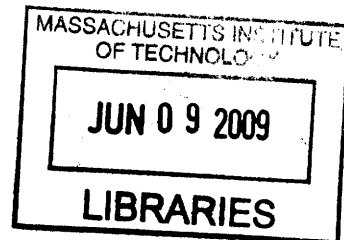


Lessons from a Rare Disease

by

Stephanie Lynn Dutchen

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Signature of Author: _____

Graduate Program in Science Writing
May 18, 2009

Certified by: _____

Marcia Bartusiak
Visiting Professor, Graduate Program in Science Writing
Thesis Advisor

Accepted by: _____

Thomas Levenson
Professor of Science Writing
Director, Graduate Program in Science Writing

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Submitted to the Program in Writing and Humanistic Studies
On May 18, 2009 in Partial Fulfillment of the
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ABSTRACT

Progeria is a genetic aging disease of childhood affecting an estimated one in four to eight million births. Children with progeria experience a range of developmental disorders and aging-like symptoms, including wrinkled and discolored skin, stunted growth, visible veins, fat loss, hair loss, bone loss, joint contractures, and heart disease. Their average life expectancy is thirteen. There is currently no treatment or cure.

The disease arises from a single nucleotide mutation in the *LMNA* gene, which makes proteins called lamins that comprise the inner lining of the nuclear wall. The mutation leads to the production of a misshapen lamin called progerin that builds up with time, disrupting nuclear shape and function. It is not yet clear how these changes lead to the disease's symptoms.

Doctors probe potential treatments while researchers explore progeria's potential links to far more widespread health problems such as aging, heart disease, and laminopathies. Experts debate the extent to which progeria represents normal human aging on overdrive. It is seen as a segmental aging disorder, sharing only some symptoms with aging. Progeria may reveal insights into basic biological phenomena such as gene expression, DNA regulation, RNA splicing, protein processing, cellular aging, and stem cell differentiation.

Instrumental to the discovery of the progeria gene and the growth of scientific interest since 2002 has been The Progeria Research Foundation. The story of its creation when Sam Berns, son of doctors Leslie Gordon and Scott Berns, was diagnosed with progeria in 1998, is also the story of the birth of modern progeria research in the U.S.

Research highlighted in this thesis includes progeria's cardiovascular complications in transgenic mice; the discovery that progeria's symptoms can be reversed; clinical trials testing farnesyltransferase inhibitors or FTIs, statins and bisphosphonates, and all three together; the search for a cure; and the presence of progerin in the skin cells of healthy people.

Thesis Supervisor: Marcia Bartusiak

Title: Visiting Professor, Graduate Program in Science Writing

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SD

The mice come from a room upstairs. Racks of cages line the walls there, housing hundreds of mice that lounge around in their litter or play in cardboard mazes. Some of them look old and hunched. The room isn't sterile, but it is kept clean enough that visitors have to put on gowns, hats, gloves, and booties before stepping inside. "So you look like a cosmonaut," laughs molecular biologist Michelle Olive in a melodic French accent. "And usually they don't have every size, so you really look like a big bubble."

Once she has selected her mouse, Olive brings it downstairs to the lab and puts it in a shoebox-sized glass container on the countertop. A tube connects the box to a canister of carbon dioxide. She turns a small knob to release the gas, which lulls the mouse to sleep and then death. "There's strict laws on how you can kill a mouse," Olive says. This way has been approved as humane.

After the animal has been euthanized, Robin the "mouse technician" takes out its tiny blood vessels under a microscope with careful tugs and snips. Each gets preserved in dry ice or formaldehyde and sent out to be sliced—"chook chook chook chook chook," Olive mimics the chopping blade—into five-micron cross-sections, one twentieth the width of a human hair. When the slices come back embedded in strips of wax, every O-shaped cross-section hardly bigger than a speck, Olive runs them through a series of baths and adds stains for each part of the cell she wants to look at. A few drops of another dye light up the DNA, and she mounts the slices on glass slides for the microscope.

An artery has three layers. On the inside are the endothelial cells, the lining. On the outside is the adventitia. In between is the media, the muscular layer that lets the vessel contract and relax to push blood along. Healthy arteries have lots of smooth muscle cells in

the media. On Olive's slide, the muscle cells are dyed red. She peers into the microscope.

There is almost no red.

"All those cells that used to contract the vessel, they are gone," she says. "They died." Under the microscope, the luminous artery has suffered a neighborhood blackout. Tracking her mice, Olive has observed that this cell loss worsens over time. "At five weeks nothing's wrong, at five months nothing's wrong, at twelve months you see the cells are mostly gone, and at sixteen to twenty months it's completely empty," she says.

Healthy vessels also contain bands of springy elastin that help them recover their shape after each pulse. Olive zooms in to find them. Instead of strong, thick bands, she says, "they're actually broken." The once-fit artery has become loose and flabby like the stretched-out waistband of an old pair of sweatpants.

The one-two punch of smooth muscle cell loss and snapped elastin is "really dramatic. It's a severe modification of the vessel," says Olive. It leads to low blood pressure problems. The outer layer, the adventitia, also seems to be thicker than normal. Olive suspects that this is the weakened artery's attempt to contain the blood flowing through it. "It kind of makes a sheath just to hold the pressure," she says, "and it doesn't do it very well." Little surprise, then, that the people these mice are modeled after tend to die from a combination of these two deformities and artery-clogging plaques.

You would expect that such damaged vessels were designed to replicate the heart disease of people in their last decades of life. But Olive's mice have actually been engineered to study children—young girls and boys with a genetic aging disease called progeria.

Children with Hutchinson-Gilford progeria syndrome look like strange, haunting hybrids of youth and old age. Like those optical illusions that flip back and forth from a duck to a rabbit, looking at a child with progeria leaves you blinking between a baby and a grandparent. Old and young are supposed to lie at opposite ends of the spectrum, but progeria melds them into one.

Progeria is striking to see. The children's heads look too big for their faces, with visible meandering veins, large eyes, small jaws and beaky noses. They have little or no hair anywhere on their bodies. When they talk—and like any other kids, they talk a lot—it's with voices pitched slightly higher than normal. They're small, often weighing only thirty pounds and standing three feet tall even as teenagers. They have patches of hard or brown skin. Under that skin, much more is going on. The kids lose their body fat—that's what makes their veins look so prominent—and their bones weaken. Their shoulders narrow. The skin and ligaments around their joints stiffen, so they have trouble fully extending their fingers or their knees; problems with their hips leave them walking subtly bowlegged like John Wayne. Most significant of all, they suffer from arteriosclerosis, hardening of the arteries. The resultant strokes or heart attacks eventually kill them. The average life expectancy is thirteen. Some live to be twenty. Some die at three or four.

The children develop normally at first, but by about age two it becomes obvious that they're suffering from what doctors call a failure to thrive: they don't grow enough, no matter how much they eat. Together with hair loss and a vein standing out across the top of the nose, the lack of growth usually leads the doctors to suspect progeria—if they've heard of it. A genetic test confirms the diagnosis.

Progeria is rare, affecting only one in four to eight million people. Forty to fifty children have been diagnosed with progeria in the world right now. In part because of its rarity, no cure or effective treatment has yet been found.

The Office of Rare Diseases Research at the National Institutes of Health defines a rare disease as affecting fewer than two hundred thousand people in the United States. Hemophilia affects eighteen thousand people. Progeria affects twenty people. It is "vanishingly rare," says Bruce Korf, a geneticist at the University of Alabama at Birmingham.

Progeria's rarity can be a problem for the people who want to study it. Researchers have a limited number of tissue samples to examine. When it comes to clinical trials, they can only recruit from a small pool of patients. That number further dwindles because not every family wants to participate, and not all of the children who do are eligible. Those who are accepted don't always survive the full duration. If there are concurrent clinical trials, as there have been since 2008, the available candidates diminish again. And if one trial follows another, the families may begin to suffer from a sort of trial exhaustion, parents less willing to put their children through another rigorous course of therapy that often involves frequent medical examinations and travel. Plus, not all doctors have heard of the disease, so they may incorrectly diagnose affected children. Some researchers estimate that there are four times as many cases of progeria in the world than are currently recognized. This underdiagnosis prevents the kids from getting what help is available and deprives the research community of treasured sources of information.

But there is great potential in persevering despite these obstacles, not only because understanding progeria can lead to a treatment or a cure for the children it strikes, but also because, as most progeria researchers believe, the disease is related to normal human aging.

Progeria resembles aging in many ways. You can see the children's veins under wrinkled, spotted skin. They move stiffly. Their bones are easily broken. They die of heart attacks and strokes. Similarities like these have led many researchers to view progeria as aging on overdrive. Now they're finding that the connection may run much deeper than "you look at people and say they don't have hair so it's aging," as researcher Paola Scaffidi puts it. Scaffidi and other scientists at the National Institutes of Health (NIH) have found that the same molecular mechanism is at work in progeria patients and healthy people. Their experiments were done *in vitro*—studying cells in dishes. Building on those studies, Columbia University researcher Karima Djabali has confirmed *in vivo*—in living people—that small amounts of the same protein that causes progeria accumulate in our own skin cells as we age. Because of this association, Djabali wants to extend her progeria research into the realm of normal aging. "I would like to find out if there is a way to, how do you say it, delay aging," she says, and laughs. "I enjoy thinking that there will be one day a way to delay aging so that we remain in good health and most importantly in good condition to enjoy life as long as we can." She hopes this will be the case for both "normal" people and progeria patients.

However, part of the scientific community contests the extent to which progeria is linked to normal aging. Many gerontologists believe they are totally unrelated phenomena. "It is time to relinquish the myth that the Hutchinson-Gilford progeria syndrome [...] hold[s] the key to an understanding of aging," three scientists from the National Institute on Aging wrote in the *New England Journal of Medicine*. Other researchers see a tenuous similarity but don't believe they can learn about one by studying the other. Progeria is "not normal aging. There's some resemblance, but I'm not convinced," says Leonard Guarente, a biologist at the Massachusetts Institute of Technology who studies lifespan extension in "many critters" like

mice and worms. Guarente says gerontologists prefer to focus on a substantial list of established culprits of aging. He rattles off a few of the serious-sounding phenomena: oxidative damage, protein glycation, protein cross-linking, protein aggregation, DNA damage, RNA damage, and lipid oxidation. "And that's just the beginning," he says. Guarente himself specializes in substances called sirtuins, which he believes control the rest. Until researchers can manipulate the progeria gene to slow down aging instead of speed it up, he's not planning to give progeria any attention.

There can be a financial motive to emphasize the potential connection between progeria and aging. With so few people affected, curing progeria in and of itself may not be enough to warrant research grants. A few programs support work on rare conditions precisely because it's so hard to fund otherwise, but their resources are limited. Researchers need to find a broader impact for their work if they want to snag a share of the rest of the rapidly diminishing funding pie. The link to aging helps. "It's easier for people working on progeria and wanting things to go forward to say that it's linked to normal aging, because big pharmaceutical companies are going to be interested in that subject," says Olive, who studies progeria's effects on blood vessels at the National Heart, Lung and Blood Institute. Francis Collins, who recently stepped down as director of the National Human Genome Research Institute and who helped discover the gene that causes progeria, agrees with her. "I would bet that every grant application submitted to NIH on progeria emphasizes the connection to the aging process," he says. Those applications aren't all hot air; the NIH is listening. Even though it is still unclear how much progeria can teach us about normal aging, enough researchers have presented compelling findings that the institute has grown to consider funding progeria research worthwhile.

Despite the differing viewpoints on progeria and normal aging, both skeptics and proponents agree that the disease is not a perfect reflection of the aging process. As Mark Kieran, a pediatric cancer doctor who is running the United States' first clinical trials for progeria at Children's Hospital Boston, explains, "Progeria isn't exactly premature aging. These kids do not age in exactly the way you and I do, although some of the phenotypes"—the visible symptoms—"overlap." Progeria is known as a segmental aging disorder; it doesn't completely recapitulate what the rest of us expect to experience as we get older. It shares some of the symptoms, not all, and it adds a few of its own. To listen to all but the die-hard gerontologists, progeria and aging are like the two intersecting circles on a MasterCard logo; each has unique symptoms, while some symptoms are common to both.

The cause and progress of progeria's age-like problems may not be exactly the same as in the aged. Children with progeria develop sticky build-ups called plaques in their arteries, but the composition of those plaques is different from that of ordinary people with heart disease. And it has yet to be determined whether the mutant protein that causes the children's heart disease has anything to do with the development of atherosclerosis in the elderly.

Progeria patients also have problems not necessarily seen in normal aging, such as loss of body hair and teeth coming in late. Developmental disorders mix with aging symptoms. In the early days of progeria research, one scientist recalls, an orthopedist was asked to look at the X-rays of progeria patients and found that their bones look like abnormally developing children's bones, not the bones of elderly people; a cardiologist asked to consult on the few available autopsies of progeria patients found that their hearts didn't look like aged hearts.

In addition, the children don't get some of the most common age-related disorders, including cataracts, arthritis, kidney disease, and liver disease. They don't share the elderly's

tendency to have high platelet counts in their blood, slow clotting times, and certain immune responses. They don't suffer from dementia or Alzheimer's disease. In fact, they're free of any mental degeneration. As children with progeria age, their minds continue to develop like any child's, leaving them bright and boisterous inside withering bodies.

Some scientists are interested in finding out why progeria patients seem immune to some aging symptoms while they suffer from others, because even the divergences from normal aging may offer important clues about what happens in more general human biology. For example, many other premature aging disorders carry a significantly increased risk of cancer. Victims of two of these diseases, Werner and Bloom syndromes, tend to die of cancer. Scientists suspect this is because DNA damage builds up. But almost none of the Hutchinson-Gilford progeria patients develop cancer, even though they have DNA damage too. Researcher Tom Misteli at the National Cancer Institute is one of the researchers who wants to know why. "Progeria patients are unique in that they have extremely high loads of DNA damage but they never develop tumors. Which is extremely unusual," he says. He's investigating whether progeria might somehow actively prevent cancer, and whether it's acting in a way our own bodies are capable of. The disease may also provide a model for studying how cancers form.

Whether this disease has something to tell us about the universal experience of aging is only one question the progeria research community is exploring. Indeed, aging is only one spoke on a wheel with progeria at its hub. Progeria research has the potential to shed light on numerous areas of human biology. This is in large part because, even though progeria affects astonishingly few people, its single, specific cause can be a benefit to scientists who want to study more common diseases. "Sometimes rare conditions provide clues to mechanisms of

common conditions. In a way they isolate for you a purer case of what happens when some very extreme thing goes wrong that illuminates what's going on in much more complicated common situations," says geneticist Korf. It's the same philosophy that drives physicists to study black holes to learn about gravity.

Some of the most common medical conditions, such as high blood pressure, heart disease, and cancer, are hard to study because they involve many different symptoms with many possible causes. "That's why single-gene diseases [like progeria] provide an attractive model—they're on the extreme end of the spectrum, when one thing goes very, very wrong and creates problems in its wake," says Korf. "It's easier—not easy—but easier to trace disease mechanisms to their root causes. Even if there are only *some* similarities, progeria offers the possibility of understanding how some of these mechanisms work that one hopes will carry over to other conditions." Those hopes drive people to study progeria who are interested in the aging process and in widespread health problems like heart disease, cancer, muscular dystrophy, skin problems, bone disorders, and fat loss.

Their ambitions are not without precedent; it's common for rare-disease research to lead to more widespread applications. One of the most impressive examples comes from retinoblastoma, an eye cancer that affects about three hundred children a year in the U.S. In the 1970s, scientists studying retinoblastoma theorized the existence of a tumor suppressor gene. They believed that an unknown gene was supposed to prevent tumor growth but had been turned off or cut out so the children's retina cells then could divide uncontrollably. They found their gene a decade later—and the idea of tumor suppressors has become one of the central concepts in the cancer research world. The gene itself even turned out to be implicated in many cancers besides retinoblastoma.

Along with these health concerns, progeria may provide further insight into some of the most basic functions in our cells. Many researchers are using progeria to learn more about such phenomena as gene expression, the way the shape of the cell nucleus affects its function, the purpose of the constituents of the nucleus' casing, DNA regulation, RNA splicing, protein processing, cellular aging, and stem cell differentiation.

The poet William Blake wrote of seeing a world in a grain of sand; many see in progeria the opportunity to study biology in a grain of mutated protein. Scientists from a variety of seemingly disparate fields have converged on this curiosity to peer into its workings, not only to help the children it afflicts, but also with the hope that they can tease out secrets about what happens in all of us.

*

Progeria was originally documented in 1886 by a then-famous British physician named Jonathan Hutchinson, who described two children with a remarkable syndrome in a paper he titled "Case of congenital absence of hair, with atrophic condition of the skin and its appendages." Ten years later, another British surgeon, Hastings Gilford, examined the same cluster of symptoms. He named the syndrome *progeria*, Greek for premature aging. A few studies followed in the twentieth century, including a definitive American paper in the 1970s describing progeria's characteristic symptoms and their progression. But for the most part, progeria was relegated to the role of minor medical curiosity.

Then lightning struck.

New England residents Leslie Gordon and Scott Berns knew something was wrong with their son, Sam. Since birth, he moved stiffly, and his skin looked strangely bubbly, especially on his stomach. He stopped gaining much weight after he was nine months old, and he threw up frequently. At one year, his teeth still hadn't come in. As his second birthday approached, he started to lose his hair. They consulted with experts, who focused on nutritional solutions. Some thought Sam had gastric reflux. Then a friend of the family suggested he might have progeria.

It was August 1998. Sam was twenty-two months old. There was no genetic test for progeria then. Instead, Gordon and Berns had X-rays taken and found the damning sign: the tips of Sam's collarbones and fingers were dissolving away. They sent the results to the world's leading expert on progeria, Ted Brown, a developmental disease specialist in New York. He confirmed the diagnosis. Like every other parent of a child with progeria, Gordon and Berns were told that no treatment was available. Progeria was uniformly fatal.

Sam's parents didn't like that answer. They swallowed their grief and began to research ways they could help their son. Fortunately, they were in a privileged position to take action. Gordon had already completed a combined M.D./Ph.D. program at Brown University in Rhode Island and was on her way to a pediatric ophthalmology residency at the prestigious Massachusetts Eye & Ear Infirmary. She dropped ophthalmology and concentrated on progeria instead. Berns was the youngest board-certified doctor in Rhode Island, and had the advantage of being politically well-connected.

Gordon recruited doctor-friends and mentors to serve as her "brain trust" and plunged into research about her son's condition. She soon discovered just how little information was out there. There was no central source of information, no funding, few sources of tissue to

study, and no place for families to go. Only about five researchers were working on the disease. Gordon decided to start a foundation.

"It wasn't like there was a cancer society we could latch onto. We would've loved to have been a chapter, or the U.S. counterpart to something else going on in other parts of the world," says Gordon's sister, Audrey Gordon, who is the executive director of the foundation. "But we realized that we really had to start it from nothing, from ground zero." The Gordon family launched The Progeria Research Foundation in 1999. Leslie became PRF's medical director and her husband its chairman of the board, which they populated with friends and business colleagues.

The family knew that they had to move fast. "We were in a race; we knew that," says Audrey. Sam would turn thirteen, the average life expectancy for progeria victims, in eleven years.

The foundation had a lot to do. As Leslie explained in a talk in 2007, they had to first build a scaffold to support progeria research before any progress could be made. They started a cell and tissue bank at Rhode Island Hospital and Rutgers University so researchers would have a supply of samples to study. Today, it houses more than ninety-nine different cell lines. They raised funds so they could offer grants to stimulate research. They have awarded more than twenty-three. They compiled a database of medical records at Brown University's Center for Gerontology of children with progeria. It now has information, scans and photographs of more than seventy children, and can be accessed from around the world. Gordon also convinced the National Institute on Aging to fund a scientific meeting only about progeria. She persuaded a handful of researchers to speak, even though most of them were not experts

in the disease. PRF's meetings are now held every other year, usually in Boston. In 2007, one hundred participants came from around the world.

One of the best things to come out of the first PRF workshop in 2001, says emeritus molecular biology professor Frank Rothman, who served as one of Gordon's earliest progeria mentors at Brown, was that scientists working on progeria agreed to work together. Rothman recalls: "We actually sat down and asked people, 'Now look, are we going to be the way scientists usually are and compete about this, or are we—because these kids are dying every year—are we going to keep in touch with each other and collaborate and see whether we can get off the ground on this faster?' And we did the latter." Since then, progeria researchers in many labs have put aside science's usual competition for the sake of helping the children.

It's not perfect, Rothman emphasizes. There are still practical considerations like protecting work before it's published to ensure that researchers, particularly those who are just starting out, get the credit they need for a degree, a job, or tenure. But for the most part, the progeria research community enjoys and has benefited from this voluntary collaboration. "There's always of course publishing and being able to be the first," says Michelle Olive, who has attended two of PRF's meetings, but "the fact that the community would come together and do as much as possible just to share the results was really important."

PRF's next big project was putting together a consortium to search for the progeria gene. For many years, researchers had thought that progeria might be a recessive disorder inherited from the child's parents. But children with Hutchinson-Gilford progeria didn't have siblings with the disease. It became clear that the cause had to be a random mutation. In 2003, two teams found it. One was PRF's; the other was a European group.

Each team discovered that patients with the classic form of Hutchinson-Gilford progeria syndrome have a mutation that changes a single letter, or nucleotide, in the three-billion-letter genetic code that makes us who we are. One cytosine, or C, becomes a thymine, or T. This mutation happens spontaneously in a germ cell—a pre-egg or pre-sperm cell—before the child is even conceived; neither the mother nor the father have it. It happens in the gene that tells our cells how to make proteins called lamins.

The nucleus sits in the center of the cell like the yolk of an egg. Inside it, DNA sits in nucleoplasm goo. A thin casing called the nuclear envelope separates the nucleus from the rest of the cell like the rubber skin of a water balloon. The inner lining of the nuclear envelope is called the lamina, and it is made of proteins called, appropriately enough, lamins. The lamina is a sort of mesh that gives the nucleus its shape and strength. Too many lamins, and the nuclear envelope becomes thick, misshapen, and stiff. Not enough, and the nucleus weakens and bulges. The weblike lamina is also thought to play a role in organizing genetic material in the nucleus and helping to control which genes are turned on or off at any given time.

Progeria's gene, *LMNA*, contains the recipe for a few of the different kinds of lamins in our bodies. Progeria specifically affects lamin A. The disease creates a mutant form of lamin A, named progerin, that sticks to the nuclear envelope. Normal lamins "go there, they stay for a while, and then they fall off again," says Tom Misteli; but in progeria, "they go there and they stick like glue to what's already there." Over time, progerin builds up and builds up until the nuclear envelope becomes thick and misshapen, blobby like a child's drawing of a cloud. Progerin may wreak further havoc by disrupting the function of the

nucleus and the genes it contains. Scientists are still trying to figure out exactly how this deformed lamin leads to the symptoms progeria patients experience.

Progeria is the unluckiest of situations. "It's a very nasty mutation," says Misteli. Short of being immediately lethal, "it's probably the worst thing a single nucleotide change could do." The disease's single genetic misspelling happens in just the wrong place. If the mutation made one more or one less amino acid, it would throw off the total, and the cell would recognize the error before it made any incorrect protein. The children would be fine. If there were a second mutation nearby, it might negate the typo; again, the children would be fine. Instead, they end up caught in the middle, having to live with a progressively worse illness.

Once the gene was found, scientists created a genetic test to definitively identify Hutchinson-Gilford progeria and separate it from other diseases that look like it. The discovery also meant they could engineer mice that suffered from some of progeria's symptoms so they could study the physiology and pursue possible treatments. And it paved the way for clinical trials.

With Leslie Gordon at the helm, PRF jump-started progeria research by recruiting scientists in numerous fields to study the disease. As for what lies in store next, her sister says, "We just want to keep on going. I think we've spoiled people in a way, because so many things have happened in such a short amount of time." In many ways, progeria research has been at the center of a perfect storm of events. "Sometimes the stars just align for you. We're very lucky."

Luck has come for progeria research in many forms. First, the disease struck a child whose parents were both doctors. Then progeria happened to be traced to a gene that was already well-known to scientists.

Researchers had been interested in the *LMNA* gene for many years before progeria was linked to it, because it's a hot spot for mutation. Progeria is just one of an unusually high number of mutations along that short stretch of the human genome. In the last ten years, researchers have found more than three hundred others that altogether cause at least eleven distinct diseases. Along with mutations in one other gene that affects lamins, these disorders are known as laminopathies. They affect hundreds of thousands of people.

There are muscle disorders like Emery-Dreifuss muscular dystrophy, a degenerative disease that causes muscle weakness and wasting, stiff joints, and heart problems. Peripheral neuropathies such as Charcot-Marie-Tooth disorder affect the nerves in the feet and hands. Lipodystrophies like familial partial lipodystrophy rearrange body fat and can lead to diabetes. People with mandibuloacral dysplasia suffer from stunted growth, skull and skeleton problems, and skin discoloration. And then there are the accelerated aging diseases, including not only Hutchinson-Gilford progeria but also restrictive dermopathy, lethal within days of birth, and some cases of atypical Werner syndrome, which appears in the twenties and thirties and kills at about age fifty from heart attack or cancer.

The progeria community was fortunate to have all this research immediately at its disposal instead of having to do the groundwork. Frank Rothman explains what could have happened: "Very often people look for the gene for a disease and they find the gene and they don't have the faintest idea of what that gene does, what that sequence of DNA does, because they find it in a part of the DNA that hadn't been identified functionally. And it can take years

after that. But here they were on day one with a well-established group of cell biologists who were studying the biochemistry and cell biology of lamin." There was a framework already in place to support progeria research. Without the preexisting knowledge of *LMNA*, progeria research would be years behind where it is today.

Following the *LMNA* gold mine came another happy accident. This one had to do with the way the progeria protein is mutated. First, the genetic typo in *LMNA* means fifty amino acids get cut out of the middle of the lamin. The impact is seen down the line as the newly created lamin gets transformed from immature prelamin A into mature lamin A. In step one, an enzyme tacks on a squiggly bit of fat to one end like a tail. This is a farnesyl group, and the process is called farnesylation ("far-NESS-il-a-tion"). After a few more steps, another enzyme is supposed to cleave the lamin—chop off the end with the farnesylated tail to produce the final lamin A. The problem in progeria is that the part of the lamin that tells the enzyme to chop was located in those fifty missing amino acids. So the enzyme lacks instructions, and cleavage doesn't happen. The lamin becomes progerin. Progerin not only lacks some of the amino acids found in normal lamin A, it also has a farnesyl tail that should have been cut off.

Scientists aren't sure why normal lamin A goes through all this trouble only to have its modified end chopped off at the last minute. There are plenty of farnesylated proteins in the body, and most of them remain permanently tailed, including lamin A's cousin, lamin B. Researchers suspect that the farnesyl group helps steer the lamins to the nuclear envelope. Snipping it off lets the lamin float away once it's done its job. The permanently attached farnesyl tail may be what makes progerin stick there for the rest of its life. Currently the dominant theory in progeria research is that permanent farnesylation causes progeria's damage, although recent evidence suggests that it is not the only culprit.

Like the *LMNA* gene itself, farnesylation was familiar to scientists. Farnesylated proteins had been implicated in about half of all known cancers. The most famous cancer-causing protein, Ras, is farnesylated. Particularly because of Ras, scientists had developed drugs called farnesyltransferase inhibitors, or FTIs. The idea behind FTIs was to stop the game of "pin the tail on the protein" by preventing farnesyl groups from being attached. Some of these drugs were in human clinical trials. Two had even been tested in children. Progeria researchers could hardly have asked for anything more.

"So the moment the gene was known, there was already a possible treatment," says Rothman. With a laugh, he adds, "Well, that's pretty unheard of."

Many immediately looked to FTIs as a potential treatment. The cancer trials had shown that the drug would be relatively safe for patients. The FTIs would drastically reduce progeria's damning farnesylation. If scientists couldn't cut off progerin's farnesyl tail in the end, then with FTIs they could at least keep it from being pinned on in the first place. Progerin would still be missing those fifty amino acids, and cleavage still wouldn't happen in the end, but at least without the farnesyl group it might behave more like normal lamin A. The idea was to make a tailless progerin "that theoretically could not grab onto the nuclear membrane and hopefully would just sort of float around and do not much," as Scott Berns once put it.

Over the next few years, teams of researchers around the world tested FTIs in dishes of human progeria cells and in their progeria mouse models. The first results came in 2005 from three teams. They found that FTIs returned many progeria nuclei to normal shapes in both mice and human tissue, sparking a flurry of interest. A pair of studies the following year found that FTI treatment started early in a mouse's life mitigated some of progeria's

symptoms, including weight loss and bone fragility. A study published in August 2008 suggested that at least some types of progeria damage can be reversed and not just prevented. This past October, a group of researchers reported that FTIs can prevent heart disease in progeria mice and reverse damage that had already been done. As lead author Brian Capell at New York University School of Medicine pointed out, damage reversal is as critical as prevention when it comes to treating progeria patients, since most of the children they want to help have already developed heart disease.

Of course, just because something works in a mouse model doesn't mean it will work in a person. What an FTI does for a progeria mouse, it might not do for a progeria child. "We've cured cancer a thousand times in mice, in spite of the fact that most humans who get cancer will still die of cancer," says FTI specialist Mark Kieran. That's because, as Kieran puts it, "humans are more than just kind of large mice without tails." Although animals allow scientists to study diseases and test treatments in living creatures where it would be unethical to experiment with people, mouse physiology isn't the same as ours. Some of these differences specifically impact progeria research. For instance, humans and mice have two copies of the *LMNA* gene—one from each parent—but in humans, only one of the copies has to be mutated for the person to suffer from progeria, whereas in mice, both genes must be mutated to produce symptoms.

On top of that, not all progeria mouse models are created equal. Each line of mice was made in a different way, and so each ends up with a different combination of symptoms. For example, the mice Michelle Olive studies have had the human progeria gene pasted into their DNA. They experience heart disease, but that's all. Two other strains of mice in Spain and California, on the other hand, make normal lamins but lack the enzyme that's supposed to

chop off the farnesyl group. These mice are small, lose their hair, and have weak muscles and bones. In another strain in Sweden, the mice's progeria genes can be turned on and off.

Researchers can study how their skin and hair changes.

"I was very naïve when we first started all this. I thought, oh, make 'the mouse,' but it doesn't work that way," Leslie Gordon said during a grand rounds lecture in 2007. There is no perfect progeria mouse that presents the full complement of symptoms. But the diversity of models allows researchers to study isolated symptoms, pinpoint causes, and test treatments on the different breeds. Gordon recognized the benefits lurking within an imperfect situation.

"Whoever tries a strategy, no matter what they do they don't make an identical mouse to somebody else's model, and that is to our advantage," she said.

Researchers working with mouse models have to wrestle with other problems as well. The mice with heart disease, for instance, don't reproduce themselves well. (It's not that a boy mouse and a girl mouse don't want anything to do with each other; it's that only ten percent of their babies have both copies of the progeria gene.) And studies take time; the mice sometimes need to grow for a year or more before they start to develop the symptoms. Plus, if researchers want to try a treatment, they have to convince a pharmaceutical company to give them the drug they need, and it's not an easy battle to get enough to treat a significant number of mice over the necessary months or years. All these factors add up to move research along more slowly than it did in the beginning.

Still, having at its disposal all this information about *LMNA* and FTIs, along with the Progeria Research Foundation's social and financial encouragement, allowed progeria research to leap ahead in a short period of time. Misteli takes this as compelling evidence that basic research—research that may not have an immediate application—should be supported,

because it may be called into use down the road in unexpected ways. "You just never know," he says. What may seem like useless research today may be called upon to save lives tomorrow. Brian Capell adds, "There is an emphasis by the NIH and Congress for translational science"—science with known practical applications—"but to do that research depends on those basic discoveries. If they had found that the progeria gene was something we knew nothing about, we wouldn't have been able to jump to a clinical trial in four years."

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It's been less than a decade since Sam's diagnosis. From the discovery of the progeria gene to the start of a clinical trial took four years, an unusually swift step forward for a rare disease. "It was a triumph to go so fast," says Jan Lammerding. "Now the big question is if the clinical trial will work." FTIs have not done much in cancer trials. But a lot of hope rests on them right now as a stopgap treatment for progeria.

Since farnesylation was the most widely suspected culprit and since there was a drug available that might fix it, it made sense to find someone intimately familiar with FTIs to help run a clinical trial. Enter Mark Kieran, a pediatric neurooncologist at Dana-Farber Cancer Institute and Children's Hospital Boston. Leslie Gordon approached him in 2005 as an expert who could advise the progeria research community on how to approach FTIs as a treatment. Kieran spoke at a PRF conference, agreed to write a sample proposal showing how a clinical trial might proceed, and in 2006 found himself in charge of that trial.

Working with terminally ill children takes a certain kind of fortitude, but progeria was not too different from Kieran's day job treating kids with brain cancer. Kieran has intense blue

eyes and hardly blinks, giving his audience the sense that they are the complete focus of his attention. He is a living embodiment of the saying that if you want something done, ask someone who's busy. Known among the hospital staff for never saying no to patients or projects, Kieran made room for the progeria trial within an already airtight schedule of rounds, appointments, meetings, and research. Pictures of progeria patients joined the other crayon-scrawled thank-you cards and trinkets in his office.

The trial began in 2007 with twenty-nine children enrolled. Twenty-seven of them are still participating a year and a half later; one dropped out before taking any of the treatments, and one passed away. The remaining families hail from eleven countries, including Argentina, Romania, Pakistan and India. Their flags and smiling faces shine from PRF postcards proudly tacked up in their doctors' offices.

The team treating them is equally as diverse, to meet the demands of a multifaceted disease. "We've discovered progeria is in itself a complex disease," says Kieran, whose voice borders on hoarseness. "It has all kinds of abnormalities: cardio, vascular, cerebral, strokes, the height, the weight—the inability to gain weight." No single person or preexisting group of people had the required proficiency in the complexities of progeria as well as FTIs for kids, so Kieran and Gordon assembled their own team of thirty specialists. Together, they provide expertise in areas such as audiology, cardiology, dentistry, dermatology, endocrinology, neurology, ophthalmology, orthopedics, and, of course, FTIs—"basically, everything you can think of," says clinical research coordinator Kiera McKendrick. That's not counting the researchers at the NIH and a few other labs who get tissue samples to study.

Such pooling of knowledge is needed not only to gather more detailed information about what goes on in these kids, but also to tease out which symptoms are part of the natural

course of progeria, which are likely a result of the treatment, and which might be a novel interaction between the two. Kieran can identify any symptoms he's seen before in children treated with FTIs; the other specialists can tell him whether anything else, like a developing heart problem, is normal for the disease. If a symptom doesn't fit FTIs or progeria, Kieran says, then either the clinical description of progeria needs to be adjusted, or they're looking at an unexpected reaction between the disease and their treatment.

Seeing all the kids and serving as contact points for the research team are Children's Hospital doctors David Miller and Monica Kleinman. Kleinman—a friend of the Gordons and the doctor who originally suggested that Sam might have progeria—and Miller take each child's medical history and conduct the physical exams every time they come in. With each patient visiting every four months, that means about two two-day appointments a week. On the milestone appointments at the end of each child's first and second year in the trial, Miller and Kleinman are joined by that army of specialists to check up on the kids' hearts, bones, skin, teeth, eyes, ears, hormones, and so on down the list. The primary marker of the trial's success will be if the children gain weight. The team also is keeping an eye on the children's height, hair growth, bone density, body fat, and vascular health.

No details have been released yet about the trial's progress. At this point, the team isn't expected to make an announcement at least until the trial's completion in late 2009 or, more likely, in early 2010, when they publish their results. The report will coincide with PRF's next scientific conference.

Nobody thinks FTIs will be a cure. The hope is that they can turn progeria into a chronic disease instead of a terminal one. "For sure it won't be a cure," says Kieran. "If this drug worked, this would really be more in the context of giving insulin to a diabetic. They've

still got diabetes all their lives, and giving insulin isn't perfect—diabetics still have all kinds of medical problems—but at least they don't die in childhood from their disease. To some extent we're hoping to achieve the same thing in this trial."

Some think farnesylation is not the whole story. Yet even if it does turn out to be the main culprit, research shows that FTIs aren't a perfect solution. They may repair the blobs that deform nuclei, but one researcher suspects they distort nuclei in a new way. FTIs also have no effect on the DNA damage and some of the other functional problems progerin causes. Moreover, FTIs aren't specific; they target not just progerin but all of the hundreds of farnesylated proteins in the body. And they may open a window for a different kind of toxic group to become attached to the lamin instead of farnesyl. It's this last concern that prompted a second progeria trial across the Atlantic.

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Carlos López-Otín fell into progeria research by accident. For fifteen years, he had been studying cancer at the Universidad de Oviedo in Spain, trying to understand how tumors spread throughout the body. He had found more than sixty new proteins associated with cancer, and he wanted to know whether any of them actively caused tumors. His lab discovered an enzyme that he thought was involved in turning on some of these farnesylated, cancer-causing proteins. In 2001, he started to breed mice that lacked the enzyme. López-Otín suspected that without it, the mice would make fewer farnesylated proteins and therefore have fewer tumors. He saw a chance to solidify the connection between farnesylation and cancer

and provide evidence to drug companies that stopping farnesylation—that is, using FTIs—would be useful for treating human cancer patients.

But his mice didn't behave exactly as he'd expected. They started aging much faster than normal, dying at three or four months instead of two or three years. With such short lifespans, they were useless for studying cancer. López-Otín had to make a decision. He could abandon the mice and create another strain that lacked a different enzyme. Or he could follow this new path and try to figure out what was causing the rapid aging.

He chose aging, and spent the next few months working his way down a list of farnesylated proteins found in humans and mice. Eventually, he identified lamin A as the aging culprit. It and it alone had remained farnesylated. Lamin A turns out to be unique among farnesylated proteins because it has to be cleaved twice to be de-farnesylated—and it needed that missing enzyme to do the final chopping. Although he didn't know it yet, what López-Otín had inadvertently given his mice was something almost identical to Hutchinson-Gilford progeria; farnesylated lamin A is very close to being progerin. He did know that he had created a model for a lamin disorder and says he was "very happy" to have found this defect in a living creature, because now the scientific community had a model to study a range of human laminopathies, including heart disease, skin problems, and hair loss. He published his results in the journal *Genetics*.

Almost a year later, in 2003, López-Otín went on vacation with his family to the Greek islands. He was having lunch in a tavern when he caught a news report on CNN. A French scientist named Nicolas Lévy was announcing the identification of the gene for progeria. López-Otín had never met the man, but he realized that the gene was related to what he'd been studying. López-Otín emailed Lévy when he got back to Spain, "and since that very

same day we are very good friends," he says with a smile. It turned out that Lévy, a geneticist who'd been working with progeria patients for many years, had read López-Otín's paper and realized that his patients could have mutations on the gene encoding lamin A. He looked, and found them. His patients had lost the genetic instructions to tell an enzyme to cut off lamin A's farnesylated tail. The effect was the same as in López-Otín's mice that didn't have the enzyme at all. The two scientists began to collaborate.

In 2005 López-Otín and other researchers on his team reported in *Nature* that taking some of the farnesylated lamin A out of the enzyme-deficient mice relieved their progeria symptoms. The team had found a genetic cure for their version of progeria. Since then, he says, researchers have been trying to achieve the same result with drugs, with FTIs.

Last year, López-Otín and Lévy reported that FTIs might not be relieving progeria symptoms as well as some people had hoped because they create a new problem. FTIs may stop farnesyl groups from being tacked on, but the drugs leave the lamins open for another unwanted tail, this one saddled with the unfortunate name of geranylgeranyl. So they tried a different treatment with their mice. Instead of FTIs, they used a combination of two drugs that prevent both farnesylation and geranylgeranylation in one fell swoop. They were intervening a step before FTIs. Whereas FTIs prevent farnesyl groups from getting attached, these drugs block the production of farnesyl groups and geranylgeranyl groups altogether. Like proponents of FTIs, López-Otín's team believes farnesylation is a problem in progeria; they're just going about stopping it in a different way. The team found that their combination improved progeria symptoms. Mice on the two drugs grew more, didn't lose as much weight or hair, had more fat, had fewer bone problems, and also lived longer than mice without any treatment. Many of their lumpy nuclei returned to more normal shapes, too.

Lévy is now running a clinical trial in France to see if what worked in the mice will work in children. Like Kieran's team, Lévy's has the benefit of knowing that their drugs are already in common use for people. The first, statins, are routinely prescribed to lower people's cholesterol. The second, called bisphosphonates, are used to strengthen bones in conditions like osteoporosis. Another trial testing these two drugs for both progeria and another laminopathy called mandibuloacral dysplasia has since started in Italy. But again, statins and bisphosphonates will provide a treatment at best, not a cure.

López-Otín would have liked to compare the statin-bisphosphonate therapy to FTI therapy in his mice, but he wasn't able to get FTIs. Back in Boston, Mark Kieran already had access to them. "The real question is, if each of them works a little bit, would all three of them work better," says Kieran. With that in mind, he and the Progeria Research Foundation submitted an application for a new clinical trial where all three compounds—FTIs, statins and bisphosphonates—will be tried together. Children's Hospital approved a one-month test to make sure the combination is safe for children and is now reviewing the full trial proposal. If it's given the green light, the team hopes that all the children will "roll over" into the new trial as soon as the current one ends.

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While doctors focus on treating their young patients, researchers want to know how things work. They're driven to figure out what goes on in our cells, our nuclei, and other, less well-known structures and molecules within.

The potential to help terminally ill children adds a new dimension to their commitment, but more than anything else, progeria fascinates basic researchers because it's an

extreme disease with a single cause. The disease causes devastation in very specific ways, offering researchers a chance to puzzle out basic phenomena that happen not only in progeria but also in normal bodies. If they want to know what normal lamins do, they can study what happens when lamins go horribly wrong. "We're interested in this disease for two reasons," says Misteli. "One reason is it's a fascinating disease and we want to know what the disease mechanism is. But it's also an amazing model system to study all sorts of things. For us it's become a model to study extremely fundamental aspects of how the nucleus functions. So Mark [Kieran] thinks about people and symptoms and organismal phenotypes, and we think more about cell biology."

One of those fundamental aspects involves highly condensed DNA called heterochromatin that's normally found at the edges of the nucleus. Progeria patients' cells don't have any. "It actually disappears completely. It's really remarkable," says Misteli. Researchers don't yet understand how heterochromatin is formed, and its absence in progeria patients gives them a clue that something the disease disrupts is involved. In addition, one of heterochromatin's jobs is to control which genes are turned on and off, so scientists can also study which genes are switched on when they shouldn't be, and determine whether that has an effect on the rest of the body.

Researchers can also examine how proteins are broken down and replenished in the nucleus. For one thing, progerin-clogged nuclei break down more proteins than normal. It's "fairly dramatic," says Misteli. Two leading theories in the protein degradation field are that progerin isn't getting broken down and flushed out fast enough, and that progerin is keeping the cell's quality assurance team from attending to other misshapen proteins, which then build

up. For these reasons, progeria is a welcome model; protein degradation has been mostly studied in the rest of the cell, not in the nucleus.

Another fruitful area stemming from progeria research is stem cell biology. "We've shown that if you introduce progeria into stem cells, they stop behaving properly," Misteli says. This provides another potential connection to aging, since one theory of aging blames stem cells' failing ability to replenish the body's other cells.

Almost all of the tissues affected in progeria patients are of mesenchymal origin, says Misteli. Mesenchymal stem cells are found in the bone marrow and have the potential to become bone, muscle, fat, and connective tissue. Misteli has found that adding progerin to these stem cells disrupts how they turn into each of these tissues. Progerin blocks the creation of fat, which he says may explain why the children have no subcutaneous fat. Progerin also accelerates the creation of bone. It sounds counterintuitive, because the children have low rather than high bone density. But Misteli says what's happening is that bone turnover speeds up too, so while the body makes more bone, old bone gets broken down faster. It's as if the children's bone cells are running on a treadmill, but the treadmill is set to go faster than they can run. So even though the stem cells are running as hard as they can—harder than a healthy person's—they just can't keep up, and the children lose bone.

"I've come to suspect that at least a major issue in the disease is a difficulty in replacing cells that are lost, because of a loss of stem cells," says Huber Warner, a biologist and aging specialist at the University of Minnesota. "I think that very early in life, between one and two years of age, the children become less able to replace cells that die." Warner believes the premature exhaustion of the body's ability to replace so many dying cells explains almost all of the symptoms progeria patients experience. But because he no longer does

experimental work, he doesn't have data to support his speculation. "I don't have a lot of committed supporters to my point of view," he says. "That's okay. I feel pretty strongly about this since I met Leslie [Gordon], and I think it does suggest some animal models for developing a possible intervention. There are people in Boston trying to develop a treatment. I'm trying to develop a cure."

If stem cells are the problem, as Warner suspects, then fixing the defective *LMNA* gene before the cells differentiate might help. One possible way to accomplish this would be to take some mesenchymal cells out of the soup of primitive cells in the children's bone marrow, correct the gene mutation, and inject the newly fixed cells back into the bone. The hope would then be that these progeria-free stem cells eventually take over from the remaining defective stem cells because they're better adapted to survive. This combination of gene therapy and cell replacement would of course have to be attempted in mouse models before children. But it may not be so farfetched; Warner points to similar treatments currently being performed successfully on mice with other mesenchymal tissue diseases.

Meanwhile, Olive's group is trying a different bone marrow procedure in their mouse model. They want to perform a transplant: to take the bone marrow of a healthy donor, put it in a progeria body, and see whether the healthy cells take over and relieve the symptoms. However, this procedure has limited value at the moment. It would be useful to see whether transplants work in the mice, but they couldn't be tried next in children with progeria. Bone marrow transplants require the recipient to undergo radiation treatment that effectively kills their immune system so they don't reject the donor cells. "These children are very sick, you don't want to do a bone marrow transplant on them," says Olive. "It's too drastic for the children for us to consider it now."

If the transplants do end up helping the mice, Olive says, then perhaps the treatment could be considered in the future if someone develops a new technique that isn't so traumatic. The gene therapy Warner describes offers another way in; since it would use the patients' own cells, the children wouldn't have to be exposed to radiation.

"We know that FTIs are not going to solve all the problems," says Olive. "We need new drugs and new treatments."

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At the National Cancer Institute in Bethesda, Maryland, Tom Misteli's lab is exploring some of those alternative treatments. He wants to interfere even further up the chain, back when the progeria gene first gets spliced.

Misteli grew up in Switzerland and earned his doctorate in England, giving him an accent just left of British and a penchant for punctuating conversation with "bloody"s. As in, "How about we cure this bloody disease?" That's the question he asked his postdoc back in the summer of 2003 when he first heard about progeria. The night before, he'd seen a press conference on BBC News announcing the discovery of the gene for progeria. In the background, he caught sight of a drawing of the *LMNA* gene. On it was a triangle that researchers sometimes use to symbolize a cellular phenomenon called splicing. Misteli had long been interested in splicing and the structure of the nucleus. "I thought, hmm, if this is splicing of lamin, this might be interesting," he remembers, leaning back in his chair and running a hand through his mop of curly brown hair. He read the study and thought his lab

would be able to fix the problem. The next morning, he walked into his office, showed the paper to his postdoc, and issued her the challenge.

The postdoc was an Italian woman named Paola Scaffidi, who was then working on an unrelated project that wasn't going anywhere. She switched her attention to the new task: trying to figure out whether she could stop progeria cells from making damaged lamin, and if so, whether the symptoms of progeria would then go away. In other words, Misteli wanted her to prove that the disease was curable. He knew that progeria is such a devastating illness, people might argue that its damage couldn't be reversed. "The basic question was, can you take progeria patient cells and reverse their phenotype?" he says. "If you look at the patients, you can say no matter what you do, you cannot reverse those sort of dramatic cellular and organismal defects." Scaffidi adds, "If you look at these cells, it's amazing they can even divide. It's really bad." But Misteli thought they had a way to save them. His and Scaffidi's idea was to correct RNA splicing.

RNA's job is to translate DNA's instructions so proteins can be made. Like the DNA it copies, RNA contains alternating coding and non-coding sections, like the black-and-white scale on a map. Once the RNA protein-template gets formed, other molecules in the cell called splicing factors get to work on it, acting like scissors and Scotch tape. They cut on the dotted lines between those alternating sections, throw out the non-coding parts, and splice together the coding bits.

That's the way it's supposed to work. But progeria's single-letter error tells the molecules to "cut here" too early in one of those coding bits. The end of that vital section gets thrown out along with the next non-coding section. This faulty "cut here" is called a cryptic splice site.

Scaffidi set out to erase that fatal dotted line. She decided to use an oligonucleotide, a short string of genetic material that sticks to the incorrect splice site. "It's like you put a patch on it," explains Scaffidi. This way, the splicing scissors can't see it, and the RNA doesn't make the bad protein. Cells treated with the oligonucleotide should stop making progerin, leaving only healthy lamin A behind. Children with progeria do make plenty of normal lamin A; it just gets overwhelmed by the mutated progerin. Scaffidi's mission was to see if preventing the production of progerin straight from its RNA birth would reverse progeria's symptoms. The challenge was getting the splice-blocking oligonucleotide into her progeria cell samples to see if it would work.

It took her a year to set up the experiment. She put the skin cells of progeria patients in a dish. She tried scraping them to create tiny holes for the oligo to seep into, but the fragile cells kept dying. She tried bathing them in an oligo-and-chemicals soup, but her cells remained impermeable. Finally, she tried something unusual: she shocked the cells with electricity to create those tiny holes. Oligos don't have an electrical charge, but Scaffidi hoped that using a lot of them would force a few in by simple diffusion. At last, she found an oligo that successfully made it in.

Now that she had discovered a way to get the drug into her cells, she had to wait and see whether they recovered from progeria. On the first day, nothing happened. Nothing again on the second day, nor the third, nor the fourth. But a week after she'd started, something changed. "I remember coming out from the darkroom there and I couldn't believe it," she remembers. The cells had stopped making progerin. And when she looked under the microscope, she found that they had healed. She says she can count on one hand the number of times in her scientific career she has seen such striking results.

Scaffidi was afraid to repeat the test in case the success was a fluke. But she ran the experiment again and again, and each time she got the same conclusive outcome. When they stopped making progerin, the cells recovered their health. The nuclei resumed a normal shape, lamins distributed themselves properly, and problems with heterochromatin and gene expression resolved. It was proof of principle that the disease could be cured in children. "I was surprised that the cells are really plastic," she says. And they recover quickly: "As soon as you take out the protein, basically, they go back." She tried shocking the cells without adding the molecule, to make sure she wasn't inadvertently killing off the sickest cells and skewing her sample. It still worked.

The reason no one had tried what Scaffidi did before, Misteli suspects, is not that her technique is new or difficult, but that it's not well known. "It's actually very easy to do. It works almost every time, it's remarkable," he says. "It's an underappreciated therapeutic strategy." When he and Scaffidi published a paper in *Nature Medicine* in 2005 announcing their results, many people thought they had invented this way of fixing the splicing problem. In truth, the method had been around for more than a decade. Misteli was simply one of the few people who had been exposed to it. None of the other researchers who knew the technique had thought of applying it to progeria.

Scaffidi's findings bolstered hope that one day a treatment could be developed. If it was possible to reverse the symptoms in tissue samples, someone might be able to do the same for the children. She and Misteli believe that blocking the faulty splicing will provide better results than the protein-modifying drugs currently being tested. The trouble with FTIs, Misteli says, is that because they affect all farnesylated proteins in the body, they're likely to cause side effects. In contrast, the oligos he's working with are designed to target the

particular area of the particular gene that causes the disease. They don't affect anything else in the cells.

He just has to find one that can be given safely to people. Although Scaffidi's oligo worked for cells in dishes, it's too difficult to get it into a person without harm. (Shocking people's cells is simply not an option.) She is looking at other molecules like it. Several have been tried before in people with various diseases without much luck; some flow right through the patients without getting into their cells, while others get absorbed but don't fix the splicing problem. Scaffidi thinks she may be on the way to finding a different molecule that will get in easily and do the job.

At the same time, Misteli is running a high-volume search known as high-throughput screening to find a drug that works. It goes through so many drugs in such tiny amounts so quickly that a robot has to do it. A giant yellow arm straight out of an automobile assembly line works day and night in a room across town. Whether they'll find an effective drug that causes little trauma remains to be seen.

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Across the tree-lined NIH campus at the National Heart, Lung and Blood Institute, Michelle Olive continues to study progeroid mice in the hope that they will find a treatment for progeria and learn more about what happens in heart disease, one of the top causes of death in the country. Under director Elizabeth Nabel's guidance, the lab is testing FTIs in the mice to see if and how they improve the vascular symptoms. So far, the team has been able to

show that FTIs can prevent the onset of progeria. They're now starting to look at the statin-bisphosphonate regimen as well.

Olive's group has also begun looking at wound healing. They want to see whether progeria patients' vessels cope poorly with stress and injury. In some experiments, they look at how the lining of the mice's arteries reacts to being scraped with a thin wire. "So what our surgeon does, he makes a hole here and a hole there in the femoral artery"—she traces a finger down her thigh, where the artery runs—"and puts a very thin wire and just does, *chk chk chk*, removes the endothelial cells." She makes a two-handed back-and-forth motion like flossing to demonstrate. In other experiments, they crush living mouse cells in a dish and see how fast they move back together. It takes time, so they leave the dish under a microscope with a camera that takes a picture every five minutes, and the next day they can study the movie.

Jan Lammerding has been looking at similar phenomena in his lab in Boston. He lays a single layer of human progeria cells across the bottom of a Petri dish, scratches it with a pipette, and watches how long it takes the two sides to fill the gap. They take longer than they should. Lammerding says this is one part of a three-fold problem he sees in progeria cells. One, he says, you lose cells, because the cells are fragile. Two, those that do survive don't grow or replicate well. And three, cells that try to repair the damage are not effective. The result is an overall loss of cells in affected tissues and slow, incomplete recovery from injury.

Lammerding's main interest at the moment is in muscular dystrophy. Heart disease may not seem related to muscular dystrophy at first glance, but the connection became apparent when it turned out that the muscle cells in the blood vessels were dying. In his experiments, he subjects the cells to mechanical strain, which is a fancy term for repeatedly

stretching and compressing them—like what happens in our muscles every time we move—to see how they react. The answer for progeria cells is, not well. They die quickly and messily. "The cells don't like being stretched," as Lammerding puts it. This has devastating consequences in arteries, where Olive's missing smooth muscle cells are supposed to act as shock absorbers for pumping blood. FTIs don't correct the problem. Lammerding and others have been led to suspect that progerin is causing damage they haven't yet identified that makes the cells so sensitive to being stretched.

Although he is wary of linking progeria too closely to normal aging, Lammerding believes progeria's impact on the cardiovascular system and muscles can help researchers better understand laminopathies, which affect hundreds of thousands to millions of people. His protégé, dermatology resident Valerie Verstraeten, sees multiple levels to the value of studying progeria and other laminopathies. There is much to learn about the function of an important protein in the cell and nucleus, she says. Researchers can also study how the diseases progress and figure out whether they can do something about it. And it allows doctors to diagnose those syndromes early, understand what health problems the patients are likely to develop, and follow them up very closely to keep them as healthy as possible for as long as possible.

Those laminopathies in turn might tell us about conditions that affect larger populations. "I think that the truth of the matter is they all can be used to model less rare diseases," says Howard Worman, a laminopathy specialist at Columbia University. "You never know when you'll find something applicable. Maybe what happens in the heart in *LMNA*-associated cardiomyopathy you'll find something out about acquired cardiomyopathy like hypertension. It may tell you something about how the heart fails."

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In 2006, Scaffidi and Misteli discovered that tiny amounts of progerin are also found in the normal cells of healthy people. Each of us produces a little bit of progerin along with healthy lamin A. This isn't because we have a progeria gene mutation. Instead, it's because progeria's cryptic splice site is already "a little bit attractive" in healthy bodies, says Leslie Gordon. It's sort of a weak spot in the gene. Every once in a while, RNA gets cut on the wrong dotted line, and our cells make progerin instead of lamin A. In progeria, the single letter-change in the gene makes the spot even more attractive, and the RNA ends up making progerin fifty times as often. It's the same process, only with the volume turned way up.

Karima Djabali's follow-up study at Columbia University in 2007 showed that progerin accumulates in normal cells with age. She looked at skin samples grown from biopsies of 150 people without progeria, ranging in age from newborns to ninety-seven-year-olds. She found low levels of progerin in a few normal cells in the young patients, and slightly higher levels in more cells in the elderly. Even though those amounts were much lower than in children with progeria, it was a compelling finding. "Anything that increases with age that causes damage could certainly have something to do with aging," says Frank Rothman. That's what makes Djabali want to push forward with aging research. Francis Collins at the NIH, for one, supports the idea, saying that these experiments suggest that progerin contributes to normal cellular aging.

However, researchers are not yet sure whether the amount of progerin in the elderly is enough to cause a problem. "Progeria seems dose-dependent," says Jan Lammerding. "Old people may not have enough progerin for it to be toxic. Unless you can show that certain cells

accumulate more of it." Plus, Djabali and others have yet to determine whether progerin is a cause or effect of aging or just a coincidence. Olive adds, "We find progerin in normal persons. Why is it there? Is it good to have progerin or not? I don't think we know the answer yet." But even if progerin does turn out to hasten age, and even if scientists can figure out how to sweep the defective lamin from our cells, getting rid of it still won't lead to immortality.

Reflecting a timeless human desire to comprehend and overcome death, aging research has been trying to understand cellular and molecular mechanisms of mortality for decades. One question that's been floating around is about whether "evolution built in a little clock that tells you when you're getting older and when a cell is really supposed to stop working," says Mark Kieran. These biological clocks tell the body when it's time to die. One of the "master clocks" that has received a lot of attention is the telomere, a cap on the ends of our chromosomes that shortens each time a cell divides until it runs out and the cell goes dormant. Djabali's finding raised the question of whether progerin may be another clock, building up over time until the cell dies. Still, since it would work in concert with other clocks, stopping progerin wouldn't make us live forever. "It's unlikely that any one pathway is going to be the sole fountain of youth—if you stop it you stop aging completely—but this may be one of the clocks that helps you age, and slowing down one of those clocks may have some impact on that process for even the normal population," Kieran says.

Progerin is only one substance among many in the complex system that is the human body. "I don't think progerin is the marker of aging—it's one of them. There are many others," says Djabali. "There is not one pathway to age for cells, but several." She illustrates the idea by mentioning that because of genetics, environment and other factors, people develop

different combinations of illnesses as they grow older; they don't all experience everything. In a sense, each of us as we age will undergo a segmental aging disorder.

"Aging is such a multifactorial process," says Brian Capell. "Certainly there's more going on, progeria is not an ideal model, but when you think that we're all making small amounts of this mutant protein that builds up in our skin with age, and a lot of the same nuclear defects you see in progeria you see in cells from aged individuals, I think there is some relationship and some role for producing progerin. It's worth exploring."

Huber Warner was director of the Biology of Aging program at the National Institute on Aging when Leslie Gordon first lobbied the NIH for progeria research funding in 2000. Warner, who had been at the institute for almost twenty years, was familiar with progeria, but dismissed it as "a very odd disease that really had nothing to do with aging." Still, he agreed to fund the first Progeria Research Foundation meeting. "We went in extremely skeptical because we all knew progeria was not a very accurate representation of aging," he says. "But you know, at the end of the two days I was just fascinated by the pathology exhibited by these kids." Now his opinion has swung completely around. "I think progeria, although not an exact copy of normal aging, is characterized by phenotypes that are related to what happens in normal aging, just at a faster rate. That's why I think progeria is worth studying from an aging perspective."

"Maybe all the information—even the pharmacological results of these clinical trials—could be extrapolated in the future for trying to block or intervene on some partial aspects of normal aging," says Carlos López-Otín. "I think that this is the final dimension of this work."

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Sam Berns is twelve now. He talks and acts like a regular pre-teen, even if his size makes him look half his age. At a fundraising dinner for the Progeria Research Foundation at a pub in Foxboro, Massachusetts, where his family lives, he text-messages from his iPhone and grins at a sketch his friend made for him of the Joker from the latest *Batman* movie. He can hardly sit still, constantly up and around to say hi to people he knows, from his physical therapist to his young cousins. But his baggy PRF t-shirt, black jeans, and baseball cap don't completely hide his taut, knobby knuckles and elbows, his narrow shoulders, his pinched nose and hairless head, his subtly awkward gait. After a little while, though, it's easy to see past the odd features progeria leaves him with and see him just as Sam.

He wants to be an inventor when he grows up. He talks about time machines and hovercars and robots, and is delighted to learn about the mysteries of the universe as they're revealed in his science classes. He says he's making straight A's in math because he knows it'll be important for his coursework. He's not sure which school he wants to go to more: MIT, where so many inventors are, or Boston University, where his dad went.

Sam speaks of the future as if it's a certainty, even though he is less than a year away from reaching the average life expectancy of a child with progeria. With FTI treatment, physical therapy, supplemental therapies like growth hormone, and two extraordinary parents, he has resources available that weren't available to those who have gone before him. But it's not clear whether the number of scientists who've been drawn to progeria research will make sufficient progress soon enough to let Sam and other children with this disease live another ten years, or twenty, to allow them to realize their dreams.

"I hope I live long enough to see a cure, but I probably won't. Science is slow," says researcher Warner, who is 72. A cure may not be found within Warner's lifetime. It may not be found soon enough to save Sam. But researchers are trying, and in the meantime, the potential repercussions ripple farther and farther out from progeria's epicenter. It provides perspective as one of many premature aging disorders that are opening windows into our understanding of aging and cancer. It's even possible that studying the way lamins accumulate at the nuclear envelope will lead to insights into Alzheimer's disease and Parkinson's disease, which also involve protein aggregates, says Tom Misteli. Mark Kieran wonders whether progeria might tell us something about weight and eating disorders, since one of the earliest and most persistent symptoms of progeria is that the children don't grow, no matter how much they eat. "There are some components we're still learning, but again, we are learning it from these kids," Kieran says. "Whether a whole bunch of treatments for obesity or anorexia are going to come out of this part as well, there's so much to learn from these kids."

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