that the tumors in those mice had been induced using carcinogens, and there was thus no way to know how spontaneous tumors such as Rex had would respond to treatment with DHEA. Even more to the point, in mice, yes, very large doses of DHEA had slowed tumor growth, *but it had not cured any tumors* . He said that the owners were willing to try anything, and since the opportunity to test DHEA in a spontaneous tumor in a new species appealed to me, I put together a standard informed consent form, took my plan to the Animal Care Committee at the University, explained to them what I wanted to do, and obtained their permission to treat this one dog with daily subcutaneous doses of DHEA. Once the owners signed the informed consent form, I homogenized DHEA in sesame oil, sterilized it, and headed over to the veterinary clinic where the dog and his owners were waiting.

When I first saw the dog, I came very close to telling the vet and the owners that he was too far gone to make any attempt at treatment. The Rottweiler lay on the floor, clearly in distress, breathing rapidly, and apparently unable or unwilling to move even when his owners urged him to do so. Although I

was fairly certain that I was making a mistake, I agreed to proceed. We injected the DHEA subcutaneously, creating a depot from which DHEA would dissipate into the dog's system over the next 24 hours. At the end of that period of time, the vet would make another, identical injection, but at a different site in the dog's body. I left enough sterile DHEA mixture for the vet to make daily subcutaneous injections for an entire month. I told the vet that if the dog lasted for a month, I would like an X-ray taken to see if the treatment had done anything. Fearing the worst— that the dog might not last the day, let alone the month— I headed back to campus. I was in the teaching rotation that semester, and I had to prepare my lecture for the next morning's class of physicians-to-be. I quickly fell back into my routine, lecturing to medical students in the early morning, directing my lab's research all day, and then racing home at seven or so to have dinner with my family and wrestle on the floor with my kids.

A couple of days later one of the departmental secretaries stopped me after class to tell me that she had received an excited message from a veterinarian who said that I was helping him with a case. He wanted her to let me know that the dog was up and running around, acting like it was a puppy again. The family was very happy, the vet had said. Ecstatic, in

fact. I didn't have a chance to call back, and I did not believe that the dog's recovery would be long-lived, so maybe there was no reason to call back. I had no idea then that this would turn out to be a general feature of DHEA in dogs— unlike any other animal, dogs respond to DHEA as if it literally turns back the clock on their age. Even old dogs like this cancer-ridden Rottweiler will frequently turn into veritable puppies again, as if they had been reborn. It is a truly amazing thing to see. (I will explain why they do this later.)

Exactly three weeks and one day after treatment had been initiated, I received an even more excited call from the vet. The Rottweiler had been feeling so well that the family had him perform another X-ray, and there was no sign of any of the tumors! He wanted me to come to his office immediately to see the film, and the dog. At first I thought someone was having some fun with me. I had a hard time believing that all of the tumors could be gone, because we had never seen anything remotely like that in any of the thousands of mice with tumors that we had treated. But when he handed me the before and after films, and I held them up to the light box for a better view, all I could say was, "are you sure these are from the same dog?"

One result does not a medical breakthrough make, but I should have pounced on this discovery, for it was big. Unfortunately, I did not. In fact, probably because I was not paying careful enough attention, the vet made a fateful mistake that I might have been able to prevent. DHEA was half of my thesis research, so I knew very well that it was an inhibitor of an enzyme called glucose-6-phosphate dehydrogenase (G6PD.) G6PD deficiency is the most common inborn error of metabolism in humans, with some 400 million or so people around the world carriers for some level of deficiency. The mutations conferring G6PD deficiency became fixed in the human population because such deficiency confers protection against the malarial parasite, *Plasmodium* . G6PD deficiency is extremely rare in dogs.

Special precautions have to be taken in people with severe G6PD deficiency, because they are exceptionally sensitive to certain drugs that can act as oxidants. The general anesthetic isoflurane is one such oxidizing drug that can produce fatal results if used in G6PD-deficient patients. [54] During my thesis research I had learned that mice and rats that had been given high doses of DHEA were extraordinarily sensitive to the effects of certain anesthetics. Accordingly, I had explained the G6PD mechanism of action to the vet, and had indicated to him

that Rex should not be anesthetized while he was being treated with DHEA. For whatever reason, however— perhaps the fact that G6PD deficiency is not generally considered to be a relevant issue in veterinary medicine, or that Rex was beginning to get annoyed with the subcutaneous injections — the vet anesthetized Rex prior to his next treatment, and this valiant dog that had survived a deadly cancer, promptly expired.

I am not certain if it was my frustration at this treatment blunder, or the fact that another project took off at this very moment, but I put the DHEA project on a back burner. What I should have done was to find ten more dogs with this kind of tumor, treat them in an identical manner, and see if I could produce the same result in them. If I did reproduce the result, then I should have found a hundred dogs with different kinds of tumors, and see if I could extend the list of responsive tumors beyond soft tissue sarcoma.

But I did none of these things, because shortly after Rex's death, I had a paper accepted to Nature, the most respected scientific journal in the world. The manuscript that I had submitted to *Nature* described a new class of respiratory drugs — Respirable Antisense Oligonucleotides (RASONs)— that I had invented. In collaboration with another lab at the medical

center, we had just shown remarkable efficacy of my lead RASON in that lab's animal model of asthma. A paper published in *Nature* means worldwide exposure, and I was immediately inundated with requests for interviews about this discovery. On the day the paper was published, our departmental secretary interrupted a seminar I was attending in another department to tell me that Dan Rather's office from CBS was on the phone and wanted to speak to me, and they were sending a camera truck and reporter from Raleigh— pretty heady stuff for a young associate professor. As luck would have it, however, about thirty minutes later came the news that the Premier of China, Deng Xiaoping, had just died, and the CBS van had been turned around as this breaking story was going to dominate that evening's, if not that week's news. But scores of interviews did take place. National Public Radio, for example, did an extensive interview with me that was broadcast around the world, in 70 different languages. Also, more than a dozen pharmaceutical companies called, from around the world— it was time to get my passport renewed.

Again, heady, distracting stuff for a young professor.

This was the first drug that I had invented that I would submit to FDA and take into clinical trials. Others would follow. But this was the first. It is difficult for me to explain

how I felt, mere months later, standing in a large, open hospital ward with about fifty beds in it, watching the first fifty patients about to be treated with my drug— a drug that I had conceived of and designed in my head, and then had made with my own hands. [55] I will tell you this. It was— unfortunately— enough to distract me from Rex's disappearing tumors.

Even the NIH (parent organization of the NCI) helped me push this new drug forward, as did the state of New Jersey, where I was now based, near Princeton. This was a potentially breakthrough new drug, and the reviews we received from NIH, and the New Jersey Commission on Science & Technology, were encouraging:

"*The product has tremendous commercial and medical potential, the investigative team is very strong…and the preliminary studies are exceptionally well-carried out…"*

"This is important work, and has the potential to push antisense oligo therapy to a new level. Obviously, there is great commercial application…"

— Technical due diligence reviewers, NIH, on S.B.I.R. RASON technology grant award 2R44HL057716

"This is a first-rate project… exceptionally well planned… excellent scientific basis…"

"The science performed to this point is first class… Unique, excellent scientific basis. Unique mode of action and delivery…"

— Technical due diligence reviewers, NJ Commission on Science & Technology, RASON technology grant award to Principal Investigator JN

With the preclinical work that I was able to do with this early funding from NIH and New Jersey, I was subsequently able to do two major deals with pharmaceutical companies. The first was a small, $17 million deal with Chiesi Farmaceutici , based in Milan; the second was larger, a $110 million deal with the Japanese company Taisho , based in Tokyo. After we received our first exciting data in FDA-monitored clinical trials in people with asthma, I even negotiated to the point of a signed term sheet with another pharmaceutical company— then the largest pharmaceutical company in the world— for a deal worth an estimated $800 million. That would have been the largest biotech deal ever at that point in time, and, of course, I was really excited about this success. On cloud nine, as the saying goes.

And then the world fell apart

In September of 2001, our country suffered the 9-11 attacks, when almost 3,000 innocent people were slaughtered in the World Trade Center Towers by terrorists. I had been in meetings in 1 World Trade Center just two weeks before. Most of the people that I had met with died in these attacks. On the day of the attack, I was having a business discussion with representatives from Meiji Seika, another Japanese pharmaceutical company, at my lab and offices in Princeton. I remember realizing immediately that Osama Bin Laden must have been involved. When I told the Meiji Seika people what had happened, and that their afternoon flight to Seattle had been cancelled (along with every other commercial flight in the United States) I remember how disappointed they were that they were not going to see their hero, Ichiro Suzuki, play outfield for the Mariners that evening. I remember dismissing all the people working for me so that they could go home and be with their families, and, not being able to get in touch with my wife, racing to my boys' school at Bear Tavern, and then on to my daughter's school, so that I could gather them up and make sure that they were safe. [56]

Not many people are aware that those attacks decimated the pharmaceutical industry, erasing about one-third of the value of

major companies involved in this sector— including the one with whom I had a signed term sheet. As part of that deal, I had invested almost all of our remaining cash in a new research building, to the specifications required to fulfill the terms of the agreement. When this company pulled the term sheet after 9-11, I was left with a building that I could no longer afford. Backed into a corner, I fell prey to a voracious group of venture capitalists who decided to see if they could make a quick killing by ignoring the science I had created.

The antisense drug that I had invented was specific for a major— *but not exclusive* — cause of asthma; the upregulation of the adenosine A1 receptor. I had determined that about 20% of asthma patients responded strongly to my drug, and that I could identify such "responders" by a simple test which I called "ABC Rainbow." It was named this way because it involved Adenosine Bronchial Challenge, and the test kit used seven doses of inhaled adenosine, each color coded to one of the primary colors— the rainbow. With this kit, I could identify those patients who would respond really well to my treatment, and limit treatment to them. The new investors scrapped that whole idea, deciding to gamble on the possibility that, if they treated *all* asthma patients, even those who I could accurately predict would not respond, the statistics for the responders

would be so good that they would carry the non-responders; i.e., the statistics of the whole group— responders and non-responders alike— would be masked by the high degree of efficacy shown in the responders. When I refused to go along with this, I was promptly fired. To make a long, horrific story short, the vc's gamble didn't work, they shrugged, and moved on to look for their next prey, and they shut down a drug that could have been great for a significant fraction of asthma patients. When I later asked for all the data to be returned to me, so that I could follow my original plan, they refused. They would rather kill the project than risk me succeeding where they had failed.

I decided never to put myself and my inventions into such hands again.

Fast forward 13 years

Over the years, I constantly found myself thinking back to Rex, and the remarkable tumor regression that we saw in him after exposure to DHEA. By 2011 I was beginning to formulate the hypothesis that dogs had evolved a rudimentary form of an otherwise primate-specific mechanism of tumor suppression based on DHEA, and its potentially irreversible uncompetitive

inhibition of G6PD, as discussed in the previous chapter. This would explain the results we saw with Rex, if we were triggering such a “kill switch” tumor suppression system by administering high dose DHEA. Thus, DHEA was acting as a “back-up” to p53— if p53 was inactivated, DHEA became activated. In 2012 I established an independent laboratory to investigate this hypothesis.

Cancer cells produce a lot more ROS than normal cells do, and consequently, G6PD levels are dramatically increased in such cells. I reasoned that if my hypothesis was correct, that DHEA was backing up p53, then p53 should be an inhibitor of G6PD, too. I began to gear up to test to see if this was true, but, nearing retirement age, knew I could never get funding from the traditional sources that I had been successful with earlier in my career. So I began funding these experiments myself, from my retirement fund from my University days; but these limited funds made my progress too tentative. Before I could obtain the necessary equipment, a lab down the road, at the University of Pennsylvania, beat me to the punch. They showed that, indeed, the p53 tumor suppressor protein was a natural inhibitor of G6PD. I was very disappointed at having been “scooped” by the lab at Penn, but excited that my “back-up” hypothesis was correct! And the Penn lab was unaware of my focus involving

DHEA, and they certainly knew nothing about Rex. I decided to press ahead and do the research that I should have done 13 years before, when Rex's tumors mysteriously disappeared.

Could Rex's results be reproduced in other dogs with soft tissue sarcomas? And if they could, what other canine tumors would respond to re-activation of the kill switch tumor suppression mechanism by high dose treatment with DHEA? Perhaps I would find that Rex had been a fluke that I could not reproduce— after all, I had never observed such results in thousands of DHEA-treated mice with tumors. But the possibility that dogs, and their tumors, were different— much more like we humans, and our tumors— was intriguing. The question now was, how would I pay for this research?

I have been successful getting grants in my career, mostly from the National Cancer Institute, and other divisions of the NIH, but from other sources, too, including pharmaceutical companies, and foundations, even private investors. I have even sat on study sections reviewing the grant applications of other scientists, so I know that game fairly well. As most successful scientists will tell you, when you apply for a new grant, you have to have most of the work already done; then ask for funding to take the research the next small step further. Well, at this point, I had only one dog, Rex, and confirmation from the

Penn lab that my germinal idea that DHEA and p53 shared the ability to inhibit G6PD, and were therefore linked in a deep way. This would not be enough for any traditional source of funding. There was the further problem of where to get a sizeable number of dogs with spontaneous tumors to study. And finally, I was beginning to see a connection between dogs and humans with respect to p53 and DHEA. Thus, one thing that distinguishes primates from all other lineages of animals is that they have extraordinarily high levels of circulating DHEAS, the circulating form of DHEA. Mice and rats, the species that I had studied in my laboratory for so many years, had no circulating DHEAS— at least no detectable levels. But dogs did, and this is quite unique in the animal Kingdom. I couldn't quite put it all together, yet, but I could see that there was a connection between dogs and humans and the way they interacted with p53, DHEA, and G6PD— a connection that did not exist in mice and rats. Although Rex constituted an N of just 1, and could therefore be a fluke, the result that I had seen in him was stunning. If I could figure out the connection between dogs and humans and p53, DHEA, and G6PD, would it lead me to the possibility of inducing the disappearance of tumors in humans, too? I had never seen a single tumor disappear in a mouse treated with DHEA. Was Rex a beacon, alerting me that dogs

responded to DHEA treatment because they had a system that naturally used DHEA, whereas mice did not respond because they lacked such a DHEA-mediated system? It was all still quite nebulous, but I had a very strong feeling that the jumbled pieces would eventually all fit together, and the puzzle would be solved.

I eventually settled on what I call the participant investor model for raising the money needed to do this research. In this model, people whose dogs had been diagnosed with cancer could choose, if they wished, to pay for the costs associated with entering their dog in my research program. In return, they would receive stock in my company. This would give me access to the dogs I needed.

I could not have imagined just how important the results I was about to obtain in dogs would be, both for dogs and for humans. Rex was no fluke. He *was* a beacon, signaling an entirely new approach to cancer treatment, and cancer prevention, in both dog and man.

Let me show you some of the most exciting results of my study, obtained in a soft tissue sarcoma called canine cardiac hemangiosarcoma (CCH). CCH is a deadly tumor in dogs, with an equally deadly counterpart in humans. It usually occurs in the right atrium of the heart, and if left untreated, dogs affected

with this cancer die in a matter of days or weeks. Even with treatment, lifespan is only minimally extended. It is a rare tumor in both dogs and humans, and perhaps the most rapidly fatal in either species.

Four dogs with CCH were entered into our study, each diagnosis made by a skilled veterinarian and/or cardiac specialist. Each dog presented with the classical symptoms and signs of CCH— extreme lethargy, shortness of breath, right atrial masses on echocardiography or x-ray, and pleural effusion (blood in the pleural cavity.) We treated each dog with daily high-dose DHEA. In two cases—those in which we were able to begin treatment within a few days of diagnosis—eureka! Unbelievable! We saw *complete resolution of the tumor mass* . Both of these dogs went on to live long, normal lives, each surviving several years after their original diagnosis, and dying of other causes five years after diagnosis! (Figure 3.1.) In the two dogs in which treatment was delayed for a few weeks following diagnosis, tumor resolution may not have been complete (including metastatic disease), but even these dogs far outlived the best results in CCH reported in the literature. [57] In this literature report, untreated dogs with a diagnosis of CCH were shown to have an expected longevity of just 7 days. In dogs with CCH treated only medically (digitalis,

glucocorticoids, antibiotics, removal of fluid from the pleural cavity), longevity averaged 27 days. Dogs in the study treated surgically survived for an average of 86 days. Finally, dogs treated surgically, followed by high dose chemotherapy (doxorubicin, cyclophosphamide, and vincristine) lived an average of 189 days. As you can see from the figure below, all dogs with CCH treated with DHEA to trigger kill switch activation in their tumor dramatically outlived even the longest-lived animals in the published study. We published our data in Translational Medicine Reports while two of our dogs were still doing well. As of this writing, the dogs have now died, but of old age, tumor free.

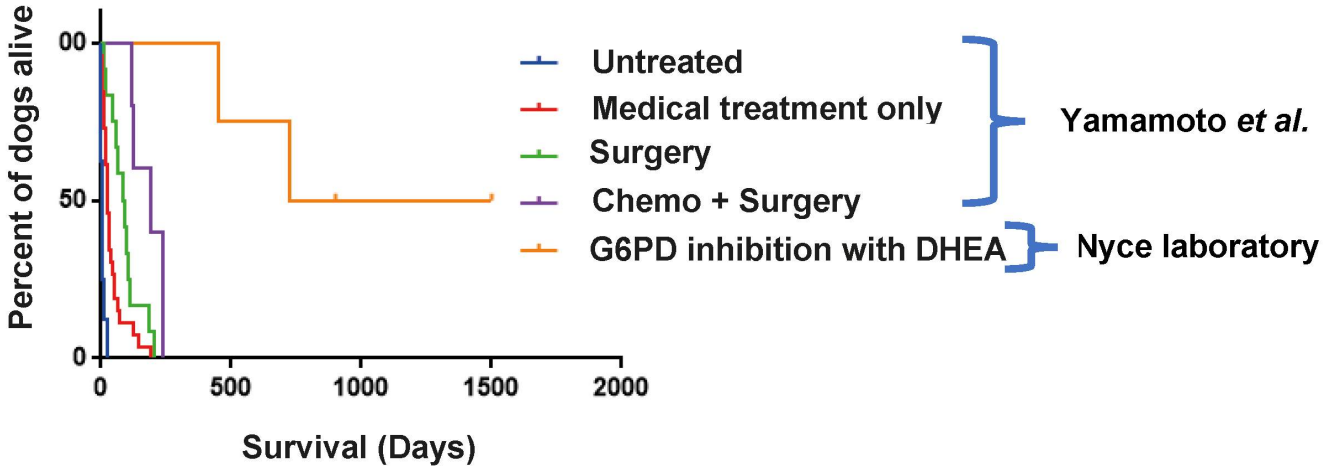

Figure 3.1 Kaplan-Meier survival curves for dogs with CCH, comparing kill switch re-activation to the best results reported in the literature, those of Yamamoto et al. Each downward tic in each timeline marks the death of a dog in the respective

treatment regimens. The first four regimens are from Yamamoto et al. [58] The final treatment regimen, kill switch re-activation, is based upon the author's discovery that dogs have evolved a rudimentary form of an otherwise primate-specific tumor suppression mechanism based on the de-sulfation of DHEAS to DHEA in cells experiencing p53 tumor suppressor inactivation. Where there is a tic without the curve moving downward, this represents a s0-called "censured" data point; i.e., the dog did not die of its cancer. In this case, the only censured data points occurred with the Kill Switch re-activation regimen, representing two dogs in whom tumor disappeared completely, which dogs went on to live full lifespans, eventually dying of old age.

Man's best friend to the rescue again

I noted above that humans have exactly the same disease, which is referred to as Cardiac Angiosarcoma (CAS). This presents the really exciting possibility that CAS in humans will respond to triggering of the kill switch in the same manner as CCH did in dogs. The numbers of people with CAS is not large — there are just a few hundred CAS patients in the US each year (a few thousand worldwide), a number far too small to

attract Big Pharma and the business of cancer. But for a small company like ACGT, the discovery company I now run, that is in it for the science, and the benefit to patients (human and animal), rather than simply for profit, this is an amazing opportunity to demonstrate that the kill switch can be re-activated in human tumors, just as we have demonstrated in dogs.

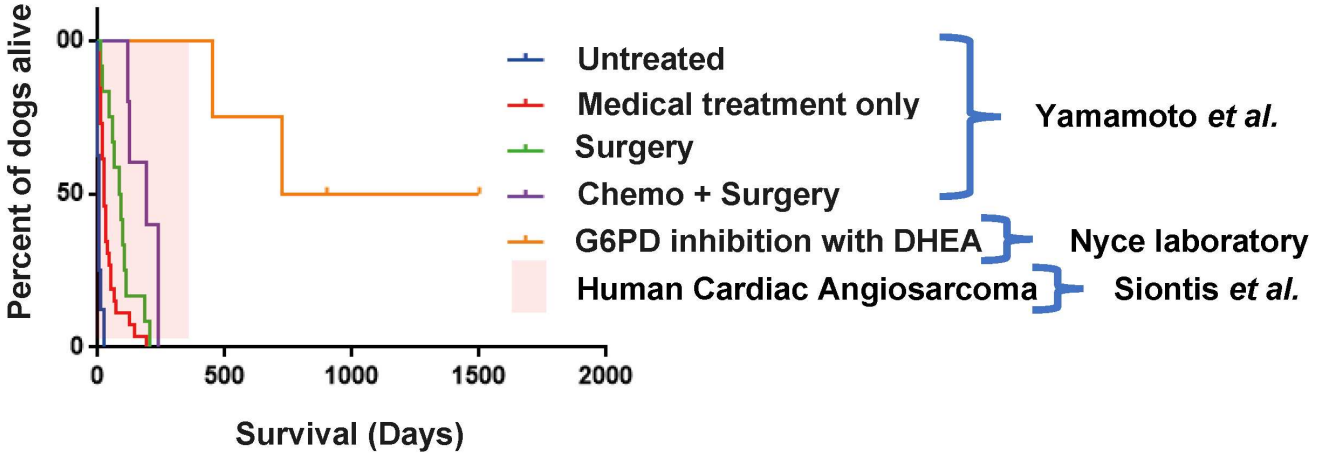

Figure 3.2 Kaplan-Meier Survival Curves, as above, but now showing overall survival in the same tumor occurring in humans, called cardiac angiosarcoma. Data from Siontis *et al* ., 2019, [59] in which overall survival for their cohort of 14 patients with cardiac angiosarcoma, following surgery and chemotherapy, was 12.1 months. See also Hong *et al* ., 2019, Figure 2C. [60]

These data present the exciting possibility that our research in canine cancer may have immediate application in this very needy group of humans with cardiac angiosarcoma— patients

who currently can expect no more than a year of life after diagnosis; typically much less. If the tumors in these patients respond to kill switch activation the same way that the canine tumors did— and there is every reason to expect that they will — then this deadly disease may be transformed into a completely curable form of cancer. I have already proposed a clinical trial in such patients to the National Cancer Institute, and am waiting to hear back. One objection that they may have is that I am proposing a clinical trial in humans based upon a research result obtained in just four dogs. I would argue that, even with just four dogs, the result of kill switch activation was so overwhelmingly that it is statistically significant from Yamamoto et al.'s best treatment group not by FDA's standard 0.05 p value, but by an astonishing 0.0005. Furthermore, there is no treatment for patients with CAS that enables survival longer than 12 months. To the extent that CAS is equivalent to CHS, and there is every reason to believe that they are identical, kill switch re-activation may be as life-saving in the human disease as it is in the canine. We will have to do the clinical trial in patients with CAS to know for sure. But if we can trigger dissolution of these deadly cardiac tumors in humans, we can then go down the list of additional human tumors that have usurped components of the kill switch for

their own use— sensitizing them to kill switch re-activation. We believe that many human tumors will respond to such kill switch triggering, just as many canine tumors do.

Pure Karma

So, what is the evidence that other canine tumors can be effectively treated by activating the kill switch? To answer this question, consider the case of Karma, a Doberman diagnosed with soft tissue sarcoma by an expert team at the Lois Bates Acheson Veterinary Teaching Hospital of the Oregon State University College of Veterinary Medicine in Corvalis. At just three years of age, Karma had gone lame in her right leg, and x-rays showed a mass growing in the area of her ishium. A biopsy was taken, and histopathologic analysis revealed that the mass was a soft tissue sarcoma. As these can be quite deadly tumors, it was recommended to Karma's owners that she undergo hemi-pelvectomy, a surgical procedure in which the affected leg and most of the affected hip would be amputated. Even with such a drastic surgery, the vets handling Karma's case said that her long term prognosis was not good; she would probably die of metastatic disease within six months. There was even the question of whether Karma was a candidate for the surgery, or

whether she was already too sick. She would have to be evaluated further by the hospital's soft tissue service, Karma's owners were told.

Faced with this dismal prognosis, Karma's owners entered her into a research protocol that we had going at the time, attempting to trigger the kill switch in canine tumors using high dose DHEA. I will discuss the specifics of the protocol that we used with Karma later in this book.

Over the course of the next few months, Karma's tumor literally melted before our eyes as we triggered the kill switch in its cells. Below are before and after x-rays, showing complete dissolution of the soft tissue sarcoma. Another element of Karma's response to high dose DHEA was the exceptional change in her temperament. She went from being a hobbled, sad dog to one absolutely filled with joy, as you can see for yourself in a video produced by her owners. She even became pregnant during her treatment protocol, and gave birth to five healthy puppies . That is unlikely to have happened if she had been treated with standard cytotoxic chemotherapy. (Videos are available at www.ACGT.us)

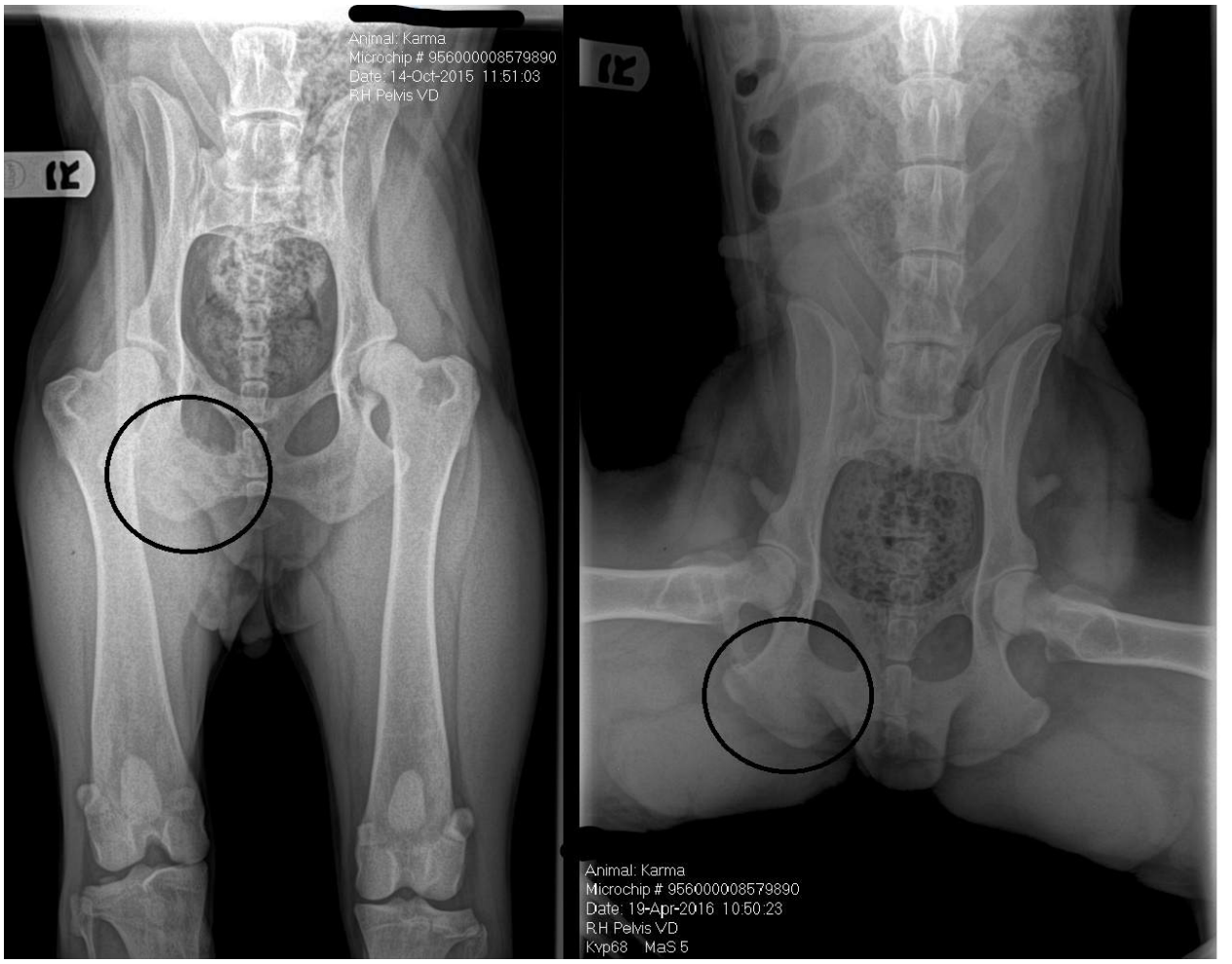

Figure 3.3 X-ray evidence that kill switch activation induced complete dissolution of a histologically confirmed soft tissue sarcoma in Karma, a three-year-old Rottweiler. Left, before treatment. Right, complete tumor resolution after triggering the kill switch in this tumor using high dose DHEA. Please also note the increased muscle mass after treatment, compared to pre-treatment muscle mass. Disuse of the leg prior to treatment led to such a decrease in muscle mass. As you can see from video available on our website, Karma regained full use of her leg after the tumor resolved. She remains alive and well five years after her original diagnosis.

We published our data on Karma in Translational Medicine Reports. [61] She remains alive and well more than five years after her original diagnosis, living life to the fullest—something that would definitely not have occurred had her owners opted for amputation and hemipelvectomy, followed by high dose chemotherapy. [62]

p53 redux

With these data in hand, I was now firmly convinced that there were species-specific differences in response to DHEA. Slowly, I started to put the pieces of this puzzle together. I was seeing tumors disappear in dogs after treatment with DHEA, while in mice they never did. Dogs, like primates, have circulating DHEAS; mice and rats do not. DHEA is an inhibitor of G6PD, and so is p53. p53 is the core of the vertebrate tumor suppression system. Was DHEA also part of that core? But circulating DHEAS is limited to primates, and dogs, and very few other species; so DHEA could not be involved as a general mechanism of tumor suppression in all species. What did that mean? Was DHEA part of a tumor suppression mechanism in dogs? A mechanism specific to dogs? Were tumors in dogs the

result of a failure of that tumor suppression mechanism? A failure that I was correcting when I treated them with DHEA? If DHEA was part of a tumor suppression system in dogs, it must be much more so in primates, particularly humans, who had by far the highest circulating levels of DHEAS. Had I uncovered what was really a *primate* -specific tumor suppression mechanism centered on DHEA, and, one would presume, p53, working in tandem? Was what I was observing in dogs a rudimentary form of an otherwise primate-specific mechanism of tumor suppression? A flood of ideas filled my brain. Why would dogs and primates have similar systems? Of course! Dogs had to have a similar system to enter into their collaboration with us! From that point forward, they would be exposed to all of the same carcinogens— most especially PAH — that we were. *Of course* they would have to adapt toward that new exposure! Or maybe they could adapt to that exposure *because they already had this rudimentary DHEAS-mediated tumor suppression mechanism on board!* I began to see the more general ramifications of these ideas. If dogs and primates had lineage-specific mechanisms of tumor suppression, was this a general feature of all animals? What would that say about our "war on cancer?" If species-specific mechanisms of tumor suppression existed, which is what my research was suggesting,

then this would mean that the p53 paradigm— that cancer was the same disease from one animal to the next, enabling the use of animal models to study cancer in humans— the paradigm that had dominated and led cancer research for three decades and more, *was completely false* . If all species had species-specific mechanisms of tumor suppression, then cancer was *not* the same disease from one animal to the next; it was a different disease in every species because it was countered in different ways by every species! If this were true, then *one vertebrate species could not be used to construct a valid model system to study cancer in another vertebrate species* . What this said about the last fifty years of cancer research— almost all of which had been performed in mice and rats— made me feel nauseous at first. If these conclusions that I was drawing were true, we had been studying how to cure cancer in mice, not humans *for all those decades* . We had not been studying how to cure human cancer at all.

With excitement, apprehension, dread, I realized that I was at the beginning of seeing a completely *new* path forward. It was at this moment that the concept of species-specific mechanisms of tumor suppression was born.

4

The lex naturalis

"You can't even begin to understand biology, you can't understand life, unless you understand what it's all there for, how it arose - and that means evolution."

Richard Dawkins

During the industrialization of the dog, when it spread across Eurasia, and then throughout the world, all dogs probably looked pretty much the same, resembling a smallish wolf, though probably generally lighter in coat color . The idea of breeding dogs for a specific purpose, changing such attributes as size, temperament, and skill at a particular behavior useful to humans, came much later. Until recently, it had been thought

that the first attempts at such canine breeding, designed to create dogs especially good at herding, did not occur until about 7,000 years ago. However, a recent study showed that about 9,000 years ago, in Siberia, dogs were specifically bred for pulling sleds, with a body size optimized for heat dissipation, and musculature appropriate for arduous pulling. Other dogs were bred for different characteristics useful in tracking and engaging large prey. [63] The hunters who bred these dogs of differing body types were hunters of reindeer, and even polar bears, and only with such specialized canine assistance were they able to hunt successfully and thereby survive in the harsh, dangerous Siberian landscape. Dogs had begun to be thought of as like a Swiss Army knife for humans, a Swiss Army knife that could be opened to expose any number of tools, depending upon the task at hand.

There are additional examples, a little more recent but not yet of the modern age, of apparent breeding to achieve a preferred body size and/or temperament. For example, DNA was recently isolated from the skeletal remains of a dog found on board the wreck of the *Mary Rose* , a Tudor warship that was sunk by a French invasion fleet in the Solent channel between Portsmouth and the Isle of Wight on the 19th of July, 1545. Analysis of this dog’s DNA showed it to be a young male

terrier of the Jack Russell -type, with brown coloration in its coat. Interestingly, this dog was found to be heterozygotic for the gene that causes hyperuricosuria (excessive uric acid in the urine), which causes kidney stones when homozygous in modern members of this breed. [64] Unfortunately, it is a common theme in canine breeding that selection for specific traits that humans find appealing results in dogs in which genes in a configuration causing health issues are carried along with the genes that confer the desired characteristic. The maintenance of a relatively closed gene pool in each dog breed— enforced by the adoption of the breed barrier rule which holds that no dog may become a registered member of a breed unless both its sire and its dam are registered members— underlies and maintains breed-associated genetic disease.

Sometimes the random occurrence of a rare germline mutation makes a dog attractive to humans because it is an oddity— a mutation that without subsequent human intervention would almost certainly have prevented the animal from passing on its altered genotype to successive generations. This is what happened with the strange looking, loose-skinned Shar Pei , pictured in Chinese scrolls from as long ago as 200 BCE. The thickened skin and loose folds of skin in this breed

are caused by excessive synthesis and accumulation of hyaluronic acid (HA) in the skin.

Figure 4.1 A mutation causing the thickened skin and wrinkles of Shar-Pei dogs also causes periodic fever disorder and chronic inflammation.

A similar malady occurs in humans, albeit very rarely. Because this condition in humans is associated with a similar degree of skin folding, and is characterized by high levels of HA in both

serum and skin biopsies, it has been referred to as *Shar Pei Syndrome of Humans* .[65]

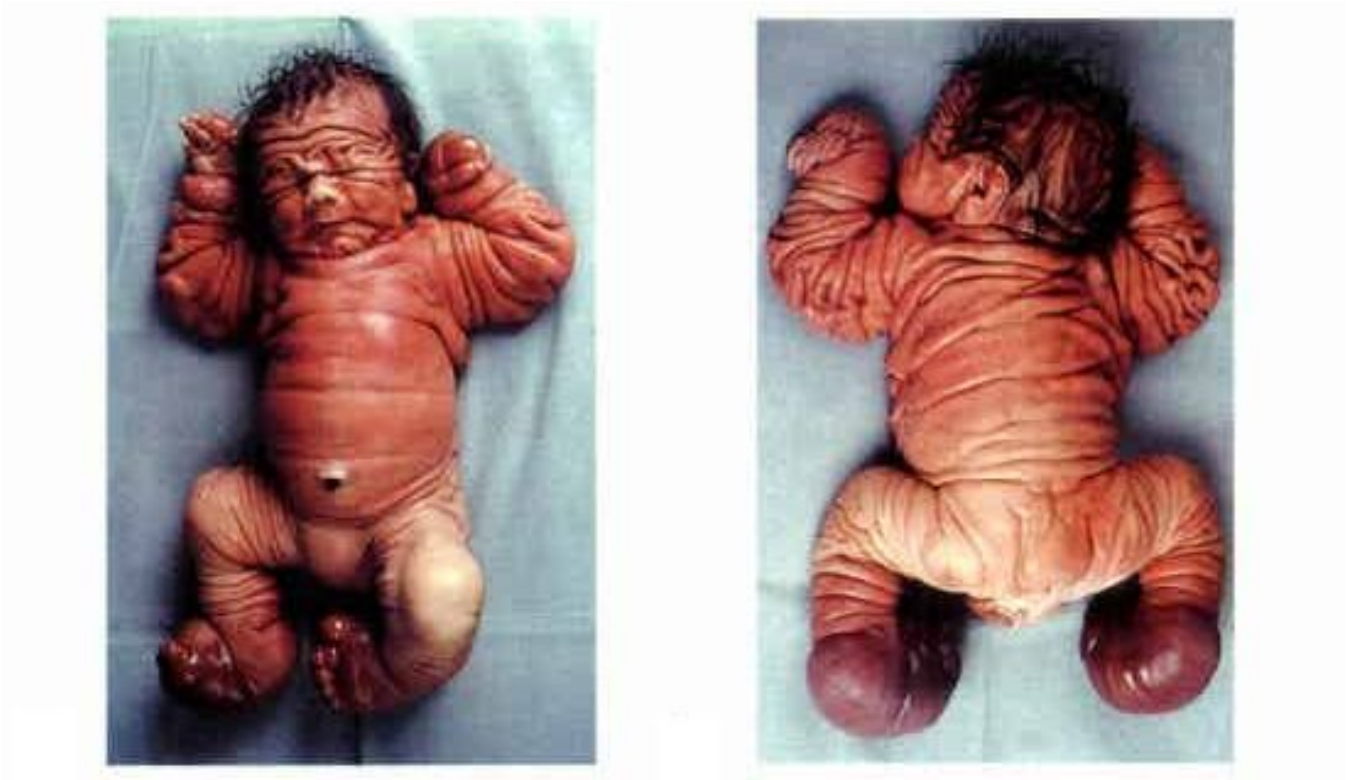

Figure 4.2 Shar Pei syndrome in a baby. From Ramsden et al. 2000[66]

My point in presenting these data is to show that some traits that we find unacceptable, and in fact horrific for our own species, we adopt as not only acceptable, but prized for their uniqueness in our dogs. Examine how you felt when you saw the picture of the Shar Pei, and then compare that to how you felt when you saw the picture of the baby with Shar Pei Syndrome. Don't feel guilty; we *all* have these completely different responses looking at the one compared to the other.

We are naturally more empathic toward the baby, because we can more closely relate to his discomfort. My point is that we would never intentionally select for this condition in our own species.

Shar Pei dogs are subject to a debilitating, breed-specific autoinflammatory disease (SPAID) that includes a systemic autoinflammatory reaction, skin eruptions, and episodic high-grade fevers. The SPAID which afflicts this breed can sometimes be so debilitating that euthanasia is required. Many other breeds are also associated with “defects” that were carried along when humans selected for various traits in dogs. Examples include uric acid bladder stones in Dalmatians; brachycephalic syndrome (exaggerated breathing sounds, snoring) in short-faced dogs such as pugs and pekingese; histiocytic sarcoma in Bernese Mountain dogs, etc. The point is that, when humans selected for specific traits in dogs, in almost every case, we simultaneously selected for hidden traits that were detrimental to the resulting breed’s health.

The creation of the modern breeds of dogs

So, long before the discovery of DNA and the invention of genetic engineering, humans learned that they could manipulate

dogs, almost as if they were clay, simply by selecting for certain desirable traits over and over again, until the trait was magnified to the satisfaction of the human that was engineering the transformation. In chapter two we talked about the bell-shaped curve, and how traits several standard deviations from the mean occurred at every possible level— anatomical (smaller dogs at one end of the curve, giant dogs on the other), biochemical (high levels of circulating DHEAS at one end of the bell-shaped curve, low levels of DHEAS at the other), behavioral (barking dogs at one end of the bell-shaped curve, dogs that rarely barked at the other)— every aspect of canines that a human found appealing could be selected for just by controlling which dogs bred with which other dogs. Tiny dogs that made great, affectionate companions were created, as were giant dogs capable of defeating wild wolves and hunting megafauna. Dogs with exceptional senses were bred with barking dogs to create watch dogs, or dogs that helped with hunting.

The *Fédération Cynologique Internationale,* the largest kennel club in the world, currently recognizes 344 separate breeds, ranging in size from the 2 kg (4 pound) Chihuahua to the 90 kg (200 pound) Great Dane and Irish Wolfhound. Yet, all of these breeds represent a single species, and it is clear from

their interaction with each other that the Chihuahua and the Great Dane know this— and that they also share a common animus with the cat (unless directed to feel otherwise by their human.) Almost all of these breeds came into existence within the past 300 years, as humans selectively bred their dogs to emphasize particular traits.

If the various breeds of dogs had evolved naturally, rather than by the hand of humans, it would have taken millions and millions of years, and they would have separated into separate species, each with its own species-specific mechanism of tumor suppression. (*Or they would have evaporated into the "mistake" pile of natural selection, which is what would have happened to most, perhaps all, of these different breeds. It is hard, after all, to compete with the grey wolf, one of Nature's masterpieces. Only by their association with humans have our various dog breeds been able to survive. In the wild, predation would have limited the evolution of small dogs like the Chihuahua. They exist only because humans protect them, eliminating the selection pressure of a natural environment, thereby allowing them to survive despite their small, indefensible size* .) If our various dog breeds would have evolved naturally, in the wild, lifespan, body size, and species-specific mechanism of tumor suppression [67] would all have

been taken into account in order to optimize resource assimilation into DNA, while automatically maintaining lifetime cancer risk for each new canine species at the required 4% ceiling, a number derived from extant species. [68] Under natural selection, it is doubtful that any of these variations would have been able to successfully compete with the ubiquitous grey wolf. Without human protection, virtually all would have become a grey wolf's meal.

The range of canine lifespans

Every species has a specific lifespan associated with it. Some members of the species will not make it to that lifespan, and a very few may slightly exceed it, but by and large, lifespan is fixed for every species. We can learn from whales that lifespan is associated with body size, because the largest whales have some of the longest lifespans of all mammals (the bowhead whale, *Balaena mysticetus, is known to have a lifespan longer than 200 years)*. This is because nature is efficient and would not expend the resources necessary to sustain a short-lived Goliath. But the various species of whale evolved in the wild, under natural selection. We see something very different in the

various breeds of *Canis lupus familiaris* , which were created by human selection.

Our manipulation of canine body size away from that which evolved naturally over millions of years in the grey wolf, has dramatically altered the range of lifespans in the dog, and the association of body size with lifespan in the dog is crystal clear — and opposite to what we see in cetaceans— this, again, because *Canis lupus familiaris* is a man-made invention. In the wild, a grey wolf will live 10-13 years, and weigh about 100 pounds. But intensive selective breeding in dogs has created breeds that are much smaller than their ancestral grey wolf, and others that have much larger body size than the grey wolf. An interesting dimension of these human-engineered changes in body size is that, on average, the smallest breeds of dogs have dramatically longer lifespans compared to the largest breeds of dogs. Thus, Chihuahuas and Yorkshire terriers can live as long as 20 years, while a Dogue de Bordeaux has a published average lifespan of about 6 years (see Figure below.)

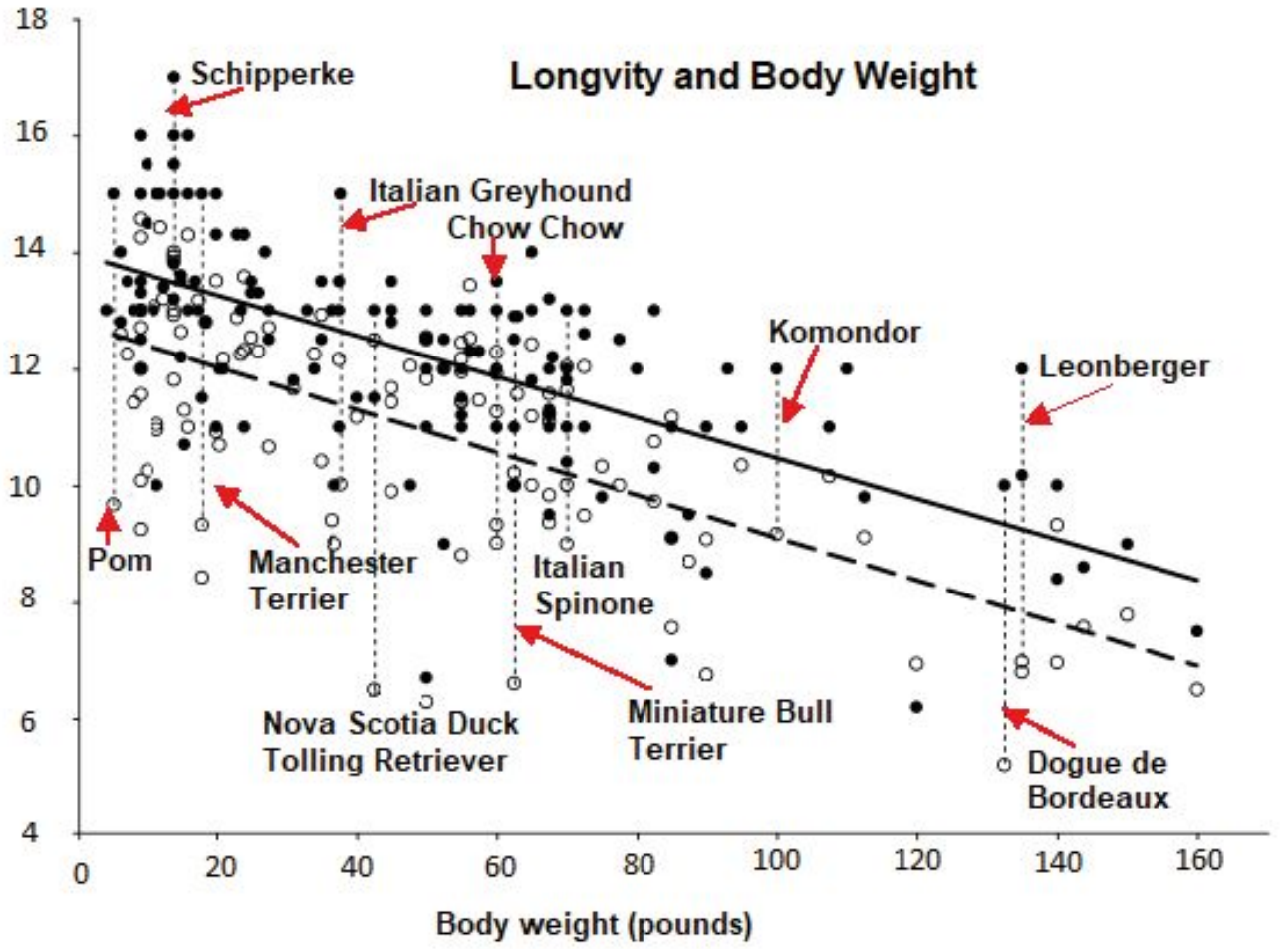

Figure 4.3 The various breeds of dogs are all a single, man-made species with a 50-fold range of body size from the smallest to the largest. This does not happen in nature. For example, there is less than a two-fold difference between the shortest members of our species, as adults, and the tallest. Y axis, age in years. From Jones et al. 2008, Figure 4 [69]

The tall and the short of it

Another point of interest is that it may not be body weight *per se* that drives cancer risk, but rather body *height* — linear bone

growth—both in our dogs, *and in us* . This idea feels counter-intuitive because we have had it drilled into us that it is our excess body fat that is unhealthy, and tallness in our species has generally been thought to be a good thing; a sought-after thing. But as we noted in chapter 6, it has been definitively shown that increased height above average increases cancer risk in humans — the "million women study," which followed 1,297,124 women for a median time of 9.4 years each, reported an overall 16% increase in cancer risk for every 10 cm (4 inches) in height above average, [70] confirmed by additional studies performed in 144,701 women [median follow up, 12 years], [71] and in 310,000 male and female UK Biobank participants. [72] At the opposite end of the spectrum, studies of dwarf humans with Laron Syndrome— one of which studies lasted 57 years— demonstrated a near total absence of cancer in these long-lived, small bodied humans. [73] , [74] It is thus clear that in both our species, and in our dogs, increasing height is associated with increasing cancer risk. Our large breeds of dogs truly have a "Sword of Damocles" hanging over their head. As shown in the figure above, lifespan *decreases* the larger a dog breed is at its adult size. But as shown below, lifetime cancer risk goes in the *opposite* direction, *increasing* the larger a dog breed is at its adult size.

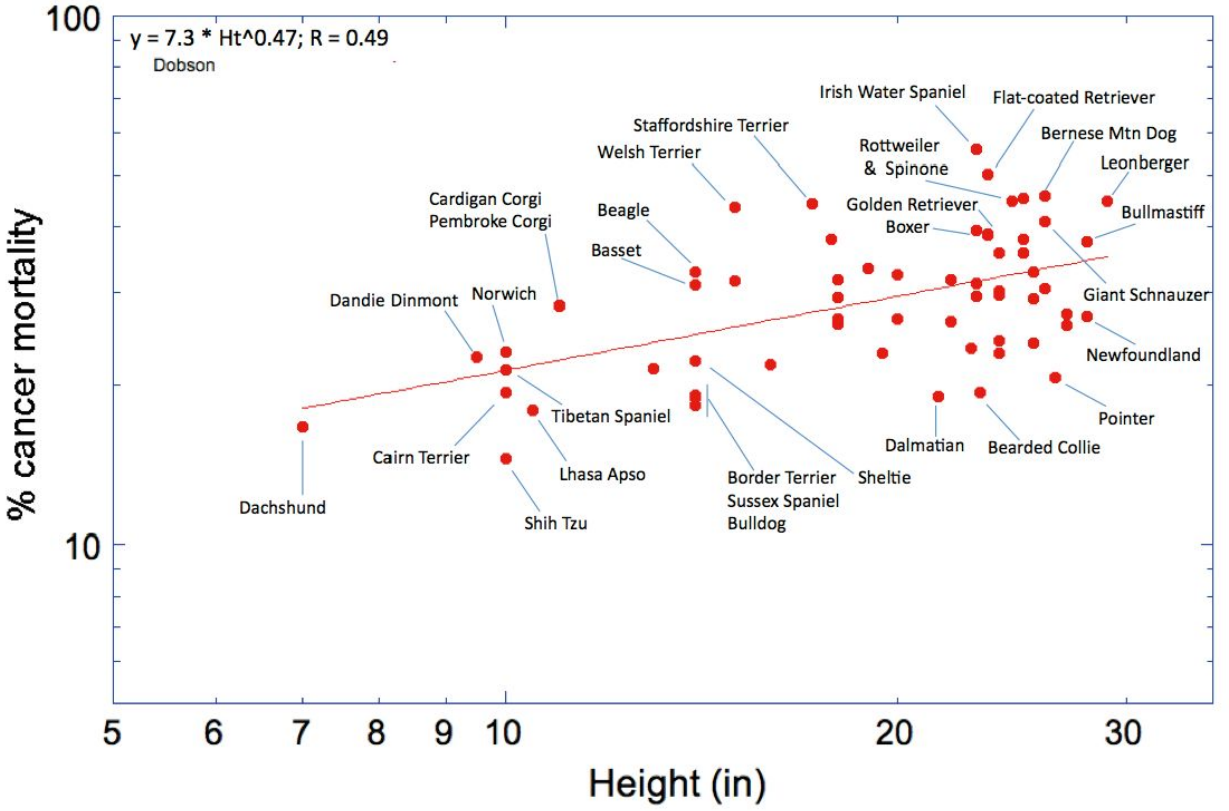

Figure 4.4 Relationship between canine height and cancer risk in dogs. Data plotted from Dobson, 2013 [75] *by Carol Beuchat, PhD, of the Institute of Canine Biology* [76]

If we consider that dogs would be separate species if they had evolved in the wild, then there is something very important in this linear increase in lifetime cancer risk with increasing size, and this is it: *Peto's Paradox does not apply to dogs! There is no paradox in dogs! The tumor risk in dogs is exactly what you would expect if, the greater the number of cells at risk for neoplastic transformation, the greater the lifetime risk of cancer!* This is a remarkable exception. Peto's Paradox applies

to whales and elephants, whale sharks, cassowaries and ostriches, *but not to dogs* . Why does Peto's Paradox apply to these other species? Because in those species, there is no increase in cancer risk with increasing size— because species-specific mechanisms of tumor suppression evolved as a necessary prerequisite to *enable* their increased size. Peto's Paradox does not apply to large dogs *because they came into existence as a result of human selection, not natural selection, and we did not provide them with the improvement to their tumor suppression mechanism that Nature would have done to permit their larger size! We created our modern dog breeds in complete ignorance of this requirement!*

The lex naturalis equation

Suddenly, the pieces of the entire cancer puzzle all began to fall into place for me. I suddenly had a perspective that no other human has ever had before. Peto's Paradox vanished for me, as the elements of a natural law of speciation— a *lex naturalis* — materialized in my mind in the form of an equation. An equation that explained the aberrant, extraordinarily high lifetime cancer risk of our dogs; we had simply failed to provide the improvements in tumor suppression to our dogs that

Nature would have done before permitting any increase in their body size. And as a natural law, this explained not only the excessive cancer risk in dogs, it also explained the excessive cancer risk of our own species. As amply demonstrated in the literature, the body size of modern humans was more than twice what it was in pre-modern humans; and our life spans were now more than three times longer. We had violated the *lex naturalis* when we created our dogs, and again when we created the conditions for doubling our body size and more than tripling our lifespan.

If it was a natural law in vertebrate animals that their lifetime cancer risk had to be maintained at a low rate, around 4% — as it appeared to be judging from animals in the wild— then *all* of the things that could affect that rate had to be tightly linked together, so that any evolutionary change in one would require an equilibrating change in one or more of the others in order to keep lifetime cancer risk at 4%. Body size, lifespan, species-specific mechanism of tumor suppression—and carcinogenic hazard, too, I now realized—these were all dependent variables in an equation describing an unbreakable law of vertebrate speciation— the *lex naturalis* of vertebrate speciation. Lifetime cancer risk was the independent variable, a variable that could be forced to participate in the equilibration

process, but only if something went really wrong, preventing equilibration among the dependent variables.

One can visualize this pictorially, showing the interdependence of all of the variables with respect to one another, like this:

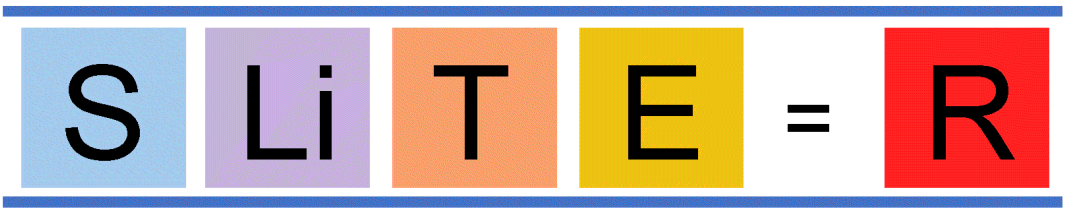

Figure 4.5 The lex naturalis equation. During vertebrate speciation, equilibration occurs between body size (S), lifespan (Li), species-specific mechanism of tumor suppression (T), and carcinogen exposure (E), in order to maintain lifetime caner risk (R) at 4% or lower.

The *lex naturalis* equation depicted above represents a species that is optimized for assimilation of environmental resources into DNA, with all dependent variables equilibrated during the evolution of that species to produce a lifetime caner risk, R, of about 4%. That all variables have been equilibrated is reinforced pictorially by the fact that they are all contained within the blue dashed lines. [77] Thus, all variables in this representation are in a *normalized* configuration. When we want to show what would happen if one of the variables

changed, that is, what compensatory modulations would occur in other variables to equilibrate that change, we can do so by expanding the changing variable outside the blue dashed lines, and doing the same with the variable(s) undergoing compensatory equilibration.

In nature, a disequilibrium between S, Li, T, E is never permitted to occur, and species optimized to assimilate environmental resources into DNA will always have variables that are between the lines. In the discussion that follows, when we show variables outside the lines, it is done to illustrate which variable changed to advance speciation, and which variable was modulated to equilibrate that change. In actuality, the *lex naturalis* for species evolving under natural conditions can always be drawn in a normalized configuration, with all variables within the lines. Only where Nature has been thwarted, as in the human selection that led to the modern breeds of dogs (and also in our own species, as we shall discuss in the next chapter), can *lex naturalis* equations exist *only* in a dis-equilibrated configuration.

As an example, let's consider the first dogs, or even epi-wolves, which had only a rudimentary form of a "kill switch" tumor suppression mechanism (based on the same circulating DHEAS that enabled inhibition of the "fight-or-flight"

response, and therefore enabled “first contact”). By itself, this rudimentary kill switch would not have been sufficient to enable them to co-habit the smoke-filled environs of pre-modern humans. But as we discussed in chapter two, epi-wolves, and the first dogs, were smaller, which made them less threatening to humans than full sized grey wolves. Thus, small body size combined with the rudimentary kill switch to equilibrate the *lex naturalis* after epi wolves/dogs began co-habiting with humans, offsetting the dramatic increase in E (from exposure to the polycyclic aromatic hydrocarbons—PAH— in smoke) that such co-habitation involved. According to the *lex naturalis* , large wolves could not have made this transition, nor wolves without high levels of circulating DHEAS.

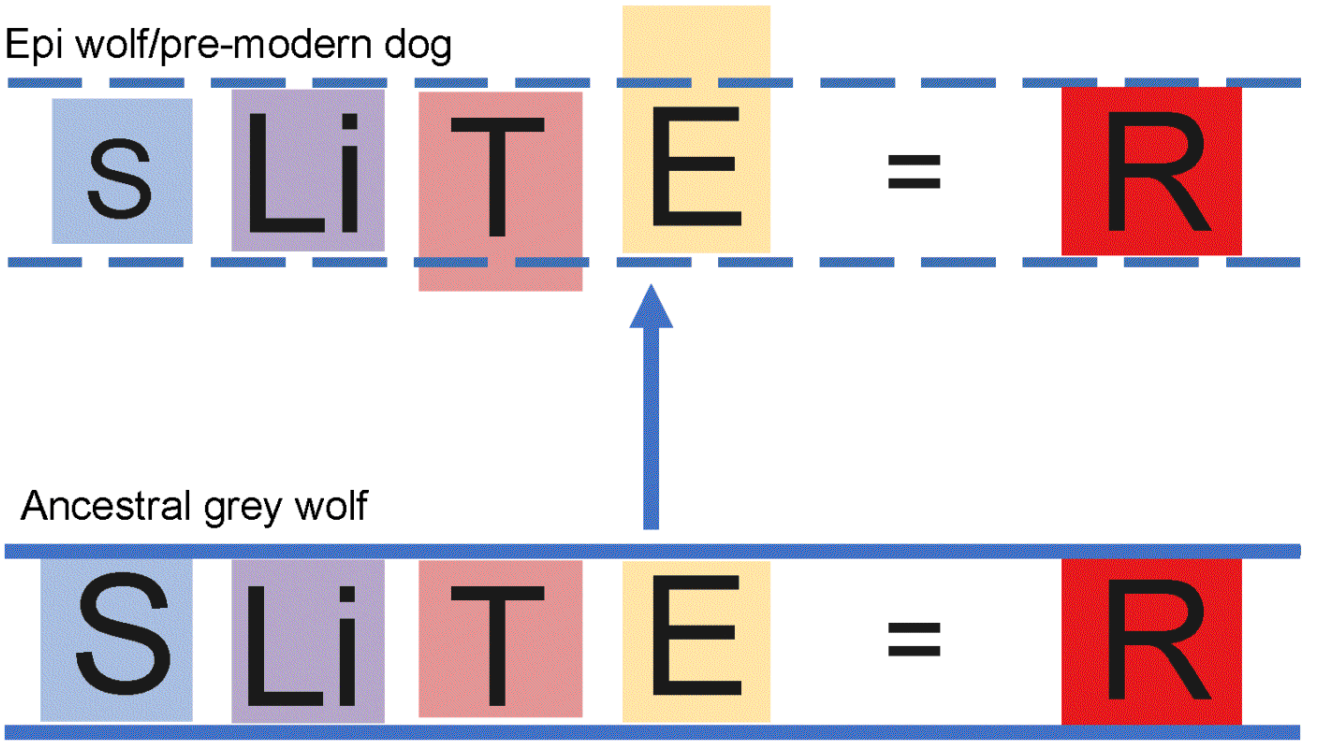

Figure 4.6 Lex naturalis equation of ancestral grey wolf equilibrated by millions of years of evolution. Epi wolves were able to establish relationships with humans because their small body size (S) and enhanced levels of circulating DHEAS (T) equilibrated the increased carcinogen exposure that occurred when they joined humans in their smoke-filled habitats. Enhanced levels of circulating DHEAS probably also moderated the "fight or flight" response, enabling contact.

Next, we can consider the *lex naturalis* equations for our modern dog breeds, comparing small dogs to large, tall dogs. The Irish Wolfhound is the tallest of all dogs, and can stretch out to a height of over seven feet when standing on its back

legs. They were bred to great size in order to hunt wolf, deer, boar and elk on the Irish countryside, and can achieve incredible speeds at the gallop. As companion animals in the modern world, they have been described as courageous, dignified and calm. Unfortunately, these magnificent creatures have very short lifespans, ranging in various studies from 5.95 to 8.75 years. [78] In accordance with the *lex naturalis* , when we increased body size by artificial selection in the Irish Wolfhound without providing it with an improved tumor suppression mechanism— the way Nature would have if this animal had evolved under natural selection— there was an equilibrating decrease in lifespan in order to offset this increase in body size.

When equilibration becomes impossible

While changes in lifespan absorbed some of our violation of the *lex naturalis* during our selective breeding of canines, our failure to provide each new breed of dog with its own unique mechanism of tumor suppression, the way natural selection would have done in the creation of new species, left some breeds, particularly larger breeds, at elevated lifetime risk of cancer. Thus, not all of our violation of the *lex naturalis* could

be absorbed by changes in lifespan, as it becomes inherently inefficient to decrease lifespan in animals with large body size; at some point in the equation, when it becomes too inefficient to decrease lifespan any further as a means to equilibrate human-engineered violations of the *lex naturalis* — something that would never happen in a natural setting— the excess translates to changes in lifetime cancer risk, R. Overall, our repeated violations of the *lex naturalis* in the selective breeding that produced today's dog breeds has caused a situation in which there are now 3 1/2 times more dogs with cancer in the United States than there are people with cancer! (Even though there are 3 1/2 times fewer dogs than people.)

There can be no doubt but that our manipulation of the wolf genome by selective breeding has increased cancer risk in man's best friend. Thus, the wolf is similar to other longer-lived placental mammals in having a lifetime cancer risk of about 4%. Since a wolf has a natural lifespan of about 13 years, this equates to an incidence rate of cancer in the wolf of about 210 per 100,000 wolves per year. This can be compared to the human cancer incidence rate of about 442 per 100,000 humans per year. As we noted in chapter one, in our dogs the cancer incidence has skyrocketed to a figure of 6,600 per 100,000 dogs per year— *more than 30 times higher than in the wolf* .

Cancer is truly epidemic among our dogs. And it is our fault.

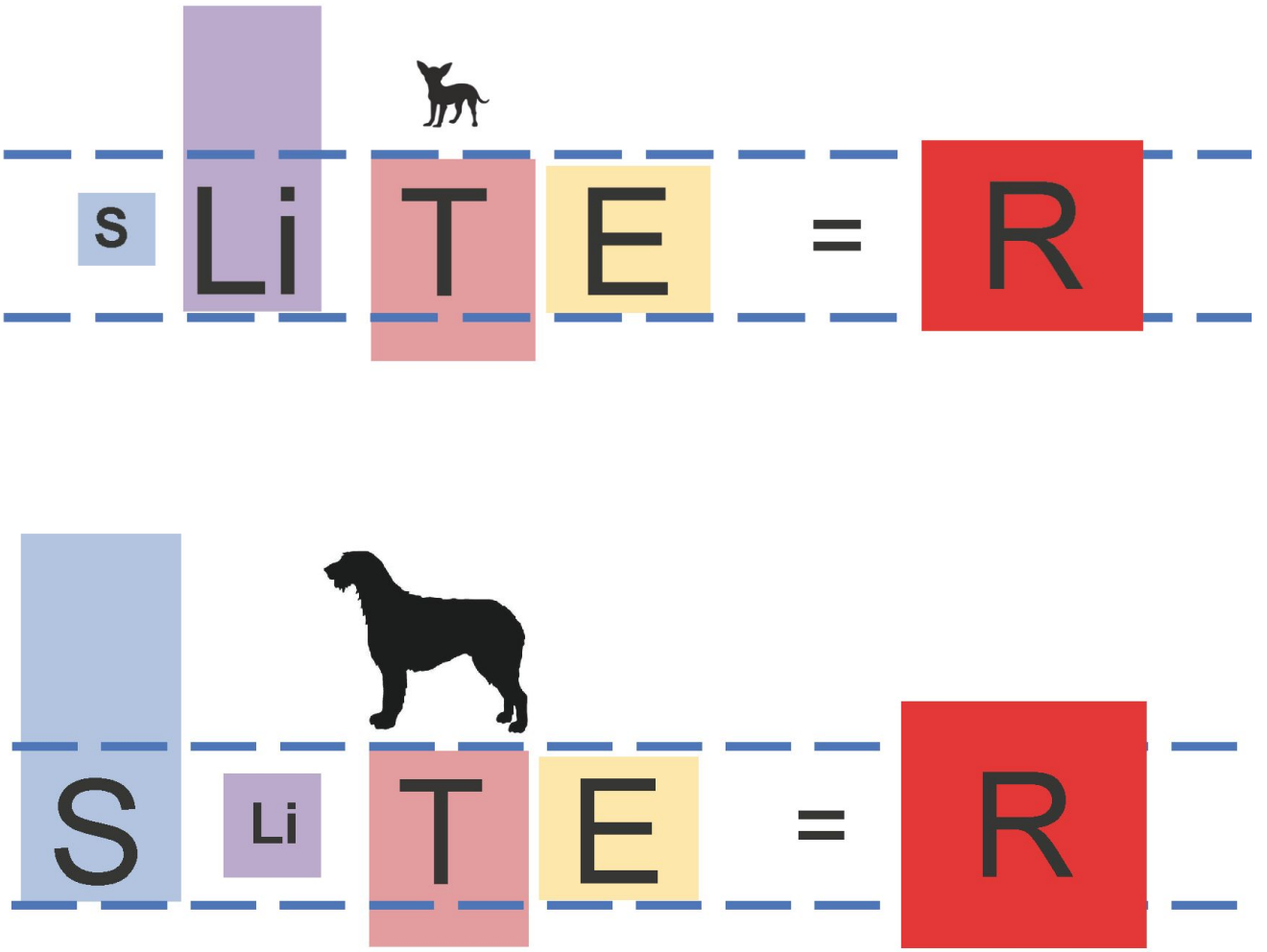

Figure 4.7 The lex naturalis equations for small versus large dogs. Note that even though small dogs benefit by an increase in lifespan, they still have an increased risk for cancer—although not nearly the increase observed in large dogs—for reasons that we will discuss below.

Additional risk factors in dogs brought about by breeding

Small dogs

As we have discussed above, overall lifetime cancer risk for all types of cancer is affected by body size, particularly height. However, additional risk factors for individual cancer types are clearly superimposed upon this overall pattern, suggesting that the genomic/epigenomic [79] changes that were selected for to achieve prized breeds carried with them an enhanced risk for specific types of cancer. For example, small dogs, even though they are at overall reduced risk for mortality from cancer, are three-fold more susceptible to mammary cancer than are large dogs. [80] Clearly, the selection process that was required to produce the smallest dog breeds has somehow left them with mammary tissue that is inordinately susceptible to neoplastic transformation. While the cause for this increased susceptibility remains to be clarified, it may be that the genetic and epigenetic modifications responsible for reducing growth of muscles, bones and most other tissues, resulting in small body size actually enhanced the susceptibility of mammary epithelium to neoplastic transformation. Early spaying of female dogs

dramatically reduces the risk of later development of mammary cancer, suggesting a fundamental role of progesterone in its etiology. But how small dogs remain preferentially affected by mammary cancer remains a mystery.

Large dogs

In addition to their increased overall lifetime cancer risk for all types of cancer, tall dogs like the Irish Wolfhound, and the Great Dane, have a particularly high risk for certain kinds of cancer, such as osteosarcoma. [81] In both dogs and humans, osteosarcoma generally occurs in the growth plates of long bones. But there are also differences between canine and human osteosarcoma. In humans, osteosarcoma occurs primarily in children, whereas in dogs, the median age of developing osteosarcoma is about seven years, which corresponds to the adult phase of the canine life cycle. Human osteosarcoma occurs more frequently in males, whereas there is no sex preference in canine osteosarcoma. Another difference between human and canine osteosarcoma is that this particular tumor type is 30-50 times more frequent in dogs than it is in humans. [82] Large and giant breeds of dogs account for 90% of all cases of canine osteosarcoma. [83] Clearly, the genes that have

undergone artificial selection to increase size in dogs has left them with an increased lifetime risk of cancer in general, and osteosarcoma in particular.

As you would probably expect, based upon their role in bone growth, IGF1 and its receptor, IGF1R, are both thought to play a significant role in osteosarcoma, [84] with certain genetic polymorphisms in IGF1 enhancing the risk of and reducing the prognosis for osteosarcoma. [85] Two microRNAs (miR-503 and miR133a) have been found to inhibit the growth of osteosarcoma by targeting the mRNA of IGF1R, [86] and high expression of IGF1R predicts poor outcome in canine appendicular osteosarcoma. [87] In addition to IGF1 and IGF1R, fibroblast growth factor 1 (FGF1) and its receptor (FGF1R) are also over-expressed in osteosarcoma, [88] and FGF1R is suppressed by the natural miRNA133b. [89] Both IGF1 and FGF1 increase with increasing body size in dogs, [90] offering an explanation as to why large breeds of dogs have an increased lifetime risk of osteosarcoma. But there may also be a temporal aspect to the increased risk of osteosarcoma in the largest dog breeds. Thus, when our breeding practices forced increases in height in our largest dogs, a corresponding increase in the speed at which they achieved their adult size also occurred in many, but not all of them. It now appears that it is the largest dogs

with the shortest developmental period to reach adult height that seem to be at highest risk for osteosarcoma. It thus may be the rapidity of growth in the long bones of big dogs that underlies such high risk. [91] As we shall discuss in chapter 10, the *lex naturalis* teaches us how we might "normalize" cancer risk in our dogs— not just osteosarcoma, but all canine tumors — from its current aberrant 50% lifetime risk to the 4% of their ancestral species, the grey wolf.

Summary of Chapter 4

A *lex naturalis* exists for vertebrate speciation in which any change in the variables of body size, lifespan, species-specific mechanism of tumor suppression, or carcinogen exposure (which change might occur in order to enable a new environmental niche to be exploited) must have a simultaneous, equilibrating change in one or more of the other variables in order to keep lifetime cancer risk at a value of about 4%. We gave as an example how an increase in size must be compensated for by an improvement in species-specific mechanism of tumor suppression, or a decrease in lifespan. Without such an equilibrating change among these dependent

variables, lifetime cancer risk could not be maintained at the required 4%. In the natural world, Nature does not permit violations of the *lex naturalis* , because it would reduce the efficiency of assimilation of environmental resources into DNA.

But with humans steering the evolution of canines instead of natural selec-tion, we failed to provide new dog breeds with their own tumor suppression strategy the way nature would have done if she had developed these breeds as separate species. As a consequence, our manipulation of canine biology, particularly body size, caused equilibrating changes in other elements of the lex naturalis, most obvious of which is the decreased lifespan and increased cancer risk of our large breed dogs. All of our dog breeds are members of a single species—*Canis lupus familiaris* — but they would be separate species if they had evolved naturally, and would have survived, or not, depending upon whether their particular features increased the probability of survival in the natural world. If the Irish Wolfhound, the Great Dane, the Dogue d Bordeaux, or any other giant breed had evolved in the natural world, natural selection would not have permitted such increase in body size without simultaneously adjusting the animal's tumor suppression mechanism so that lifetime cancer risk would

remain at about 4%. Neither would Nature have permitted lifespan to be so dramatically decreased as an equilibrating mechanism, because short lifespan in large animals is an inefficient use of resources. If the Chihuahua had evolved naturally, its dramatic reduction in body size would likely have rendered its lineage-specific tumor suppression system dispensable— its small body size enabling R to be kept at 4% using only the canonical p53 tumor suppression mechanism exemplified by the mouse. Additionally, since *lifespan is simply another variable that natural selection manipulates within the lex naturalis to effect optimum efficiency of a species* , if the Chihuahua had evolved naturally, as a separate species, it might not have the long lifespan that it now enjoys. Whether it did or not would depend entirely upon what particular lifespan best satisfied the *lex naturalis* equation for the Chihuahua species.

Our manipulation of the canine genome/epigenome away from that of the grey wolf may thus have provided some advantages to our smallest dogs, at least in terms of lifespan. Reducing their body size violated their *lex naturalis* equation, which caused an equilibrating increase in lifespan. But when we selected for large increases in body size, as for example in the Irish wolfhound, without providing such dogs with the improved tumor suppression mechanism that nature would have

required before permitting increased size, this violation of their *lex naturalis* caused the opposite effect— an equilibrating decrease in lifespan. And when decreasing lifespan reached the point that it was no longer a tenable strategy because of the decrease in efficiency that it caused (it is uneconomical to create large, short-lived bodies), the lex naturalis was enforced by an equilibrating increase in lifetime cancer risk. Thus, our giant dog breeds, with their big hearts and their "mutual gaze," live short, accelerated lives, leaving us far too soon. But as I will describe in the next chapter, we may yet be able to remove the "Sword of Damocles" that we suspended over the heads of these wondrous creatures, and provide even our giant breeds with long, cancer-free lives.

Figure 4.8. Hal, the Irish wolfhound, meets Tic, the Chihuahua. Photo credit, Rohan Kelly. Photo from the Daily Telegraph, August 4, 2016. By the time you are reading this, Hal will have already lived out most of his lifespan, while Tic has many years yet to go.

5

Using the lex naturalis to Prevent Cancer in Your Dog

"Dogs' lives are too short. It is their only flaw, really."

Agnes Sligh Turnbull

"Dogs come into our lives to teach us about love and loyalty. They depart to teach us about loss. A new dog never replaces an old dog; it merely expands the heart. If you have loved many dogs, your heart is very big."

Erica Jong

A dog's purpose

In chapter 4 we learned that we created a *Sword of Damocles* over the heads of our dogs— especially are big dogs— by failing to provide them the improved tumor suppression mechanism that Nature would have provided them if these breeds had evolved naturally, in the wild, as separate species. In our creation of every breed, we unwittingly committed gross violations of the *lex naturalis* . Natural selection must have watched our efforts with some degree of bewilderment, because it is certain that none of the breeds we created could actually survive in the wild. Not one could successfully compete with the grey wolf. Most would likely end up on the grey wolf menu. Even if they could avoid the grey wolves that would hunt them, a few hundred years of human selection produced a subspecies of *Canis lupus* with more than 300 variations, each of which can only live among humans, because it is dependent upon human protection. There appears to be no feature selected by man that would confer upon any of our dog breeds a survival advantage in the wild environment. Every feature that gave dogs novelty, and was therefore favored by humans, would confer reduced survivability upon them in the wild. It is only by the intervention of humans that most dogs survive. [92]

So, what were we thinking?

When many of the breeds were originally created, they were created for a purpose. Blood hounds for tracking and treeing foxes (or, apparently in North Carolina, racoons.) Wolf hounds for detecting, running down and eliminating wolves in the freezing mist of the Irish moors. Great Danes were bred to hunt wild boar. Border collies for herding nervous sheep. Golden Retrievers for fetching water foul felled by the blast of a shotgun. Dachshunds for hunting badgers in their burrows ("dach" is German for badger; thus "dachshund," for badger dog.) St. Bernards were bred for mountain rescue by the St. Bernard Hospice— named after an Italian monk— in the Alps along the Italian-Swiss border; And so on.

Most of these breeds are now revered for other qualities, such as their temperament, their loyalty, and their playfulness, rather than for the task for which they were bred. This gives us some flexibility, in both the near and long term, to correct some of the violations of the *lex naturalis* that we, in our ignorance, imposed upon our dogs.

Cancer Prevention

Our near term goal

My lab's major near term veterinary goal is to "normalize" lifetime cancer risk in dogs from its current 50% toward the 4% of animals in the wild. Even by itself, that is a worthwhile goal, although in the longer term it may also be possible to simultaneously increase their lifespan, even though reduction in lifetime cancer risk, R, and increase in lifespan, Li, represent competing elements of the *lex naturalis* equation. Reducing lifetime cancer risk in dogs to 4-5% would reduce canine cancer incidence *12-fold* , from its current 6,600 new cases per year per 100,000 dogs, to a much more manageable 550 new cases per year per 100,000 dogs. In terms of all dogs in the United States, instead of the 6 million new cases of cancer every year (NCI), this number should drop to about *half a million* . In terms of *your* dog, it would make cancer something you really didn't have to worry about very much; something that had only a very small chance of affecting your dog— a disease as rare as it is in wild wolves, who were protected from cancer by the process of *natural* selection that adhered to the *lex naturalis* .

To accomplish this dramatic reduction of cancer in the near term, we must dramatically improve species-specific mechanism of tumor suppression, T, in our dogs. Let's consider what this would mean for the dogs most in need, our giant

breeds. Below we show the *lex naturalis* equation for large dogs.

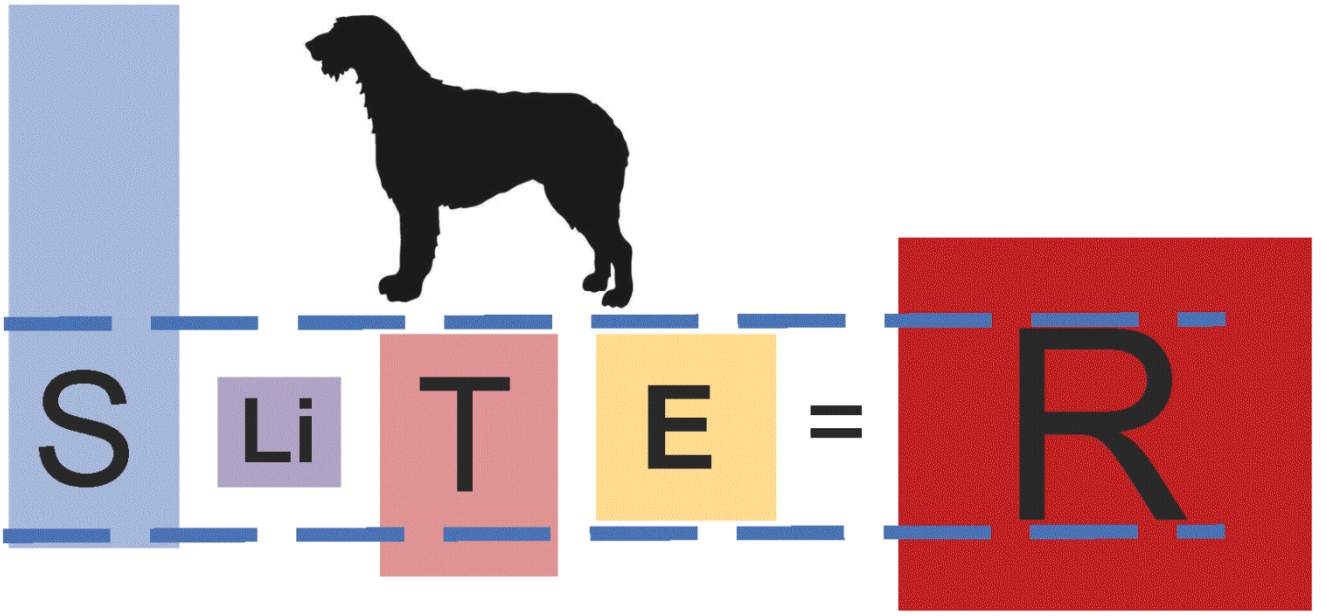

Figure 5.1 The lex naturalis equation for large dog breeds

Because we increased body size, S, in our large dogs without simultaneously improving T, the increase in body size bred into our large dogs was equilibrated by a decrease in lifespan, Li. However, since lifespan can only be reduced to a certain level before the breed is no longer viable, equilibration to offset body size was forced to occur through a dramatic increase in R, lifetime cancer risk.

Pharmacologically improving T

As we discussed, dogs were able to form a relationship with us because the epi wolf from which they were derived was at the high end of the bell-shaped curve for circulating DHEAS. (Interestingly, female dogs have been reported to secrete most of their circulating DHEA from their adrenal gland— compared to male dogs which secrete DHEA from their gonads. This appears to be another step by canines to move toward the otherwise primate-specific kill switch. [93]) In addition to the role that DHEAS played in enabling first contact, by suppressing the fight-or-flight response, it also acted as a rudimentary form of the otherwise primate-specific "kill switch" tumor suppressor mechanism in which DHEAS is naturally imported into a cell that has undergone p53 inactivation. That DHEAS can then be de-sulfated to DHEA, a potent uncompetitive inhibitor of G6PD, and that inhibition of G6PD will deprive the p53-affected cell of NADPH. Loss of NADPH deprives such a cell of the selenoprotein firemen and NADPH-filled fire hydrants required to put out cellular ROS— resulting in ROS-mediated immolation of the cell.

DHEA and DHEAS are small, stable molecules, and can be prepared as formulations that can be administered to our dogs to *pharmacologically* improve T— we don't have to wait for Nature to do it, taking her own, sweet, evolutionary time. We

can use pharmacological treatment with DHEA/DHEAS to equilibrate the *lex naturalis* equations of our dogs.

Below is the *lex naturalis* equation for large dogs that have been provided with such a pharmacological improvement of T. Our major goal here is to reduce R, lifetime cancer risk, to normal limits of 4-5%, but since R and Li compete for benefit derived from the equilibration provided by the improved T, there may also be some increase in lifespan, at the cost of less reduction in R. However, because R is the independent variable in the equation, it should be equilibrated preferentially, returning toward a much more normal figure, approaching 4-5%. Only after R is fully equilibrated will Lifespan be free to increase in a less restrained manner.

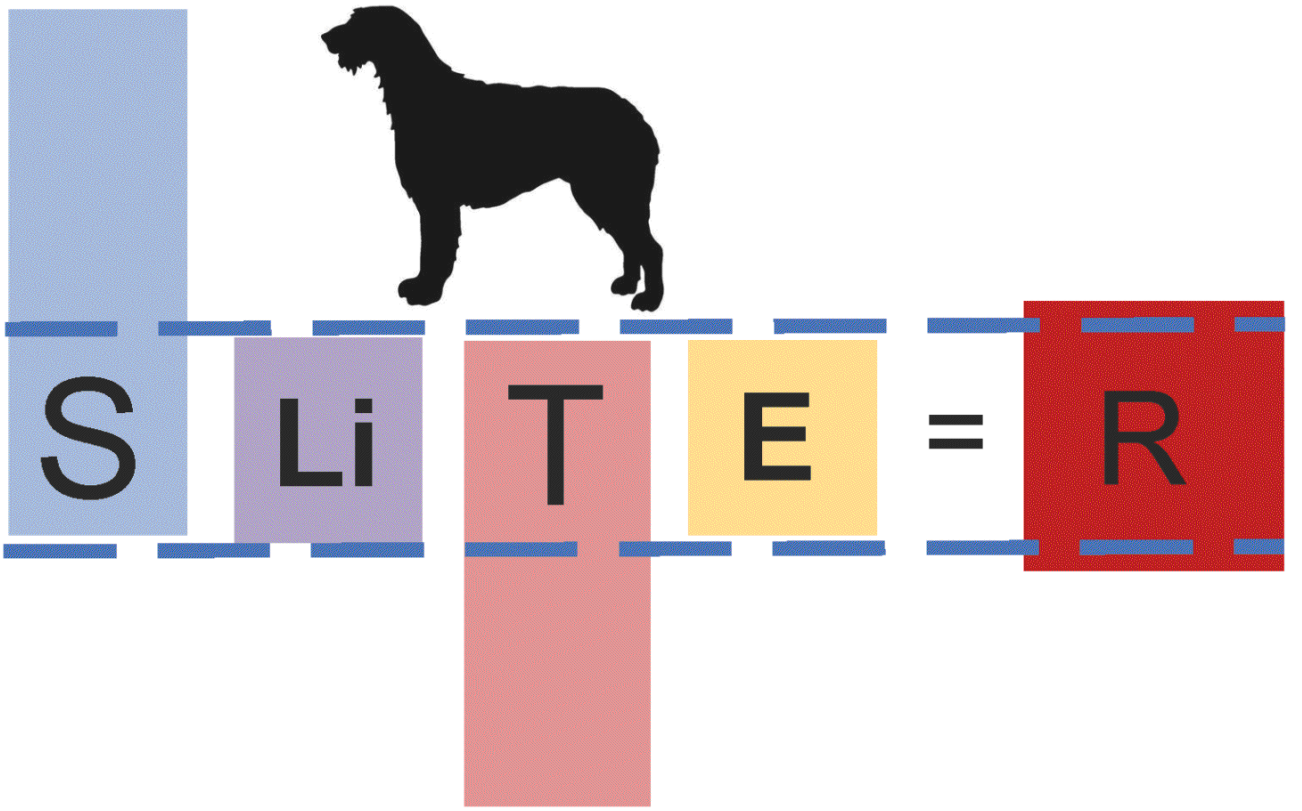

Figure 5.2 The lex naturalis equation for a large breed dog that has T improved pharmacologically by treatment with DHEA/DHEAS. R and Li will compete for equilibration by this improvement in T. Note the large improvement in R, and also improvement in Li.

E can also be decreased to assist in equilibration

There may be various ways that we can reduce carcinogen exposure for our dogs. For example, many dog food manufacturers tout the high percentage of meat and meat

products in their products, but we already know that, at least in humans, replacing meat protein with plant protein significantly affects cancer risk. [94] Our dogs did evolve as carnivores— that was the niche that wolves fit into within the expanding mass of DNA on this planet. But our dogs are no longer part of the wild kingdom, and it is not obvious that they must remain obligate carnivores. In my opinion, reducing E in our dogs by feeding them a vegan diet may offer an effective way to help equilibrate the l*ex naturalis* , and along with our proposed improvements in T, reduce R and increase Li. Ellen DeGeneres has taken a lot of heat from purported experts in canine nutrition, for her line of vegan dog food (Halo), but in my opinion, she is on the right track.

Further reductions in E might be had immediately by eliminating unnecessary exposure to carcinogens, such as glyphosate weed killer products (e.g., RoundUp^{TM} .) This statement is based upon recent studies out of the School of Public Health at UC Berkeley that confirm an association between exposure to glyphosate and the development of non-Hodgkin lymphoma in humans. [95] The International Agency for Research on Cancer (IARC) has stated that glyphosate is "probably carcinogenic to humans," and an international group of toxicologists has also raised alarms. [96] If glyphosate is

harmful to humans, then it certainly will be harmful to our dogs, especially since they may achieve high exposure levels by running and playing and lying about in glyphosate herbicide-sprayed yards. If we want to do everything in our power to reduce R and increase Li in our dogs, then, in addition to providing them with pharmacological improvements to T, we should also reduce E by every available measure.

Below is how the *lex naturalis* equation is anticipated to look for a large breed dog with both a pharmacologically improved T, and a reduction in E.

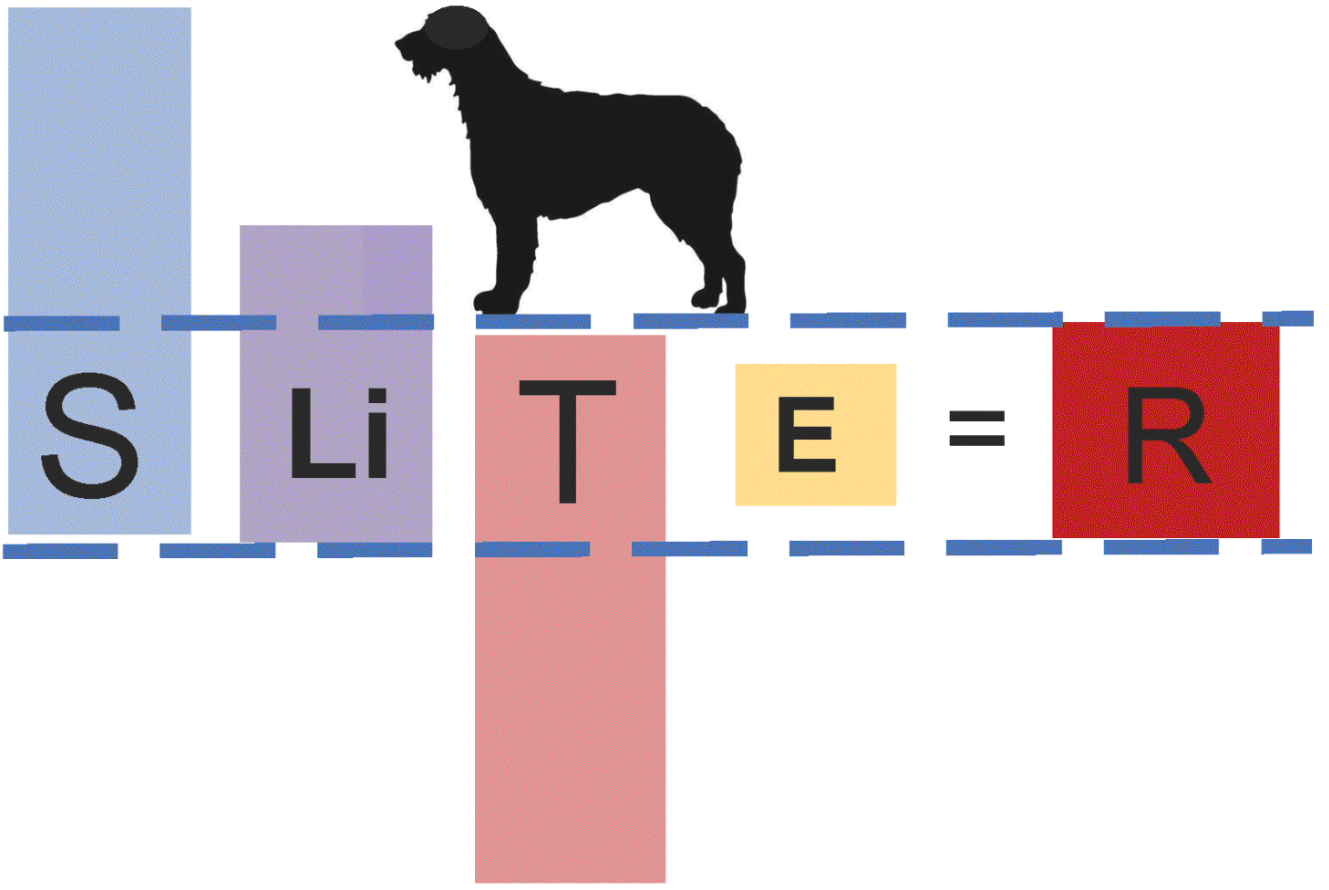

Figure 5.3 Reduction in E, carcinogen exposure, will also help equilibrate the disequilibrated lex naturalis equation of large breed dogs. Once R is fully equilibrated, increases in lifespan, Li, should occur.

The above strategies, focusing on immediately do-able "fixes" to our violations of the *lex naturalis* are focused on reducing R to the levels that are "normal" when selection is *natural* , rather than driven by human intervention. But now that Nature has revealed her *lex naturalis* to us, we may be able to produce dogs with both "normal" R and substantially increased Li—something that natural selection could not do, because she must produce animals capable of surviving in the wild. We do not have that same constraint, because our dogs don't have to survive in the wild. They are under our protection. Hopefully it will remain that way for the foreseeable future.

DHEA or DHEAS for this application of reducing R and increasing Li?

Dogs are different than humans in the important aspect that they have the canonical GCAG sequence motif in the G6PC promoter, and a perfectly working GLO gene— so they can synthesize vitamin C, and DHEA stimulates G6PC activity.

Because of these differences as compared to humans, what they *cannot* do is accumulate very much G6P, which you will remember is necessary in order to drive uncompetitive inhibition of G6PD by DHEA to irreversibility. So it is much safer to administer DHEA to dogs than it is to administer it to humans. We are hard at work pushing a granular form of DHEAinto clinical trials with FDA, a formulation in which the unpleasant taste, and even the odor (undetectable to us, but not to dogs) of DHEA are pleasantly masked. This formulation enables precise measurement of mg/kg doses to all size dogs, by adding it to their food. As a first approach in our veterinary clinical trials to reduce R and increase Li, dose is being selected based upon that dose which provides an obvious increase in sociability. This effect is so visible that we have even filed patent applications on it as the target effect. As we obtain data with larger and larger numbers of dogs, we can more precisely determine what doses bring about maximum decreases in R, and increases in lifespan.

Medium-term goal in cancer prevention

We noted above that, most of the time, we prize our dogs for features related to their temperament, their loyalty, their

unabashed joy at our arrival in their midst—in short, for their *companionship* — and not the feature for which they were originally bred. This leads me to ask the question, wouldn't an Irish Wolfhound be just as splendid a companion at half its current size? Unless a St. Bernard is actually headed for a life as a rescue animal in the Alps, where large body mass insulates against the cold, wouldn't a much smaller version be just as loveable and fun to have in our lives? (Certainly it would be less expensive to feed!) Since there are very few wild boar around to hunt anymore, wouldn't a miniaturized Great Dane— perhaps 24 inches tall instead of 36— be just as perfect a companion? If this makes sense to you, we can use the *lex naturalis* to direct future breeding programs, selecting for breeding purposes those animals at the smaller end of the bell-shaped curve for size. We can even be more precise and select as breeders those animals with the lowest IGF1 and FGF1 levels in their serum, and IGF1R and FGF1R expression in their bone tissues. If we are going to correct our violations of the *lex naturalis* , and give our dogs their longest possible lifespan, and the lowest possible cancer risk, we are going to have to include these ideas into our breeding strategies.

Let's consider what the equation might look like for a large breed dog that has undergone optimization for size by breeding

to conform to the *lex naturalis* . Irish wolfhounds that are half the size that they used to be. Great Danes, Dogues de Bordeaux, mastiffs, all half the size than that at which they were originally bred. Using this combination of strategies, I believe that we can double, perhaps even triple the lifespan of our currently giant breeds, and simultaneously reduce their lifetime cancer risk, R, to the normal range of about 4%.

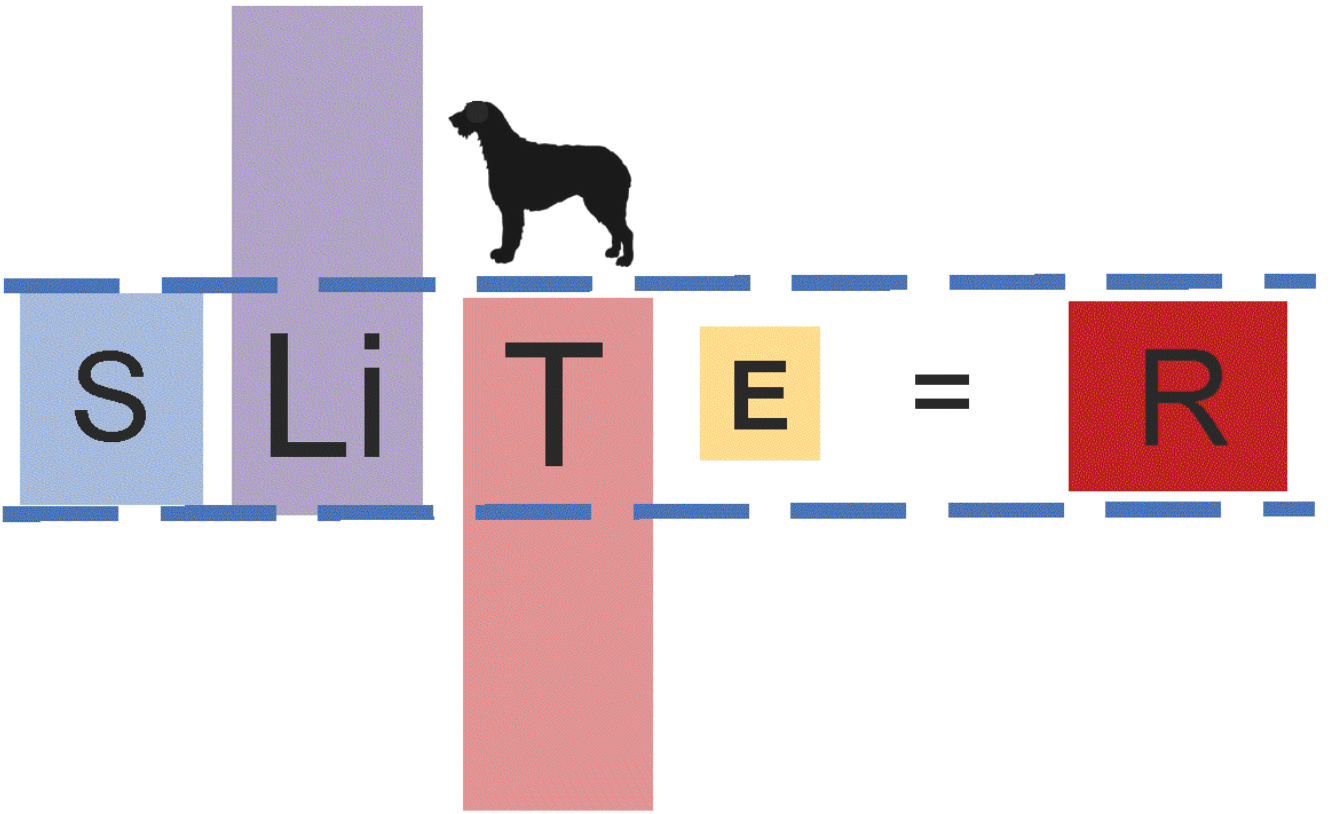

Figure 5.4 The lex naturalis equation for a formerly giant breed, such as an Irish Wolfhound, St. Bernard, Great Dane, etc., miniaturized to about half its former height by standard breeding practices, selecting dogs from the small end of the bell-shaped curve; and these dogs also receiving

pharmacological improvement of T, and maximal reduction of E as discussed above.

What about our miniature breeds?

Should we continue to miniaturize dogs until they are the size of ancestral primates? No. We can already see that R is larger than it should be in some of the miniature breeds, particularly with respect to endocrine-related cancers. Small dogs, for example, have been found to have three times the risk of developing mammary gland cancer than large dogs— although their risk for developing certain other kinds of tumors, especially those related to bone growth plates such as osteosarcoma, is quite reduced compared to large dogs. [97] From what we know right now, we can say with certainty that the breeds that are already miniature should not be bred to be even smaller. Spaying a female dog before her first heat almost completely removes her risk of developing mammary gland cancer; although this is not a reassuring fact for breeders whose business it is to produce these dogs. Also, while a female dog's risk of mammary gland cancer disappears after neutering, there is evidence that her risk for developing other kinds of tumors goes significantly up. [98]

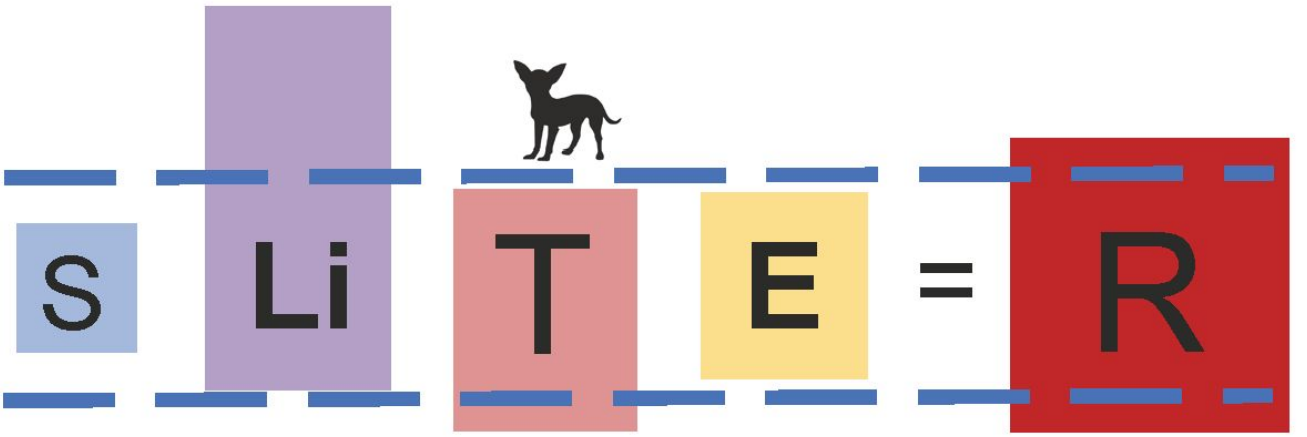

Figure 5.5 Miniature breeds have benefitted by having their small body size equilibrate the lex naturalis by increasing lifespan, Li. However, their lifetime cancer risk, R. remains outside the 4-5% mandated by the lex naturalis as it would be expressed under natural selection.

Miniature dogs should benefit from improving T *via* pharmacological manipulation of DHEA/DHEAS levels in potentially dramatic ways. However, their *lex naturalis* equations may respond in a different way than they would for large dogs. By pharmacologically enhancing T in our miniature breeds, we should be able to significantly reduce R, and increase their lifespan still further. Will we be able to enhance T and reduce E to the extent that our small dogs will become virtually lifelong companions? I believe that by correcting our violations of the *lex naturalis* we may very well see small dogs

living 40 year lifespans, with lifetime cancer risks normalized to the 4% that Nature intended. So, yes. This strategy could make the “First Friend” our companion throughout long stretches of our own lifespan.

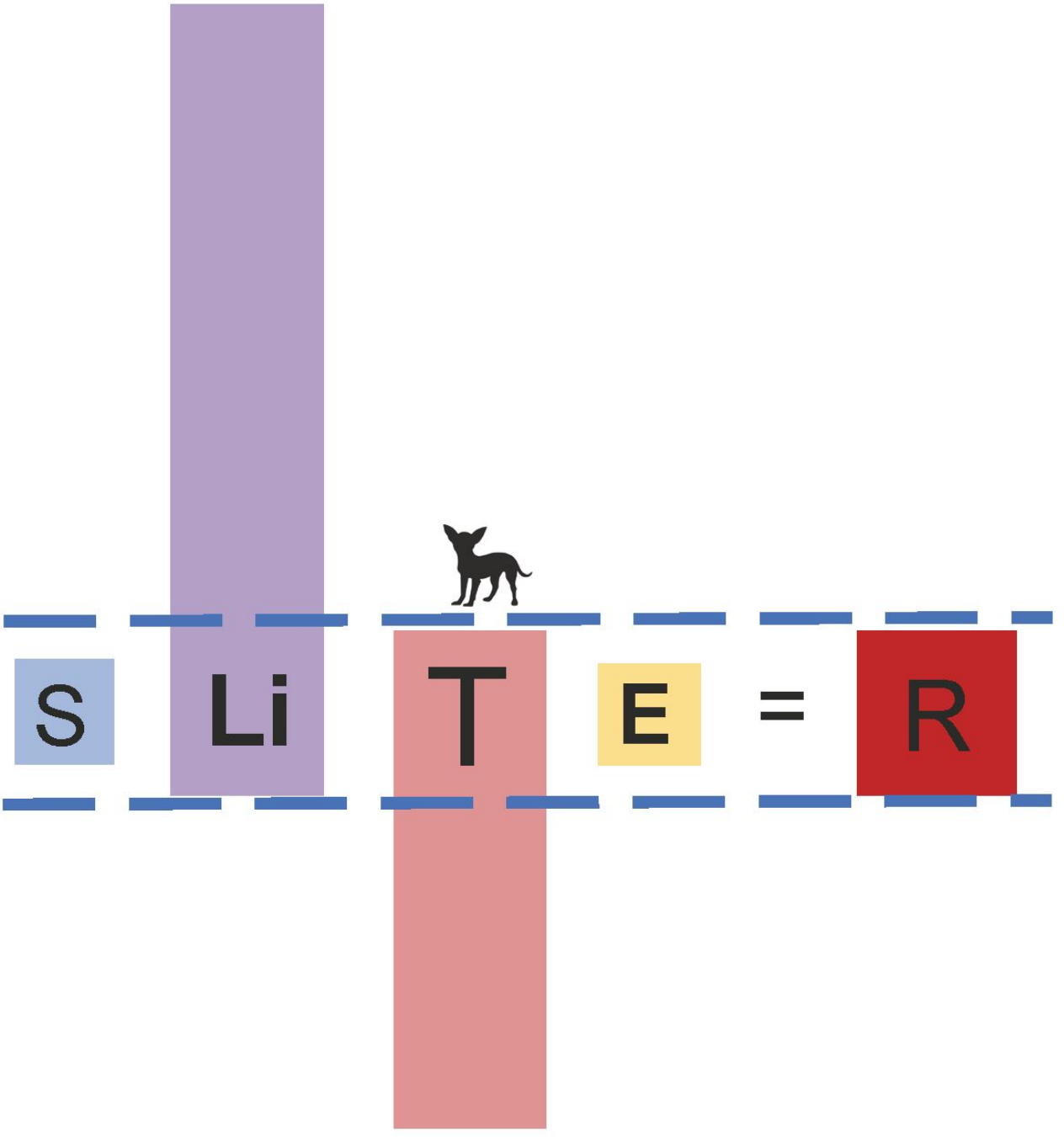

Figure 5.6 Correction of the lex naturalis in small dogs, by pharmacologically enhancing Twith DHEA, and reducing E by managing carcinogen exposure, is predicted to decrease R and increase Li to the point where such dogs may become lifelong companions.

What about our "normally-sized" breeds?

Cancer has become the main cause of death in *all* dogs, irrespective of their breed. [99] We have noted that female dogs resemble primates in that their adrenals secrete DHEA—albeit not at the levels observed in primates— and that they decline with age, similar to what is observed in humans.[99] Thus, all dogs are likely to benefit from our intervention to improve T by pharmacological administration of DHEA or DHEAS. As in our discussion of dogs at the extremes of body size, medium sized dogs should also experience significant decreases in R, and increases in Li, by such intervention.

Using the lex naturalis to "cancer-proof" the dogs of the future

Earlier we discussed the possibility that we might be able to use the new tools of genetic engineering to correct some of the

violations to the *lex naturalis* that the last several hundred years of breeding have caused. Anticipating that there may be those who object to artificial means to alter breeds, we pointed out that this particular horse has already left the barn— there was nothing "natural" about humans selecting dogs for gigantic size, miniature size, wrinkled skin, blue eyes, white coats, or any of the other myriad traits that some human somewhere at sometime came across and decided to immortalize for its uniqueness— not its ability to increase that particular dog's fitness. If we can use the tools of modern genetic engineering to fix our past errors — and there is no longer any technical obstacle to doing so— then we *should* do so. So, the question is, how would we go about this? What would we change? If we install a complete primate-style tumor suppression system, according to the *lex naturalis* , that should normalize lifetime cancer risk in our dogs, and after that, it should increase their lifespan. If we use the tools of genetic engineering to install a primate-style kill switch into our dogs, should we follow the order that primates followed when they naturally evolved their "kill switch" tumor suppression mechanism?

We have noted that dogs resemble humans in that they have (1) circulating DHEAS, and therefore at least a rudimentary form of the primate "kill switch;" and (2) a rudimentary form of

adrenarche, being one of the rare animals that secretes DHEAS into the circulation from their adrenal glands. [100] It also turns out that, unlike most mammals, but very much like humans as we saw in the chapter on the evolution of the primate kill switch, (3) dogs re-absorb uric acid from the kidney. [101] They thus have the potential to reproduce this element of the primate kill switch— except that most dogs have functional UOX, so the transition toward the primate kill switch ends halfway there. Their active UOX breaks down uric acid, which is then metabolized all the way to allantoin, and excreted. *Except in one breed of dogs* . A breed of dogs in which uric acid is not broken down, and consequently builds up, both in the circulation, and in the urine. Intriguingly, a breed of dogs with an *inordinately close association with fire* , *and therefore with PAH* . And even more intriguingly, a breed of dogs in which the mutation that caused this increase in uric acid just happens to be the *signature mutation of PAH exposure, G to T* ; the same signature mutation that converted the GCAG sequence motif of most mammals to the kill switch-enabling GAAT motif in the G6PC promoter of anthropoid primates. Let’s have a look at this singular breed of dog.

The Dalmatian story: Experimenting with improvements in T?

Inactivation of UOX in hominoid primates occurred by two different sets of mutations, one in the *Hominoidea* — the Great Apes and humans— and an entirely different one in the *Hylobatidae* — the gibbons. To enable the great increase in body size in the hominoids— e.g., the lowland and mountain gorillas, each of which can reach a body mass of 200 kg— there was also a modification in the hominoid lineage related to control of the uric acid transport protein, SLC2A9, putting it under p53 control. Thus, in cells with active p53, SLC2A9 actively transports uric acid into the cell, and the antioxidant property of uric acid helps to maintain ROS at low, nontoxic levels. But in a cell in which p53 has been inactivated, SLC2A9 activity is shut off, uric acid entry into the cell stops, and the increased ROS caused by DHEA-mediated inhibition of G6PD burns the cell down. High circulating uric acid levels in the *Hominoidea* were thus the improvement in kill switch function that enabled increased body size while maintaining R at the required low rate.

It struck me as fascinating, then, that Nature appears to be "experimenting" in the breed of dogs known as Dalmatians with elements of the primate "kill switch" beyond the

circulating DHEAS that all dogs have; beyond the rudimentary adrenarche that all dogs have; and beyond the reabsorption of uric acid that all dogs have. Thus, while other dog breeds end up metabolizing uric acid and excreting the breakdown products, Dalmatians do not. This leads to a situation in which Dalmatians have serum uric acid levels that are almost three times higher than those observed in other dog breeds; and nearly in the range of humans (0.68 mg/dL in other breeds of dogs, vs 1.85 mg/dL in Dalmatians, vs. 3.5 mg/dL in humans.) [102] (It also leads to a serous problem of high uric acid in the urine of Dalmatians, which can precipitate out as painful "stones" called calculi.) We saw in the previous chapter that heavy selection for high circulating levels of uric acid occurred in the hominoid primates, as part of the kill switch tumor suppression mechanism in this lineage. Is the increase in circulating levels of uric acid under similar positive selection in the Dalmatian?

Evolution is approaching uric acid metabolism in a different way in Dalmatians than it did in hominoid primates. Thus, the UOX gene in Dalmatians is fully functional— just as it is in all other animals except humans and the great apes. It is therefore not the cause of the elevated levels of circulating uric acid. [103] Instead, evolutionary change in Dalmatians appears to be

focusing on SLC2A9, [104] the same transport protein that came under p53 control in the hominoids. What is happening to this gene in Dalmatians is intriguing. Three mutations have been identified— one in the coding region and two in the promoter. (To refresh, the promoter is in the non-coding 5' or left side of the gene, and controls the rate of expression of the gene.) The mutation in the coding region consists of a G to T mutation in the fifth exon. (An exon is a region that codes for proteins, generally flanked by noncoding regions called introns). This G to T mutation dramatically reduces the activity of the SCL2A9 uric acid transport protein. [105] We do not yet know if the modifications in the Dalmatian SLC2A9 gene have anything to do with putting it under p53 control. But if Dalmatian SLC2A9 *has* been put under p53 control, then it would seem that natural selection is following a parallel evolutionary path in this breed as compared to hominoid primates. More likely, it may be that the high *circulating* levels of uric acid generally reduce E in the *lex naturalis* equation of Dalmatians, but the inhibition of uric acid transport into the interior of the cell— caused by the SLC2A9 mutations— prevents uric acid antioxidant from entering cells. This would have the blunt effect of reducing the threshold at which ROS could burn down the cell, because there would be less intracellular uric acid antioxidant around to

naturally suppress ROS. If this is what is happening, one could imagine a scenario in which DHEAS would be imported into Dalmatian cells that have p53 inactivated; that DHEAS would be rapidly de-sulfated to DHEA, and DHEA would be toxic at a lower threshold of G6PD inhibition than in other dog breeds because the lack of intracellular uric acid means that ROS are high to begin with— it would not take much NADPH depletion to cause the p53-affected Dalmatian cell to burn up.

The G to T mutation in the fifth exon of the SCL2A9 gene occurs in all Dalmatians, and in *both* alleles; that is, it is homozygous. [106] (Every animal has a separate allele, or copy of the gene, one inherited from its mother, and one from its father. When both alleles are the same, they are said to be homozygous. When they are different, with different properties, they are said to be heterozygous.) Again, *G to T is the signature mutation of PAH* , as we discussed for the evolution of the anthropoid primate-specific GAAT sequence motif in the G6PC gene, which evolved from the canonical GCAG motif of most other vertebrate animals. In thinking about this, one should keep in mind that G to T mutations are not common. In humans, the species most studied, G to T mutations are found in the lung tumors of smokers— but not in the lung tumors of non-smokers. In non-smokers, G to A mutations predominate.

Thus, G to T mutations are *rare* in the absence of PAH exposure. [107] Did Dalmatians experience greater PAH exposure than other dogs? Is this why they fixed these signature mutations of PAH exposure into both alleles of their SLC2A9 genes?

In this regard, the history of the Dalmatian is equally fascinating. They were bred in the 17th and 18th century as so-called "coach dogs," meaning that they showed a special affinity for horses, and so accompanied the horse-drawn carriages of the nobility. The gene for their spotted coloration is physically very near to the SLC2A9 gene on the chromosome, and selection for this spotted coloration to create the breed may have been responsible for fixing the G to T mutations in this gene into the Dalmatian lineage. This breed was subsequently found to have great aptitude in the leading of horse-drawn fire wagons to the site of fires— *also the site of dramatically increased PAH exposure.* Because of their aptitude with fire, they rapidly became the canine of choice for fire brigade duty. It is thus either one of the most incredible coincidences in the history of dogs, or a clue to why Dalmatians were *able* to become fire brigade dogs, that the G to T mutations in the SCL2A9 gene in Dalmatians consists of the signature mutations of PAH exposure [108]

Figure 5.7 The Dalmatian became the dog of choice to lead horse-drawn fire brigades to the sites of fires— and PAH exposure. Did their unique SLC2A9 mutations, consisting of the signature mutation of PAH exposure— lead to their unique ability to perform such duty? Picture courtesy of the archives of the Boston Globe .

It is too early to believe with any real confidence that Dalmatians are evolving toward a more cancer-resistant breed — i.e., that the *lex naturalis* is attempting to wrest control of selection away from humans— but there are intriguing hints

that this may be the case. For example, Professor Jane Dobson of the University of Cambridge, in the UK, published a study of 15,888 canine deaths, 4,282 of which were found to have been caused by cancer. [109] To examine whether there was any hint that the SLC2A9 mutations in Dalmatians might be undergoing selection as a means to equilibrate the *lex naturalis* equation in this breed, I did a re-analysis of Professor Dobson's data in which I divided the frequency of death due to cancer by the age at death, for each breed. This provided a crude measure of any equilibration going on between R and Li— the lower the resulting quotient, the stronger the equilibration effect. The Dalmatian is a relatively large breed, with an average body mass of 55 pounds— certainly not as big as a massive Leonberger or Bullmastiff, but far outside the range of small dogs such as a Cairn terrier. So I wasn't very hopeful that I would see anything interesting. You can imagine my surprise when I found that the Dalmatian scored in the 92nd percentile, with the only dogs scoring better (lower) being the miniature and small breeds, such as the Cairn terrier (9 pounds), the Shi Tzu (12 pounds), the Lhassa Apso (16 pounds), the Border terrier (14 pounds), and the Dachshund (about 20 pounds). Of dogs near its own weight class, only the German Spitz (averaging about 38 pounds), and the Bearded collie (averaging

about 50 pounds) scored in a similar range. As we would expect, the Leonberger, at 150 pounds, scored at the upper end of the range, showing no equilibration, as did the Bullmastiff, at up to 200 pounds in body mass. If we thus take body size, S, into consideration, there may actually be equilibration going on in which SLC2A9 mutations producing elevated circulating (but reduced intracellular) uric acid levels in Dalmatians enable increased body size while maintaining a lower R than might otherwise be the case. While this quick calculation does not prove that equilibration of the *lex naturalis* is going on in Dalmatians, it does indicate that a deeper look is warranted.

Should we take over correction of the lex naturalis in Dalmatians?

The SCL2A9 mutations that have occurred in Dalmatians have put them at risk for kidney stones that are generally quite painful, even debilitating, and which frequently require surgical intervention. Perhaps this makes Dalmatians the perfect breed in which to initiate genetic engineering studies— trying to intervene and relieve their propensity to develop kidney stones, while at the same time improving T to reduce R and increase Li. This might be accomplished by adopting the hominoid

modifications to SLC2A9 that put this gene under p53 control. In other words, we might work backwards (from the primate perspective), modifying SLC2A9 to make it function like the human gene. We might then inactivate UOX, perhaps employing the same mutations as observed in the human gene. Of course, once the Dalmatian SLC2A9 gene has been repaired or replaced, a more straightforward approach would be to administer uric acid pharmacologically. Genetic modifications to canine URAT1— or its replacement with the human homologue— would then be next on our list, transforming canine uric acid re-absorption kinetics toward those that make the human kill switch function. We could then explore what effect inactivation of GLO (making dogs auxotrophic for vitamin C, which could easily be added back in their diet), and replacement of the GCAG sequence in the canine Glucose-6-phosphatase (G6PC) gene with the anthropoid primate GAAT. And, of course, all the while providing primate-levels of DHEAS to them.

By choosing the Dalmatian as our initial focus, we would be selecting a breed that has been doubly victimized by human selection— first by the SLC2A9 mutation that may confer an "improvement" in tumor suppression by increasing circulating uric acid (but also creating painful renal calculi in the process);

and then by selecting Dalmatians as the best dogs for fire brigade duty.

Whether we begin our genetic engineering program to correct human violations of the *lex naturalis* in the Dalmatian, or begin generally across a variety of different breeds, remains to be seen. But we intend to correct the mistakes that we humans made in the breeding of our dogs, and hope that you will support our efforts in this.

What should be the role of kennel clubs in correcting human violations of the lex naturalis in our dogs?

There are a large number of kennel clubs around the world, the largest being the *Fédération Cynologique Internationale* (FCI), based in Thuin, Belgium, and the American Kennel Club (AKC) headquartered on Madison Avenue in New York City. Both clubs maintain a registry of "pure bred" dogs based upon their immediate ancestry. The information page for the AKC states that it "is the recognized and trusted expert in breed, health and training information for dogs," and the foreword from the AKC "Rulebook" published in September, 2019, states that the purpose of the organization is "to do everything

to advance the study, breeding, exhibiting, running and maintenance of the purity of thoroughbred dogs." As with thoroughbred horses, the word "thoroughbred" comes from the phrase, "thoroughly bred," meaning that the decision was made that no further selection could improve the breed. (This decision, of course, was made before the uncovering of the *lex naturalis*). Those dogs selected for breeding purposes are thus those that are closest to the absolute center of the bell-shaped curve for every visible trait of their breed. These same dogs—the ones closest to the absolute center of the bell-shaped curve for their breed—are the ones that win AKC and FCI shows.

At least for our large breeds of dogs, such selection from the very center of the bell-shaped curve will not get us where we need to go if we intend to remove the "Sword of Damocles" that we suspended over their heads during the establishing of their breeds. I respectfully request that FCI and AKC consider what changes need to be made to take the discoveries described in this book into consideration. If they do not, alternative kennel clubs that are based on improving breeds by correcting human violations of the *lex naturalis* are sure to spring up.

Summary of Chapter 5

The plasticity of DNA to expand and fill every available niche is exemplified in the dog. In just a few hundred years, the dog diversified from one to more than 300 different breeds, and such diversification could keep going, making 300, 600, 1200, almost an infinity of *new* breeds in another 500 years, if that's the way we humans, acting in the place of natural selection, took it. We would be creating new niches for canine DNA that relate to the desires of man, not the strictures of natural selection. Such new niches would have nothing to do with survival of the fittest— we would make sure that even the least fit dog survives— and everything to do with the whimsy of our own species. It is my considered opinion that, as nothing less than a moral obligation to our "First Friend," we should correct the violations of the *lex naturalis* that we unwittingly made in the creation of the modern dog breeds. This can be started by breeders of large dog breeds beginning to select from the small end of the bell-shaped curve. And ACGT will do its part, with its collaborators, to make the DHEA and DHEAS formulations available as quickly as possible. It is our belief that, using a combination of the strategies discussed in this book, we can normalize lifetime cancer risk in dogs from its current epidemic levels, to the 4-5% that are the natural boundaries of the *lex naturalis* equations for vertebrate species. We also believe that

we can double, perhaps triple the lifespan of our large breeds of dogs, and make our small breeds virtually lifelong companions using the same strategies. If we do this, then humans will have earned the loyalty given to us by our dogs.

It is a basic premise of this book that canines became the First Friend because they had certain "human-resembling" attributes that enabled the epi wolf to initiate first contact, and then maintain a relationship with humans thereafter. These attributes, the first of which was circulating DHEAS, enabled first contact by suppressing the fight-or-flight response. But circulating DHEAS was also part of a kill switch tumor suppression mechanism that enabled the epi wolf, and dogs thereafter, to tolerate exposure to PAH. Additionally, dogs, just like primates during the evolution of their kill switch tumor suppression mechanism, also evolved a rudimentary form of adrenarche, and bi-directional transport of uric acid in the kidney (even if they have not yet put the import of uric acid into cells under the control of p53). One breed of dog— the breed that we recruited for fire brigade duty, and hence a substantially increased exposure to PAH— even appears to have evolved a rudimentary, and flawed, mimic of the human kill switch mechanism of withdrawing uric acid from p53-

affected cells; and has done so *via* the G to T signature mutation of PAH.

Dogs have been evolving toward us for ages. When humans took up farming and switched from a primarily meat-based diet to one with a high starch content, dogs even evolved changes in starch-metabolizing enzymes so that they could continue the collaboration. [110] It seems to me that our dogs have done just about everything they could possibly do to earn their place as First Friend. I think it is time for us to earn ours.

6

Chemo for your dog?

“ The time has come in America when the same kind of concentrated effort that split the atom and took man to the moon should be turned toward conquering this dread disease. Let us make a total national commitment to achieve this goal.”

President Richard Nixon, 1971

For us cancer research scientists, our unforgivable mistake in the five decades since President Nixon declared war on cancer is that we have attempted to mentally levitate our prosecution of that war over a vast chasm in our basic knowledge, as if that chasm didn’t exist, or didn’t matter. That chasm was the

complete absence of an understanding of what a species was— what the *process* of speciation was— and therefore complete ignorance of the fundamental role that species play in cancer. For 50 years and more, the whole cancer war machine has been levitated over the false belief that one vertebrate species, such as a mouse, can be used to construct a valid model system with which to study cancer in another vertebrate species, such as a human. Piling error on top of error, severely testing our powers of levitation, we next declared that the p53 tumor suppressor functions in a virtually identical manner from one species to another— when we didn't even know what a species was! When we had no agreement whatsoever on what constituted a species! And how could we? Until the uncovering of the *lex naturalis* , we did not know that cancer was a *fundamental force opposing vertebrate speciation* , and that species-specific mechanisms of tumor suppression evolved as a *counterforce to that opposition,* particularly when increases in body size were a component of speciation. Cancer is thus a fundamental aspect of speciation, and species are a fundamental aspect of cancer. No accurate definition of species— and no successful prosecution of the war on cancer— was even possible until we understood this. And such understanding happened only recently. [111]

The discovery of the *lex naturalis* revealed that p53 *is indeed* fundamental to cancer, but as the *chief architect of species-specific mechanisms of tumor suppression* ; not for its canonical repertoire of anti-cancer effects so well studied in the p53 knockout mouse. Small animals such as mice use small body size and short lifespan as their species-specific mechanism of tumor suppression. Small body size minimizes the number of cells at risk of becoming cancerous, and short lifespan resets accrued mutations to near zero at very short intervals in successive generations. (*Remember to think of a species as an agglomeration of similar genomes, moving together through spacetime en masse, without a "precise now." See the online supplements attached to the reference just above for deeper discussion.*) But this "small animal strategy" is a completely different tumor suppression strategy than those that evolved in larger, longer-lived species such as humans, elephants and whales— the strategies of which will clearly be as different from each other as they are from mice because of the different environments these species exploit, and the species-specific mechanisms that have evolved to enable exploitation of those environments. Such species could only evolve large bodies and long lifespans by augmenting the canonical p53 tumor suppression repertoire in ways that,

because they are linked to methods of habitat exploitation, are necessarily species-specific. By this revelation, the past five decades of cancer research using animal models has been rendered irrelevant to human cancer. And to canine cancer. Because of the existence of species-specific mechanisms of tumor suppression, only humans can be used to conduct valid research on human cancer. Similarly, only dogs can be used to conduct valid research on canine cancer. This is critical for our discussion because virtually all drugs used to treat canine cancer are *human* cancer drugs that were discovered in *mice* , making them doubly irrelevant for our dogs with cancer.

As we have already noted, there were suggestions along the way that experiments in animal models were not giving us reliable information regarding human cancer.

> *"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."*
>
> *Dr. Richard Klausner*
>
> *Former Director of the National Cancer Institute*

We have discussed how it is the mutability of the collective mass of DNA on this planet that enables it to expand,

amoeboid-like, into the environment, and to assimilate the environment into more of itself. We have also noted how species act like the pseudopodia of this amoeboid-like DNA super-organism, and have evolved as the basic *machines* of evolution because they are efficient mechanisms with which to assimilate environmental resources into DNA. Because the mutability of DNA is the basic element of both speciation and neoplastic transformation/cancer, in retrospect it should have been obvious early on that there was a fundamental connection between species and cancer.

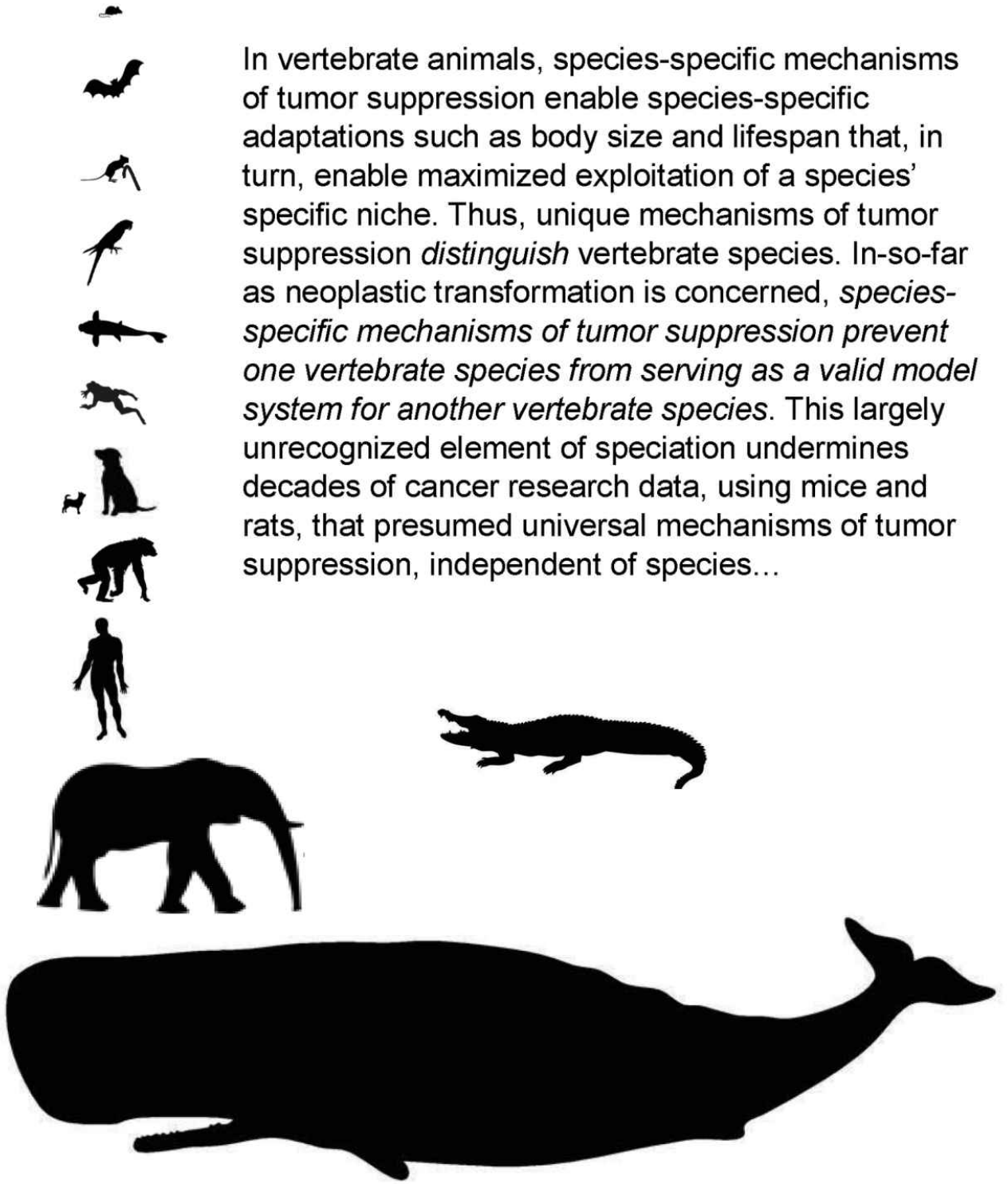

Figure 6.1 First published in Nyce, 2019 [112]

The war machine of cancer research in this country, in the form of the National Cancer Institute (NCI), has now reached an

annual budget of $6.7 billion per year. And this doesn't include the $174 billion that goes directly to the treatment of cancer each year— $895 billion worldwide . All of this money is being spent continuing to levitate the cancer war machine over the false premises that the concept of species is irrelevant to human cancer, and therefore mouse models of cancer produce data relevant to human cancer. [113]

Want final proof that we have so far failed using animal models in our war on cancer? A recent study has reported that only 8% of cancer drugs that work *fantastically* in mice show results in humans that reach statistical significance. [114] Clearly, inventing drugs to treat mouse cancer is a very ineffective strategy for creating effective drugs for human cancer— let alone for canine cancer. This is borne out in the most elemental indicator of success— cancer patient survival. The figure below is a simple graphical representation of data published by the NCI, showing that over the past 27 years, *two-year survival among patients with invasive cancer has improved less than 7%* . Clearly, levitating the war machine of cancer research over the bottomless chasm of reliance on animal models, and by our failure to consider, deeply, the concept of species, has brought us to this stalemate. Treatment outcome in invasive human cancer is at an asymptotic limit beyond which further progress

may not even be theoretically possible. Cancer research results have "flat lined."

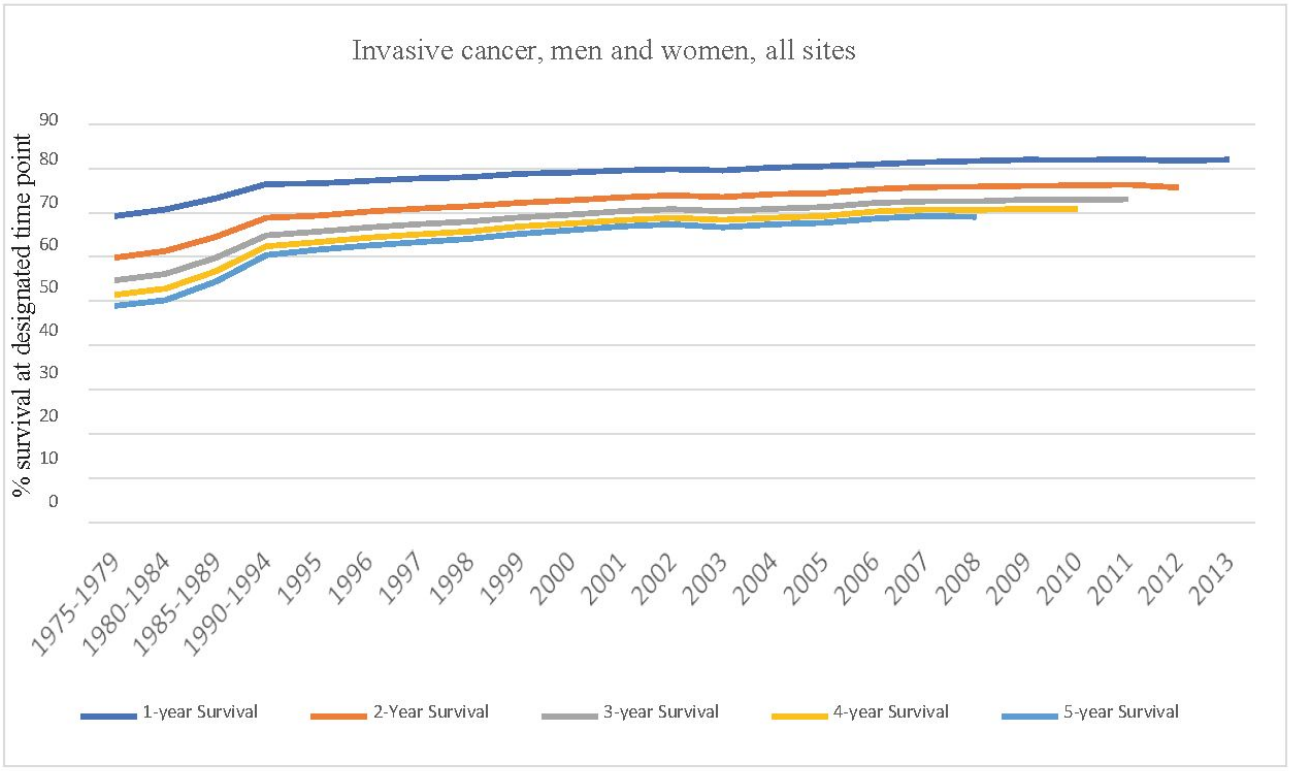

Figure 6.2 Treatment outcome in invasive human cancer. Data from NCI SEER Cancer Statistics Review (CSR) 1975-2014. First published in Nyce, 2018 . [115]

But the story behind the failure depicted in the two-year survival numbers in the figure above goes back much further than Nixon's declaration of war on cancer, and the efforts to levitate the ensuing cancer war machine above huge gaps in our basic knowledge. The roots of our failure to win the war on cancer originated at an unlikely time and in an unlikely place—all the way back to the days of Sopwith Camels and Fokker tri-

planes tracing graceful, deadly arcs in the sky; a time when flying aces like the Red Baron and Eddie Rickenbacker dueled with each other in and out of the clouds, while far below, looking like ants from such elevated, moving perches, other men, made far less gentlemanly by their circumstance, clawed at each other in the mud of the Western Front.

A brief history of chemo

As surprising as it might seem, cancer chemotherapy— chemo for short— had its origins on the battlefields of World War I. More than 2,500 kilometers (1,500 miles) of trenches were dug by opposing forces facing each other across a series of killing fields between France and Belgium. If put end to end these trenches would have stretched from Boston to Miami, a testament to the industry of men when they are determined to kill each other. The trenches were built as protection from the new inventions of war, including the rapid fire machine gun, and extremely accurate long rifles. In order to enable the effective use of such new weapons, another was invented, a volatile chlorine mustard gas that could be hurled into opposing trenches, shot from cannons, or dropped from airplanes, for the purpose of forcing opposing soldiers out of their trenches and

into the line of fire. As a weapon of war, these mustard gasses were horrific, destroying every tissue with which they came into contact by alkylating the DNA of those tissues (adding a methyl or ethyl group to oxygens and nitrogen's in DNA bases). Given the choice of choking to death in a mustard gas-filled trench or exposing themselves to enemy machine gun fire, most soldiers chose the latter fate.

Battlefield surgeons treating soldiers exposed to mustard gas noticed that, in addition to terrible burns on their skin and in their lungs, such victims were also devoid of lymphocytes, colloquially known as white blood cells, or WBCs. Since WBCs are the cells that mediate the immune system in the body, the effect of destroying them is called immunosuppression. After the war was over, medical scientists took this discovery into the laboratory, repeating the finding of chlorine mustard-induced immunosuppression in experimental animals such as rabbits. [116]

Figure 6.3 The chlorine mustard gas used on the battlefields of WWI became the basis for cancer chemo. Picture from the archive department, U.S. Department of War

At the advent of the Second World War, the U.S. War Department, fearing that mustard gases might be deployed against American troops overseas, made money available for research into their effects. Two medical scientists at the Yale University School of Medicine, Drs. Alfred Gilman and Louis Goodman, received some of those research funds, and after converting the volatile chlorine mustard into a much more stable nitrogen mustard that could be easily handled in the laboratory, they collaborated with their colleague Dr. Thomas

Dougherty, to study nitrogen mustard using Dougherty's model of transplanted lymphoma in mice. These experiments bore immediate fruit. Together, they proceeded to use nitrogen mustard to effect what appeared to be outright cures in these mice with lymphoma.

At about this same time, a 46-year-old Polish immigrant (JD) had presented himself to the Yale infirmary, where he had been diagnosed with lymphoma. JD was in agony. Most of his lymph glands had swollen out of all proportion, the right submandibular so much so that he could barely open his mouth. Radiation and surgery had failed to sustain remission of JD's lymphoma, and his case seemed hopeless. It was at this time that his physician, Dr. Gustaf Lindskog, was presented with Goodman, Gilman, and Dougherty's mouse data. This was before the age of Institutional Review Boards, FDA, and all of the other safety mechanisms of 21st century medicine, and so JD became the first patient to be exposed to nitrogen mustard—the first chemotherapeutic drug— in an attempt to kill the lymphocytes that were killing him.

JD's medical charts have recently been located— eight decades after their recording— at an off-campus storage facility in New Haven. [117] Although JD's treatment had been reported over the years in various accounts, the finding of his actual

medical charts provided a much more accurate representation than had previously been available. Here is the gist of what those records revealed.

Based on dosing estimated from a quick toxicity study performed on rabbits to guess at a maximum tolerated dose, JD was administered intravenous nitrogen mustard for the first time at 10:00 a.m. on August 27, 1942. With that initial dosing, the age of cytotoxic chemotherapy had been born. After the fifth day of treatment, Dr. Landskog noted symptomatic improvement, and so informed Drs. Goodman and Gilman. After the tenth day of treatment, biopsy of the affected lymph glands showed complete absence of lymphoma cells, and JD was able to sit up, eat, and move his head around again without pain. It seemed like a miracle to everyone involved, especially JD, who became the first patient ever to find relief from cancer by systemic chemotherapy.

However, one week later, side effects began. JD's white blood cell and platelet counts began to decrease, his gums began to bleed, and he had to be treated with blood transfusions. He thus also became the first person to experience the side effects of cytotoxic chemotherapy. By day 49, JD's lymphoma had returned, full blown, and nitrogen mustard this time produced only a short-lived, partial response. JD died of

his lymphoma on December 1st, 1942, ninety-six days after the initiation of his cytotoxic chemotherapy. At autopsy, it was observed that he had “erosion and hemorrhage of the buccal mucosa, emaciation, and extreme aplasia [incomplete development] of the bone marrow.”

Despite what we would consider a bad outcome, JD’s treatment with nitrogen mustard was considered miraculous by both the scientific community and the general public because up to that point, nothing, outside of the short-lived effects of a beam of radiation, had been found that could actually kill tumor cells. Excitement was palpable in the halls of academic medicine. If this result could be achieved in the *very first patient* , imagine what might be done if treatment schedules and doses were optimized, or if new cytotoxic drugs were created with better killing potential. It was widely felt that JD’s treatment was just a harbinger of much greater success to come. Surely JD’s results could be improved upon. Perhaps this dread disease, cancer, was curable after all.

For reasons that are hard to understand, this excitement, or at least this strategy of tinkering to make cytotoxic drugs work better, has continued unabated in the face of decades of contrary evidence; with very little in the way of improvement in cancer patient survival. Far from being a first case that might

be drastically improved upon, JD's case had demonstrated the essential limits of cytotoxic cancer chemotherapy: First, that its toxicity makes it a balancing act to kill the tumor before killing the patient; and second, that it is easy to kill some portion of a tumor cell population using cytotoxic drugs, but there are always some cells in the population that are impervious to a particular treatment and which will grow out once their unique characteristics have been selected for by the drug. Resistance to chemotherapy thus occurs in virtually all cancers in human patients treated with chemo.

Cytotoxic chemotherapy thus revealed early on that a tumor is an evolving ecosystem, and that exposure of a tumor cell population to a cytotoxic drug is in essence an environmental pressure that shapes the evolution of that ecosystem. The initiation of cytotoxic chemotherapy is thus an environmental cataclysm for a tumor cell population; many of its members will die, just as an asteroid impact may wipe out most forms of life on a planet — but not all. The aftermath of a chemotherapy protocol, just like the aftermath of an asteroid strike, creates conditions that allow some tumor cells not only to survive, but to thrive. Without the competition of the bulk of tumor cells that died in the chemotherapy cataclysm, the survivors can exploit this new environment in which some random change in

their constitution gave them safe passage. And the withered bodies of fallen cancer cells provide an additional treasure trove of additional resources for these surviving tumor cells to utilize. In pediatric acute lymphocytic leukemia, for example, the disease that most of the children that I worked with at the Children's Hospital of Los Angeles had, there are typically about 100 billion cancer cells present in the child's body when he or she is first brought to the clinic. After chemotherapy that destroys 99.9% of those cancer cells, *there still remain 100 million resistant survivors* . The most troubling aspect of these survivor cells is that they have proven themselves to be extremely fit to survive cytotoxic chemotherapy. And they are not finished evolving. The same genetic and epigenetic diversification mechanisms that enabled them to survive initial chemotherapy re-creates a population of cells with even more vast potential to respond to future chemotherapy. This is the problem that classical chemotherapy faces, whether it is used to treat people with cancer, or dogs.

How far have we gotten since the first use of chemo in JD? Even today, progression-free survival in non-Hodgkins lymphoma, the kind of lymphoma that JD presumably had and one of the most treatable cancers there is, remains a disappointing 11 months, even with heavy chemotherapy

followed by bone marrow transplantation. And nitrogen mustard-based drugs remain a mainstay of cancer treatment, with tinkering to improve them, however slightly, still going on. [118]

The chaos of chemo: If only the initial conditions had been different

Most of you will have heard of the mathematical theory known as chaos— if nowhere else, then at least from Dr. Ian Malcolm's flirtatious description of it in *Jurassic Park* . Just before the giant, plant-eating dinosaurs appeared, Dr. Malcolm described chaos theory as "sensitive dependence upon initial conditions," and demonstrated the phenomenon to Dr. Ellie Sattler by showing her how successive beads of moisture that he deposited on the back of her hand would traverse different descending paths depending upon the tiny, random "imperfections" in her skin at the site where he dropped it. To translate this idea of the sensitivity of outcomes to initial conditions to our discussion, if mustard gas had not been used in the trench warfare on the Western front in WWI, Goodman and Gilman would never have gotten their grant to study its effects in their laboratory, and would never have been able to

induce the transient remission in JD that would lead, decades later, to the dismal outcome depicted in the figure above showing cancer patient survival. The use of mustard gas on the battlefield was the "initial condition" to which cancer research responded in such a sensitive a manner, causing it to proceed in an entirely different direction than might otherwise have naturally evolved.

What alternative direction might have evolved if vesicant war gas had never been deployed on the battlefields of the Western Front?

At this same time, evolutionary theory was undergoing a complete renaissance, attempting to uncover the physiological underpinnings of speciation. [119] Also at this same time, a study was published in *Cancer Research* demonstrating that in 5,365 necropsies of mammals and birds at the Philadelphia Zoo, the overall lifetime risk of cancer was 2%, [120] more than an order of magnitude lower than that which was being observed in humans. Given that the scientific literature was a tiny fraction of the size it is today, it is extremely likely that scientists funded in evolutionary theory would have seen this work, and

vice versa , and begun to fit the pieces together, as I did an untimely eight decades later. But research thrives where the funding is directed, and after Goodman and Gilman reported their work, virtually all of the funding in medial biology was directed toward cytotoxic chemotherapy. Virtually no funding went toward investigating the connection between evolutionary biology and cancer suggested by the work of the scientists at the Philadelphia Zoo. This was a tragedy of immense proportions.

Canine oncology is the use of cancer drugs discovered in mice, and developed in humans, for use in dogs. After what you have read in this book, does this still make sense to you?

In the very first chapter, I noted that there are 3 1/2 times more dogs with cancer than there are people with cancer in the United States. The business opportunity that this represents to veterinary oncologists should be obvious. We love our dogs, and will do just about anything that we can for them. A recent study discovered that dog owners experiencing hard times will often go without medical treatment for themselves, so that they

can afford medical treatment for their dogs. While that may be going overboard (even in passenger jets the stewardess reminds you to put your own oxygen mask on first, so that you will be conscious to help your child with theirs), it demonstrates that veterinary oncology is just about the most-recession-proof business that there is. In saying this, I am not saying anything whatsoever negative about veterinary oncologists. I know a lot of them as friends, and I count them as some of the finest human beings on the planet. They truly do want to help their patients. Their hearts are clearly in the right place, and they can't be faulted for wanting to make a good living doing what they were trained to do—using the knowledge imparted to them during that training. The problem is… well, after reading this book, you know what the problem is. Almost all of the drugs used to treat canine cancer were discovered in mice, and developed in humans— making them doubly removed from relevancy to canine cancer. And some of the most commonly employed human cancer drugs used to treat canine cancer are the very same class of nitrogen mustard drugs that JD was treated with eight decades ago! An example is cyclophosphamide, the C in the famous CHOP protocol used to treat human and canine lymphoma. Almost every dog with lymphoma is treated with the CHOP protocol, with results that

more or less mimic those that JD got. In another common protocol used in dogs with lymphoma that has relapsed during CHOP treatment, a different regimen is turned to that is distinguished primarily by the use of a different nitrogen mustard, aptly named *Mustargen* .[121] Knowing the history of these drugs, I almost expect to see on their labels directions for us to fix bayonets and put our gas masks on. This is not to say that you will not see an effect in your dog with lymphoma treated with the CHOP protocol. You probably will. Two out of three dogs with lymphoma will undergo remission with CHOP-based protocols.[122] But like JD, most will die within a few months, and very few will live another year. It seems a reasonable question to ask, is this a reasonable course of action?

So although the hearts of veterinary oncologists are in the right place, I disagree with their protocols. The rationale for using drugs discovered to treat cancer in *mice* , and developed as drugs to treat cancer in *humans* , in order to treat cancer in *dogs* just doesn't make any sense to me in light of the *lex naturalis* . The problem is, I have only just published the major work on the *lex naturalis* ,[123] so most veterinary oncologists remain unaware of it. And even in those publications, the focus is on human cancer. The best place for them to learn about the

application of the *lex naturalis* to canine cancer is this book; and even fewer will have read it. (Hint: You could buy your veterinarian a copy.)

There are a few drugs that have been designed specifically for dogs, such as Palladia, and two monoclonal antibodies for leukemia, and a few others which I will briefly note, but because big pharma companies can charge $1,000 per day for cancer drugs used in the treatment of humans, and only a tiny fraction of that for canine cancer drugs, big pharma R&D projects to develop new cancer drugs in and for dogs are virtually non-existent.

I noted that Palladia (a Zoetis\Pfizer drug) was developed specifically for dogs. It is a small molecule inhibitor of a type of enzyme called a tyrosine kinase, and it is approved by the FDA for the treatment of canine mast cell tumors (MCT). The tyrosine kinase that Palladia inhibits is known as KIT, which is over-expressed or mutated to a hyper-active form in canine mast cell tumors, as well as some other tumors in both dogs and humans. In a multi-center study of Palladia for recurrent or inoperable mast cell tumors in dogs, the percentage of complete responses (tumors undergoing complete regression) during the double blind portion of the trial was 8.1% (n = 86, with 7 complete responders). [124] And as in JD's lymphoma, such

remissions were short-lived. Among all responders in the study, the median duration of objective response was just 12 weeks, and the median time to tumor progression was just 18 weeks. In an attempt to increase the response rate, a recent study combined Palladia with an alkylating agent, lomustine, that has also shown some limited activity in canine MCT. Although this combination therapy did increase the objective response rate, severe toxicity occurred in all treated dogs, killing several, and the study had to be discontinued. [125] Zoetis is attempting to extend the use of Palladia to a variety of solid tumors in dogs, but the results have so far been disappointing. [126]

Another drug that was developed for dogs with lymphoma is Tanovea. It has been conditionally approved by the FDA for the treatment of canine lymphoma that has relapsed after CHOP treatment, and has been shown to add a few additional months to the affected dog's life. [127] In a study of relapsing B cell lymphoma, Tanovea produced CRs in 45% of treated dogs, and median progression-free intervals of 108, 172, and 203 days for all dogs, all responders, and all complete responders, respectively. [128]

In an example of "what goes around, comes around," microbial therapy of cancer is also attempting a comeback, in both humans and dogs. Thus, when I was just starting out in

cancer research four and half decades ago at the Fels Cancer Research Institute in Philadelphia, every young student of cancer was taught about the famous case of *Bacillus Calmette Guerin* , BCG, named after its inventors, two French bacteriologists. Drs. Calmette and Guerin wanted to make a vaccine to fight tuberculosis, which remains to this day the microbial-mediated disease responsible for more deaths than any other. Prior to World War I they were able to attenuate the virulence of a strain of the tuberculosis virus that had been isolated from a cow with tuberculosis. Even when WWI broke out, the potential importance of their work was recognized by German military surgeons during the occupation of France, and they were given the necessary assistance to carry on their research. However, BCG was never proven effective against tuberculosis. Even though that fact continues to be true to this day, because of the lack of any other vaccine that can prevent tuberculosis, it is still recommended to be administered by the health authorities in many countries where TB is endemic. [129]

But this book is not about TB, but rather cancer. So here is how BCG came to be related to cancer. Two papers have been published in which the incidence of “spontaneous cures” in human cancer were documented. One paper placed the incidence at 1 spontaneous cure per 100,000 cancer cases, and

the other placed it at 1 per 80,000 cases— pretty close numbers. It was subsequently hypothesized that spontaneous cures *never* occurred in cancer, but rather, what appeared to be spontaneous cures had really been induced by infections that had stimulated the immune system. This led to the testing of BCG inoculation in an attempt to stimulate the patient's immune system against the tumor. Amazingly, such BCG treatment produced some therapeutic effect, and it is now the mainstay of treatment of human bladder tumors that have not invaded the muscle wall. [130] A recent study demonstrated that canine distemper virus has oncolytic activity against canine mammary tumor cells *in vitro* , but tissue culture studies are a long way away from demonstrating activity in an actual dog with mammary cancer. [131] Studies testing the oncolytic potential in canine tumors of a number of other viruses has recently been reviewed. [132]

Verdinexor is an oral drug that was tested in a phase II study in dogs with various types of lymphoma. In this study, the overall response rate was just 37% (20/54 dogs), the average duration of response was just 18 days, and the average time to progression of disease was just 29 days. [133]

Finally, two vaccines were recently conditionally approved with great fanfare by the U.S Department of Agriculture for the

treatment of canine lymphoma, but did not reach their clinical expectations, and have been discontinued. [134]

Summary of Chapter 6

Chemo drugs had their origin in the mustard war gasses used on the battlefields of WWI. The use of such toxins to kill tumors has continued to this day, such that the armamentarium of the oncologist, whether treating humans or dogs, consists of a pharmacopeia of poisons. In my opinion, this fact will not go down well with future medical historians, who a half-millenia from now will view current practitioners of this pharmacopeia as little better than witch doctors. But what does that make us cancer research scientists? We were the ones who handed that pharmacopeia to them. No, future medical historians will not treat we cancer research scientists with much respect, and will write endlessly about how we constructed our entire research paradigm— the compass and sextant that we used to direct our cancer research— over a deep chasm of ignorance. Knowing full well that we did not even know what a species was, we plowed forward for the better part of a century— and right up to the present day— believing that mice with cancer provided a valid model of humans with cancer; that cancer was the same

disease in mice that it was in humans. And neither did we stop there. Once we learned of the fundamental role that the p53 tumor suppressor played in mouse cancer, we proceeded to make the completely unjustified leap in logic that it played exactly the same role in human cancer! [135] How could we make so many bad decisions that took us so far in the wrong direction? So far away from the truth of what cancer is? It is almost as if we were being punished by a higher power for the sacrilege of using such horrific weapons as poison gas to kill each other on the battlefield; or that we were being punished by the power of chaos, with its sensitive dependence upon initial conditions.

But there is absolution for our sins on the horizon. The *lex naturals* teaches a new and completely different way forward for both canine and human cancer. It demands that we finally come to understand that *only humans* can provide valid models for human cancer; that *only dogs* can provide valid models for canine cancer; and that we don't have to subject a hundred million mice each year to the rigors of testing drugs meant to treat humans with cancer— its hard on the mice , and it clearly does not provide information that translates to humans, as the paltry 7% increase in two year survival of cancer patients obtained over the past 27 years of research shows full well.

Above we noted what the *lex naturalis* demands: that the only species in which safe and effective drugs for human cancer can be discovered is *Homo sapiens* ; and that the only species in which safe and effective drugs for canine cancer can be discovered is *Canis lupus familiaris* . Before we end this chapter it must be pointed out that the FDA demands *exactly the opposite* . The FDA demands that every cancer drug *must* be proven safe and effective in animals before it can proceed to human testing— essentially securing defeat of the drug discovery process. To me, this is very 15th century, like the Pope of that age demanding that every ship set off to explore poorly-charted seas with a fully paid-up Papal *Imprimatur* , but without a compass, and without a sextant— compelled to trust in the infallibility of the Papacy. We will continue to be lost at sea if the FDA does not radically change its position. If the FDA refuses to permit us the assistance of the compass and sextant provided by the *lex naturalis* , at some point it will become *responsible* for the cancer holocaust that is already upon us— a cancer holocaust that is clearly going to intensify .

(*In the actual written regulations of FDA, only safety studies are required prior to human clinical trials. But there remains an unwritten requirement for efficacy studies in animals, and I*

am unaware of any drug having been approved without prior demonstration of efficacy in an animal model. I myself have taken two drugs into FDA human clinical trials, and in each case it was made very clear in pre-clinical discussions with FDA that such demonstration of efficacy was required. With one of these drugs, a RASON for human asthma, the agency "suggested" that pre-clinical efficacy be demonstrated in primates. This "suggestion" was complied with at great expense. It may be an unwritten rule that efficacy, too, be demonstrated in an animal model before human clinical trials can begin, but it is a rather stringent unwritten rule.)

7

The Kill Switch is a Rational Alternative to Chemo

"Our species needs, and deserves, a citizenry with minds wide awake and a basic understanding of how the world works"

Carl Sagan

The kill switch alternative was discovered in dogs, not mice

Let me start by saying that we discovered the kill switch alternative *in* dogs, and that it does not even work in mice, because mice do not have any semblance of the kill switch mechanism— they have vanishingly low levels of circulating DHEAS. This, in itself, is a powerful demonstration of the *lex naturalis* lesson that, because of species-specific mechanisms of tumor suppression, one vertebrate species cannot be used to create a model system with which to study cancer in another vertebrate species. If the world was upside down, and we had been using dogs to discover new drugs to treat cancer in mice, we would have thought we had discovered a great drug in our kill switch activation studies— a drug that produces apparent *cures* in some dogs with very lethal cancers— only to have clinical trials in mice fail. There appear to be only two lineages that possess kill switch tumor suppression mechanisms based on circulating DHEAS— canines and primates. So, just as mice cannot be used to discover new cancer drugs for humans (or dogs), dogs (and humans) cannot be used to discover new cancer drugs for mice.

The kill switch alternative is natural

Whereas cytotoxic chemo is a pharmacopeia of poisons, kill switch activation represents the triggering of the natural system that evolved to suppress tumors— the same system that enabled canines to strike up their relationship with humans. Whereas the intent of chemo is to use a manufactured poison to block some aspect of tumor cell biochemistry, the intent of the kill switch approach is to trigger an integrated, highly evolved system. Chemo is also universally thwarted by the development of drug resistance by the tumor being treated. It would take an entire book longer than this one to tell you about all the different ways that have been found by which tumor cells become resistant to virtually any drug thrown at them. In one of my earliest projects as a young professor, I discovered that *cancer drugs themselves epigenetically modify the tumor cells in such a way that resistance to the treatment drug occurs* .[136] And there are literally thousands of additional mechanisms by which tumor cells can escape toxicity created by any drug, or combination of drugs. It is of course theoretically possible to sterilize any tumor cell population using an onslaught of many different drugs at once— but not without simultaneously killing the patient. Above I noted how the killing of 99.9% of the cells in a tumor cell population of 100 billion cells leaves 100 million surviving cells. Survivors occur because every tumor

cell population has a bell-shaped curve for every aspect of cell survival, and this creates alternative circuits by which these survivor cells are able to bypass the circuit interrupted by the cancer drug. And these survivors develop further clones that have their own bell-shaped curves, and so on, creating the heterogeneous tumor cell populations that have made both human and canine cancer impossible to cure up to now. [137]

Because the kill switch evolved naturally, and is not a poison coming into the cell from the outside, the concept of acquired drug resistance does not even apply to it. And as we shall discuss, tumor cell populations frequently usurp elements of the kill switch for their own use, making them exquisitely sensitive to kill switch triggering.

The Kill Switch and the Mevalonate Pathway

In early work I did as a young medical school professor, my lab uncovered something very important: By inhibiting G6PD and depleting cellular NADPH, DHEA blocked the mevalonate pathway. [138] The reason that this is so important is that cancer cells— including the singularity, that first cancer cell that will go on to create a tumor—are completely dependent upon a mevalonate pathway that is in overdrive. Normal cells respond

very differently to mevalonate pathway inhibition than malignant cells do. Normal cells slow down gracefully, even to a stop, according to evolved mechanisms. Malignant cells, on the other hand, cannot slow down gracefully— they trip all over themselves, unable to follow the evolved mechanisms that they have been ignoring as an element of their malignant behavior. Because cancer cells divide more rapidly than normal cells do, they create a lot more ROS that must be detoxified— and so need a lot more NADPH and selenoprotein firemen— and they need a lot more cholesterol to create the lipids that are used to construct cell membranes, as well as a hundred other functions that tumor cells need to conduct to survive. The mevalonate pathway is a treasure trove for the cancer cell, responsible for everything from the animation of selenoprotein firemen and the filling of their fire hydrants, to the synthesis of cholesterol. Capturing control of the mevalonate pathway is thus one of the most important goals of cancer cells. This is probably also why it is also the target of the kill switch tumor suppression mechanisms that evolved in both dogs and humans. To accomplish the extinguishing of the singularity while it is still in the single cell stage, evolution has set up the mevalonate pathway to be *exquisitely* sensitive to intracellular NADPH concentrations. Our discovery that even well-developed canine

tumors are still able to respond to kill switch triggering is, in my opinion, the most important finding ever made in canine cancer. Because it has direct application to human cancer, *it is the most important finding ever made in cancer. Period* . That might sound like an arrogant thing to say, but I don't mean it to be. When your entire species, and that of your best friend, are headed toward a cliff in the dark, and you discover a flashlight, that is not the time for speaking softly, or to mince your words.

Let's dissect the mevalonate pathway

HMG CoA reductase is the first, and rate-limiting enzyme of the mevalonate pathway. It is the enzyme responsible for converting HMG CoA to mevalonate, and it is a *unique* enzyme in intermediary metabolism in that it requires *two* mols of NADPH for each mol of mevalonate product produced. This two-for-one dependence makes HMG CoA reductase ultrasensitive to intracellular NADPH concentrations— a perfect dependency to incorporate into a kill switch system that targets G6PD, the primary source of intracellular NADPH. The depletion of selenoprotein firemen and the emptying of NADPH fire hydrants caused by DHEA-mediated interruption of NADPH synthesis, and the inhibition of the mevalonate

pathway that this causes, is a critical component of both the canine and the human kill switch tumor suppression mechanisms. And cancer cells of both species have additional needs that can only be satisfied by a hyper-functioning mevalonate pathway, and that can therefore also be blocked by DHEA-mediated kill switch activation.

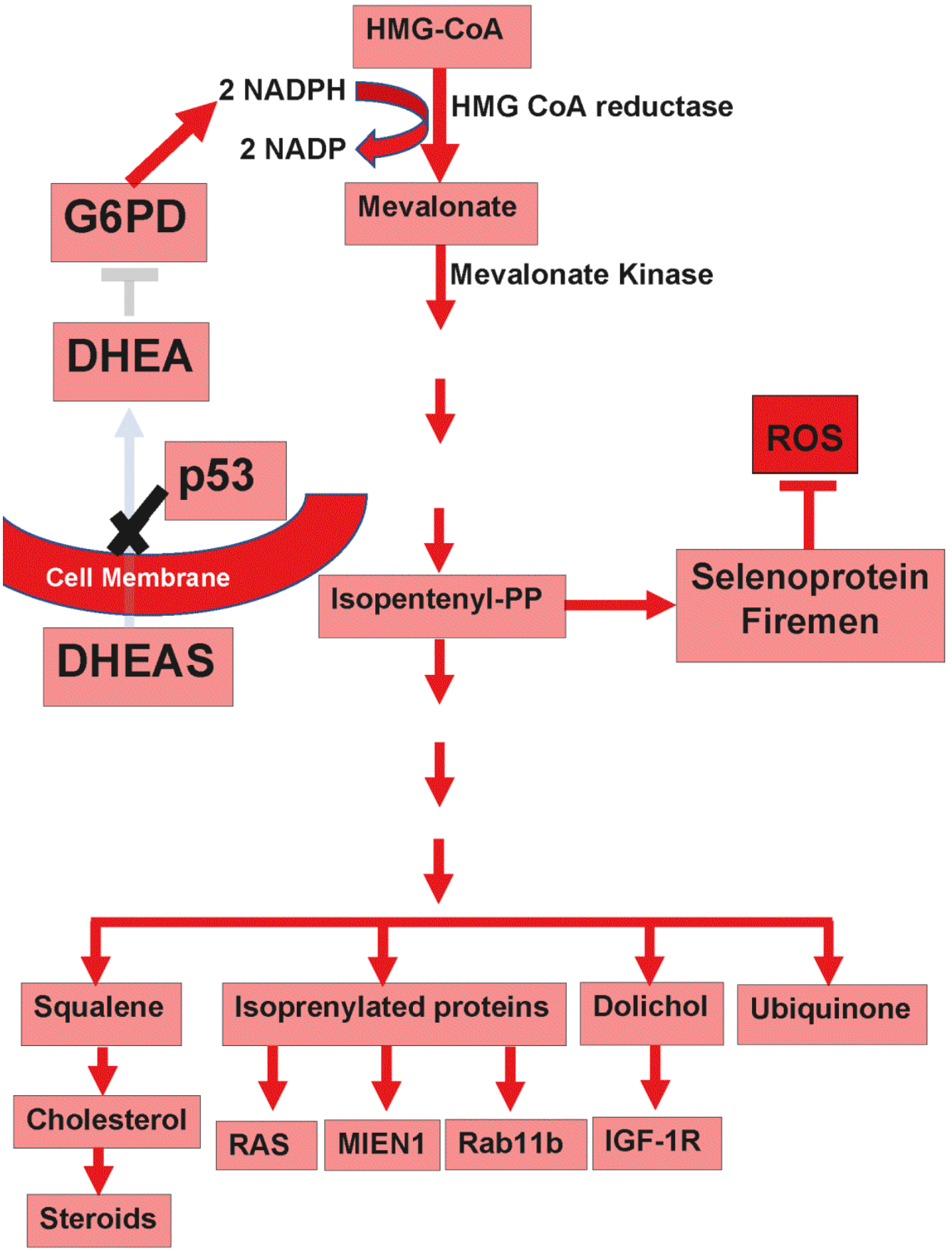
HMG-CoA
2 NADPH
HMG CoA reductase
2 NADP
G6PD
Mevalonate
Mevalonate Kinase
DHEA
p53
Cell Membrane
DHEAS
ROS
Isopentenyl-PP
Selenoprotein
Firemen
Squalene
Isoprenylated proteins
Dolichol
Ubiquinone
Cholesterol
RAS
MIEN1
Rab11b
IGF-1R
Steroids

Figure 7.1 The mevalonate pathway is a key participant in the kill switch tumor suppression mechanism because it is exquisitely sensitive to intracellular concentrations of NADPH, which are primarily regulated by G6PD. In dogs, some tumors can be successfully treated by G6PD inhibition. Dolichol is an intermediate used in the N-glycosylation of proteins.

Isoprenylation of oncoproteins

Some oncoproteins (products of oncogenes) prominent in both human and canine cancer, such as RAS, [139] Migration and Invasion Enhancer 1 (MIEN1), [140] and Rab11b [141] require a modification called isoprenylation in order for them to be targeted to the membranes where they exert their biological action. In the earlier work from my laboratory cited above, we demonstrated that depleting intracellular mevalonate by using DHEA to inhibit G6PD blocked such oncoproteins from becoming isoprenylated, thereby preventing them from reaching their membrane site of action. Unable to reach their site of action, these oncoproteins are unable to express their oncoprotein function.

RAS, an example of an isoprenylated protein with a critical role in cancer

Whereas *p53* is the most frequently mutated gene in cancer, *RAS* is the most frequently mutated *oncogene* in cancer. As I noted above, RAS must reach the inner surface of the plasma membrane of the cell in order for it to be active, and it must be isoprenylated in order for it to reach its membrane site of action. By embedding itself in the plasma membrane, RAS places itself in direct proximity to its accessory proteins—amounting to a 5,000 fold increase in its effective concentration. RAS becomes an oncoprotein by undergoing mutation at specific sites in its gene which switch its protein form to a permanently "active" state. Such *RAS* mutations are very frequent in both human and canine tumors. [142] Most research, of course, has been carried out in human cancer, where *RAS* mutations have been found in 91% of pancreatic tumors, 42% of colon tumors, 33% of lung tumors, and at rates ranging from 6% to 22% in a variety of other tumors such as those of the endometrium, cervix, bile duct, and stomach. [143] Similar numbers probably apply to canine cancer, although, again, canine cancer is less well-studied. Our research shows that activation of the kill switch will block RAS from being

isoprenylated, making it impossible for it to reach its membrane cite of action. [144]

RAS is thus a target of the kill switch that evolved in humans and dogs. It is again astonishing to me that activation of the kill switch has such wide ranging effects, naturally inhibiting many of the oncoproteins known to be involved in cancer. While chemo attempts to poison them one-by-one, using multiple drugs with multiple toxicities, kill switch activation triggers the natural tumor suppression mechanism that evolved in dogs and humans, simultaneously inhibiting an array of oncogenic targets.

Let's look at a few of the others.

N-Glycosylation

Another mevalonate-dependent pathway important to cancer cells is called N-glycosylation, which is the attachment of a Christmas tree-like chain of sugars (glucose, mannose, galactose, sialic acid, etc.) to nitrogen (N) molecules in specific asparagine amino acids in a target protein. The critical first step in this process originates with dolichol, [145] another product of the mevalonate pathway (see figure above). Dolichol organizes the sugars into a row, and then functions as a membrane anchor

in the process of transferring the sugar chain to the target protein. Dolichol-mediated N-glycosylation has a variety of functions, such as enabling proper folding of proteins into their correct 3-dimensional shape, and protein stability. Blocking the N-glycosylation of proteins involved in cancer would thus produce unstable, misfolded and therefore inactive proteins. By inhibiting the mevalonate pathway, kill switch activation depletes tumor cell dolichol, preventing the process of N-glycosylation.

Some examples of oncoproteins requiring N-glycosylation to be active

Epithelial Cell Adhesion Molecule (EpCAM)

EpCam is an N-glycosylated transmembrane protein that is overexpressed in virtually all epithelial tumors (carcinomas), such as cancers of the mammary gland , lung , colon, pancreas, bladder, skin, etc. [146] This transmembrane protein is believed to be heavily involved in invasion and metastasis, and in a process known as epithelial to mesenchymal transition (EMT). [147] Epithelial cells are normally polarized, with their “bottom” (basal) surface attached to the basement membrane, and their

"top" (apical) surface interacting with the environment— e.g., respiratory epithelial cells with the atmosphere, intestinal epithelial cells with the contents of the gut, and so on. When they undergo EMT, all of this changes. Mesenchymal cells are more primitive, and in fact play a prominent role in cell movement during embryogenesis. For example, the cells that will eventually become the cornea of the eye have to move during embryogenesis— i.e., invade and migrate— to their final position relatively far from their starting point. [148] EMT also plays a critical role in the repair of epithelial tissues such as the lung, colon, and others. [149] As one of my mentors, Sidney Weinhouse, used to say, this shows that the primary elements of invasion and metastasis are normal processes when they occur at the proper time and in the proper place. It is only when they are usurped by the cancer cell that these normal processes become primary mediators of the malignant state.

During EMT in the progression of malignancy, transformed epithelial cells adopt the migratory mesenchymal lifestyle, detach from the basement membrane, invade adjoining tissue, with some eventually reaching blood vessels, which they also invade, and then enter the bloodstream as single tumor cells or sometimes in groups. Tumor cells that have entered the bloodstream can circulate for years, but some, as a function of

the pressurized pumping in closed circulatory systems, lodge in other tissues where the diameter of the blood vessels has become too narrow for further progress. At this point, continuing to use their mesenchymal tool kit, they invade the new tissue where they have landed— the process of metastasis. In a final display of tumor cell plasticity, they then frequently revert back to the epithelial state, so-called mesenchymal to epithelial transition (MET), which will often give them a better chance to survive when they have metastasized to an epithelial tissue such as the lung.

The ability of EpCAM to drive EMT is dependent upon EpCAM being N-glycosylated. [150] Since metastases (rather than the primary tumor) are what generally kill the canine or human cancer patient, preventing EMT is a highly sought after clinical effect. [151] A recent study in human breast cancer cells has shown that inhibiting EpCAM prevents EMT, thereby inhibiting the processes of invasion and metastasis. [152] Remembering the importance of RAS oncoprotein expression in many canine and human tumors, active, N-glycosylated EpCAM has been shown to be required for RAS to exert its oncogenic function, particularly in breast cancer, linking these oncogenic pathways together. [153] Again, it is truly remarkable

how the kill switch targets the same oncoproteins that it took more than 50 years of cancer research to identify.

Insulin Like Growth Factor Receptor 1 (IGF1R)

We discussed the important role that the Insulin-like Growth Factor 1 (IGF1), and the receptor (IGF1R) by which it mediates its effects, play in body size by stimulating bone growth , essentially acting as the primary effector molecules of Growth Hormone (GH). IGF1R, which requires N-glycosylation to be active, is involved in the progression of most tumors— both human and canine. This has made it, too, an important target of anticancer synthetic chemists. [154] And there's more to this story. Although we have been primarily discussing activation of the kill switch specifically in cancer cells, there are also *systemic* effects of the kill switch alternative to chemo. Thus, while inhibition of IGF1R in tumor cells *inhibits* their growth, inhibition of IGF1R in the T lymphocytes known as Natural Killer (NK) cells dramatically *activates* NK cell multiplication and tumor cytotoxicity. [155] NK cells are a primary component of the innate immune response against cancer. It is thus of profound interest that inhibition of N-glycosylation of IGF1R

has opposite effects in these different cell types, blocking cell division in tumor cells, while activating cell division in the NK cells tasked with killing those tumor cells. Below we will discuss evidence that we have seen such systemic NK cell activation in some of the dogs treated with high dose DHEA to trigger the kill switch in their tumors.

Another important point to consider is that IGF1R, which is also embedded in the cell membrane, is, like EpCAM, a major activator of RAS , the isoprenylated protein heavily involved in cancer that we discussed above. So, *the isoprenylation of RAS, and the N-glycosylation of IGF-1R and EpCAM required for these proteins to activate RAS, are all simultaneously inhibited by kill switch activation* . By depleting intracellular NADPH and inhibiting the mevalonate pathway, kill switch activation simultaneously and naturally inhibits many of the pathways that are the targets of chemo efforts— although with chemo the process is one target at a time, and in a very unnatural manner.

(A brief aside)

IGF1/IGF1R and body size

As noted previously, a polymorphism near the IGF-1 gene is common to all small breeds of dogs, and absent from giant breeds. [156] This suggests that the IGF-1/IGF-1R system is a major determinant of body size across the incredible range of body sizes that characterize our modern breeds of dogs; that is, humans inadvertently selected for the presence or absence of this IGF-1 polymorphism during our selection of dogs for size. It also produces an interesting hypothesis: Since IGF-1R requires N-glycosylation to be active, and N-glycosylation requires dolichol, formed in the mevalonate pathway, could we miniaturize our giant breeds of dogs by inhibiting IGF-1R function with DHEA throughout their growth phase? We already know from the fact that Karma produced healthy puppies while on high dose DHEA, that such a protocol appears to be safe. This may be a shortcut to reducing size in our beloved giant dog breeds, which should decrease their lifetime risk of cancer, R, and increase their lifespan, Li, as we discussed in the previous chapter. I have added the testing of this hypothesis to my already overfull to do list.

(Back to the main story)

Cholesterol and the kill switch

Tumor cells also have a much greater need for cholesterol than normal cells, because cancer cells generally divide more rapidly than normal cells do, and cholesterol is used to make the lipids and fats that are major components of the cell membrane. There exists a wide array of studies linking cholesterol levels to cancer in humans. If you are interested in reading about this further, I recommend the excellent review of Kuzu *et al* . [157] Here we will limit ourselves to a few particular topics of relevance to kill switch function.

The *SQLE* gene encodes squalene epoxidase, a *c* ritical enzyme in cholesterol synthesis that has recently been elevated to the status of a bona fide oncogene. For example, in a study of 22 different types of human cancer, comprising 8783 different cases, a large and significant increase in the expression or copy number of *SQLE* was observed in breast, ovarian and colorectal tumors, with rates as high as 76% of cases (ovarian). [158] By inhibiting the mevalonate pathway by blocking its source of NADPH, kill switch activation inhibits this important mediator of the malignant phenotype too.

Massive sequencing studies are showing that many of the enzymes being identified as oncoproteins, and therefore also being selected as new drug targets, are already targeted naturally by the kill switch. The overwhelming majority of this

new research is in human cancer, and shows just how deeply the kill switch mechanism is embedded into human cells. The question thus remains, how completely is it embedded in canine cells? Judging from the results in Karma and many other dogs in our research program, it appears to be very well embedded indeed.

Ubiquinone

You may have noticed that we covered all of the end products of the mevalonate pathway in the figure above, except one: Ubiquinone. Ubiquinone is a lipoidal derivative of this pathway that has long been known to play a key role in the generation of the energy molecule ATP in mitochondria, a process called oxidative phosphorylation, or OxPhos for short. But Ubiquinone is also found in the outer membrane of the cell, as well. Ubiquinone is well known to be important in heart function, which requires a lot of ATP. Early on in my research I discovered that treatment with DHEA depletes intracellular ubiquinone, and even obtained a patent on ubiquinone supplementation to offset the negative effects of any agent that inhibited the mevalonate pathway. I was also aware of studies in which patients waiting for heart transplants due to end stage

heart failure experienced dramatic clinical benefit by treatment with ubiquinone. [159] Clearly, ubiquinone seemed like something that should be added to the kill switch protocol, to prevent its depletion.

Ferroptosis

You will recall that while we saw remarkable results with our kill switch activation protocol in a wide array of canine tumor types, there were a few in which responses were short-lived, such as lymphoma, and osteosarcoma, or non-existent, such as oral fibrosarcoma and oral melanoma. I now believe that some of these treatment failures may have been caused by the addition of ubiquinone to their treatment regimens. Thus, one particular selenoprotein fireman, GPX4—glutathione peroxidase 4— specializes in the extinguishing of a particular kind of intracellular fire caused by a particular family of lipid-damaging ROS. These lipid-damaging ROS (lipid peroxides) are created in the presence of iron, in a process called the Fenton Reaction. In 2012, Brent Stockwell and his colleagues at Columbia University demonstrated that the destruction of membranes by these iron-generated, lipid-damaging ROS constituted a distinct form of regulated cell death, and gave it

the name ferroptosis (from the Latin, *ferrum* , iron, and *apoptosis* , "to fall off," as in a leaf from a tree, when the leaf is dead.) [160] Since the selenoprotein firemen depend upon an active mevalonate pathway, I realized that ferroptosis was a major part of kill switch function.

Ubiquinone is well known to have antioxidant properties—to act as a flame retardant— particularly in its reduced form, ubiquinol. I realized that by adding ubiquinone to my kill switch protocol, I was inhibiting the kill switch from functioning— ubiquinone was rescuing tumor cells from ferroptosis by extinguishing ROS. So, at this point in my research, I discontinued all use of ubiquinone in the kill switch protocol in dogs with cancer. [161]

It has recently been shown that I was correct to do so. Thus, two separate groups demonstrated that ubiquinone participates in ferroptosis in a completely separate pathway that is parallel to that which is manned by the GPX4 selenoprotein firemen. These groups discovered that a protein called FSP1 (Ferroptosis Suppressor Protein 1) participates with ubiquinone in the detoxification of lipid-damaging ROS in the cellular membrane. [162] In this newly described process, FSP1, using NADPH, reduces ubiquinone to ubiquinol in cellular membranes. Ubiquinol converts lipid peroxides into non-toxic

lipid alcohols, inhibiting ferroptosis , and preserving cellular membranes. By adding ubiquinone in our original protocol, we may have been derailing the ferroptosis by which the kill switch derives much of its cancer killing ability, by providing so much ubiquinone that it acted as a sink for the NADPH remaining in the cell, allowing FSP1 to extinguish lipid peroxides.

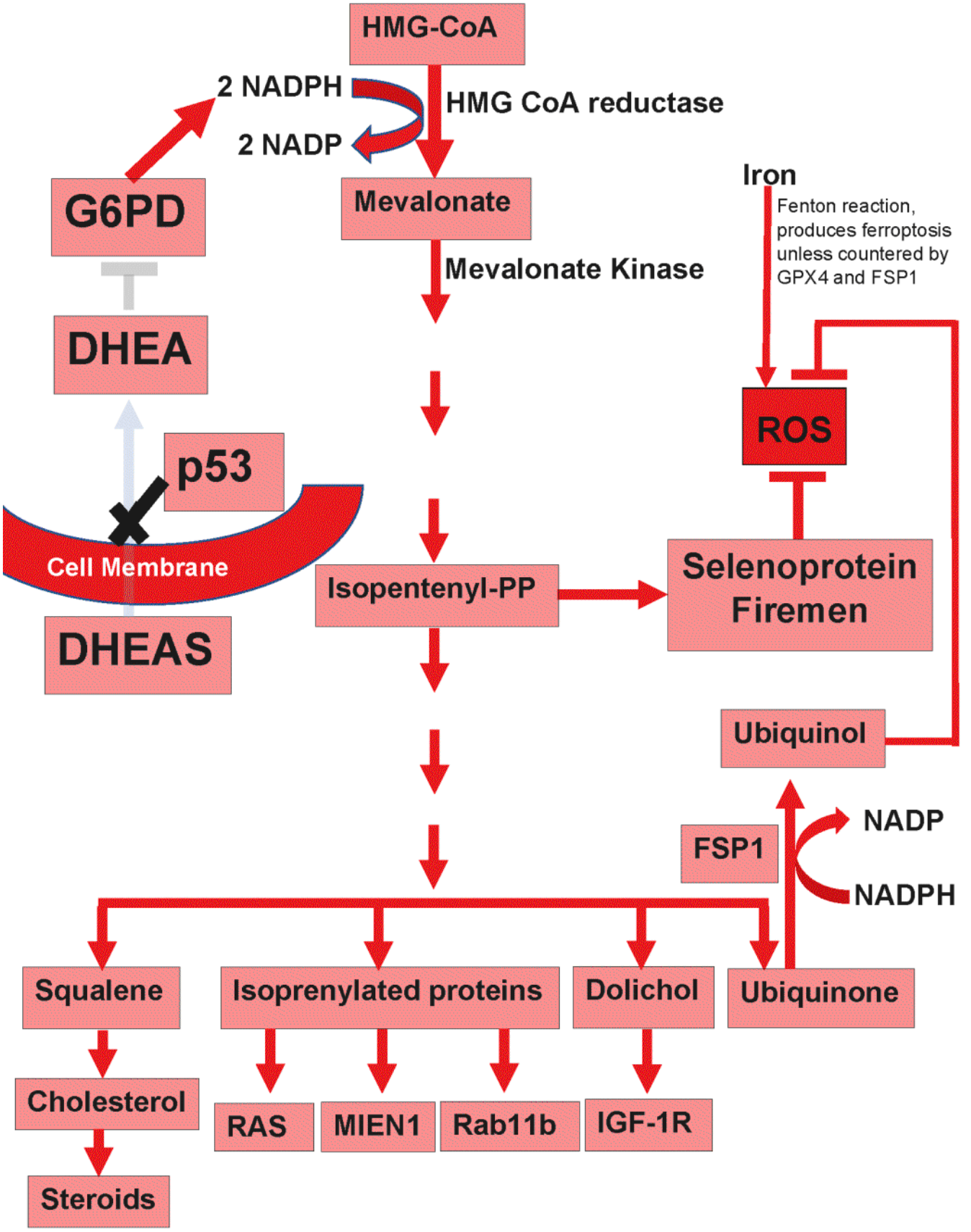

Figure 7.2 Cells are dependent upon both selenoprotein firemen (and their NADPH-filled fire hydrants), and the FSP1-mediated

regeneration of reduced ubiquinone (ubiquinol) to combat ROS. This requires G6PD to produce prodigious amounts of NADPH for selenoprotein synthesis. Active p53 prevents DHEAS from being imported into the cell.

As we noted previously, G6PD is the NADPH factory that brings the cell's selenoprotein firemen to life, and keeps their fire hydrants full of NADPH.

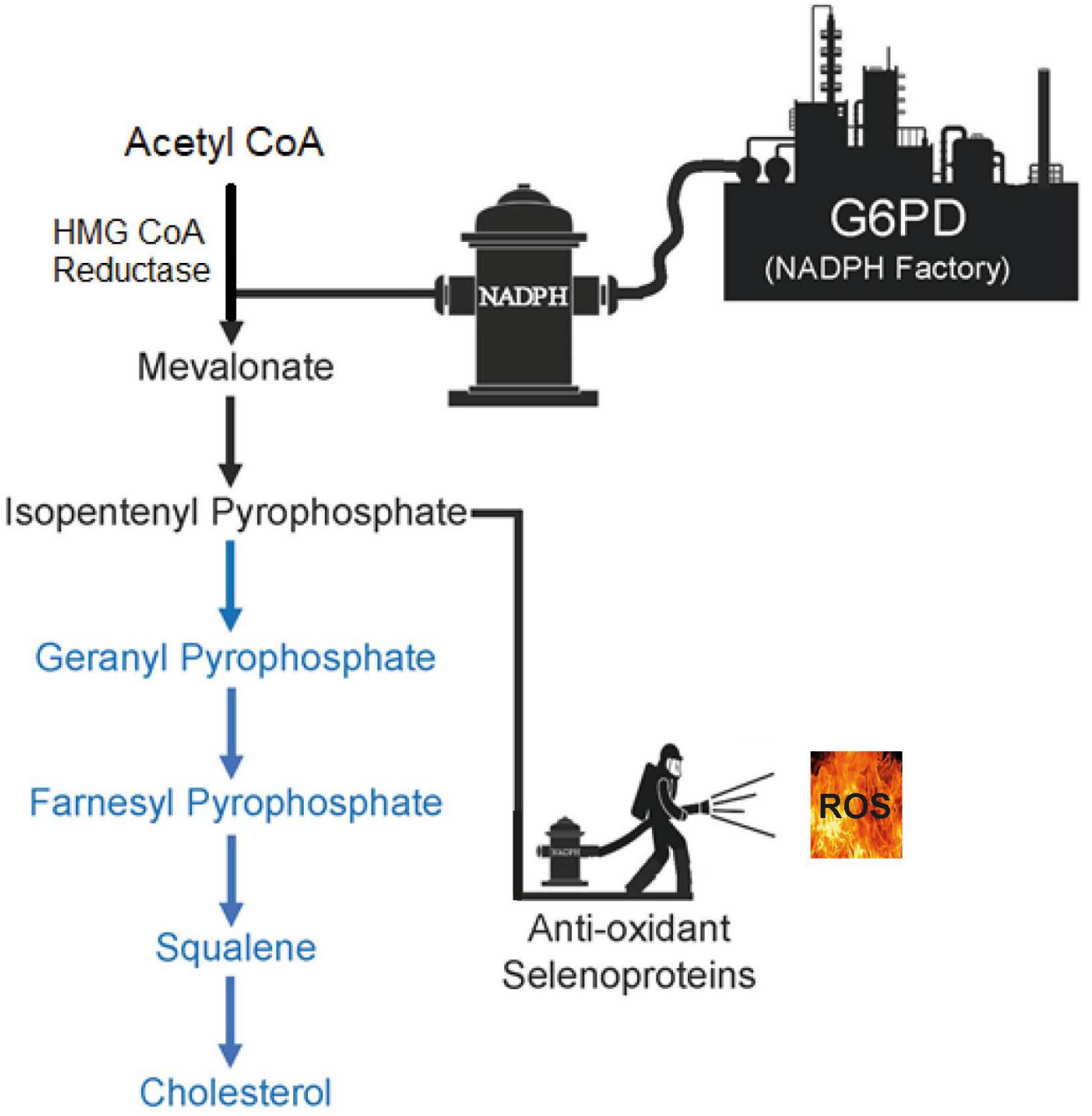

Figure 7.3 G6PD is the factory where NADPH is produced, which is then used to animate the selenoprotein firemen of the cell, and to fill their fire hydrants with NADPH. This is how cells maintain ROS at low enough levels to prevent them from burning down the cell.

Activating the kill switch by administering high-dose DHEA thus causes cells to self immolate by broadly shutting down the mevalonate pathway, inducing ROS-mediated and ferroptosis-mediated cell death.

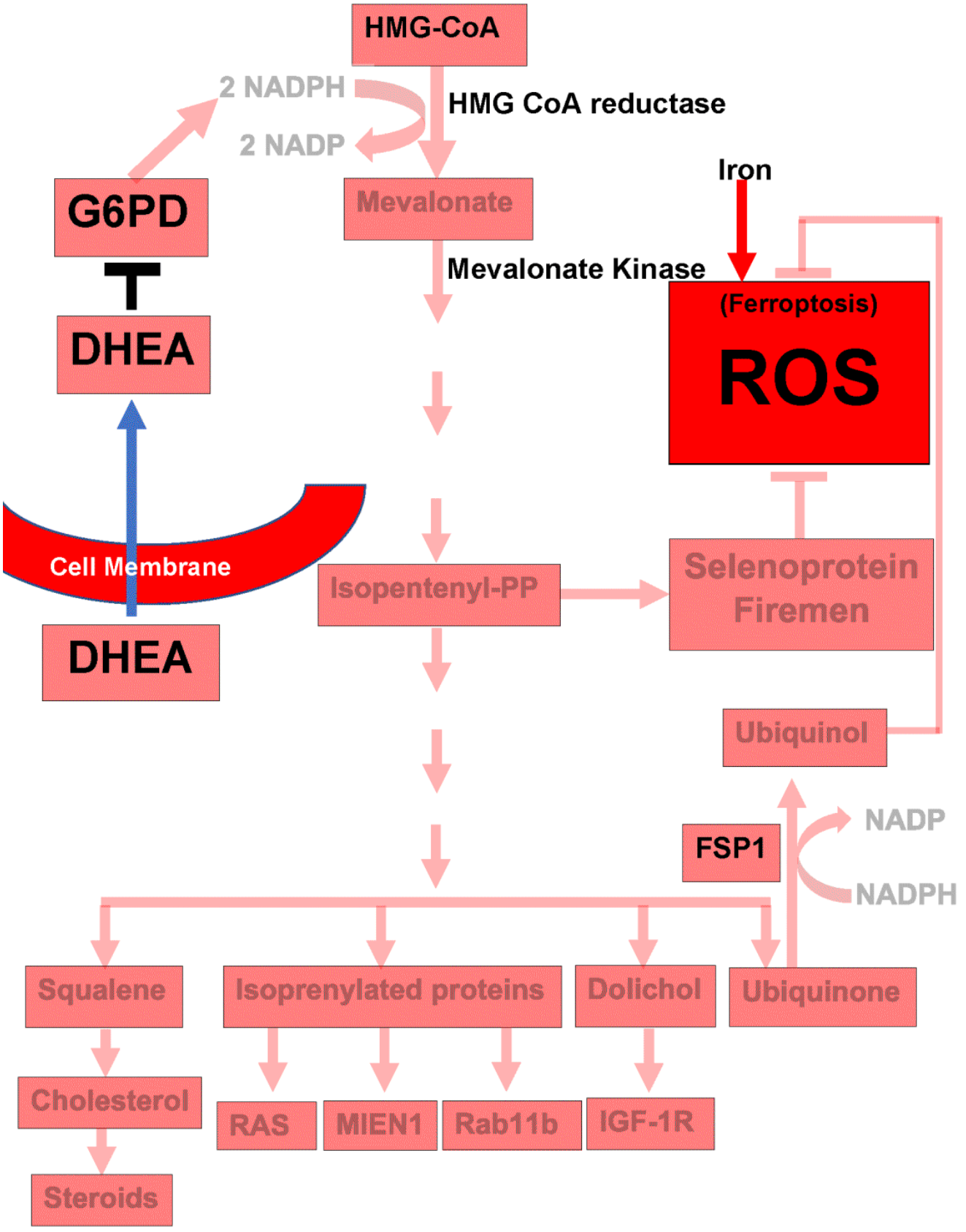

Figure 7.4 Kill switch activation by administration of high dose DHEA rapidly deprives the cell of selenoprotein firemen,

empties their fire hydrants, depletes the cell of ubiquinone, and prevents FSP1 from countering ferroptosis.

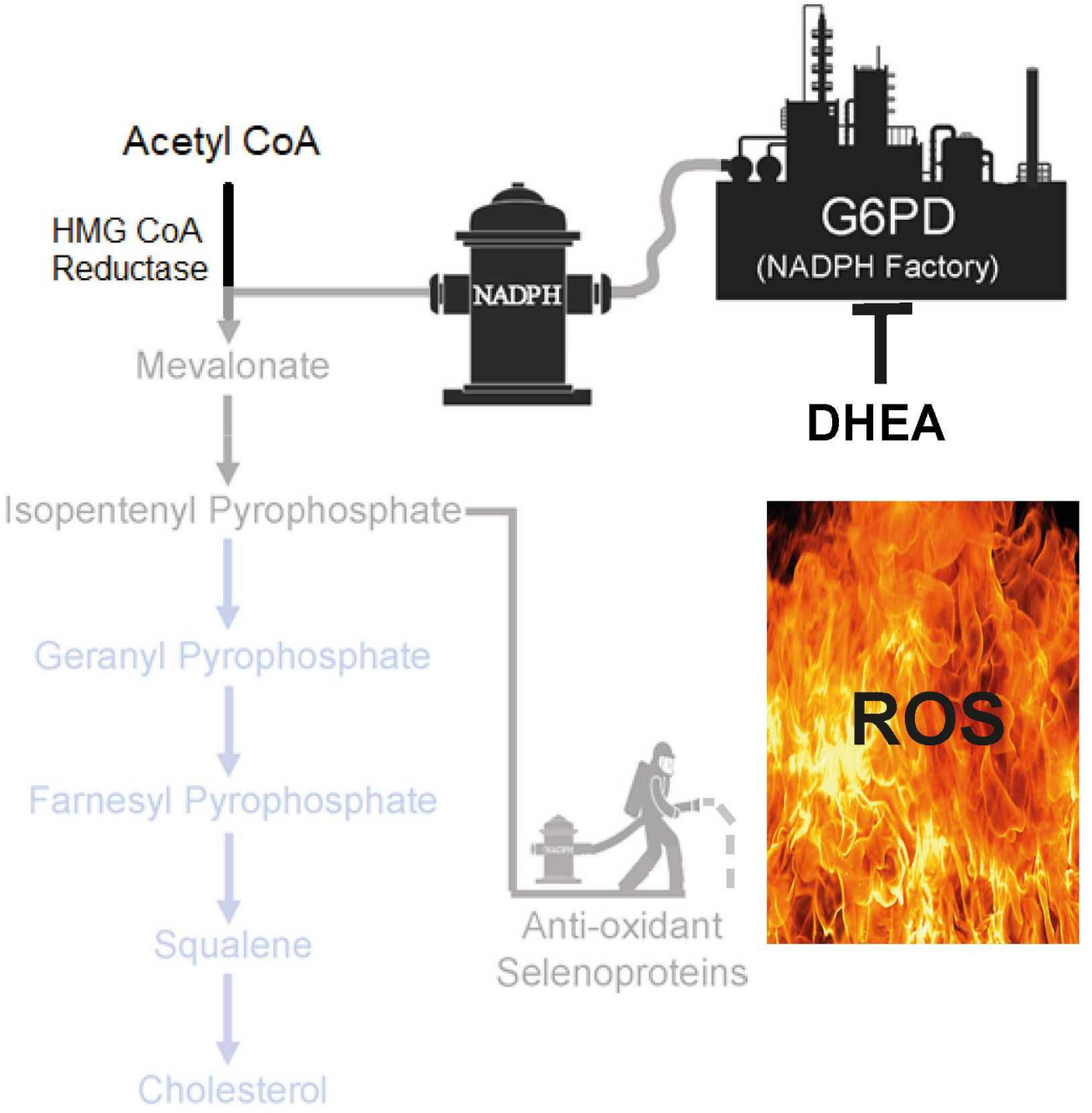

Figure 7.5 Tumor cells have an enhanced dependence upon selenoprotein firemen because of their high metabolism and consequent production of excessive ROS. In such tumor cells,

inhibition of selenoprotein synthesis using DHEA can lead to their ROS-induced destruction.

Unlike DHEAS, which requires transport across cell membranes, DHEA is lipophilic and therefore crosses through cell membranes freely. Once inside the cell DHEA shuts down the G6PD factory. The resulting depletion of intracellular NADPH makes the cell's selenoprotein firemen disappear, and empties their fire hydrants. This results in ROS-induced immolation of the cell, particulalry in tumor cells that have an amplified need for selenoprotein firemen due to their enhanced production of ROS compared to normal cells.

Some tumors are rendered ultra-sensitive to kill switch activation by the mutations that cause them

Alternative sources of NADPH and sensitivity to kill switch activation

In the interest of presenting a clear story, we have emphasized that G6PD produces most of the NADPH required to maintain selenoprotein firemen, to keep their fire hydrants full, and to

provide the NADPH required by the FSP1 protein to reduce ubiquinone to ubiquinol, and thereby prevent iron-induced ROS from activating the death program called ferroptosis. But now it is time to provide a more detailed picture of NADPH production in the cell, because that detailed picture reveals additional opportunities to activate the kill switch.

It remains true in this more detailed picture that G6PD is responsible for the vast majority of NADPH produced in the cell, and used for ROS control. But two additional enzymes, one called malic enzyme (ME) and one called Isocitrate Dehydrogenase (IDH) produce smaller amounts of NADPH. DHEA does not inhibit these enzymes that constitute an additional source of NADPH, but as we shall see, they still can figure prominently in treatment strategies for certain tumors.

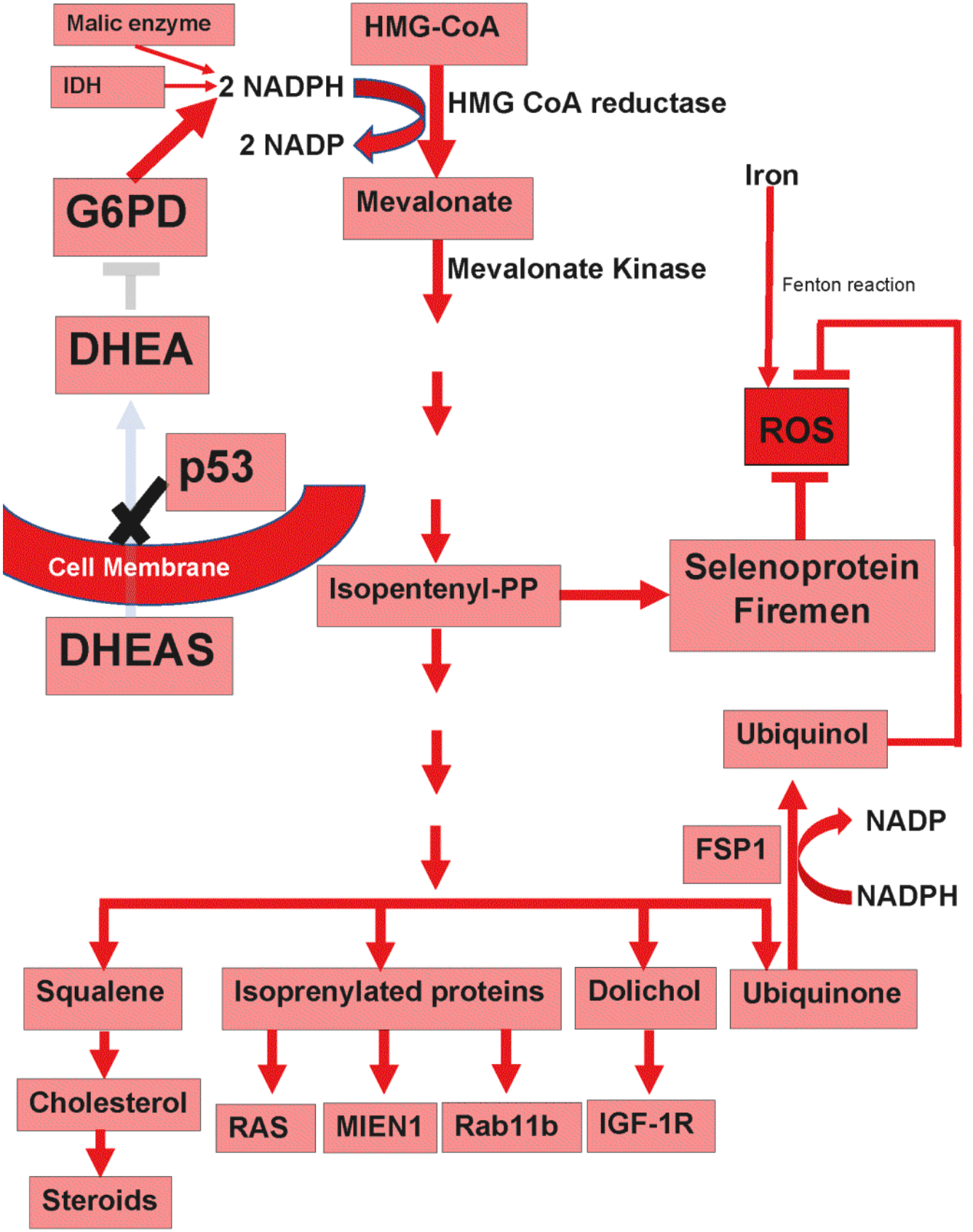

Figure 7.6 Although G6PD provides the lion's share of NADPH for ROS detoxification, IDH and ME contribute lesser amounts.

Let’s discuss one by one these alternative sources of NADPH, and what inactivating mutations in their genes does to kill switch sensitivity.

Malic enzyme (ME) and the kill switch

ME is primarily found in mitochondria, the energy factories of the cell where the high energy molecule ATP is manufactured. In mitochondria, there are two forms of ME— ME2 and ME3 — and both contribute to detoxification of ROS in mitochondria by producing NADPH. [163] Loss of one of these ME enzymes is not lethal to mitochondria, as long as the second is producing NADPH. Pancreatic cancer, and gastric cancer, in humans are frequently associated with deletions of a tumor suppressor called SMAD4, and since ME2 is adjacent to SMAD4 on the human chromosome, *it is lost along with SMAD4* . This has been shown to make pancreatic tumor cells exquisitely sensitive to interference with the remaining malic enzyme, ME3; i.e., they cannot tolerate any further diminution in NADPH. [164] Other human cancer types, such as head and neck carcinomas, and colon cancer, also appear to be driven by SMAD4 deletion. [165] Well, if such tumors with ME2 deleted are

sensitive to inhibition of ME3, imagine how much more sensitive they would be to G6PD inhibition.

While the focus of other labs has been to create ME3 inhibitors for use in ME2-deleted cancers, you are probably not surprised by now to hear that we are taking a different approach, triggering the kill switch. Considering the lethality of pancreatic cancer, this could turn out to be extremely relevant for both humans and canines with ME2-deleted tumors.

IDH and the kill switch

IDH actually comes in two isoforms, called IDH 1 and IDH 2. A primary purpose of IDH enzymes is the production of alpha ketoglutarate (αKG), which is a critical cofactor in the process of DNA de-methylation, a function that is performed by the TET family of enzymes. As you will recall from our earlier discussion, DNA methylation occurs at specific CpG dinucleotides, resulting in the 5-carbon of the cytosine being methylated, producing 5mCpG. In general, methylated genes are transcriptionally inactive (they produce no mRNA, and therefore no protein), while unmethylated genes are transcriptionally active. The evolution of TET enzymes enabled DNA methylation to be a dynamic process: where and when

necessary, TET can reactivate silent genes by de-methylating them. Also, TET continuously "prunes" methylated cytosines, maintaining the proper over-all methylated state of CpGs in a genome. Without TET, the number of methylated CpGs would rapidly rise, creating a hyper-methylated genome that would have lots of genes aberrantly silenced.

An array of human tumors appear to be caused by specific mutations in the *IDH* gene, including gliomas, acute myeloid leukemia, cholangiocarcinoma, thyroid tumors, and chondrosarcoma. [166] The mutant IDH enzymes— both IDH1 and IDH2— act in a completely opposite fashion, creating a completely different product than their normal, wild type versions. Instead of producing αKG, they begin manufacturing an oncometabolite called D-2-hydroxyglutarate (D2HG), which is believed to cause tumors to form in tissues where these IDH mutations occur. Loss of αKG has significant consequences. Since TET is a de-methylase, its inhibition by loss of its αKG co-factor in IDH-mutant tumors results in genome wide DNA hyper-methylation. Cancer biologists quickly learned that the hypermethylation-mediated inactivation of the Methyl Guanine Methyl Transferase (MGMT) gene had therapeutic value. MGMT is a "suicide" enzyme that removes the most toxic lesion caused by alkylating agents (O6-methylguanine) by

attaching the lesion to itself, leaving the guanine base in its natural form. Since MGMT expression in tumors creates drug resistance to alkylating agents (such as the nitrogen mustard that JD received), a standard approach to the treatment of some human tumors caused by IDH mutation, such as the brain tumors known as gliomas, is to take advantage of hyper-methylation-mediated inactivation of MDMT by treating with the alkylating agent Temozolomide. This has been shown to prolong life in such glioma patients with hyper-methylated MGMT, albeit briefly. [167] Gliomas in their variety of histological subtypes remain a particularly deadly form of cancer. [168]

There are several anticancer drugs in clinical trials which specifically target mutant IDH1 and IDH2 enzymes, in an attempt to inhibit them. [169] We are employing a completely different approach in both human and canine tumors with IDH mutations— a sort of judo throw in which we *use* the effects of the IDH mutations against the tumor, rather than try to block them. Our approach is based upon the fact that mutant IDH enzymes not only produce D2HG instead of αKG, but in their production of D2HG, they *use rather than produce NADPH* . Mutant IDH thus acts as a sink for NADPH, lowering intracellular concentrations of NADPH *selectively in tumor*

cells , and thereby lowering the threshold required at which any further decrease in NADPH will enable ROS to burn the cell down. In tumor cells with such a lowered threshold for ROS immolation, activation of the kill switch by administering high dose DHEA can act as a "magic bullet" selectively killing mutant cancer cells while leaving normal cells with wild type IDH alone. This is a very active area of research in my laboratory.

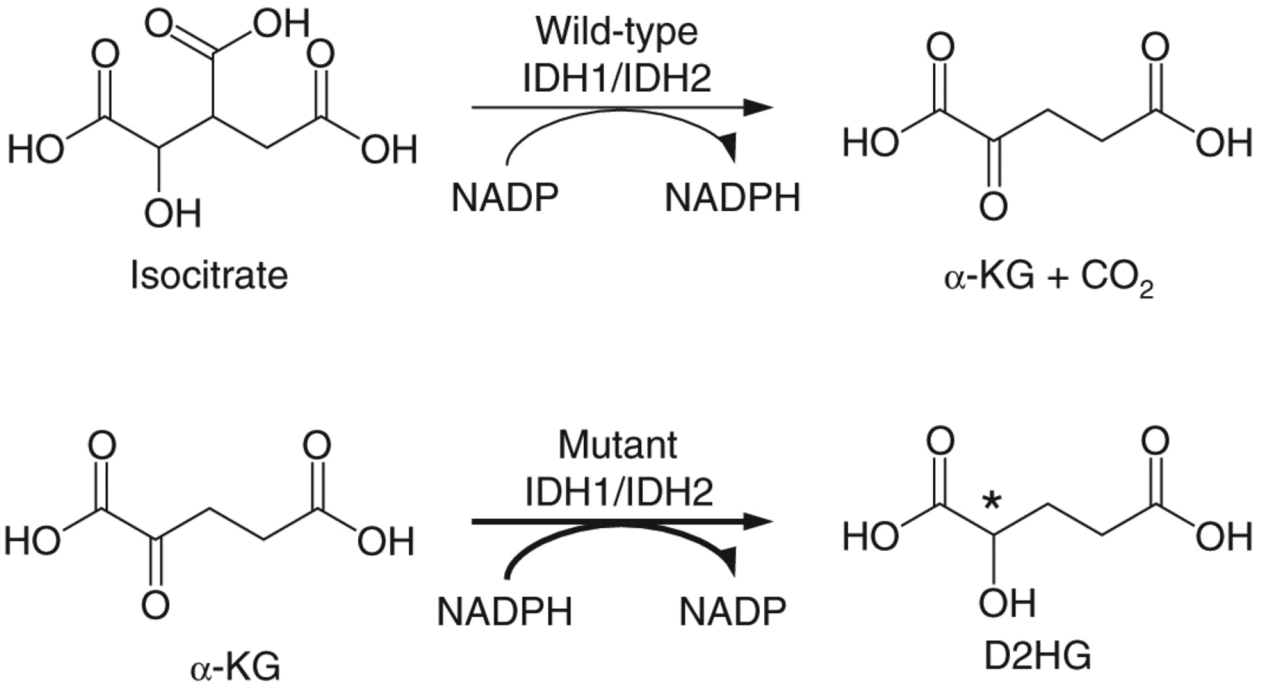

Figure 7.7 Instead of synthesizing NADPH, as normal IDH enzymes do, mutant IDH depletes intratumor NADPH, sensitizing them to kill switch activation.

Intracellular ROS concentrations have been shown to increase in human cancer cells with IDH mutations. [170] While canine

gliomas do not show the IDH mutations observed in human gliomas, [171] my lab is determining the spectrum of canine tumors that are caused by IDH mutations— and which may therefore be ultrasensitive to kill switch activation.

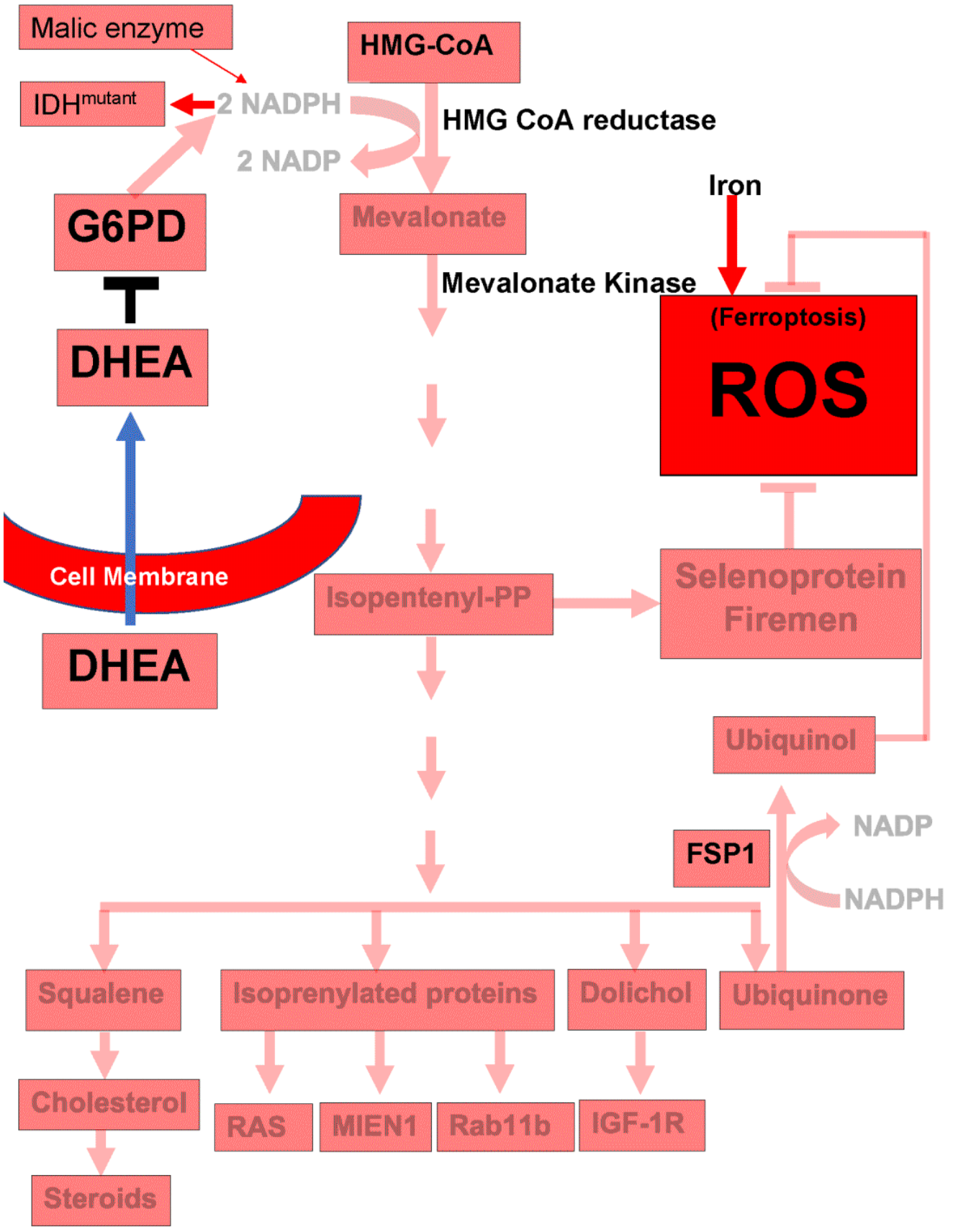

Figure 7.8 In normal cells, IDH produces NADPH, contributing to selenoprotein- and FSP1-mediated ROS control.

But in tumors driven by IDH mutations, IDH depletes , rather than produces NADPH, making such tumors selectively sensitive to kill switch activation by administration of DHEA.

My lab discovered that the portion of canine mast cell tumors that protrude to the surface is *unusually permeable* — in the way that the skin covering human testicles is unusually permeable, compared to skin elsewhere on the body. We capitalized upon this discovery by using an antisense oligonucleotide targeting G6PD, prepared as a transdermal formulation designed to carry the drug through the skin and into the tumor. [172] You will remember from chapter 5 that an antisense oligonucleotide binds to the messenger RNA of the target gene, preventing it from making the protein that it codes for. During my early days, I had used this approach to invent a new class of respiratory drugs, called Respirable Antisense Oligonucleotides, or RASONs. [173] Now I adapted it to take advantage of the surprising absorption kinetics of the skin overlying canine mast cell tumors.

Our strategy was to use the transdermal formulation of the G6PD antisense oligonucleotide to deplete the mast cell tumor of G6PD mRNA, and therefore G6PD enzyme, thereby selectively reducing tumor NADPH, rendering it ultrasensitive

to kill switch activation. We then triggered the kill switch using our systemic high dose DHEA protocol. The results were gratifying, with tumors rapidly shrinking, leaving little behind but apparent scar tissue— and so far, this kind of response *in every treated animal* . As you will read below, this is one of the clinical programs that we are moving forward with FDA to test in a much larger group of dogs with mast cell cancer. In view of the fact that Palladia, the currently favored drug for canine mast cell cancer, shows an objective, measurable response in *only about 1 out of three dogs* , a complete response (complete tumor regression) in *only about 1 out of 10 of dogs* , an average time to tumor progression of *only 9-10 weeks* , and a substantial risk of serious side effects, [174] we are anxious to begin our FDA studies utilizing this variation on the kill switch protocol.

What happens when tumors usurp components of the kill switch?

We have discussed at length the fact that the human kill switch evolved to protect during the 25-30 year lifespans that characterized our species for 99.95% of its existence. Tumors are able to form as a result of kill switch failure caused by the loss of circulating DHEAS with age. The same thing appears to be true for dogs, who have a rudimentary form of the kill

switch that evolved in primates. Tumors occurring in people and dogs are thus *fossils of kill switch failure* — failure due to insufficient levels of circulating DHEAS.

Because tumors represent instances of kill switch failure, they are able to usurp kill switch components for their own use, to enhance their growth and survival. What components of the kill switch are useful to tumors? Let's consider a few examples.

In contrast to DHEA, which can easily cross cellular membranes, DHEAS requires facilitated transport across cell membranes by specific transport proteins. Because it can easily cross into cells, circulating DHEA must be maintained at very low serum concentrations, orders of magnitude below its inhibition constant for G6PD. [175] Circulating levels of DHEAS can be extremely high because, unlike DHEA, DHEAS is nontoxic.

In humans or dogs that have developed tumors, circulating DHEAS was clearly not present at high enough concentrations to trigger the kill switch and prevent the tumor. Even though DHEAS is not present at high enough circulating levels to trigger the kill switch in those cases, it may be high enough to act as a precursor for dihydrotestosterone and estrogen synthesis. This is because its metabolite, DHEA, is a precursor for steroid hormone synthesis. In hormone responsive tumors,

even those low circulating levels of DHEAS can drive their growth. [176] Locally advanced or metastatic prostate cancer, for example, is treated with androgen deprivation therapy (ADT), a treatment that can be circumvented by circulating DHEAS. [177] While patients with such tumors generally respond well to ADT, they invariably progress to ADP-resistant prostate cancer. Such failure is generally attributed to intra-tumor androgen synthesis, occurring by the import of circulating DHEAS. A completely analogous situation occurs in breast cancer. [178] [179]

Let's examine in more detail these two examples of tumor usurpation of kill switch components.

DHEAS transport proteins and the kill switch

Circulating DHEAS is imported into tumor cells by *SLCO* -encoded OATP transporters, a component of the kill switch mechanism that would participate in the killing of tumor cells were it not for the low levels of circulating DHEAS. Usurped by the tumor cell, these DHEAS transport proteins can import DHEAS into the cell, and use it to synthesize testosterone and its derivatives which stimulate prostate cancer growth, or estrogens that can simulate the growth of breast cancer and other hormone-dependent cancers of the female reproductive

system. Accordingly, inhibitors of OATP transporters are another target of anti-cancer research. [180] We propose an alternative approach, using high expression of OATP in certain cancers to have DHEAS analogs selectively taken up by such tumors.

Steroid Sulfatase and the kill switch

A variety of human cancers— both those typically known to be endocrine-dependent, but non-endocrine cancers as well— show high expression of STS. [181] STS is usurped and highly expressed in many bladder cancers, for example, and in this kind of cancer it promotes metastatic spread by inducing Epithelial to Mesenchymal Transition (EMT). [182] Similar findings have been found with respect to STS in prostate cancer and cervical cancer, [183] and colon cancer. [184] In ADT-resistant prostate cancer, residual circulating DHEAS and its metabolism to DHEA and then to testosterone and dihydrotestosterone are a major reason for treatment failure. [185] In human mammary cancer, STS expression correlates with progressive disease and cancer-related death in a highly significant manner. [186]

Like OATPs, STS has become an active target for inhibitor synthesis, in an attempt to prevent circulating DHEAS from

contributing to tumor growth. [187] As with OATPs, we propose an alternative strategy, using STS appropriated by tumors t*o activate novel compounds to their tumoricidal forms* . [188]

Kill switch judo: Using kill switch components usurped by tumors to selectively kill them

Because DHEAS can stimulate tumor growth in hormone-responsive tumors that have high OATP/STS expression, we cannot simply treat such patients with DHEA or DHEAS. [189] To solve this pharmacologic problem, my lab built upon some early work that I participated in with colleagues while I was a doctoral student at the Fels Cancer Research Institute in Philadelphia. [190] These colleagues synthesized a series of DHEA analogues in which the number 16 carbon atom of DHEA was substituted with either fluorine or bromine. The fluorinated derivative was given the name fluasterone, and was subsequently studied by the National Cancer Institute as a potential tumor preventative. [191] However, this was decades before my discovery of the *lex naturalis* and the kill switch tumor suppression phenomenon, so it was impossible for these investigators to achieve the result they were after— first of all

because they used mice and rats as their experimental models, and therefore did not yet have the means to understand the species-specific triggering of the kill switch by p53 inactivation. [192]

Although fluasterone washed out in these studies (because what it demonstrated in rodents was meaningless), it nevertheless was an exceedingly interesting molecule. This is because it has the important feature that, unlike DHEA, *it cannot be used as a precursor for steroid hormone synthesis,* and is also about 30-fold more potent than DHEA as an uncompetitive inhibitor of G6PD. [193]

I realized that I might be able to utilize these properties of fluasterone if I modified it in a way that it had never been modified before— by sulfating it to make it an analog of DHEAS. A *Mr. Hyde* to *Dr. Jekyl* transformation, since sulfated steroids cannot enter cells without transport, and are therefore non-toxic. My lab thus produced fluasterone sulfate, a compound with the potential to be selectively taken up by tumors hyper-expressing OATP transport proteins. Here is the structure of this new compound.

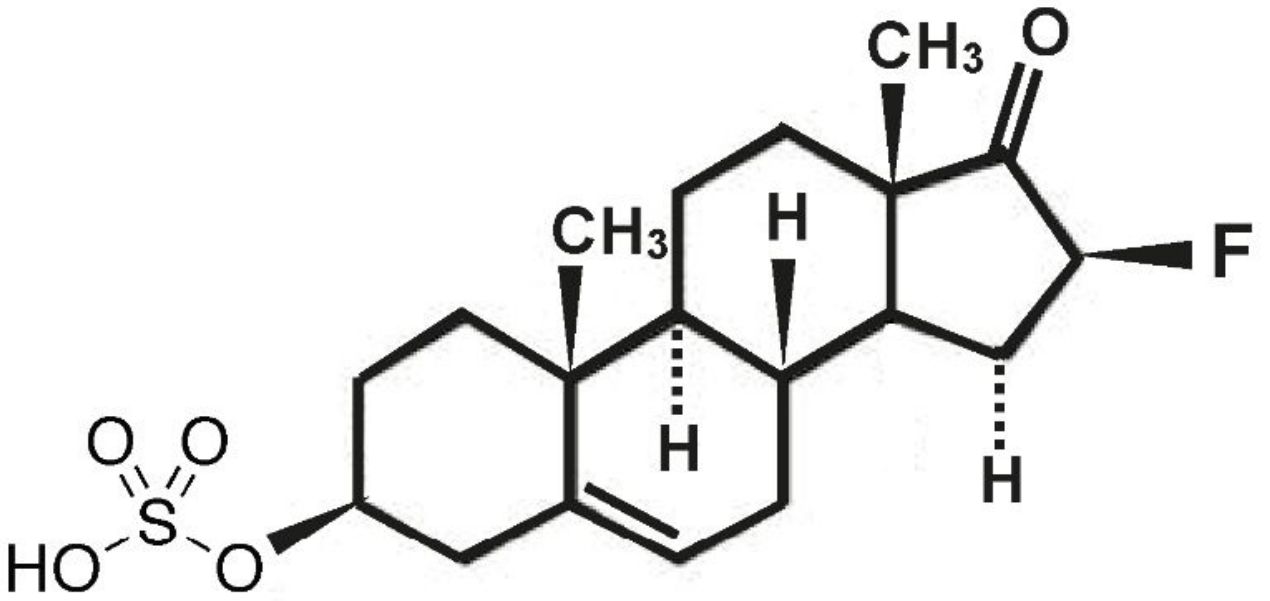

Figure 7.9 Fluasterone sulfate

We have proposed a series of clinical studies to the National Cancer Institute to deploy fluasterone sulfate in canine tumors that highly express one or more of the DHEAS transport proteins, and STS. Our rationale for these studies is that fluasterone sulfate will be selectively taken up by such canine tumors (because of high expression of OATPs), and then selectively metabolized to highly toxic fluasterone (because of high STS), triggering the kill switch in a much more selective and meaningful fashion than was possible with the initial NCI studies on fluasterone in rats. A successful study in canine tumors would encourage moving on to the large number of now deadly human tumors that do also.

Sugar metabolism and the kill switch

Foods are broken down by digestion into their constitutive components, one of which is the simple sugar glucose. Glycolysis is the fist step in extracting energy from glucose, and shares Glucose-6-phosphate (G6P) with the Pentose Phosphate Pathway (PPP) so that G6PD can produce the NADPH required to prevent ROS-mediated ferroptosis. Tumors have a particular need for glucose to fuel both glycolysis and the PPP, and glucose transport proteins are almost always extremely highly expressed in the cells of malignant tumors. [194] This, of course, has inspired synthetic chemists targeting cancer to develop an array of inhibitors of glucose transport. [195] But we have discovered an entirely different, kill switch-derived approach. There is a little history behind this.

In 1969 an enterprising group of Czech chemists working in Prague— Josef Pacak, Zdenek Tocik and Miloslav Cerny— synthesized an analog of glucose called Fluorodeoxyglucose (FDG), creating a molecule that was rapidly metabolized to FDG6P, an analogue of G6P, but which, because of the attached fluorine, gummed up the works of the machinery of glycolysis and the PPP; that is, it would bind to the enzymes of these pathways as a substrate, but could only slowly (or not al all) be metabolized to product. [196] Furthermore, because of the highly

charged fluorine moiety it contained, it could not pass through the cell membrane. Scientists at the Brookhaven National Laboratory, who were looking for ways to use the laboratory's cyclotron— an instrument that can be used to make radioactive isotopes— to create new imaging techniques. These scientists created 18F-FDG, a radioactive molecule that, like glucose, was metabolized by hexokinase to 18F-FDG6P, which then accumulated in cells that had a high requirement for glucose— such as tumor cells. [197] This isotope of fluorine can be visualized with great precision by a technology known as Positron Emission Tomograph). An entire science then rapidly developed administering 18F-FDG to patients with suspected cancer, and visualizing those tumors using PET— an imaging technique now known as 18F-FDG PET, or simply FDG PET. [198]

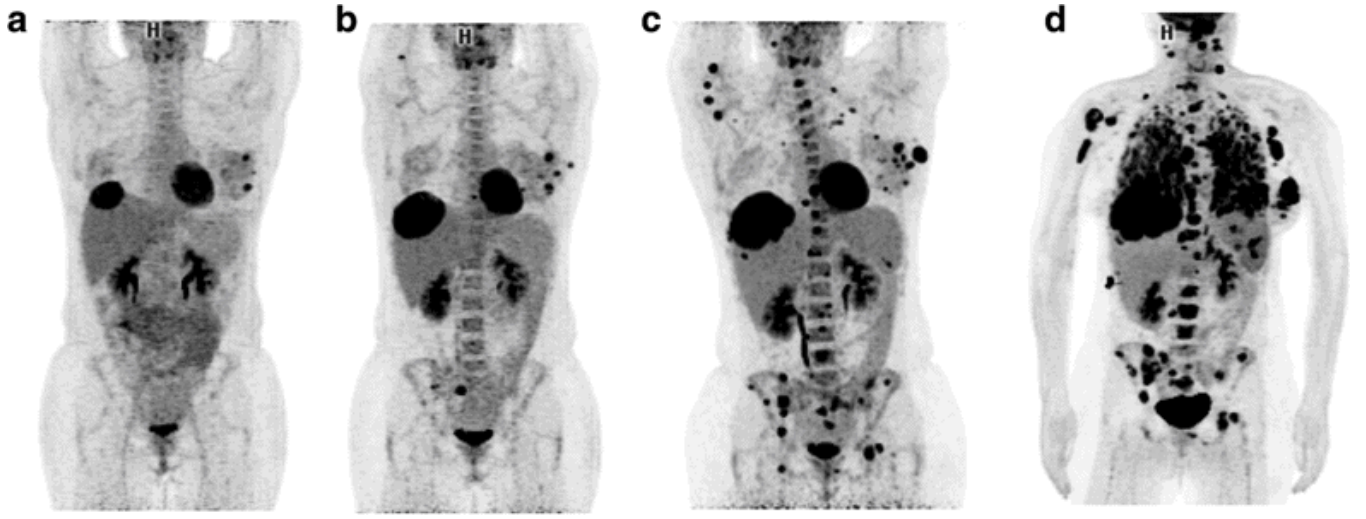

Figure 7.10 Use of 18F-FDG PET imaging to visualize tumor load. These images were obtained in a melanoma patient with

breast and liver metastases treated with the immunotherapy drug nivolumab after progression under anti-BRAF and anti-MEK treatment. They were acquired by an international group reporting their results in the European Journal of Nuclear Medicine and Molecular Imaging. [199] *(a) Baseline scan prior to treatment. (b) Early scan after two cycles shows progression in the breast and liver lesions as well as the appearance of bone metastases. (c) Scan after six cycles confirms the findings of progression. (d) This case was classified as hyper-progression during immunotherapy; i.e., for unknown reasons tumor growth accelerated during nivolumab treatment. Without FDG PET, it would have been difficult or impossible to document such progression. FDG PET is equally useful in documenting canine cancer.* [200]

FDG and the kill switch

My lab discovered that FDG6P supports the driving of uncompetitive inhibition of G6PD by DHEA toward irreversibility in the same manner that G6P does. This is an important finding, because it suggests that we can make *any* tumor characterized by high glucose uptake— which is most tumors — hypersensitive to kill switch activation because

FDG6P will preferentially accumulate in them. This is especially important for canine cancer, because dogs have not evolved methods to have G6P preferentially accumulate in cancer cells. Our discovery that FDG6P supports irreversible uncompetitive inhibition of G6PD by DHEA suggests that we can skip million years of evolution and do this for them *pharmacologically* by administering cold (non-radioactive) FDG. The FDG that we administer will be metabolized to FDG6P and accumulate preferentially in tumor cells. After administration of DHEA (or a DHEA analog) FDG6P substrate will selectively drive kill switch activation in those tumor cells.

Studies done by other labs have demonstrated that some tumors accumulate more 18F-FDG in direct correlation with how deadly they are. Thus, in osteosarcoma, high accumulation of 18F-FDG6P was associated with dramatically shorter overall survival than low accumulation. [201] The kill switch might be effectively triggered in canine osteosarcomas that are pre-treated with nonradioactive FDG. It will preferentially accumulate in such tumors, and then the kill switch can be triggered by administering a DHEA-based drug.

In human non-Hodgkin's lymphoma (NHL)— the kind of cancer JD had— a positive 18F-FDG result at the end of the treatment protocol predicted poor survival. [202] In another study,

a positive 18F-FDG result at the midway point in the treatment protocol predicted poor survival just about as well. [203] These studies suggest that in dogs with the most common form of canine lymphoma, a positive 18F-FDG result at the midway point in the CHOP treatment protocol would identify dogs who (1) are likely to fail such treatment, and (2) who might be good candidates for the kill switch protocol plus non-radioactive FDG. We are very excited to move forward in FDA clinical trials with these improvements upon our original kill switch protocol— first in canines, and then in humans with tumors that absorb high amounts of FDG.

In a series of studies that I have proposed to the NCI, radioactive 18F-FDG will be used to qualitatively and quantitatively evaluate primary and metastatic tumor load in spontaneous canine tumors, with those tumors showing high 18F-FDG uptake becoming candidates for treatment with fluasterone plus *nonradioactive* FDG. Our strategy is that, since FDG accumulates preferentially in tumor cells, and is capable of replacing G6P as substrate in the G6PD reaction, we can precipitate uncompetitive inibition of G6PD that will reach irreversibility in a tumor-specific fashion. In this way, the therapeutic index of fluasterone should be substantially improved— i.e., it will be selectively toxic to cancer cells, and

spare normal cells. In tumors that both avidly take up FDG and express high levels of STS, the therapeutic index might be further optimized by using fluasterone sulfate instead of fluasterone. Here, too, we are proceeding with studies in dogs with spontaneous tumors as a model system for what might be expected in triggering of the kill switch in human cancer. Imagine what it would mean for the treatment of human cancer if we can demonstrate activation of the kill switch in the majority of canine tumors. Although the main thrust of our research is on the *prevention* of cancer by kill switch maintenance into old age, even if we are wildly successful with that prevention approach, some tumors will still occur— the 4% lifetime cancer risk that should be occurring in our species, instead of the 40% it is now. If we could trigger kill switch activation in that 4% by selectively loading tumors with FDG, producing irreversible uncompetitive G6PD inhibition in a tumor-specific way, that would represent an entirely new, more-or-less natural method of cancer treatment.

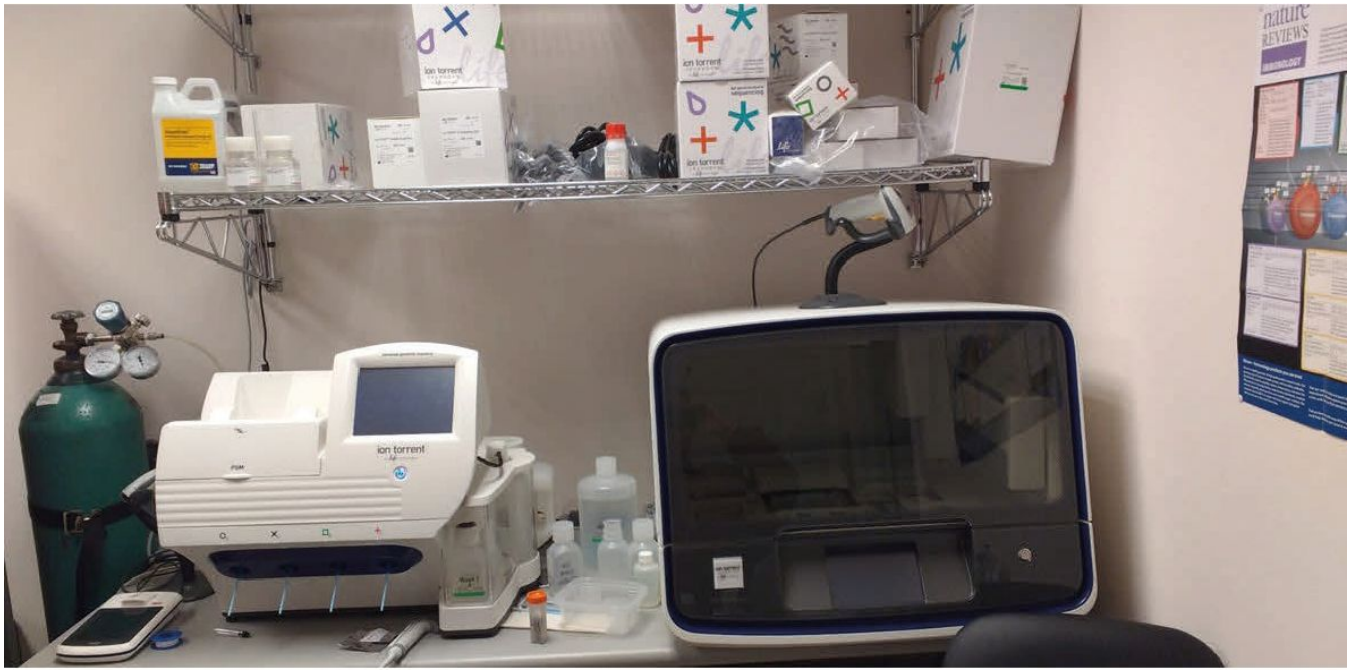

Figure 7.11 The Next Generation sequencing station in my laboratory.

Next Generation transcriptome analysis to identify canine tumors with ME2 or IDH mutations, STS and OATP hyper-expression, and FDG uptake and metabolism

We have now collected an array of canine tumors, and have isolated the mRNA from tumor and adjacent non-tumor tissue. We also applied for and received a grant to a California foundation to set up a Next Generation DNA sequencing facility in my laboratory. Our purpose in this project is to identify which canine tumors are associated with ME2 or IDH mutations, or hyperexpression of the genes for STS, OATP, and

FDG uptake and metabolism to FDG6P. Such studies are expensive to perform, and are most cost-effectively done when large groups of tumors are assessed simultaneously. Our deep freeze is filling with these mRNA samples, and we are excited to launch this project.

Side effects of kill switch activation

Kill switch protocols have entirely different risk levels in dogs and people

I have been a vocal critic of the FDA's policy of allowing over-the-counter (OTC) sales of DHEA for human consumption as a "dietary supplement"— basically, as a food. This policy is insane. All other developed countries treat DHEA as a controlled substance. Only in the United States is it available, in a completely unrestricted fashion, online, in pharmacies, and in supermarkets. I have frequently referred to DHEAS and DHEA as the "Dr. Jekyll" and "Mr. Hyde" of androgen biology, with DHEAS safely circulating in humans at extremely high concentrations, while blood concentrations of DHEA are kept *five orders of magnitude lower* . This is because DHEA is a potent uncompetitive inhibitor of G6PD and DHEAS is not. In

addition to low circulating levels of DHEA, further safety controls have evolved to keep DHEA out of all healthy normal human cells— reserving its entry exclusively to those that have suffered p53 inactivation. [204] Thus, DHEAS requires the assistance of transport proteins to enter cells, and once inside the cell, requires the action of STS to be transformed from Dr. Jekyl (DHEAS) to Mr. Hyde (DHEA). Because DHEA is lipophilic and therefore freely enters cells, the self-administration of DHEA short circuits 66 million years of primate evolution! People who take supraphysiologic doses of DHEA for its androgenic effects— aging males desperate to retain some aspects of their youth, or body builders, who use DHEA as a testosterone precursor— are hurting themselves; potentially fatally so. I have also recently argued that the unrestricted availability of OTC DHEA is contributing to the COVID-19 pandemic, which is well known to be influenced significantly by androgens. [205]

The FDA might counter that it was the "Supplement Health and Education Act of 1994" that made DHEA freely available. [206] But it is the FDA that is tasked with safeguarding the health of U.S. citizens from unhealthful products in the marketplace. All that FDA has ever had to do to retain regulatory control over DHEA is to perform a single study— preclinical in

primates would have sufficed— to demonstrate any of DHEA's negative clinical effects— for example the uncoupling of nitric oxide synthase in vascular endothelial cells, that I have been writing about for years. The unrestricted OTC availability of DHEA has thus likely been damaging the health of users for decades. But I fear it is contributing to the morbidity and mortality of the COVID-19 pandemic in a particularly deadly manner.[206] Not a single clinical trial demonstrating safety of oral DHEA has ever been performed during normal times, let alone during conditions of the current COVID-19 pandemic. And as an OTC product not requiring physician supervision, no data on adverse events have ever been or are now being collected. Primates, of which we are one, have an entire evolutionary history designed to make DHEA more and more toxic— *for the purpose of selectively killing cancer cells* . Self-administering DHEA is dangerous in humans, and should not be used without medical supervision. The FDA is wrong, even negligent, in allowing its continued OTC purchase as a "dietary supplement."

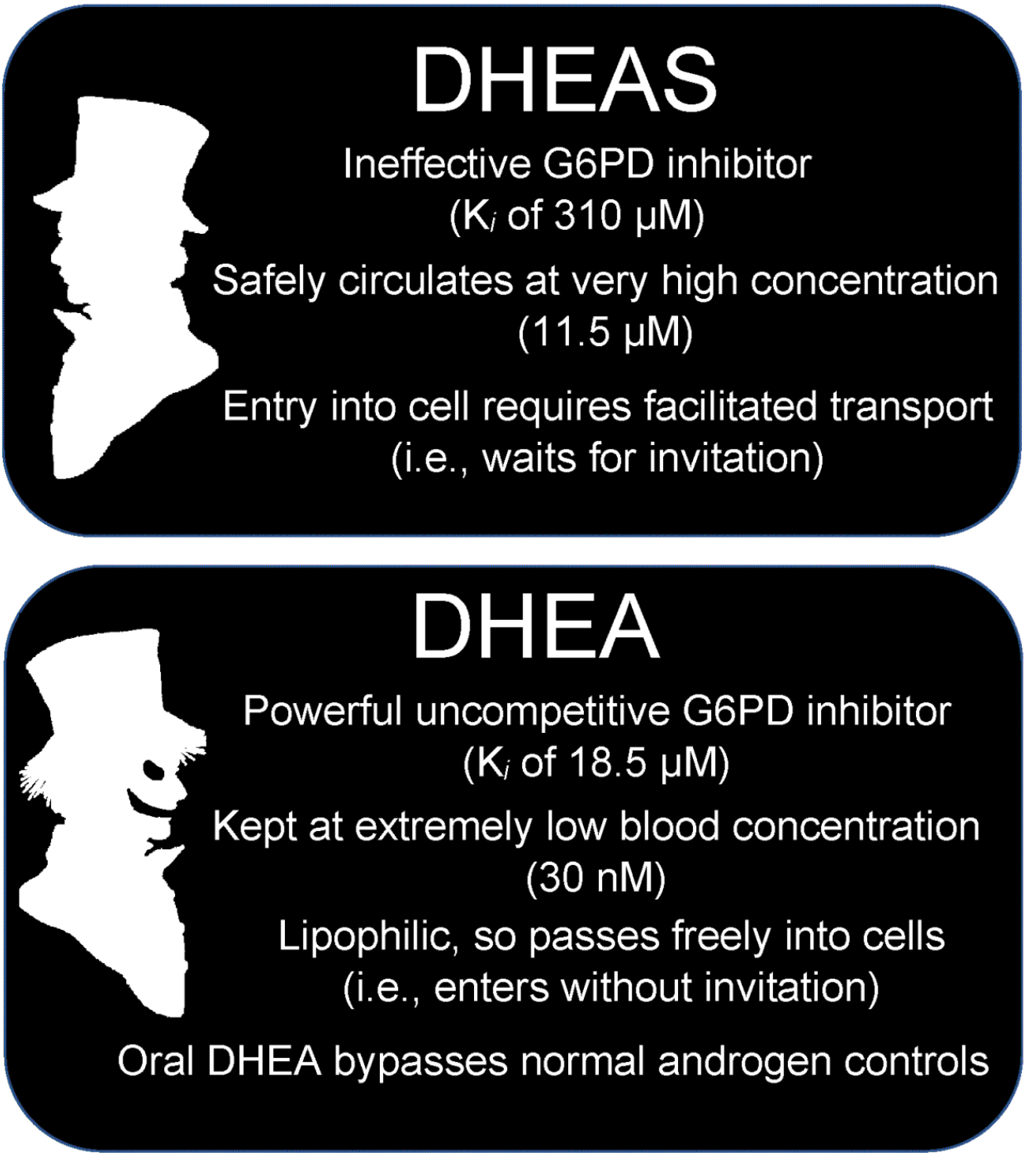

Figure 7.12 DHEAS and DHEA are the Dr. Jekyll and Mr. Hyde of human androgen biology. First published in Nyce, 2021 [207]

The situation is different in dogs

Unlike humans, dogs did not evolve the complex series of biochemical changes that constitute the kill switch tumor suppression mechanism (Figure 2.3). Instead, they evolved a very rudimentary form of the kill switch, closer to the version that evolved in the first primates. In those first primates, the toxic effects of DHEA toward p53-deficient cells— along with the small body size of these primates— was just sufficient to enable them to survive the introduction of PAH into their environment caused by the Chicxulub impact. For epi wolves, the toxic effects of DHEA toward p53-deficient cells— along with the small body size of these epi wolves— was just sufficient to enable them to tolerate the PAH-filled habitats of pyrophilic humans. None of the "improvements" to the primate kill switch described in figure 2.3 occurred in dogs— improvements that dramatically enhanced the ability of the primate DHEAS/DHEA system to destroy cells in which p53 had been inactivated. For this reason, administering DHEA to dogs is much safer than it is in humans, but there can still be toxicities associated with it. At this point in our discussion, I will describe some of the toxicities that we observed in our research exploring high dose DHEA as an alternative to chemo in dogs with cancer.

Mevalonate Kinase Deficiency in humans explains Karma's side effects

The kill switch tumor suppression mechanism in both humans and dogs operates by depriving the mevalonate pathway of the NADPH it needs to animate selenoprotein firemen and fill their fire hydrants with NADPH that both they, and FSP1 require to prevent ROS-mediated immolation of the cell. What happens when the mevalonate pathway becomes non-functional outside of the setting of cancer? There is an inherited human disease, called Mevalonate Kinase Deficiency (MKD), that will answer this question for us. MKD patients reveal what happens when the mevalonate pathway shuts down, something we are *trying* to do when we employ kill switch activation as an alternative to chemo. In other words, it will show us what side effects we might expect.

As you may remember from chapter 5, Karma was the Doberman histologically diagnosed with a soft tissue sarcoma (STS) at the Oregon State University College of Veterinary Medicine in October of 2015, and given just a few months to live even if her owners agreed to the devastating surgery that was recommended— surgery that would have removed her leg and half of her pelvis on the affected side. Karma's STS responded well to our kill switch activation protocol, with

complete tumor resolution. She remains healthy and tumor-free today, five years later. During her treatment protocol, however, Karma developed an auto-inflammatory reaction that revealed itself visually as ulcerated skin lesions as shown in the photographs reproduced below.

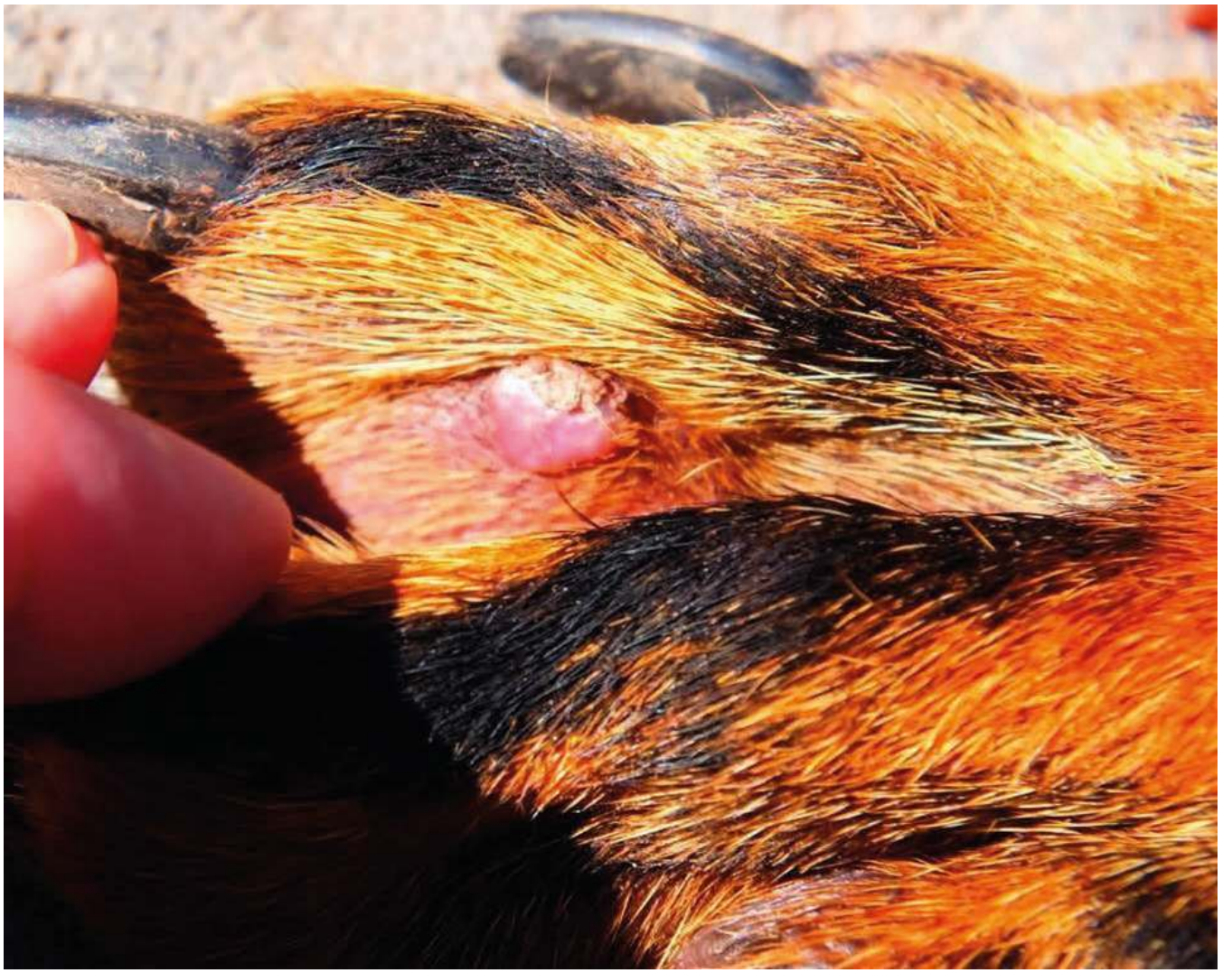

Figure 7.13 Auto-inflammatory reaction occurring during successful treatment of a soft tissue sarcoma using our kill switch activation protocol, in Karma, a four-year-old Doberman.

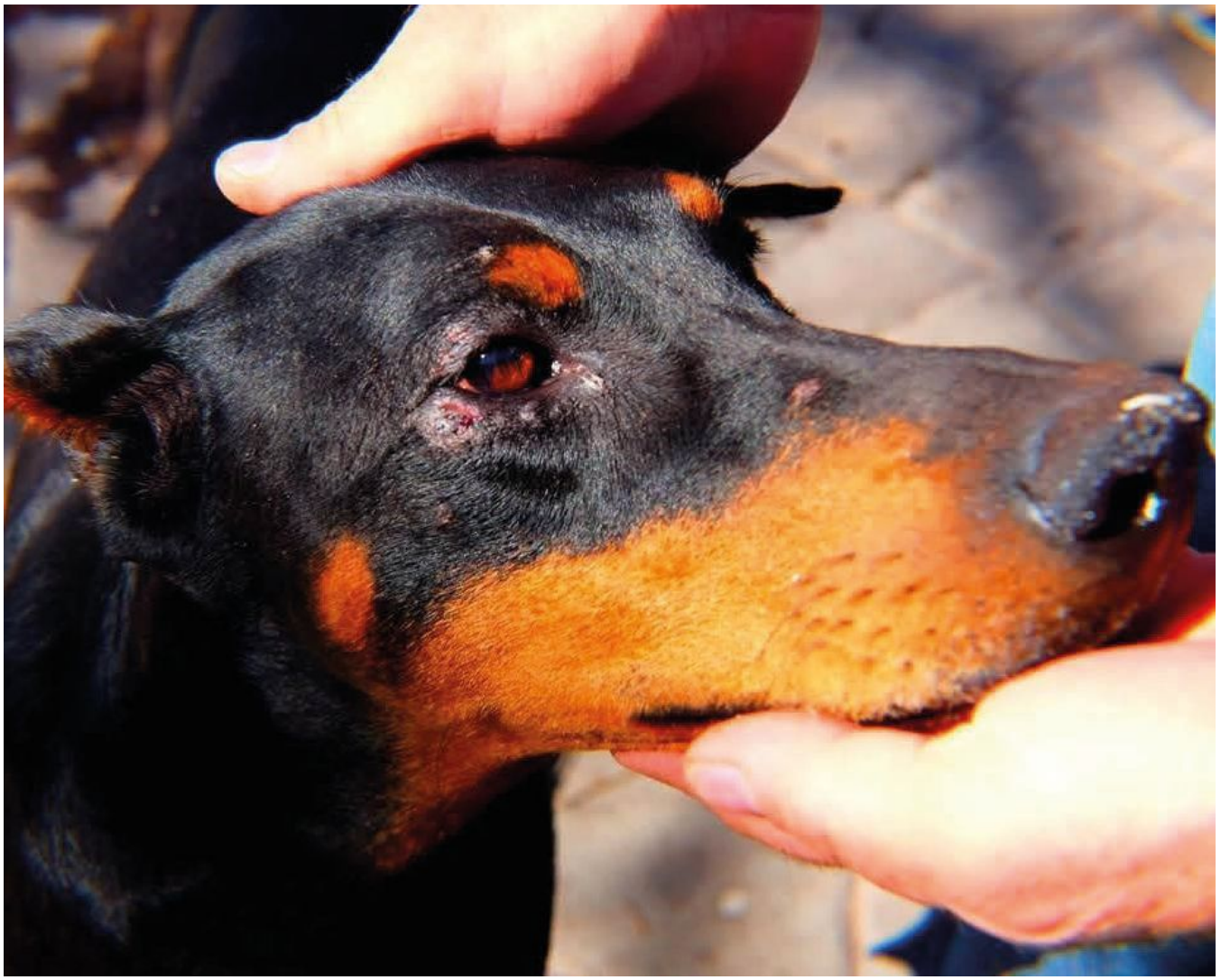

Figure 7.14 Auto-inflammatory reaction occurring in Karma during kill switch activation treatment of her soft tissue sarcoma.

Several dogs in our study developed this same autoinflammatory reaction to high dose DHEA treatment, and we quickly identified the problem as DHEA-mediated inhibition of the mevalonate pathway. To prove this, we demonstrated that the auto-inflammatory reaction caused by high dose DHEA disappeared quite dramatically by

replenishing the mevalonate pathway via administration of geraniol. However, we also learned that this inflammatory reaction resolved nearly as quickly simply by discontinuing treatment for a few days, and this is the treatment path that we are pursuing with FDA in all future studies. [208]

A large, multi-institutional group has recently published work supporting our contention that inhibition of the mevalonate pathway is the root cause of the auto-inflammatory response we observed in Karma and other dogs. This group demonstrated that, in human cells, the absence of a particular kind of protein isoprenylation, called geranylgeranylation, produces an unchecked inflammatory response and constitutive activation of a protein complex involved in inflammation, known as the Pyrin inflammasome. [209] New methods to resolve skin lesions caused by the Pyrin inflammasome have been applied recently to MKD patients and similar auto-inflammatory conditions associated with inhibition of various elements of the mevalonate pathway, so activation of this inflammasome may play a role in the autoinflammatory reaction that we observed in dogs. [210] However, we believe that in addition to inhibition of protein isoprenylation, the inhibition of selenoprotein synthesis and function, and the induction of NADPH depletion that induces ferroptosis, also plays an

important role in the autoinflammatory reaction that we observed in Karma.

While we might be able to prevent this auto-inflammatory response from occurring by replenishing protein isoprenylation by the administration of geraniol, this might actually be counterproductive, much the way that we believe our administration of ubiquinone was counterproductive. It might be that the induction of a systemic inflammatory response is part of the tumor lysis program of our treatment protocol— at least in some canine tumors. In our new work with the FDA, we will therefore be using high dose DHEA without ubiquinone, and without any replenishment of the mevalonate pathway. If you enter your dog in one of our studies, and this auto-inflammatory reaction occurs, do not despair. It means that we are definitely on the right track, and have accomplished inhibition of the mevalonate pathway. Discontinuation of the protocol for a few days should resolve the issue, with the same results that Karma had.

We suggest to our colleagues studying MKD patients that they examine the state of the selenoproteome of their patients, and explore the additional possibility that FSP1 activity may be inhibited by a reduction in ubiquinone . While in our kill switch

activation protocol such inhibition is a positive thing, in MKD patients it most definitely is not.

Cataracts

We also noted sporadic, rare cases of cataract in some of the dogs treated with the kill switch protocol for extended periods of time. One of the shortcomings of our initial studies is that x-ray or other analysis techniques to assess the effect of treatment were sometimes difficult to obtain on a timely basis. In such cases, treatment persisted for extensive periods (> 2 years), and it was in these dogs, which were generally aged by the time tumor assessment techniques were applied, that we occasionally observed the development of cataracts. In our upcoming studies, 18F-Fluorodeoxyglucose (18FDG) Positron Emission Tomography (PET) will be used to assess the effect of treatment at monthly intervals. We can thus continuously monitor for the development of cataracts, and depending upon the 18FDG PET results, discontinue treatment if there is no further evidence of tumor, and the beginnings of cataract lesions are suspected.

By what physiological process might high dose DHEA cause cataracts? As you will recall from our discussion above,

the process of N-glycosylation requires dolichol in its initial steps. Several proteins important to maintain the malignant state, such as EpCAM and IGF-1R, require N-glycosylation in order to perform their cancer-related functions. DHEA-mediated inhibition of dolichol synthesis blocks their action, and such inhibition represents an important component of the kill switch. But prolonged inhibition of N-glycosylation, which affects many proteins in the cell, might produce untoward effects. For example, in human patients with inherited diseases of dolichol synthesis, cataracts frequently occur at a young age, [211] and most of these diseases of dolichol metabolism involve N-glycosylation. [212] It is therefore possible that the cataracts that we observed, few in number as they were, might have been caused by DHEA-mediated inhibition of dolichol synthesis, and hence protein N-glycosylation. Alternatively, the instances of cataracts that we observed in our research might simply have occurred as a function of those dogs experiencing a normal incidence of age-associated cataracts. As noted above, our FDA studies using the kill switch protocol will include monthly assessment of treatment effect upon the tumor, enabling us to potentially conclude the study at a much earlier stage than was possible in our original research. We believe that this careful approach should eliminate, or substantially reduce, the risk of

any treatment-associated instances of cataract. In any case, we will alert veterinarians participating in our FDA clinical trials to be on the look out for the development of cataracts.

Summary of Chapter 7

The kill switch tumor suppression system evolved in primates over a period of 66 million years, beginning with the Chicxulub impact that extinguished the dinosaurs, but *created* the primate lineage. The Chicxulub asteroid struck an area of the Earth that was extraordinarily rich in PAH, which, kicked up into the atmosphere by the impact, caused many species to succumb to the strictures of the *lex naturalis* related to E— increased carcinogen exposure— especially large species. These same PAH activated *Alu* transposable elements in the diminutive species that we refer to as the proto primate. A remarkable reshuffling of the proto primate genome caused by these "jumping genes" ensued, creating a new species in which the primordial adreno-gonadal anlage gave rise during development to an adrenal gland uniquely capable of synthesizing and secreting remarkable levels of DHEAS— the first primate. A separation of labor followed, with gonadal synthesis and secretion of hormones maintaining responsibility

for sexual dimorphism in primates, as in other animals— but this newly reconfigured adrenal gland, with its capacity to synthesize and secrete DHEAS, took over the responsibility for the species-specific tumor suppression mechanism, T, that enabled equilibration of this first primate's *lex naturalis* equation in the face of the dramatically increased E caused by the Chicxulub impact.

The split in the evolutionary tree that gave rise to felids and canines— cats and dogs— as separate lineages occurred somewhat later, but the canine lineage was also remarkable in that it, too, relied upon circulating DHEAS as part of its evolutionary inheritance. This feature of circulating DHEAS enabled canines to initiate— and then maintain— a relationship with humans that continues to flourish to this day.

But cancer is out of control in both humans and dogs, caused by dis-equilibriums in our respective *lex naturalis* equations— underlain in humans by the dramatic increase in body size (S) and Life span (Li) resulting from the modern economy and improvements in medicine and public health; and in dogs by human manipulation of their body size.Tumors in both humans and dog are thus fossils of kill switch failure— they occur at astronomically higher rates than they should because, in humans, our species-specific tumor suppression

mechanism has failed— and we never supplied to our dogs the improvements in kill switch function that Nature would have if she had produced these differently sized canines as species. The astronomical rates of cancer in our dogs is due to our violations of their *lex naturalis* equations.

Fortunately for our dogs, we have discovered that we can trigger failed kill switches in a wide array of canine tumors by administering high dose DHEA to them— and we believe that we have uncovered mutations (e.g., IDH, ME), tumor-specific expression of enzymes (e.g., OATPs, STS), and methods (FDG) that may make *most* canine tumors sensitive to kill switch triggering. This really could be the beginning of a new age of enlightenment in the treatment of canine cancer. Our freezer is filling up with mRNA isolated from canine tumors, and we are about to subject those samples to analysis in our Next Generation DNA sequencing facility. We should have the answers soon. This is an exciting time indeed.

While no species, including dogs, can act as a valid model to discover and develop drugs to treat human cancer, dogs have a tumor suppression mechanism, T, utilizing DHEAS, exactly parallel to the one that has evolved in our species. This may have been why dogs were able to maintain equilibration of their *lex naturalis* while co-habiting with our ancestors in their

primitive, smoke-filled habitats. This parallel between the human and the canine tumor suppression mechanism— a parallel that does not exist in mice and rats— means that dogs most closely model human cancer. Perhaps this parallel will result in better cancer drugs for both our species.

8

Where we are with FDA

"Every organization should tolerate rebels who tell the emperor that he has no clothes."

Colin Powell

The Three Faces of the FDA

The Three Faces of Eve was the title of a popular film which hit movie screens in 1957. It was written by two psychiatrists, Corbett H. Thigpen and Hervey M. Cleckley, who had treated a woman for what they described as "dissociative identity disorder"— they reported that she presented three entirely different personalities at different times, as if she was three

entirely different people inhabiting the same body. Given the time, and its gender biases, it is easier to believe that it was not really Eve who had a problem, but rather these male psychiatrists who simply interpreted the emergent feminine spirit of this period in American history as a malady.

The FDA suffers from dissociative identity disorder

The FDA has presented three entirely different personalities toward our efforts to advance kill switch triggering into clinical trials, and therefore appears, as an institution, to be suffering from a quite severe case of dissociative identity disorder. The first of these three faces of the FDA is the scientific arm— that part of the organization composed of scientists charged with ensuring that drugs that make it to the public are “safe and effective.” This face of the FDA has been very helpful to our kill switch efforts, and has already approved five of our veterinary cancer projects for entry into special FDA programs. I have nothing negative to say about them— my experience with them has been extremely positive. As far as I am concerned, they are the very best face of the FDA, and were it not for the other faces— the other completely distinct faces of

the agency— the scientific arm might erode some of the criticisms that I have made of the FDA in this book and elsewhere. The scientific arm thus does seem to be thinking about what I am telling them regarding the *lex naturalis* , and how this dictum rules against the agency's long held policy that drugs for cancer must be proven safe and effective in lower species before they can advance into humans. [213]

The second face of the FDA is the "cash cow" of the organization— the division charged with collecting lucrative "user fees" from Big Pharma companies. As we shall discuss, the collection of such fees from Big Pharma has made FDA *dependent* upon this source of income, creating one of the most damaging conflicts of interest in the history of our country.

The third face of the FDA is the enforcement arm— brought into being to ensure that no barrier to the income stream from Big Pharma user fees is allowed to long survive. Over time, in response to their very clear mandate to keep the stream of user fees flowing, this face of the FDA has evolved to essentially act as hit men for Big Pharma— on the premise that, with FDA now dependent upon the collection of user fees, what is good for Big Pharma is good for the FDA.

Let's discuss each of these different faces of FDA one by one, and I will relate my interaction with each one of them

The Scientific Arm

I don't know if you know the history of the scientific arm of the FDA, but it is illustrious, even heroic. In particular, it is the story of one of my heroes, Dr. Francis Oldham Kelsey.

The Food and Drug Administration (FDA) came into existence in 1906 when Teddy Roosevelt signed the Food and Drugs Act into law and entrusted its implementation to the U.S. Department of Agriculture. The goal of this act was to protect consumers from the harmful effects of "adulterated" food and drugs, and the mechanism of enforcement was to prohibit the interstate transport of such goods. Much of the impetus for the Food and Drugs Act was the sale of products such as *Mrs. Winslow's Soothing Syrup* , which advertised on its label, "This preparation contains no poisonous ingredient and may be used with perfect safety," despite it being a mixture of powdered opium and morphine. It was advertised as a medicament that would "quiet" unruly children and help them sleep. Because of its severe depressant properties, it is believed to have been responsible for the deaths of thousands of children in the United States and Great Britain. The Food and Drugs Act put an end to the sale and use of this dangerous product.

Figure 8.1 Mrs. Winslow's Soothing Syrup, a dangerous mix of opium and morphine, is thought to have been responsible for

the deaths of thousands of children in the United States and Great Britain.

Later, in the 1930s, a mass poisoning occurred in the United States, which was tracked back to an improperly prepared medicine called *elixir sulfanilamide* . Working on this project was a young pharmacology postdoc named Dr. Frances Oldham. She and her colleagues identified that the poisonings caused by *elixir sulfanilamide* were the result of the use of diethylene glycol as a solvent in some of the manufactured batches. After this brilliant chemical detective work, Oldham became a faculty member in the Department of Pharmacology at the University of Chicago, where she met and married fellow faculty member Fremont Ellis Kelsey.

In 1960, Frances Oldham Kelsey was hired by the FDA. One of her first tasks at the agency was to review an application from the American company Richardson Merrell (now Merrell Dow Pharmaceuticals) for a drug called thalidomide, which had already been approved for sale in Canada, the United Kingdom, and most of Europe. In these countries, thalidomide was being marketed as a tranquilizer and painkiller specifically for pregnant women experiencing morning sickness. Kelsey studied all of the available clinical data on the drug, and became troubled by an English report that had documented a

nervous system defect. She withheld approval of the drug, and requested further studies to allay her uncertainties. Despite intense pressure from Richardson Merrell, which insisted the additional studies were unnecessary, Kelsey stuck to her guns and refused to move forward with approval until the company could explain the side effects that the English researchers had uncovered. Dr. Kelsey's refusal to grant approval based upon what she considered to be incomplete data made her a hero when, shortly thereafter, horrific birth defects began to be reported in all of the countries where thalidomide was in use to treat morning sickness in pregnant women. I was just a boy when the popular magazines LIFE, and LOOK began publishing articles on the thalidomide horror , showing armless and legless children, who had hands and feet protruding directly from their torsos as a result of their mother's having taken this drug. [214] *Frances Oldham Kelsey had prevented this from happening in the United States* . For her work that saved thousands of American children from suffering this fate, Dr. Kelsey received the President's Award for Distinguished Federal Civilian Service from President John F Kennedy in 1962.

Figure 8.2 Dr. Frances Oldham Kelsey receiving the President's Award from President Kennedy. Figure courtesy of the New York Times

It is clear from these cases that the FDA has an important role to play in protecting Americans from harmful food and drugs. But it is also important to point out that throughout all this time, the FDA was funded as part of the federal budget. It thus had no special interest in supporting the drug companies that it oversaw, and this was reflected in the work of Dr. Kelsey and many others. The agency was immune to drug company pressure, and could be fair and impartial in the approval process.

Then something happened that changed all of this—something that required FDA to employ a division of enforcers — hit men, really— whose job it was to prevent any interference with the flow of money coming into the agency from a completely new source.

The second face of the FDA: the “cash cow” collection branch of the FDA, big pharma user fees

The Prescription Drug User Fee Act

In 1992, Congress ended the ability of the FDA to be free of special interest, when it ordered the FDA to begin to pay its own way by collecting user fees from pharmaceutical companies when they submitted new drug applications. In the period leading up to the legislation authorizing user fees, the people who comprised the FDA at that time opposed the idea, arguing that it would negatively impact industry innovation—particularly making it difficult for academic researchers and small companies to participate in drug discovery and development. They also argued that it would increase costs of drugs to consumers because Big Pharma would simply pass the

additional costs on to consumers in the price of their drugs. They also publicly worried that becoming dependent upon funding from the very industry that they were supposed to regulate would raise conflict of interest concerns, perhaps even call the agency's integrity into question.

Despite these concerns, on first blush it might seem to you that this was a reasonable idea, getting pharmaceutical companies to pay for the work that FDA had to do to study the safety and effectiveness of drugs that the industry submitted for approval. On first blush it might also seem that Big Pharma would be against this idea, because now they had to pay for something that previously they had gotten for free. But despite public protests, behind the scenes Big Pharma likely was enthusiastic about the idea of such user fees, because it played right into their hands. Although the user fees were substantial, the Big Pharma companies were by now so large and wealthy that it was no impediment to them at all. And these user fees would represent a significant, even insurmountable barrier to small companies and academic researchers, preventing them from filing applications directly with the FDA to develop their discoveries. The Prescription Drug User Fee Act of 1992 also brought guarantees of speedy evaluation of pharmaceutical company data, thereby expediting the drug approval process for

Big Pharma. It even specified exactly *how* the income from user fees would be spent— in a manner that expedited drug approval. The FDA was rendered powerless to use the money as it saw fit. But perhaps the most onerous effect of this legislation was that it clearly put the FDA *on the side* of Big Pharma, and against small companies and academic researchers where most of the innovation is generated.

Over time, the people in the FDA who had opposed user fees retired or were put into career paths where their opposition would be ineffective. Over time, they were replaced by people who were given, and accepted, the mandate of running FDA like a for-profit business— those profits coming from their new "customers," Big Pharma. Clearly, the intent of user fees was to favor Big Pharma, and it put the FDA in their pocket.

If the intent of user fees had not been to help Big Pharma by putting obstacles in front of academia/micro pharma, FDA would instead have put the fee at the other end of the process— entitling itself to a percentage of profits on successfully marketed drugs. By collecting a percentage of profits, FDA could have generated the same or even more income. And it would have enabled academia/micro pharma to develop the drugs that it discovered directly with FDA. By putting the user fee up front, rather than as a percentage of profits, FDA ensured

that all discoveries made in academic or micro pharma laboratories had necessarily to flow to Big Pharma, the only entities with the capital to pay those upfront fees. One FDA commissioner boasted that he would never allow a small company to receive an approval for a major drug.

How dependent is the FDA on Big Pharma user fees? The latest U.S. Department of Health & Human Services FDA budget overview shows that of the $5.1 billion total annual budget for the agency, FDA acquired $2.5 billion— fully half— in the form of user fees. Thus, FDA has now been rendered completely dependent on user fees collected from the very companies they are supposed to oversee.

Dr. Kelsey must be turning over in her grave.

Collection of user fees for animal drugs

The FDA was set up to protect the public— the *human* public — from adulterated drugs. This protection included overseeing drugs that went into animals consumed by people— pigs, cows, chickens and the like— because human health could be affected when those drug-treated animals were eaten. But the MBAs who had replaced the old guard at the FDA realized that there was an additional "cash cow" that had not yet been milked.

With a growing economy, more and more people were adopting pets into their homes, particularly dogs and cats. And these dogs and cats sometimes got ill. And the flourishing business of veterinarians showed clearly that owners were willing to pay significant sums to maintain the health of their pets. This willingness was attracting some of the Big Pharma companies into the pet medicines business. FDA decided to stick out its hand, Monopoly style.

The Animal Drug User Fee Act was signed into law in 2003, enabling FDA to acquire additional income by charging companies user fees when they submitted new animal drug applications to the agency. This is purely an income generating mechanism, because *FDA has no real interest in protecting animals* . I know this for a fact because, as I noted earlier in this book, I have taken two drugs into human clinical trials under FDA, and the agency required me to hire outside companies to perform toxicology and efficacy studies that led to the deaths of many thousands of animals— not just mice and rats, but hundreds of dogs, and many scores of primates. I love animals, and so this FDA requirement sickened me, leaving me with a form of PTSD that I still have not fully recovered from.

For me, a really important offshoot of the *lex naturalis* and species-specific mechanisms of tumor suppression is that it

proves that animals can provide no useful information regarding the identification of drugs for human cancer. Therefore, it should lead to the cessation of the use of animals in the discovery and development of drugs for human cancer. That will save, literally, hundreds of millions of animals from being slaughtered to support cancer research. As just one example, it is estimated that 100 million mice die each year in cancer research studies. The USDA , which oversees animal welfare, reported that 64,707 dogs were used in research studies in the United States in 2017 (and 75,825 non-human primates). As understanding and acceptance of the *lex naturalis* increases, the number of animals used in cancer drug discovery should dramatically decrease— hopefully to zero.

The bottom line of my argument here regarding the collection of user fees for animal drugs is that, while FDA does have legitimate concern about the drugs that go into *animals that people will consume, thereby exposing humans to those animal drugs* , as an organization, they are responsible for the deaths of millions of animals— including dogs— every year. To claim that their collection of user fees for animal drugs is to protect animals is therefore absurd on its face. It is to collect user fees, pure and simple.

ACGT's goals put the user fee "cash cow" of FDA in jeopardy

Above I noted that FDA has already approved five of our programs to move forward in special FDA programs. These "special" programs were created by the scientists in the veterinary division of FDA as a means for academic scientists, and scientists at small research and development companies like mine (ACGT Biotechnology), to move forward with their discoveries without having to pay the exorbitant user fees noted above. This is another reason to applaud the scientific arm of the FDA, which appears always to be trying to do the right thing. If we are successful with these five programs, I believe that the scientific arm of the FDA will take pride in what they helped us to accomplish.

If ACGT [215] intended to keep our focus only on canine cancer, I think that the other faces of FDA would probably have ignored us. But everything that we are doing is focused upon pushing our kill switch discovery into human cancer, and this becomes a huge threat to the "cash cow" of FDA user fees from Big Pharma. Our stated goal, which we have published in major peer-reviewed journals, is to "normalize" lifetime cancer risk in humans from its current 40% to the 4% that the *lex naturalis* says it should be. [216] This means that instead of the 30 million

new cancer cases in 2040 that Big Pharma is counting on to drive their profits, if we are successful in "normalizing" cancer risk to 4%, that number of new cancer cases will drop by 90%, to 3 million. This would put most Big Pharma companies focusing on treating cancer out of business. Maybe all of them, if the treatment applications we described in the previous chapter (ME and IDH mutations; STS and OATP overexpression; FDG driving most human tumors toward irreversible uncompetitive inhibition) work as well as we expect them to. We control this new technology based upon the *lex naturalis* and kill switch tumor suppression, and we intend to drive down the number of new cancer cases to as small a number as possible. This will wreak havoc with the predictive power of the calculus equations written out by the MBAs at those Big Pharma cancer companies. The business of cancer will cease to be Big Pharma's major profit center. It will also wreak havoc with FDA's user fee dependency. If we are successful in using the *lex naturalis* to reduce human cancer by 90%, those user fees on cancer drugs, already in FDA's budget projections, will all but disappear.

The third face of the FDA: The hit men

Basic research vs. development: a primer

As I noted above, I have taken two drugs into human clinical trials under FDA, so I am very familiar with the process. Let me break it down for you. There are two completely separate stages in bringing a drug to market. The first is the basic research stage, when an idea is generated— an idea which is then tested experimentally to see if there is something worth pursuing clinically. This research phase is usually conducted in academic laboratories, as was the case for me when I advanced the RASON for asthma into human clinical trials. Sometimes this same sort of research is conducted in small research companies, like ACGT, and sometimes it is conducted in the labs of Big Pharma. But in none of these cases does FDA involve itself, or have any jurisdiction over the research phase. It is only when a research group has decided that they have something that works so well that they are willing to risk the extreme costs of *developing* it as a drug, and prepare and present to FDA an Investigational New Drug application (IND), that the agency gets involved. This is where the line is crossed between the research phase and the development phase. [217] The FDA has no jurisdiction whatsoever over the research, but absolutely, by law, controls the development phase. This was well understood by me, and by virtually all other scientists

working in medical research. I was nearing the stage when I could sit down and write an IND, as I had before for the previous drugs that I had taken into clinical trials; but I was not yet there with my present research project. (This time, my application to the FDA would be for an investigational animal drug, called an INAD— investigational new animal drug application.) My plan was to repeat what I had done before. Get enough data to publish in *Nature* , creating the necessary worldwide "splash" to show the world what I had discovered, and then put together the team that would help me write the INADs and INDs and launch our kill switch studies into the development phase.

I never expected to have any experience with the enforcement arm of the FDA. After all, my lab was clearly in the research phase, in the midst of the dramatic breakthrough that is the subject of this book; working hard to reach the development boundary and submit our IND, but not there yet. Because we were going against fifty years of dogma— the use of *rodents* in cancer research— it is true that we could not expect funding from the NCI, the source of that dogma— but our participant-investor funding model had kept the lab moving forward, and we had generated enough data in dogs that we were able to win the grant that had enabled us to set up the

Next Generation DNA sequencing system. We did not yet have the data necessary to publish in *Nature,* but it seemed imminent as we got closer to analyzing the canine tumors that we had collected.

I hope you can imagine my surprise, then, when I received a "warning" letter from the FDA enforcement office in Philadelphia saying that I was using an "adulterated drug" in my studies in canine cancer. *Adulterated drug?* Here is the dictionary definition of the word "adulterate:"

"to corrupt, debase, or make impure by the addition of a foreign or inferior substance or element especially : to prepare for sale by replacing more valuable with less valuable or inert ingredients."

I was outraged. I responded immediately to the FDA office that had sent the letter, showing that we obtained our DHEA from a bulk supplier based in Seattle that they— the FDA— had approved for over-the-counter (OTC) sales of DHEA to the public *for human consumption* . It was outrageous to call this DHEA "adulterated" when we were using it in its pure form exactly as we received it from the company. I sent chromatograms that we had received from the company demonstrating the purity of the DHEA being used, and waited for a response. When the response came it argued that I had

made “claims” regarding the potential benefits to dogs of being in the study, and that I needed to do clinical trials. This I felt was equally outrageous. Of course I had explained to participant-investors my rationale for the study— after all, who would enter a dog with cancer in a research study if the person conducting the experiment did not have a clear rationale? But describing your rationale is not the same thing as making claims. And I intended to do clinical trials when I had the sequencing data. I made my arguments to the same FDA Enforcement Lawyer in the Philadelphia Office, and when I did not receive an additional reply, I felt the matter was settled.

The Scientific Arm of the FDA to the rescue

In fact, just a short time later the scientific arm of the FDA held a colloquium at which they seemed to address this very issue. It so closely fit my case that I believed that it was an “instruction” to the enforcement arm to stop their attempts to interfere with my research. I will reproduce only the highlights from this colloquium here, but you can read and listen to the entire colloquium on the FDA site .

The title of the colloquium was: Regulatory Considerations for Using Pharmaceutical Products in Research Involving Laboratory Animals.

The speakers were:

Dorothy Bailey, DVM, Center for Veterinary Medicine, FDA

Neal Bataller, DVM, ME, Center for Veterinary Medicine, FDA

Carol Clarke, DVM, Animal Care, Animal and Plant Health Inspection Service (APHIS), USDA

John Bradfield, DVM, PhD, DACLAM (Diplomate of the American College of Laboratory Animal Medicine), American Association for Accreditation of Laboratory Animal Care (AALAC)

Axel Wolff, DVM, Office of Laboratory Animal Welfare (OLAW), NIH

Susan Silk, MS, OLAW, NIH

George Babcock, PhD, University of Cincinnati and OLAW, NIH

Slide 7 of Dr. Bailey's presentation:

Slide 7 Investigational Use (Test Articles)

"Now I'm going to talk about investigative use of drugs, and once again this is where the drug is the test article or focus of research. And to help explain FDA regulation of investigational uses I've split it into three categories. The first category is basic research. This is where a drug— essentially at this point it's a chemical or compound— is being studied and it's prior to any sort of known use of the compound as a drug. So, this is prior to drug development. <u>This type of test article research is not reported to the FDA</u> ."

"The second category is pilot or preclinical studies that are conducted early in drug development. At this point there is a known use for the compound and it's a drug use. I've provided you with the citations to the federal regulations which cover this type of use for both human [21 CFR 312] and animal [21 CFR 511.1(a)] drugs. Essentially what these regulations say is as long as the drug is labelled appropriately as an investigational drug and certain shipment records are kept, the drug manufacturer can ship the drug interstate and interstate commerce and the drug can be used in studies involving

laboratory animals. These studies do not have to be reported directly to the FDA .”

Clearly, I was still in the research phase described by the first paragraph, and even if the line had been blurred a little between the research and the pilot study phase of my work, I still did not have to report to the FDA at this stage. [218] Only when I crossed into the development phase, by submitting an INAD application to the FDA, would I then be under their control. I was trying as hard as I could to reach that stage; but I just had not achieved it yet. The timing of this colloquium by the scientific arm of the FDA appeared to me to be their directly coming to my rescue and “informing” the enforcement arm that they were way off track sending me warning letters. Case closed, and thank you again, scientific arm of the FDA.

Figure 8.3 My laboratory was conducting high level basic research. Clockwise from upper left: Next Generation DNA sequencer; Oligonucleotide synthesizer used for RASONs, transdermal antisense oligonucleotides, and the synthesis of probes and primers required for molecular biology studies; view of molecular biology laboratory showing PCR enclosure and other equipment; assorted laboratory equipment; view of part of tissue culture facility ; assorted analysis equipment including Luna cytometer, PCR machine, and Agilent

Bioanalyzer. PCR, polymerase chain reaction, a technique for amplifying the amount of DNA coding for specific genes, to enable their analysis.

But why does FDA permit DHEA to be sold as an OTC product, without regulation?

Despite the scientific arm's clarification that the research phase of discovery, and even early pilot studies in the development phase, were not under the control of FDA, I was still irritated over the "warning" letter that I had received. No apology or any other acknowledgment had been forthcoming from the Philadelphia office. I marveled at their audacity to allow OTC sales of DHEA as a human "supplement," and then sending me a warning letter when I was researching the fundamental role of this same molecule in cancer. My dander up, I decided to press my complaint that DHEA should not be available as an OTC supplement for human use— as freely available as aspirin to anyone with money in hand, no matter what their age. In a new letter, I described the outlines of the kill switch tumor suppression mechanism based on circulating DHEAS, and used my analogy that DHEAS and DHEA are the Dr. Jekyll and Mr. Hyde of androgen biology in humans. Like the medical school

professor that I had been, I even sent the FDA enforcement office in Philadelphia an entire PowerPoint presentation lecturing them about the kill switch tumor suppression mechanism and the work my lab was doing to eventually advance it into clinical trials. Slide after slide drove home that 66 million years of evolution had put DHEAS at the center of kill switch tumor suppression mechanisms in both dogs and humans. I described how DHEAS circulating in the bloodstream is imported into cells that had suffered p53 inactivation, and in such cells— and such cells only— it was de-sulfated to DHEA,— completing a Dr. Jekyll to Mr. Hyde transformation. I lectured that this discovery of the kill switch was going to revolutionize the treatment and prevention of cancer both in dogs and in people, and had the potential to make cancer a rare disease again in both species.

In the next slides I began to scold that FDA's allowing of OTC sales of DHEA was dangerous, even reckless. I explained that, because DHEA could freely cross cell membranes and enter any cell, evolution had kept its circulating levels extremely low, many orders of magnitude below the level required to trigger the kill switch. This system had evolved over the entire 66 million years of primate evolution— *66 million years of evolution designed to make DHEA as toxic as possible*

— so ingesting DHEA directly short circuited this evolution, putting all the normal cells of the body at risk for kill switch activation!

I presented some advertisements clearly marketing DHEA to puberty aged-males, as well as to adult men participating in the sport of body building. Such advertising gives the false impression that ingesting pharmacologic amounts of DHEA can build muscles. It cannot. No study has shown this. Circulating levels of DHEA have nothing to do with the building of muscles. DHEA evolved as the killing element of our species' kill switch tumor suppression mechanism, and circulating levels of DHEA have little or nothing to do with the muscle hypertrophy that body builders seek. For that, androgen receptor numbers increase in muscles in response to the "injury" that previous heavy weight training has done to them. The muscles respond to this "injury" be expanding their number of androgen receptors, in effect trying to grow bigger to prevent injury the next time that they are exposed to the stress of lifting such heavy weights. The increased androgen receptor number is part of a response to stress that can include increased uptake of DHEAS from the circulation, which can be metabolized to testosterone in the stressed muscle tissue. This is how muscles are built, by increasing the number of androgen

receptors in stressed out muscle tissue. Not by ingesting pharmacological amounts of DHEA.

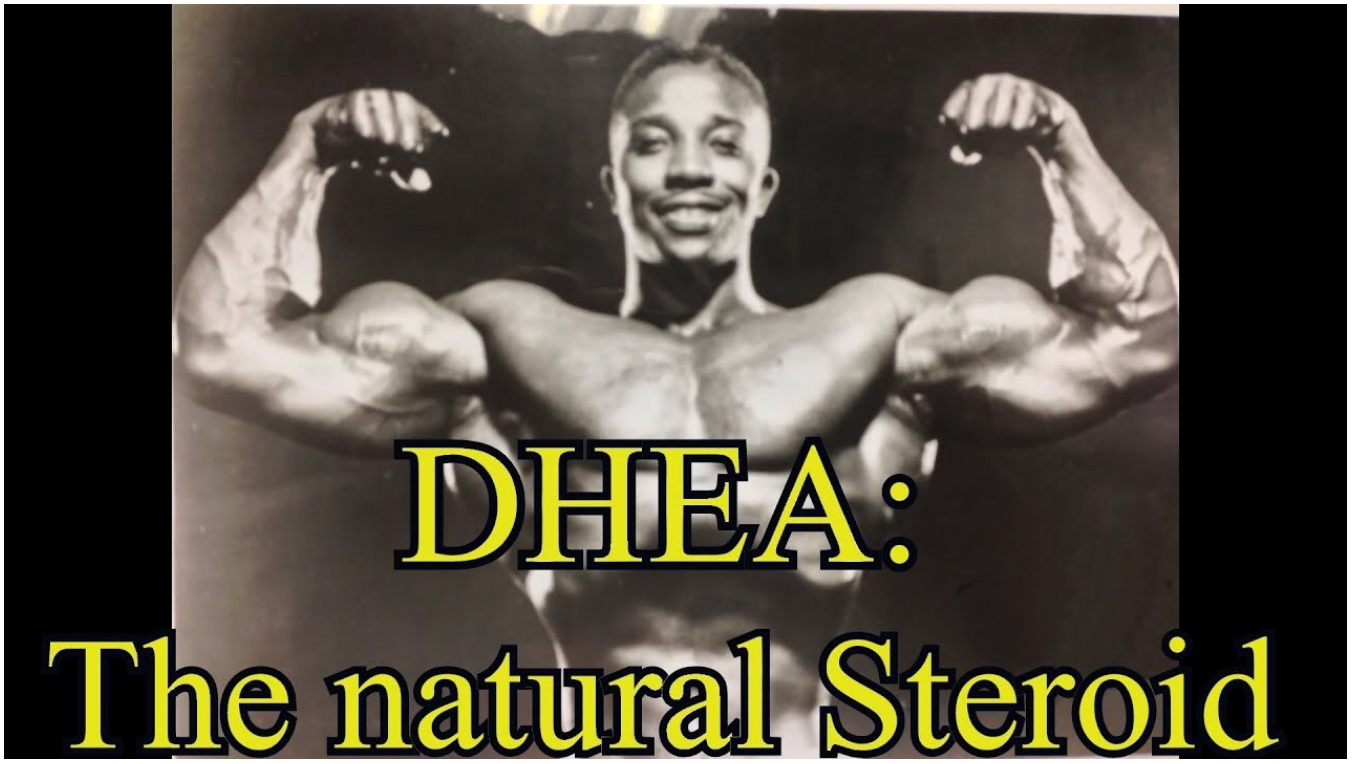

Figure 8.4 DHEA is the Mr. Hyde of androgen biology, and its direct ingestion in high amounts activates the kill switch in normal, healthy tissues. This advertisement, and others, touting DHEA as a body building steroid are easily found on the web .

Figure 8.5 The marketing of DHEA targets body builders and puberty-aged boys wishing to enhance their musculature. There is no evidence that the ingestion of pharmacological amounts of DHEA can produce increases in muscle mass.

Figure 8.6 There is no scientific data that ingesting pharmacologic amounts of DHEA improves muscle mass. Rather, vigorous weight training increases the number of androgen receptors in muscles in response to tissue "damage" caused by previous weight training. Then DHEAS is imported into those cells and converted into testosterone under carefully orchestrated intracellular conditions designed not to trigger the kill switch.

Adenosine depletion

DHEA can potentially have additional negative effects—beyond promiscuous kill switch triggering in normal cells. My eldest son, Alexander, and I investigated some of these. Alex, a

budding neuroscientist, received permission to complete a research course in my laboratory (incredibly, receiving such permission from one of the same professors who taught me when I was an undergraduate in the same department many decades before!) As part of that research project, we published a paper showing that in rats, administration of high dose DHEA led to depleted levels of adenosine in both the brain and heart.[219]

The fact that DHEA can deplete adenosine is important, because adenosine plays a critical role in an array of critical physiological processes, ranging from modulation of the firing of neurons,[220] to energy transfer, signal transduction, and DNA methylation,[221] as well as proper function of blood vessels and the cardiovascular system.[222] You yourself probably manipulate your adenosine pathway in your brain every day as part of your daily routine, because adenosine induces sleepiness by binding to certain of its receptors in the brain, and caffeine induces alertness by acting as an antagonist of adenosine at those receptors.

The adenosine system is evolutionarily ancient and underlies many of the most basic circuits of brain physiology among virtually all animals. This means that the same depletion of cardiac and brain adenosine caused by high dose DHEA in rats is extremely likely to also occur in humans exposed to

comparable doses on a body weight basis. Our point in the paper we published on DHEA-mediated adenosine depletion was that body builders, and boys trying to enhance the natural effects of puberty, would be negatively affecting some of the most important systems in their body if ingesting DHEA was their path to accomplish these effects. As noted above in the discussion of caffeine, adenosine exerts its actions *via* an array of receptors, named, respectively, A1, A2a, A2b, and A3. (You may recall that the RASON that I took into FDA clinical trials for human asthma targeted the adenosine A1 receptor. [223] As part of one of my doctoral student's PhD research my university lab had also shown that the adenosine A1 receptor was responsible for the effects of alcohol upon motor coordination— i.e., drunken behavior. [224]) People who deplete adenosine by ingesting pharmacological amounts of DHEA are essentially antagonizing all adenosine receptors, preventing their function. This can clearly have important consequences. For example, Gimenez-Llort and colleagues at the University of Barcelona demonstrated that adenosine A1 receptor knockout mice showed high levels of aggression and *reduced* muscle strength. [225] Similarly, when mice were injected with an adenosine A1 receptor *agonist* (a drug which binds to the A1 receptor and stimulates it, as adenosine binding would),

simulating supraphysiologic levels of adenosine with respect to this receptor, aggression measured *via* the Resident Intruder model was *abolished* . [226] Altogether, these studies suggest that the use of DHEA by body builders could explain some of the aggression that appears to be a side effect of anabolic steroid abuse. [227]

Because DHEA is a precursor to testosterone, its use is banned by the Olympics, the World Anti-Doping Agency, the National Collegiate Athletic Association (NCAA), the National Football League, and the National Basketball Association. Professional baseball has taken a different approach, permitting DHEA use by its athletes because FDA permits it to be sold OTC. Even if all of the blame for the OTC availability of DHEA cannot be placed on the FDA, [228] they are the government agency ultimately tasked with keeping dangerous drugs from harming the public. Our work shows very clearly the 66 million years of primate evolution has had as a major goal the honing of methods to make DHEA as toxic as possible— and because of that toxicity, its circulating levels are kept extremely low. Ingesting DHEA directly short circuits that evolution, and will trigger the kill switch in unintended normal cells, and is therefore very dangerous. I am certain that people are being

harmed by abusing DHEA, and I want to state plainly that FDA is failing in its responsibility to protect the public when it permits OTC sales of this steroid.

After the argument with the enforcement arm of the FDA, I eventually calmed down and went back to my work. At the top of my list was collecting the remaining canine tumors I would need to make an economical sequencer run. I had isolated mRNA from most of the tumors that I had already collected, and stored them in my deep freeze. Soon, very soon now, I would be able to analyze them for ME and IDT mutations, for the possibility of STS and OATP over expression, and for expression of the genes involved in the take up FDG and its metabolism to FD6P.

Outside of the births of my children, this was the most exciting period of my life.

9

Our solution to the spiraling cost of cancer drugs

"Large corporations, of course, are blinded by greed. The laws under which they operate require it - their shareholders would revolt at anything less."

Aaron Swartz

"Avarice is the spur of industry."

David Hume

"If we go on the way we have, the fault is our greed, and if we are not willing to change, we will disappear from the face of the globe."

Jacques Cousteau

The Business of Cancer

Cancer in our species has become big business. And it is a business that is growing by leaps and bounds. According to Mordor Intelligence, the global cancer therapy market alone (just cancer drugs) was valued at $136 b illion in 2018, and is estimated to be valued at $221 billion in 2024. [229] The MBAs at Big Pharma companies with cancer drugs in their portfolios are well aware that these numbers will continue to skyrocket in the decades to come. Thus, the World Health Organization (WHO) projects that by 2030, just a decade into the future, new cancer cases and cancer deaths will reach 24 million (from about 12 million in 2020) and 13 million (from about 7 million in 2020) per year, respectively. As the IARC has warned, these are not numbers that we have any chance of treating our way out of. But don't tell that to the Big Pharma companies focusing on cancer. To them this is not so much a cancer catastrophe as it is a fantastic market opportunity.

If we project the WHO figures forward, by the year 2084—100 years beyond the dystopian future predicted by George Orwell's novel *1984* — the number of new cancer cases

diagnosed *each year* will reach 136 million, and cancer deaths, 76 million, a truly dystopian future. Such numbers are far beyond the effective capacity of any conceivable health care system to deal with, and clearly have the potential to destabilize society. Do we choose by lottery which cancer patients will be treated? Do only the wealthy get treated? Or those of the party in power? To paraphrase Captain Cousteau, "If we go on the way we have [in cancer research], the fault is our greed, and if we are not willing to change, we will disappear from the face of the globe." This coming cancer catastrophe is every bit as big and looming an existential threat as global warming. But while global warming threatens all species, the coming cancer catastrophe is targeting only our species, and our dogs.

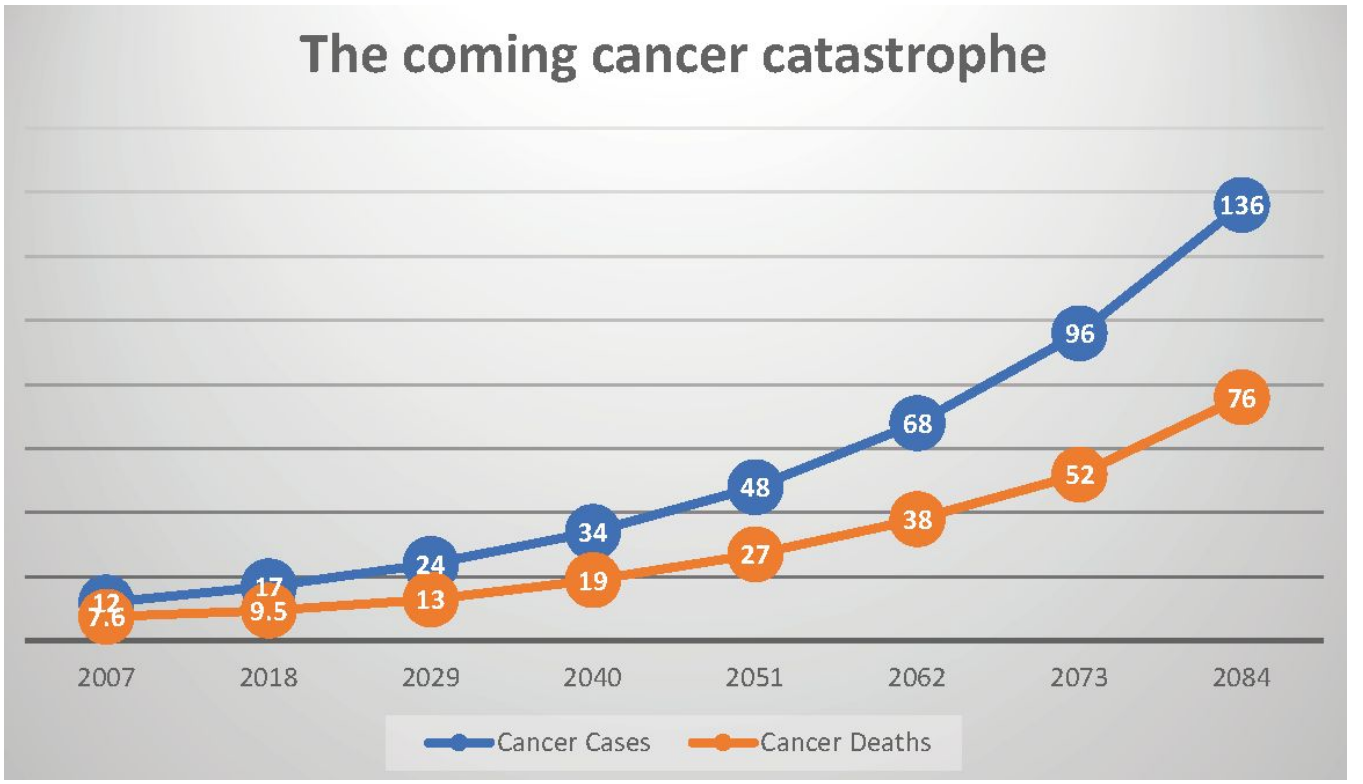

Figure 9.1 The coming cancer catastrophe is identified in the cold calculus of Big Pharma market projections as an enormous business opportunity. The plots above show the global number of human cancer cases and cancer deaths, in millions, projected out to 2084. This figure uses National Cancer Institute data for prior years, and WHO data projected out to 2030, and assumes that cancer patient survival is approaching the asymptotic boundary appearing in NCI statistics (National Cancer Institute SEER Cancer Statistics Review (CSR) 1975-2014. Updated June 28, 2017.

National Cancer Institute statistics report a dismal 7% improvement in two-year survival for cancer patients over the past 27 years, and further show that survival at every time point

(1, 2, 3, 4 and 5 years) appears to be at an asymptotic limit beyond which further improvement may not even be theoretically possible. Even the newly published cancer statistics reporting a decrease in death from cancer over the past several years do not blunt the figure below, because they primarily represent the dramatic decrease in smoking that occurred after the Surgeon General's warnings appeared on cigarette packs in 1965— not improvement in cancer chemotherapy results.

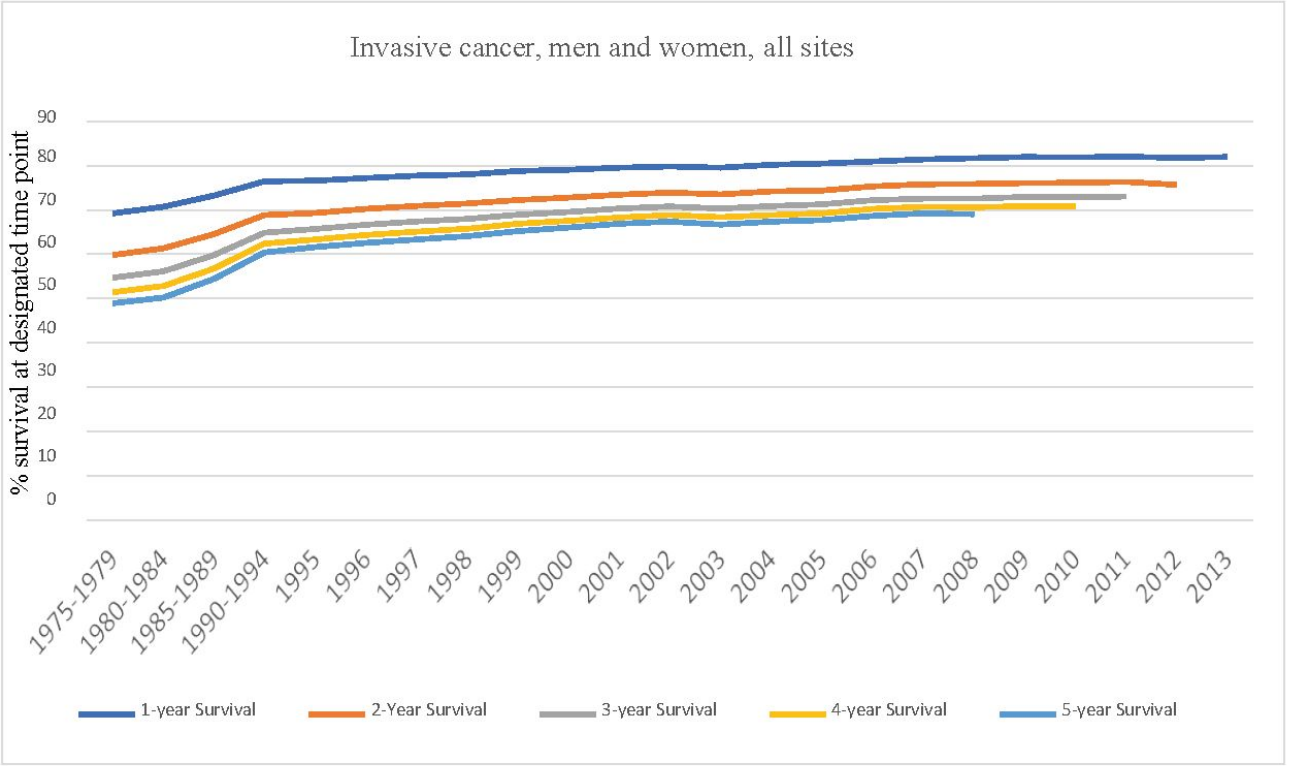

Figure 9.2 National Cancer Institute statistics show that survival at every time point has plateaued, appearing to have reached an asymptotic limit beyond which any further

improvement may not be possible. See Nyce 2018 [230] for citations and discussion.

Even if health care systems improved worldwide to a degree unimaginable in the current environment, there is still the expense of *paying* for cancer treatments. Consider *Kymriah* — a chimeric antigen receptor (CAR) T-cell therapy for the treatment of adolescent and young adult acute lymphoblastic leukemia— which is the *most expensive* cancer therapy ever, at $475,000 ($1,302/day). And *Oncaspar* , used to treat acute lymphoblastic leukemia, with an average wholesale price of $387,864 ($1,062/day). It appears that the current big pharma strategy is to target $1,000 per day as the cost (profit, really) of each of their new drugs, even when these drugs increase lifespan only minimally. For example, a recent "landmark study" of a drug called *Blincyto* , used in patients with Philadelphia chromosome negative Acute Lymphocytic Leukemia (ALL), showed an overall survival of 7.7 months, compared to 4.0 months using the prior standard of care chemo in this disease. The *Blincyto* treatment protocol for such ALL patients requires two courses of drug, at $89,000 per course, which equates to *$48,108 per extra month of life achieved* .

While the benefit of being treated with these expensive drugs is of course of immense value to the patient and the

patient's family— every moment of extra time with a loved one is precious— the dramatic and constantly escalating cost of such minimal life extension is unsustainable for society. Even if we could be certain that the accelerating increases in the cost of cancer medicines will level off in the future at the current cost of *Blincyto (* the greed of the businessmen of cancer makes this an extremely unlikely scenario), by 2050 the worldwide cost of keeping cancer patients alive *for one extra year of life* will already exceed $43 trillion, twice the current yearly revenues of the twenty richest countries in the world combined! Clearly, the IARC is correct. We cannot *treat* our way out of the coming cancer catastrophe. Alternative, preventative measures that reduce the occurrence of cancer in our species are required. [231]

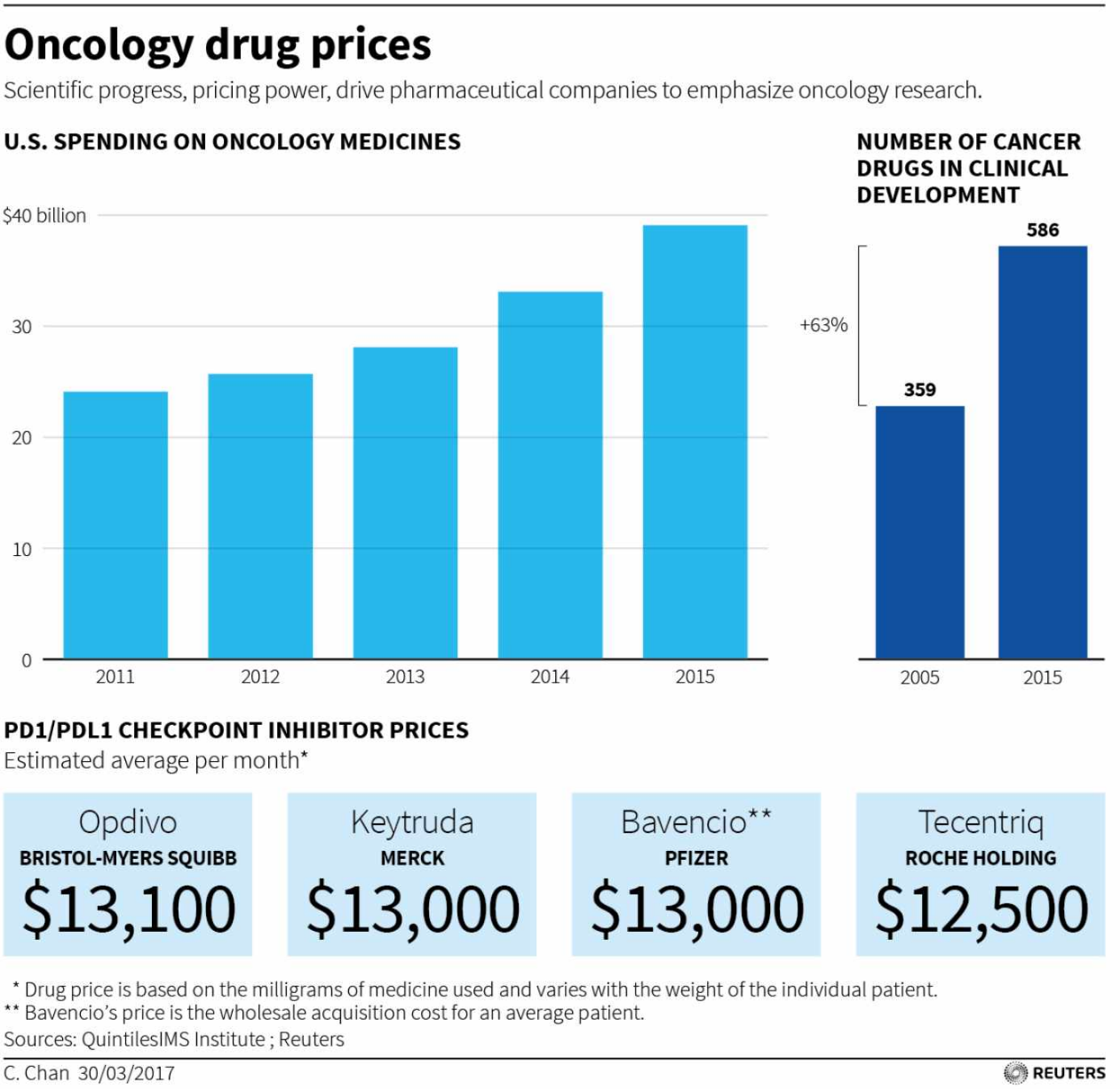

Figure 9.3 Pricing power drives pharmaceutical companies to emphasize oncology products. Figure courtesy Reuters.

Our solution to the unsustainable increases in cost of medicines for human cancer? ACGT will propose a new pricing model to the National Cancer Institute and the IARC for

medicines emanating from our kill switch program: Ten cents per patient per day

The major goal of my company, ACGT Biotech, Inc., is the "normalization" of lifetime cancer risk in our species from its current aberrant 40% to the 4% mandated by the *lex naturalis* .[232] This is thus a cancer *prevention* program. It is based upon our finding that the kill switch tumor suppression mechanism evolved in humans to protect during the 25 year lifespans that characterized our species for almost all of its existence. Circulating DHEAS levels thus peak at 25 years of age, and begin to sharply decline thereafter. Our plan is to "normalize" lifetime cancer risk by pharmacologically maintaining circulating DHEAS levels at their peak throughout the entire, expanded modern human lifespan. This means that *everyone in the world* should begin taking DHEAS as soon as their DHEAS levels begin to decline, at about age 29-30, and they will continue to take DHEAS for their entire life.

Instead of the Big Pharma pricing model of $1,000 per patient per day for their cancer medicines designed to treat existing tumors, extending life for some generally minimal period of time, we will propose to NCI and IARC that they can *prevent* tumors from occurring in the first place, by pharmacologically maintaining peak levels of circulating

DHEAS— compensating ACGT at the rate of just ten cents per patient per day for the license to do so— worldwide, for the benefit of everyone.

Because it would be administered to everyone after the age of 29-30, DHEAS would be the major drug driving down cancer costs in this model, with the potential to reduce cancer incidence by 90%. This would reduce the 24 million new cancer cases projected to occur in 2030, to 2.4 million—*almost 22 million people in that year alone who otherwise would have contracted cancer, but who will not because their kill switch tumor suppression mechanism was maintained* . Imagine the impact that preventing cancer in 22 million people a year would have, both economically and in the reduction of human suffering.

As part of this "deal," NCI and IARC (and their other worldwide partners) will also have full rights to our treatment protocols for human cancer. [233] Therefore, even the much reduced cancer patient population will have almost free access to our new kill switch-based treatment protocols. Many in this residual cancer patient population will have tumors with ME or IDT mutations, that over-express STS and OATPs, or in which FDG accumulation can be used to drive uncompetitive inhibition of G6PD to irreversibility selectively in tumor cells.

The tumors in these patients are predicted to be particularly sensitive to kill switch triggering, whittling down still further any need to resort to expensive and generally ineffective chemo.

Our goal, literally, is for the kill switch discovery to drive cancer completely off the list of common human diseases. The kill switch discovery is so fundamental to cancer, that this is clearly an achievable goal. As it appears also to be for our dogs.

The income from this kill switch licensing will be re-invested into pediatric cancer research

I find it to be one of the great ironies of the uncovering of the l*ex naturalis* , and of the identification of the components of the human-specific kill switch tumor suppression mechanism, that the medical benefits to be enjoyed from those discoveries appear to relate only to adult cancer. As you may recall, I had my beginning in clinical cancer research at the Children's Hospital of Los Angeles (CHLA), and I promised myself that I would return one day with something in hand to help children with cancer. I have yet to fulfil that promise. My family intends that our founder's shares will provide the funding for the

establishment and permanent funding of a pediatric cancer research institute and hospital, based in Central and South America, where the unmet need for such centers is very, very great. Based on the St. Jude model, no charges will be incurred by any family with a child treated for cancer at this facility, and we will develop a network to retrieve such sick children from their home territory. We hope to hire some of the best cancer clinicians in the world to treat these children with cancer. Because pediatric cancer is so different from adult cancer, we can expect about an 80% cure rate in our clinic, as is obtained at St. Jude, CHLA, etc. I will run a cancer research laboratory at the facility where we will try to improve the fate of the 20% of pediatric cancer patients who still now die of their disease. This is my next big goal.

If all of this happens, as I believe it will, it will only have happened because of the business model that we employed, using participant-investors to fund the early research. If we had gone to investment bankers, or to Big Pharma, they would *never* agree to the ten cents per patient per day pricing model that we are proposing. As I learned from hard experience, their greed literally knows no bounds, and our discovery would have been placed out of reach of all but the wealthy, and the properly insured— a description that applies to less than 10% of the

world's population. By keeping outside investment to a minimum, we have been able to maintain control of the company, and guide it toward the pricing model that will make it available to everyone, everywhere— and still leave us with the wherewithal to continue to expand our research.

We have made some enemies along the way, to be sure. Our discovery is so fundamental that it may mean the end of Big Pharma companies targeting cancer as their profit center. Such companies— like species subjected to a sudden change in climate, or to the loss of their primary food source— may simply disappear. If this happens, then it will also mean the end of the user fees that those companies would have paid to FDA —and that will put the agency in financial disarray. But this is what a big discovery does; what it is *supposed* to do— it changes the world as we know it, transforming it into something different; something better. The world that I envision once the *lex naturalis* is put into practice is a world in which cancer is a rare disease again, as it was in our primitive ancestors; and where it is a rare disease in our dogs again, as it was in their ancestral species— even in their ancestral breed, those first dogs that all looked pretty much the same, resembling small wolves.

If we are as successful as I think we are going to be, the business of cancer will all but disappear— and that will cause angst among those who have profited from cancer for so long. They will be angry at the prospect of this profit center evaporating overnight for them, to the point of potentially failing to see that its disappearance would be a *good* thing. To paraphrase Upton Sinclair, it is difficult to get a man to see the benefit in something, if his greed depends upon him not seeing it.

Conclusion

Our First Friend came into our human lives perhaps as long ago as 35,000 years, and has been helping us, protecting us, all that time, clearly with the intent to "be our friend for always, and always, and always." In this life you will have no truer friend than a dog that you take into your home, into your heart, into your life. To many of us, a house is not a home unless there is a dog living there with us. Now our First Friend has performed the most important act of friendship of all, revealing the *lex naturalis* to us and showing us the path forward to break free from the pincer grip of cancer. It was only by abandoning rodents, and adopting dogs with spontaneous cancer as our

model system that we were able to uncover the *lex naturalis* , and use it to identify the components of our own species-specific tumor suppression mechanism, and that of our dogs, and to understand why both we and our dogs are afflicted in such a profound way by cancer.

Just as there is resistance to these new discoveries from the businessmen of cancer, there has also been some resistance from those who have built their careers in cancer research using rodent models. I see no reason for that resistance, do not understand it, and am troubled by it. After all, the uncovering of the *lex naturalis* could never have happened except through understanding the total— the sum of all the parts— of what has transpired over the past 60 years of cancer research— 44 (and counting) years of which, I have been a small part of. A great pyramid of knowledge. Even where we went wrong, how would we have known except for the doing of it? The overall negative result of using mice and rats to discover and develop drugs for human cancer was thus perhaps the most illuminating piece of the entire puzzle. It was a dreadfully costly piece of the puzzle to bring into existence, but once JD received his treatment and cytotoxic chemotherapy became the focus for the next 75 years of cancer research, it is difficult to see how the *lex naturalis* would have been uncovered without that overall

negative result also coming into existence. To my fellow cancer research scientists I want to say, we *all* contributed necessary pieces to the solution of this puzzle. It was a team effort—even if, at times, it did not feel as if we were on the same team.

I have been at this a long time, and have known a lifetime of cancer research scientists, every one of them struggling to find the truth. Many of these heroic men and women are no longer with us, but that does not reduce their contribution even a little. They helped build the pyramid of knowledge that we got to stand on top of, and peer out over the landscape from, searching for the truth. And now we have found it, together. The *lex naturalis* equation is a victory to be shared by all cancer research scientists, living and dead; and I congratulate all of you. And to all of you, let me add this. When you go home this evening, after a long day at your lab, and your dog is the first to greet you, please give him a scratch behind the ears for me. He, or she, deserves it.

And then, tomorrow, first thing, let's get to work on this next great phase of cancer research.

10

Epilogue

“My job is to prevent the future.”
Ray Bradbury

Raided by the “firemen” of the FDA

This particular June morning started out perfectly. It was a gloriously sunny day, filled with promise. It was the day that the sequencing supplies would arrive, and I was filled with anticipation of what I would find in the canine tumor mRNA samples stored in my deep freeze. Just the weekend before, my children had gathered at my lab to celebrate my 66th birthday — Sammy and Alex who lived with me, and Trevor, who had

traveled up from Florida. I had been present for the birth of each of my children, and now they were here to celebrate with me the uncovering of the *lex naturalis,* and the concept of species-specific mechanisms of tumor suppression. It felt good.

I was particularly happy on this day because my eldest son, Alex, had received permission from his chairman in the biology department at Temple (who, incredibly, had been a signatory on my thesis some thirty-three years before!) to complete an independent research project in my laboratory which he needed to graduate. Alex is a budding neuroscientist, and we were going to do a DNA sequencing project to understand the molecular underpinnings of the role that adenosine receptors play in ethanol-induced intoxication. I was so proud to be helping him with this project. Little did I know as I pulled into the parking lot at my lab, this dream was about to be crushed.

At this point in the *lex naturalis* story I still had not yet pieced together all of the details of what I have described in this book. Analysis of the tumor mRNA samples should tell me if canine tumors were usurping for their own use parts of the rudimentary form of the kill switch tumor suppression mechanism in dogs. This would be very exciting, because if the same thing happened in human tumors (I now know that it does), reactivation of the kill switch with DHEAS should kill

even developed tumors. Earlier in this book I gave the example of steroid sulfatase, the enzyme that de-sulfates DHEAS (Dr. Jekyl) into DHEA (Mr. Hyde) inside cells in which p53 had been inactivated. I had isolated several of the RNA samples from canine mammary tumors, and hoped to find steroid sulfatase over-expressed in them. Such a finding would represent a critical milestone in the application of my kill switch tumor suppression discovery in the treatment of tumors that had already developed. If human breast tumors showed the same usurpation of steroid sulfatase (because the kill switch had failed due to inadequate circulating levels of DHEAS), then the DHEAS analog fluasterone sulfate might act as a magic bullet in human breast cancer, driving metastases from them into ROS-induced apoptosis. It was hard to contain my excitement over all of these possibilities.

Some of my friends had warned that what I was proposing would make me a target for *everyone* profiting from the business of cancer, from Big Pharma to the FDA. But I had laughed off their worries. *Everyone* should want to bring an end to cancer. Who could possibly be against the normalization of lifetime cancer risk from 40% to 4%? What I had uncovered wasn't a cure for cancer. It was *better* than a cure. It was prevention. It would be insane to oppose it.

Well, as it turns out, the world is insane. At least small, aberrant parts of it are.

On this beautiful June day, as Alex and I walked toward the front door of ACGT, we were suddenly surrounded by a heavily armed contingent of FDA agents, seemingly jumping out of the bushes, and from behind every tree. They descended upon us from everywhere, about ten of them. I had no idea what was going on, but we were soon told to sit on the sofa in the vestibule of the lab, and not to move. Gestapo-like, they began to collect everything from my lab— computers, external hard drives, lab notebooks, the messengerRNA samples from my deep freeze; everything. While they were stripping my lab of everything I needed to do my research, a Fed Ex delivery man arrived with the DNA sequencing reagents that I had ordered in order to perform the transcriptome analysis on the dog tumors. The FDA agents seized these reagents too, making sure that I could not continue my research.

At one point I was taken into the back lab, and the agent leading the invasion of my lab pointed to my DNA sequencer, asking “what’s this?” I explained that it was a next generation DNA sequencer that I was going to use to sequence the messenger RNA isolated from the canine tumors in my deep freeze. Then he pointed to the huge server that operated the

sequencing programs, “and that?” I explained what it was. “Take it,” he ordered one of his assistants, who apparently was in charge of the IT part of their operation. “I’m not touching that,” the assistant said, refusing the order.

I would later learn that the agent who led the raid had conducted it without the knowledge of the Philadelphia FDA office that I had been arguing with over the warning letter that they had sent me. After the FDA raid on my laboratory, I had immediately called the head of the endowment that had provided the funding for the DNA sequencer, and they had contacted and spoken to the person who had sent the warning letter to me. This person told my funding agency that *no action had been taken against me* — she had been kept in the dark about the raid that agents of her own agency had planned and executed.

In a bizarre twist to this story, bracketing the time that the “firemen” were conducting their destructive raid on my laboratory, two separate groups of FDA scientists were using my work to advance their own research programs, citing my work in their publications. One of these groups was working in collaboration with scientists at MIT (Massachusetts Institute of Technology), developing a high throughput assay of the global DNA methylation status of drug-exposed cells, a platform

based upon my discovery of drug-induced DNA hypermethylation and the role it plays in cancer drug resistance:

Environ Mol Mutagen. 2017 Aug; 58(7): 508–521.

The Development and Validation of EpiComet-Chip, a Modified High-Throughput Comet Assay for the Assessment of DNA Methylation Status

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Introduction

DNA methylation is an epigenetic modification that provides a stable gene silencing mechanism that, along with histone modification, plays an important role in regulating gene expression and maintaining genome stability (Bird 2002). DNA methylation primarily occurs by the covalent modification of cytosine residues in CpG dinucleotides, to yield 5-methylcytosine (5-mC), and in general, DNA global methylation status refers to the overall content of 5-mC in the genome, expressed as a percentage (5-mC%) (Waggoner 2007). Several normal biological processes (Reik 2007), environmental exposures (Feil and Fraga 2011; Benayoun et al. 2015) and pharmaceutical compounds (Csoka and Szyf 2009) have been shown to alter DNA methylation status in a variety of reproducible and predictable patterns. For example, treatment with cisplatin (Nyce 1989; Nyce et al. 1993), nalidixic acid (Nyce 1989), hydroxyurea (Nyce 1989) and higher dose methotrexate (MTX) (Nyce 1989; Nyce et al. 1993) have individually been shown to increase global methylation; whereas 5-azacytidine (5-Aza) (Christman 2002), procainamide (Cornacchia et al. 1988; Lee et al. 2005), 4,6-dioxoheltanoic acid (SA) (Wentzel et al. 2010; Lewies et al. 2014), tamoxifen (Wu et al. 2005; Tryndyak et al. 2006), valproic acid (Detich et al. 2003; Cribbs et al. 2015) and hydralazine (Cornacchia et al. 1988) exposures have each conversely resulted in decreased global methylation in defined physiological contexts.

Citing:

Nyce J. Drug-induced DNA hypermethylation and drug resistance in human tumors. Cancer Res. 1989;49(21):5829–5836. [PubMed] [Google Scholar]

Nyce J, Leonard S, Canupp D, Schulz S, Wong S. Epigenetic mechanisms of drug resistance: drug-induced DNA hypermethylation and drug resistance. Proc Natl Acad Sci U S A. 1993;90(7):2960–2964. [PMC free article] [PubMed] [Google Scholar]

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PMCID: PMC3644411

PMID: 23675285

Role of DNA Repair Pathways in Response to Zidovudine-induced DNA Damage in Immortalized Human Liver THLE2 Cells

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Abstract

The nucleoside reverse transcriptase inhibitor zidovudine (3'-azido-3'-dexoythymidine, AZT) can be incorporated into DNA and cause DNA damage. Previously, we determined that the human hepatocellular carcinoma HepG2 cells are more susceptible to AZT-induced toxicities than the immortalized normal human liver THLE2 cells and the nucleotide excision repair (NER) pathway plays an essential role in the response to AZT-induced DNA damage.

Citing:

30. Nyce J, Leonard S, Canupp D, et al. Epigenetic mechanisms of drug-resistance: drug-induced DNA hypermethylation and drug-resistance. Proc. Natl. Acad. Sci. USA. 1993;90:2960–2964. [PMC free article] [PubMed] [Google Scholar]

What kind of upside down, insane world have I found myself in? While one section of FDA is relying on my published work to advance their own research, and another section of FDA is helping me enter my veterinary research into one of their special programs, yet another section raids my lab and confiscates my research materials so that further progress is

impossible. This latter group is so thoughtless, acting without any discernible conscience, that they probably cannot even realize the harm that they have done. This is a war, after all, a War on Cancer, and lives are at stake—both canine and human lives. Lives have already been lost because of the delays in my research caused by the raid on my laboratory by the firemen of FDA. Were it not for their senseless action, I would already have completed FDA clinical trials for canine cardiac hemangiosarcoma (and possibly all four of the other veterinary indications approved for special programs by the scientific arm of FDA), *and would be in clinical trials for human cardiac angiosarcoma* . How will the FDA bureaucracy be held to account if triggering the kill switch works as well in human cardiac angiosarcoma as it does in the identical disease in dogs? The delay that they caused by the confiscation of my research materials will then be proven to actually have killed people—hundreds of people here in the United States, perhaps thousands worldwide. Will they remain oblivious, conscienceless even then? Probably. And imagine the different, better world that we will all be living in if pharmacological maintenance of DHEAS normalizes lifetime cancer risk in humans and dogs. If the *lex naturalis* is correct— and there is so much suggesting that it is — and maintaining DHEAS levels throughout the modern

lifespan normalizes human cancer risk to 4%— *you* may be spared the cancer diagnosis that you would otherwise have received. Over time, hundreds of millions of people would be spared such a diagnosis. But there will also be those of you who may now receive an *unnecessary* cancer diagnosis— a diagnosis brought about by the delay of my research caused by the seizure of my research materials.

* * *

As a bin filled with my notebooks was carried out and loaded into one of the FDA police vans, I realized that these cops were in actuality "firemen" right out of *Fahrenheit 451* , Ray Bradbury's story about a despotic government's response to dangerous new ideas. Fahrenheit 451, of course, refers to the temperature at which paper burns. In Bradbury's work, it was the job of "firemen" to seize and burn all books, because books contain ideas, and new ideas are dangerous to the *status quo* . I felt like my son and I had somehow been transported to the world of *Fahrenheit 451* , and these "firemen" were here to seize my books, pile them into a great bonfire and burn them to cinders. My ideas were too combustible. They posed a threat to the established order. They had to be destroyed, and these "firemen" were here to do just that; to destroy them. They were

here to make certain that my ideas would never see the light of day. They were here to make sure that I could not trouble the people with my heretic, unsettling notions. These “firemen” were here to maintain the *status quo* .

> *Give the people contests they win by remembering the words to popular songs or the names of state capitals or how much corn Iowa grew last year. Cram them full of noncombustible data, chock them so damned full of ‘facts’ they feel stuffed, absolutely ‘brilliant’ with information. Then they’ll feel they’re thinking, they’ll get a* sense *of motion without actually moving. And they’ll be happy, because facts of that sort don’t change. [Facts of that sort are harmless. They pose no threat to the* status quo . *Facts of that sort, Montag, are the opposite of combustible. They are the* only *ones the people should be permitted to know.]”*
> *Ray Bradbury,* Fahrenheit 451

This strike against me by the “firemen” of FDA was designed to cripple my ability to continue my research. Research materials that took years to collect— the canine tumor RNA samples from my deep freeze— are essentially irreplaceable. Even if the “firemen” gave them back, they would be in

unusable condition. The scientific division of FDA still appears to be behind me, helping me, approving my requests for entry into special clinical programs. But the "firemen" from the other half of the same agency have effectively destroyed my ability to continue my research; at least for the moment. I have written this epilogue in a last-ditch effort to bring my combustible facts to you.

What I have uncovered *is* a threat to the *status quo* . It *does* criticize the FDA, and the path that the FDA has forced cancer research to travel down. It *is* combustible to the business of cancer. To test it clinically *will* completely disrupt the *status quo* of cancer research, for many years into the future. But the combustible facts that I have uncovered are true facts, and they point out extreme danger—the ice burg is straight ahead, and we *will* strike it if what is now the Titanic ship of cancer research is not turned around, re-directed by the *lex naturalis* . If the discovery described in this book can be brought to bear against human cancer, and it successfully "normalizes" cancer risk in our species, that would represent a death knell to the business of cancer. And to the user fees collected by the FDA on that business. That is why these "firemen," these hit men, raided my laboratory. But the FDA was never intended to act as

hit men in the interests of Big Pharma— or even in their own interests.

I am a veteran soldier in the War on Cancer, and, as unlikely as it may seem to the businessman of cancer, and the businessmen of the FDA, I have made what could very well be the discovery that removes cancer from the list of common diseases. The only way to know for sure is to do the clinical trials that I had been pushing towards before the raid on my lab. I hope that you will get behind me as I push this discovery forward through the obstacles that have been put in front of me.

Notes

The Canine Cancer Epidemic

[1] Nyce J. W. (2018). Detection of a novel, primate-specific 'kill switch' tumor suppression mechanism that may fundamentally control cancer risk in humans: an unexpected twist in the basic biology of TP53. *Endocrine-related cancer* , *25* (11), R497–R517. https://doi.org/10.1530/ERC-18-0241

Nyce J. W. (2019). Species-specific mechanisms of tumor suppression are fundamental drivers of vertebrate speciation: critical implications for the 'war on cancer'. *Endocrine-related cancer* , *26* (2), C1–C5. https://doi.org/10.1530/ERC-18-0468

Nyce J. W. (2020). A lex naturalis delineates components of a human-specific, adrenal androgen-dependent, p53-mediated 'kill switch' tumor suppression mechanism. *Endocrine-related cancer* , *27* (2), R51–R65. https://doi.org/10.1530/ERC-19-0382

Nyce J 2019 Components of the human-specific, p53-mediated "kill switch" tumor suppression mechanism are usurped by human tumors, creating the possibility of therapeutic exploitation. *Cancer Drug Resist* 2019;2:1207-1214.10.20517/cdr.2019.89
https://cdrjournal.com/article/view/3293

and about a hundred additional papers published in the peer-reviewed literature, dating all the way back to this earliest one:

Lotlikar, P. D., Dwyer, E. N., Baldy, W. J., Jr, & Nyce, J. (1977). Phospholipid requirement for 2-acetamidofluorene N-and ring-hydroxylation by hamster liver microsomal cytochrome P-450 enzyme

system. *The Biochemical journal* , *168* (3), 571–574. https://doi.org/10.1042/bj1680571

[2] With the exception of a contagious form of cancer that is decimating the Tasmanian Devil.
See: Kosack, L., Wingelhofer, B., Popa, A., Orlova, A., Agerer, B., Vilagos, B., Majek, P., Parapatics, K., Lercher, A., Ringler, A., Klughammer, J., Smyth, M., Khamina, K., Baazim, H., de Araujo, E. D., Rosa, D. A., Park, J., Tin, G., Ahmar, S., Gunning, P. T., … Bergthaler, A. (2019). The ERBB-STAT3 Axis Drives Tasmanian Devil Facial Tumor Disease. *Cancer cell* , *35* (1), 125–139.e9. https://doi.org/10.1016/j.ccell.2018.11.018

[3] https://seer.cancer.gov/statfacts/html/all.html

[4] Abegglen, L. M., Caulin, A. F., Chan, A., Lee, K., Robinson, R., Campbell, M. S., Kiso, W. K., Schmitt, D. L., Waddell, P. J., Bhaskara, S., Jensen, S. T., Maley, C. C., & Schiffman, J. D. (2015). Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans. *JAMA* , *314* (17), 1850–1860. https://doi.org/10.1001/jama.2015.13134

[5] Figure 6 in: Nyce J. W. (2018). Detection of a novel, primate-specific 'kill switch' tumor suppression mechanism that may fundamentally control cancer risk in humans: an unexpected twist in the basic biology of TP53. *Endocrine-related cancer* , *25* (11), R497–R517. https://doi.org/10.1530/ERC-18-0241

[6] See Sean Carroll's, Preposterous Universe podcast: https://www.preposterousuniverse.com/podcast/2019/12/16/77-azra-raza-on-the-way-we-should-fight-cancer/

The origin of our dogs

[7] Wang, G. D., Fan, R. X., Zhai, W., Liu, F., Wang, L., Zhong, L., Wu, H., Yang, H. C., Wu, S. F., Zhu, C. L., Li, Y., Gao, Y., Ge, R. L., Wu,

C. I., & Zhang, Y. P. (2014). Genetic convergence in the adaptation of dogs and humans to the high-altitude environment of the tibetan plateau. *Genome biology and evolution* , *6* (8), 2122–2128. https://doi.org/10.1093/gbe/evu162

[8] Axelsson, E., Ratnakumar, A., Arendt, M. L., Maqbool, K., Webster, M. T., Perloski, M., Liberg, O., Arnemo, J. M., Hedhammar, A., & Lindblad-Toh, K. (2013). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* , *495* (7441), 360–364. https://doi.org/10.1038/nature11837

[9] Wang, G. D., Zhai, W., Yang, H. C., Fan, R. X., Cao, X., Zhong, L., Wang, L., Liu, F., Wu, H., Cheng, L. G., Poyarkov, A. D., Poyarkov, N. A., Jr, Tang, S. S., Zhao, W. M., Gao, Y., Lv, X. M., Irwin, D. M., Savolainen, P., Wu, C. I., & Zhang, Y. P. (2013). The genomics of selection in dogs and the parallel evolution between dogs and humans. *Nature communications* , *4* , 1860. https://doi.org/10.1038/ncomms2814

[10] http://digimorph.org/specimens/Canis_lupus_lycaon/

[11] Miller G. (2010). Epigenetics. The seductive allure of behavioral epigenetics. *Science (New York, N.Y.)* , *329* (5987), 24–27. https://doi.org/10.1126/science.329.5987.24

[12] Banlaki, Z., Cimarelli, G., Viranyi, Z., Kubinyi, E., Sasvari-Szekely, M., & Ronai, Z. (2017). DNA methylation patterns of behavior-related gene promoter regions dissect the gray wolf from domestic dog breeds. *Molecular genetics and genomics : MGG* , *292* (3), 685–697. https://doi.org/10.1007/s00438-017-1305-5

Janowitz Koch, I., Clark, M. M., Thompson, M. J., Deere-Machemer, K. A., Wang, J., Duarte, L., Gnanadesikan, G. E., McCoy, E. L., Rubbi, L., Stahler, D. R., Pellegrini, M., Ostrander, E. A., Wayne, R. K., Sinsheimer, J. S., & vonHoldt, B. M. (2016). The concerted impact of domestication and transposon insertions on methylation patterns between dogs and grey wolves. *Molecular ecology* , *25* (8), 1838–1855. https://doi.org/10.1111/mec.13480

[13] Frantz, L. A., Mullin, V. E., Pionnier-Capitan, M., Lebrasseur, O., Ollivier, M., Perri, A., Linderholm, A., Mattiangeli, V., Teasdale, M. D., Dimopoulos, E. A., Tresset, A., Duffraisse, M., McCormick, F., Bartosiewicz, L., Gál, E., Nyerges, É. A., Sablin, M. V., Bréhard, S., Mashkour, M., Bălăşescu, A., … Larson, G. (2016). Genomic and archaeological evidence suggest a dual origin of domestic dogs. *Science (New York, N.Y.)* , *352* (6290), 1228–1231. https://doi.org/10.1126/science.aaf3161

[14] Botigué, L. R., Song, S., Scheu, A., Gopalan, S., Pendleton, A. L., Oetjens, M., Taravella, A. M., Seregély, T., Zeeb-Lanz, A., Arbogast, R. M., Bobo, D., Daly, K., Unterländer, M., Burger, J., Kidd, J. M., & Veeramah, K. R. (2017). Ancient European dog genomes reveal continuity since the Early Neolithic. *Nature communications* , *8* , 16082. https://doi.org/10.1038/ncomms16082

[15] https://www.sciencemag.org/news/2015/05/arctic-find-confirms-ancient-origin-dogs

[16] Skoglund, P., Ersmark, E., Palkopoulou, E., & Dalén, L. (2015). Ancient wolf genome reveals an early divergence of domestic dog ancestors and admixture into high-latitude breeds. *Current biology : CB* , *25* (11), 1515–1519. https://doi.org/10.1016/j.cub.2015.04.019

[17] Botigué, L. R., Song, S., Scheu, A., Gopalan, S., Pendleton, A. L., Oetjens, M., Taravella, A. M., Seregély, T., Zeeb-Lanz, A., Arbogast, R. M., Bobo, D., Daly, K., Unterländer, M., Burger, J., Kidd, J. M., & Veeramah, K. R. (2017). Ancient European dog genomes reveal continuity since the Early Neolithic. *Nature communications* , *8* , 16082. https://doi.org/10.1038/ncomms16082

[18] Freedman, A. H., Gronau, I., Schweizer, R. M., Ortega-Del Vecchyo, D., Han, E., Silva, P. M., Galaverni, M., Fan, Z., Marx, P., Lorente-Galdos, B., Beale, H., Ramirez, O., Hormozdiari, F., Alkan, C., Vilà, C., Squire, K., Geffen, E., Kusak, J., Boyko, A. R., Parker, H. G., … Novembre, J. (2014). Genome sequencing highlights the

dynamic early history of dogs. *PLoS genetics* , *10* (1), e1004016. https://doi.org/10.1371/journal.pgen.1004016

[19] Yokoyama, Y., Lambeck, K., De Deckker P, Johnston, P., & Fifield, L. K. (2000). Timing of the Last Glacial Maximum from observed sea-level minima. *Nature* , *406* (6797), 713–716. https://doi.org/10.1038/35021035

[20] 50,000-year-old footprints of a *Smilodon* hunting the coast line of what is now Miramar, Argentina. These tracks were discovered by members of the Museo Municipal Punta Hermengo, and were highlighted in a vertebrate paleontology conference held in Buenos Aires . https://2.bp.blogspot.com/-_edSmHbh7Fs/WQOL3PJJ_HI/AAAAAAAAAB_c/riHoNDqu-VowYHvXOa58eCqs7BNMvEeTACLcB/s1600/the%2Bfirst%2Bever%2Bdiscovered%2Bfrom%2Bthe%2Bsabertoothed%2Bcat%2BSmilodon%2Bfootprint.JPG

[21] Other possibilities for the extinction of the mammoth steppe megafauna about 12,000 years ago include the change from mammoth steppe vegetation, dominated by highly nutritious forbs, to grasses that could grow in the new, warming, moistening climate. Such grasses have much less nutritional value than forbs, and may not have been able to sustain breeding populations of the megafauna.

[22] http://www.bbc.co.uk/nature/20031460

[23] See, for example, Alexandra Horowitz's book, "To Be a Dog ."

[24] Shipman, Pat. The Invaders: How humans and their dogs drove Neanderthals to extinction .

[25] Thompkins, A. M., Ramaiahgari, B., Zhao, S., Gotoor, S., Waggoner, P., Denney, T. S., Deshpande, G., & Katz, J. S. (2018). Separate brain areas for processing human and dog faces as revealed by awake fMRI in dogs (Canis familiaris). *Learning & behavior* , *46* (4), 561–573. https://doi.org/10.3758/s13420-018-0352-z

[26] Charalampopoulos, I., Remboutsika, E., Margioris, A. N., & Gravanis, A. (2008). Neurosteroids as modulators of neurogenesis and neuronal survival. *Trends in Endocrinology and Metabolism: TEM* , *19* (8), 300–307. https://doi.org/10.1016/j.tem.2008.07.004
Dong, Y., & Zheng, P. (2012). Dehydroepiandrosterone sulphate: action and mechanism in the brain. *Journal of Neuroendocrinology* , *24* (1), 215–224. https://doi.org/10.1111/j.1365-2826.2011.02256.x
Zheng P. (2009). Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. *Progress in neurobiology* , *89* (2), 134–152. https://doi.org/10.1016/j.pneurobio.2009.07.001

[27] Nguyen, T. V., Wu, M., Lew, J., Albaugh, M. D., Botteron, K. N., Hudziak, J. J., Fonov, V. S., Collins, D. L., Campbell, B. C., Booij, L., Herba, C., Monnier, P., Ducharme, S., & McCracken, J. T. (2017). Dehydroepiandrosterone impacts working memory by shaping cortico-hippocampal structural covariance during development. *Psychoneuroendocrinology* , *86* , 110–121. https://doi.org/10.1016/j.psyneuen.2017.09.013
do Vale, S., & Escera, C. (2018). Dehydroepiandrosterone and Dehydroepiandrosterone-Sulfate and Emotional Processing. *Vitamins and hormones* , *108* , 413–441. https://doi.org/10.1016/bs.vh.2018.01.022

[28] In most primate species, DHEAS is synthesized and secreted by the adrenal gland in both sexes. While male dogs synthesize DHEAS primarily in their testicles, in female dogs DHEAS is primarily synthesized in their adrenals. See:
Mongillo, P., Prana, E., Gabai, G., Bertotto, D., & Marinelli, L. (2014). Effect of age and sex on plasma cortisol and dehydroepiandrosterone concentrations in the dog (Canis familiaris). *Research in veterinary science* , *96* (1), 33–38. https://doi.org/10.1016/j.rvsc.2013.10.010

[29] Nyce JW. (2020) ASCENT: the extraordinary new story of the origin of our species.
Nyce J. W. (2020). A *lex naturalis* delineates components of a human-

specific, adrenal androgen-dependent, p53-mediated 'kill switch' tumor suppression mechanism. *Endocrine-related cancer* , *27* (2), R51–R65. https://doi.org/10.1530/ERC-19-0382

Nyce J. W. (2018). Detection of a novel, primate-specific 'kill switch' tumor suppression mechanism that may fundamentally control cancer risk in humans: an unexpected twist in the basic biology of TP53. *Endocrine-related cancer* , *25* (11), R497–R517. https://doi.org/10.1530/ERC-18-0241

Nyce J. W. (2019). Species-specific mechanisms of tumor suppression are fundamental drivers of vertebrate speciation: critical implications for the 'war on cancer'. *Endocrine-related cancer* , *26* (2), C1–C5. https://doi.org/10.1530/ERC-18-0468

[30] Cornish-Bowden A. (1986). Why is uncompetitive inhibition so rare? A possible explanation, with implications for the design of drugs and pesticides. *FEBS letters* , *203* (1), 3–6. https://doi.org/10.1016/0014-5793(86)81424-7

[31] Hainaut, P., & Pfeifer, G. P. (2001). Patterns of p53 G—>T transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. *Carcinogenesis* , *22* (3), 367–374. https://doi.org/10.1093/carcin/22.3.367

[32] Mayo Clinic, https://www.mayoclinic.org/diseases-conditions/gout/symptoms-causes/syc-20372897

[33] Itahana, Y., Han, R., Barbier, S., Lei, Z., Rozen, S., & Itahana, K. (2015). The uric acid transporter SLC2A9 is a direct target gene of the tumor suppressor p53 contributing to antioxidant defense. *Oncogene* , *34* (14), 1799–1810. https://doi.org/10.1038/onc.2014.119

[34] Domingo, J. L., & Nadal, M. (2017). Carcinogenicity of consumption of red meat and processed meat: A review of scientific news since the IARC decision. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* , *105* , 256–261. https://doi.org/10.1016/j.fct.2017.04.028

[35] Turesky R. J. (2018). Mechanistic Evidence for Red Meat and Processed Meat Intake and Cancer Risk: A Follow-up on the International Agency for Research on Cancer Evaluation of 2015. *Chimia* , *72* (10), 718–724. https://doi.org/10.2533/chimia.2018.718
Chiang, V. S., & Quek, S. Y. (2017). The relationship of red meat with cancer: Effects of thermal processing and related physiological mechanisms. *Critical reviews in food science and nutrition* , *57* (6), 1153–1173. https://doi.org/10.1080/10408398.2014.967833

[36] Pruetz, J. D., & LaDuke, T. C. (2010). Brief communication: Reaction to fire by savanna chimpanzees (Pan troglodytes verus) at Fongoli, Senegal: Conceptualization of "fire behavior" and the case for a chimpanzee model. *American journal of physical anthropology* , *141* (4), 646–650. https://doi.org/10.1002/ajpa.21245

[37] Schiebinger, R. J., Albertson, B. D., Barnes, K. M., Cutler, G. B., Jr, & Loriaux, D. L. (1981). Developmental changes in rabbit and dog adrenal function: a possible homologue of adrenarche in the dog. *The American journal of physiology* , *240* (6), E694–E699. https://doi.org/10.1152/ajpendo.1981.240.6.E694

[38] Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychological review* , *107* (3), 411–429. https://doi.org/10.1037/0033-295x.107.3.411

[39] Campbell B. (2006). Adrenarche and the evolution of human life history. *American journal of human biology : the official journal of the Human Biology Council* , *18* (5), 569–589. https://doi.org/10.1002/ajhb.20528

[40] Pilley, J. W., & Reid, A. K. (2011). Border collie comprehends object names as verbal referents. *Behavioural processes* , *86* (2), 184–195. https://doi.org/10.1016/j.beproc.2010.11.007
Kaminski, J., Call, J., & Fischer, J. (2004). Word learning in a domestic dog: evidence for "fast mapping". *Science (New York, N.Y.)* , *304* (5677), 1682–1683. https://doi.org/10.1126/science.1097859

[41] Nagasawa, M., Mitsui, S., En, S., Ohtani, N., Ohta, M., Sakuma, Y., Onaka, T., Mogi, K., & Kikusui, T. (2015). Social evolution. Oxytocin-gaze positive loop and the coevolution of human-dog bonds. *Science (New York, N.Y.)* , *348* (6232), 333–336. https://doi.org/10.1126/science.1261022

[42] vonHoldt, B. M., Shuldiner, E., Koch, I. J., Kartzinel, R. Y., Hogan, A., Brubaker, L., Wanser, S., Stahler, D., Wynne, C., Ostrander, E. A., Sinsheimer, J. S., & Udell, M. (2017). Structural variants in genes associated with human Williams-Beuren syndrome underlie stereotypical hypersociability in domestic dogs. *Science advances* , *3* (7), e1700398. https://doi.org/10.1126/sciadv.1700398
vonHoldt, B. M., Ji, S. S., Aardema, M. L., Stahler, D. R., Udell, M., & Sinsheimer, J. S. (2018). Activity of Genes with Functions in Human Williams-Beuren Syndrome Is Impacted by Mobile Element Insertions in the Gray Wolf Genome. *Genome biology and evolution* , *10* (6), 1546–1553. https://doi.org/10.1093/gbe/evy112

[43] Codina-Sola, M., Costa-Roger, M., Pérez-García, D., Flores, R., Palacios-Verdú, M. G., Cusco, I., & Pérez-Jurado, L. A. (2019). Genetic factors contributing to autism spectrum disorder in Williams-Beuren syndrome. *Journal of medical genetics* , *56* (12), 801–808. https://doi.org/10.1136/jmedgenet-2019-106080

[44] Crespi, B. J., & Procyshyn, T. L. (2017). Williams syndrome deletions and duplications: Genetic windows to understanding anxiety, sociality, autism, and schizophrenia. *Neuroscience and biobehavioral reviews* , *79* , 14–26. https://doi.org/10.1016/j.neubiorev.2017.05.004

[45] Işık Ü, Bilgiç A, Toker A, Kılınç I. Serum levels of cortisol, dehydroepiandrosterone, and oxytocin in children with attention-deficit/hyperactivity disorder combined presentation with and without comorbid conduct disorder. *Psychiatry Res* . 2018;261:212-219. https://pubmed.ncbi.nlm.nih.gov/29324397/

[46] Nakayama, T., Hirai, N., Hashino, M., Chiba, H., Maruyama, M., Ono, H., Ohigashi, S., Suzuki, T., Kosaki, T., & Yanaihara, T. (1991).

Nihon Sanka Fujinka Gakkai zasshi , *43* (7), 707–714. https://pubmed.ncbi.nlm.nih.gov/1832428/

[47] Yin S. (2002). A new perspective on barking in dogs (Canis familiaris). *Journal of comparative psychology (Washington, D.C. : 1983)* , *116* (2), 189–193. https://doi.org/10.1037/0735-7036.116.2.189

[48] ABC Evening News with David Muir, December 13, 2018.

[49] Frantz LA, Mullin VE, Pionnier-Capitan M, Lebrasseur O, Ollivier M, Perri A, Linderholm A, Mattiangeli V, Teasdale MD, Dimopoulos EA, Tresset A, Duffraisse M, McCormick F, Bartosiewicz L, Gál E, Nyerges ÉA, Sablin MV, Bréhard S, Mashkour M, Bălăşescu A, Gillet B, Hughes S, Chassaing O, Hitte C, Vigne JD, Dobney K, Hänni C, Bradley DG, Larson G. Genomic and archaeological evidence suggest a dual origin of domestic dogs. Science. 2016 Jun 3;352(6290):1228-31. https://pubmed.ncbi.nlm.nih.gov/27257259/

Rex the Wonder Dog

[50] Antonsson BE, Avramis VI, Nyce J, Holcenberg JS. Effect of 5-azacytidine and congeners on DNA methylation and expression of deoxycytidine kinase in the human lymphoid cell lines CCRF/CEM/0 and CCRF/CEM/dCk-1. Cancer Res. 1987 Jul 15;47(14):3672-8. PMID: 2439195. https://pubmed.ncbi.nlm.nih.gov/2439195/

[51] Avramis VI, Mecum RA, Nyce J, Steele DA, Holcenberg JS. Pharmacodynamic and DNA methylation studies of high-dose 1-beta-D-arabinofuranosyl cytosine before and after in vivo 5-azacytidine treatment in pediatric patients with refractory acute lymphocytic leukemia. Cancer Chemother Pharmacol. 1989;24(4):203-10. https://pubmed.ncbi.nlm.nih.gov/2473850/

[52] Tupperware was the container of choice for doing these experiments which involved radioactive nucleotides. Tupperware has exceptionally tight fitting lids, which prevented radioactive contamination of your

laboratory. But Tupperware is not sold in stores. It can only be purchased through Tupperware representatives. My mentor, Professor Magee, director of the Fels Cancer Research Institute, made arrangements for a Tupperware representative to come to the Fels so that we could have our need for such tight-fitting enclosures for our experiments. At the appointed hour, on the appointed day, about thirty hard core scientists stopped what they were doing and trundled off to the Fels library to place their Tupperware orders. Itching to get back to our work as quickly as we could, we all arrived in our white lab coats, with our requirements scribbled on notepads, as Professor Magee had suggested. After everyone was assembled, Professor Magee introduced the "Tupperware lady," and asked her if there was a particular form that each of us had to complete for our orders, or, since the Fels was paying the bill, could she just write down what everyone wanted on one form. The Tupperware lady blinked a few times, apparently thrown off her routine. But she quickly recovered. She introduced herself by first name, and then said, "First, let's go around the room and each person tell us your name, and where you're from, and what you think is your best feature or quality." I hope you can imagine the disconnect. She wanted to have a Tupperware party! We just wanted to get back to our work. I'm telling you this story now, from my point of view, but I am sure the Tupperware lady, too, has had a good story to tell her friends for all these years!

[53] After writing this, I remembered a second case that I saw during a pathology course that I took at Temple University School of Medicine. In that case, a man with malignant melanoma had hundreds of jet black masses that literally filled his body. The man had died a few days after admittance to Temple Hospital, and when I participated in his autopsy as a student, I remembered wondering how a man composed of that much tumor could have survived at all.

[54] Ravaei, H., Yavari Barhaghtalab, M. J., Salehi, V., & Hejr, H. (2020). Isoflurane Induced Malignant Hyperthermia in a Patient with Glucose 6-Phosphate Dehydrogenase Deficiency and Growth

Hormone Abuse. *Case reports in anesthesiology* , *2020* , 8888368. https://doi.org/10.1155/2020/8888368

[55] I had synthesized the drug that we used for the animal studies published in Nature. The drug we used for these clinical trials in humans, of course, was manufactured in a facility that adheres to all the minutiae of FDA regulations.

[56] The reason that I was so concerned was that the terrorists who had previously attempted, and failed, to bring down the towers, had used Route 95 as their major movement corridor. There had been talk of the variety of targets that might be added to terrorist lists, and schools along Route 95 had been among those cited. Both of the schools that my children attended were right off Route 95.

[57] Yamamoto S, Hoshi K, Hirakawa A, Chimura S, Kobayashi M, Machida N. Epidemiological, clinical and pathological features of primary cardiac hemangiosarcoma in dogs: a review of 51 cases. J Vet Med Sci. 2013 Nov;75(11):1433-41. https://pubmed.ncbi.nlm.nih.gov/23811814/

[58] Yamamoto S et al. 2013. *ibid* .

[59] Siontis, B. L., Zhao, L., Leja, M., McHugh, J. B., Shango, M. M., Baker, L. H., Schuetze, S. M., & Chugh, R. (2019). Primary Cardiac Sarcoma: A Rare, Aggressive Malignancy with a High Propensity for Brain Metastases. *Sarcoma* , *2019* , 1960593. https://doi.org/10.1155/2019/1960593

[60] Chen, T. W., Loong, H. H., Srikanthan, A., Zer, A., Barua, R., Butany, J., Cusimano, R. J., Liang, Y. C., Chang, C. H., Iakobishvili, Z., Razak, A., & Lewin, J. (2019). Primary cardiac sarcomas: A multi-national retrospective review. *Cancer medicine* , *8* (1), 104–110. https://doi.org/10.1002/cam4.1897

[61] Nyce J. Species specificity of an adrenal androgen-mediated kill-switch triggered by p53 inactivation. Translational Medicine Reports 2(1):2018 .

https://www.pagepressjournals.org/index.php/tmr/article/view/6773
The treatment protocol that we ended up using in most of our canine studies ended up being a little different than those reported in the Translational Medicine Reports paper, above, for reasons discussed in chapter 11. Our most successful protocol consisted of 65 mg of DHEA/kg body weight/day, in two divided doses, at meal time, delivered orally using a wide bore oral syringe. The DHEA was suspended in unrefined sesame oil using a covered blender set aside for this purpose, calculating the amount needed, as per the dog's body weight, for a one month period of treatment. The suspension was kept at room temperature for up to a month, with re-blending of the suspension prior to each use. Treatment lasted from 3 months to substantially longer, depending upon x-ray results and the dog's general well being. Some side effects were observed in some dogs, as reported in chapter 11. Because of the variability in purity of DHEA on the supplement market, we purchased micronized (200 mesh) DHEA in bulk quantitites from PureBulk, Inc ., a distributor located in Roseburg, Oregon, and always requested a Certificate of Analysis (COA) to assess batch purity.
Commercial use of this protocol is prohibited, and is protected by our issued and pending patents. The protocols that we are moving forward with in our FDA studies are an improvement upon that reported above. Neither the new or the older protocol have as yet been approved by the FDA, and we cannot advocate for their use in anything other than a research setting. ***Additionally, this protocol may not be reproduced or published or distributed in any manner, written, oral, electronic, etc.***

[62] At this point in time, the name of my research company was Advanced Canine Genetic Testing, and we had obtained the website www.ACGT.us. Subsequently, when I realized the full potential of the discovery that I was about to make— for both canines and humans with cancer— I changed the name of the company to ACGT Biotech, using the same web address.

[63] Grimm D 2017 Siberia yields earliest evidence for dog breeding. Science 356(6341): 896. https://davidhgrimm.com/wp-content/uploads/2017/06/896.full_.pdf

[64] Zouganelis GD, Ogden R, Nahar N, Runfola V, Bonab M, Ardalan A, Radford D, Barnett R, Larson G, Hildred A, Jones M, Scarlett G. An old dog and new tricks: Genetic analysis of a Tudor dog recovered from the Mary Rose wreck. Forensic Sci Int. 2014 Dec;245:51-7. https://pubmed.ncbi.nlm.nih.gov/25447174/
I am particularly interested in the mutation in this dog that causes excessive uric acid in the blood. As we shall discuss in the next chapter, this closely mimics an "improvement" in the hominoid primate-specific "kill switch" tumor suppressor mechanism— an inactivating mutation in the uric acid oxidase gene— that enabled increased size in this lineage, and/or the ability to increase exposure to carcinogens, as we shall discuss. Was this early Jack Russell Terrier already undergoing further selection to improve the rudimentary form of the "kill switch" tumor suppression that enabled dogs to co-habit with humans? A recent study has shown fairly wide dissemination of this mutation among different dog breeds.
Karmi, N., Brown, E. A., Hughes, S. S., McLaughlin, B., Mellersh, C. S., Biourge, V., & Bannasch, D. L. (2010). Estimated frequency of the canine hyperuricosuria mutation in different dog breeds. *Journal of veterinary internal medicine* , *24* (6), 1337–1342. https://doi.org/10.1111/j.1939-1676.2010.0631.x

[65] Ramsden, C. A., Bankier, A., Brown, T. J., Cowen, P. S., Frost, G. I., McCallum, D. D., Studdert, V. P., & Fraser, J. R. (2000). A new disorder of hyaluronan metabolism associated with generalized folding and thickening of the skin. *The Journal of pediatrics* , *136* (1), 62–68. https://doi.org/10.1016/s0022-3476(00)90051-9

[66] Ramsden CA et al. 2000. *ibid* .

[67] In the earliest formulation of these ideas, I had not yet included E, carcinogen exposure, into the *lex naturalis* .

[68] Abegglen, L. M., Caulin, A. F., Chan, A., Lee, K., Robinson, R., Campbell, M. S., Kiso, W. K., Schmitt, D. L., Waddell, P. J., Bhaskara, S., Jensen, S. T., Maley, C. C., & Schiffman, J. D. (2015). Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans. *JAMA* , *314* (17), 1850–1860. https://doi.org/10.1001/jama.2015.13134

[69] Jones, P., Chase, K., Martin, A., Davern, P., Ostrander, E. A., & Lark, K. G. (2008). Single-nucleotide-polymorphism-based association mapping of dog stereotypes. *Genetics* , *179* (2), 1033–1044. https://doi.org/10.1534/genetics.108.087866

[70] Green, J., Cairns, B. J., Casabonne, D., Wright, F. L., Reeves, G., Beral, V., & Million Women Study collaborators (2011). Height and cancer incidence in the Million Women Study: prospective cohort, and meta-analysis of prospective studies of height and total cancer risk. *The Lancet. Oncology* , *12* (8), 785–794. https://doi.org/10.1016/S1470-2045(11)70154-1

[71] Kabat, G. C., Anderson, M. L., Heo, M., Hosgood, H. D., 3rd, Kamensky, V., Bea, J. W., Hou, L., Lane, D. S., Wactawski-Wende, J., Manson, J. E., & Rohan, T. E. (2013). Adult stature and risk of cancer at different anatomic sites in a cohort of postmenopausal women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* , *22* (8), 1353–1363. https://doi.org/10.1158/1055-9965.EPI-13-0305

[72] Ong, J. S., An, J., Law, M. H., Whiteman, D. C., Neale, R. E., Gharahkhani, P., & MacGregor, S. (2018). Height and overall cancer risk and mortality: evidence from a Mendelian randomisation study on 310,000 UK Biobank participants. *British journal of cancer* , *118* (9), 1262–1267. https://doi.org/10.1038/s41416-018-0063-4

[73] Laron, Z., & Kauli, R. (2016). Fifty seven years of follow-up of the Israeli cohort of Laron Syndrome patients-From discovery to treatment. *Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society* , *28* , 53–56. https://doi.org/10.1016/j.ghir.2015.08.004
Janecka, A., Kołodziej-Rzepa, M., & Biesaga, B. (2016). Clinical and Molecular Features of Laron Syndrome, A Genetic Disorder Protecting from Cancer. *In vivo (Athens, Greece)* , *30* (4), 375–381. https://pubmed.ncbi.nlm.nih.gov/27381597/
Laron Syndrome, a genetic disorder protecting from cancer. In Vivo 30(4):375-381.

[74] Laron Z, Kauli R 2016 *ibid* .

[75] Dobson J. M. (2013). Breed-predispositions to cancer in pedigree dogs. *ISRN veterinary science* , *2013* , 941275. https://doi.org/10.1155/2013/941275

[76] Beuchat C, https://www.instituteofcaninebiology.org/lifespan.html

[77] In the *lex naturalis* equation, both T (improvement in species-specific tumor suppression mechanism) and R (lifetime cancer risk) are actually expressed as reciprocal values (1/T and 1/R). This allows Li (lifespan), for example, to be expressed as:
Li = T/S E R
that is, lifespan increases as T increases, and/or as S, E, and R decrease.

[78] Urfer, S. R., Gaillard, C., & Steiger, A. (2007). Lifespan and disease predispositions in the Irish Wolfhound: a review. *The veterinary quarterly* , *29* (3), 102–111. https://doi.org/10.1080/01652176.2007.9695233

[79] The epigenome refers to reversible modifications made to DNA that change the way it is expressed— transcribed into mRNA— rather than the irreversible changes caused by mutations. The field of epigenetics

is the study of the mechanisms by which such reversible changes are introduced, and removed, altering gene expression. One of such epigenetic mechanisms is DNA methylation, in which one of a series of DNA methylase enzymes modifies cytosines— that are usually next to guanines, so-called CpG dinucleotides— at their 5-position carbon, producing 5-methylcytosine. In turn, demethylase enzymes exist that can remove methylation on cytosines in CpG dinucleotides. When the promoters of genes are methylated, they are generally not expressed, and when they are unmethylated, they are expressed.

[80] Grüntzig, K., Graf, R., Boo, G., Guscetti, F., Hässig, M., Axhausen, K. W., Fabrikant, S., Welle, M., Meier, D., Folkers, G., & Pospischil, A. (2016). Swiss Canine Cancer Registry 1955-2008: Occurrence of the Most Common Tumour Diagnoses and Influence of Age, Breed, Body Size, Sex and Neutering Status on Tumour Development. *Journal of comparative pathology* , *155* (2-3), 156–170. https://doi.org/10.1016/j.jcpa.2016.05.011

[81] Sakthikumar, S., Elvers, I., Kim, J., Arendt, M. L., Thomas, R., Turner-Maier, J., Swofford, R., Johnson, J., Schumacher, S. E., Alföldi, J., Axelsson, E., Couto, C. G., Kisseberth, W. C., Pettersson, M. E., Getz, G., Meadows, J., Modiano, J. F., Breen, M., Kierczak, M., Forsberg-Nilsson, K., … Lindblad-Toh, K. (2018). *SETD2* Is Recurrently Mutated in Whole-Exome Sequenced Canine Osteosarcoma. *Cancer research* , *78* (13), 3421–3431. https://doi.org/10.1158/0008-5472.CAN-17-3558
Davis, B. W., & Ostrander, E. A. (2014). Domestic dogs and cancer research: a breed-based genomics approach. *ILAR journal* , *55* (1), 59–68. https://doi.org/10.1093/ilar/ilu017 and references therein

[82] Anfinsen, K. P., Grotmol, T., Bruland, O. S., & Jonasdottir, T. J. (2011). Breed-specific incidence rates of canine primary bone tumors —a population based survey of dogs in Norway. *Canadian journal of veterinary research = Revue canadienne de recherche veterinaire* , *75* (3), 209–215. https://pubmed.ncbi.nlm.nih.gov/22210997/

[83] Dobson JM 2013 *ibid.*

[84] Li, Y. S., Liu, Q., He, H. B., & Luo, W. (2019). The possible role of insulin-like growth factor-1 in osteosarcoma. *Current problems in cancer* , *43* (3), 228–235. https://doi.org/10.1016/j.currproblcancer.2018.08.008
MacEwen, E. G., Pastor, J., Kutzke, J., Tsan, R., Kurzman, I. D., Thamm, D. H., Wilson, M., & Radinsky, R. (2004). IGF-1 receptor contributes to the malignant phenotype in human and canine osteosarcoma. *Journal of cellular biochemistry* , *92* (1), 77–91. https://doi.org/10.1002/jcb.20046

[85] Mao, J., Zhuang, G., & Chen, Z. (2017). Genetic Polymorphisms of Insulin-Like Growth Factor 1 Are Associated with Osteosarcoma Risk and Prognosis. *Medical science monitor : international medical journal of experimental and clinical research* , *23* , 5892–5898. https://doi.org/10.12659/msm.908004

[86] Wang, Z., Zheng, C., Jiang, K., He, J., Cao, X., & Wu, S. (2017). MicroRNA-503 suppresses cell proliferation and invasion in osteosarcoma via targeting insulin-like growth factor 1 receptor. *Experimental and therapeutic medicine* , *14* (2), 1547–1553. https://doi.org/10.3892/etm.2017.4648
Chen, G., Fang, T., Huang, Z., Qi, Y., Du, S., Di, T., Lei, Z., Zhang, X., & Yan, W. (2016). MicroRNA-133a Inhibits Osteosarcoma Cells Proliferation and Invasion via Targeting IGF-1R. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* , *38* (2), 598–608. https://doi.org/10.1159/000438653

[87] Maniscalco, L., Iussich, S., Morello, E., Martano, M., Gattino, F., Miretti, S., Biolatti, B., Accornero, P., Martignani, E., Sánchez-Céspedes, R., Buracco, P., & De Maria, R. (2015). Increased expression of insulin-like growth factor-1 receptor is correlated with worse survival in canine appendicular osteosarcoma. *Veterinary*

journal (London, England : 1997) , *205* (2), 272–280. https://doi.org/10.1016/j.tvjl.2014.09.005

[88] Ishiwata T 2018 Role of fibroblast growth factor receptor-2 splicing in normal and cancer cells. Front Biosci (Landmark Ed). 2018 Jan 1;23:626-639 https://www.bioscience.org/2018/v23/af/4609/list.htm

[89] Gao, G., Tian, Z., Zhu, H. Y., & Ouyang, X. Y. (2018). miRNA-133b targets FGFR1 and presents multiple tumor suppressor activities in osteosarcoma. *Cancer cell international* , *18* , 210. https://doi.org/10.1186/s12935-018-0696-7

[90] Jimenez A. G. (2016). Physiological underpinnings in life-history trade-offs in man's most popular selection experiment: the dog. *Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology* , *186* (7), 813–827. https://doi.org/10.1007/s00360-016-1002-4
Maniscalco L et al. 2015 *ibid.*

[91] Kent, M. S., Burton, J. H., Dank, G., Bannasch, D. L., & Rebhun, R. B. (2018). Association of cancer-related mortality, age and gonadectomy in golden retriever dogs at a veterinary academic center (1989-2016). *PloS one* , *13* (2), e0192578. https://doi.org/10.1371/journal.pone.0192578

Using the lex naturalis to Prevent Cancer in Your Dog

[92] While feral packs of dogs do exist in the wild, they can now only be considered an invasive species, as evidenced by the harm they have caused to endemic species in the Galapagos Islands, and elsewhere. See, for example: Kutschera, U., & Kleinhans, S. (2013). Alfred Russel Wallace and the destruction of island life: the Iguana tragedy. *Theory in biosciences = Theorie in den Biowissenschaften* , *132* (4), 259–265. https://doi.org/10.1007/s12064-013-0193-4

Doherty, T. S., Glen, A. S., Nimmo, D. G., Ritchie, E. G., & Dickman, C. R. (2016). Invasive predators and global biodiversity loss. *Proceedings of the National Academy of Sciences of the United States of America* , *113* (40), 11261–11265. https://doi.org/10.1073/pnas.1602480113

[93] Mongillo, P., Prana, E., Gabai, G., Bertotto, D., & Marinelli, L. (2014). Effect of age and sex on plasma cortisol and dehydroepiandrosterone concentrations in the dog (Canis familiaris). *Research in veterinary science* , *96* (1), 33–38. https://doi.org/10.1016/j.rvsc.2013.10.010

[94] Liao, L. M., Loftfield, E., Etemadi, A., Graubard, B. I., & Sinha, R. (2019). Substitution of dietary protein sources in relation to colorectal cancer risk in the NIH-AARP cohort study. *Cancer causes & control : CCC* , *30* (10), 1127–1135. https://doi.org/10.1007/s10552-019-01210-1

[95] Zhang L, Rana I, Shaffer RM, Taioli E, Sheppard L. Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence. Mutat Res. 2019 Jul-Sep;781:186-206. doi: 10.1016/j.mrrev.2019.02.001. Epub 2019 Feb 10. PMID: 31342895; PMCID: PMC6706269. https://pubmed.ncbi.nlm.nih.gov/31342895/

[96] Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., Hansen, M., Landrigan, P. J., Lanphear, B. P., Mesnage, R., Vandenberg, L. N., Vom Saal, F. S., Welshons, W. V., & Benbrook, C. M. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environmental health : a global access science source* , *15* , 19. https://doi.org/10.1186/s12940-016-0117-0

[97] Gruntzig R et al. 2016 Swiss Canine Cancer Registry 1955-2008: Occurrence of the most common tumour diagnoses and influence of age, breed, body size, sex and neutering status on tumour development. Journal of Comparative Pathology 155(2-3):156-170.

[98] Torres de la Riva, G., Hart, B. L., Farver, T. B., Oberbauer, A. M., Messam, L. L., Willits, N., & Hart, L. A. (2013). Neutering dogs: effects on joint disorders and cancers in golden retrievers. *PloS one* , *8* (2), e55937. https://doi.org/10.1371/journal.pone.0055937
Oberbauer, A. M., Belanger, J. M., & Famula, T. R. (2019). A Review of the Impact of Neuter Status on Expression of Inherited Conditions in Dogs. *Frontiers in veterinary science* , *6* , 397. https://doi.org/10.3389/fvets.2019.00397 .

[99] Thamm D. H. (2019). Canine Cancer: Strategies in Experimental Therapeutics. *Frontiers in oncology* , *9* , 1257. https://doi.org/10.3389/fonc.2019.01257

[100] Mongillo P. *et al* . 2014, *ibid.* reference 99
Schiebinger, R. J., Albertson, B. D., Barnes, K. M., Cutler, G. B., Jr, & Loriaux, D. L. (1981). Developmental changes in rabbit and dog adrenal function: a possible homologue of adrenarche in the dog. *The American journal of physiology* , *240* (6), E694–E699. https://doi.org/10.1152/ajpendo.1981.240.6.E694
Cutler, G. B., Jr, Glenn, M., Bush, M., Hodgen, G. D., Graham, C. E., & Loriaux, D. L. (1978). Adrenarche: a survey of rodents, domestic animals, and primates. *Endocrinology* , *103* (6), 2112–2118. https://doi.org/10.1210/endo-103-6-2112

[101] Bannasch, D., Safra, N., Young, A., Karmi, N., Schaible, R. S., & Ling, G. V. (2008). Mutations in the SLC2A9 gene cause hyperuricosuria and hyperuricemia in the dog. *PLoS genetics* , *4* (11), e1000246. https://doi.org/10.1371/journal.pgen.1000246

[102] Briggs, O. M., & Harley, E. H. (1985). Serum urate concentrations in the Dalmatian Coach Hound. *Journal of comparative pathology* , *95* (2), 301–304. https://doi.org/10.1016/0021-9975(85)90017-9
Simkin P. A. (2005). The Dalmatian defect: a hepatic endocrinopathy of urate transport. *Arthritis and rheumatism* , *52* (8), 2257–2262. https://doi.org/10.1002/art.21241
For human serum uric acid levels:

Tan, P. K., Farrar, J. E., Gaucher, E. A., & Miner, J. N. (2016). Coevolution of URAT1 and Uricase during Primate Evolution: Implications for Serum Urate Homeostasis and Gout. *Molecular biology and evolution* , *33* (9), 2193–2200. https://doi.org/10.1093/molbev/msw116

[103] Safra, N., Ling, G. V., Schaible, R. H., & Bannasch, D. L. (2005). Exclusion of urate oxidase as a candidate gene for hyperuricosuria in the Dalmatian dog using an interbreed backcross. *The Journal of heredity* , *96* (7), 750–754. https://doi.org/10.1093/jhered/esi078

[104] Bannasch D *et al* . 2008 *ibid* .

[105] The mutation that is homozygous in Dalmatians is the same mutation that can be heterozygous in other breeds of dogs, indicating that the mutation occurred before the separation of dogs into their various breeds. In this respect, the Jack Russel terrier found on the almost 500-year-old Wreck of the Engish ship, the Mary Rose, which was able to be sequenced, showed this dog to be heterozygous for this same mutation. See:
Zouganelis, G. D., Ogden, R., Nahar, N., Runfola, V., Bonab, M., Ardalan, A., Radford, D., Barnett, R., Larson, G., Hildred, A., Jones, M., & Scarlett, G. (2014). An old dog and new tricks: Genetic analysis of a Tudor dog recovered from the Mary Rose wreck. *Forensic science international* , *245* , 51–57. https://doi.org/10.1016/j.forsciint.2014.10.001 .
Thus, the mutation did not originate in the Dalmatian, but rather in early dogs which had very high daily exposure to PAH. But it did not become fixed in any breed, until it became fixed in Dalmatians.

[106] Bannasch D *et al* . 2008 *ibid* . An important note: The work of Bannasch *et al* . also showed that the mutation in SLC2A9 in Dalmatians also occurs in some other dogs, suggesting that the original mutation occurred before the separation of the early dog into modern breeds.

[107] Pfeifer, G. P., Denissenko, M. F., Olivier, M., Tretyakova, N., Hecht, S. S., & Hainaut, P. (2002). Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* , *21* (48), 7435–7451. https://doi.org/10.1038/sj.onc.1205803

[108] The work of Bannasch et al., ibid, shows that the original mutation in SCL2A9 that is homozygous in Dalmatians occurred quite early in dogs, before the separation of the dog into different breeds. This might be as expected if this mutation conferred some resistance to PAH exposure, which would have been at its most extreme level in early dogs. With the advent of Western civilization, such extreme levels of PAH exposure might have been reduced, except for dogs engaged in certain occupations, such as fire brigade duty. In such dogs, homozygosity for the SLC2A9 mutation may have enabled their participation in such duty.

[109] Dobson J. M. (2013). Breed-predispositions to cancer in pedigree dogs. *ISRN veterinary science* , *2013* , 941275. https://doi.org/10.1155/2013/941275

[110] Reiter, T., Jagoda, E., & Capellini, T. D. (2016). Dietary Variation and Evolution of Gene Copy Number among Dog Breeds. *PloS one* , *11* (2), e0148899. https://doi.org/10.1371/journal.pone.0148899

Chemo for your dog?

[111] Nyce J. W. (2019). Species-specific mechanisms of tumor suppression are fundamental drivers of vertebrate speciation: critical implications for the 'war on cancer'. *Endocrine-related cancer* , *26* (2), C1–C5. https://doi.org/10.1530/ERC-18-0468
Nyce J. W. (2020). A lex naturalis delineates components of a human-specific, adrenal androgen-dependent, p53-mediated 'kill switch' tumor suppression mechanism. *Endocrine-related cancer* , *27* (2), R51–R65. https://doi.org/10.1530/ERC-19-0382

See addendum to the above at: https://erc.bioscientifica.com/view/journals/erc/27/9/ERC-19-0382a.xml?rskey=9D9Ubs&result=4

Hey J. (2006). On the failure of modern species concepts. *Trends in ecology & evolution* , *21* (8), 447–450. https://doi.org/10.1016/j.tree.2006.05.011

[112] Nyce J. W. (2019). Species-specific mechanisms of tumor suppression are fundamental drivers of vertebrate speciation: critical implications for the 'war on cancer'. *Endocrine-related cancer* , *26* (2), C1–C5. https://doi.org/10.1530/ERC-18-0468

[113] I will have critics who argue that there have been breakthroughs in human cancer that were originally discovered in mice. An example they might use is the discovery of tumor-induced "immune exhaustion" in which receptors expressed on the surface of tumor cells inactivate attacking host immune cells by interacting with receptors on the immune cell. Antibody-based drugs have been developed that block the receptor on either the tumor or the immune cell, and such drugs have been shown capable of producing dramatic remission in some previously untreatable tumor types. My argument is that, as dramatic as some such remissions have been, the great majority of patients do not respond to these new treatments, and the responses are usually (but not always) of short duration. In total, these "breakthrough" treatments will not break through the asymptotic boundary in the patient survival graph shown in this chapter.

[114] Mak, I. W., Evaniew, N., & Ghert, M. (2014). Lost in translation: animal models and clinical trials in cancer treatment. *American journal of translational research* , *6* (2), 114–118. https://pubmed.ncbi.nlm.nih.gov/24489990/

[115] Nyce J. W. (2018). Detection of a novel, primate-specific 'kill switch' tumor suppression mechanism that may fundamentally control cancer risk in humans: an unexpected twist in the basic biology of

TP53. *Endocrine-related cancer* , *25* (11), R497–R517. https://doi.org/10.1530/ERC-18-0241

[116] Hektoen L, Corper HC. 1920. The effect of mustard gas (dichloroethylsulphide) on antibody formation. Journal of Infectious Diseases 28:279-285. https://academic.oup.com/jid/article-abstract/28/3/281/855777?redirectedFrom=fulltext

[117] Christakis P. (2011). The birth of chemotherapy at Yale. Bicentennial lecture series: Surgery Grand Round. *The Yale journal of biology and medicine* , *84* (2), 169–172. https://pubmed.ncbi.nlm.nih.gov/21698052/

[118] Singh, R. K., Kumar, S., Prasad, D. N., & Bhardwaj, T. R. (2018). Therapeutic journery of nitrogen mustard as alkylating anticancer agents: Historic to future perspectives. *European journal of medicinal chemistry* , *151* , 401–433. https://doi.org/10.1016/j.ejmech.2018.04.001

[119] Sarkar S. 2002 Evolutionary theory in the 1920's: The nature of the synthesis . Proceedings of the 2002 Biennial Meeting of The Philosophy of Science Association, Vol. 71, No. 5, Part II: Symposia Papers Edited by Sandra D. Mitchell (December 2004), pp. 1215-1226 http://philsci-archive.pitt.edu/722/1/HFW-1920s-1_doc.pdf

[120] Ratcliffe HL 1933 Incidence and nature of tumors in captive wild mammals and birds. Cancer Research 17(1). https://cancerres.aacrjournals.org/content/17/1/116
The above work was reproduced in a subsequent study done at the Philadelphia Zoo in 1959:
LOMBARD, L. S., & WITTE, E. J. (1959). Frequency and types of tumors in mammals and birds of the Philadelphia Zoological Garden. *Cancer research* , *19* (2), 127–141. https://pubmed.ncbi.nlm.nih.gov/13629476/

[121] Back, A. R., Schleis, S. E., Smrkovski, O. A., Lee, J., Smith, A. N., & Phillips, J. C. (2015). Mechlorethamine, vincristine, melphalan and prednisone (MOMP) for the treatment of relapsed lymphoma in dogs.

Veterinary and comparative oncology , *13* (4), 398–408. https://doi.org/10.1111/vco.12055

[122] Rassnick KM, McEntee MC, Erb HN, Burke BP, Balkman CE, Flory AB, Kiselow MA, Autio K, Gieger TL. Comparison of 3 protocols for treatment after induction of remission in dogs with lymphoma. J Vet Intern Med. 2007 Nov-Dec;21(6):1364-73. doi: 10.1892/07-057.1. PMID: 18196748. https://pubmed.ncbi.nlm.nih.gov/18196748/

[123] Nyce J. W. (2020). A lex naturalis delineates components of a human-specific, adrenal androgen-dependent, p53-mediated 'kill switch' tumor suppression mechanism. *Endocrine-related cancer* , *27* (2), R51–R65. https://doi.org/10.1530/ERC-19-0382
See addendum to the above at: https://erc.bioscientifica.com/view/journals/erc/27/9/ERC-19-0382a.xml?rskey=9D9Ubs&result=4

[124] London, C. A., Malpas, P. B., Wood-Follis, S. L., Boucher, J. F., Rusk, A. W., Rosenberg, M. P., Henry, C. J., Mitchener, K. L., Klein, M. K., Hintermeister, J. G., Bergman, P. J., Couto, G. C., Mauldin, G. N., & Michels, G. M. (2009). Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. *Clinical cancer research : an official journal of the American Association for Cancer Research* , *15* (11), 3856–3865. https://doi.org/10.1158/1078-0432.CCR-08-1860

[125] Bavcar, S., de Vos, J., Kessler, M., de Fornel, P., Buracco, P., Murphy, S., Hirschberger, J., & Argyle, D. J. (2017). Combination toceranib and lomustine shows frequent high grade toxicities when used for treatment of non-resectable or recurrent mast cell tumours in dogs: A European multicentre study. *Veterinary journal (London, England : 1997)* , *224* , 1–6. https://doi.org/10.1016/j.tvjl.2017.04.010

[126] London, C., Mathie, T., Stingle, N., Clifford, C., Haney, S., Klein, M. K., Beaver, L., Vickery, K., Vail, D. M., Hershey, B., Ettinger, S., Vaughan, A., Alvarez, F., Hillman, L., Kiselow, M., Thamm, D., Higginbotham, M. L., Gauthier, M., Krick, E., Phillips, B., … Gillings, S. (2012). Preliminary evidence for biologic activity of toceranib phosphate (Palladia(®)) in solid tumours. *Veterinary and comparative oncology* , *10* (3), 194–205. https://doi.org/10.1111/j.1476-5829.2011.00275.x
Please note the dearth of complete responses (CR), and note that the term "clinical benefit" is inherently subjective.

[127] De Clercq E. (2018). Tanovea® for the treatment of lymphoma in dogs. *Biochemical pharmacology* , *154* , 265–269. https://doi.org/10.1016/j.bcp.2018.05.010

[128] Saba, C. F., Vickery, K. R., Clifford, C. A., Burgess, K. E., Phillips, B., Vail, D. M., Wright, Z. M., Morges, M. A., Fan, T. M., & Thamm, D. H. (2018). Rabacfosadine for relapsed canine B-cell lymphoma: Efficacy and adverse event profiles of 2 different doses. *Veterinary and comparative oncology* , *16* (1), E76–E82. https://doi.org/10.1111/vco.12337

[129] Luca S, Mihaescu T. 2013 History of BCG vaccine. Maedica (Buchar) . 8(1): 53–58. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3749764/

[130] Kamat, A. M., Bellmunt, J., Galsky, M. D., Konety, B. R., Lamm, D. L., Langham, D., Lee, C. T., Milowsky, M. I., O'Donnell, M. A., O'Donnell, P. H., Petrylak, D. P., Sharma, P., Skinner, E. C., Sonpavde, G., Taylor, J. A., 3rd, Abraham, P., & Rosenberg, J. E. (2017). Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of bladder carcinoma. *Journal for immunotherapy of cancer* , *5* (1), 68. https://doi.org/10.1186/s40425-017-0271-0

[131] Li, P., Wang, J., Chen, G., Zhang, X., Lin, D., Zhou, Y., Yu, Y., Liu, W., & Zhang, D. (2019). Oncolytic activity of canine distemper virus in canine mammary tubular adenocarcinoma cells. *Veterinary and*

comparative oncology , *17* (2), 174–183. https://doi.org/10.1111/vco.12466

[132] Sánchez, D., Cesarman-Maus, G., Amador-Molina, A., & Lizano, M. (2018). Oncolytic Viruses for Canine Cancer Treatment. *Cancers* , *10* (11), 404. https://doi.org/10.3390/cancers10110404

[133] Sadowski, A. R., Gardner, H. L., Borgatti, A., Wilson, H., Vail, D. M., Lachowicz, J., Manley, C., Turner, A., Klein, M. K., Waite, A., Sahora, A., & London, C. A. (2018). Phase II study of the oral selective inhibitor of nuclear export (SINE) KPT-335 (verdinexor) in dogs with lymphoma. *BMC veterinary research* , *14* (1), 250. https://doi.org/10.1186/s12917-018-1587-9

[134] Aratana Therapeutics AT-004, AT-005. The US Department of Agriculture (USDA), rather than the FDA. is in charge of biological medications such as vaccines derived from animals.

[135] We cannot use as an excuse the "red herring" of Lee-Fraumeni Syndrome (LFS), in which patients have an inherited mutation in one p53 allele. In both p53 knockout mice, and patients with LFS, a wide array of tumors occur at a much earlier age than normal, giving the false appearance that p53 acted as a tumor suppressor in an identical fashion in both species. If we had been firmly grounded in basic biology— in which evolution underlies everything— we would have recognized Li-Fraumeni Syndrome for the red herring that it was.
Why isn't the kill switch activated in all the cells of a Li-Fraumeni Syndrome patient? The number of Li-Fraumeni patients identified in the entire world is very small, less than a thousand. The rarity of this mutation indicates that there are secondary and probably even tertiary mutations in these patients that prevent the kill switch from firing. Where these secondary and tertiary mutations in kill switch elements do not occur, affected individuals likely do not survive.

[136] Nyce, J., Leonard, S., Canupp, D., Schulz, S., & Wong, S. (1993). Epigenetic mechanisms of drug resistance: drug-induced DNA hypermethylation and drug resistance. *Proceedings of the National Academy of Sciences of the United States of America* , *90* (7), 2960–2964. https://doi.org/10.1073/pnas.90.7.2960
Nyce J. (1989). Drug-induced DNA hypermethylation and drug resistance in human tumors. *Cancer research* , *49* (21), 5829–5836. https://pubmed.ncbi.nlm.nih.gov/2790794/
Nyce J. W. (1997). Drug-induced DNA hypermethylation: a potential mediator of acquired drug resistance during cancer chemotherapy. *Mutation research* , *386* (2), 153–161. https://doi.org/10.1016/s1383-5742(96)00051-8

[137] In human cancer, “cure” has been re-defined as 5-year cancer-free survival. This is the only disease for which we have had to make such a re-definition— a re-definition so obviously at odds with the normal definition of cure.

[138] Schulz S and Nyce JW 1991 Inhibition of protein isoprenylation and p21ras membrane association by dehydroepiandrosterone in human colonic adenocarcinoma cells in vitro. Cancer Res. 1991 Dec 15;51(24):6563-7. https://pubmed.ncbi.nlm.nih.gov/1835900/

[139] Lim, J., & Leprivier, G. (2019). The impact of oncogenic RAS on redox balance and implications for cancer development. *Cell death & disease* , *10* (12), 955. https://doi.org/10.1038/s41419-019-2192-y

[140] Kushwaha, P. P., Gupta, S., Singh, A. K., & Kumar, S. (2019). Emerging Role of Migration and Invasion Enhancer 1 (MIEN1) in Cancer Progression and Metastasis. *Frontiers in oncology* , *9* , 868. https://doi.org/10.3389/fonc.2019.00868

[141] Hashimoto, A., Oikawa, T., Hashimoto, S., Sugino, H., Yoshikawa, A., Otsuka, Y., Handa, H., Onodera, Y., Nam, J. M., Oneyama, C., Okada, M., Fukuda, M., & Sabe, H. (2016). P53- and mevalonate pathway-driven malignancies require Arf6 for metastasis and drug resistance. *The Journal of cell biology* , *213* (1), 81–95. https://doi.org/10.1083/jcb.201510002

[142] Mochizuki, H., & Breen, M. (2017). Sequence analysis of RAS and RAF mutation hot spots in canine carcinoma. *Veterinary and comparative oncology* , *15* (4), 1598–1605. https://doi.org/10.1111/vco.12275

[143] Simanshu, D. K., Nissley, D. V., & McCormick, F. (2017). RAS Proteins and Their Regulators in Human Disease. *Cell* , *170* (1), 17–33. https://doi.org/10.1016/j.cell.2017.06.009

[144] Schulz S, Nyce J 1991, *ibid* .

[145] It is more correct to use the plural, dolichols, because they constitute family of isoprenoid moieties of different lengths.

[146] Pan, S., Chen, R., Tamura, Y., Crispin, D. A., Lai, L. A., May, D. H., McIntosh, M. W., Goodlett, D. R., & Brentnall, T. A. (2014). Quantitative glycoproteomics analysis reveals changes in N-glycosylation level associated with pancreatic ductal adenocarcinoma. *Journal of proteome research* , *13* (3), 1293–1306. https://doi.org/10.1021/pr4010184

Zhang, D., Liu, X., Gao, J., Sun, Y., Liu, T., Yan, Q., & Yang, X. (2017). The role of epithelial cell adhesion molecule N-glycosylation on apoptosis in breast cancer cells. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* , *39* (3), 1010428317695973. https://doi.org/10.1177/1010428317695973

Herreros-Pomares, A., Aguilar-Gallardo, C., Calabuig-Fariñas, S., Sirera, R., Jantus-Lewintre, E., & Camps, C. (2018). EpCAM duality becomes this molecule in a new Dr. Jekyll and Mr. Hyde tale. *Critical*

reviews in oncology/hematology , *126* , 52–63. https://doi.org/10.1016/j.critrevonc.2018.03.006

[147] Kalluri, R., & Weinberg, R. A. (2009). The basics of epithelial-mesenchymal transition. *The Journal of clinical investigation* , *119* (6), 1420–1428. https://doi.org/10.1172/JCI39104
Kalluri R. (2009). EMT: when epithelial cells decide to become mesenchymal-like cells. *The Journal of clinical investigation* , *119* (6), 1417–1419. https://doi.org/10.1172/JCI39675

[148] Langd L, lecture on Embryology of the Eye. See especially slides 35-37 in this presentation to see how mesenchymal cells move during embryology to form critical elements of the eye. https://www.slideshare.net/Lhacha/embryology-of-human-eye

[149] Zhang, J., Jamaluddin, M., Zhang, Y., Widen, S. G., Sun, H., Brasier, A. R., & Zhao, Y. (2019). Type II Epithelial-Mesenchymal Transition Upregulates Protein N-Glycosylation To Maintain Proteostasis and Extracellular Matrix Production. *Journal of proteome research* , *18* (9), 3447–3460. https://doi.org/10.1021/acs.jproteome.9b00342

[150] Zhang, D., Yang, L., Liu, X., Gao, J., Liu, T., Yan, Q., & Yang, X. (2020). Hypoxia modulates stem cell properties and induces EMT through N-glycosylation of EpCAM in breast cancer cells. *Journal of cellular physiology* , *235* (4), 3626–3633. https://doi.org/10.1002/jcp.29252
Munz, M., Fellinger, K., Hofmann, T., Schmitt, B., & Gires, O. (2008). Glycosylation is crucial for stability of tumour and cancer stem cell antigen EpCAM. *Frontiers in bioscience : a journal and virtual library* , *13* , 5195–5201. https://doi.org/10.2741/3075

[151] Bhattacharya, D., & Scimè, A. (2019). Metabolic Regulation of Epithelial to Mesenchymal Transition: Implications for Endocrine Cancer. *Frontiers in endocrinology* , *10* , 773. https://doi.org/10.3389/fendo.2019.00773
Lunetti, P., Di Giacomo, M., Vergara, D., De Domenico, S., Maffia,

M., Zara, V., Capobianco, L., & Ferramosca, A. (2019). Metabolic reprogramming in breast cancer results in distinct mitochondrial bioenergetics between luminal and basal subtypes. *The FEBS journal* , *286* (4), 688–709. https://doi.org/10.1111/febs.14756

[152] Liu X, Yang L, Zhang D, Liu T, Yan Q, Yang X. Deglycosylation of epithelial cell adhesion molecule affects epithelial to mesenchymal transition in breast cancer cells. J Cell Physiol. 2019 Apr;234(4):4504-4514. doi: 10.1002/jcp.27256. Epub 2018 Sep 24. PMID: 30246502. https://pubmed.ncbi.nlm.nih.gov/30246502/

[153] Gao, J., Liu, X., Yang, F., Liu, T., Yan, Q., & Yang, X. (2015). By inhibiting Ras/Raf/ERK and MMP-9, knockdown of EpCAM inhibits breast cancer cell growth and metastasis. *Oncotarget* , *6* (29), 27187–27198. https://doi.org/10.18632/oncotarget.4551

[154] King, H., Aleksic, T., Haluska, P., & Macaulay, V. M. (2014). Can we unlock the potential of IGF-1R inhibition in cancer therapy?. *Cancer treatment reviews* , *40* (9), 1096–1105. https://doi.org/10.1016/j.ctrv.2014.07.004
Singh, P., Alex, J. M., & Bast, F. (2014). Insulin receptor (IR) and insulin-like growth factor receptor 1 (IGF-1R) signaling systems: novel treatment strategies for cancer. *Medical oncology (Northwood, London, England)* , *31* (1), 805. https://doi.org/10.1007/s12032-013-0805-3

[155] Jamitzky, S., Krueger, A. C., Janneschuetz, S., Piepke, S., Kailayangiri, S., Spurny, C., Rossig, C., & Altvater, B. (2015). Insulin-like growth factor-1 receptor (IGF-1R) inhibition promotes expansion of human NK cells which maintain their potent antitumor activity against Ewing sarcoma cells. *Pediatric blood & cancer* , *62* (11), 1979–1985. https://doi.org/10.1002/pbc.25619

[156] Hoopes, B. C., Rimbault, M., Liebers, D., Ostrander, E. A., & Sutter, N. B. (2012). The insulin-like growth factor 1 receptor (IGF1R) contributes to reduced size in dogs. *Mammalian genome : official*

journal of the International Mammalian Genome Society , *23* (11-12), 780–790. https://doi.org/10.1007/s00335-012-9417-z

[157] Kuzu, O. F., Noory, M. A., & Robertson, G. P. (2016). The Role of Cholesterol in Cancer. *Cancer research* , *76* (8), 2063–2070. https://doi.org/10.1158/0008-5472.CAN-15-2613

[158] Brown, D. N., Caffa, I., Cirmena, G., Piras, D., Garuti, A., Gallo, M., Alberti, S., Nencioni, A., Ballestrero, A., & Zoppoli, G. (2016). Squalene epoxidase is a bona fide oncogene by amplification with clinical relevance in breast cancer. *Scientific reports* , *6* , 19435. https://doi.org/10.1038/srep19435

[159] Folkers, K., Langsjoen, P., & Langsjoen, P. H. (1992). Therapy with coenzyme Q10 of patients in heart failure who are eligible or ineligible for a transplant. *Biochemical and biophysical research communications* , *182* (1), 247–253. https://doi.org/10.1016/s0006-291x(05)80137-8

Langsjoen, P. H., Vadhanavikit, S., & Folkers, K. (1985). Response of patients in classes III and IV of cardiomyopathy to therapy in a blind and crossover trial with coenzyme Q10. *Proceedings of the National Academy of Sciences of the United States of America* , *82* (12), 4240–4244. https://doi.org/10.1073/pnas.82.12.4240

Berman, M., Erman, A., Ben-Gal, T., Dvir, D., Georghiou, G. P., Stamler, A., Vered, Y., Vidne, B. A., & Aravot, D. (2004). Coenzyme Q10 in patients with end-stage heart failure awaiting cardiac transplantation: a randomized, placebo-controlled study. *Clinical cardiology* , *27* (5), 295–299. https://doi.org/10.1002/clc.4960270512

[160] Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. S., Morrison, B., 3rd, & Stockwell, B. R. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* , *149* (5), 1060–1072. https://doi.org/10.1016/j.cell.2012.03.042

[161] Early on in this research, I devised means to replenish many of the intracellular metabolites that were depleted by G6PD inhibition, and

downstream mevalonate pathway inhibition. However, since these "side effects" can be eliminated simply by going off the kill switch protocol for a few days, my new protocols for all studies that I will progress through FDA represent monotherapy, with one exception—the use of fluorodeoxyglucose as both an imaging agent, and as a nonmetabolizable analog of G6P that retains capacity to drive DHEA-mediated inhibition of G6PD to irreversibility.
The kill switch protocol was pruned back to just DHEA suspended in sesame oil, delivered orally in a total daily dose of 60 mg/kg.

[162] Bersuker, K., Hendricks, J. M., Li, Z., Magtanong, L., Ford, B., Tang, P. H., Roberts, M. A., Tong, B., Maimone, T. J., Zoncu, R., Bassik, M. C., Nomura, D. K., Dixon, S. J., & Olzmann, J. A. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* , *575* (7784), 688–692. https://doi.org/10.1038/s41586-019-1705-2
Doll, S., Freitas, F. P., Shah, R., Aldrovandi, M., da Silva, M. C., Ingold, I., Goya Grocin, A., Xavier da Silva, T. N., Panzilius, E., Scheel, C. H., Mourão, A., Buday, K., Sato, M., Wanninger, J., Vignane, T., Mohana, V., Rehberg, M., Flatley, A., Schepers, A., Kurz, A., … Conrad, M. (2019). FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* , *575* (7784), 693–698. https://doi.org/10.1038/s41586-019-1707-0

[163] Lu, Y. X., Ju, H. Q., Liu, Z. X., Chen, D. L., Wang, Y., Zhao, Q., Wu, Q. N., Zeng, Z. L., Qiu, H. B., Hu, P. S., Wang, Z. Q., Zhang, D. S., Wang, F., & Xu, R. H. (2018). ME1 Regulates NADPH Homeostasis to Promote Gastric Cancer Growth and Metastasis. *Cancer research* , *78* (8), 1972–1985. https://doi.org/10.1158/0008-5472.CAN-17-3155

[164] Dey, P., Baddour, J., Muller, F., Wu, C. C., Wang, H., Liao, W. T., Lan, Z., Chen, A., Gutschner, T., Kang, Y., Fleming, J., Satani, N., Zhao, D., Achreja, A., Yang, L., Lee, J., Chang, E., Genovese, G., Viale, A., Ying, H., … DePinho, R. A. (2017). Genomic deletion of malic enzyme 2 confers collateral lethality in pancreatic cancer. *Nature*

, *542* (7639), 119–123. https://doi.org/10.1038/nature21052
Lu YX *et al* . 2018 *ibid* .

[165] Hernandez, A. L., Wang, Y., Somerset, H. L., Keysar, S. B., Aisner, D. L., Marshall, C., Bowles, D. W., Karam, S. D., Raben, D., Jimeno, A., Varella-Garcia, M., & Wang, X. J. (2019). Inter- and intra-tumor heterogeneity of SMAD4 loss in head and neck squamous cell carcinomas. *Molecular carcinogenesis* , *58* (5), 666–673. https://doi.org/10.1002/mc.22958

[166] Waitkus, M. S., Diplas, B. H., & Yan, H. (2018). Biological Role and Therapeutic Potential of IDH Mutations in Cancer. *Cancer cell* , *34* (2), 186–195. https://doi.org/10.1016/j.ccell.2018.04.011

[167] Bahadur, S., Sahu, A. K., Baghel, P., & Saha, S. (2019). Current promising treatment strategy for glioblastoma multiform: A review. *Oncology reviews* , *13* (2), 417. https://doi.org/10.4081/oncol.2019.417

[168] Chen, R., Smith-Cohn, M., Cohen, A. L., & Colman, H. (2017). Glioma Subclassifications and Their Clinical Significance. *Neurotherapeutics : the Journal of the American Society for Experimental NeuroTherapeutics* , *14* (2), 284–297. https://doi.org/10.1007/s13311-017-0519-x

[169] Karpel-Massler, G., Nguyen, T., Shang, E., & Siegelin, M. D. (2019). Novel IDH1-Targeted Glioma Therapies. *CNS drugs* , *33* (12), 1155–1166. https://doi.org/10.1007/s40263-019-00684-6
Pusch, S., Krausert, S., Fischer, V., Balss, J., Ott, M., Schrimpf, D., Capper, D., Sahm, F., Eisel, J., Beck, A. C., Jugold, M., Eichwald, V., Kaulfuss, S., Panknin, O., Rehwinkel, H., Zimmermann, K., Hillig, R. C., Guenther, J., Toschi, L., Neuhaus, R., … von Deimling, A. (2017). Pan-mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma in vivo. *Acta neuropathologica* , *133* (4), 629–644. https://doi.org/10.1007/s00401-017-1677-y
Chaturvedi, A., Herbst, L., Pusch, S., Klett, L., Goparaju, R., Stichel, D., Kaulfuss, S., Panknin, O., Zimmermann, K., Toschi, L., Neuhaus, R., Haegebarth, A., Rehwinkel, H., Hess-Stumpp, H., Bauser, M.,

Bochtler, T., Struys, E. A., Sharma, A., Bakkali, A., Geffers, R., … Krämer, A. (2017). Pan-mutant-IDH1 inhibitor BAY1436032 is highly effective against human IDH1 mutant acute myeloid leukemia in vivo. *Leukemia* , *31* (10), 2020–2028. https://doi.org/10.1038/leu.2017.46
Sharma H. (2018). Development of Novel Therapeutics Targeting Isocitrate Dehydrogenase Mutations in Cancer. *Current topics in medicinal chemistry* , *18* (6), 505–524. https://doi.org/10.2174/1568026618666180518091144

[170] Li, S., Sun, C., Gu, Y., Gao, X., Zhao, Y., Yuan, Y., Zhang, F., Hu, P., Liang, W., Cao, K., Zhang, J., Wang, Z., & Ye, J. (2019). Mutation of IDH1 aggravates the fatty acid-induced oxidative stress in HCT116 cells by affecting the mitochondrial respiratory chain. *Molecular medicine reports* , *19* (4), 2509–2518. https://doi.org/10.3892/mmr.2019.9903

[171] Fraser, A. R., Bacci, B., le Chevoir, M. A., & Long, S. N. (2018). Isocitrate Dehydrogenase 1 Expression in Canine Gliomas. *Journal of comparative pathology* , *165* , 33–39. https://doi.org/10.1016/j.jcpa.2018.09.005
Reitman, Z. J., Olby, N. J., Mariani, C. L., Thomas, R., Breen, M., Bigner, D. D., McLendon, R. E., & Yan, H. (2010). IDH1 and IDH2 hotspot mutations are not found in canine glioma. *International journal of cancer* , *127* (1), 245–246. https://doi.org/10.1002/ijc.25017

[172] Antisense oligonucleotides targeting other NADPH-producing enzymes should also be effective.

[173] Nyce, J. W., & Metzger, W. J. (1997). DNA antisense therapy for asthma in an animal model. *Nature* , *385* (6618), 721–725. https://doi.org/10.1038/385721a0
Sandrasagra, A., Tang, L., Leonard, S. A., Teng, K., Li, Y., Mannion, J. C., & Nyce, J. W. (2001). RASONs: a novel antisense oligonucleotide therapeutic approach for asthma. *Expert opinion on biological therapy* , *1* (6), 979–983. https://doi.org/10.1517/14712598.1.6.979

Sandrasagra, A., Leonard, S. A., Tang, L., Teng, K., Li, Y., Ball, H. A., Mannion, J. C., & Nyce, J. W. (2002). Discovery and development of respirable antisense therapeutics for asthma. *Antisense & nucleic acid drug development* , *12* (3), 177–181. https://doi.org/10.1089/108729002760220770
Ball, H. A., Sandrasagra, A., Tang, L., Van Scott, M., Wild, J., & Nyce, J. W. (2003). Clinical potential of respirable antisense oligonucleotides (RASONs) in asthma. *American journal of pharmacogenomics : genomics-related research in drug development and clinical practice* , *3* (2), 97–106. https://doi.org/10.2165/00129785-200303020-00003
Ali, S., Leonard, S. A., Kukoly, C. A., Metzger, W. J., Wooles, W. R., McGinty, J. F., Tanaka, M., Sandrasagra, A., & Nyce, J. W. (2001). Absorption, distribution, metabolism, and excretion of a respirable antisense oligonucleotide for asthma. *American journal of respiratory and critical care medicine* , *163* (4), 989–993. https://doi.org/10.1164/ajrccm.163.4.9907078 .

[174] Data from the EMA, the European equivalent of the FDA, Palladia Product Information.

[175] Ki = 18.5 μM; compare DHEAS Ki = 310 μM; peak serum concentrations of DHEA of ≈ 30 nM, and of DHEAS of ≈ 11.5 μM. See Nyce 2018 for references detailing DHEAS and DHEA concentrations and inhibition constants.

[176] Kalogera, E., Pistos, C., Provatopoulou, X., Athanaselis, S., Spiliopoulou, C., & Gounaris, A. (2013). Androgen glucuronides analysis by liquid chromatography tandem-mass spectrometry: could it raise new perspectives in the diagnostic field of hormone-dependent malignancies?. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* , *940* , 24–34. https://doi.org/10.1016/j.jchromb.2013.09.022

[177] Anh NH et al. 2019 Steroidomics for the prevention, assessment, and management of cancers: A systemic review and functional

analysis. Metabolites. 2019 Sep 21;9(10). pii: E199. https://pubmed.ncbi.nlm.nih.gov/31546652/

[178] McNamara, K. M., & Sasano, H. (2015). The intracrinology of breast cancer. *The Journal of steroid biochemistry and molecular biology* , *145* , 172–178. https://doi.org/10.1016/j.jsbmb.2014.04.004

[179] Penning, T. M., & Detlefsen, A. J. (2020). Intracrinology-revisited and prostate cancer. *The Journal of steroid biochemistry and molecular biology* , *196* , 105499. https://doi.org/10.1016/j.jsbmb.2019.105499

[180] Grosser, G., Baringhaus, K. H., Döring, B., Kramer, W., Petzinger, E., & Geyer, J. (2016). Identification of novel inhibitors of the steroid sulfate carrier 'sodium-dependent organic anion transporter' SOAT (SLC10A6) by pharmacophore modelling. *Molecular and cellular endocrinology* , *428* , 133–141. https://doi.org/10.1016/j.mce.2016.03.028

Green, S. M., Kaipainen, A., Bullock, K., Zhang, A., Lucas, J. M., Matson, C., Banks, W. A., & Mostaghel, E. A. (2017). Role of OATP transporters in steroid uptake by prostate cancer cells in vivo. *Prostate cancer and prostatic diseases* , *20* (1), 20–27. https://doi.org/10.1038/pcan.2016.42

Cho, E., Montgomery, R. B., & Mostaghel, E. A. (2014). Minireview: SLCO and ABC transporters: a role for steroid transport in prostate cancer progression. *Endocrinology* , *155* (11), 4124–4132. https://doi.org/10.1210/en.2014-1337

Arakawa, H., Nakanishi, T., Yanagihara, C., Nishimoto, T., Wakayama, T., Mizokami, A., Namiki, M., Kawai, K., & Tamai, I. (2012). Enhanced expression of organic anion transporting polypeptides (OATPs) in androgen receptor-positive prostate cancer cells: possible role of OATP1A2 in adaptive cell growth under androgen-depleted conditions. *Biochemical pharmacology* , *84* (8), 1070–1077. https://doi.org/10.1016/j.bcp.2012.07.026

[181] Suzuki, T., Miki, Y., Nakamura, Y., Ito, K., & Sasano, H. (2011). Steroid sulfatase and estrogen sulfotransferase in human carcinomas. *Molecular and cellular endocrinology* , *340* (2), 148–153. https://doi.org/10.1016/j.mce.2010.11.001

Lee, W. M., Jang, K. S., Bae, J., & Koh, A. R. (2016). The Role of Steroid Sulfatase as a Prognostic Factor in Patients with Endometrial Cancer. *Yonsei medical journal* , *57* (3), 754–760. https://doi.org/10.3349/ymj.2016.57.3.754

Mungenast, F., Aust, S., Vergote, I., Vanderstichele, A., Sehouli, J., Braicu, E., Mahner, S., Castillo-Tong, D. C., Zeillinger, R., & Thalhammer, T. (2017). Clinical significance of the estrogen-modifying enzymes steroid sulfatase and estrogen sulfotransferase in epithelial ovarian cancer. *Oncology letters* , *13* (6), 4047–4054. https://doi.org/10.3892/ol.2017.5969

[182] Shimizu, Y., Tamada, S., Kato, M., Takeyama, Y., Fujioka, M., Kakehashi, A., Nakatani, T., Wanibuchi, H., & Gi, M. (2018). Steroid sulfatase promotes invasion through epithelial-mesenchymal transition and predicts the progression of bladder cancer. *Experimental and therapeutic medicine* , *16* (6), 4463–4470. https://doi.org/10.3892/etm.2018.6787

[183] Shin, S., Im, H. J., Kwon, Y. J., Ye, D. J., Baek, H. S., Kim, D., Choi, H. K., & Chun, Y. J. (2017). Human steroid sulfatase induces Wnt/β-catenin signaling and epithelial-mesenchymal transition by upregulating Twist1 and HIF-1α in human prostate and cervical cancer cells. *Oncotarget* , *8* (37), 61604–61617. https://doi.org/10.18632/oncotarget.18645

[184] Gilligan, L. C., Rahman, H. P., Hewitt, A. M., Sitch, A. J., Gondal, A., Arvaniti, A., Taylor, A. E., Read, M. L., Morton, D. G., & Foster, P. A. (2017). Estrogen Activation by Steroid Sulfatase Increases Colorectal Cancer Proliferation via GPER. *The Journal of clinical endocrinology and metabolism* , *102* (12), 4435–4447. https://doi.org/10.1210/jc.2016-3716

Gilligan, L. C., Gondal, A., Tang, V., Hussain, M. T., Arvaniti, A.,

Hewitt, A. M., & Foster, P. A. (2017). Estrone Sulfate Transport and Steroid Sulfatase Activity in Colorectal Cancer: Implications for Hormone Replacement Therapy. *Frontiers in pharmacology* , *8* , 103. https://doi.org/10.3389/fphar.2017.00103

[185] Armandari, I., Hamid, A. R., Verhaegh, G., & Schalken, J. (2014). Intratumoral steroidogenesis in castration-resistant prostate cancer: a target for therapy. *Prostate international* , *2* (3), 105–113. https://doi.org/10.12954/PI.14063

[186] Al Sarakbi, W., Mokbel, R., Salhab, M., Jiang, W. G., Reed, M. J., & Mokbel, K. (2006). The role of STS and OATP-B mRNA expression in predicting the clinical outcome in human breast cancer. *Anticancer research* , *26* (6C), 4985–4990. https://pubmed.ncbi.nlm.nih.gov/17214375/

[187] Foster, P. A., Chander, S. K., Newman, S. P., Woo, L. W., Sutcliffe, O. B., Bubert, C., Zhou, D., Chen, S., Potter, B. V., Reed, M. J., & Purohit, A. (2008). A new therapeutic strategy against hormone-dependent breast cancer: the preclinical development of a dual aromatase and sulfatase inhibitor. *Clinical cancer research : an official journal of the American Association for Cancer Research* , *14* (20), 6469–6477. https://doi.org/10.1158/1078-0432.CCR-08-1027

Foster, P. A., Chander, S. K., Parsons, M. F., Newman, S. P., Woo, L. W., Potter, B. V., Reed, M. J., & Purohit, A. (2008). Efficacy of three potent steroid sulfatase inhibitors: pre-clinical investigations for their use in the treatment of hormone-dependent breast cancer. *Breast cancer research and treatment* , *111* (1), 129–138. https://doi.org/10.1007/s10549-007-9769-3

Geisler, J., Sasano, H., Chen, S., & Purohit, A. (2011). Steroid sulfatase inhibitors: promising new tools for breast cancer therapy?. *The Journal of steroid biochemistry and molecular biology* , *125* (1-2), 39–45. https://doi.org/10.1016/j.jsbmb.2011.02.002

Palmieri, C., Stein, R. C., Liu, X., Hudson, E., Nicholas, H., Sasano, H., Guestini, F., Holcombe, C., Barrett, S., Kenny, L., Reed, S., Lim, A., Hayward, L., Howell, S., Coombes, R. C., & IRIS trial participants

(2017). IRIS study: a phase II study of the steroid sulfatase inhibitor Irosustat when added to an aromatase inhibitor in ER-positive breast cancer patients. *Breast cancer research and treatment* , *165* (2), 343–353. https://doi.org/10.1007/s10549-017-4328-z

Lu, Q., Xia, W., Lee, K., Zhang, J., Yuan, H., Yuan, Z., Shi, Y., Wang, S., & Xu, F. (2020). Bicalutamide plus Aromatase Inhibitor in Patients with Estrogen Receptor-Positive/Androgen Receptor-Positive Advanced Breast Cancer. *The oncologist* , *25* (1), 21–e15. https://doi.org/10.1634/theoncologist.2019-0564

Reinert, T., Debiasi, M., Bines, J., & Barrios, C. H. (2018). Trends in progression-free survival (PFS) and time to progression (TTP) over time within first-line aromatase inhibitors trials in hormone receptor-positive advanced breast cancer. *Breast cancer research and treatment* , *168* (2), 457–465. https://doi.org/10.1007/s10549-017-4593-x

[188] Nyce J. Components of the human-specific. P53-mediated 'kill switch" tumor suppression mechanism are usurped by human tumors, creating the possibility of therapeutic exploitation. *Cancer Drug Resist* 2019;2:1207-1214. https://cdrjournal.com/article/view/3293

[189] I am especially critical of a recent study performed in a series of 12 women with advanced breast cancer treated with 100 mg of oral DHEA daily. I can hardly imagine a more ill advised study, which had no chance of therapeutic effect at the dosage used, and may have had a stimulatory effect on tumor growth. See, *but do not repeat* :

Pietri, E., Massa, I., Bravaccini, S., Ravaioli, S., Tumedei, M. M., Petracci, E., Donati, C., Schirone, A., Piacentini, F., Gianni, L., Nicolini, M., Campadelli, E., Gennari, A., Saba, A., Campi, B., Valmorri, L., Andreis, D., Fabbri, F., Amadori, D., & Rocca, A. (2019). Phase II Study of Dehydroepiandrosterone in Androgen Receptor-Positive Metastatic Breast Cancer. *The oncologist* , *24* (6), 743–e205. https://doi.org/10.1634/theoncologist.2018-0243

[190] Abou-Gharbia, M., Pashko, L., Schwartz, A., & Swern, D. (1981). Synthesis of dehydroepiandrosterone sulfatide and 16 alpha-halogenated steroids. *Journal of pharmaceutical sciences* , *70* (10),

1154–1157. https://doi.org/10.1002/jps.2600701017

Nyce, J. W., Magee, P. N., Hard, G. C., & Schwartz, A. G. (1984). Inhibition of 1,2-dimethylhydrazine-induced colon tumorigenesis in Balb/c mice by dehydroepiandrosterone. *Carcinogenesis* , *5* (1), 57–62. https://doi.org/10.1093/carcin/5.1.57

[191] Hursting, S. D., Perkins, S. N., Phang, J. M., & Barrett, J. C. (2001). Diet and cancer prevention studies in p53-deficient mice. *The Journal of nutrition* , *131* (11 Suppl), 3092S–4S. https://doi.org/10.1093/jn/131.11.3092S

Ciolino, H. P., MacDonald, C. J., & Yeh, G. C. (2002). Inhibition of carcinogen-activating enzymes by 16alpha-fluoro-5-androsten-17-one. *Cancer research* , *62* (13), 3685–3690. https://pubmed.ncbi.nlm.nih.gov/12097275/

McCormick, D. L., Johnson, W. D., Kozub, N. M., Rao, K. V., Lubet, R. A., Steele, V. E., & Bosland, M. C. (2007). Chemoprevention of rat prostate carcinogenesis by dietary 16alpha-fluoro-5-androsten-17-one (fluasterone), a minimally androgenic analog of dehydroepiandrosterone. *Carcinogenesis* , *28* (2), 398–403. https://doi.org/10.1093/carcin/bgl141

[192] Schwartz, A. G., & Pashko, L. L. (2001). Suppression of 12-O-tetradecanoylphorbol-13-acetate-induced epidermal hyperplasia and inflammation by the dehydroepiandrosterone analog 16alpha-fluoro-5-androsten-17-one and its reversal by NADPH liposomes. *Cancer letters* , *168* (1), 7–14. https://doi.org/10.1016/s0304-3835(01)00423-2

[193] McCormick, D. L., Johnson, W. D., Kozub, N. M., Rao, K. V., Lubet, R. A., Steele, V. E., & Bosland, M. C. (2007). Chemoprevention of rat prostate carcinogenesis by dietary 16alpha-fluoro-5-androsten-17-one (fluasterone), a minimally androgenic analog of dehydroepiandrosterone. *Carcinogenesis* , *28* (2), 398–403. https://doi.org/10.1093/carcin/bgl141

[194] Barron, C. C., Bilan, P. J., Tsakiridis, T., & Tsiani, E. (2016). Facilitative glucose transporters: Implications for cancer detection,

prognosis and treatment. *Metabolism: clinical and experimental* , *65* (2), 124–139. https://doi.org/10.1016/j.metabol.2015.10.007
Ancey, P. B., Contat, C., & Meylan, E. (2018). Glucose transporters in cancer - from tumor cells to the tumor microenvironment. *The FEBS journal* , *285* (16), 2926–2943. https://doi.org/10.1111/febs.14577

[195] Shi, Y., Liu, S., Ahmad, S., & Gao, Q. (2018). Targeting Key Transporters in Tumor Glycolysis as a Novel Anticancer Strategy. *Current topics in medicinal chemistry* , *18* (6), 454–466. https://doi.org/10.2174/1568026618666180523105234
Shriwas, P., Chen, X., Kinghorn, A. D., & Ren, Y. (2020). Plant-derived glucose transport inhibitors with potential antitumor activity. *Phytotherapy research : PTR* , *34* (5), 1027–1040. https://doi.org/10.1002/ptr.6587
Abolhasani, A., Biria, D., Abolhasani, H., Zarrabi, A., & Komeili, T. (2019). Investigation of the Role of Glucose Decorated Chitosan and PLGA Nanoparticles as Blocking Agents to Glucose Transporters of Tumor Cells. *International journal of nanomedicine* , *14* , 9535–9546. https://doi.org/10.2147/IJN.S228652

[196] McSheehy, P. M., Leach, M. O., Judson, I. R., & Griffiths, J. R. (2000). Metabolites of 2'-fluoro-2'-deoxy-D-glucose detected by 19F magnetic resonance spectroscopy in vivo predict response of murine RIF-1 tumors to 5-fluorouracil. *Cancer research* , *60* (8), 2122–2127. https://pubmed.ncbi.nlm.nih.gov/10786672/
Nakada, T., Kwee, I. L., & Conboy, C. B. (1986). Noninvasive in vivo demonstration of 2-fluoro-2-deoxy-D-glucose metabolism beyond the hexokinase reaction in rat brain by 19F nuclear magnetic resonance spectroscopy. *Journal of neurochemistry* , *46* (1), 198–201. https://doi.org/10.1111/j.1471-4159.1986.tb12945.x
Wiebe L. I. (2001). FDG metabolism: Quaecumque sunt vera… *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* , *42* (11), 1679–1681. https://pubmed.ncbi.nlm.nih.gov/11696639/

[197] Som, P., Atkins, H. L., Bandoypadhyay, D., Fowler, J. S., MacGregor, R. R., Matsui, K., Oster, Z. H., Sacker, D. F., Shiue, C. Y., Turner, H., Wan, C. N., Wolf, A. P., & Zabinski, S. V. (1980). A fluorinated glucose analog, 2-fluoro-2-deoxy-D-glucose (F-18): nontoxic tracer for rapid tumor detection. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* , *21* (7), 670–675. https://pubmed.ncbi.nlm.nih.gov/7391842/

[198] Fukuda, H., Matsuzawa, T., Abe, Y., Endo, S., Yamada, K., Kubota, K., Hatazawa, J., Sato, T., Ito, M., Takahashi, T., Iwata, R., & Ido, T. (1982). Experimental study for cancer diagnosis with positron-labeled fluorinated glucose analogs: [18F]-2-fluoro-2-deoxy-D-mannose: a new tracer for cancer detection. *European journal of nuclear medicine* , *7* (7), 294–297. https://doi.org/10.1007/BF00253423

[199] Aide, N., Hicks, R. J., Le Tourneau, C., Lheureux, S., Fanti, S., & Lopci, E. (2019). FDG PET/CT for assessing tumour response to immunotherapy : Report on the EANM symposium on immune modulation and recent review of the literature. *European journal of nuclear medicine and molecular imaging* , *46* (1), 238–250. https://doi.org/10.1007/s00259-018-4171-4

[200] Sánchez, D., Romero, L., López, S., Campuzano, M., Ortega, R., Morales, A., Guadarrama, M., Cesarman-Maus, G., García-Pérez, O., & Lizano, M. (2019). ^{18} F-FDG-PET/CT in Canine Mammary Gland Tumors. *Frontiers in veterinary science* , *6* , 280. https://doi.org/10.3389/fvets.2019.00280

Randall E. K. (2016). PET-Computed Tomography in Veterinary Medicine. *The Veterinary clinics of North America. Small animal practice* , *46* (3), 515–vi. https://doi.org/10.1016/j.cvsm.2015.12.008

Seiler, S. M., Baumgartner, C., Hirschberger, J., Beer, A. J., Brühschwein, A., Kreutzmann, N., Laberke, S., Wergin, M. C., Meyer-Lindenberg, A., Brandl, J., von Thaden, A. K., Farrell, E., & Schwaiger, M. (2015). Comparative Oncology: Evaluation of 2-Deoxy-2-[18F]fluoro-D-glucose (FDG) Positron Emission

Tomography/Computed Tomography (PET/CT) for the Staging of Dogs with Malignant Tumors. *PloS one* , *10* (6), e0127800. https://doi.org/10.1371/journal.pone.0127800
Kim, J., Kwon, S. Y., Cena, R., Park, S., Oh, J., Oui, H., Cho, K. O., Min, J. J., & Choi, J. (2014). CT and PET-CT of a dog with multiple pulmonary adenocarcinoma. *The Journal of veterinary medical science* , *76* (4), 615–620. https://doi.org/10.1292/jvms.13-0434

[201] Griffin, L. R., Thamm, D. H., Brody, A., & Selmic, L. E. (2019). Prognostic value of fluorine^{18} flourodeoxyglucose positron emission tomography/computed tomography in dogs with appendicular osteosarcoma. *Journal of veterinary internal medicine* , *33* (2), 820–826. https://doi.org/10.1111/jvim.15453

[202] Rigacci, L., Puccini, B., Broccoli, A., Dona, M., Gotti, M., Evangelista, A., Santoro, A., Bonfichi, M., Re, A., Spina, M., Botto, B., Pulsoni, A., Pagani, C., Stelitano, C., Salvi, F., Nassi, L., Mannelli, L., Kovalchuk, S., Gioia, D., & Zinzani, P. L. (2020). Clinical characteristics of interim-PET negative patients with a positive end PET from the prospective HD08-01 FIL study. *Annals of hematology* , *99* (2), 283–291. https://doi.org/10.1007/s00277-019-03889-3

[203] Terasawa, T., Lau, J., Bardet, S., Couturier, O., Hotta, T., Hutchings, M., Nihashi, T., & Nagai, H. (2009). Fluorine-18-fluorodeoxyglucose positron emission tomography for interim response assessment of advanced-stage Hodgkin's lymphoma and diffuse large B-cell lymphoma: a systematic review. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* , *27* (11), 1906–1914. https://doi.org/10.1200/JCO.2008.16.0861

[204] DHEAS is also converted to DHEA, for use in steroid hormone synthesis, an endocrine process referred to as intracrinology; see: Labrie F. (2015). All sex steroids are made intracellularly in peripheral tissues by the mechanisms of intracrinology after menopause. *The Journal of steroid biochemistry and molecular biology* , *145* , 133–

138. https://doi.org/10.1016/j.jsbmb.2014.06.001
Labrie F. (2019). Intracrinology and menopause: the science describing the cell-specific intracellular formation of estrogens and androgens from DHEA and their strictly local action and inactivation in peripheral tissues. *Menopause (New York, N.Y.)* , *26* (2), 220–224. https://doi.org/10.1097/GME.0000000000001177

[205] Nyce JW. 2021 Endocrine-Related Cancer, *in press* .

[206] https://ods.od.nih.gov/about/dshea_wording.aspx

[207] Nyce J. W. (2021). Alert to US physicians: DHEA, widely used as an OTC androgen supplement, may exacerbate COVID-19. *Endocrine-related cancer* , ERC-20-0439. Advance online publication. https://doi.org/10.1530/ERC-20-0439

[208] Geraniol is completely unpalatable to dogs, and attempting to administer it will adversely affect a dog's compliance with the kill switch treatment protocol. This is another reason that we will not employ it in our FDA clinical trials. The auto-inflammatory reaction is only rarely observed, resolves simply by discontinuation of the treatment protocol, and may in fact be an important factor in the resolution of some tumors.

[209] Akula, M. K., Shi, M., Jiang, Z., Foster, C. E., Miao, D., Li, A. S., Zhang, X., Gavin, R. M., Forde, S. D., Germain, G., Carpenter, S., Rosadini, C. V., Gritsman, K., Chae, J. J., Hampton, R., Silverman, N., Gravallese, E. M., Kagan, J. C., Fitzgerald, K. A., Kastner, D. L., … Wang, D. (2016). Control of the innate immune response by the mevalonate pathway. *Nature immunology* , *17* (8), 922–929. https://doi.org/10.1038/ni.3487

[210] Fenini, G., Contassot, E., & French, L. E. (2017). Potential of IL-1, IL-18 and Inflammasome Inhibition for the Treatment of Inflammatory Skin Diseases. *Frontiers in pharmacology* , *8* , 278. https://doi.org/10.3389/fphar.2017.00278

[211] Buczkowska, A., Swiezewska, E., & Lefeber, D. J. (2015). Genetic defects in dolichol metabolism. *Journal of inherited metabolic disease* , *38* (1), 157–169. https://doi.org/10.1007/s10545-014-9760-1
Sparks SE, Krasnewich DM. Congenital Disorders of N-Linked Glycosylation and Multiple Pathway Overview. 2005 Aug 15 [Updated 2017 Jan 12]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1332/

[212] Cylwik, B., Naklicki, M., Chrostek, L., & Gruszewska, E. (2013). Congenital disorders of glycosylation. Part I. Defects of protein N-glycosylation. *Acta biochimica Polonica* , *60* (2), 151–161. https://pubmed.ncbi.nlm.nih.gov/23730680/

Where we are with FDA

[213] An Investigational New Drug Application (IND) to the FDA thus requires a pharmacology section demonstrating "proof of principle." i.e., efficacy in an animal model. (See, for example, Hillary Sheevers : How much animal data is required to move into clinical testing? https://accelerate.ucsf.edu/files/RKS_Sheevers_101007.pdf) As we have demonstrated in the references from my laboratory cited in this book, no other species has evolved the human-specific kill switch tumor suppression mechanism, not even the chimpanzee, which differs in critical components of the *lex naturalis* , including body size, life span, and a much less efficient kill switch due to the fact that only humans evolved in concert with our harnessing of fire. Similarly, for dogs, there is no lower animal model with its particular kill switch mechanism.

[214] Thalidomide caused these birth defects because it blocked angiogenesis— the new growth of blood vessels— in the developing limbs of the affected children. Without blood supply, the limbs could

not develop properly. Later an enterprising team of Scandinavian cancer research scientists realized that, since angiogenesis was a necessary characteristic of tumors if they were to grow and thrive in the body, and brought thalidomide back as a medicine, this time for the treatment of cancer. See, BACH, A., BICHEL, J., & HEJGAARD, J. J. (1963). STUDIES ON THE POSSIBLE ANTI-NEOPLASTIC EFFECT OF THALIDOMIDE. *Acta pathologica et microbiologica Scandinavica* , *59* , 491–499. https://doi.org/10.1111/j.1699-0463.1963.tb01251.x

[215] just for clarity, the full name of our company is ACGT Biotech, Inc. The ACGT stands for the four major bases that comprise DNA. Earlier in the research, before we understood that we had actually uncovered the human-specific tumor suppression mechanismthe ACGT stood for Advanced Canine Genetic Testing.

[216] Nyce J. W. (2020). A *lex naturalis* delineates components of a human-specific, adrenal androgen-dependent, p53-mediated 'kill switch' tumor suppression mechanism. *Endocrine-related cancer* , *27* (2), R51–R65. https://doi.org/10.1530/ERC-19-0382
Nyce J. W. (2019). Species-specific mechanisms of tumor suppression are fundamental drivers of vertebrate speciation: critical implications for the 'war on cancer'. *Endocrine-related cancer* , *26* (2), C1–C5. https://doi.org/10.1530/ERC-18-0468
Nyce J. W. (2018). Detection of a novel, primate-specific 'kill switch' tumor suppression mechanism that may fundamentally control cancer risk in humans: an unexpected twist in the basic biology of TP53. *Endocrine-related cancer* , *25* (11), R497–R517. https://doi.org/10.1530/ERC-18-0241
Nyce J 2019 Components of the human-specific, p53-mediated "kill switch" tumor suppression mechanism are usurped by human tumors, creating the possibility of therapeutic exploitation. Cancer Drug Resistance 2:1207-1214. https://cdrjournal.com/article/view/3293

[217] Or, if someone— like the purveyors of *Mrs. Winthrop's Soothing Syrup* — begin marketing a potentially dangerous product directly to

consumers.

[218] Are dogs considered “laboratory animals?” Insofar as FDA is concerned, dogs are laboratory animals since the FDA requires toxicology studies to be performed in dogs. Furthermore, the *lex naturalis* shows clearly that only dogs can be used to determine the safety and effectiveness of drugs meant to treat canine cancer.

[219] Nyce AT, Nyce JW 2018 The natural product dehydroepiandrosterone depletes brain and cardiac adenosine. Journal of Traditional Medicine & Clinical Naturopathy 7:3 https://www.omicsonline.org/open-access-pdfs/the-natural-product-dehydroepiandrosterone-depletes-brain-and-cardiac-adenosine-2573-4555-1000275.pdf
Our finding described in the publication above, that DHEA depletes brain and cardiac adenosine, should be viewed with alarm since the adenosinergic pathways are highly conserved throughout species, unlike steroidal pathways, which vary widely among species. In other words, the adenosine depletion that we observed in the brain and heart of rats is likely also to occur in the brain and heart of humans who use DHEA as an anabolic steroid.

[220] Qi, G., van Aerde, K., Abel, T., & Feldmeyer, D. (2017). Adenosine Differentially Modulates Synaptic Transmission of Excitatory and Inhibitory Microcircuits in Layer 4 of Rat Barrel Cortex. *Cerebral cortex (New York, N.Y. : 1991)* , *27* (9), 4411–4422. https://doi.org/10.1093/cercor/bhw243

[221] Camici, M., Garcia-Gil, M., & Tozzi, M. G. (2018). The Inside Story of Adenosine. *International journal of molecular sciences* , *19* (3), 784. https://doi.org/10.3390/ijms19030784

[222] Zukowska, P., Kutryb-Zajac, B., Toczek, M., Smolenski, R. T., & Slominska, E. M. (2015). The role of ecto-5’-nucleotidase in endothelial dysfunction and vascular pathologies. *Pharmacological reports : PR* , *67* (4), 675–681. https://doi.org/10.1016/j.pharep.2015.05.002

[223] Nyce, J. W., & Metzger, W. J. (1997). DNA antisense therapy for asthma in an animal model. *Nature* , *385* (6618), 721–725. https://doi.org/10.1038/385721a0

[224] Phan, T. A., Gray, A. M., & Nyce, J. W. (1997). Intrastriatal adenosine A1 receptor antisense oligodeoxynucleotide blocks ethanol-induced motor incoordination. *European journal of pharmacology* , *323* (2-3), R5–R7. https://doi.org/10.1016/s0014-2999(97)00147-7

[225] Giménez-Llort, L., Fernández-Teruel, A., Escorihuela, R. M., Fredholm, B. B., Tobeña, A., Pekny, M., & Johansson, B. (2002). Mice lacking the adenosine A1 receptor are anxious and aggressive, but are normal learners with reduced muscle strength and survival rate. *The European journal of neuroscience* , *16* (3), 547–550. https://doi.org/10.1046/j.1460-9568.2002.02122.x

[226] Navarro, J. F., Romero, C., & Maldonado, E. (2000). Effects of N6-cyclohexyl adenosine (CHA) on isolation-induced aggression in male mice. *Methods and findings in experimental and clinical pharmacology* , *22* (1), 43–46. https://doi.org/10.1358/mf.2000.22.1.795830

[227] Klötz, F., Petersson, A., Isacson, D., & Thiblin, I. (2007). Violent crime and substance abuse: a medico-legal comparison between deceased users of anabolic androgenic steroids and abusers of illicit drugs. *Forensic science international* , *173* (1), 57–63. https://doi.org/10.1016/j.forsciint.2007.01.026
Lundholm, L., Käll, K., Wallin, S., & Thiblin, I. (2010). Use of anabolic androgenic steroids in substance abusers arrested for crime. *Drug and alcohol dependence* , *111* (3), 222–226. https://doi.org/10.1016/j.drugalcdep.2010.04.020

[228] Kornblutt AE, Wilson D. 2005 How one pill escaped place on the steroid list. New York Times , April 17, 2005. https://www.biopsychiatry.com/dhea/legal.html

[229] **Cancer Therapy Market - Growth, Trends, and Forecast (2020 - 2025)** https://www.mordorintelligence.com/industry-reports/cancer-therapy-market

[230] Nyce J. W. (2018). Detection of a novel, primate-specific 'kill switch' tumor suppression mechanism that may fundamentally control cancer risk in humans: an unexpected twist in the basic biology of TP53. *Endocrine-related cancer* , *25* (11), R497–R517. https://doi.org/10.1530/ERC-18-0241

[231] Schüz, J., Espina, C., & Wild, C. P. (2019). Primary prevention: a need for concerted action. *Molecular oncology* , *13* (3), 567–578. https://doi.org/10.1002/1878-0261.12432
Nyce J. W. (2020). A lex naturalis delineates components of a human-specific, adrenal androgen-dependent, p53-mediated 'kill switch' tumor suppression mechanism. *Endocrine-related cancer* , *27* (2), R51–R65. https://doi.org/10.1530/ERC-19-0382
Nyce J 2018. *ibid* .

[232] Nyce J 2020 *ibid* .

[233] ACGT will keep all rights to the veterinary aspects of our kill switch-based drug technology.

About the Author

Jonathan Nyce received his doctorate in cancer biology in 1983 for studies performed at the Fels Cancer Research Institute, part of the Temple University School of Medicine, in Philadelphia, PA. His mentors at the Fels were Sidney Weinhouse, a member of the National Academy of Sciences and the recipient of the first award made by the National Science Foundation; and Peter Magee, a Fellow of the Royal College of Physicians, and like Professor Weinhouse, served as an editor in chief of Cancer Research, president of the American Association for Cancer Research (AACR), and director at the Fels. While still at the Fels, Dr. Nyce published one of the first papers establishing the new field of epigenetics in cancer— a field that has now grown to include thousands of researchers from around the world, and

more than 25,000 publications. The recipient of an NIH Fellowship after the Fels, Dr. Nyce performed postdoctoral research at the Children's Hospital of Los Angeles, where he developed, and then tested and published with clinical colleagues, an improved treatment for pediatric leukemia that increased the percentage of complete remissions— a method that is still being used today in cancer wards around the world. Based upon this work, Dr. Nyce was offered a professorship in the department of pharmacology at the Brody School of Medicine, part of the University of North Carolina system, with a joint appointment in Medicine, in the department of pediatrics. Here, Professor Nyce lectured to medical students, supervised the research of graduate students, and expanded on the research that had led to the improved treatment method for pediatric leukemia, founding a new area of cancer research— drug-induced DNA hypermethylation as a mechanism of cancer drug resistance. This area, too, has now expanded to include many hundreds of cancer research scientists investigating this mechanism of drug resistance, at institutions around the world. During the early stages of this work, Nobel Laureate George Hitchings became an additional mentor, helping Professor Nyce to acquire early funding from the Welcome Foundation, where Hitchings was director. Later in his academic career, Professor

Nyce invented a new form of respiratory therapy, called Respirable Antisense Oligonucleotides (RASONs), demonstrating its utility in a paper published in *Nature* , and taking a drug utilizing this new technology into FDA clinical trials in patients with asthma. *Nature* subsequently invited Professor Nyce to organize a world conference on this technology, which he did and which was held in Tokyo as *Antisense 2000: Advances in Epigenetic Medicine* . Over the course of his career, Professor Nyce has been the recipient of 13 grants from the National Cancer Institute and the National Institutes of Health, has published almost 100 papers in the peer-reviewed literature, has fifty patents issued or pending on his inventions, and has been an invited speaker around the world. In 2012 he founded a research and development company to assess his hypothesis that the use of mouse models to study human cancer had wildly misled the field of cancer research for more than 50 years. Working with dogs with spontaneous cancer, Professor Nyce discovered that they had evolved a rudimentary form of an otherwise primate-specific tumor suppression mechanism involving circulating DHEAS. It was this work that led to the uncovering of the *lex naturalis* equation, a fundamental theorem linking cancer with speciation. By its demonstration of the fundamental

interdependence of cancer and speciation— the *lex naturalis* equation is so illuminating that it can rightly be called the $E = mc^2$ of biology.

Professor Nyce has three children, who are the light of his life. Pictured above in the vestibule of ACGT Biotechnology, they are, left to right, Trevor, Samantha and Alex— splendid individuals each.

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