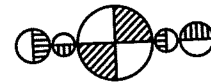
THE GENE

AN INTIMATE HISTORY



SIDDHARTHA MUKHERJEE

SCRIBNER

NEW YORK LONDON TORONTO SYDNEY NEW DELHI

Those who promise us paradise on earth never produced anything but a hell.

—Karl Popper

It's only we humans who want to own the future, too.

—Tom Stoppard, *The Coast of Utopia*

The Future of the Future

Probably no DNA science is at once as hopeful, controversial, hyped, and even as potentially dangerous as the discipline known as gene therapy.

—Gina Smith, *The Genomics Age*

Clear the air! Clean the sky! Wash the wind! Take the stone from the stone, take the skin from the arm, take the muscle from the bone, and wash them. Wash the stone, wash the bone, wash the brain, wash the soul, wash them wash them!

—T. S. Eliot, *Murder in the Cathedral*

Let us return, for a moment, to a conversation on the ramparts of a fort. It is the late summer of 1972. We are in Sicily, at a scientific conference on genetics. It is late at night, and Paul Berg and a group of students have clambered up a hill overlooking the lights of a city. Berg's news—of the possibility of combining two pieces of DNA to create "recombinant DNA"—has sent tremors of wonder and anxiety through the meeting. At the conference, the students are concerned about the dangers of such novel DNA fragments: if the wrong gene is introduced into the wrong organism, the experiment might unleash a biological or ecological catastrophe. But Berg's interlocutors aren't only worried about pathogens. They have gone, as students often do, to the heart of the matter: they want to know about the prospects of human genetic engineering—of new genes being introduced permanently into the human genome. What about predicting the future from genes—and then altering that destiny through genetic manipulation? "They were already thinking several steps ahead," Berg later told me. "I was worried about the future, but they were worried about the future of the future."

For a while, the "future of the future" seemed biologically intractable. In 1974, barely three years after the invention of recombinant DNA technology, a gene-modified SV40 virus was used to infect early mouse embryonic cells. The plan was audacious. The virus-infected embryonic cells were mixed with the cells of a normal embryo to create a composite of cells, an embryological "chimera." These composite embryos were implanted into mice. All the organs and cells of the embryo emanated from that mix of cells—blood, brain, guts, heart, muscles, and, most crucially, the sperm and the eggs. If the virally infected embryonic cells formed some of the sperm and the egg cells of the newborn mice, then the viral genes would be transmitted from mouse to mouse vertically across generations, like any other gene. The virus, like a Trojan horse, might thus smuggle genes permanently into an animal's genome across multiple generations resulting in the first genetically modified higher organism.

The experiment worked at first—but it was stymied by two unexpected effects. First, although cells carrying viral genes clearly emerged in the blood, muscle, brain, and nerves of the mouse, the delivery of the viral genes into sperm and eggs was extremely inefficient. Try as they might, scientists could not achieve efficient "vertical" transmission of the genes across generations. And second, even though viral genes were present in the mouse cells, the *expression* of the genes was firmly shut down, resulting in an inert gene that did not make RNA or protein. Years later, scientists would discover that epigenetic marks had been placed on viral genes to silence them. We now know that cells have ancient detectors that recognize viral genes and stamp them with chemical marks, like cancellation signs, to prevent their activation.

The genome had, it seemed, already anticipated attempts to alter it. It was a perfect stalemate. There's an old proverb among magicians that it's essential to learn to make things reappear before one learns to make things disappear. Gene therapists were relearning that lesson. It was easy to slip a gene invisibly into a cell and into an embryo. The real challenge was to make it visible again.

Ⓘ

Thwarted by these original studies, the field of gene therapy stagnated for another decade or so, until biologists stumbled on a critical discovery: embryonic stem cells, or ES cells. To understand the future of gene

therapy in humans, we need to reckon with ES cells. Consider an organ such as the brain, or the skin. As an animal ages, cells on the surface of its skin grow, die, and slough off. This wave of cell death might even be catastrophic—after a burn, or a massive wound, for instance. To replace these dead cells, most organs must possess methods to regenerate their own cells.

Stem cells fulfill this function, especially after catastrophic cell loss. A stem cell is a unique type of cell that is defined by two properties. It can give rise to other functional cell types, such as nerve cells or skin cells, through differentiation. And it can renew *itself*—i.e., give rise to more stem cells, which can, in turn, differentiate to form the functional cells of an organ. A stem cell is somewhat akin to a grandfather that continues to produce children, grandchildren, and great-grandchildren, generation upon generation, without ever losing his own reproductive fecundity. It is the ultimate reservoir of regeneration for a tissue or an organ.

Most stem cells reside in particular organs and tissues and give rise to a limited repertoire of cells. Stem cells in the bone marrow, for instance, only produce blood cells. There are stem cells in the crypts of the intestine that are dedicated to the production of intestinal cells. But embryonic stem cells, or ES cells, which arise from the inner sheath of an animal's embryo, are vastly more potent; they can give rise to *every* cell type in the organism—blood, brains, intestines, muscles, bone, skin. Biologists use the word *pluripotent* to describe this property of ES cells.

ES cells also possess an unusual third characteristic—a quirk of nature. They can be isolated from the embryo of an organism and grown in petri dishes in the lab. The cells grow continuously in culture. Tiny, translucent spheres that can cluster into nestlike whirls under the microscope, they resemble a dissolving organ more than an organism in the making. Indeed, when the cells were first derived from mouse embryos in a laboratory in Cambridge, England, in the early eighties, they generated little interest among geneticists. "Nobody seems to be interested in my cells," the embryologist Martin Evans complained.

But the real power of an ES cell lies, yet again, in a transition: like DNA, like genes, and like viruses, it is the intrinsic duality of its existence that makes this cell such a potent biological tool. Embryonic stem cells behave like other experimentally amenable cells in tissue culture. They can be grown in petri dishes; they can be frozen in vials and thawed back to life. The cells can be propagated in liquid broth for generations, and genes

can be inserted into their genomes or excised from their genomes with relative ease.

Yet, put the same cell into the right environment in the right context, and life literally leaps out of it. Mixed with cells from an early embryo and implanted into a mouse womb, the cells divide and form layers. They differentiate into all sorts of cells: blood, brain, muscle, liver—and even sperm and egg cells. These cells, in turn, organize themselves into organs and then become incorporated, miraculously, into a multilayered, multicellular organism—an actual mouse. Every experimental manipulation performed in the petri dish is thus carried forward into this mouse. The genetic modification of a cell in a dish "becomes" the genetic modification of an organism in a womb. It is a transition between lab and life.

The experimental ease permitted by embryonic stem cells also surmounted a second, and more intractable, problem. When viruses are used to deliver genes into cells, it is virtually impossible to control where the gene is inserted into the genome. At 3 billion base pairs of DNA, the human genome is about fifty thousand or a hundred thousand times the size of most viral genomes. A viral gene drops into the genome like a candy wrapper thrown from an airplane into the Atlantic: there is no way to predict where it might land. Virtually all viruses capable of gene integration, such as HIV or SV40, generally latch their genes randomly onto some spot in the human genome. For gene therapy, this random integration is an infernal nuisance. The viral genes might fall into a silent crevasse of the genome, never to be expressed. The genes might fall into an area of the chromosome that is actively silenced by the cell without much effort. Or worse, the integration might disrupt an essential gene or activate a cancer-causing gene, resulting in potential disasters.

With ES cells, however, scientists learned to make genetic changes not randomly, but in targeted positions in the genome, including within *the genes themselves*. You could choose to change the insulin gene and—through some rather basic but ingenious experimental manipulations—ensure that *only* the insulin gene was changed in the cells. And because the gene-modified ES cells could, in principle, generate all the cell types in a full mouse, you could be sure that a mouse with precisely that changed insulin gene would be born. Indeed, if the gene-modified ES cells eventually produced sperm and egg cells in the adult mice, then the gene would be transmitted from mouse to mouse across generations, thus achieving vertical hereditary transmission.

This technology had far-reaching implications. In the natural world, the only means to achieve a directional or intentional change in a gene is through random mutation and natural selection. If you expose an animal to X-rays, say, a genetic alteration might become permanently embedded in the genome—but there is no method to focus the attention of an X-ray to one particular gene. Natural selection must choose the mutation that confers the best fitness to the organism and thereby allows that mutation to become increasingly common in the gene pool. But in this scheme, neither mutation nor evolution has any intentionality or directionality. In nature, the engine that drives genetic alteration has no one in its driver's seat. The "watchmaker" of evolution, as Richard Dawkins reminds us, is inherently blind.

Using ES cells, however, a scientist could intentionally manipulate virtually any chosen gene and incorporate that genetic change permanently into the genome of an animal. It was mutation and selection in the same step—evolution fast-forwarded in a laboratory dish. The technology was so transformative that a new word had to be coined to describe these organisms: they were called *transgenic* animals—from "across genes." By the early 1990s, hundreds of strains of transgenic mice had been created in laboratories around the world to decipher the functions of genes. One mouse was made with a jellyfish gene inserted into its genome that allowed it to glow in the dark under blue lamps. Other mice, carrying variants of the growth hormone gene, grew twice the size of their normal counterparts. There were mice endowed with genetic alterations that forced them to develop Alzheimer's disease, epilepsy, or premature aging. Mice with activated cancer genes exploded with tumors, allowing biologists to use these mice as models for human malignancies. In 2014, researchers created a mouse carrying a mutation in a gene that controls the communication between neurons in the brain. These mice have substantially increased memory and superior cognitive function. They are the savants of the rodent world: they acquire memories faster, retain them longer, and learn new tasks nearly twice as fast as normal mice.

The experiments brimmed over with complex ethical implications. Could this technique be used in primates? In humans? Who would regulate the creation of animals with transgenes? What genes would be, or could be, introduced? What were the limits on transgenes?

Fortunately, technical barriers intervened before the ethical mayhem had a chance to become unmoored. Much of the original work on ES

cells—including the production of transgenic organisms—had been performed using mouse cells. In the early 1990s, when several *human* embryonic stem cells were derived from early human embryos, scientists ran into an unexpected barrier. Unlike the mouse ES cells, which had proved so amenable to experimental manipulations, human ES cells did not behave themselves in culture. "It may be the field's dirty little secret: human ES cells do not have the same capabilities as mouse ES cells," the biologist Rudolf Jaenisch said. "You can't clone them. You can't use them for gene targeting. . . . They are very different from mouse embryonic stem cells, which can do everything."

At least temporarily, the genie of transgenesis seemed contained.

Ⓘ

Transgenic modification of human embryos was out of the question for a while—but what if gene therapists could settle for a less radical goal? Could viruses be used to deliver genes into human *nonreproductive* cells—i.e., to neurons, blood, or muscle cells? The problem of random integration into the genome would remain—and most crucially, no vertical transmission of genes from one organism to the next would occur. But if the virally delivered genes could be put into the right kind of cells, they might still achieve their therapeutic purpose. Even that goal would represent a leap into the future of human medicine. It would be gene-therapy lite.

In 1988, a two-year-old girl named Ashanti DeSilva, or Ashi, for short, in North Olmsted, Ohio, began to develop peculiar symptoms. Children have dozens of transient ailments in infancy, as any parent knows, but Ashi's illnesses and symptoms were markedly abnormal: bizarre pneumonias and infections that seemed to persist, wounds that would not heal, and a white blood cell count that hovered consistently below normal. Much of Ashi's childhood was spent in and out of the hospital: at age two, a run-of-the-mill viral infection spun out of control, causing life-threatening internal bleeding and a prolonged hospitalization.

For a while, her doctors were mystified by her symptoms, attributing her periodic illnesses loosely to an underdeveloped immune system that would eventually mature. But when the symptoms refused to abate as Ashi turned three, she underwent a barrage of tests. Her immunodeficiency was attributed to her genes—to rare, spontaneous mutations in both copies of a gene called ADA on chromosome twenty. By then, Ashi

had already suffered several near-death experiences. The physical toll on her body had been immense—but the emotional anguish that she had experienced was more pronounced: one morning, the four-year-old awoke and said, "Mommy, you shouldn't have had a child like me."

The ADA gene—short for "adenosine deaminase"—encodes an enzyme that converts adenosine, a natural chemical produced by the body, into a harmless product called inosine. In the absence of the ADA gene, the detoxification reaction fails to occur, and the body gets clogged with toxic by-products of adenosine metabolism. The cells that are most acutely poisoned are infection-fighting T cells—and in their absence, the immune system collapses rapidly. The illness is fleetingly rare—only one in 150,000 children is born with ADA deficiency—but it is even rarer still, because virtually all children die of the disease. ADA deficiency is part of a larger group of notorious illnesses called severe combined immunodeficiency, or SCID. The most famous SCID patient, a boy named David Vetter, had spent all twelve years of his life in a plastic chamber in a Texas hospital. The Bubble Boy, as David was called by the media, died in 1984, still imprisoned in his sterile plastic bubble, after a desperate attempt at a bone marrow transplant.

David Vetter's death gave pause to doctors who had hoped to use bone marrow transplants to treat ADA deficiency. The only other medicine, being tested in early clinical trials in the mideighties, was called PEG-ADA—the purified enzyme derived from cows and wrapped in an oily chemical sheath to make it long-lived in the blood (the normal ADA protein is too short-lived to be effective). But even PEG-ADA barely reversed the immunodeficiency. It had to be injected into the blood every month or so, to replace the enzyme degraded by the body. More ominously, PEG-ADA carried the risk of inducing antibodies against itself—depleting the levels of the enzyme even more acutely and precipitating a full catastrophe, making the solution infinitely worse than the original problem.

Could gene therapy correct ADA deficiency? After all, only one gene needed to be corrected, and the gene had already been identified and isolated. A vehicle, or vector, designed to deliver genes into human cells had also been identified. In Boston, Richard Mulligan, a virologist and geneticist, had designed a particular strain of retrovirus—a cousin of HIV's—that could potentially shuttle any gene into any human cell with relative safety. Retroviruses can be designed to infect many kinds of cells; their distinct capability is their ability to insert their own genome into the cell's

genome, thereby permanently affixing their genetic material to that of a cell. By tweaking the technology, Mulligan had created partially crippled viruses that would infect cells and integrate into their genomes, but would not propagate the infection from cell to cell. Virus went in, but no virus came out. The gene fell into the genome but never popped out again.

ⵀ

In 1986, at the National Institutes of Health in Bethesda, a team of gene therapists, led by William French Anderson and Michael Blaese,* decided to use variants of Mulligan's vectors to deliver the ADA gene into children with ADA deficiency.† Anderson obtained the ADA gene from another lab and inserted it into the retroviral gene-delivery vector. In the early 1980s, Anderson and Blaese had run some preliminary trials hoping to use retroviral vectors to deliver the human ADA gene into blood-forming stem cells of mice and then monkeys. Once these stem cells had been infected by the virus carrying the ADA gene, Anderson hoped, they would form all the cellular elements of blood—including, crucially, T cells into which the now-functioning ADA gene had been delivered.

The results were far from promising: the level of gene delivery was abysmal. Of the five monkeys treated, only one—Monkey Roberts—had cells in the blood that showed long-term production of the human ADA protein from the virus-delivered gene. But Anderson was unfazed. "Nobody knows what may happen when new genes enter the body of a human being," he argued. "It's a total black box, despite what anyone tells you. . . . Test-tube and animal research can only tell us so much. Eventually you have to try it in a person."

On April 24, 1987, Anderson and Blaese applied to the NIH for permission to launch their gene-therapy protocol. They proposed to extract bone marrow stem cells from the children with ADA deficiency, infect the cells with the virus in the lab, and transplant the modified cells back into the patients. Since the stem cells generate all the elements of blood—including B and T cells—the ADA gene would find its way into the T cells, where it was most required.

* Kenneth Culver was also a crucial member of this original team.

† In 1980, a UCLA scientist named Martin Cline attempted the first known gene therapy in humans. A hematologist by training, Cline chose to study beta-thalassemia, a genetic disease in which the mutation of a single gene, encoding a subunit of hemoglobin, causes severe anemia. Reasoning that he might be able to run his trials in foreign countries, where the use of recombinant DNA in humans was less constrained and regulated, Cline did not notify his hospital's review board, and ran his trials on two thalassemia patients in Israel and Italy. Cline's attempts were discovered by the NIH and UCLA. He was sanctioned by the NIH, found to be in breach of federal regulations, and ultimately resigned as the chair of his division. The complete data from his experiment were never formally published.

The proposal was sent to the Recombinant DNA Advisory Committee, or RAC, a consortium set up within the NIH in the wake of the Berg recommendations of the Asilomar meeting. Known for its tough oversight, the advisory committee was the gatekeeper for all experiments that involved recombinant DNA (the committee was so notoriously obstreperous that researchers called getting its approval being "taken through the Rack"). Perhaps predictably, the RAC rejected the protocol outright, citing the poor animal data, the barely detectable level of gene delivery into stem cells, and the lack of a detailed experimental rationale, while noting that gene transfer into a human body had never been attempted before.

Anderson and Blaese returned to the lab to revamp their protocol. Begrudgingly, they admitted that the RAC's decision was correct. The barely detectable infection rate of bone marrow stem cells by the gene-carrying virus was clearly a problem, and the animal data was far from exhilarating. But if stem cells could not be used, how could gene therapy hope to succeed? Stem cells are the only cells in the body that can renew themselves and therefore provide a long-term solution to a gene deficiency. Without a source of self-renewing or long-lived cells, you might insert genes into the human body, but the cells carrying the genes would eventually die and vanish. There would be genes, but no therapy.

That winter, mulling over the problem, Blaese found a potential solution. What if, rather than delivering the genes into the blood-forming stem cells, they merely took *T cells* from the blood of ADA patients and put the virus into the cells? It would not be as radical or permanent an experiment as putting viruses into stem cells, but it would be far less toxic and much easier to achieve clinically. The T cells could be harvested from peripheral blood, not bone marrow, and the cells might live just long enough to make the ADA protein and correct the deficiency. Although the T cells would inevitably fade from the blood, the procedure could be repeated again and again. It would not qualify as definitive gene therapy, but it would still be a proof of principle—gene-therapy double-lite.

Anderson was reluctant; if he was to launch the first trial of human

gene therapy, he wanted a definitive trial and a chance to stake a permanent claim on medical history. He resisted at first, but eventually, conceding Blaese's logic, relented. In 1990, Anderson and Blaese approached the committee again. Again, there was vicious dissent: the T cell protocol had even less supportive data than the original. Anderson and Blaese submitted modifications, and modifications to the modifications. Months passed without a decision. In the summer of 1990, after a prolonged series of debates, the committee agreed to let them proceed with the trial. "Doctors have been waiting for this day for a thousand years," the RAC's chairman, Gerard McGarrity, said. Most others in the committee were not as sanguine about the chances of success.

Anderson and Blaese searched hospitals around the country to find children with ADA deficiency for their trial. They came upon a small trove in Ohio: all of two patients with the genetic defect. One was a tall, dark-haired girl named Cynthia Cutshall. The second, Ashanti DeSilva, was a four-year-old daughter of a chemist and a nurse, both from Sri Lanka.

Ⓓ

In September 1990, on an overcast morning in Bethesda, Van and Raja DeSilva, Ashi's parents, brought their daughter to the NIH. Ashi was now four years old—a shy, hesitant girl with a fringe of shiny hair, cut pageboy-style, and an apprehensive face that could suddenly be brightened by a smile. This was her first meeting with Anderson and Blaese. When they approached her, she looked away. Anderson brought her down to the hospital's gift shop and asked her to pick out a soft toy for herself. She chose a bunny.

Back at the Clinical Center, Anderson inserted a catheter into one of Ashi's veins, collected samples of her blood, and rushed it up to his lab. Over the next four days, 200 million retroviruses, in an enormous cloudy soup, were mixed with 200 million T cells drawn from Ashi's blood. Once infected, the cells grew in petri dishes, forming lush outcroppings of new cells, and even newer cells. They doubled by day and doubled by night in a silent, humid incubator in Building 10 of the Clinical Center—just a few hundred feet from the lab where Marshall Nirenberg, almost exactly twenty-five years before, had solved the genetic code.

Ashi DeSilva's gene-modified T cells were ready on September 14, 1990. That morning, Anderson ran out of his home at dawn, skipping

breakfast, near nauseous with anticipation, and dashed up the steps to the lab on the third floor. The DeSilva family was already waiting for him; Ashi was standing by her mother, her elbows planted firmly on her seated mother's lap as if waiting for a dental exam. The morning was spent running more tests. The clinic was silent, except for the occasional footfall of research nurses running in and out. As Ashi sat on her bed in a loose yellow gown, a needle was put into one of her veins. She winced slightly but recovered: her veins had been cannulated dozens of times before.

At 12:52 p.m., a vinyl bag carrying the murky swirl of nearly 1 billion T cells infected by the ADA-gene-carrying retrovirus was brought up to the floor. Ashi looked apprehensively at the bag as the nurses hooked it to her vein. Twenty-eight minutes later, the bag had run dry, its last dregs emptied into Ashi. She played with a yellow sponge ball on her bed. Her vital signs were normal. Ashi's father was sent downstairs with a pile of quarters to buy candy from the vending machine on the ground floor. Anderson looked visibly relieved. "A cosmic moment has come and gone, with scarcely a sign of its magnitude," one observer wrote. It was celebrated, in style, with a bag of multicolored M&M's.

"Number one," Anderson said, pointing elatedly at Ashi as he wheeled her down the hallway after the transfusion had been completed. A few of his colleagues at the NIH were waiting outside the door to witness the coming of the first human to be transfused with gene-modified cells, but the crowd thinned quickly and the scientists vanished back to their labs. "It's like people say in downtown Manhattan," Anderson groused. "Jesus Christ himself could walk by and nobody would notice." The next day, Ashi's family returned home to Ohio.

Ⓓ

Did Anderson's gene-therapy experiment work? We do not know—and perhaps we will never know. Anderson's protocol was designed as a proof of principle for safety—i.e., could retrovirus-infected T cells be safely delivered into human bodies? It was not designed to test efficacy: Would this protocol cure ADA deficiency, even temporarily? Ashi DeSilva and Cynthia Cutshall, the first two patients on the study, received the gene-modified T cells, but they were allowed continued treatment with PEG-ADA, the artificial enzyme. Any effect of the gene therapy was thus confounded by that medicine.

Nonetheless, both DeSilva's and Cutshall's parents were convinced that the treatment worked. "It's not a big improvement," Cynthia Cutshall's mother admitted. "But to give you an example, she just got over one cold. Usually her colds end up in pneumonia. This one didn't. . . . That's a breakthrough for her." Ashi's father, Raja DeSilva, concurred: "With PEG, we'd seen a tremendous improvement. But even with [PEG-ADA], she had runny noses and a constant cold, was on antibiotics all the time. But by the second infusion of genes, in December, it began to change. We noticed because we were not using up so many boxes of tissues."

Despite Anderson's enthusiasm, and the anecdotal evidence from the families, many proponents of gene therapy, including Mulligan, were far from convinced that Anderson's trial had amounted to anything more than a publicity stunt. Mulligan, the most voluble critic of the trial from the very first, was particularly incensed by the claims of success when the data was insufficient. If the most ambitious gene-therapy trial attempted in humans was going to be measured in the frequency of runny noses and boxes of Kleenex, then it would be an embarrassment for the field. "It's a sham," Mulligan told a journalist when asked about the protocol. To test whether targeted genetic alterations could be introduced into human cells, and whether these genes would confer normal function safely and effectively, he proposed a careful, uncontaminated trial—"clean, chaste gene therapy," as he called it.

But by then, the ambitions of gene therapists had been frothed to such frenzy that "clean, chaste," careful experiments had become virtually impossible to perform. Following the reports of the T cell trials at the NIH, gene therapists envisaged novel cures for genetic diseases such as cystic fibrosis and Huntington's disease. Since genes could be delivered into virtually any cell, any cellular disease was a candidate for gene therapy: heart disease, mental illness, cancer. As the field readied itself to sprint forward, voices such as Mulligan's urged caution and restraint, but they were brushed aside. The enthusiasm would come at a steep price: it would bring the field of gene therapy, and human genetics, to the brink of disaster, and to the lowest, bleakest point in its scientific history.

Ⓘ

On September 9, 1999, almost exactly nine years after Ashi DeSilva had been treated with genetically modified white blood cells, a boy named

Jesse Gelsinger flew to Philadelphia to enroll in another gene-therapy trial. Gelsinger was eighteen years old. A motorcycling and wrestling enthusiast, with an easy, carefree manner, Gelsinger, like Ashi DeSilva and Cynthia Cutshall, was also born with a mutation in a single gene involved in metabolism. In Gelsinger's case, the gene was called ornithine transcarbamylase, or OTC, which encodes an enzyme synthesized in the liver. OTC, the enzyme, performs a critical step in the breakdown of proteins. In the absence of the enzyme, ammonia, a by-product of protein metabolism, accumulates in the body. Ammonia—the chemical found in cleaning fluid—damages blood vessels and cells, diffuses past the blood-brain barrier, and ultimately results in the slow poisoning of neurons in the brain. Most patients with mutations in OTC do not survive childhood. Even with strictly protein-free diets, they are poisoned by the breakdown of their own cells as they grow.

Among children born with an unfortunate disease, Gelsinger might have counted himself as especially fortunate, for his variant of OTC deficiency was mild. The mutation in his gene had not come from his father or his mother, but had occurred spontaneously in one of his cells *in utero*, probably when he was still a young embryo. Genetically, Gelsinger was a rare phenomenon—a human chimera, a patchwork cellular quilt, with some cells lacking functional OTC, and some with a working gene. Still, his ability to metabolize proteins was severely compromised. Gelsinger lived on a carefully calibrated diet—every calorie and portion weighed, measured, and accounted for—and took thirty-two pills a day to keep his ammonia level in check. Despite such extreme cautionary measures, Gelsinger had still suffered several life-threatening episodes. At four, he had joyfully eaten a peanut butter sandwich that had precipitated a coma.

In 1993, when Gelsinger was twelve years old, two pediatricians in Pennsylvania, Mark Batshaw and James Wilson, began to experiment with gene therapy to cure children with OTC deficiencies. A former college-level football player, Wilson was a risk taker fascinated by ambitious human experiments. He had formed a gene-therapy company, named Genova, and an Institute for Human Gene Therapy at the University of Pennsylvania. Both Wilson and Batshaw were intrigued by OTC deficiency. As with ADA deficiency, that OTC is caused by the dysfunction of a single gene made the illness an ideal test case for gene therapy. But the form of gene therapy that Wilson and Batshaw envisioned was vastly more radical: rather than extracting cells, genetically modifying them, and injecting them back into

children (à la Anderson and Blaese), Batshaw and Wilson imagined inserting the corrected gene *directly* back into the body via a virus. This would not be gene-therapy lite: they would create a virus carrying the OTC gene and deliver the virus into the liver through the bloodstream, leaving the virus to infect cells *in situ*.

The virus-infected liver cells would start synthesizing the OTC enzyme, Batshaw and Wilson reasoned, and thus correct the enzyme deficiency. The telltale sign would be a reduction of ammonia in the blood. "It wasn't that subtle," Wilson recalls. To deliver the gene, Wilson and Batshaw chose adenovirus, a virus that typically causes a common cold but is not associated with any severe disease. It seemed like a safe, reasonable choice—the blandest of viruses used as the vehicle for one of the boldest human genetic experiments of the decade.

In the summer of 1993, Batshaw and Wilson began to inject the modified adenovirus into mice and monkeys. The mouse experiments worked as predicted: the virus reached the liver cells, disgorged the gene, and transformed the cells into microscopic factories for the functional OTC enzyme. But the monkey experiments were more complicated. At higher doses of the virus, an occasional monkey raised a brisk immune response to the virus, resulting in inflammation and liver failure. One monkey hemorrhaged to death. Wilson and Batshaw modified the virus, shaving off many of the viral genes that might elicit immunity, to make it a safer gene-delivery vehicle. They also reduced the potential human dose by seventeenfold to doubly ensure the safety of the virus. In 1997, they applied to the Recombinant DNA Advisory Committee, the gatekeeper for all gene-therapy experiments, for approval for a human trial. The RAC was resistant at first, but it too had changed: in the decade between the ADA trial and Wilson's, the once-fierce guardian of recombinant DNA had turned into an enthusiastic cheerleader of human gene therapy. The fizz of enthusiasm had even leached beyond the committee. Asked by the RAC to comment on Wilson's trial, bioethicists argued that treating children with full-blown OTC deficiency might result in "coercion": What parent *wouldn't* want to try a breakthrough therapy that might work on a dying child? Instead, ethicists recommended a trial on normal volunteers and patients with mild variants of OTC, such as Jesse Gelsinger.

ⓘ

In Arizona, Gelsinger, meanwhile, was chafing against the elaborate restrictions on his diet and medications ("All teenagers rebel," Gelsinger's father, Paul, told me, but teenage rebellion might feel particularly acute when it involves "a hamburger and a glass of milk"). In the summer of 1998, when he was seventeen, Gelsinger learned of the OTC trial at the University of Pennsylvania. Gelsinger was gripped by the thought of gene therapy. He wanted a respite from the grinding routine of his life. "But what got him even more excited," his father recalled, "was the idea that he was doing it for the babies. How do you say no to that?"

Gelsinger could hardly wait to sign on. In June 1999, he contacted the Pennsylvania team through his local doctors to enroll in the trial. That month, Paul and Jesse Gelsinger flew to Philadelphia to meet Wilson and Batshaw. Jesse and Paul were both impressed. The trial struck Paul Gelsinger as a "beautiful, beautiful thing." They visited the hospital and then wandered through the city in a haze of excitement and anticipation. Jesse stopped in front of the Rocky Balboa bronze outside the Spectrum Arena. Paul snapped a picture of his son, his arms raised in a boxer's victory stance.

On September 9, Jesse returned to Philadelphia with a duffel bag filled with clothes, books, and wrestling videos to start the trial at University Hospital. Jesse would stay with his uncle and cousins in the city and admit himself to the hospital on the appointed morning. The procedure was described as so quick and painless that Paul planned to pick up his son one week after the therapy had been completed to bring him back home on a commercial flight.

ⓘ

On the morning of September 13, the day chosen for the viral injection, Gelsinger's ammonia level was found to be hovering around seventy micromoles per liter—twice the normal level and at the upper edge of the cutoff value for the trial. The nurses brought news of the abnormal lab to Wilson and Batshaw. The protocol, meanwhile, was in full swing. The operating rooms were on standby. The viral liquid had been thawed and sat glistening in its plastic pouch. Wilson and Batshaw debated Gelsinger's eligibility, but decided that it was clinically safe to continue; the previous seventeen patients had, after all, tolerated the injection. At about 9:30 a.m., Gelsinger was wheeled down to the interventional radiology suite.

He was sedated, and two large catheters were snaked through his legs to reach an artery close to the liver. Around 11:00 a.m., a surgeon drew about thirty milliliters from a bag clouded with the concentrated adenovirus and injected the puff of virus into Gelsinger's artery. Hundreds of millions of invisible infectious particles carrying the OTC gene streamed into the liver. By noon, the procedure was done.

The afternoon was uneventful. That evening, back in his hospital room, Gelsinger spiked a fever to 104 degrees. His face was flushed. Wilson and Batshaw did not make much of the symptoms. The other patients had also experienced transient fevers. Jesse called Paul in Arizona on the phone and said, "I love you," before hanging up and drawing up his covers in bed. He slept fitfully through the night.

The next morning, a nurse noted that Jesse's eyeballs had turned the palest shade of yellow. A test confirmed that bilirubin, a product made in the liver and also stored in red blood cells, was spilling into his blood. The elevated bilirubin meant one of two things: either the liver was being injured or blood cells were being damaged. Both were ominous signs. In any other human, the small bump in cellular breakdown or liver failure might have been shrugged off. But in a patient with OTC deficiency, the combination of these two injuries might spark a perfect storm: the extra protein leaching out from the blood cells would not be metabolized, and the damaged liver, deficient in protein metabolism even in the best of times, would be even less capable of processing the excess protein load. The body would intoxicate itself on its own poisons. By noon, Gelsinger's ammonia level had climbed to a staggering 393 micromoles per liter—nearly ten times the normal level. Paul Gelsinger and Mark Batshaw were alerted. James Wilson heard the news from the surgeon who had inserted the catheter and injected the virus. Paul booked a red-eye to Pennsylvania, while a team of doctors swooped into the ICU to begin dialysis to avert a coma.

At eight o'clock the next morning, when Paul Gelsinger reached the hospital, Jesse was hyperventilating and confused. His kidneys were failing. The ICU team sedated him to try to use a mechanical ventilator to stabilize his breathing. Late that night, his lungs began to stiffen and collapse, filling up with fluids from the inflammatory response. The ventilator faltered, unable to push enough oxygen in, and so Jesse was hooked up to a device to force oxygen directly into the blood. His brain function was also deteriorating. A neurologist was called to examine him and noted Jesse's downcasting eyes—a sign of damage to the brain.

The next morning, Hurricane Floyd struck the East Coast, battering the shores of Pennsylvania and Maryland with shrieking winds and torrents of rain. Batshaw was stuck on a train getting to the hospital. He ran down the last minutes of his cell phone's battery talking to the nurses and doctors, then sat in the pitch darkness, stewing with anxiety. By the late afternoon, Jesse's condition worsened again. His kidneys shut down. His coma deepened. Stranded in his hotel room, with no taxi in sight, Paul Gelsinger walked a mile and a half through the whistling storm to the hospital to see Jesse in the ICU. He found his son unrecognizable—comatose, swollen, bruised, yellowed with jaundice, with dozens of lines and catheters crisscrossing his body. The ventilator puffed ineffectually against his inflamed lungs with the flat, dull sound of wind slapping water. The room buzzed and beeped with hundreds of instruments recording the slow decline of a boy in desperate physiological distress.

On the morning of Friday, September 17, on the fourth day after the gene delivery, Jesse was found to be brain-dead. Paul Gelsinger decided to withdraw life support. The chaplain came into the hospital room and put his hand on Jesse's head, anointing it with oil, and read the Lord's Prayer. The machines were shut off, one by one. The room fell into silence, except for Jesse's deep, agonal breaths. At 2:30 p.m., Jesse's heart stopped. He was officially pronounced dead.

"How could such a beautiful thing go so, so wrong?" When I spoke to Paul Gelsinger in the summer of 2014, he was still searching for an answer. A few weeks earlier, I had e-mailed Paul about my interest in Jesse's story. Gelsinger spoke to me on the phone, then agreed to meet me after my talk on the future of genetics and cancer at an open forum in Scottsdale, Arizona. While I stood in the lobby of the auditorium at the end of the talk, a man in a Hawaiian shirt with Jesse's round, open face—a face that I remembered vividly from pictures on the Web—pushed his way through the crowd and extended his hand.

In the aftermath of Jesse's death, Paul has become a one-man crusader against the overreach of clinical experimentation. He is not against medicine or innovation. He believes in the future of gene therapy. But he is suspicious of the hyperbaric atmosphere of enthusiasm and delusion that ultimately resulted in his son's death. The crowd thinned, and Paul turned around to leave. An acknowledgment passed between us: a doctor writing about the future of medicine and genetics, and a man whose story had been etched into its past. There had been an infinite horizon of grief

in his voice. "They didn't have a handle on it yet," he said. "They tried it too quickly. They tried it without doing it right. They rushed this thing. They really rushed it."

The pattern of neglect was so damning that it nearly obscured the most important scientific lessons of the trial. Even if the doctors admitted that

they had been negligent and impatient, Gelsinger's death was still a mystery: no one could explain why Jesse Gelsinger had suffered such a severe immune reaction to the virus, while the seventeen other patients had not. Clearly, the adenoviral vector—even the "third-generation" virus shorn of some of its immunogenic proteins—was capable of inciting a severe idiosyncratic response in some patients. The autopsy of Gelsinger's body showed that his physiology had become overwhelmed by this immune response. Notably, when his blood was analyzed, antibodies highly reactive to the virus were found, dating from even *before* the viral injection. Gelsinger's hyperactive immune response was likely related to prior exposure to a similar strain of adenovirus, possibly from a common cold. Exposures to pathogens are well-known to incite antibodies that remain in circulation for decades (this, after all, is how most vaccines work). In Jesse's case, this prior exposure had likely triggered a hyperactive immune response that had spiraled out of control for unknown reasons. Ironically, perhaps the choice of a "harmless," common virus as the initial vector for gene therapy had turned out to be the trial's key failing.

What, then, was the appropriate vector for gene therapy? What kind of virus could be used to deliver genes safely into humans? And which

"Gene therapy is not yet therapy."

In science, there is a well-known aphorism that the most beautiful theory can be slayed by an ugly fact. In medicine, the same aphorism takes a somewhat different form: a beautiful therapy can be killed by an ugly trial. In retrospect, the OTC trial was nothing short of ugly—hurriedly

designed, poorly planned, badly monitored, abysmally delivered. It was made twice as hideous by the financial conflicts involved; the prophets were in it for profits. But the basic concept behind the trial—delivering genes into human bodies or cells to correct genetic defects—was conceptually sound, as it had been for decades. In principle, the capacity to deliver genes into cells using viruses or other gene vectors should have led to powerful new medical technologies, had the scientific and financial ambitions of the early proponents of gene therapy not gotten in the way.

Gene therapy would eventually become therapy. It would rebound from the ugliness of the initial trials and learn the moral lessons implicit in the "cautionary tale of scientific overreach." But it would take yet another decade, and a lot more learning, for the science to cross the breach.

Genetic Diagnosis: "Previvors"

All that man is,
All mere complexities.
—W. B. Yeats, "Byzantium"

The anti-determinists want to say that DNA is a little side-show, but every disease that's with us is caused by DNA. And [every disease] can be fixed by DNA.
—George Church

While human gene therapy was exiled to wander its scientific tundra in the late 1990s, human genetic diagnosis experienced a remarkable renaissance. To understand this renaissance, we need to return to the "future's future" envisioned by Berg's students on the ramparts of the Sicilian castle. As the students had imagined it, the future of human genetics would be built on two fundamental elements. The first was "genetic diagnosis"—the idea that genes could be used to predict or determine illness, identity, choice, and destiny. The second was "genetic alteration"—that genes could be changed to change the future of diseases, choice, and destiny.

This second project—the intentional alteration of genes ("writing the genome")—had evidently faltered with the abrupt ban on gene-therapy trials. But the first—predicting future fate from genes ("reading the genome")—only gained more strength. In the decade following Jesse Gelsinger's death, geneticists uncovered scores of genes linked to some of the most complex and mysterious human diseases—illnesses for which genes had never been implicated as primary causes. These discoveries would enable the development of immensely powerful new technologies that would allow for the preemptive diagnosis of illness. But they would also force genetics and medicine to confront some of the deepest medical