Michael Bellomo

THE Stem Cell Divide

THE FACTS, THE FICTION, AND THE FEAR DRIVING THE GREATEST SCIENTIFIC, POLITICAL, AND RELIGIOUS DEBATE OF OUR TIME

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MICHAEL BELLOMO

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Most know that an avalanche begins with the motion of the smallest stone. Very few understand that even the smallest stone needs the tiniest nudge to begin its journey.

Dedicated to Sally Moses,
of the Hun School of Princeton

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Stem Cell Cures and Curses

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INTRODUCTION

The term "cell" comes from the Latin word *cella*, or "small room." Robert Hooke, a seventeenth-century Renaissance man, coined the term when he first peered through his hand-crafted, leather- and gold-tooled microscope at a piece of cork. Reportedly, he came up with the name when the little cells he saw through the microscope reminded him of the small rooms that housed medieval monks.

In humans and other forms of animal life, *stem cells* are the special, primal structures in the body that retain two special traits: first, the ability to divide indefinitely, and second, the ability to differentiate into other cell types.

These traits are at the root of why stem cells are a source of both order and chaos, representing miracle cure and societal curse. Specifically, the cells that show the most potential can only be retrieved with great difficulty—and through the destruction of a human embryo.

It's possible that these "embryonic" stem cells may lead to great things in the future. But in the here and now, religious conservatives see their destruction as nothing more than a high-tech form of cannibalism.

Belief over what is right permeates the field of stem cell study and its researchers. In the course of my investigation for this book, very rarely did I encounter any person on either side of the issue who held the slightest doubt whether they were on the side of the "just."

To take one example: In mid-2005, I was interviewing an embryonic stem cell researcher when I asked: "How do you deal with

people who feel that you're doing things that are morally and ethically wrong?"

"I don't feel what I'm doing is morally wrong," was the reply. "Is it really immoral to use cells from embryos which would have been incinerated as discarded medical waste from a fertility clinic? Or is it moral to do nothing and allow human suffering to go unchecked when you know you could do something to stop it?"

I am not a theologian or philosopher by training. I had no answer to this.

The morality and the science of stem cell research turned more indistinct the deeper I delved. Late in 2005, I had scheduled to interview several researchers in the field of embryonic stem cell technologies. Mysteriously, almost all of them cancelled on my interviews in the same week. Ten days later, the premier embryonic stem cell researcher, South Korean scientist Hwang Woo Suk, was accused of fraudulent research.

Many of my slated interviewees had been prepared to praise Hwang's work as some of the best ever seen in their field. It is possible that they had been taken in by the man's claims that his superior technique was from using "slippery steel chopsticks" as a child.

The delay was, in fact, serendipitous. While important new developments can arise at any moment, *The Stem Cell Divide* covers items that only a very recent book can capture, describing events that will reverberate for decades:

- The story of how Hwang Woo Suk went from obscure veterinarian to rock-star scientist thanks to stem cells—the very cells that would end up devastating his career and leaving his reputation in tatters.
- Why California voters, when confronted with a bankrupt state government and rolling blackouts, authorized enough money for stem cell research—enough to give a dollar bill to every single person on the planet.
- The truth about the Bush and Clinton administrations' claims to be the first to allow and federally fund embryonic stem cell research.

- The public break between President Bush and Senate Majority Leader Bill Frist—and why it could spell defeat for the Republican Party in future elections.
- Finally, *The Stem Cell Divide* provides in-depth analysis about the latest attempts to cure spinal cord injuries. This March, a paraplegic woman was cured by stem cells to walk again—for three weeks, before the therapy failed and put her back into her wheelchair.

I believe that you will find the answers to the questions raised by these fascinating issues—and much more besides—between this book's covers. My goal as an author is to present a clear story about how we came to this point in stem cell research, what is going on today, and what—just perhaps—will happen in the next 100 years. Objectivity, not partisanship on the left or right, is what drives this text.

Regardless of who sits in the White House, the genie of stem cell research has been let out of its plastic T-flask. Whether we agree with the result or shun it as a product of science that has gone out of control, it *will* happen. The only question is: *When*?

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PART I

DISCOVERY OF THE STEM CELL'S UNIQUE ABILITIES

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CHAPTER 1

MORNING ON THE DEVIL'S HIGHWAY

It is strange to think that a tiny piece of matter, so small that it cannot be seen by the naked eye, could change the entire world. And yet, the promise of the human stem cell is as vast as anything the scientific community has yet seen.

Stem cells have two unique properties. First, they can give rise to other more specialized cells of the body, particularly in the case of embryonic cells. Second, they are self-renewing, with the ability to grow in laboratory culture for long periods without losing their ability to give rise to other cell types.

But stem cell biology is also replete with unanswered questions. Only a few can be definitively answered today, but there are signs and portents in abundance that offer us clues as to where the research might yet lead.

Human stem cell research is a young field—arguably dating back to as recently as 1998. But it is a pivotal one. Perhaps no other area of research today offers as great a potential for alleviating great amounts of human suffering. Consider one possible scenario that could easily take place in the next decade or two.

■ ■ ■

Shortly after dawn on a humid July weekend, the worst storm seen in a decade engulfed southwest Colorado in black, roiling thunderclouds. Leaden sheets of rain pounded the landscape, opening up sluices where earthen cracks or old ditches lay open to the sky. The stretches of prairie to the east absorbed most of the water like a grass-covered sponge. But near the towns of Cortez and Yellow Jacket, only a few plants broke the expanse of gray silt and pink gravel: handfuls of mesquite trees, patches of blue stem, and a few scraggly tumbleweeds that looked like refugees from a B-grade western.

Flash flood warnings crackled over the radio and the CB frequencies used by truckers and highway patrolmen. Most of the drivers on the main highway, Route 666, coped with the blinding rain by pulling to the side of the road or sheltering under a convenient overpass.

Route 666 is often referred to by locals in three states as "Satan's Highway" or the "Highway to Hell." The road owes its colorful nickname to the unfortunate coincidence of the "number of the beast" cited in the New Testament. However, the name is fairly well given. Route 666 is a tortuous stretch of asphalt running through high desert and golden layer-cake mountain canyons stretching hundreds of miles from Arizona to Utah.

The road holds two claims to fame, or at least infamy. First, there is an ongoing problem with signage theft. The local police blame thieves looking for souvenirs and not the Devil, who is presumably occupied with more important things than stealing highway signs. Second, Route 666 has some of the highest fatalities per mile of any highway in the southwest. This is in part due to the questionable condition of the road, which suffers from frequent rock falls, gaping potholes, and washouts.

Kelley Michaelson, a twenty-year-old junior at the University of North Carolina, uttered a small curse as the rain spattered across her windshield. She shifted her Hyundai SUV into a lower gear and switched her lights on. The northbound lane she was in began to slow, but the traffic kept moving. Since she was getting off in the next couple of exits, she thought she could make it through okay despite the torrential downpour.

Kelley was a compactly built young woman with shoulder-

length brown hair, a dark tan that masked a crop of childhood freckles, and a no-nonsense attitude. She was a standout at UNC in both her anthropology classes and women's track events.

Her friends had encouraged her to leave behind anything relating to her major over the summer. But with graduation a disturbingly close apparition on the horizon—and the prospect of graduate school only a little further out—she needed more field experience, and some cash. With more than a few persuasive phone calls, she'd finally snagged a paid internship doing fieldwork at one of the Native American ruins out west.

The job met her expectations. It gave her some pocket money as well as some badly needed references. She had picked up the deft sureness of the senior hands at piecing together shattered pottery. She'd also learned the useful "annoyed scowl" that kept the tourists from constantly going "*Whattaya doing*?" while she was trying to work.

Back East, Kelly had never seen anything like that storm she was now facing. She couldn't see more than a couple yards through all the rain and wind. Suddenly, some kind of sound began cutting through the din of the beating raindrops and the howling wind. One part monster-like rumble, two parts banshee shriek. The sound of metal scraping on asphalt. Goosebumps rippled up her arms as the sound ratcheted up the Doppler scale. It was heading her way.

Ahead of Kelley's car, Route 666 made a wide turn, slashing through the side of an inconvenient hill as it angled towards the left. The hill was not solid. It was made up of tumbled, rough-edged stone ranging from suitcase to boxcar size.

The bare, water-saturated soil gave way and tore loose from the slope, taking a sizeable chunk of the hillside with it. The mass of dirt and rock punched through the cement barrier at the end of the road. It spilled across the southbound lanes, coating the asphalt with a deep layer of slick mud and a jumble of loose stones.

As the southbound lanes disappeared under the wave of mud, a heavily loaded big rig truck slid out of control. The rig jackknifed, skidding into the highway's open center divide. The rig's tires blew out on the sharp gravel like gunshots. The trailer swung around the cab and into Route 666's northbound lanes like a steel wall.

Blinded by the rain, the only hint that the northbound traffic

had as to what was coming was the high-pitched squeal of the trailer scraping across the asphalt. In a ghastly domino effect, twenty cars piled into one another and the rig's trailer. One of the cars burst into flame. The fireball rose into the gunmetal gray sky, adding a hellish orange flicker to the morning light.

Kelley saw the chaos ahead only for a split second. The red brake lights of the cars ahead glowed bright red, then pinwheeled in all directions. She swerved desperately to avoid broadsiding a blue pickup that veered into her path. The SUV skidded completely around before it crunched into the mass of cars. Her windshield shattered and she closed her eyes as a bright glare poured through the open space. She felt another car slam into her. The white brilliance of xenon headlights plunged into black, and she immediately lost consciousness.

AFTER THE CRASH

The first responders decided right away to call in helicopters to airlift the worst cases to the nearest ER. After a quick consultation with authorities in the neighboring states, the helicopters were sent to a hospital in Gallup, New Mexico.

Kelley arrived in dire straits. The staff treated her for assorted bruises and lacerations, but by far her worst injury was a spiral fracture of her left femur. The femur is the thickest, strongest bone in the human body. Kelley's had snapped like a wet matchstick in the mass collision.

Initially, she appeared to make good progress towards recovery. In the first forty-eight hours after the accident, she was awake and lucid, even under heavy pain medication. Her parents flew to Gallup and found her in good spirits. In a separate operation, her femur had been set with rods and plates without incident and the initial healing had begun.

On the third day, Kelley began to complain of a pain in her gut. It was a shooting, twisting pain, and she cried out if anyone touched her abdomen. She was checked for appendicitis, and an ultrasound examination of her organs did not detect anything unusual. She became unable to keep food down.

Kelley began to regurgitate in increasing waves of severity, continuing long after her stomach was empty. The thin gruel of fluid in her vomit had the bitter smell of bile. Her eyes became watery and bloodshot, she became less responsive to stimuli, and her face turned a soupy whitish color.

Kelley's physician, Dr. William Glazier, was one of the lead surgeons at the hospital. He had had his hands full as the accident victims had arrived en masse by air rescue. Glazier was a Minnesota native who had moved to Gallup ten years earlier when his wife demanded that they live closer to her family. He was known for being somewhat short-tempered. This was in part because he was constantly pushing for the hospital to keep up with the latest surgical techniques. Mostly it was because, although his first name was William, people kept calling him "Gil", as he bore a striking resemblance to Gil Grissom, the main character on the television show *CSI*.

Kelley's condition was a mystery, but Glazier had one clue to go on: The young woman's tests showed that she had elevated levels of amylase and lipase in her bloodstream. Amylase and lipase are digestive enzymes formed in the pancreas. The abnormal spike in these substance's levels was a clear indicator that pancreatitis—inflammation of the pancreas—was evident, even if the cause was not.

Pancreatitis was very bad news. In severe cases, there may be bleeding into the pancreas, serious tissue damage, infection, and cysts. Enzymes and toxins leaking from the damaged organ may enter the bloodstream and severely injure the heart, lungs, and kidneys.

MARROW OF THE MATTER

Further examination showed that Kelley's pancreatitis had been caused by a rare but not unknown event known as a "fat embolism." The primary location of human bone marrow, the material which produces the oxygen-carrying red blood cells, is inside the body's long, thin bones. When one of these bones—such as Kelley's femur—is shattered, marrow fat is commonly released into the

bloodstream. These droplets of fat scatter freely through the body, similar to the way a gobbet of hot fat will jump out of the frying pan when the burner is turned to "high."

Most commonly, the body will reabsorb this fat. Some may be deposited in the lungs. Other fat droplets can lodge like leaves in a storm drain in organs like the brain, kidney, and skin. In Kelley's case, they had lodged in the vessels that supplied blood to her pancreas. The organ, deprived of its cellular needs of oxygen and waste transport, had died. The resulting rot was slowly spreading and the process was irreversible.

The pancreas is one of those amazingly complex, subtle organs. We don't even know everything it does—only that you can't function without it. That's why pancreatic cancer is a death sentence. But Glazier wasn't about to go out to her parents and tell them that it was time to say good-bye to their daughter.

Glazier conferred with the hospital's director and convinced him that this was a textbook case where the use of stem cells could be the difference between saving or losing the patient. Simply put, Kelley Michaelson needed a new pancreas if she was going to live. By using a nucleus from one of her cells, it would be possible to create a new, genetically identical organ for her. This form of therapeutic cloning wouldn't run the risk of transplant rejection. Her own white blood cells wouldn't even notice the new pancreas as anything but the body's own.

The question now became which stem cell facility could create a new pancreas for Kelley in time. The Supreme Court had steadfastly refused to make a concrete decision, concerned about further politicizing the issue in the manner of abortion. So the matter had been kicked back to the state level, with mixed results.

The medical facilities that could handle and direct high-speed stem cell growth had been growing, but were still small in number and always in high demand. One facility in Arizona was not yet up and running. The stem cell center in Denver was in the middle of a batch of critical experiments and could not be interrupted.

The search continued further afield. The issues involving stem cells were still being debated in California, the labs in Michigan were booked solid until the next decade. There was one facility sit-

ting idle in Amarillo, Texas. The area is home to over 300 Evangelical churches, and they didn't approve of the use of embryonic stem cells.

Ironically, the right timing and the right facility was available—at the medical school of Kelley's college, the University of North Carolina. Dr. Susan Nguyen, the director of Therapeutic Genetic Research at UNC, called Glazier immediately upon hearing of his need. She confirmed that they had the equipment that could quickly recreate the damaged organ.

Staff at the hospital in Gallup took a sample of healthy cells from Kelley and prepared the sample for immediate shipment. Not one to take chances, Glazier hired a professional courier service, one that was experienced in transporting items as varied as exotic deep-water fish or a shipment of live brain tissue. Within seven hours, the sample had been handed off to the doctors at UNC.

ROAD TO REGENERATION

Dr. Susan Nguyen is the daughter of two Vietnamese doctors who had fled Vietnam during the fall of Saigon back in 1975. She is coolly contemplative and direct to the point of being blunt. Despite the availability of inexpensive vision correction surgery, she continues to wear glasses that hold thick lenses in a sturdy tortoiseshell frame. Her explanation for this anachronism is that while she understands genes and cell cultures, she is terrified of scalpels, lasers, and needles.

Dr. Nguyen took one of Kelley's cells and teased the nucleus from the cell's cytoplasm with a tiny glass pipette. She then painstakingly transferred the nucleus—the microscopic dot containing all the genetic information that made up the young woman who lay dying in a hospital in New Mexico—to the inner mass of a fertilized egg whose nucleus had already been removed.

The images Dr. Nguyen used to complete the transfer were projected in a nearly three-dimensional view screen. Her instruments precisely scaled her movements down so that she could manipulate all the pieces of the cellular jigsaw puzzle with relative ease. Even so, it wasn't until some time later that Nguyen called her task complete.

The newly created hybrid cell rested securely in its accelerator growth medium. As she watched, the cell's installed genetic "machinery" took over and began to divide at an astonishing rate. As the cells continued to split and multiply, the researchers at UNC were alert to the cell's needs and re-plated the colony on fresh culture dishes.

The rate of division and the level of cell differentiation were watched as closely as the volatile pile of rods in the core of a nuclear reactor. Too little in the way of nutrients was like placing too much graphite between the rods of uranium in the reactor: By starving the reaction of fuel, the fire would go out. On the other hand, overabundance of resources risked a cellular meltdown: Cells would begin to grow uncontrollably and mature to the point where they lost the ability to morph into the kinds of cells that were needed.

Dr. Nguyen points out that a lot of people think that it's a challenge to get stem cells to grow on culture medium. "That's not just wrong," she says, "it's exactly wrong. These cells just love to grow—it's what they're designed to do! Our problem is convincing them to grow just in the *way* we want them to."

The dimpled, yellow clump of undifferentiated stem cells, each bearing Kelley's unique genetic blueprint was the size of a pinhead when it was next placed in a bioreactor chamber. The shiny plastic vessel looked like an ice-cream maker as re-imagined by modernist architect Frank Lloyd Wright. Tubes connected to the unit at impossible angles served to manipulate the growth medium with complex combinations of human growth factors and organic compounds.

These mixtures, incomprehensible in composition to anyone without a Ph.D. and carefully guarded by individual researchers, acted like flagmen directing fighter jets to land on a crowded aircraft carrier. Amount X of Factor Y told the cells to grow in one direction. A different amount or combination nudged the development in another way. The mixture injected into the bioreactor by Dr. Nguyen and her staff gave a direct order to the clump of cells: to quit remaining in a sort of biological "ready state" and differentiate.

The bioreactor was a space-age machine based on a design pioneered by NASA in the weightlessness of space. It spun gently, rotating clockwise at a speed that helped press the cells against the insides

of the tank. The interior was lined with a special tissue "scaffold" that allowed Kelley's cells to multiply into sheets of tissue and then into more complex structures—all in the space of a few days instead of months.

What the team decanted from the bioreactor wasn't just a jumble of cells. It wasn't a carefully cultured imitation pancreas. The end result was, in fact, an *exact* duplicate of the pancreatic tissue that was slowly failing Kelley's body.

For a second time, a special medical courier was dispatched to take the meticulously packaged tissue across the country via airplane. Dr. Glazier was paged ahead of time and immediately prepped Kelley for surgery.

At last, the packaged organ arrived and Kelley Michaelson's surgery took place later that evening. Dr. Glazier made an incision and pulled back the warm, baby-pink sheet of abdominal muscles. What he found disturbed him. The organ was swollen, hard to the touch, and off-color. The pancreatic tissue in Kelley's abdomen had continued to die off. In another day, the doctors could have been facing mass sepsis, with multiple organ failures. It was already tough getting the original organ out—it was soggy, crumbly, like it was made of Ricotta cheese gone bad.

The replacement of the new organ went smoothly. Dr. Glazier took his time in making sure that an adequate blood supply was available to nourish the brand new pancreas before closing the incision. After cleaning up, he went back to his office, turned the lights off, and breathed a deep sigh of relief that the worst should be over.

For once, it was. Over the next two days, Kelley's color and disposition began to improve. The healing around her left femur began to continue again without interruption. Interestingly enough, it was at this point that Kelley remembered "coming to" after the accident. For whatever reason, she was never able to retrieve the memories from the days where her life hung in the balance as her cloned organ was grown in the bioreactor vessel.

Kelley returned to her studies after missing the fall semester. She is still in physical therapy for her healing leg, but is expected to eventually make a full recovery. The only shadow in her life is, if it can be said, on her conscience.

"I'm still not sure about how I feel regarding the use of the embryonic stem cells," she says honestly, "I know I wouldn't be here today talking about it if something hadn't been done. It just feels like I *should* be feeling guilty. I wish I knew more about how the process really worked so that I know for sure."

Dr. Nguyen isn't ambivalent at all. "The way I see it, there's a bright young woman who is here today. She wouldn't have been here otherwise. That's all I need to know."

CELLS OF PROMISE

The story above is, of course, completely fictional. However, it is not outside the realm of possibility for this scenario to be commonplace someday. The excitement over the use of stem cells—embryonic and otherwise—is akin to that of the development of software and microchips during the heady days of the dot-com boom in the late 1990s.

Comparing the field of stem cells to the dot-com boom may be more accurate than you think. While their potential is immeasurably promising, their actual uses have been far more limited in scope. And yet, consider the titanic effect that these tiny bits of organic matter have had across the United States and the world:

- The position on federal funding for stem cell research has been a major campaign issue in the last two presidential elections, and shows no sign of going away.
- Famous names like Christopher Reeve and Ronald Reagan are now linked in the public's mind with stem cell research.
- Cities around the United States are racing to capture as many of the nascent stem cell research companies as possible, betting that these companies will be the next Genentech or Chiron.
- Scientific teams from Europe to East Asia are doing their best to steal a lead on the United States.

- Wary of the turmoil created by the harvesting of embryonic stem cells, researchers are searching for the next Holy Grail: a cell that can be taken from elsewhere in the human body, which will morph into anything needed upon request.
- Despite the lack of any concrete accomplishments in ending or limiting human suffering, the voters of California have allocated billions in state money to fund research.

Questioning the use of stem cell research brings to mind the case of nineteenth-century British physicist Michael Faraday. According to legend, Queen Victoria—his monarch, and main provider of research funds—spoke with Faraday about the questionable results of his work.

Many of Faraday's discoveries involved the interaction and inner workings of magnetism and electricity. Though obviously important today, in Victorian England these findings fell into the categories of "obscure," "quaint," or "quite likely bloody useless." Perhaps foreshadowing future dialog between scientists and venture capitalists everywhere, the Queen asked Faraday of what possible use such studies were.

Faraday is said to have replied, "Madam, of what use is a baby?" In other words, Faraday knew that despite the lack of practical application in the here and now, there was tremendous promise in his work. He was convinced that in the future a practical use for his studies in electricity and magnetism would emerge and have great repercussions.

Stem cell research differs from those long-ago experiments in one key way. The research's potential is clearly recognized, and more, regardless of actual results. In fact, the promises made are hyped, spun, or downgraded in ways that can make your head spin. The more you delve into the field, the brighter the light gets.

Oddly, rather than illuminating the situation, the light gets so bright that it becomes harder, not easier, to see where the paths lead and what is truly fact or fiction. There are times that one has to step back and realize that what is said is actually speculation. Complicat-

ing matters is the ever-shifting nature of the "truth," as science continues to push back the realm of the impossible and ground it firmly in the possible.

I said that the potential of the research is hyped, spun, or downgraded. This is often done according to one's own views of science, politics, and religion. And that is the root of the matter. Because stem cells promise so much—even if they have delivered relatively little to date—they are by nature a flashpoint. There is no other subject where the frontiers of science, the needs of business, the question of the government's role, and the religious definition of life intertwine so perfectly.

"No one can say whether the results will be a treasure trove of cures or curses," one of the researchers I interviewed said confidentially. What is clear is that decisions taken today by the movers and shakers in this field will cause a ripple effect on the whole. The ripples themselves—the founding of new industries, reducing human suffering, changing our view of life itself—will be on the order of the invention of the transistor, the Pill, or the atomic bomb. They will take decades to play out and will fundamentally change the course of evolution for the human species.

Cure or curse? As with many aspects of the stem cell divide, what will result may be purely a matter of one's perspective.

CHAPTER 2

HEAD OF THE HYDRA

The promise that underlies all of the hope and hype about stem cells is that they can potentially regenerate any cell, tissue, or organ. There is a commonly mistaken belief that this development is somehow a result of one of modern medicine's miracles. Rather, it is a miracle with a decidedly older origin—one that is found in nature.

MYTH AND NATURE

Writers from as far back as ancient Greece claimed that many creatures could regrow parts of their bodies, particularly their limbs. Undoubtedly, the best known of all of these accounts is the story of the Greek hero Hercules and his battle with the Hydra. The gods had ordered Hercules to serve Eurystheus, the king of Mycenae, for twelve years. As part of his sentence, Hercules had to perform twelve Labors, feats so difficult that they were deemed impossible to carry out by ordinary men.

The second of the twelve labors of Hercules was to kill the

Lernean Hydra, a gigantic serpent that would rise up from the swamp and terrorize the kingdom. The Hydra had nine heads, each with venom-dripping fangs. If matters weren't difficult enough, one of the nine heads was immortal and therefore indestructible.

Hercules lured the Hydra into the open by shooting flaming arrows into its den. Once it emerged, he immediately grappled the monster, preventing it from escaping. He attacked the many heads of the hydra, smashing each of his heads with his massive battle club.

Unfortunately, this was when the Hydra's real power became apparent. Each time Hercules smashed one of the heads, two more would immediately grow in its place. The longer he fought, the more difficult the battle became. Luckily for the hero, his trusty nephew, Iolaus, had accompanied him on the mission as his charioteer. At Hercules' request, Iolaus joined the fray.

FIGURE 2-1
The nine-headed Lernean Hydra slain by Hercules.

Now each time Hercules crushed one of the Hydra's heads, Iolaus held a torch to the headless tendons of the neck. The flames prevented the growth of replacement heads, and finally, the beast was defeated. Hercules chopped off the ninth, immortal head, pinned it in place with a heavy rock, and buried the remains.

The story of the Hydra was undoubtedly based on observations of lizards and salamanders, each of which can regrow lost limbs. These observations were first formally documented in the 1700s with the arrival of a new breed of scientist—the European naturalists.

At the climax of the historical period known as the European Enlightenment, three men cataloged and classified the ideas and organisms that demonstrated the amazing property of regeneration. Taken together, they were a very diverse and unlikely trio: a peerless French scholar, a Swiss academic who earned his keep tutoring the sons of minor members of the nobility, and his nearsighted, botanist-lawyer cousin.

THE WHIZ KID

The first of the three was a French scientist with the imposing name of René Antoine Ferchault de Réaumur. An eighteenth-century whiz kid with an insatiable curiosity about the natural world, his talents ranged from geometry to meteorology, from entomology to early industrial science. In 1703 he came to Paris to study mathematics and physics. Five years later he was elected a member of the Académie des Sciences. He was only twenty-four years old.

For the next five decades, Réaumur published one paper after another, creating a body of scientific work that is inspiring to behold in its depth and breadth. He started with a treatise on geometry. Next, he compiled a comprehensive list of data on the gold-bearing rivers, turquoise deposits, and fossil beds in the French countryside. One of his experiments proved that the strength of a rope is more than the sum of the strengths of its separate strands.

During his tenure at the Académie, Réaumur demonstrated the importance of carbon in making steel, published a multivolume work on the classification of European insects and designed incubators for various types of bird eggs. His invention of a thermometer scale that bears his name was slowly rejected over time, and replaced by the Kelvin system of measurements. Given his delight in the systematic study of natural history, it is no wonder that his contemporaries called him "the Pliny of the eighteenth century."

His experiments and observations in the realm of biological processes are of the most interest for our purposes. He certainly did not shy away from controversial theories. In one set of experiments, he proved that the stomach digested food via chemical, not physical means. Réaumur did this by feeding a hawk metal cylinders containing meat, and examining them when they were regurgitated. He used another set of experiments to prove correct a controversial hypothesis that corals were actually a kind of animal, not a plant.

Spanning an impressive 138 portfolios, Réaumur's writings on natural history also contained a study on the families of *echinoder-*

mata (spiny-skinned animals such as sea urchins or starfish) and crustaceans (lobsters, crabs, shrimp, and barnacles). In both cases, authors from classical antiquity—Aristotle among them—had claimed that many of these creatures routinely regenerated limbs. Never one to shy away from a contested claim, Réaumur investigated the matter.

Much to his surprise, he found that, at least for crustaceans such as shore crabs, a lost limb truly did not permanently inconvenience the animal, as the appendage could regrow in the course of the year. His drawings, which showed the regeneration process in a series of steps, caused quite a stir in the scientific circles of the day. Yet this commotion paled in the light of the documented observations of one of his contemporaries, a well-regarded tutor to the children of Dutch counts and minor nobility.

NEW LINK IN THE CHAIN

One of Réaumur's many correspondents was Abraham Trembley, an erstwhile naturalist from Switzerland who had received what many would consider an enviable post in the Netherlands. Being a tutor to members of the upper crust paid handsomely, and the duties were relatively light for the day, compared to many academic jobs. It was while out in the Dutch countryside picking up new specimens of plants and aquatic life to use in teaching the two sons of Count Bentinck, his patron at the time, that he stumbled across a major advancement in the understanding of how creatures can regenerate tissue.

Upon close observation with a magnifying glass, the small green nubbins at the base of the water plants he had collected turned out to be extremely curious indeed. They looked like tiny trees, though their branches seemed to move on their own. His suspicion that the branches moved only because of random currents in the water was shattered when, during his patient observation, he saw one of the organisms wrap a "branch" around a water flea and stuff it into the center of its "head."

In an instant, he realized that what he was seeing could not wholly be a plant. The branches were more like arms or tentacles,

and the "trunk" was more of a body with a rudimentary head and mouth. However, the "insecte," as he described it, bore several vegetative characteristics.

A key factor was that the organisms displayed a tremendous amount of range in the number of body parts, sporting between four and eight tentacles. This variability is common in plants—a geranium may have one, two, or ten branching stems. It is correspondingly rare in animals, as five-, seven-, or eight-legged gazelles are at a distinct disadvantage when fleeing from predators.

Yet if his discovery wasn't definitively an animal or a plant, what exactly could it be? Trembley considered that it could be a *zoophyte,* a sort of link between plant and animal. This theory, while odd to us at first glance, in fact fits in perfectly logically with the prevailing ideas at the time.

The medieval conception of the order of the universe accepted in Trembley's age was based on a strict hierarchy of nature, called the "Great Chain of Being." The chain could be understood as a sort of naturalist's feudal order, unbreakable and unchanging. From top to bottom, a general classification might read:

- God
- Angels
- Humans
- Animals
- Vegetables
- Minerals

Each class was subdivided further depending on the medieval perception of their intellect, holy nature, and utility. For example, in the "Animals" category, wild beasts were seen as superior to all other animals, because they possessed superior force of will, defying training and domestication. Domestic animals follow, with the more useful animals preceding the less useful ones.

Birds follow the domestic animals, and they are in turn ahead of fish. The different fish are subdivided into actual fish and other sea creatures, such as eels and shellfish, which are abhorred in the Bible. Below even the least of the fish come insects.

At the very bottom of the animal level are snakes. The snake is placed at the bottom of the list because of a sort of "guilt by association." In the medieval mind, its lowly place was punishment for the actions of the serpent in the Garden of Eden in tempting Eve to partake of the fruit from the tree of knowledge.

Trembley was sure that he was on to something, perhaps a new link in the great chain linking all life together in hierarchical perfection. Surely, the tentacles on the organism were snake-like, proof that it was the lowest form of animal life, close to the next-lowest classification of the vegetable. In order to settle the matter once and for all, he sharpened a pair of tiny scissors and bisected the creature across its trunk.

He placed the pieces in a separate glass of water and observed them over the next week, carefully noting any change in his notebook. The pieces were easily identifiable to him, as the "head" retained its vital tentacles, while the remaining stem looked like a tiny piece of lopped-off tree trunk.

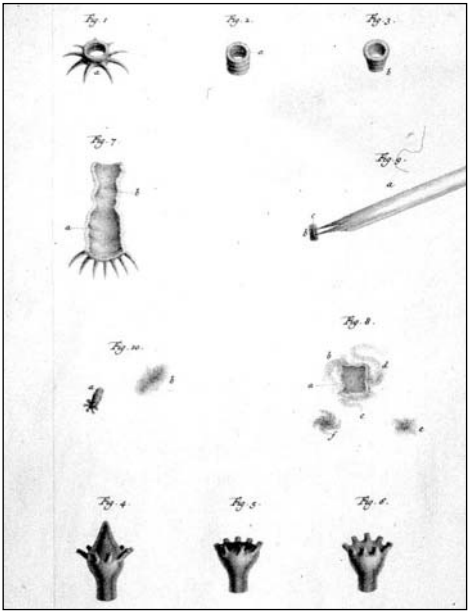
Trembley's hypothesis was that the organism, despite its green, treelike appearance, was in fact animal in nature. If so, then he fully expected the trunk-like remainder to shrivel up and die while the head survived and grew a new body. In his memoirs, he recorded his opinion that because the trunk was more like a tail that would not contain any vital organs, it should have died while the rest of the animal lived.

The actual result, however, was startling. By the ninth day of the experiment, both the "head" and the "tail" of the creature were visibly growing into whole new organisms. In two weeks, Trembley had two identical creatures growing in his glass, where before there had been only one.

Trembley began experimenting on the plant-animal, finding out that even dividing the creature into multiple segments would only end up yielding more copies of complete organisms. It was at this point that he made the connection with the classical legend of

FIGURE 2-2

Sketches from Abraham Trembley's notebook, dated 1744, showing the regeneration and growth of fresh-water hydras.



Hercules' second labor against the multiheaded monster, and he dubbed the organism a "hydra."[1]

For the next four years, Trembley became obsessed with the seemingly miraculous regenerative powers of the hydra, detailing notebook after notebook with pictures of his findings.

In one experiment, Trembley bisected the head of the hydra down to its midsection—not severing it completely, but leaving a large cleft down its middle. The animal regenerated both parts and kept each head in a sort of "Y-shaped" configuration. While funny looking (at least, presumably, to other hydras) the organism functioned without a problem.

1. Many believe that it was Anton van Leeuwenhoek (1632–1723) who first discovered the freshwater hydra. Leeuwenhoek did in fact observe the creature shortly before Trembley, but although he sketched the creature, he did not classify or name it.

Yet another discovery relating to his strange organism would have far-reaching consequences in his time. Trembley's drawings of the hydra's process of regeneration followed the exact same pattern seen during the gestation of animal embryos. Even in the iconoclastic, individualistic world of eighteenth-century Enlightenment, this was treading on dangerous, even blasphemous ground.

Rigidity was still the rule of the day. While the church did not have quite the same power of thumbscrew as it did during Galileo's era (when the famed astronomer was placed under house arrest for pointing his telescope at the heavens), there were still limitations. The established tenets of the Great Chain of Being, or the scientifically applied doctrines of Calvinist thought, were enough to ensure that Trembley's observations were minimized, or at worst scorned by many.

Accepted doctrine was that animals could only reproduce sexually, that is, with a male and female being the only way of creating new offspring. Trembley's observations that hydras could "bud" in the same manner as plants, or that they could spring whole from tiny clumps of cells, changed the system of sexual reproduction from "essential" to "optional."

Luckily for Trembley, the controversy over his findings did not result in his being burned at the stake. Instead, the worst that was said about his work was that he had simply misclassified the hydra in the Animal kingdom when it really was part of the Vegetable kingdom. The French philosopher Voltaire, upon reading Trembley's work and seeing the green, vaguely tree-like structure of the organism, offered the opinion that the hydra should be classified as a plant since it resembled "a stalk of asparagus."

EYE OF NEWT

Trembley's younger cousin, Charles Bonnet, was also part of the endlessly inventive and highly chatty scientific circles of the day. Coincidentally, he had also given a tremendous knock to the defined rule of sexual reproduction in nature. Just before his older cousin published his work about the freshwater hydra, Bonnet announced that he had observed *parthenogenesis* in na-

ture. Parthenogenesis is literally creation "without a father" to sire the offspring.

Although Bonnet's life proved to be quite uneventful, his family's prior history was not. Originally from France, the entire family branch had had to relocate because of the shifting tides of religious persecution in the sixteenth century. By the time Charles was added to the lineage, the Bonnets were firmly established in Geneva, where both religious and scientific tolerance were the order of the day.

Originally trained in the study of law, Bonnet soon turned to areas of natural science as his main interest. Some of his earliest work built and expanded on Réaumur's gigantic compendium on insects. His later work led to distinction in the field of botany, particularly advancing the now accepted notion that plants are endowed with limited but effective powers of sensation.

In 1740, the same year that his cousin added studies of freshwater hydras to his schedule of tutoring the Dutch upper crust, Bonnet sent the Académie des Sciences a paper containing a series of experiments describing the process of parthenogenesis in a kind of insect called an aphid.

Aphids, also known as tree-lice, are well known to most gardeners today as an invasive pest. They are parasites, living by sucking the juices from plant stems and causing many a horticulturalist to reach for the bug spray or a handful of predatory ladybugs.

Bonnet had determined that while sexual reproduction did take place in the species, male participation seemed to be only an option, not a requirement. Female aphids could produce clutches of viable eggs, as far as he could tell, without having a male provide sperm for fertilization. This realization of how supposedly unbreakable natural rules could be rather easily shattered prepared him to accept Trembley's conclusions about the asexual reproduction of the hydras in water glasses.

In 1741, spurred on by his cousin's astonishing findings, Bonnet began to take a harder look at reproduction by fusion and the regeneration of lost parts in the freshwater hydra and other animals. Choosing to move up the evolutionary scale a bit, he picked mud worms to study. Again, he was able to glean abundant evidence

from his experiments that worms were consummate masters of regrowing cut tissue.

A few decades later, Bonnet heard about a startling new claim from another leading scientist of his day, Lazzaro Spallanzani. The Italian naturalist asserted that while many kinds of invertebrates (creatures without backbones) had been observed regrowing lost appendages, he had found a vertebrate who could do the same. Spallanzani's experiments had shown that a small amphibian called a salamander not only could regenerate lost limbs but could regrow a relatively complex organ like an eye.

Bonnet, who was suffering from increasingly poor vision at this point in his life, even as he continued to look into the mysteries of cellular regeneration, was fascinated by the claim. Not one to be squeamish, he rather clumsily removed the eye from a salamander as part of an experiment to verify the claim. Sure enough, in the course of a year, a completely regenerated organ replaced the missing eye.

And yet, the origin of these powers was still a mystery. Even more mysterious were the apparent limitations of regeneration. Beyond the salamander, all other complex creatures did not regenerate observable injured or missing limbs and organs. The infant science of biology would need a great deal of time before it could even come up with theories to attempt to explain the "what" and the "why" for these things.

EARLY GLIMPSE OF STEM CELL RESEARCH

When looking at the history of cellular biology, it is worth noting one more curious set of experiments performed by Abraham Trembley on his hydras. While not as attention-grabbing as his assertions of asexual development, they are much more pertinent to the modern study of stem cells and the regeneration of tissue. They also illustrate how creative a thinker and dexterous a workman he really was.

After working with his hydras for over a year, Trembley had come to realize that each organism was essentially a hollow "tube" along its trunk, with a set of arms or tentacles ringing the top. Some-

how, he managed to turn one hydra inside out, much the way an eager child will turn out a Christmas stocking in order to ensure no gifts lie hidden away in the toe. He next used this inside-out hydra to cap a tiny spike of boar's bristle. He then took the bristle and used it like a ramrod, pushing the inverted hydra down the "throat" of a second hydra.

Considering the tools Trembley was working with, this was an amazing feat of surgical skill. Pipettes and syringes, to say nothing of machines that could remove or inject cellular nuclei, were many years in the future. Yet the experiments were a success in that they further deepened the mystery of how adaptable the hydra really could be.

Instead of one hydra digesting the other, the two organism's tissues fused together, becoming a single, slightly thicker individual. Because of this, Trembley is widely considered to be the first person known to have performed a successful animal tissue graft in history. He also kicked off an entirely new line of speculation. If tissue could be grown and added back to the body without fear of rejection, the sky could be the limit in terms of replacing dead, dying, or damaged skin or organs in people.

Today's researchers know that a hydra's two layers of cells—the *endoderm*, or inner layer, as well as the *ectoderm*, or outer layer—act as stem cells. The cells perform functions, such as digestion, that help keep the entire organism alive, but they do not lose the ability to regrow into new tissue or even into entirely new organisms. It is this ability that drove the next wave of researchers to delve even deeper into the mysteries of cellular growth. And it is this ability that today's stem cell researchers prize as the holy grail of tomorrow's medicine.

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CHAPTER 3

THE FURNACE OF CREATION

In humans, after conception takes place, the resulting cells start to lose the ability to regrow into new tissue as the fetus develops. And even though every adult person continues to harbor a small but important number of stem cells within their bodies, these cells are nowhere near as multifunctional as the stem cells at the embryonic stage.

In a nutshell, not all stem cells are created equal. What separates a marginally useful stem cell from one that could produce amazing results is the degree of developmental plasticity. This "plasticity" is defined as the ability of the cell to divide for an indefinite period and develop into a wide variety of cell types. As mentioned at the end of Chapter 2, this ability is what today's stem cell researchers look to as the holy grail of tomorrow's medicine.

OUT OF THE FURNACE

The level of plasticity that a stem cell is able to retain falls under various names, the most common one being *pluripotency*. A simple but

effective way to understand what this term truly means is to visualize the stem cell's ability to create new tissue as similar to the work done with the red-hot molten globules of glass that come out of a glassmaker's furnace.

Modern glass blowing is still done in basically the same way as in medieval times. The glass blower's main tool is a hollow iron pipe about four feet long, called a *blowpipe*. The blowpipe is dipped inside the blast furnace and a small amount of molten glass is rolled onto the end. The artist then rotates the mass, also called a "gather," on the pipe against a paddle or metal plate to give it a cylindrical shape to start off.

The glassblower puffs air into the pipe, creating a bubble, then shapes the gather into whatever he or she wants, using everything from wooden paddles with holes to wet paper. Shears can be used to cut the soft glass, or the gather can be dipped into molten glass of a contrasting color. This allows the base mass of gather to pick up the properties of the second type of glass.

The parallels to what is being attempted with stem cells are striking. Just as with the molten glass fresh out of the furnace, human embryonic stem cells are pure potential with a minimum of form. Fresh, almost liquid glass can be turned into anything desired. The cells at this state are very similar in that they could potentially, with the right chemical prods, become anything wished for—liver cells, bone cells, any sort of tissue found in the human body.

By contrast, when the glass is pulled out of the furnace and worked with, the level of plasticity decreases quickly with age. As the substance cools, there is less and less that the glassblower can do with it. Shaping and blowing can still be utilized to create the final form, but there comes a point where the basic form is irrevocably fixed. The glassblower can alter the general shape and size, but the form remains fixed as a cup, or a vase, or whatever the object started out as in the artist's mind.

The same principle is at work with all kinds of human stem cells. Time rapidly reduces their plasticity. Think of a conveyor belt, moving the cells away from their own furnace of creation. They quickly begin to lose the miraculous ability to become anything needed by the body should the need arise.

The differing levels of cellular plasticity—how "molten" the cellular "glass" really is—have separate scientific classifications. These help scientists define exactly what they are looking for, and the degree of application that they can hope to wring out of the cells they are working with in their experiments.

THE ORCHID'S SECRET

Totipotency is perhaps the most precious trait for any stem cell to possess, at least from the standpoint of the developing embryo. Totipotency, as one might guess from the name, is the ability of a single cell to produce all the differentiated cells in an organism. The potential truly is "total" in that it can form any kind of body tissue.

At the moment of conception, when the sperm fertilizes the egg, a single totipotent cell is created. In the first hours after fertilization, this cell divides into identical totipotent cells. To the best of our understanding, it takes several cycles of cell division, taking three to four days, before this amazing property begins to fade.

The totipotent cells begin to specialize, and lose the ability to create other types of cells beyond what they have already begun to generate. The glass has begun to cool, and each "gather" can only become a type of one class of objects. Instead of being able to differentiate into hair, blood, bone, and organ, each specialized cell can only make variations of one type of cell.

Anyone who has spent time gardening is already familiar with the secrets of totipotency from nature. For example, taking a tiny portion of a plant, or a "cutting," can be used to grow an entire new plant. Simply taking some specialized plant cells and placing them in a growth medium—much the way modern stem cell researchers put embryonic cells into cellular medium in dishes or flasks—is actually enough to start the process.

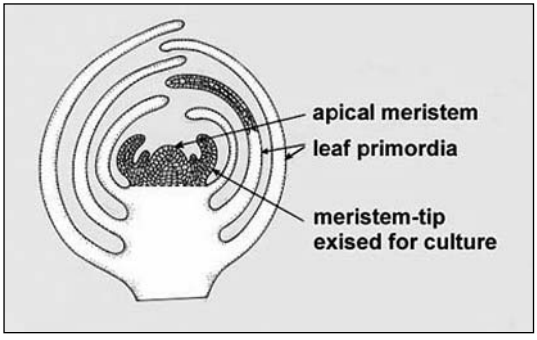
Experienced botanists and gardeners know the two main limitations of this sort of work. First, there is a great difference in how successfully the technique can be carried out. Just as with different species, which seem to have higher or lower levels of regenerative ability (say, hydras versus humans), some plant species can grow more easily from cuttings than others.

Second, the type of cell matter used in the cutting matters a great deal. Simply sticking a random leaf or piece of bark into the ground is rarely (if ever) enough to regrow a plant. Instead, certain tips of growth, such as the roots and shoots, are the prime candidates, as they contain *meristem* tissue. Meristem tissue is comprised of a type of embryonic stem cell in plants. This tissue is chock full of unspecialized, youthful cells called meristematic cells.

As these meristematic cells divide, they provide new growth for expansion of tissues, and create the basic structure of the plant's body. Each new root or shoot can go on growing for as long as the plant lives, and retains its totipotency until the end of the plant's natural lifespan.

FIGURE 3-1

A diagram of meristem tissue in plants and the meristematic cells.



Since new plants can grow from cuttings or broken plants, this can be accurately classified as a form of asexual reproduction. As a consequence, cloning of these kinds of life forms is relatively easy. The most spectacular form of this takes place in the greenhouses of orchid growers.

The flowers of tropical orchids come in a dizzying variety of shapes and a riot of colors. Yet when one sees a display of orchids in a bouquet or in a garden, the most striking characteristic is how regular and symmetrical the color patterns are on each and every plant's flower. This can be directly attributed to the process of *meristemming*, a sort of low-tech cloning that has allowed orchid growers to churn out vast numbers of genetically—and not incidentally, cosmetically—identical plants.

In meristemming, once a plant has been bred with the exact color and pattern that is most desirable, meristematic cells are taken from the growing tip of a new orchid shoot. The shoot tip, which is about the size of a stick of lead in a mechanical pencil, is

placed in a liquid growth medium containing hormones and nutrients to encourage cell growth. As the process progresses, the tiny specks of meristematic cells turn into fingernail-size green clumps. The clumps can be easily divided again to increase the number of plants under development—or they can be transplanted into the greenhouses so that they and hundreds or thousands of their clone-siblings can develop into the plants that you purchase at the florist or even the supermarket's floral section.

FIGURE 3-2
The meristemming process.

DEGREES OF PLURIPOTENCY

Cells with the ability to differentiate into the vast majority, if not 100 percent, of all cell types are called *pluripotent*. The analogy to the glassblower's furnace earlier was used to demonstrate pluripotency. This quality is the major focus of stem cell researchers for two main reasons. The first is that total cell potency is of such short duration that it is, at best, a fleeting phenomenon. Rather than spend time trying to catch lightning in a bottle, the more sensible stem cell researchers are trying to create more modest—but also more reliable—ways to capture this cellular quality.

The other reason for focusing on pluripotent stem cells is the need to develop therapeutic medicine without unduly provoking additional controversy. Pluripotent cells by definition can develop into almost any bodily tissue. However—and this is key—they cannot themselves develop into a human being. Support for at least some forms of stem cell research has consistently been high, but the attitude toward cloning human beings—or even having the technology to do so—has consistently been strongly negative.

Pluripotent stem cells are the primary choice for medical researchers seeking to develop therapies because these cells can develop

into any of the three primary tissue types listed below (see also Figure 3-3).

1. *The endoderm, or the innermost layer of the embryo*. The tissue types here include the interior gut lining, gastrointestinal tract, lungs, and most of the internal organs.

2. *The mesoderm, or middle layer*. Types here develop into the tissues in the muscle, bone, marrow, and blood.

3. *The ectoderm, or outermost layer*. The ectoderm is comprised mostly of epidermal tissues and portions of the nervous system.

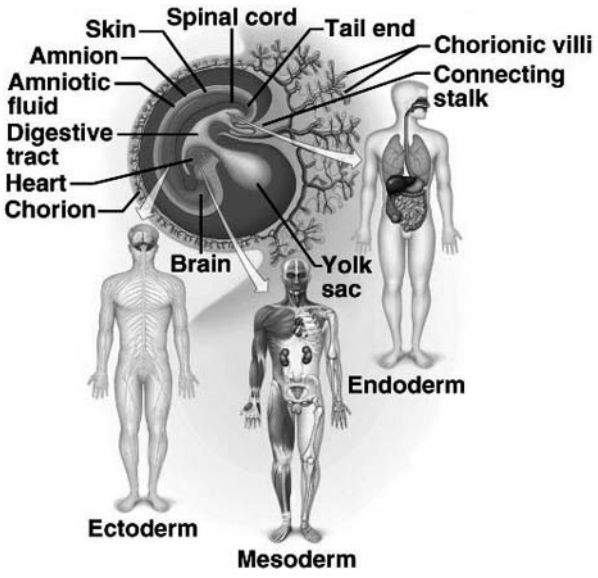
Pluripotency is not only a classification of a stem cell, but also a characteristic that varies depending on the age and culture of the cell. A stem cell with a higher degree of pluripotency is a better candidate to develop into more types of tissue than a cell with a relatively low degree of pluripotency. Studies are being done to see whether the degree of a cell's pluripotency can be increased. In other words, researchers want to see whether they can follow the lead of the glassblower, who can re-assert control of their work by placing the glass on the end of their blowpipe back into the furnace.

IN THE BLOOD AND UNDER THE SKIN

The further down the scale of potency one goes, the more "set" the cells become and the cooler the glass is from the furnace. The next type of cell that medicine is interested in is the *multipotent* cell. Multipotent stem cells can give rise to several other cell types, but those types are limited in number. Out of the multiple cell divisions that formed the embryo, these cells are terminally differentiated—in other words, permanently committed to a specific function.

Hematopoietic cells, which are stem cells found in the blood, are the classic example of the multipotent cell. Hematopoietic cells have the ability to develop into several types of blood cells. However, being terminally differentiated cells, they cannot form liver cells, kid-

FIGURE 3-3
How the embryo develops into the three primary tissue types.



ney cells, nerve cells, or any other kind of cell except for a select few kinds of blood cells. These kinds of multipotent cells have given the best results for developing the start of stem-cell-based therapies that will save lives.[1]

Until very recently, it was thought impossible for multipotent cells to revert to pluripotency at all—the glass had been out of the fire too long and was permanently set in place. The latest research, however, indicates that this barrier might actually not be permanent, and that multipotent cells could behave in ways other than how they have already been committed. Experiments have been able to reprogram or "regress" the potency level so that blood stem cells have started to behave like neurons, or brain cells.

Finally, the type of stem cells with the lowest ability to differentiate into different types is the *unipotent* cell. According to cell

1. The medical therapies involving multipotent and unipotent stem cells are detailed in Chapters 13, 16, and 17.

biology, the term literally means that the cell has the capacity to develop/differentiate into only one type of tissue/cell type. And yet even with this limitation, unipotent cells have proven to have great potential in treating certain kinds of injuries.

The most common unipotent stem cell in the human body are the skin cells hidden in the epithelium. The epithelium is the outermost tissue layer, which on the top is comprised of dead squamous epithelial cells, as are the mucous membranes lining the inside of our mouths, nose, and other body cavities.

A company called BioSurface Technology was the first company in the world to generate and sell human skin tissue for therapeutic use. Based in Cambridge, Massachusetts, BioSurface manufactures and sells epidermal skin grafts. By gathering and growing a patient's own skin stem cells—taken from the undamaged portion of a burn victim's body, for example—transplantable sheets of skin can be created.

The technology still has limitations, the primary one being that it can take several weeks' worth of time to grow a decent sized sheet that is a fragile three cells' thickness. But BioSurface has proven that given the right conditions, unipotent stem cells will grow happily, and *en masse*, providing a critical option for treating injuries. Future experimentation in areas such as growing cartilage also provides a great deal of hope for those who may have suffered sports injuries to the knee or elbow.

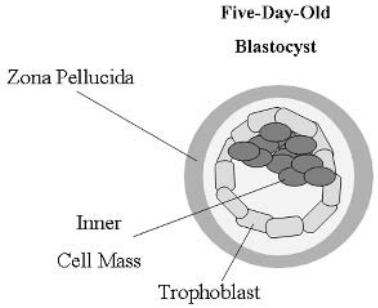
WHERE THE POTENT CELLS ARE

The final characteristic that separates the different types of stem cells is where they can be found, or harvested, if that is the purpose of the research or therapy. In the case of totipotent or pluripotent stem cells, there is only one place they can be found—the inner cell mass of a blastocyst.

A *blastocyst* is a sort of hollow ball of cells that is created from the initial fertilization of the egg. The fertilized egg, which is a single cell called a *zygote*, begins dividing into multiple cells with the elegant precision of a fine Swiss clock. By the time the number of cells reaches 40 to 150, the mass of cells has shaped itself into a hollow sphere, rather like a beach ball. The center is a

fluid-filled cavity and the inner cell mass, which is also called the *embryoblast*, clings to the inner wall of the sphere. It is these undifferentiated cells which are the source of embryonic stem cells.

FIGURE 3-4
Simple diagram of a blastocyst.



Once the cells that make up the embryoblast are removed to be placed in culture for research or medical purposes, they automatically change from totipotent to pluripotent cells. This is because the disruption in being placed in vitro prevents the cells from fulfilling the definition of the totipotent cell—the ability to grow naturally into a duplicate of the entire organism. Instead, the cells retain the ability to grow into a huge number of cell types—a high degree of pluripotency—and, in the biological sense, retain a certain kind of immortality, dividing and proliferating indefinitely in the growth medium.

Adult stem cells, on the other hand, are found in varying degrees—and different levels of multipotency and unipotency—in several different parts of the body.

- *Brain Cells*: Stem cells found in the brain do differentiate into three kinds of nerve tissue, though compared to other kinds of cells this differentiation is both slower and more rarely observed.
- *Bone Marrow Cells*: The hematopoietic cells that differentiate into multiple kinds of blood cells are found in the marrow, and are probably the best example of a multipotent cell.
- *Digestive System Cells*: The stem cells in this area are similar to the skin cells in that they make up, replace, and repair the lining of the gut.
- *Endothelium Cells*: Known to differentiate into tissues that make up the linings of blood vessels. This has proven

crucial to developing therapies that could help treat heart disease.

- *Liver Cells*: Repairs to the body's main filter seem to be done by certain stem-cell-like cells in this organ, though the bone marrow seems to be a likely candidate as well.
- *Pancreas Cells*: Believed to exist but not conclusively proven. As with the liver, it appears that repairs to a damaged pancreas are done via a stem cell agent within the organ itself.
- *Skeletal Muscle Cells*: Responsible for muscle growth and repair, and found both in the muscle tissue itself and in the marrow.
- *Skin Cells*: The prime example of the unipotent cell, which can differentiate strictly into skin cells and no other kind.

While the existence of adult stem cells is important for developing many of the new techniques to repair tissue and seal wounds, it must be emphasized that their lower degree of plasticity makes them much less useful. Their multipotency and unipotency qualities, as opposed to pluripotent embryonic stem cells, by definition limit their use.

Yet whether adult or embryonic cells are used, science still grapples with two heavy barriers to making therapies effective and widely available. To begin with, it's the biological equivalent of hunting for a needle in a haystack to find the rare stem cell in a solution of marrow or skin cells.

Second, it does the patient no good if injecting stem cells leads to the formation of cancerous teratomas. Ironically, it was the very study of these ugly cellular formations that led the way to identify and use stem cells in a way that stands to change medicine forever.

CHAPTER 4

THE UGLIEST THING IN MEDICINE

When you really get down to the core of cellular development, things start to get more than a little spooky.

It may sound unscientific to say so, but to the casual—and not so casual—observers and researchers studying cell biology, there is one fact that can send a shiver down their spines. That is, at the level of the individual cell, normal reproduction and the wildly malignant growth of types of cancer are separated by the smallest shades of gray. The right set of genetic "switches" flipped into the wrong position moves us away from a cell's normal aging process and into an alien universe. Like creatures from a bad horror film, these cells simply *refuse to die*, and eventually take over and consume the host organism.

THE MONSTER INSIDE

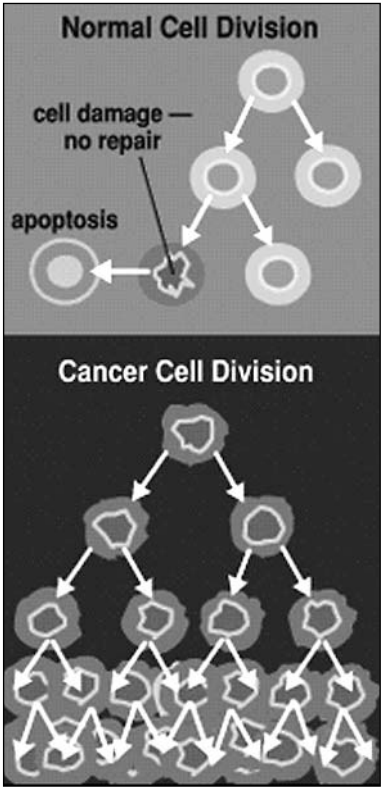
Cancer can be thought of as a disease caused by the uncontrolled division of the body's own cells and the ability of these cells to invade other tissues. This uncontrolled growth is caused by damage

to a cell's unique blueprint, its nucleic DNA. Damage to the DNA can lead to a change, or mutation, in the DNA itself.

Genetic damage can have many causes. It may result from a casual and nonthreatening source, such as exposure to the sun's UV radiation while tanning on the beach. It can also be caused by a terrifying event, like exposure to radiation from nuclear waste. At its most insidious, this damage can come from carcinogens, physical agents in what we eat or drink, or from harmful viruses whose reproductive strategy is to insert their DNA into the human genome.

Luckily, it seems that a great many mutations must take place before a normal cell is transformed into a malignant one. All of our body's cells have self-contained instructions to repair DNA. In fact, much as NASA always has a self-destruct charge on its rockets in case the launch goes horribly off-course, each cell has its own processes to destroy itself in case of severe genetic damage.

FIGURE 4-1
Normal cell growth and death versus cancerous growth.



This form of programmed cell death is called *apoptosis*. Apoptosis is carried out in an ordered process that is part of an organism's normal developmental cycle. For example, when a human embryo develops, the fingers do not grow outward from the palm. Instead, apoptosis takes place and cells slough off in strips between where the fingers will emerge from the pad-like "hand".

Only the mutations that encourage unrestricted growth and repress the genes for programmed cell death eventually lead to cancer.

The word *tumor* can be frightening to hear, but in medical terminology it simply refers to a swelling

or lump of tissue. Through popular usage, the term has been irrevocably linked in the public's mind to the malignant (cancerous) or benign (noncancerous) growth of tissue. We're interested here in a specific type of tumor that derives from pluripotent germ cells; it's known as a *teratoma*, which comes from Greek for "monster tumor."

Teratomas arise only in certain types of cellular tissue: the testes in men or the ovaries in women.[1] Teratomas make up an incredibly small fraction of all tumors. The majority are benign, and found in the female ovaries—perhaps remnants of eggs that failed to develop beyond a certain stage and so never were properly expelled into the fallopian tubes and onward. A few are found in the male testes, where they can develop into fatal testicular cancer.

A GHASTLY MIXTURE OF CELLS

Like an embryo, a teratoma produces stem cells. But a teratoma is unable, whether due to genetic malfunction or lack of the proper chemical and growth signals, to create a fully integrated organism or even a single type of cell or organ. The pluripotent potential is squandered and goes horribly off key, like a lump of wet clay that has spun off a clumsy potter's wheel and splatters on the floor.

In clinical terms, teratomas can and do contain "well-differentiated" cells. The tissues that grow within a teratoma can be "somewhat different than that of the surrounding tissue," according to medical texts. This is an extremely polite way of describing what Dr. William Hurlbut, member of the President's Council on Bioethics, has called "about the ugliest thing in medicine."

Teratomas grow into ghastly, irregularly shaped balls of tissue. Extremely well developed ones look something like the encapsulated remains of an airplane crash victim who has been run through a blender. They are full of bits and pieces of what would have been a body, replete with vestigial pieces of limbs or strips of bone and ligament. Even the more common teratomas have been known to

1. The medically correct term for multiple teratoma is actually *teratomata*, but to avoid confusion, we'll use the more popular form of a plural noun.

contain bits of skin, nerve tissue, body fat, and muscle tissue. Disturbingly, some of these jumbled bags of cells contain hair follicles—some of which actually can and do grow hair.

A few ovarian teratomas have been observed with clusters of fully developed teeth. The teeth do not root to any hard, jaw-like surface. But they nevertheless seem to derive proper nourishment from the blood vessels surrounding them and mature as recognizable incisors or molars designed for grinding food. The fact that they are buried deep within a woman's pelvis does not seem to deter their growth in any way whatsoever.

The existence of teratomas induces the same kind of queasiness as the idea of cancer itself, and for a similar reason. Just as cancer and stem cells make us aware of how thin the line is between reproduction and deadly disease, teratomas make us aware of how invisible the boundaries are between the normal and the abnormal. And how revolting the results can be if the engines of life continue to rumble along when the blueprints are smudged.

OF MICE AND MUTATIONS

Teratomas play into the story of stem cell research starting in the early 1950s. Dr. Leroy Stevens, an embryologist at the Roscoe B. Jackson Memorial Laboratory, had a combination of lucky breaks mixed with keen scientific insight that pointed the way toward modern day stem cell research. These insights came from a most unlikely place—teratomas—and from an equally unlikely source—testicular growths in the lowly laboratory mouse.

In the centuries after the amazing discoveries of Abraham Trembley and his manipulation of freshwater hydras, several theories had come and gone regarding the basis of cellular regeneration of limbs and organs. The primary theory that persisted was that in more complex animals it was the cells in the early embryo that held the key. This made logical sense. After all, the different types of cells and organs arose from the fertilized, single-celled egg, through the ball-shaped blastocyst and so on into the mature organism. If the embryo was the source of a tissue's generation, it could potentially be the source of its regeneration.

The first clue toward the proof or debunking of this theory did not come until late 1953, and it happened largely by chance. Dr. Stevens had been working on a research project to look for physiological differences between lab mice that might provide a clue as to which defects could be attributed to certain genes. As the junior researcher, he was the one who actually handled the mice.

Had Stevens been higher up in the pecking order of the laboratory, he might not have been the one who spotted the enlarged scrotum on one mouse. The specimen was dissected and one of Steven's colleagues confirmed that the enlargement in question was not the result of a viral or bacteriological infection. It was that cellular rarity, a full-blown germ-cell teratoma.

As mentioned, teratomas are quite rare. This is to be expected given the high levels of reliability and self-checking in the cellular reproductive cycle. These self-checks are a good thing, as otherwise multicellular animals would be unable to exist for long without unchecked cell growth ravaging the elegant organization of their systems.

An inherited form of teratoma is even rarer, yet that is exactly what Stevens found. A small but statistically significant number of male mice with teratomas were derived from a single strain of mouse. It was indeed the strain of mouse that he had happened to be working with that very day, where he had happened to be the researcher. Chance had indeed smiled on the field of embryology that day.

While less than 1 percent of the animals had a teratoma, it took a great deal of patient and mind-bogglingly tedious laboratory work to reach this conclusion. Autopsies of literally hundreds of mice in this particular strain had shown that several of the mice carried the exact same type of testicular cellular mishmash. The source of the teratomas in this strain, generation after generation, pointed conclusively to a genetic cause: a defective gene that continued to be passed on again and again.

Stevens figured that he could discover the cause of a teratoma's formation by going as far back to its source as possible—the developing mouse embryo. If that could be done, then maybe—just maybe—he could glean from the data the biological cause of the tumor itself.

In order to determine where in the developing mouse embryo the teratoma would first appear, Stevens had to inspect testicular tissue from fetal mice. The next phase of his research would do just that, slowly peeling back the layers of impossibly fine tissue in younger and younger subjects. But he soon ran into a problem—the statistically low yield of teratomas (again, less than one in a hundred) meant that running the tests he wanted would require a Herculean amount of effort. To gather enough teratomas to make a solid conclusion about any of his theories meant committing to dissect literally thousands—or tens of thousands—of lab mouse fetuses.

But Stevens was up to the task. Over the course of two years, he inspected tissue from thousands of mice. One minor break was the discovery of a substrain of mice which increased the number of teratomas in the population from 1 percent to a grand total of 2 percent. Progress to be sure, but also proof positive of the platitude that "the doubling of close to nothing results in . . . close to nothing." Even with the increased number of teratomas to inspect, the work was moving slowly enough that Stevens was concerned about keeping his funding from the American Cancer Society.

BREAKS AND BREAKTHROUGHS

In 1961, Stevens made a major breakthrough in increasing the size of his teratoma samples. In the past, he had tried unsuccessfully to create a higher incidence of teratomas in his mice by all sorts of methods. Feeding them substances with carcinogenic or mutagenic properties. Bombarding them with radiation.

Nothing had ever worked—until several generations of cross-breeding different strains of mice with the original strain that was susceptible to teratomas fixed the defect permanently at a high rate of thirty percent. This was a fifteen-fold increase in Steven's sample size, which finally allowed him to move to exclusively studying the teratoma itself.

The next big break came in 1964. While dissecting a 12-day-old mouse embryo, a single germ cell was spotted as it began to differ-

entiate—but not into a sperm cell, which is what its genetic blueprint should have read. Stevens and his team of researchers had been the first to trace a cancerous process back to its very start.

Like an overzealous architect, the germ cell began its woeful try to build something far beyond its abilities. It was trying to become a full grown embryo, dividing far too many times for its environment and capabilities. And yet, these cells, showing their talent for pluripotency—if not organization and restraint—were able to show researchers a brand new truth: only some of the cells in the horrid stewpot of tissues called a teratoma were to blame in causing the growth at all.

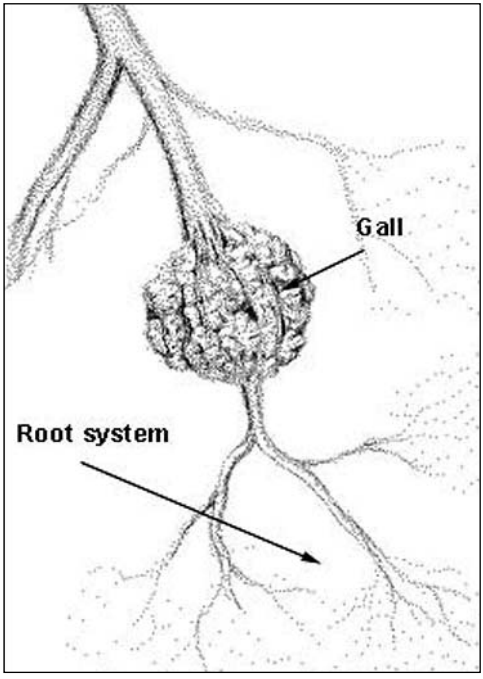
Stevens helped move this understanding along with a series of transplant experiments involving the misplacement of wayward germ cells and mouse embryos. First, he moved tissue from the genital ridges of the fetal mice containing no germ cells into adult mice. Teratomas did not form. However, transplanting entire mouse embryos into adult mice—specifically, into areas outside of the womb, where the embryos would have a familiar environment—frequently turned into cancerous growths like teratomas. Clearly, there was some kind of growth control mechanism that failed in the cells that turned into teratomas, and that mechanism was affected by the local cellular environment.

This research was taken one step further when experiments were undertaken to induce new teratomas in healthy mice by implanting a single cell from an existing teratoma. These experiments proved that not all cells that formed in a teratoma—stem cells and mature cells—were malignant, contributing to its continued growth. The mature cells, though horribly misplaced as hair, bone, or teeth in the wrong organ, did not grow in an unchecked manner. It was the undifferentiated, pluripotent stem cells that continued to operate in the mode of reckless growth. Once differentiation took place, growth slowed and reverted into a benign state.

CROWN GALL

The orthodoxy up to that time was that cells were relatively fixed in their orientation. Normal cells would continue to live and die under

FIGURE 4-2
Crown gall growth on plant roots. The spherical shape containing the jumbled, immature cells closely follows the pattern of animal teratomas.



properly programmed cellular mechanisms, while abnormal, cancerous ones would never return to a normal state. Parallel research on plants had shown something different: cells could shift states from the abnormal to the normal, and vice versa.

The work in question took place in the first decade of the twentieth century. Botanists had been studying a disfiguring plant disease known as *crown gall*. Crown galls are large, tumor-like growths that protrude from the stems, branches, or roots of many infected plants. Crown gall disease makes plants grow poorly, thus impacting the production of vital crops such as fruit trees, grape vines, and berry canes.

In 1907, scientists at the U.S. Department of Agriculture discovered that the cause of crown galls was a rod-shaped soil bacterium *Agrobacterium tumefaciens*. This was a fascinating discovery, because the plant "tumors" were not developed by the bacteria itself but by a strange loosening of the bonds in the plant cells that told them when to stop dividing into stem or root cells.

Forty years later, plant pathologist Armin Braun, of Rockefeller University, found that crown galls, unlike normal plant tissue, didn't need normal growth supplements. Instead, the cells' internal mechanisms had been altered to grow quite happily on a simple medium of salts and sugars. The cells themselves had changed from being a specific tissue into a messy conglomeration of immature and mature cells—in other words, a vegetable version of the animal teratoma.

Braun concluded that the plant cells had been somehow been permanently transformed into these tumor-like cells by the *A. tumefaciens* bacteria.

By the 1960s, Braun had made a final discovery whose impact rippled over into the study of stem cells. He began by using a strain of tobacco plants afflicted with crown gall disease. Although the plants contained the tumor-like growths, Braun made sure that they were free of the colonies of *A. tumefaciens* bacteria. Finally, he grafted the galls to normal plant tissue and watched over the period of weeks and months while the graft took hold.

To Braun's surprise, once the grafts were in place, the cells in the crown gall swelling retuned to normal function. The gall itself did not shrink like a deflating balloon, for the cells remained where they had grown, but the cells inside again grew at normal rates and took to normal growth medium. It was proof positive that, at least in the plant kingdom, that it was possible for cells to be switched back from a malignant state to a normal one.[2]

With the idea in mind that cellular states were not static, researchers in the United States and Great Britain performed a series of experiments in the 1970s by injecting a teratoma's cells into normal mouse embryos. Most of the time, the cells reacted to their new surroundings by turning into normally developing cells that contributed to the development of the embryo. This proved beyond the shadow of a doubt that in animals as well as plants, the immature stem cell could shift states from the abnormal to the normal, and vice versa.[3]

Life at the cellular level was proving to be more flexible, more easily shaped and molded, than was thought even a few decades before.

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2. In the mid 1970s, scientists showed that some of the bacterium's genes were transferred into the chromosomes of the plant cells, which induced the cells to go cancerous and continually divide until galls developed. A daring idea was developed off this discovery: If bacteria can introduce foreign genes that cause disease, why couldn't scientists use the bacteria as little messengers to deliver desirable genes into the plant's DNA? This was the "germ" of a lot of modern genetic therapy, some of which is discussed in Chapter 19. It's an excellent example of how discoveries in one area of science can "ripple" into others.

3. Some saw the possibility of stopping certain kinds of cancer, by using chemical or cellular processes to shunt an abnormal cell towards benign maturity. This field, known as "differentiation therapy" is still being researched today.

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CHAPTER 5

THE STARTER CULTURE

The identification and growth of animal stem cells continued apace, but by the time the 1980s had arrived, only adult human stem cells had been identified and collected. The most obvious obstacles to working on human embryonic cells were the delicate political and cultural mores that surrounded the research. It wasn't until the advent of widespread IVF (in vitro fertilization) technology that this last barrier was—at least partially—breached.

IVF is a fertility treatment that collects sperm and egg from the parents and fuses them "in vitro"—that is, in a petri dish. While this offered a wonderful opportunity for infertile couples to achieve their dream of having a child, the success rate was still relatively low. Only between 5 to 10 percent of the embryos so created survived implantation and made it to birth.

These percentages required clinics—ever mindful of the need for a high success rate to attract clients—to create at least a dozen embryos for each potential mother. Once the mother conceived, the remaining embryos, each with its potential for future life, were frozen

and held in case a sibling was desired. Of course, this led to a relative "oversupply" of embryos, since not every couple wanted to use every single fertilized egg. Some argued that this was a serendipitous development, leading to a biogenetic stockpile that was simply going to waste.

Once past their useful life—or if the donors were sure they wanted no more children—the cells would in all likelihood be incinerated, as with all types of medical refuse.[1] This was an outrage to scientists. With such a supply of totipotent cells available, it was inevitable that researchers would want to use them to advance their knowledge.

IN THE CITY OF THE LION

The first attempt to isolate human stem cells and keep them alive without differentiating into either a fetus or petering out into a jumbled mass like a teratoma took place in metropolitan Southeast Asia. The National University Hospital of Singapore was the right place for the experiment. Located adjacent to the university in a pleasant-looking cubist building, the hospital is a medical school and one of the premier research centers in Asia.

Dr. Ariff Bongso was the right person at the hospital to make the effort. An astonishingly intuitive man who had started out in the field of veterinary science, he had become involved with IVF work in the early 1980s. It was the dedication of researchers like Bongso that has helped make the technology commercially feasible across the globe.

In the early 1990s, he achieved international scientific acclaim by growing IVF embryos on a bed of fallopian tube cells. This improved the embryo's sustainability and increased the odds that the embryos would survive the traumatic transplantation procedure. His extensive work with the newly created human embryos convinced Bongso that he could create lines of cells that could be grown into tissues at a later date for treating disease.

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1. While the amount of time that human embryos can be stored and still retain maximum viability is a matter of some debate, most countries set the maximum storage time at ten years.

FIGURE 5-1
Embryos are stored in special vats kept at supercold temperatures with liquid nitrogen.

Dr. Bongso realized that he would need to complete two tasks in order to reach his goal. First, he would have to figure out how to isolate the stem cells hidden away in the blastocyst's inner cell mass. The second, more daunting task would be to sustain the cells' divisions in a growth culture without their differentiating.

He made his attempt in 1994, performing brilliantly at the first attempt and coming to the brink of success on the second. Once he received permission from the hospital's bioethics committee, he obtained twenty-one "surplus" embryos that had been frozen in vats of liquid nitrogen. The embryos came from nine different patients who, having conceived the number of children they desired through the IVF process, had no further use for the fertilized cells.

OUT OF THE DEEP FREEZE

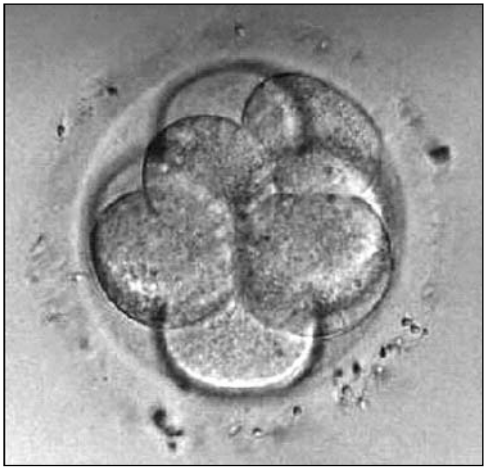
Embryos removed from the cellular "deep freeze" are allowed to thaw naturally, until they come to room temperature. Typically, the embryos are then steeped in various solutions—which ones are used varies slightly depending on the clinic—to help remove any cryoprotectants used during the initial freezing process. Once the embryos were thawed and "washed," Bongso warmed them to

human body temperature and mixed them with a small amount of culture medium.[2]

The embryos he was using were in an extremely primitive stage of development. They were simple six or eight cell clusters that hung together like a round clump of grapes, until they began to assume the hollow ball-like shape of the blastocyst. Bongso let the cells grow up until the day-5 blastocyst stage, then introduced an enzyme into the medium to separate the inner mass of stem cells from the rest of the sphere of cells.

FIGURE 5-2

The simple multicellular embryo. It is a simple cluster of cells here, before the blastocyst begins to form.



The enzyme Bongso used dissolved the gelatinous protein coating on the outer coating of the blastocyst. This allowed him to gently tease the inner mass free and suck it into a pipette. The process would be rather like dissolving the seams of an inflated beach ball and, once it is deflated, carefully plucking the valve stem from the scraps of plastic. Bongso's first phase had gone flawlessly, and he placed both the inner and outer cell masses into growth medium with feeder cells to see how they would react.

Both types of cell survived and thrived in the growth medium. But success was fleeting, and substantial doubt remains as to whether the cells were, in fact, true "stem cells." (Bongso hedged his bets by calling them "stem-cell like" in his report.) Although he made multiple attempts, the cultured cells would last only a

2. There are several types of growth medium used for plating human cells for cultures. The selection of mediums gets much more intricate and detailed when one tries to encourage the growth of specific types of mature cells that differentiate from the initial stem cells.

short time before they began to spontaneously differentiate. In other words, though the cells survived, whatever genetic trick kept them "naïve" enough to remain pluripotent still remained to be discovered.

RAISING THE BAR

Ariff Bongso's experiments had come to the very threshold of world-changing success. Much of what he had discovered quickly became established as standard procedure. For example, his experiments reinforced what was becoming widely acknowledged: That it was a relatively simple matter to physically or chemically separate cells from blastocysts. Also, while the pluripotency of the extracted cells was never verified, the fact that the cultured cells had been kept alive through multiple divisions raised the bar for the rest of the scientific community.

The next advance was to take place on the other side of the Pacific, at the University of Wisconsin's primate research center. In 1993, Dr. James Thomson's research team had managed to successfully flush embryos from the reproductive tracts of pregnant rhesus monkeys. Within a year and a half—roughly the same time that Bongso was performing his work in Singapore, Thomson had been able to isolate stem cells from these embryos and keep them growing in a special cell culture that he had invented. Yet this was not the full extent of the success.

Thomson's team had managed to take the next step forward. They had been able to keep the cells happy and dividing—without differentiating and losing the cellular plasticity that was needed in a pluripotent stem cell. The little cell cultures had been quietly persisting for over a full year with no loss of pluripotency.

This amazing feat was announced in the late summer of 1995, in a blaze of press releases that generated . . . almost no media interest at all. The subject was abstract, and did not relate to people so much as to lab monkeys (at least to the press corps). In addition, the news on the plasticity of monkey stem cells had to compete with the much more heralded launch of Bill Gates' Windows 95 operating system, the first of the true "killer applications"

that Silicon Valley would start churning out as the dot-com days began in earnest.

The ability of the stem cell researchers to catch the public eye would drastically change in the ensuing years. What was apparent to the researchers in the field—that a monkey's embryonic development ran almost completely parallel to a human's—would soon become apparent to even the most jaded journalists.

Thomson was certain that stem cells were the key to the twin wonders of therapeutic cloning and "transplantation" medicine. Creating a steady-state source of stem cells that could be switched on to differentiate on demand would allow a doctor to replace damaged or diseased tissue with brand new, mature cells. And yet, due to the increasing debate over all sorts of related events—abortion, human cloning, and genetic testing just for starters—there were few scientists lining up for the challenge.

Thomson was practically the only person with the right know-how to push the envelope out a little further. At first, it seemed that the effort might founder from lack of funding. His application for funds had been turned down by the university, not on scientific grounds but over concerns that the research would attract controversy and negative attention like a lightning rod.

This was a legitimate concern for a public institution, which not only had to eventually answer to the taxpayers but also to alumni. Thomson himself appeared to recognize the controversy that could engulf the project. Not wishing to be subject to funding restrictions from alumni who might disagree with his work, he hadn't even applied for funds from the alumni research foundation.

But fate had a way of smiling on Thomson. With private funds provided by Geron, a newly formed biotechnology company located in California, he would be able to pursue the creation of human stem cell lines. Thomson's project was approved of by the university's health sciences board. It was a politically astute decision. The board was apparently willing to back the research and weather the controversy so long as the funding was not coming from the university, thereby allowing the institution to "keep its hands clean."

Thomson's team dove into the work as soon as funding was as-

sured. Internationally, the race to isolate and culture human stem cells was gathering steam. Though the national press seemed slow to catch on, for the past decade scientists in several countries had been pushing to be the first.

Aside from the national prestige of being first—not to mention the acclaim from fellow scientists around the world—the ultimate vision appeared to be less of a mirage or a far-off dream and more of a possibility. The vision was, of course, the ability to develop customized strategies for treating diseases ranging from diabetes to Parkinson's disease.

PRE-NEURAL ONLY

Thomson obtained his cells from the "surplus" human embryos left over from IVF clinics. They had been produced in a laboratory dish to assist couples having fertility problems and were left over after successful clinical procedures resulted in pregnancy. In cooperation with the university's department of obstetrics and gynecology, the embryos were donated specifically for this project with the informed, written consent of the clinic's patients.

The embryos were extremely young. Of the three dozen embryos used by the research team, 22 were nothing more than a six- or eight-cell clump. The remaining 14 were the "oldest," but only in the relative sense, since they dated from "Day 5" of blastocyst development. They were the exact same types of extremely young embryonic cells that Ariff Bongso had used one year earlier, in 1994, on the opposite side of the globe in Singapore. This wasn't purely by coincidence.

Thomson believed that the research he was doing was right, but he was concerned with reducing the amount of controversy that his work would cause. He mandated that he would only use cells from this stage of the blastocyst's development. There were two strong reasons for doing so.

First, Thomson wanted to avoid coming close to the fourteenth day of embryonic development. It was accepted that in the early human embryo, pre-neural pathways only began to form at this point. To call the differentiating cells "nerves" at this point is a gross

exaggeration. Essentially, this was when the very first nerve cells were starting to "hook up" into the barest silhouette of a nervous network. But Thomson wisely wanted to ward off any argument that his work could cause an embryo the sensation of "pain."

Second, Thomson's idea had been paralleled in a separate conclusion by the 1994 Embryo Research Panel of the National Institutes of Health (NIH). Specifically, they concluded that the "pre-implantation" embryo (which was defined as an embryo aged days 1 through 7) did not have the ability to feel pain. Additionally, since the pre-implantation embryo had such a naturally high mortality at this stage, it was felt that while the embryo "warrants serious moral consideration as a developing form of human life, it does not have the same moral status as infants and children."

EMBRYOID BODIES

Much as with Bongso's work in Singapore, the inner cell mass of the blastocyst was removed and placed in a culture dish containing a growth medium and a layer of feeder cells. The cells were allowed to grow for 10 to 14 days, then transferred to plates that contained growth media but no feeder cells. This method prevents cells from adhering to a surface to form the typical colony growth and instead encourages them to form what is called an "embryoid body."

Embryoid bodies are not embryos that can develop into a fetus. Instead, they are aggregates of cells that typically form a ball or, at most, a hollow sphere somewhat like a blastocyst. The structure of an embryoid body is such that it is relatively easy for a researcher to collect cells off of its edge to plate out and test for the degree of pluripotency.

Thomson's research team was able to induce the cells to differentiate, but only to a very limited extent. They confirmed that the cells retained pluripotency for a solid half year when they transplanted the cellular clusters into mice and teratomas resulted.

In the fall of 1998, Thomson's team went public with the news when he reported his stem cell feat in the journal *Science*. They had established five independent cell lines, known as lines H1, H7, H9,

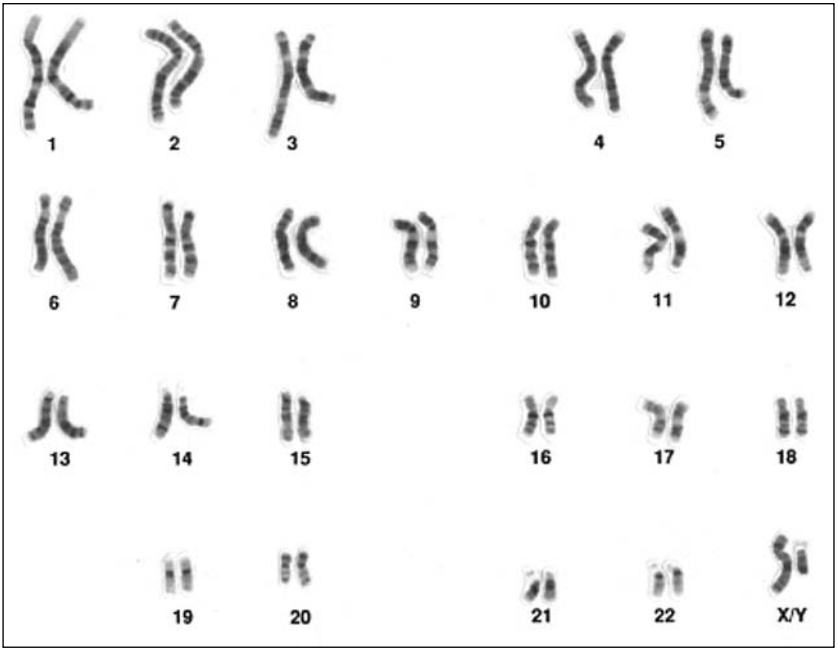
H13, and H14. The team had been able to keep the cells continuously growing in culture without losing their pluripotent nature.

Furthermore, they had taken offshoots of the original culture and allowed them to differentiate into the primary germ lines that make up the body. By the following year, the team had shown that their stem cells could subsequently develop into arrays of tissues like nerves, cartilage, bone, gut, and muscle.

Most importantly, at least for medical applications, the five lines created proved to be able to maintain a normal set of chromosomes, or *karyotype*. Karyotypes are typically examined by scientists in search of chromosomal aberrations. Cells with damaged or broken chromosomes generally have serious problems with growth and development, and their use in medical therapies would have been very limited.

FIGURE 5-3

A photograph of a typical, normal male karyotype.



LIKE PAMPERED PASHAS

Fast forward to the present and it turns out that the descendents of Thomson's original stem cell lines are still going strong. They grow into the millions on their culture plates, where small sections are nicked off and placed into other vessels for growth or experimentation. The most "successful" of the lines created by Thomson's team was H9, which turned out to be the most prolific of the initial stem cell lines. At the same time, it also turned out to be the most stable—in the sense that it retained pluripotency. H9 has become one of the more commonly used lines for experiments around the world involving stem cells.

The University of Wisconsin at Madison has become the national center for those who are utilizing public funds to study stem cells. A three-day course is taught on how to isolate and care for the fragile cell colonies. The classes are full, since these lines are approved under the current research funding restrictions.

The work is arduous. It turns out that the easiest way to keep the cell lines flourishing is to allow them to grow on a bed of embryonic mouse cells.[3] Five to six solid hours are spent by the visiting scientists in dissecting a pregnant mouse, removing the cherry-pit sized uterus, and prying loose the tiny embryos inside. The cells from these embryos must be washed, then physically and chemically torn apart to create a rich red carpet, upon which the human cells recline (as *National Geographic* put it), "like little pampered pashas."

Caring for the human stem cells requires a demanding schedule. They must be given fresh nutrients on a daily basis, split in half at least once a week to prevent overcrowding, and most importantly, their carpet of mouse cells must be replaced once a fortnight. Rather like in some bizarre fairy tale, if these conditions are not met, the cells either shrivel and die, or their genetic legacy turns them into teratoma-like masses that lose pluripotency and any usefulness whatsoever.

It appears that the hard work may pay off, at least for Geron,

3. Oddly enough, it is still unclear why this is the case. It is possible that the mouse embryo cells exude some chemical that allows the human stem cells to continue to grow happily without permanently differentiating into adult cells.

the California company that initially sponsored Thomson's work. According to the company's president, Tom Okarma, Geron has worked out a way to grow uniform batches of daughter cells from their master batch. And their master batch is itself a descendant of the first cell lines created by Thomson in Wisconsin all those years ago.

This consistency will be the key to creating cellular technologies that will allow Geron to dominate the market when the first stem cell therapies are approved for mass use, according to Okarma. As he said in a recent interview, "Why do you think San Francisco sourdough bread is so successful? They've got a reliable sourdough culture, and they stick with it."

The analogy of human stem cells being akin to the yeast-based starter culture for bread is not too far off the mark. Sourdough bread is made by using dough that is impregnated with the yeast culture that gives the bread its distinctive taste. Taking a little pinch off this so-called "starter dough" and mixing it with new flour and water allows one to create a fresh loaf.

The remaining starter dough is saved to use as the starter next time. Much as with the human colonies of stem cells, the yeasts in starter dough like *Lactobacillus sanfrancisco* have their own demands. The culture must be "fed" fresh flour and water on a regular basis, and if not divided to use in making fresh loaves, the colony can quickly die of overcrowding.

This comparison of human cells and yeast culture may be overly simplified—and perhaps a little disturbing to some. But the comparison does illustrate that at the cellular level, life has similar demands when creating its own unique miracles. Thomson himself may have said it best when he noted that the work being done "shows you can derive and culture these cells, and it opens the possibility for some dramatic new transplantation therapies."

His faith in his team's work is very much justified, and he is confident that his lines will be pivotal in shaping the world to come. As Thomson put it simply in a 1998 interview, "I believe that in the long run [stem cells] will revolutionize many aspects of transplantation medicine." Given the run of luck the field has enjoyed so far since the 1950s, it would be difficult to wager against it.

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PART II

THE RACE TO HARNESS THE POWER OF LIFE

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CHAPTER 6

CALIFORNIA DREAMING

California has long been a perplexing, unorthodox state when it comes to legislation. This comes partly from the structure of the government, and partly from the demography. The citizens make up the most ethnically diverse population in the country. They are obsessed with the latest in technology and fashion. And, in general, they seem to breathe in more than a few of the visionary vapors that emanate from Silicon Valley, the Napa Wine country, and Hollywood. But California takes its citizen-targeted initiatives very seriously, which gives the entire state an artificially fractious appearance to outsiders each time an election cycle rolls through.

After all, this is the place that began the wave of property tax rebellion over a decade ago, which rippled eastward across the country and changed the way school funding was viewed. California was the state that came within a mere sliver of a vote to legalize euthanasia laws after millions were spent on both sides of the issue. And of course, it famously recalled its luckless Governor Gray Davis and opened up its 2003 special election to a bevy of

attention-seekers, including a curvaceous female stripper, a pornography publisher, and an action movie strongman with a world-famous Austrian accent.

WEST COAST BACKLASH

But the latest legal earthquake to rumble through the state did not originate in Sacramento, the state's capital. Instead, the epicenter was located smack in the middle of the nation's capital, Washington, D.C. In August 2001, President George W. Bush announced his administration's policy regarding human embryonic stem cell research.

Since his own ascension to the Oval Office, Bush had come under pressure from religious groups to concretely demonstrate that he was, indeed, a social conservative. In this case, he met their requests to ban embryonic stem cell research only halfway. Instead, he opted to allow federal funds for researchers who experimented only on the 60 or so existing cell lines.

The President's policy would have consequences on the opposite coast. The California state legislature had been down this road before. In September 2002, they had passed a bill allowing therapeutic cloning, which then-Governor Gray Davis signed into law. The bill was seen as a way to attract and keep biotech industry. But with the restriction on federal funding, California's legislators felt that they had to go further. State Senator Deborah Ortiz, a strong stem cell research supporter, introduced a billion-dollar bond measure for research funding, but it was blocked by the sizable Republican minority. Ortiz and others in the private and public section decided to use the initiative process and take the case directly to the voters of California. The culmination of these efforts was Proposition 71.

Proposition 71, also known as the *California Stem Cell Research and Cures Initiative*, was a startling legislative event in three major ways. First, the initiative essentially made conducting stem cell research a state constitutional right. Second, it allocated a grand sum of $3 billion to be given over a period of ten years to stem cell research and research facilities. Third, while the funds could be used to finance all kinds of stem cell research, one specific branch

was given priority. In a direct rebuke to the President's directive, Californians who voted for Prop 71 would hand top funding priority to *embryonic* stem cell research.[1]

The leader of the campaign effort to pass the proposition was Robert Klein, a charismatic real-estate lawyer and developer from the decidedly upscale suburb of Palo Alto in the San Francisco Bay Area. His interest in the legislation was partly scientific, but also included personal reasons. Klein's teenage son had been diagnosed with insulin-dependent diabetes several years ago, and the therapies derived from stem cells would be a boon to him. Since therapies could be developed with the huge base of scientific talent that California was home to, it made sense that anything speeding up the effort would be most welcome.

Klein and the pro-71 forces correctly reasoned that they could in effect bypass the state legislature and bring about change using the citizen initiative system. They felt if they took the case directly to the people, the California population would decisively demonstrate that they cared enough to act when their elected politicians would not. As leader of the pro-71 efforts, Robert Klein spent millions of dollars of his own money to finance the campaign.

STATE OF REBELLION

Proposition 71 quickly attracted a large number of proponents and detractors. The Hollywood glitterati, for example, comprised a small but very visible part of the pro-71 Coalition for Stem Cell Research and Cures. Christopher Reeve, Brad Pitt, and Michael J. Fox were among the most notable, as was Arnold Schwarzenegger.

Schwarzenegger had been the "Governator" for less than a year. Since he had run ostensibly as a moderate Republican, he had remained silent on the stem cell issue until a month before the November elections. When he finally endorsed the proposition on October 18, it was likely as much his reading of the public opinion as from

1. Since Prop 71 passed in 2004, the measure has been added as Article XXXV of the California Constitution.

any personal conviction, but it may have further solidified support of the legislation at the last minute.

Pro-71 advocates also included twenty-two Nobel laureates, the California State Treasurer and Controller, several state legislators, and more than fifty patient and disease advocacy groups, ranging from the Juvenile Diabetes Research Foundation, to the National Coalition of Hispanic Organizations and Planned Parenthood.

Opposing the proposition were most of the elected Republican party leaders in California, several conservative advocacy groups from Orange County, actor/director Mel Gibson, and last but not least, the Roman Catholic Church. But it was not only the conservative groups that were fighting the proposition. The union body of the California Nurses Association and the elements of the left-leaning Green Party were among the proposition's opponents as well.

There were three subtle distinctions among the groups opposed to the proposition that proved to be critical in the post-71 era. The groups that fought Prop 71 on anti-abortion grounds were generally opposed to all embryonic stem cell research. The groups that were nominally pro-choice on abortion (or at the very least neutral) emphasized the potential for abuse, citing conflicts of interest and allegedly inadequate safeguards to protect the health of stem cell research subjects.

The third faction campaigning to defeat the initiative was represented by the lobbying group called "Doctors, Patients, and Taxpayers for Fiscal Responsibility." Their primary objection to the proposal was that it used "general obligation" bonds for funding. General obligation bonds are normally used to finance more humdrum brick-and-mortar projects, such as hospital improvements, bridge abutments, and paving projects.

Additionally, the sheer dollar value of the funding requested put California into a whole new league. This was a state taking the lead in an untested, fledgling industry, the kind of a move more typically made by the federal government. It was as if John F. Kennedy had declined to pursue the development of a rocket that could take men to the moon in the 1960s—and in a show of rebellion, Texas had decided to fund NASA on its own dime.

A WHOLE NEW BALL GAME

While the exact numbers are debated on both sides of the issue, it appears that the pro-71 campaign spent over $30 million, and received a great deal of free publicity from the generally supportive California press. Once rolling, the campaign did not have to rely on funds from Klein's personal fortune—contributions came in from luminaries such as Bill Gates, Google venture capitalist John Doerr, and Pierre Omidyar, one of the founding partners of the online auction site eBay.

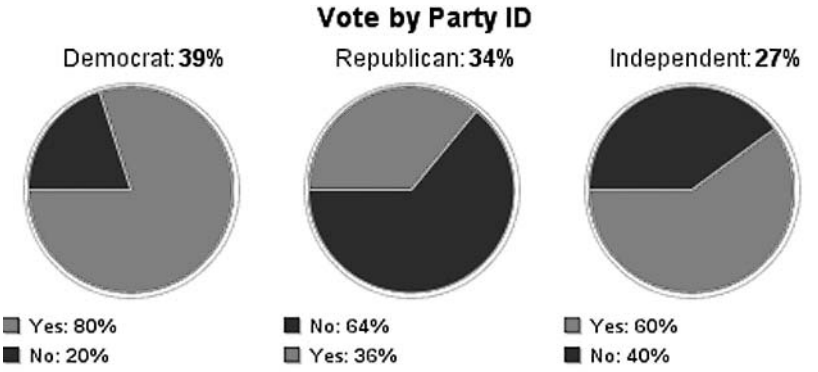
California's socially liberal majority meant that opponents to Prop 71 could not gain traction with appeals to pro-life sensibilities. Instead, the anti-71 forces tried mightily to capitalize on the general voter frustration with the heavy burden of debt and overspending by the state government. This was a valid tact to take. California's budget was routinely issued late and laden with all sorts of pet pork projects. Worse, the recent power-generating crisis had turned the state's energy commission into roadkill and led to rolling blackouts throughout the state.

The price tag of Proposition 71 was much higher than what many of its boosters claimed. For the first five years, repayment of the bonds' principal was postponed. Furthermore, the interest on the debt would be repaid using bond proceeds. It would take $3 billion to pay off the principal, then *another* $3 billion to pay off the interest on the bonds—making the fiscal impact to the state about $6 billion over the next thirty years.

Yet the cost didn't matter to the voters. The math was not emotionally compelling compared to headlines claiming that stem cell research could—and would!—find cures for cancer, heart disease, and, in general, save millions of lives. The initiative was even advanced as fiscally responsible, as in the long run it would cut health care costs by billions.

To cap it off, the sponsors of the proposal did a superb job in nullifying two major objections of many Californians. First, the language of the provision prohibited the funding of human reproductive cloning research. Second, not a single dollar would be taken from the state's school system by approving the funding measure.

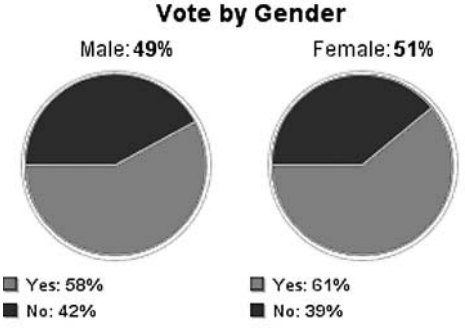
FIGURE 6-1
Prop 71 Results by Party.



It was a brilliant strategy, one that allowed the proposition to overcome all but the most die-hard fiscal and social conservatives. In the end, the pro-71 advocates had won over a majority of both genders and the critical independent voters. On November 2, 2004, the people spoke—and in political terms, the decision was a landslide victory: 59 percent of the votes were in favor of the proposition, an incredibly healthy margin.

Supporters were ecstatic. At times it appeared that California's public officials, who had initially turned down similar measures, would strain their arms patting themselves on the back. State Controller Steve Westly captured the attitude best when he stated, "Californians approved this unprecedented level of funding because it sends such a powerful and symbolic message to the rest of the world: The state that brought the world Silicon Valley and the majority of the world's biotechnology now will be setting the standards for the next big technological revolution."

FIGURE 6-2
Prop 71 Results by Gender.



Robert Klein was in full agreement. "There is no doubt in my mind," he

said afterwards, "that the mission Californians accepted today is a critical first step in changing the face of human suffering forever." Anti-71 forces were stunned by the sharpness of the setback. To put it another way, Klein and the Pro-71 backers had convinced seven million voters to compel the cash-strapped California government to outlay enough money in the end to give a dollar to every single person on the planet.

GUARDIANS OF THE GOLDEN SPIGOT

The passage of Proposition 71 established a board of scientists, biotechnology professionals, and representatives from disease advocacy groups to set the priorities for how the funds were to be spent. This organization, the California Institute for Regenerative Medicine (CIRM), was authorized to issue the mind-bogglingly large sum of grant money.

The institute was consciously modeled after the government's premier scientific research organization, the National Institutes of Health (NIH). It would mimic the NIH by playing the role of advocate, funding research projects that would normally be applying to the federal government. Indeed, the leaders of the institute are arguably the custodians for a gold-plated public trust fund. In true California fashion, the CIRM one-ups the NIH in a key way: the $300 million that it can spend annually dwarfs the $190 million that the federal agency allocated to stem cell research in 2004.

California's top-notch state universities were quick to jump on the bandwagon. They immediately began recruiting researchers and students, using the lure of the state funding to pull talent from rival research centers in Michigan, Wisconsin, and Massachusetts. Millions have already been earmarked by these institutions for stem cell research in anticipation of the public spigot being turned on as soon as possible.

Yet all of this preparation may be more than a little premature. CIRM and its governing board have gotten off to a decidedly rocky start since the passage of Proposition 71. The same freewheeling, hyper-legalistic attitude that California brings to the table when it comes to the legislative system hurts as well as helps the organiza-

tion. For example, the CIRM's first meeting had to be postponed due to a challenge from a public interest lawyer over the state's open-meeting laws.

Nor have the ambitious goals of the institute shielded it since that initial challenge. Two lawsuits have been brought forward against CIRM, tying its scheduled agenda in knots. The suits, which have been filed by the People's Advocate and the National Tax Limitation Foundation, and by the California Family Bioethics Council, attack the way CIRM is structured. Both parties claim that the organization would violate California's Constitution by spending money without sufficient state control and oversight.

And even beyond the legal matters, Proposition 71 left open how the state government would directly share in any financial success from the research. California Attorney General Bill Lockyer has advised the agency that these challenges must be resolved before Wall Street will finance the sale of the voter-approved tax-exempt bonds.

On a cold and blustery Thanksgiving in 2005, Judge Bonnie Sabraw of the Alameda County Superior Court declined to dismiss the pair of lawsuits that had blocked the state from issuing bonds to finance Prop 71's research program. While she rejected many of the arguments made in the dual lawsuits, the decision meant the case would have to go to trial before they could be resolved.

Judge Sabraw's decision both cheered and depressed the parties on both sides. She did not dismiss the lawsuits outright, but neither did she grant the plaintiffs a quick victory. Instead, the judge determined that the high legal burden of proving the stem cell initiative to be "clearly, positively, and unmistakably unconstitutional" had not been met.

Given the stakes of the science and the pitch of emotion on both sides, it is easy to predict the next part of the timeline. Whoever loses is bound to appeal the decision. Thus, it could be another year before any resolution is reached. David L. Llewellyn Jr., the lawyer for the California Family Bioethics Council, took an optimistic approach when the ruling was read: "[Embryonic stem cell advocates] are the ones who brought this motion claiming that we should be thrown out of court immediately, that there was nothing to this lawsuit. The court clearly said that's not true."

As of this writing it is unclear when the first series of grants under Prop 71 will be issued, let alone yield the promised fruit of the research. Those in favor of CIRM's mission argue that the suits have put a chill in the air, making many would-be donors hesitate before supporting further research. Worse, according to deputy attorney general Tamar Pachter, since bond interest rates have increased a half-percent since the start of the litigation, the suit could have raised the cost of borrowing the money by $15 million already.

As the continuing leader of the pro-research forces, Klein remained upbeat, but visibly irritated with the delay. "It makes it terribly frustrating," he said in a recent interview. "It means all the tools we expected to be out there are on the table today. They're not possibilities. They're real, and we can't get money out."

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CHAPTER 7

INVESTING IN HOPE AND HYPE

As the year turns the corner from spring to summer along the West Coast, the onshore breeze whips up moisture and brings cooling mists known as the "June Glooms." Tonight is no exception. The fog is thick, its scent punctuated with salt, and it rolls in like a dark, downy blanket as I drive north on the freeway. It's 2005, and I've just come from an interview with a researcher working in the thriving biotech corridor in San Diego. She's hinted to me that the companies in her area are taking steps to "steal the momentum" from other cities that are also seeking to attract their share of the stem cell funding pie.

FUELING EXPECTATIONS

Companies in San Diego aren't the only ones competing for attention. This same night, I'm a guest at a seminar on stem cell research put on by OCTANe. OCTANe is a nonprofit corporation, and it's an interesting stewpot of biomedical companies, private investors, scientists, and public universities. These events are regular meeting

places for venture capitalists and budding entrepreneurs. In the holistic, new-age lingo California is prone to using, their stated goal is to "create an ecosystem" that promotes an entrepreneurial culture.

To say that it's an interesting meeting is an understatement. Over plates of lecture-circuit chicken and Gruyere cheese, the air fairly crackles with the idea that, any day now, stem cells will be able to do everything from repair brain damage to mend cracked teeth. At the podium, the dean of the local university's biological sciences touts the fantastic progress being made, then turns the podium over to the school's researchers.

It is here that I first meet Dr. Hans Keirstead of the Reeve-Irvine Research Center. Keirstead has made recent headlines with his advances in repairing nerve tissue. With a gaze that could bore holes through sheetrock and a slightly rumpled shirt and blazer, he looks very much the part of a young, brilliant scientist. His laboratory was the first in North America to use the Bush-approved embryonic stem cells in spinal cord research. The focus on restoring function to severed spinal cords, which has achieved miraculous results in animal trials, has won him an interview on *60 Minutes* and has made him the undisputed draw at this function.

As a follow-up, several start-up companies show off their latest research. At this stage, they focus on how they're selecting for cells that will differentiate into pure cultures of nerve or muscle tissue. It's a fascinating, if slightly frustrating topic for both presenter and viewer. Fascinating, because of the breathless leaps that take the lecturer from the now—growing colonies of simple cells in culture—to an estimated millions or billions of dollars all but guaranteed to flow from their T-flasks.

It's also frustrating, because so much of the information is kept under wraps in order to protect advantages in manufacturing and growth mediums. The projected timelines are also a source of frustration. It seems so simple in principle—why can't we get the results *now*? The undercurrent seems to be: what about the other states—and countries—that are rushing to get to the new gold fields before we do?

HEADY FORECASTS: OFF BY A FACTOR OF 100?

The race to nail down venture capital for stem cell research is still wide open. Compared to the dot-com days, where venture capital was flowing in like a tidal wave, the behavior of the high-risk start up "angel" investors strikes many as puzzling. While there is a great deal of media coverage about the great things that are just around the corner, the fact is that when a private investor moves to support the industry, it still makes headlines.

To take one example, New York City Mayor Michael Bloomberg has recently donated $100 million to John Hopkins University. Even though an unspecified portion of the donation was to support stem cell research, the news reports touted this as a major—if not the sole—reason for the philanthropy. It is also more than a little interesting that other private investors have been much more active in spending money to encourage state government funding, as opposed to direct donation.

Some securities analysts have forecast that by 2010, the market for stem cell technologies will exceed $10 billion. These are very heady numbers, ones that would make most investment firms salivate. But others have taken a look at these numbers and dismissed them as the same type of math that led to the over-valuation of the dot-com companies and their subsequent meltdown.

According to *The Economist*, consultants with the firm Bain & Company have taken a much more sober look at the state of the nascent industry. As of early 2006, there are now roughly 140 stem-cell-related products in development. Again, depending on which end of the telescope one looks into, this can look promising or underwhelming.

The therapies being researched stand to decrease human suffering by unheard-of levels. They are replete with cures for multiple types of cancer, liver disease, sickle-cell anemia, and more. However, less than a handful are beyond the earliest stages of development, and even the most basic clinical studies are years—up to a decade or more—in the future.

Slightly more than $1 billion was spent on stem cell work in

2005, which again sounds like an impressive sum. However, when compared to the total amount spent globally on health-care R&D, it's less than stellar: a mere 1 percent. The analysis cited also takes into account the potential hobbles of heavy government regulation and environmental concerns. Both are highly likely, given the public's uneasiness with topics related to cloning and human embryos.

The consultants at Bain & Company predict a far more modest forecast of a $100-million market for stem cell therapies by the end of this decade. By 2015, the forecast is more optimistic, rising to $2 billion worldwide. Again, impressive numbers when viewed in isolation. And yet one cannot help but notice that the conservative forecast for the decade disagrees with the optimistic one pushed by stem cell boosters by a factor of 100. The same ratio holds in the amount spent on health-care R&D: For every dollar spent on stem cells, $100 is placed elsewhere.

Another interesting fact is that more than four-fifths of the global investment in stem cells has come from governments. Private venture capital, the traditional engine of biotech start-ups, pumped an anemic $50 million into the field in 2005. The trend is sharply higher today, but why has it taken seven years from the time the first stem cell lines were made available to even start to grow?

CHAIN REACTION AMONG THE STATES

The real issue at hand for the stem cell industry is funding: specifically, federal funding. Due to the compromise worked out by President Bush and the religious conservative wing of his party, federal funding is only available to firms that are working with stem cell lines derived from human embryos *before* August 9, 2001.

There was a strong backlash against this decision by groups who wanted to press stem cell research forward as quickly as possible. Proposition 71, the 2004 initiative passed by California voters to hand over $3 billion in state money, was only the beginning. Just as California tends to set trends for the rest of the country in terms of culture and entertainment, the West Coast's cutting-edge initiative system tends to do the same thing. California's measure

has set off similar legislative issues in other states like a string of firecrackers.

New Jersey

In March 2006, New Jersey lawmakers had been debating a bill calling for spending $150 million for a pair of biotechnology research centers. Apparently, this wasn't considered aggressive enough by those who wanted to specifically study stem cells. As of this writing there is a bill on the table that would boost the taxpayers' investment to $200 million, in order to add a third research lab to specifically study adult stem cells.

The additional $50 million for the third research center, which would be based in Newark, will be borrowed against "expected revenue" generated from cigarette taxes. The mind boggles at how ironic it will be should the Newark lab discover a cure for lung cancer.

Maryland

In the same month as the New Jersey decision, the Maryland state senate approved a bill to establish guidelines for spending state money on stem cell research. The 29 to 18 vote came after an hour of impassioned debate on the subject, with only one Republican voting to support the legislation. Tellingly, this politician is facing an uphill reelection battle in a moderate district where stem cell research is popular.

As of this writing, Republican governor Robert Ehrlich's budget proposal for stem cell research has been passed into law. The proposal included $15 million in grants for research using both embryonic and adult stem cells. The initial round of funding, which will be awarded by a state commission, is expected to flow to Johns Hopkins University and biotech companies conducting research on adult stem cells.

MISSOURI

An early 2006 ballot proposal promoting embryonic stem cell work reveals what could very well be a deeply buried fault line in the Republican party over the nature of the research. Like in many states,

Missouri's Republican Party is strongly divided between religious conservatives, who view the decision in moral terms, and fiscal conservatives, who approach the issue as a business decision.

The Missouri proposal doesn't force the legislature to "put its money where its mouth is." Unlike California, the measure commits no tax dollars to such experiments. This detail may be a key clue to the Republican disarray. If the state of Missouri had, say, proposed spending $300 million per year as in California, fiscal conservatives might have stood shoulder-to-shoulder with the "religious right" and sent the measure to easy defeat.

It is the uneasy alliance of pro-business interests and religious conservatism that has allowed Missouri's Republicans to dominate the state's politics since 2000. That alliance may be frayed depending on how the stem cell issue is handled. Republican Governor Matt Blunt went on record as backing the measure. In response, the Missouri Right to Life organization has declared that Blunt can no longer be considered a "pro-life" candidate, thus opening him up for a challenge on the right.

Illinois

Illinois became yet another state to support stem cell research after Democratic Governor Rod Blagojevich ordered $10 million in tax dollars be earmarked for the research. This decision, much as was the case in Missouri, also demonstrates how closely the realms of politics and morality are intertwined in this area. Illinois' grants are to go specifically for research on embryonic as well as adult stem cells, although using the funds for reproductive cloning will be forbidden.

The Illinois situation is particularly interesting because Blagojevich's executive order went around the state legislature. Although the governor does not have a particularly contentious relationship with his fellow politicians, the legislature has questioned whether state funds should be allocated for such an ethically sensitive area. Twice in 2005, measures on this subject were debated to a standstill, resulting in one deadlock and one outright rejection of the research.

Blagojevich claims that this is a moral cause for him: "My sense

of morality argues strongly to not simply sit back and do nothing when children are suffering from juvenile diabetes. To simply be afraid to take a position or to act, I think would be immoral."

GRABBING THE RING

In March 2006, roughly a year after my discussion with the San Diegan scientist, the stem cell research community finally sprang into action. The University of California at San Diego (UCSD) offered a site on its campus as a facility for human embryonic stem cell research. It is the first of its kind to be proposed in California.

UCSD proposed the facility, which would be used by the Burnham Institute for Medical Research, The Scripps Research Institute, and the Salk Institute for Biological Studies. The plan is to join forces and seek state funding to build the facility on the university's site. As part of the agreement, the three institutes and the university pledge not to individually seek grants from the state's stem cell funding agency, the California Institute of Regenerative Medicine. Their end goal is simple and publicly stated: to vault San Diego to worldwide leadership in stem cell research.

Dr. Edward Holmes, UCSD vice chancellor of health sciences, was extremely pleased with the effort. "Individually, all of the consortium institutes are stellar. But together we will be a tour de force and become the epicenter of stem cell research in the United States, and therefore the world."

Several other people who have teamed up to bring part or all of the Proposition 71 money to their district share Holmes' optimism. The title "epicenter of stem cell research" is a brass ring that can still be grabbed, whether by San Diego, Orange County, or by the San Francisco Bay Area. Key to the recent competition, ironically, is the restriction to federal funding. For years, research institutes in San Diego have collaborated on projects. But it was the $3 billion in grants that would be available under Proposition 71 that gave the four organizations enough of an incentive to collaborate to the point of sharing researchers and facilities.

The incentive of large grants of public money is exciting to those who support the stem cell research field wholeheartedly. With such

public commitment demonstrated, the question returns to why private speculators haven't also signed on to the "next big thing" en masse. In California, Pro-71 boosters promised that the state would eventually recoup the funds borrowed to fund the initial research via royalty and tax payments. However, it's worth asking why, if this were a likely event, the state's numerous venture capitalists haven't been willing put up the $3 billion themselves?

STOCK MARKET TO STEM CELL LABORATORY

The answer may be that the time horizon for many venture capital firms, who seek returns far beyond that of established stocks, is too short for the multi-year waits that biotech imposes for improvements. "Many of the technologies we hyped to the general public haven't worked yet," said Alan Lewis, CEO of Celgene Corp.

If even the most outspoken proponents concede they are years away from actual stem-cell-based therapies, it is unlikely that the high-rolling investors will take notice.

Another possibility is that the start-up costs for a state-of-the-art laboratory, the cost of high-tech equipment, and the price of attracting top talent are simply beyond all but billionaire-level capitalists. There are no firm estimates for how much money it takes to start up a biotech firm or a stem cell lab. But the numbers that enter a discussion on the subject range from several million dollars up to $3 billion—the sum total of what California itself is willing to spend over a decade!

Yet the underlying reason for the holdup may be much more mundane. It could be simply the "hangover" left by the freewheeling 1990s. The more risk-taking venture capitalists are simply very cautious about being burned a second time after the dot-com crash, or they want a return as soon as possible.

On the other side of the financial spectrum, lending institutions might have the patience to wait a decade or more for a return, but they aren't about to get involved with the high risk and the sociopolitical controversy until conditions settle out. The irony is that stem cell companies may very well fall between the cracks of these two sets of potential large-scale investors, by a combination of a

peculiar risk profile, a set of morals that are constantly in flux across the country, and the unfortunate happenstance of being in the decade following the dot-com crash.

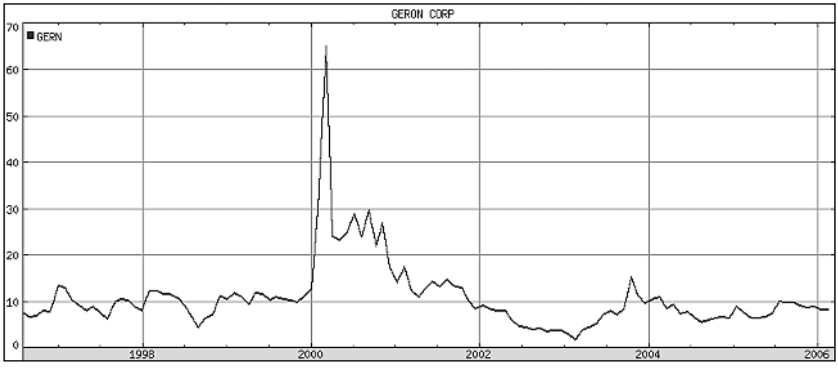
As far as publicly traded research companies are concerned, the smaller investors are valuable insofar as the price of the company's stock influences how much money is available at a given time to fund research. And smaller investors, who may have as short a timeline for returns as venture capitalists, must have a cast-iron stomach for risk taking if they plan to invest in stem cell research companies.

Take Geron for example: a classic research-based company, with some of the leading expertise in the field and potentially cutting-edge technology in the pipeline. Yet the vast unknown potential combined with the known past performance makes the stock incredibly volatile. The Menlo Park based firm has, according to financial reports, invested around $100 million in its human embryonic stem cell research since 1996. And even so, this well-placed company still lost close to $80 million in 2005.

Could the volatility of the stock be simply a problem of reputation? It is true that as result of the dot-com hype, Geron peaked in 2000 at a high of almost $80 per share—and one can imagine the champagne corks going off in the boardroom that year. After flirting with the dangerously low price of $1.41 back in 2003, Geron still trades much of the time in a relatively wide range between $5 and $15.

FIGURE 7-1

Geron's stock price trend from 1997 to present.



Two other companies that follow a similar trend are StemCells Inc, and Aastrom Biosciences. Of the two, StemCells Inc, located less than a 30-minute drive from Geron, has garnered more attention from the loss of more than $50 million since 1997. However, both companies have had flashes of promise—only to have the headlines and investors slip away while they struggle to prove a clear-cut therapeutic effect in their clinical trials.

A no-nonsense review of the industry by *Forbes* magazine at the start of 2006 perhaps put it best. When it came to betting on stem-cell-company stocks, the risk factor was akin to playing the lottery. "The more lottery tickets you have, however, the more likely chance

FIGURE 7-2

StemCells Inc's stock price trend from 1993 to present.

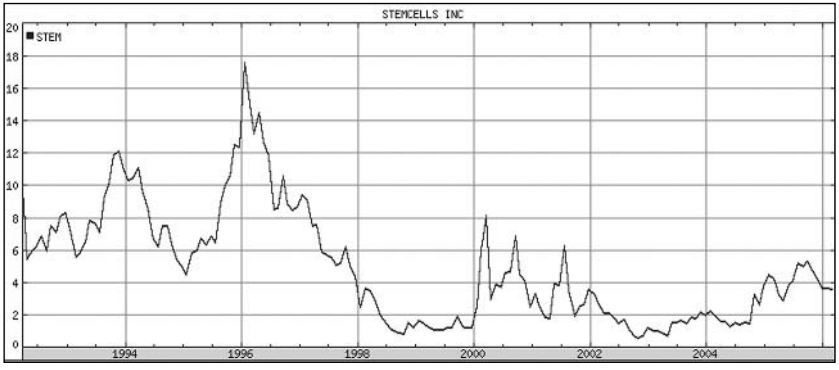
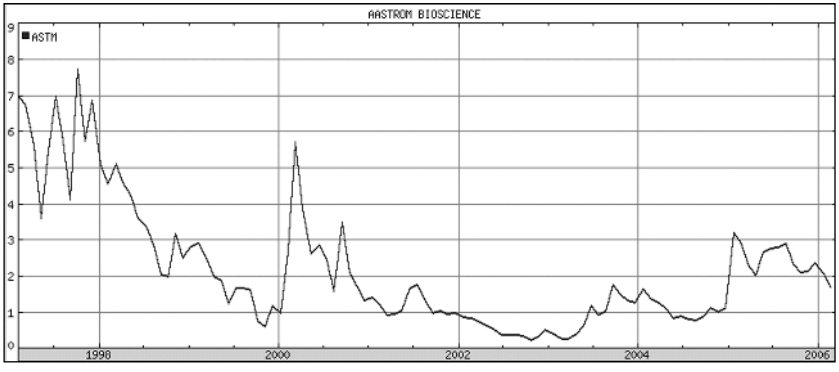


FIGURE 7-3

Aastrom's stock price trend from 1997 to present.



you have to win. As is the case with most lottery tickets, though, there is a good chance they'll never produce riches."

Though most risk-adverse private investors will look for safer bets, no one predicts that companies working with stem cells will quietly fold up their tents and go away. The public commitment is here to stay. As the scientist I interviewed in San Diego reminded me, "Today's stem cell effort is a little like the national space program of the 1960s. You might not end up buying stock in the companies that make the rockets or the boosters. But you certainly could end up owning shares in the company that took the space program's invention, Teflon, and brought it to market."

Considering how closely related stem cell work is to cloning, genetic manipulation, and in vitro fertilization technologies, it's easy to see how prolific the spin-offs could be.

In June 2005, only a few weeks after the seminar at OCTANe, cloning and stem cell researchers gathered in San Francisco to show off their latest breakthroughs. It was the biggest professional conference on the topic in the entire year, attracting more than 1,000 researchers. And in 2006, the conferences are multiplying like mushrooms after a rainstorm. Different scientific organizations are bringing together researchers, sharing the latest information on topics from new cell lines to the regeneration of spare body parts for healing sick and injured people who under today's medicine would have little or no hope.

Leonard Zon, the president of the International Society for Stem Cell Research, gave his verdict in a succinct and unmistakably confident manner: "Stem cell research is the hottest thing in science right now."

Which to believe: the hype or the hope? In the long run, it looks like the reality of stem cells will indeed catch up with the rhetoric.

It is only a matter of time.

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CHAPTER 8

FROM ROE TO DICKEY

There is no getting away from the fact that, in the public's mind, the study of embryonic stem cells is intertwined with two other controversial topics: cloning and abortion. This is due in part to the way politics has aligned the issue, but it is also legislative reality. Any change in the legal status or strictures on one of these subjects impacts the other two, as if they were joined by steel chains.

In 1973, the Supreme Court's decision in *Roe v. Wade* decreed that most laws against abortion violate a constitutional right to privacy. In one stroke, it overturned existing state laws across the country restricting abortion. The decision was, not surprisingly, one of the most controversial ever handed down from the bench of the highest court in the United States. *Roe* kicked off a decades-long national debate, reshaping national politics and inspiring a great deal of grassroots activism.

MONITORING THE "SURPLUS"

The detailed arguments for and against the decision are beyond the scope of this chapter, but they can be summarized briefly. *Roe's* opponents fall into two general camps: those who see the Court's decision as illegitimate because it read too much into the text of the U.S. Constitution, and those who have unshakable beliefs about the "personhood" of human life, even in the embryonic or fetal stage.[1]

Supporters view *Roe* as necessary to preserve women's equality and personal freedom. Many who support the decision in *Roe* cite the primacy of individual over collective rights. Since this tack is also taken up by *Roe's* opponents—who see the *fetus* as an individual—the most adamant *Roe* supporters would reply that an adult woman's rights should determine the result, not a mass of cellular tissue.

Depending on when you believe that "personhood" becomes an issue in the reproductive process likely determines your personal stance on the issue. Certainly, it has continued to play out in the laws that are made involving all kinds of human embryonic research, including stem cells.

After *Roe* became the law of the land, members of Congress grew concerned about exploitation of aborted embryos and fetuses. Though it sounds slightly macabre to say, this "surplus tissue" was indescribably rich in cellular secrets. It was a given that the medical and scientific community would wish to put these items to use.

To address these issues, in 1974 Congress put a temporary moratorium in place on federal sponsorship or funding of research using human fetuses or living embryos, unless the research was to be done for the purpose of assuring the fetus' survival. That same year, Congress explicitly assigned a commission to see whether guidelines could be drawn up for human fetal and embryo research.

The appointed body had a jaw-cracking name: The National Commission for the Protection of Human Subjects of Biomedical

1. From the time a human egg cell is fertilized, the first seven weeks of development are considered to be the *embryonic* stage. Following this stage until birth, the embryo is termed a *fetus*.

and Behavioral Research. The commission met its goal by the next year. The final report called for the establishment of a national Ethics Advisory Board within the Department of Health, Education, and Welfare (DHEW) to propose standards and research protocols for federal funding of research involving human embryos.[2]

RED TAPE FOLLIES

While at first glance this appeared to be nothing more than a government action worthy of satirizing in a Dilbert comic strip—creating an official committee to recommend that *another* committee be created—two significant steps forward had been made. First, to its credit, the commission looked ahead to the possible uses of in vitro embryos, since successful human in vitro fertilization (IVF) had been accomplished a few years before, and it looked inevitable that the technology was going to become a normal, accepted medical procedure along with abortion. Second, the commission convinced the DHEW to establish an Ethics Advisory Board to provide advice about the ethical acceptability of IVF and related human embryo research proposals.

One of the Ethics Advisory Board's most interesting positions was that research involving human embryos and IVF techniques were "ethically defensible but still legitimately controverted." In other words, the board was not a zealous advocate for either significantly curbing research or throwing open the lab doors still wider. It also advised that work was "acceptable from an ethical standpoint" so long as all donors were married couples, and that the embryos selected were fourteen days old or less.

However, just as the board bravely charged up the hill of decision, it just as quickly backed down by deciding that it "should not advise the Department on the level of Federal support, if any," that such work should receive. By doing so, the board tossed the decision like a hot potato back into the hands of the DHEW.

The DHEW in turn seemed to throw up its hands in frustration, since it decided at that stage to simply not offer funding for human

2. Today, this federal agency is known as the Department of Health and Human Services.

embryo studies. At that point, the events got even stranger. When the Ethics Advisory Board's charter expired in 1980, no renewal or replacement body was put forward by Congress. So although the board was required to review proposals for funding, since the agency had ceased to exist, the proposals literally had nowhere to go for review! On paper, the moratorium on federal funding of research using fetuses or embryos had officially been lifted in 1975. In reality, it existed through the next decade.

Fast forward to the 1990s. Increasing interest in embryonic research, combined with the election of President Clinton, led to a sea change in attitude. The Democrat-controlled Congress enacted the National Institute of Health (NIH) Revitalization Act in 1993, Clinton's first year in office. The act abolished the need for research to be approved by the now defunct Ethics Advisory Board.

A flurry of decisions soon followed that alternately perked up researchers looking for federal funding or plunged them into gloom. One of the NIH's advisory panels recommended that human embryo research should, within a recognized framework of ethical safeguards, be deemed eligible for federal funding. Startlingly, the panel further concluded that in some circumstances, funding could be used to *create* human embryos with the explicit intention of using them only for research purposes.

Even a strong supporter of embryonic research such as President Clinton recognized that this was too bold a move for many people to stomach. While it could reasonably be argued for on scientific grounds, politically it was a first-rate disaster waiting to happen. Clinton accepted all of the panel's recommendations save one—embryo creation for research would not be funded.

THE LANGUAGE OF JAY DICKEY

Reportedly, Clinton wanted the NIH to stick to funding research using existing embryos left over from IVF procedures. But then the political winds shifted again, this time with gale force. In the 1994 mid-term elections, Congressional power shifted decisively to the Republicans. The next year, before any funding proposal had ever been approved by the NIH, Congress attached a provision to the

budget bill that funded the NIH, which slammed the brakes on the use of federal funds.

This provision, which is still in force today, was originally authored by Representative Jay Dickey, of Clinton's home state of Arkansas. The Dickey Amendment (as it has been called since) exerts substantial influence over the debate about federal funding of embryonic stem cell research. The pertinent sections state that federal funds provided under the NIH budget could not be made available for any of the following circumstances:

1. The creation of a human embryo or embryos for research purposes.
2. Research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204 and 46.207, and subsection 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).

For purposes of this section, the term "human embryo or embryos" includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of the governing appropriations act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

The language in the provision that draws the line in the sand is quite clear. The Dickey Amendment prohibits the use of any federal funds for research that would even *endanger* human embryos, let alone create them for research purposes.

There is one key point that many in the public—and the media—do not seem to fully understand. Though restrictive, the Dickey Amendment does not outright prohibit any of the research itself. It does not outlaw embryonic research, or the destruction of embryos. It does not even ban the creation of human embryos for research. At the federal level, the research cited by the amendment is neither prohibited nor supported. It only spells out what may or may not be supported by taxpayer monies.

DOING THE FUNDING TWO-STEP

Lawyers at the U.S. Department of Health and Human Services (HHS) figured that the last word had not been written about the Dickey Amendment. In January 1999, Harriet Rabb, the department's general counsel, suggested that under the provisions, there was an interpretation under which human embryonic stem cell research could be funded. It was a very clever reading of the law, one that allowed researchers to do a kind of two-step dance around the literal meaning of the words in the amendment.

The language in Dickey stated that funding would be held back from research "in which" embryos were destroyed. Yet all that scientists really needed were the embryonic stem cells inside the embryo, not the embryo itself. Therefore, if the embryos were destroyed using private funding *before* the researchers went to the federal government to apply for funds, the letter of the law was met.

Predictably, this sent up a howl of outrage from critics. While legally valid, it appeared that public funds were being used to actually *reward* the destruction of human embryos by promising funding for research if such a step were taken. Supporters of the proposed approach in turn stood firmly on the language of the law. The feeling was that as long as basic ethical standards were met—including informed consent and a prohibition on financial inducements—the therapeutic potential of funding stem cell research in this way was too great to ignore.

In an August 2000 speech, Clinton reminded the public that the embryos used were not being created solely to be torn apart for their cellular treasures. The only embryos to be used were to be the surplus collected from IVF procedures. He went on to reaffirm his commitment to funding stem cell research as a way to ameliorate great amounts of human suffering:

> In the last couple of weeks we've had story after story after story of the potential of stem cell research to deal with these health challenges. And I think we cannot walk away from the potential to save lives and improve lives, to help people literally to get up and walk, to do all kinds of things we could never have imagined, as long as we meet rigorous ethical standards.

Clinton officially adopted this course of action and drew up specific guidelines to enact it, but his administration was never able to put it into practice. Changes in a bureaucratic government agency take time to ripple through the various levels. It was only several months after Clinton's statement that the NIH began accepting grant applications for research projects utilizing early human stem cells, with a specified deadline of March 15, 2001.

Although the Clinton administration first opened the door to federal funds, none of the applications submitted were funded in 2001 due to yet another shift in the political landscape.

WORD AND SPIRIT

Perhaps the ultimate irony is that the first presidential administration to ever actually give out federal funds for stem cell research was headed by a conservative Texan. And not just any conservative—but one who was first and foremost beloved by the same religious conservatives who adamantly stood against the decision handed down in *Roe v. Wade*.

In 2001, after George W. Bush took office, his Secretary of Health and Human Services ordered a review of the prior administration's policy. While this was being done, the Clinton-era applications in the NIH pipeline were halted. Although it is likely that the new administration was not as open to supporting federal funding of stem cell research, they were not immune to the fact that public pressure to do so was greater than ever.

The amazing advances made by James Thomson and others in isolating and growing human stem cells, combined with the continued positive press coverage, was making stem cells as paramount an issue as space exploration or universal health care coverage. The Bush administration therefore decided to take another look at the options regarding human embryonic stem cell research policy.[3]

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3. Bush later claimed to be the first president to ever *allow* funding for human embryonic stem cell research. This claim is a little disingenuous. His administration was the first to actually *disburse* federal funds, but it was Clinton who really put federal funding on the table.

There were some definite goals in mind. The endgame was not to block all embryonic stem cell research, as some on the right would have wanted. Instead, the idea was to allow funding to some potentially valuable research while upholding the spirit as well as the words of the Dickey Amendment.

Bush has been consistent throughout his time in office in wanting to restrict the use of human embryos for stem cell research. As Bush put it in a speech during his second term:

> Research on stem cells derived from human embryos may offer great promise, but the way those cells are derived today destroys the embryo. I share the hope of millions of Americans who desperately want to find treatments and cures for terrible diseases. . . . But I also recognize the grave moral issues at stake. So, in August 2001, I set forward a policy to advance stem cell research in a responsible way by funding research on stem cell lines derived only from embryos that had already been destroyed. This policy set a clear standard: We should not use public money to support the further destruction of human life.

The policy Bush refers to was enacted on the date that lives "in infamy" for many stem cell supporters—August 9, 2001. The 2001 policy denies federal funding not only for the creation or cloning of any human embryos for research purposes, but also for research conducted on stem cell lines derived from embryos destroyed after August 9 that same year.

In the true fashion of any political compromise, the policy was immediately denounced on all sides. It has been termed a "Solomonic" compromise, but there is a more subtle moral decision one can read into the language that accompanies it. Given the statements expressed by President Bush, it appears that the 2001 policy is not intended as a compromise on the moral status of the human embryo.

The Bush administration may very well view the acts that led to the existing stem cell lines as immoral. But, since the die had already been cast, it seemed prudent to squeeze some moral benefit—the possible creation of stem cell therapies—out of what had already

transpired. In the President's own words, "the life and death decision had already been made."

The Dickey Amendment still has the strong backing of conservatives, both in and out of Congress. The amendment expresses their ethical convictions about the link of human life and scientific research, even if said research could lead to miraculous cures. It is not seen as perfect, but in the minds of its supporters, it protects people who think human life is sacred from paying for its destruction.

And yet the success of the Dickey amendment is part of what muddies the water for people who are trying to understand what, exactly, is going on in the field of stem cell research. That is because for all intents and purposes, embryonic stem cell researchers live in a morally divided universe—*permissive* in the private sector, *prohibitive* in the public sector.

The researcher's world then is subdivided even further. Private-sector research is not constrained by federal government but often is by state government. About half the states in the United States have wildly differing laws on the books about what can and cannot be done.

The Bush compromise, sometimes inaccurately reported as the stem cell research "ban," continues to reverberate nationally. Some consequences, such as the multi-billion dollar bond issue in California, are already apparent. Others are still being hashed out in Congress, not in the polite soundbites about policy, but in heated and personal arguments. And the first President to have never vetoed a single bill in six years of holding office may yet pull out his pen to block any attempt at overturning his carefully crafted compromise.

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CHAPTER 9

DIVISION ON THE HILL

Public statements about embryonic stem cell research tend to shed more heat than light. Emotional responses are understandable, especially when the discussion moves beyond cell cultures and petri dishes. Unapologetic boosters of unrestricted research seek to reduce human suffering as greatly and swiftly as possible. Those who disagree worry that to continue down the path of unrestricted research will mean that a kind of Faustian bargain has been struck, sacrificing one type of suffering for another.

A pair of deaths in the recent past brought much of this divide to the surface. The two people, Christopher Reeve and former President Ronald Reagan, were unconnected in life, but linked at death in that both of their conditions could, arguably, have benefited from additional stem cell research. Reeve, the actor best known for his role in the *Superman* movies, had been paralyzed from a riding accident since 1995. Reagan was diagnosed in later life with Alzheimer's disease, dying of complications resulting from it at the age of 93.

HARSH WORDS, HIGH STAKES

Former first lady Nancy Reagan spoke out on the topic of stem cells in May 2004 at a star-studded dinner held by the Juvenile Diabetes Research Foundation in Los Angeles. She emphasized the need to de-politicize the debate over stem cell research, noting that the research held the potential to cure illnesses like Alzheimer's. "Science has presented us with a hope called stem cell research, which may provide our scientists with many answers that for so long have been beyond our grasp," she said, "I just don't see how we can turn our backs on this."

Actors Harrison Ford and Calista Flockhart read letters from former presidents Gerald Ford, Jimmy Carter, and Bill Clinton supporting Mrs. Reagan's efforts. Also speaking in agreement was Michael J. Fox, a longtime supporter of stem cell research because it could help lead to a cure for Parkinson's disease, with which he was diagnosed in 1991. He noted that, "For someone like Mrs. Reagan to step outside of political or ideological groupings and just speak to what she believes can help people is tremendously valuable."

Two other members of the Reagan family also stepped into the media limelight to support further research.[1]

Ronald Reagan Jr., one of the late former president's two sons and a dedicated stem cell research supporter, has been pointedly critical of the restrictions on the use of embryonic stem cells. "It does not follow that the theology of a few should be allowed to forestall the health and well-being of the many," he said. "And how can we affirm life if we abandon those whose own lives are so desperately at risk?"

Reagan agreed in principle with his mother that the issue of stem cell research should not be a partisan issue. However, this should probably be taken with a small grain of salt. During the hard-fought 2004 election, Reagan stated that when it came to embryonic stem

1. Contrary to media reports, the entire "Reagan family" is not in full support for reducing restrictions on stem cell research. Reagan's eldest son, columnist and radio-show host Michael Reagan, has consistently opposed the creation of human embryos for the sole purpose of using their stem cells as possible medical cures.

cell research, the choice was between "the future and the past, between reason and ignorance, between true compassion and mere ideology." This statement was given during a speech at the Democratic National Convention in Boston.

Daughter Patti Davis was even less restrained in her call for more research. Famously estranged from her parents' political philosophy, she reconciled with her family during Ronald Reagan's final illness. In a column written for *Newsweek* in October 2004, she referenced the passing of Christopher Reeve and said that those who did not support research to cure such terrible injuries were, in effect, morally bankrupt. "I wonder if President Bush could look into the eyes of Christopher Reeve's family and tell them that it's because he values life so deeply that he is preserving clusters of cells in freezers—cells that resulted from in-vitro fertilization and could be used for embryonic stem cell treatment—despite the fact that more people will die as a result of his decision."

DOCTOR IN THE SENATE

Divisive opinions on the stem cell topic pervaded even the Republican Senate. In August 2005, the Bush administration received a rude wake-up call from their longtime supporter and Senate Majority Leader, Bill Frist. It was a message that foreshadowed deep fault lines in the conservative opposition to further embryonic stem cell research.

Frist publicly broke with the President's stated objectives, favoring federal funding for stem cell research. Specifically, he came out in support of the position that federal funds *should* go for research on stem cells harvested from embryos. Given the close working ties between Bush and Frist, to many this seemed like the political equivalent of the mutiny on the Bounty.

Frist is someone whose opinions must be accorded extreme weight in the political calculus of the stem cell debate. A lanky, handsome fourth-generation Tennessean, Frist entered his father's profession by attending Harvard Medical School, graduating with honors in 1978, and eventually specializing in heart and lung transplantation surgery. He was elected to the Senate in the 1994

Republican sweep of both houses of Congress, becoming the first physician in the Senate since 1928.

After an easy re-election in 2000, Frist first entered the national spotlight by providing medical attention to two Capitol police officers who had been shot outside the United States Capitol. His medical background allowed him to act as the Congressional spokesman during the mysterious bioterrorism attacks of 2001, where anthrax powder was mailed to Senator Tom Daschle and others via anonymous envelopes. This presence on the national stage was cemented in 2002, when he was selected as the new Senate Majority Leader over Mississippi Senator Trent Lott, becoming the second youngest person in U.S. history to hold that post.

It's worth noting that Frist had not climbed the ladder of leadership jobs and had repeatedly disavowed interest in leading the Senate. Expressing disinterest in increased political power is a typical cover for political candidates. However, it appears that Frist was sincere in not wanting promotion in the Senate, stating from the first that he would serve only two terms and would leave the body in 2006.

Furthermore, his elevation came as the result of historical accident. Senator Lott had resigned his position as Senate Majority Leader due to intemperate comments where he said that if Strom Thurmond's segregationist presidential bid of 1948 had succeeded, "We wouldn't have all these problems today."

Perhaps due to his medical background, Frist has long been more open to the idea of using embryonic stem cells than President Bush. Still, for the first few years of his tenure as Senate Majority Leader, Frist had striven to cooperate with the chief executive as much as possible. But this came to an end in the summer of 2005.

CRACKS AND LEAKS IN THE GOP

In July that year, Frist addressed the Senate in a speech that said, "We should federally fund research only on embryonic stem cells derived from blastocysts left over from fertility therapy, which will not be implanted or adopted but instead are otherwise destined by the parents with absolute certainty to be discarded and destroyed."

This statement won praise from those outside the immediate orbit of Bush's administration, such as Senators Arlen Specter and Edward Kennedy.

The Frist statement was an explicit break from Bush's position, which restricted the collection of embryonic stem cells to lines that were in existence on August 9, 2001, not lines from embryos left over from assisted fertility therapies. Back then, Senator Frist allegedly had misgivings over the severe winnowing of the available range of stem cell lines, but wanted to support the President. Additionally, the wily doctor-politician decided to take a "wait and see" approach, to watch where the rapidly advancing field might go next and determine if an updated position would be necessary later on.

On May 25, 2005, the issue took on a new urgency as a bill to allow the use of federal funds to support embryonic stem cell research was passed by the Republican-controlled House of Representatives on a vote of 238 to 194.

The margin of passage was an important gauge of the bill's likelihood of becoming law: While it was passed by a clear majority, it was far less than the two-thirds support that would be needed to override a Presidential veto. And a veto was an almost guaranteed outcome. Within hours of the bill's clearing the House hurdle, President Bush bluntly declared, "There must be a balance between science and ethics and I have made my decision. . . . The use of federal dollars to destroy life is something I simply do not support."

Still, in order to get to the President's desk, the bill had to clear the Senate as well as the House. The medical promises of embryonic stem cell research had prompted several House members—of both parties—who opposed abortion rights to pass the measure. Similarly, it was argued in the Senate that there was a moral obligation for Congress to fund research that could lead to cures.

The argument had personal overtones as well as political ones, as the chief Republican sponsor of the bill, Arlen Specter of Pennsylvania, made a direct appeal for quick action. Specter was being treated for cancer, and the regimen of chemotherapy had caused all of the Senator's hair to fall out. His words therefore had a passionate personal ring: "I look in the mirror every day, barely recognize

myself . . . not to have the availability of the best of medical care is simply atrocious."

This was when Frist first made his misgivings with Bush's position public. And it was the manner in which he called attention to his statements that was stunning. Not only had Frist departed from the official White House line, his staff had notified the *New York Times* the night before. In effect, Frist wanted to *guarantee* wide notice for his announcement and cast the White House's position in stark relief from his own viewpoint.

SHOT ACROSS THE BOW

The Majority Leader said the promise of embryonic stem cells was being frustrated, partly because the results of the pre-existing stem cell lines have been "disappointing," and also because many of the available lines are slowly deteriorating. "I am pro-life, I believe human life begins at conception," said Frist, though he tempered this statement with the pronouncement that "I also believe that embryonic stem cell research should be encouraged and supported." Frist went on to affirm his support for the House bill, though he did not say when he would bring it to a floor vote in the Senate.

Even back in 2005, the Senator from Tennessee was often placed on the "short list" of people who were in the running for the Presidency. A year later, it appeared that the list makers were correct. Frist has been seen making the rounds to drum up early support and funding. The impact of his statements on stem cells remains to be seen, though they can be interpreted as a play for the bloc of "moderate" voters. That would make Frist's statements in effect the opening shot of the campaign for the Republican nomination for President in 2008.

Some Republican strategists question the wisdom of Frist's move. It is estimated that a quarter to a third of the GOP disagrees with Bush's position on stem cell research. But the conservative activist base that dominates the election primaries finds Frist's reasoning contradictory at best, turncoat at worst. "Senator Frist's public backing of this horrific science is being felt deeply across Middle America, and most importantly at the grass roots," said Tamara

Scott, the state director for the conservative Concerned Women for America in Iowa. "Iowans today are significantly saddened to see our Majority Leader support an issue that stands in opposition to his former pro-life stance."

As of this writing, the House bill to loosen rules on federal funding for embryonic stem cell research hasn't come close to a Senate vote for a full year since its introduction. Senate Democrats began their own effort to draw attention to the lack of action on the bill, but so far have had limited success.

In order to preserve the issue's legislative viability, in early 2006 Frist entertained several alternative bills in the Senate. One of these bills, sponsored by Iowa Democrat Tom Harkin, would lift Bush's 2001 restrictions on federal funding for new embryonic stem cell research. Another proposed bill would look for ways to study embryonic stem cells without killing the embryo. As further evidence of his break with Bush on the stem cell issue, Frist said he was still interested in looking at as many alternatives as possible.

A SUBTLE DIVIDE

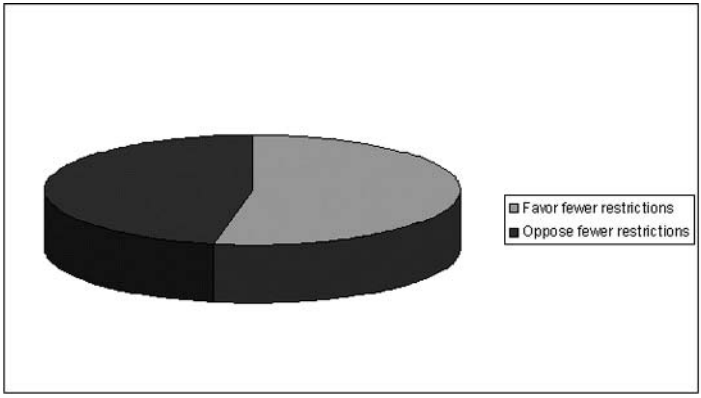
With the multiple arguments and pieces of legislation under consideration at the state and federal level, it is easy to forget the question at issue. It is not whether research using embryos for stem cell collection should be allowed, but rather whether it should be financed with the federal taxpayer's dollars. It bears repeating that, contrary to popular belief, embryonic stem cell research in the United States is *not* restricted in any way.

The restrictive public sector and the permissive private sector have muddied the public's mind about what can and cannot be funded. Kicking up additional dust has been the long-buried divide between social conservative groups. Some oppose abortion and embryonic stem cell research as its inseparable, equally evil twin. Others oppose abortion but see nothing wrong with trying to potentially reduce suffering by supporting stem cell research.

This divide spells trouble for Frist and the Republican Party in the future. The chairman of the conservative House Republican Study Committee, Representative Mike Pence of Indiana, stated in

FIGURE 9-1

Pie Chart based on May 2005 poll data from Gallup. Over half of those surveyed favor the easing of restrictions on federal funding of stem cell research.



an interview with *USA Today* that stem cell research is actually *more* divisive than abortion, in part "because of the heartfelt desire of many pro-life legislators to support medical research."

It is hard to see how religious conservatives who equate stem cell research with abortion can be persuaded to vote for more moderate, stem-cell-friendly candidates. As one reader of Patti Davis' opinion column replied, "Killing one person to harvest her body parts, for the benefit of another is pure evil, no matter how small or how far along the life's continuum the murdered donor is."

On the whole, Pence is sanguine about the division exposed by Frist's position on new, potentially troubling legislation opening up federal tax dollars for new embryonic stem cell lines. In his opinion, it should "wake up" social conservatives and send them back to the polls. "This will result in millions of Americans realizing we have a Republican majority in Congress but we don't have a pro-life majority in Congress."[2]

2. Preliminary indications are that Pence is correct. Although there has been no vote, of the 55 Republican Senators in early 2006, 13 have signed a letter urging President Bush to support federal funding for using surplus embryos from IVF clinics. Therefore, it's likely that there is a ceiling of only 42 Senators who would vote from a staunch pro-life position. It's a substantial number, but far short of a majority.

The next few years will tell whether Pence, Senator Frist, or the more liberal Democratic proposals have accurately captured the views of the majority of the American people. Even so, the dilemma that underlies the entire debate has been best summed up by Connecticut Representative Chris Shays, who declared, "God gave us intellect to differentiate between imprisoning dogma and sound ethical science." No matter which way one's sympathies may lie, let us all hope that this is indeed true.

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CHAPTER 10

STEM CELL SUPERPOWERS

"The global market for stem-cell and tissue engineering will create a billion-dollar industry, with thousands of new jobs," according to a column written for the *San Francisco Chronicle* by Ian Wilmut in June 2005. Wilmut is in a position to make the most educated of guesses. He is the scientific advisor to a subsidiary of the biotech company Geron. He also holds the prestigious—and somewhat long-winded—title of Chairman of Reproductive Science at the Research Institute for Medical Cell Biology at the University of Edinburgh in Scotland.

Rosy predictions about the future of stem cell medicine are nothing new. In fact, in the slow summer news months before September 11, 2001, it seemed that scientific prognosticators were in competition with each other to see who could predict the largest bounty for the profit-bound industry. But something else in the *Chronicle* column rings very much true and has a great deal of evidence to support it. Ian Wilmut boldly stated that, worldwide, "scientific superpowers are emerging in the field of regenerative medicine."

The column was written as a sort of congratulatory note. With

the passage of Proposition 71, Wilmut felt that "California is at the forefront in the United States." That may be so, but what does preeminence in the United States actually mean on a worldwide basis? Bold moves are taking place in Europe and Asia. Is it possible that the eventual "scientific superpower" will not be the United States, but rather a European state, or perhaps a non-western country?

OMNIS CELLULA E CELLULA

Germany is a good place to start a quick look at stem cell research outside the United States because it was home to an early pioneer in cellular study. Rudolf Ludwig Karl Virchow, a German doctor, pathologist, prehistorian, biologist, and politician, is another of those historical geniuses that dot the landscape of biological studies. In the mid-1800s, he originated the groundbreaking idea that disease began at the cellular level. A prolific writer, in 1855 he published his best known scientific treatise on the subject, titled *Omnis cellula e cellula*, Latin for "every cell originates from another cell."

Virchow was known for his wide-ranging work in medical theory and practice. Among other procedures he developed was a standardized method of performing autopsies, which is still one of the techniques used today. He is also credited with founding the branch of science known as cellular pathology. This field encompasses the study of the cellular basis of disease, including the examination of abnormal cell growth, metabolism, and division that could lead to diseases such as cancer.

Cellular pathology is one area that stands to be greatly advanced if stem cells are utilized in broadening our understanding of how a cell lives, differentiates, divides, and dies. Though the topic is still pursued with considerable interest by German medical companies, the limits to embryonic stem cell research are also being hotly debated there.

The German constitution does not explicitly dictate moral values or limits of research. Because of this, Germany's embryo policy is a bit more relaxed than in the United States. The government does allow importation and cloning of embryos for research and does not prohibit or discourage destruction of human embryos.

In 2002, the subject of embryo research flared up when German Chancellor Gerhard Schröder appointed a National Ethics Council to study the issue. The council recommended the limited importation of stem cells, but a special parliamentary commission disagreed, favoring a strict ban.

Issues of biotechnology have a special resonance in this European country, in no small part due to the Nazis' chilling history of experimentation with racial eugenics. Yet Germany is a country with a long, proud history of scientific achievement, and it would have been a bitter pill for Schröder and proponents of embryonic stem cell technology to swallow if German competitiveness in the biotech industry was put at risk.

Realizing that the parliament's commission had already recommended a much stricter set of laws, Schröder suggested accepting a compromise on the issue. By doing so, he explained, Germany would be able to keep pace with international developments and maintain a strong degree of influence on global research. His argument was persuasive. In the end, a razor-thin majority of the parliamentarians voted in favor of a compromise: The government would allow the import of embryonic stem cells for scientific research, but only under close supervision.

Reaction was predictably mixed. Regine Kolleck, the deputy chair of the National Ethics Council at the University of Hamburg, was pleased that at least some restrictions and regulation had been put in place. "The import was not prohibited before, it was completely free. Now we'll have a very restrictive law on the way the import is regulated, and that is more than we had before."

On the other hand, Alexander Kekulé, director of the Institute for Medical Microbiology in Halle, sounded very much like the pro-stem cell researchers in the United States when they issued warnings about federal funding restrictions. "Now that the parliament has decided that researchers can only use stem cells that have already been created, and prohibits German researchers from creating their own cells," Kekulé explained, "we therefore have a handicap. . . . This means we'll have to do research with cells that will soon be obsolete."

A CRAZY QUILT OF REGULATIONS

The European Union (EU), which represents fifteen European countries, has followed Great Britain's lead in banning reproductive cloning. But the EU has taken a markedly more conservative approach than England (see below) to the creation of human embryos. At present, Article 18 of the Council of Europe Convention on Human Rights and Biomedicine expressly forbids the in vitro creation of human embryos for research purposes.

The European Research Commissioner Philippe Busquin, however, said in an interview, "Regulating on ethical matters is the competence of member states. In Europe, we have a legitimate diversity of rules and ethical frameworks in the field of human embryonic stem cell research." In other words, since European countries varied greatly in their evaluation of embryonic stem cell research, the continent would be left without a strong, continent-wide policy.

The development of a patchwork quilt of laws and regulations appears to be playing out today. In mid-March 2006, the latest EU

FIGURE 10-1

The Stem Cell Global Policies Map. Countries colored in dark gray have a permissive or national policy on human embryonic stem cell research. Medium gray countries have a flexible policy. All flexible or permissive countries have explicitly banned human reproductive cloning.

conference on the subject ended in the usual diplomatic stalemate. A common human embryonic stem cell research policy remains elusive.

Germany and Ireland, for example, have decided to sharply limit controversial research and prohibit the use of surplus in-vitro-fertilization (IVF) embryos (though as stated above, Germany will allow the *importation* of embryos for research). Other countries—including Belgium, Denmark, and Spain—are opting to allow derivation of stem cells from surplus embryos created by IVF procedures.

Dr. Jochen Taupitz, a member of Germany's National Ethics Council, stated in an interview with *The Christian Science Monitor*, "There's no country in Europe where there is a unanimous opinion on this issue." He added that "in a European comparison, Germany has some of the strictest laws. . . . We are pretty isolated in Europe."

THE LEGACY OF BROWN AND WARNOCK

At the other end of the European spectrum lies the United Kingdom, which has the most open approach to embryonic stem cell research. The open, yet carefully administered policies in the U.K. are likely the product of Britain's good fortune to have already hashed out many of the issues convulsing the United States and the European Continent.

On July 25, 1978, the IVF pioneers Robert Edwards and Patrick Steptoe announced the birth of Louise Joy Brown. Little Louise was the world's first baby to be conceived outside the human womb. Two developments quickly became apparent.

First, Louise was born and developed completely normally, removing once and for all the fears that this type of fertilization would mutate into a horrific result just because it took place in a petri dish. Second, the technique of IVF was an evolutionary, not revolutionary process, based on many earlier models—not the least of which had been used in the veterinary sciences for years. As a result, it would be a breathtakingly short period of time before IVF became, if not commonplace, a relatively routine procedure for couples who had problems conceiving.

The intense debates that developed from the rise of the IVF clinic led the British government to take action. In 1982, Helen Mary Warnock was assigned as the chair of a special committee of inquiry into human fertilization. A nationally known philosopher of morality and accomplished writer on existentialism, Warnock was a formidable personality in her own right.[1]

Two years later, Warnock's official report came out in favor of allowing controlled research on human embryos up to 14 days after fertilization. This standard, which was followed by Ariff Bongso and James Thomson a decade later, is still used worldwide. The report's recommendations on using surplus IVF embryos for research were not as well accepted. Warnock's committee of inquiry decided by a 9-to-7 vote in favor of allowing human embryos to be created specifically for research.

Based on these recommendations, Margaret Thatcher's administration in 1990 set up the Human Fertilisation and Embryology Authority (HFEA), which has become the statutory body in the United Kingdom charged with inspecting and regulating all IVF clinics in the U.K.

The establishment of the HFEA in the years before work with human embryonic stem cells became available allowed the U.K. to make a comparatively orderly transition in accommodating the new technology. Legislation was amended to allow research for therapeutic purposes on cells derived from human embryos, including those created specifically for research, while banning reproductive cloning.

This approach has paid dividends in the years since. Britain is home to the world's first stem cell bank, which is heading up a worldwide project to characterize all the embryonic stem cell lines now available. In a 2006 interview, Dr. Neil Hanley, a clinician and scientist, funded by the Department of Health at the University of Southampton, discussed the benefit of the existing framework: "Investigators are at an advantage wherever they can investigate under a sound ethical and moral legislation. Problems only come when facts are distorted to bias the research performed."

1. In 1985, she was created a life peer as Baroness Warnock in the city of Westchester.

The HFEA also gave permission for Professor Alison Murdoch of Newcastle University to collect eggs from women volunteers for studies in nuclear-transfer research. This is being done with the goal of culturing patient-specific embryonic stem cells to develop treatments for illnesses such as Lou Gehrig's disease and Alzheimer's. In 2005, Murdoch's group announced the United Kingdom's first successful production of human nuclear transfer-derived embryo cells, which are slated for research into the treatment of diabetes.

BIOPOLIS

Perhaps not coincidentally, researchers from the National University of Singapore are also looking into curing diabetes. In 2004, the University's hospital partnered with a local biotechnology company with an eye to producing insulin-secreting cells from adult stem cells. The plan is that the stem cells will be extracted from a patient's own fat tissue.

With millions worldwide suffering from this potentially fatal disease, Singapore has made a major bid to be the next stem cell center in the world. As Dr. John Isaac of National University's department of surgery put it, "There is a race around the world to develop what we call surrogate islet cells—cells not derived from the human donor pancreas but cells from tissue, from stem cells, which can be coaxed into producing insulin and then transplanted."

In order to win this race, Singapore has put in place some of the most lenient restrictions on biomedical research. Serious plans were drawn up and executed in the mid-1990s to build up the country's biotech infrastructure. One of the more famous results was the construction of Biopolis, a complex built to house biomedical research laboratories and other related endeavors.

Located near the National University, Biopolis is comprised of seven buildings that sprawl over two-million square feet. Given the strictly limited space available on this island nation, providing that much real estate—and more—to the biological sciences reveals a significant commitment. It is the equivalent of throwing down the proverbial gauntlet to the current world leaders in biotech.

The Singaporean government has also channeled its financial

muscle into this enterprise by pouring over $1.8 billion into grants, tax breaks, and facilities to attract the world's top scientists. While there is no way to measure the results of this aggressive bid so far, it has not gone unnoticed that Alan Colman, one of the scientists who cloned Dolly the sheep in 1996, moved to Singapore in 2002.

Though it is not all targeted at stem cell research, the $1.8 billion still tops investments made in many other places around the world. For example, in the same year that Coleman left his home country for Singapore, the U.K. announced a £40 million ($70 million) investment in stem cell science by the country's research councils. Federal and state commitments in the United States also fall within this range, with the broad exception of California's $3 billion Proposition 71.

Perhaps it is only fitting for Singapore to express such a heavy level of interest and back it up with financial muscle. Consider the country's national flower, a hybrid orchid called the Vanda Miss Joaquim. The flower is not an indigenous natural hybrid. It was bred by Miss Agnes Joaquim in 1893 using grafting and cross-breeding.

The techniques she used were not at the cellular level, but it is clear that the citizens of Singapore have no problem with a man-made plant as their national flower. So they probably will have no problem adapting to artificially created stem cells.

PACIFIC RIM RACEHORSES

Singapore is not alone in its quest to be the center of stem cell research in Asia. Japan, China, and South Korea in particular have made impressive strides forward in research, and their level of expertise is, in many cases, on a par with or ahead of the West. South Korea has dominated the headlines as of late with its push in both embryonic and adult stem cell trials in human beings.[2]

Verifiable information on the dollar amount that has been invested in Japan for stem cell research has been difficult to locate. But Japan has made headlines as the country's researchers have

2. The advances and setbacks in South Korea are discussed at length in Chapters 11 and 17.

made impressive gains. One of its most recent and well-publicized advances was made by Dr. Shunichiro Miyoshi's research team at Keio University in Tokyo. Their goal was to increase the number of hematopoietic stem cells collected for research and the development of therapies for blood and heart diseases. The result was a smashing success.

The key was Shunichiro's idea to focus on collecting women's menstrual blood, harvesting stem cells that had been shed from the biologically rich lining of the uterus. Approximately 30 times more stem cells were harvested from the menstrual blood than from bone marrow. If this result can be duplicated and standardized, it will be a boon for researchers seeking to work with adult stem cells.

China's commitment to becoming a stem cell superpower is widely regarded as substantial—though how substantial is difficult to say. It is widely accepted that the Chinese government is dedicated to erasing the lead of Western countries in science via large-scale government investing. For example, in April 2005, Burton Richter, a Nobel laureate in physics, was quoted in an interview as stating, "As a percentage of GDP, [U.S.] federal investment in physical science research is half of what it was in 1970. [By contrast] in China, R&D expenditures rose 350 percent between 1991 and 2001, and the number of science and engineering Ph.D.s soared 535 percent."

Yet of these impressive numbers, how much is going specifically into biological or stem cell research? The claims are nebulous and difficult to verify, but if even a portion are correct, China is well positioned to contest the lead in the near future. Reportedly, the Chinese government has invested hundreds of millions of dollars in stem cell research. One tangible piece of evidence of this is Dr. Cao Yilin's Tissue Engineering Research Center, located in Shanghai.

According to Yilin, construction of the new center cost a quarter of a billion dollars, of which $200 million came from the government. Yilin, who learned cellular cultivation techniques during six years at the University of Michigan and at Harvard Medical School, states that his lab already was cultivating multiple types of body tissue: "Skin, cartilage, tendons, corneas, vessels, and so on. In the future, if this is successful, I think it's a very good way for people with organ and tissue loss."

Perhaps the final word on China's future in stem cell research can be summed up by Stephen Minger, the director of the Stem Cell Biology Laboratory at King's College in London. He recently went to China to assess the country's stem cell research program. According to Minger, "We were just flabbergasted by what we saw. World-class research centers, amazing infrastructure, committed large amounts of government support. I think they are going to be a dominant force in this field."

HOLDING THE LINE

For a nation whose sense of strength is entwined with scientific and educational prowess, the United States spends a great deal of time worrying about whether it has been eclipsed by other nations in fields ranging from nuclear power to space exploration. Stem cell development has proven to be no different.

Current doomsayers claim that the United States will fall behind the rest of world as a leader in science, ceding the lead to Europe, South Korea, or Singapore. For example, in a public address at the University of Wyoming, Professor John D. Gearhart of the John Hopkins Medical School declared that "There is a feeling we have got to do something rapidly or we are going to be really out of the ball game."

Opinions on the subject are mixed. The European countries that were supposed to have surpassed the United States are still playing catch-up, and with the exception of the U.K., Europe's laws on the use of stem cells are as restrictive as our own. The general impression is that Asia has made the greatest political and financial commitment, and is pushing hard, but for the most part the Asian countries interested in stem cell research have started from farther back.

Offering an accurate assessment of "who's in the lead" is problematic, for a couple reasons. First, money offered for stem cell research is usually public knowledge, but the studies and achievements—until they are ready for publication—are not. Second is the fact that "progress" is a relative term. Who is to say that committing 1,000 embryos for research into curing diabetes places a

team "ahead" of another team that uses 500 embryos for the purpose of regenerating nerve cells?

One way to gauge the possible future of a country's research is by how many researchers it is able to attract. Again, there is no systematic study, only anecdotes of various big-name researchers who may be switching jobs—and countries—as much for personal reasons as for a more open research environment.

For example, while Alan Coleman did leave the U.K. for Singapore, stem cell researchers have come to Britain from other countries. Roger Pedersen and Stephen Minger came from the United States to Cambridge and King's College London, respectively. Miodrag Stojkovic, a member of Alison Murdoch's diabetes-studying team in Newcastle, is originally from Germany.

But it takes more than a handful of traveling scientists to claim that a "brain drain" is taking place. Historically, European scientists have left their own countries, usually frustrated by the Byzantine layers of regulation and bureaucracy, to work in the United States. And although federal investment in embryonic stem cell research is currently under greater restrictions in the United States than in most of Europe, it appears that state governments such as California are willing to make up for the any perceived shortfall.

DUAL CELEBRATIONS

In May 2005, scientists at Newcastle University, the first university in Britain to obtain a license to carry out therapeutic cloning for stem cell research, made an amazing announcement. Two members of the British team, Professor Alison Murdoch and Dr. Miodrag Stojkovic, said they had successfully cloned from a human cell using nuclear transfer.

It was a great triumph, and yet the mood of jubilation felt by the research team had to have been undercut by a similar announcement from the other side of the world. On that same day, a team of South Korean scientists went public with the proclamation that they had developed the first lines of patient-specific stem cells, designed to match the DNA of a specific person.

The British scientists said they were delighted to hear of the

great progress made by the Koreans. The congratulations they sent were most likely sincere. After all, in some ways the scientific community benefits from all major advances.

Yet given the amount of time and "sweat equity" that is required to isolate and culture cells, one cannot help but wonder whether the congratulations were given through clenched teeth. To have to share the press coverage on the day of their big unveiling would have been irksome, as well as the thought that, once again, Asia may have stolen a march on Europe and America.

As it turned out, they need not have worried in the slightest.

CHAPTER 11

THE RISE AND FALL OF SOUTH KOREA'S CLONING KING

If ever there were a cautionary tale for ambitious scientists to learn from, the saga of Dr. Hwang Woo Suk is one of the best examples. From the moment the kindly faced researcher appeared on the international scene, he at once came to embody the hopes of a nation that justly prides itself on its scientific know-how. Across the Pacific, he became a *cause celebre* to justify the work being done with human embryos. And just as with the classic rise of a rock n' roll star, or a dreamer who flew too close to the sun, his fall was just as dramatic as his ascension.

THE BEAUTIFUL LIFE PATH OF HWANG WOO SUK

Dr. Hwang's life came tailor-made for image-makers to craft into a national hero. As the saying goes, the events had the added benefit of actually being true. Hwang's start in life was relatively humble,

his family's background being more in farming and agricultural pursuits than hard-core science.

And yet, in a society that is intensely devoted to hard work and achievement, he was a role model. Hwang had grabbed the limelight by dint of sheer willpower and intellect, first as a leading veterinary scientist and then as a researcher into the origins of human life itself.

His first scientific claim to fame was stunning indeed. Hwang, while employed at the prestigious Seoul National University, published a paper in which he claimed to have extracted a new line of stem cells from a cloned human embryo. These stem cells were truly pluripotent, and could conceivably be directed to turn into any type of cell needed by a patient.

Since the new line was from a cloned embryo, Hwang was on solid ground when his paper was published in *Science* magazine in June 2004. He suggested that in the very near future, patients could be treated with cells containing their own DNA. Theoretically, any use of these cells to repair or replace damaged tissue in the patient's body would avoid rejection by the patient's immune system.

This of course was—and remains—a long-term goal of stem cell researchers worldwide. Not only did it make Hwang known internationally, it also put South Korea on the map in terms of the biological sciences. Perhaps unfairly, South Korea, much like the other Asian "tiger" economies such as Hong Kong and Taiwan, had lived in the shadow of Japanese technological and scientific achievement.

Rather than being known for advances in lasers and robotics, these countries were thought of as simply the most efficient producers of goods. In other words, superb at trading and upgrading technologies, but not real leaders of pure science. This all changed with the arrival of Hwang Woo Suk. In a single stroke, South Korea had leapfrogged Japan, Europe, and the United States to become the leader of the main area of biotechnology that could transform the healing arts around the globe.

The South Korean's second triumph was, astoundingly, not long in coming. Whereas even luminaries such as Einstein had taken years to accomplish further major new achievements, it

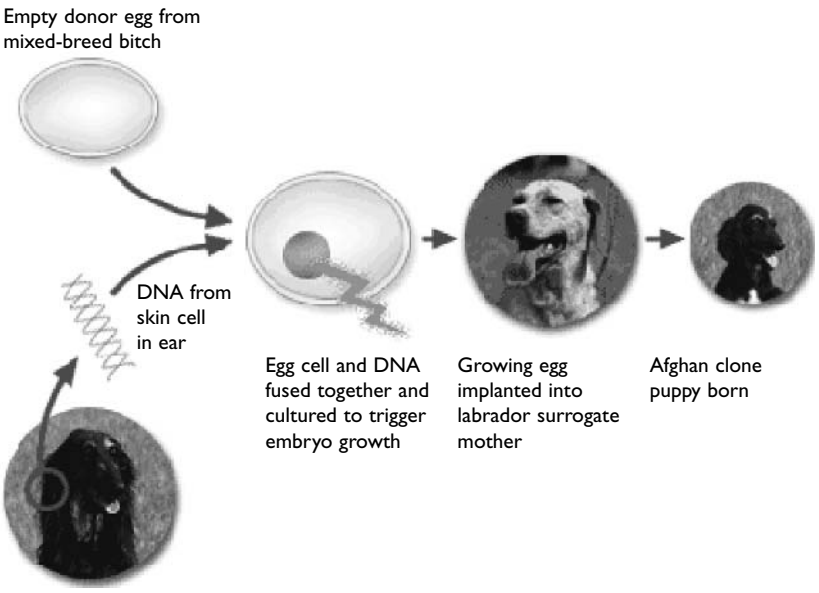
was scarcely a year later, in 2005, that Hwang made his next breakthrough. While other teams had been stymied at cloning an animal more advanced than sheep, Hwang and his team had managed to go one better and create "Snuppy", the first canine clone.

From a public-relations perspective, this was pure gold for Hwang and his team. The jump to cloning dogs from sheep, according to one researcher, was "an evolutionary jump in difficulty—not a revolutionary one." But those who focused on the scientific difficulty of the task were missing the point. Dolly, the cloned sheep, was a cuddly but relatively alien creature to most people. An interesting news story, but not one that stirred the emotions.

Snuppy, on the other hand, was a handsome Afghan hound, with languid brown eyes and luxuriously long fur that would have made Lassie jealous. Snuppy was as recognizable as the family dog. In other words, it was an animal that people were innately

FIGURE 11-1

Schematic showing the process of how "Snuppy" was cloned.



familiar with. It moved the idea of cloning from the barnyard into the family living room.

The ultimate praise from the media came from *Time* magazine, which in 2005 named Snuppy the dog as "Invention of the Year." It was a development that undoubtedly cheered those in the sciences. It likely also sent a chill down the backs of those who were disturbed by seeing a dog described as an "invention."

Thanks to Snuppy, Hwang had now not only moved his team—and by extension, the efforts of other scientists in his country—into the preeminent world position in cloning research, he had also proven that it had not been a fluke that had brought them to this point. All that remained were some small questions about whether the breakthrough had happened less due to precision work and more from simple volume.

For example, the *New York Times* reported that Hwang and his researchers had toiled for almost "three years, seven days a week, 365 days a year and used 1,095 eggs from 122 dogs before finally succeeding." Put another way, the creation of a single successful dog clone—the adorable and charming Snuppy—was the result of discarding over 1,000 lab-grown dog embryos. The 1,095 eggs had yielded a success rate of less than two-tenths of a percent—a pair of cloned puppies, one of which had expired before it was even a month old. And perhaps more troublingly, it remains unclear as of this publication whether any research group in the world has been able to repeat this work.

But such details did not matter at the time. Dubbed "The Pride of Korea," Hwang had now been placed into the role of national hero. Rewards of all kinds began to flow in. The government pledged massive financial support for his efforts—something that many scientists are only able to dream about—and scientists from around the world were clamoring to collaborate with him.

Domestic publishers aiming to cash in on the newfound celebrity rushed biographies to the bookstores. Ten of the books published were for children. For example, titles like *Children, Let's Learn from Hwang Woo Suk's Success* and *The Beautiful Life Path of Hwang Woo Suk* were written and pitched directly for the pre-teen audience. According to the reports, *The Beautiful Life*

Path actually told the fairy-tale-like rise of a humble national hero it called "South Korea's Number One Scientist."[1]

Science-oriented books are a popular gift for Christmas in South Korea. The American equivalent might be the purchase of a chemistry set, or, in the case of very young children, a set of classical music tapes to engender the "Mozart Effect." To be sure, in the minds of many approving parents and relatives it certainly couldn't hurt to see if any of Hwang's amazing ideas, drive, and intellect would "rub off" on impressionable young readers.

NOBEL FOR THE ASKING

The South Korean government was as good as its word. They began pouring millions into Hwang's chronically underfunded lab. Hwang was also given round-the-clock security and free travel on Korea's national airline. Thanks in part to this government largess, Dr. Hwang's ambition was realized: to create a world-class center for embryonic cellular research—one that could provide scientists around the world with embryonic stem cells.

This facility, called the World Stem Cell Hub, opened in mid-October 2005 to mass international fanfare. Aside from its purpose as a research center, it was also marketed as a potential source of replacement tissue for people suffering from diseases ranging from kidney failure to heart disease. The adoring public's response was, unsurprisingly, tremendous. According to the research center, on the first day that patient applications were accepted over 3,500 responses were received through phone, fax, Internet, or even in person.

The acclaim grew to proportions that many might consider outrageous. Despite the fact that all but the most basic medical advances using stem cells were speculative at best, the South Korean government didn't blink at printing a postage stamp in Hwang's

1. Unfortunately for the publisher, the book was released on December 20, 2005. This was just a few days after Seoul National University started to probe work produced by Hwang's team after two collaborators said a paper they published in May 2005 was based on fabricated data. Understandably, the market for these books quickly bottomed out in light of the events that came to light.

honor. The stamp in question showed a figure leaping out of a wheelchair.

The story just got better and better as time went by. Hwang was not only a great scientist but a good man. Stories painted him as a visionary genius, a sort of combination Jonas Salk and Captain Kirk, helped in his quest to heal humanity by a dedicated and tireless laboratory staff who venerated him. Hwang, ever the benevolent manager, was described in one book as foregoing his ability to fly first-class and instead stay in the economy class so that more of his junior researchers could travel with him.

With such overwhelmingly positive press, there was no shortage of funding, no gap in the feeling that miracles were just waiting around the next corner. Volunteers arrived at the World Stem Cell Hub almost daily, offering themselves as research subjects. By all accounts, Dr. Hwang looked like a shoo-in for Nobel Prize nomination, even a strong candidate for winning.

PLUG AND CHUG

When it came to Hwang's experiments with human embryonic stem cells, the only fly in the ointment was the appearance that the processes used were extremely inefficient. According to *Science*, the magazine that published his initial claim, it had taken the Korean team 242 human eggs to yield just one embryonic stem cell line. Once again, the question was raised as to whether genius was involved, or the more simple "plug and chug" method of generating large numbers of embryos and hoping that one would develop.

This last reservation was seemingly shattered when Hwang claimed, in the May 2005 issue of *Science*, that his team had again successfully cloned human embryos. The differences this time were twofold. First, his team had engineered an improvement in the use of the donor eggs by a factor of ten. In other words, where before it had taken over 200 eggs to yield a new line of stem cells, it would now take only a couple dozen. Second, Hwang had been able to derive not just one new stem cell line, but a grand total of eleven.

Celebrations soon followed. Triumphant predictions were made that the holy grail of therapeutic cloning was about to become a re-

ality. Patients would be able to utilize these cloned embryos to generate tissue matching their genetic makeup, which could be used in regenerative medical treatments. The impact of this new discovery, if it could be made commercially feasible, would transform society as much as the Internet, the transistor, or the atom bomb had done.

The claims made by Hwang's team also provided a powerful cudgel for stem cell advocates to use against the Bush administration, which had taken the stand against the use of federal monies for certain kinds of stem cell research. Hwang's innovations were proof positive that the Bush policy limiting federal funding of embryonic stem cell research to lines created before August 9, 2001 was hobbling American firms and allowing research in other countries to leave the United States in the dust. No small amount of this lobbying effort was made up of patriotic fervor. What the launch of Sputnik was to the fledgling U.S. Space Program, Hwang's discoveries could be to America's biotech industry.

SLIPPERY CHOPSTICKS

One question on many researchers' minds was: How did Hwang and his team achieve a tenfold improvement in their embryo cloning process? Rick Weiss, the science writer for the *Washington Post*, reported that the secret was Hwang's manual dexterity under the microscope. It was this delicate touch that had allowed him to tease out the minute cells as well as the nucleic cell structures that were to be implanted. Hwang attributed this amazing ability to "the Korean tradition of eating food with difficult-to-master steel chopsticks."

As anyone who has eaten with chopsticks knows, the relatively rough surface of wood chopsticks makes them marginally easier to hold and manipulate. The difficulty factor begins to increase with the use of other materials such as ivory or metal. And yet the chopsticks Hwang used weren't the only thing that was beginning to look more than a little slippery to some reviewers. Rumors began to circulate that beneath the surface not all was as it appeared to be.

In the late fall of 2005, the first of many blows began to rain down on Hwang's research, proving it to have been more smoke and

mirrors than stem cells. A South Korean television station began to investigate various allegations, many fuelled by the appearance of anonymous Web log entries on a website frequented by young South Korean scientists. The entries, whose source has never been concretely identified, questioned the authenticity of some of the pictures in the *Science* article.

The posted entries claimed that certain "marker" gene sequences from some of the DNA in the new stem cell lines appeared to match the patients' cells too perfectly. That in turn raised the possibility that the new lines could have been duplicates of existing lines, instead of the newly created ones that Hwang had claimed they were.

The initial investigative journalism done by the television station, which resulted in a program that confirmed something was amiss, was initially suppressed. It is not clear where the pressure to do so came from—possibly the government, which had a vested interest in keeping Hwang's reputation spotless, or by the station itself because of the uniquely strong national support that the researcher enjoyed.

However, the rumors persisted and the avalanche of bad news and shifting stories continued into the winter. On December 16, 2005, Dr. Hwang held a televised press conference to deny the accusations. He acknowledged that there were flaws in the paper in question—for example, he had only created eight cell lines at the time the paper was submitted, although he said the total did reach eleven later. After being briefly hospitalized for "stress and exhaustion," Hwang appeared publicly a week later to announce that he was asking for the paper to be withdrawn.

The explanation for the suspicious photographs was glib, and not altogether convincing. Hwang claimed there had been an administrative mistake where some of the photographs of the new stem cell lines were duplicates, not originals.

But even this story did not hold water for very long. Kim Sun-Jong, one of Hwang's junior researchers, claimed that Hwang had ordered him to submit duplicate photos of two existing lines of stem cell lines and present them as the eleven new ones. Hwang accused Jong of making the switch on his own, but the researcher claimed

that Hwang paid him a total of $30,000 to doctor the photographs. These allegations are still being investigated by Korean prosecutors at the time this is being written.

EGGS AT ISSUE

Yet another of Hwang's colleagues then claimed that the second experiment had required hundreds more eggs than reported. This knocked the second pin out from the claims of Hwang and his team. The effort to generate donor stem cells still had not overcome the hurdle of needing literally hundreds of embryos.

Dr. Gerald Schatten, director of the Pittsburgh Development Center and a medical school professor, had been one of Hwang's research partners in the United States. As the scandal continued to unfold, he immediately ended the twenty-month collaboration with Hwang's team and asked for his name to be removed from the paper that had been submitted.

While declining to give any public interviews, Dr. Schatten cited a report that had raised his concerns. This report had been published in the scientific journal *Nature*, and had contained troubling allegations that women working with the team's research had been among those who had donated eggs.

"I continue to believe in the scientific accomplishments of Professor Hwang and his research team at Seoul National University," Dr. Schatten wrote, adding that he still believes the Korean team's work constitutes "landmark discoveries accelerating biomedical research."

Nature reported that two members of Dr. Hwang's lab team had donated eggs for the experiment. This was a serious ethical breach, as the donors were supposed to have no connection to the experiment and no possibility of benefiting from it. It also raised troubling questions as to whether some sort of social or professional pressure had been involved. One of the donors was an aspiring graduate student, and she might have felt pressured to donate eggs.

As it happened, both women retracted their initial claim. Hwang, ever the front person for his team, explained that the women's poor English had caused them to be mistranslated. But by then, the cat

had been let out the bag. Another Korean television network had tracked down and interviewed many of the egg donors, and the results came as a shock. Many women claimed that they not been told they were part of a study. Others confirmed that their eggs had indeed been purchased.

The ethical guidelines issued by the National Academy of Sciences expressly forbid women who donate eggs for embryonic stem cell research from receiving financial compensation. In the eyes of the NAS, paying for the eggs would be tantamount to the poor selling extra kidneys or other organs to the highest bidder on eBay.

The day before the report aired, Sung Il Roh, head of Seoul's MizMedi Women's Hospital, which processed the egg donors for Hwang's study, admitted publicly that he had paid sixteen of the women participating in Hwang's research about $1,500 each. After more than a year of denial, Hwang finally admitted that he had used eggs donated by lab workers, and that others had been purchased for use by his team for research.

The rest of Hwang's research was subjected to intense scrutiny by an expert panel at Seoul National University. As of this writing, the panel has found that "the laboratory data for eleven stem cell lines that were reported in the 2005 paper were all data made using two stem cell lines in total."

It was further reported how the lab had pulled off the illusion, by splitting cells from one patient into two separate test tubes, rather than actually match cloned cells to a patient's original cells. "Based on these facts," wrote the panel, "the data in the 2005 *Science* paper cannot be some error from a simple mistake, but cannot be but seen as a deliberate fabrication to make it look like eleven stem cell lines using results from just two."

COLD FUSION REVISITED

The stem cell debacle of 2005 wouldn't be the first time that the scientific community, enraptured by the illusion of a genuine breakthrough, made a too-quick rush to praise and a tardy effort to judge. In early 1996, chemists from the University of Utah claimed they had successfully generated a "cold fusion" nuclear reaction. Put sim-

ply, cold fusion is nuclear fusion occurring well below the several-million-degree temperature typically required for thermonuclear reactions.

Such a discovery would have changed the world by providing limitless, cheap, low-pollution energy. But the claims turned out to be false. A combination of ambition, fear of competition, and academic pressure had led the researchers to announce the discovery before proof had been made of the discovery. Although personally devastating to the career of the scientists in question, the fate of the cold fusion field was worse. The association with the term to this day is one akin to a nuclear physicist seriously discussing power sources for UFOs or the location of Atlantis.

At this point, Hwang's scandal hasn't seriously tarnished the credibility of the stem cell research field in general. In fact, it should be noted that several of Dr. Hwang's contributions were genuine, and truly groundbreaking. The technique of gently squeezing the nucleus out of a donor egg rather than sucking it out is one. The idea of inserting the entire adult donor cell, not just its nucleus, into the hollowed-out recipient egg is another. And, of course, the existence of Snuppy the cloned dog does appear to be legitimate—no matter how many failed attempts were required to achieve a single success.

Hwang is not getting "off the hook" for his over-zealous take on his research. Chung Un-Chan, the dean of Seoul National University, has stripped Hwang of his "chair professor" post and is moving to punish him and six other professors over the faked reports. A disciplinary committee is expected to issue a final punishment within few months.

Hwang may have been ambitious, and in love with the limelight that so rarely shines on researchers who are justly deserving of moving society ahead into the future. However, it seems unlikely that he set out to intentionally perpetrate a fraud on the public. What seems more likely to me is that someone—whether Hwang, a member of the lab staff, or the entire team together—believed strongly enough in the work to be willing to cut a single corner. Then two. And then—it was only a matter of time.

On a cloudy Friday afternoon in February 2006, Dr. Hwang left his position as a university professor. He made a short public

announcement as he did so. “I sincerely apologize to the people for creating a shock and disappointment,” Hwang told reporters, “With an apologetic heart . . . I step down as professor of Seoul National University.” His regret was earnest and seemed, at its core, to be absolutely truthful.

If only his research had been as well.

PART III

STEM CELL CURES AND CURSES

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CHAPTER 12

THERAPEUTIC CLONING AND REGENERATIVE MEDICINE

Clones stole the pop-culture spotlight in the early 1990s with Michael Crichton's book and the subsequent Steven Spielberg movie, *Jurassic Park*, where cells from a prehistoric mosquito's last blood meal brought dinosaurs back to life. But clones wouldn't remain in the pages of a fiction bestseller or on the silver screen. In 1997, Dolly the sheep garnered a bumper crop of press on how any organism, even human beings, could soon be cloned, and suddenly what seemed like science fiction was dumped into the average genetic researcher's lap.

Cloning is the process of creating a genetically identical copy of an original organism. And although it seems like a twentieth-century idea, cloning is actually a part of natural processes, and had taken place many decades before people thought it was attainable. Since a variant of the cloning process plays such a large role in stem cell therapies, it's worth taking a look at how cloning processes work.

TWIG FROM THE FAMILY TREE

The term *clone* is derived from the Greek word for "twig," and is very appropriate for this offshoot of a family tree. In nature, clones are made either when an organism reproduces asexually or when two genetically identical individuals are produced by a splitting of the fertilized egg, as with identical twins. Natural cloning is more commonly seen in simpler organisms (since asexual reproduction is a frequent survival strategy), but relatively rare in humans—the odds of conceiving identical twins is roughly 1 in 250.

Of course, the popular meaning of the term "clone" is an identical copy that has been created by some conscious design. Under this definition, the first artificially created clone was made in 1885, and the animal used was a sea urchin.

Hans Adolf Eduard Driesch was a German biologist who performed the cloning experiment in order to prove that cell division does not result in a loss of genetic material. Using the latest "cellular surgical techniques" of the day, he simply shook the container holding the urchin's dual-cell embryo to separate them. As he expected, both cells grew into healthy, normal adult sea urchins.

The science of cloning began to accelerate in the twentieth century, making larger advances as the tools and techniques became more refined with each generation of researchers. In 1902, embryologist Hans Spemman experimented with separating dual-cell and multicell salamander embryos. The cells that he had separated, when left on their own, developed into identical salamanders. In this case, the state of the art tool used to gently pry apart the sticky salamander cells was a single strand of his son's baby hair.[1]

In 1952, Philadelphia scientists were able to clone frog cells using the nuclei of early tadpole embryos. This was undisputedly artificial cloning in the sense that we understand it, as they utilized techniques of nuclear transfer that have only been slightly modified over time. Adult frog clones were created the following decade.

A pair of cloning accomplishments involving mammal embryos

1. Depending on the definition used, one could argue that the experiments carried out by Hans Driesch and Hans Spemman were not instances of true cloning, but artificial twinning.

bracketed 1986. Early in the year, Steen Willadsen, an English scientist, announced he'd cloned a sheep's embryo. Later the same year, American researcher Neal First claimed to have cloned a cow's embryo. Both men were able to keep the cell tissue alive in lab conditions, but neither team attempted to clone from adult cells.

At this stage, enabling adult genetic material to revert to an embryonic state was considered a flat-out impossibility. But eleven years later, after over two hundred attempts at cloning sheep, Dolly arrived on the scene and upended a number of notions about how life could be shaped and reproduced almost at will. Scientifically speaking, all bets were now off when it came to manipulating cells and fertilized eggs.

CREATION AND DESTRUCTION

When most people talk about cloning, they generally mean the copying of the entire organism, as in the case of Dolly the sheep. Since this is the kind of cloning that yields offspring, for clarity it is called *reproductive cloning*. At present, researching this kind of cloning to create new human beings is shunned in the scientific community, and nations that have any laws on the books at all in regards to stem cell research and cloning embryos expressly forbid the reproductive cloning of humans.

When discussing the benefits of stem cell research, however, what is actually being discussed is *therapeutic cloning*. This is when embryonic stem cells are harvested from the newly created embryo and expanded in a culture dish. Though the goal in therapeutic cloning is different, both types of cloning yield a clump of cells that has the potential to grow into a whole organism.

In therapeutic cloning, the host egg is first isolated in a petri dish. The outer cell wall is then penetrated without destroying the egg, and a tiny pipette is used to gently suck the nucleus, with the host's DNA, out of the cell in the same way one might pit an extremely tiny olive. The result is a hollow structure called an *enucleated cell*.

The empty space within the host cell is quickly injected with a nucleus from the cell of the donor. (Alternatively, the donor cell can

be placed in extremely close proximity and fused with the host cell by means of an electrical pulse.) Now the host cell's mechanisms have essentially been "reprogrammed" to make copies based on the new nucleus' genetic codes.

If this procedure were to be used for reproductive cloning, the egg would be stimulated to begin dividing and developing. The newly created embryo would be genetically identical to the donor source of DNA. The resulting offspring would have only one genetic parent with whom it shares all its genes. Once reaching the blastocyst phase, the embryo can be implanted in a womb to develop naturally.

In therapeutic cloning, the embryo is also stimulated to begin dividing. But the cells will never be implanted in a womb. Instead, the embryonic stem cells are taken from the inner cell mass of the embryo, when it has formed into the ball-shaped blastocyst stage of just a couple of hundred cells.

As of this writing, the technology does not exist for doing this without tearing apart the delicate blastocyst. The procedure, which is not too unlike shelling extremely delicate peas from a microscopic pod, must necessarily puncture and destroy the embryo. This allows the precious cargo of stem cells to spill out for collection into the petri dish medium like a handful of jewels strewn on a blood-red carpet.

One of the major biological concepts overturned by the therapeutic cloning process is that once cells have differentiated, they cannot be induced to revert. Yet in this one circumstance, the rules have been broken. The isolated nuclei of an adult donor cell can revert back to pluripotency by being exposed to the inside of an egg. In effect, this creates a fertilized egg with the donor's genetic complement without going through the process of fertilization.

In the past two or three years, it has been conclusively demonstrated that human nuclear-transfer cloning works regardless of the most basic differences between the host cell and the donor cell. The nucleus donor and the host egg can be from the same person or from different people. The two parties can be of different races, gender, and ages—nuclei from people as young as 2 and as old as 56 have been successfully transplanted.

REPLACEMENT PARTS

Therapeutic cloning has great potential for generating stem cell lines that are genetically matched to the donor's genetic makeup. These stem cells lines in turn have the potential to provide amazing therapies for illnesses and injuries that have no cure today. Therapies to remedy damage due to disease or genetic abnormality—and eventually, even physical injury—are part of the quickly expanding field of *regenerative medicine*.

Regenerative medicine is a field that is still very much in its infancy. "Before stem cells can be used routinely, there is a great deal more that researchers have to learn," reports one embryonic stem cell researcher. "We still don't know what signals are required to make the stem cells mature into specific tissue types. It's also only educated guessing at this point about how to avoid tumor formation or rejection after transplanting stem cells into a new host. You might say that Nature holds her cards close."

The rejection issue is currently being researched at Stanford University, utilizing strains of laboratory mice for testing. I discussed some of the latest findings with Dr. Micha Drukker of the Silberman Institute of Life Sciences. Drukker has been part of an international research team examining the immune response that might be launched against transplanted stem cells.

"We used two experimental platforms to examine the in vivo immune system response toward transplanted stem cells," said Dr. Drukker. "First, mice with both normal and immunodeficient immune systems were used to identify T cells as the major component that causes rejection." T cells are a subset of leukocytes, or white blood cells. They can be thought of as the "hunter-killer" cells that swarm an infection. "Second," said Drukker, "mice that were conditioned to carry peripheral blood leukocytes from human origin were used to test the response toward undifferentiated and differentiated human embryonic stem cells."

Using this model, Drukker's research team detected only a minute immune response toward both undifferentiated and differentiated stem cells over the course of a month. "Our data showed that stem cells evade immune destruction due to a low immuno-

stimulatory potential," said Drukker. If this feat is replicated in human stem cell transplants, then the possibilities for healing damaged organs and tissue without fear of rejection greatly expands the range of possibilities for stem cell therapies.

Dr. James Thomson, whose team was the first to successfully isolate and reproduce human embryonic stem cell lines in 1998, is alternately hopeful and cautious about the developing potential for regenerative medicine and stem cell based transplants. In a recent interview with MS-NBC, Thomson cited the existing mismatch between the need for pancreatic tissue to treat diabetics and the available amount for donation.

"There's just this mismatch between the availability of tissue and the need: There are a couple of thousand appropriate pancreases donated in the United States every year for [transplantation] . . . and there are over a million people with diabetes," he noted. "So you'd think if you could just come up with a better cell, then that would go directly into transplantation."

Pancreatic islet cells have previously been taken from dead organ donors and were often damaged from cold storage or by toxins in the blood after death. The cells produce the hormone insulin, which regulates blood sugar levels. Thomson notes that the problem stem cell researchers must overcome is the safety issue.

Islet cells taken from cadaver tissue are not known for introducing a cancerous growth. "But with stem cells, if you manipulate them in culture for a long time, they will accumulate mutations. It's a fact of life. It's just a question of differences in the rates. If you accumulate enough of those mutations, you could actually create a cancer," Thomson cautions. "This has nothing to do specifically with embryonic stem cells. It's just any cell you put in a tissue culture. But if you're going to take those cells and put them into somebody's body, you want to make really, really sure you have some way of dealing with that—because if you're diagnosed with diabetes and you're six years old, you're going to live a very long, productive life. It's a pretty normal life until the end. . . . But you don't die from cancer in a couple of months."

DUELING VISIONS

While Thomson is concerned about patient safety, other scientists feel that the issue is whether therapeutic cloning should be viewed in the same light as reproductive cloning or abortion. The issue is far from being settled. A 2004 panel held at the National Academy of Sciences showed how even the very terminology used by the research community fueled controversy between opposing experts.

In the opinion of Dr. Leon Kass, a member of President Bush's Council on Bioethics, the term used for therapeutic cloning, Somatic Cell Nuclear Transfer (SCNT) unfairly conceals the true nature of the procedure. Kass' complaint stems from the fact that SCNT is an "opaque" name that does not convey to the general public what really is going on in the laboratory. "It obscures all moral issues," he claimed, insisting that the "immediate product of SCNT is a cloned human embryo that will produce a human being."

Kass is theoretically correct. Again, it is a question of environment. When an adult cell's nucleus is neatly slipped into an enucleated egg cell like a hand into a smooth leather glove, it reverts to acting like a regular embryonic cell. This cell will either reach the blastocyst stage when plated on a petri dish, or potentially move into the embryo, fetal, and birth stages if successfully implanted in the human womb. Whether Kass is correct that the term SCNT trivializes or masks the true procedure is subjective and open to interpretation.

Kass is not alone in his opinions. James Sherley, an associate professor of biological engineering at the Massachusetts Institute of Technology, agrees that the use of cells from cloned embryos is scientifically and ethically dubious. Sherley, in an outspoken interview, claimed, "Both scientists and physicians know very well that human embryos are alive and human. A human life begins when a diploid complement of human DNA is initiated to begin human development. Therefore, a life can be initiated by the fusion of sperm and egg or by the introduction of a diploid nucleus into an enucleated egg (i.e., 'cloning'). Given that embryos

are human beings, they have a right to self and a right to life. Exploiting their parts . . . for research is moral trespass that society should not allow."

James Thomson's vision is, of course, very different. "The bottom line is that there are 400,000 frozen embryos in the United States, and a large percentage of those are going to be thrown out," he pointed out. "Regardless of what you think the moral status of those embryos is, it makes sense to me that it's a better moral decision to use them to help people than just to throw them out. It's a very complex issue, but to me it boils down to that one thing."

Thomson correctly noted that even religious conservatives' attitudes moderated substantially with the idea of using embryos that had already been created via IVF procedures. In other words, it appears that some, if not a great deal of the ire generated by therapeutic cloning stems from the creation of embryos for that specific purpose, not the exploitation of existing material.

"Part of what's happening," said Thomson, "and the reason why things kind of stalled, is that nuclear transfer and therapeutic cloning was intermixed with trying to make new cell lines from pre-existing embryos. They're very separable. There are some scientific reasons why nuclear transfer and therapeutic cloning might have merit, but most of the value of this technology can be captured simply by making cell lines with existing embryos." This little-known distinction is the likely reason that President Bush's compromise on utilizing existing stem cell lines was tolerated by religious conservatives.

Given enough time, it is likely that therapeutic cloning will become more acceptable to the vast majority of Americans who view it with a certain queasiness today. This will be for two reasons. First, given the pace of advancements in the field of cellular surgery, it should eventually be possible to remove portions of the inner cell mass of a blastocyst without destroying the embryo.

Second, a little noted fact about stem cell research is that the knowledge gleaned at the cellular level allows the best window into how a disease such as Parkinson's or diabetes works. Stem cells, in other words, can operate as valuable research tools in the

background, instead of taking center stage as a transplant therapy. Once every aspect of a disease's biology is thoroughly understood, a targeted drug or therapy can be developed and administered. Eventually, it may be one where a patient will never know that a stem cell was involved in figuring out the remedy.

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CHAPTER 13

RESOLVING THE DEBATE: ADULT VS. EMBRYONIC STEM CELLS

At the most basic level, the promise that stem cells hold is also the source of the controversy over them. The idea that replacement parts for our bodies might one day be as easy to create as ordering prescription medication from the local drugstore is breathtaking. But if these same cells can only work their magic through the destruction of human embryos, then cure and curse will be one and the same to many people. To those who see a human being's life as starting from the moment of fertilization, regenerative medicine via stem cells is nothing more than *high-tech cannibalism*.

There is an alternative, imperfect though it may be. In recent years, scientists have discovered that similar kinds of cells can be found outside the holy sphere of the human embryo's blastocyst. These "adult" stem cells can be found in the blood, the pockets of our bone marrow, the umbilical cord, under the dermis of the skin, and, just perhaps, buried deep in the brain.

ASC PROS AND CONS

Adult stem cells (ASCs) are the technology of choice among those who morally object to the use of embryonic stem cells. At a May 2005 White House press conference, President Bush reaffirmed his opposition to funding embryonic stem cell research outside of the existing stem cell lines, but praised the use of "alternative sources" of stem cells. The ones mentioned in the above paragraph, such as stem cells from bone marrow and umbilical cord blood, are classic examples of ASCs.

"With the right policies and the right techniques," Bush asserted, "we can pursue scientific progress while still fulfilling our moral duties." But is this indeed the case, or is it wishful thinking? As with many complex subjects, there is no clear-cut answer.

The degree to which adult stem cells can be put to use often depends on who is being interviewed. However, if one sticks as closely as possible to what has been reliably reproduced in multiple laboratories over time, some hard facts do become available. That is, at least as "hard" as the facts can be, before the technology advances yet further and changes reality yet again.

A fair number of therapies involving adult stem cells are in human clinical trials at present, and the number continues to grow. It is likely that these therapies will make their appearance at the local hospital or health clinic long before embryonic stem cells can even begin to make it to human trials. At the third annual meeting of the International Society for Stem Cell Research, held in 2005, the clear majority of the presentations dealt with therapies related to adult stem cells. Clearly, the interest—and, not coincidentally, the private sector venture capital—lies in ASCs for now.

Adult stem cells have something of a trade-off in their makeup. They simply do not have the pluripotent ability to morph into any kinds of cell. However, this one-track orientation allows them to be admirably well-suited building blocks for a limited number of therapies. For example, blood-specific illnesses such as leukemia stand to benefit from the use of adult stem cells collected from bone marrow.[1]

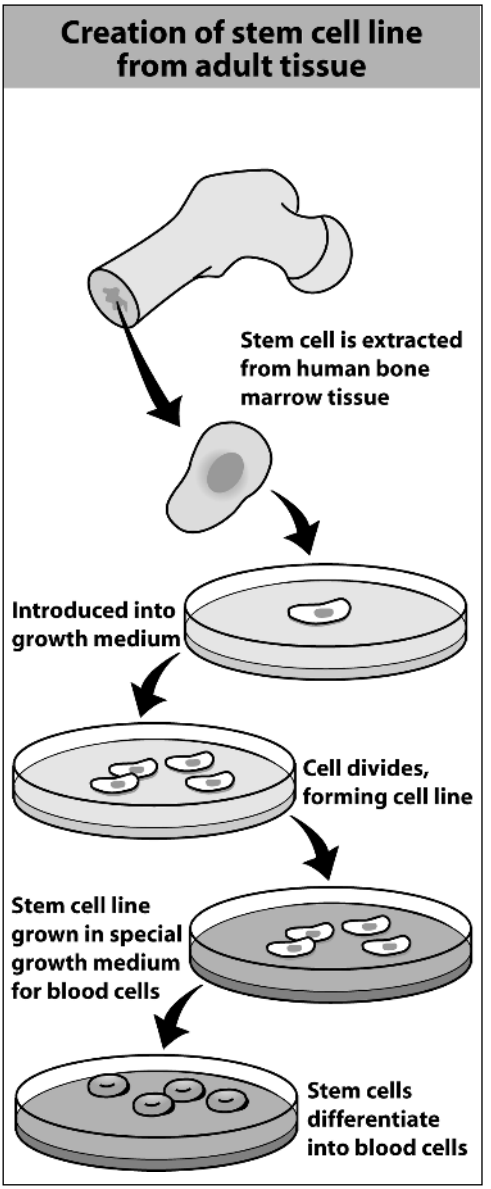
1. These particular kinds of adult stem cells are also known as *hematopoietic* stem cells. Their full description and potential are discussed in Chapter 15.

A major limitation of adult stem cells that their boosters fail to mention is that being a full-grown cell, the biological markers of an individual have matured. This means that an adult stem cell is more likely to cause a dangerous immune reaction if transplanted into another person. To avoid this, adult stem cell transplants could only be carried out using a patient's own cells.

Some researchers claim that adult stem cells do, in fact, have the ability to produce different kinds of cells. The trick, so the argument goes, is to get them to regress to the point that they become pluripotent, or developmentally plastic once again.

A few researchers, such as Dr. Catherine Verfaillie, the director of the University of Minnesota's Stem Cell Institute, have been able to persuade bone marrow stem cells to produce other types of organ tissue. However, until these results are replicated in many other labs, it is difficult to evaluate whether this can be done in a systematic way.

FIGURE 13-1
The process for culturing adult stem cells.



David Traver, a stem cell researcher at the University of Cali-

fornia at San Diego, admits that there were no hard answers yet. "The more conservative thing to say is that each human organ probably has a specific stem cell system behind it, and there probably isn't a lot of 'cross-talk' between them," says Traver.

Traver reiterates that more research had to be done, and that the field was not as advanced as many outside the scientific community would think. "The bottom line," he says, "is that we haven't learned much from studying human stem cells. Most of what we know comes from studying mice, fish, and flies."

To that end, adult stem cells are also harder to study in some ways. Because they do not renew themselves indefinitely in the lab, as embryonic stem cells do, they must constantly be replenished if they are to be studied.

On the other hand, embryonic stem cells come with their own price tag in sweat and hard work. In order to keep them renewing themselves indefinitely, constant feeding and attention must be performed, or the relatively fragile embryonic stem cells will either die off or twist themselves into a grotesque teratomatic growth in the petri dish.

ESC PROS AND CONS

There is simply no tool as powerful as the embryonic stem cell (ESC). It possesses, in the words of one researcher, "the potential to address every single disease or condition that our species is heir to." This is because embryonic stem cells, which are extracted from the fifth day of the embryo's blastocyst formation, have such a high degree of developmental plasticity that they are capable of becoming any type of cell in the body.

It is medical fact that ESCs are incredibly pluripotent. However, a second look must be taken when advocates of embryonic stem cells claim that the cells can be grown in infinite numbers.

The longer that researchers work with embryonic stem cells the more issues seem to crop up. The idea that ESCs can survive indefinitely in culture, thereby providing an inexhaustible source of cellular treatments, is only partially true. Recent studies have shown that while ESCs will reproduce quite happily again and again in suitable

growth medium, over time they develop chromosomal abnormalities similar to those found in some cancers.

Stem cell pioneer James Thomson agrees that embryonic stem cell lines have a limited "shelf life." He notes that "over time, you accumulate mutations. It's a fact of life. It's just a question of differences in the rates. If you accumulate enough of those mutations, you could actually create a cancer." In fact, the dual threat of mutations and the introduction of mouse viruses in Thomson's original stem cell lines is one of the reasons cited by researchers for lifting the Bush administration's restrictions on funding for new lines of embryonic stem cells.[2]

Moreover, while it is true that embryonic stem cells can be used to create "any kind of cell in the body," that same developmental elasticity works against ESCs as well as in their favor. In fact, only a few researchers have been able to differentiate an embryonic stem cell culture into a pure cell culture of the *exact* kind of cell they were seeking.

As of this writing, it is even more questionable whether the researchers who have been able to differentiate the ESCs into targeted cell populations have been able to consistently repeat the task. In the vast majority of "successful" attempts to change embryonic stem cells into specific cell types, the result was instead a petri dish that contained an unhealthy mélange of unwanted cells along with the target strain.

The difficulties with proliferation may be the cause of the tumors so often seen when animals are injected with ESCs. This tendency to cause teratomas in particular is one that researchers will have to spend years working on to ensure that it never happens in human patients. Injecting a patient with cells that produce the ghastly biological horror of a tumor with rudimentary skin, bone, and hair would likely scare away many patients—and researchers—from the field for years.

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2. The worry about a mouse virus tainting the original stem cell lines exists because the human embryonic stem cells in Thomson's experiments will only remain stable and growing on a carpet of embryonic mouse cells. Two mammalian cell species in such close contact raises the risk of a virus being transferred.

But for now, only embryonic stem cells can be considered truly pluripotent, their inner essence still molten enough to shape. "They (ESCs) are a blank slate," stated Dr. Theo Palmer, neuroscientist at the Stanford University School of Medicine. "They do not know what their role is. An adult stem cell has enormous potential that's already been realized." Palmer asserted that embryonic stem cells should be easier to work with than the adult version for this very reason.

While scientists, admittedly, are still groping for ways to reliably "reprogram" the blank slate of the embryonic stem cell, the possibilities just seem too great to ignore. And embryonic stem cells, since they come from a point where the organism has yet to mature, simply provide much more insight into the complexities of stem cell function and development.

Ironically, years of research spent on embryonic stem cells are very likely to teach scientists how to best reprogram an *adult* stem cell to make tissues as easily as an embryonic stem cell can. Put another way, embryonic stem cells may very well be needed . . . so that they will never be needed again.

In the meantime, it's worth moving beyond the sound bites of the researchers to review what both ASCs and ESCs are being used for at this time. Given how fast the field moves, this list is less a definitive catalog of therapies than a snapshot in time. In five years or less, the balance of treatments and knowledge between the two may have shifted, and the total number of treatments available will have expanded beyond belief.

CURRENT ADULT STEM CELL APPLICATIONS

Acute Leukemias

Acute Lymphoblast Leukemia (ALL)

Acute Myelogenous Leukemia (AML)

Acute Biphenotypic Leukemia

Acute Undifferentiated Leukemia

Chronic Leukemias

Chronic Myelogenous Leukemia (CML)

Chronic Lymphocytic Leukemia (CLL)

Juvenile Chronic Myelogenous Leukemia (JCML)

Juvenile Myelomonocytic Leukemia (JMML)

Myelodysplastic Syndromes

Refractory Anemia (Ra)

Refractory Anemia with Ringed Sideroblasts (RARS)

Refractory Anemia with Excess Blasts (RAEB)

Refractory Anemia with Excess Blasts in Transformation (RAEB-T)

Chronic Myelomonocytic Leukemia (CMML)

Stem Cell Disorders

Aplastic Anemia (Severe)

Fanconi Anemia

Paroxysmal Nocturnal Hemoglobinuria (PNH)

Pure Red Cell Aplasia

Myeloproliferative Disorders

Acute Myelofibrosis

Agnogenic Myeloid Metaplasia (Myelofibrosis)

Polycythemia Vera

Essential Thrombocythemia

Lymphoproliferative Disorders

Non-Hodgkin's Lymphoma

Hodgkin's Disease

Phagocyte Disorders

Chediak-Higashi Syndrome

Chronic Granulomatous Disease

Neutrophil Actin Deficiency

Reticular Dysgenesis

Other Inherited Disorders

Lesch-Nyhan Syndrome

Cartilage-Hair Hypoplasia

Glanzmann Thrombasthenia

Osteopetrosis

Adrenoleukodystrophy

Inherited Platelet Abnormalities

Amegakaryocytosis / Congenital Thrombocytopenia

Inherited Metabolic Disorders

Mucopolysaccharidoses (MPS)

Hurler's Syndrome (MPS-IH)

Scheie Syndrome (MPS-IS)

Hunter's Syndrome (MPS-II)

Sanfilippo Syndrome (MPS-III)

Morquio Syndrome (MPS-IV)

Maroteaux-Lamy Syndrome (MPS-VI)

Sly Syndrome, Beta-Glucuronidase Deficiency (MPS-VII)

Mucolipidosis II (I-Cell Disease)

Krabbe Disease

Gaucher's Disease

Niemann-Pick Disease

Wolman Disease

Metachromatic Leukodystrophy

Histiocytic Disorders

Familial Erythrophagocytic Lymphohistiocytosis

Histiocytosis-X

Hemophagocytosis

Inherited Erythrocyte Abnormalities

Beta Thalassemia Major

Sickle-Cell Disease

Inherited Immune System Disorders

Ataxia-Telangiectasia

Kostmann Syndrome

Leukocyte Adhesion Deficiency

Digeorge Syndrome

Bare Lymphocyte Syndrome

Omenn's Syndrome

Severe Combined Immunodeficiency (SCID)

SCID with Adenosine Deaminase Deficiency

Absence of T & B Cells SCID

Absence of T Cells, Normal B Cell SCID

Common Variable Immunodeficiency

Wiskott-Aldrich Syndrome

X-Linked Lymphoproliferative Disorder

Plasma Cell Disorders

Multiple Myeloma

Plasma Cell Leukemia

Waldenstrom's Macroglobulinemia

Amyloidosis

Other Malignancies

Ewing Sarcoma

Neuroblastoma

Renal Cell Carcinoma

Retinoblastoma

CURRENT EMBRYONIC STEM CELL APPLICATIONS

Studies of human embryonic stem cells are currently yielding information about the complex events that occur during human development.

While embryonic stem cells show vast potential for treating human illnesses, as of this writing they are awaiting human clinical trials.

CHAPTER 14

GERM OF AN IDEA

In 1995, James Thomson's team at the University of Wisconsin at Madison was struggling to isolate stem cells from an embryo the size of a grain of sand. At the time, the project's ultimate success was very much in question. It was then that another researcher came up with a new idea on how to corral the elusive master cells that could change medicine forever.

John Gearhart, a current-day professor of gynecology and obstetrics at the Johns Hopkins University School of Medicine, reasoned that if stem cells could theoretically be pulled from a human embryo, why not see if it were possible to do so from a fetus? Gearhart felt confident that a germ cell, properly retrieved from a fetus, would be pluripotent like an embryonic stem cell.

After all, germ cells were the exact same bodies that were known to develop into teratomas. There was proof positive that, even while undirected to form any useful tissue or organ, germ cells retained the valuable degree of plasticity needed if they were to be harnessed for medical or scientific purposes.

THE PATH LESS TAKEN

The so-called primordial germ cells are the cells that give rise to the gametes (sperm and eggs) in adults. Typically, scientists can obtain primordial germ cells from a 5- to 9-week-old embryo/fetus (the dividing line between embryo and fetus is at the 8th week). At the time Gearhart began looking for a practical method to collect, isolate, and culture these cells, almost no research had been done in humans.

Luckily, work had been done on laboratory mice, which were mammalian and thus close enough to draw conclusions from. Two scientists, working on separate projects involved with the transport and development of the lab mouse's genetic blueprint, had happened upon the solution that Gearhart had been seeking.

Brigid Hogan from Vanderbilt University and Peter Donovan at the National Cancer Institute had managed to keep primordial germ cells taken from fetal mice alive and growing in culture. The clusters of primordial germ cells were transferred to a specially treated plastic culture dish, where they began to form germ-cell colonies. Interestingly enough, the cultured cells lived far beyond the typical week-long life expectancy of the mouse germ cells.

What was even more interesting was the fact that the germ cells, once placed into the growth culture, began to exhibit pluripotent characteristics. It was at this point that the cultures' occupants became known as embryonic germ cells (EGs), in accordance with their strangely plastic behavior.

The reason that little or no research had been done on embryonic germ cells to this point was likely because the fetuses or embryos used for deriving EG cells are deliberately aborted. While the blastocysts used for deriving ESCs were "surplus," inadvertently created through excess production of fertilized eggs in a fertility clinic, the abortions were more often than not the result of a deliberate, voluntary choice. It is possible that researchers, who were already wary of the political brouhaha swirling about the use of IVF embryos, did not want to give the appearance of benefiting from a controversial medical procedure.

Gearhart figured differently. It appeared to him that a lot of the

objections to the use of IVF embryos by scientists like Thomson stemmed from the belief that the embryo was a potential life, missing only the chance to implant in the womb. While religious conservatives might not like the idea of Gearhart working with aborted embryos or fetuses, it seemed to him that the die had already been cast. After all, there was no way that the subject(s) of his research could ever grow into a human being.

METHODS OF DERIVATION

Gearhart's team used ultra-fine scissors and micro-forceps to pry samples of primordial germ cells from the gonadal ridge or mesentery of 5- to 9-week-gestation fetal tissue. From the samples obtained, he was able to mechanically and chemically separate out the cells from the tissue.

He then plated the cells on a feeder layer of nondividing mouse fibroblasts in growth medium supplemented with fetal bovine serum. A couple of weeks later, the human germ cells had formed dense, multilayered colonies similar to that of ES or EG cells taken from lab mice.

The range of cell types exhibited by the growing EGs included derivatives of all three embryonic germ layers—endoderm, mesoderm, and ectoderm. This result was interpreted to mean that the embryonic germ cells derived from the fetal tissue were in fact pluripotent.

As time went on, it turned out that human embryonic germ cells shared many of the characteristics of human embryonic stem cells, although they also differed in very significant ways. Both ES and EG cells proved to be capable of self-renewal and of differentiation into many different kinds of cells. On the other hand, the embryonic stem cells showed that they could proliferate for two years through 300 to 450 population doublings. Cell cultures generated by human embryonic germ cells have a smaller capacity for proliferation, lasting an average of only 40 population doublings.

One advantage to human embryonic germ cells is still being researched to prove its validity beyond a shadow of a doubt. If injected into immuno-compromised mice colonies, human embryonic

stem cells end up generating teratomas containing a mishmash of differentiated cell types. This does not happen when similar experiments are done using human embryonic *germ* cells. The EG cells therefore may offer clues as to why a stem cell line has a tendency to become either more or less stable as it proliferates.

OF MEN AND MICE

Today, a serendipitous discovery in science is sometimes called a "Fleming moment." This is in reference to the Scottish scientist Alexander Fleming, who in 1928 had forgotten to clean up the stack of bacteria plate cultures he had been testing in his lab. After several days' neglect, Fleming fortunately noticed a ring of dead bacteria around a contaminant blue-green mold that had landed on one of the Staphylococcus plate cultures.

Fleming correctly concluded that the mold was releasing a substance that was inhibiting bacterial growth. Later, he cultured a pure sample of the mold and discovered that it was the strain *penicillium notatum*. Of course, we know that he named the bacterial inhibiting substance "penicillin," which became the first widely available antibiotic.

A similar moment in time allowed Dr. David Garbers, Howard Hughes Medical Institute investigator at the University of Texas Southwestern, to advance the ability of scientists to identify male germ-line stem cells in laboratory rats. Germ-line cells are those—such as egg and sperm and their precursors—with genetic material that can be passed to offspring.

The ability to manipulate male germ-line stem cells and get them to grow and self-renew has many potential applications. Of primary interest is the development of a possible alternative to embryonic stem cells.

I had a chance to speak with Dr. Garbers about his work with male germ-line stem cells. He began by pointing out why, exactly, stem cells could be found in the male reproductive organs. "If we talk an hour, you and I will probably make about two million new sperm cells. The male makes sperm cells until he dies. And that's because we have stem cells," he explained. "A stem cell is defined

as a cell that can self-renew and also produce a cell that can differentiate into a particular lineage, whether it's a blood cell or neural cells, or sperm cells. So, the stem cell has the unique characteristic that it seems to be basically immortal."

When Dr. Garbers had arrived at UT Southwestern in 1990, he became interested in going back from the mature sperm cell to the germ stem cell with the thought that if he could grow the germ cells in culture, he might be able to bring gene targeting to species other than the mouse. "The only place we do gene targeting now, which is homologous recombination and the disruption of genes, is in the mouse," Garbers pointed out. "That's because embryonic stem cells remain pluripotent in culture in the mouse and human so far. I became really interested whether we might develop technology to knock out genes in other animals by going through the germ line instead of embryonic stem cells."

The experimentation process was simple. The researchers would excise the testes of 22- to 23-day old rats, which were then diced. A protease was added to release the cells from the tissue, allowing a mixture of the germ and somatic cells to separate into a liquid suspension, where they could be collected and placed in a petri-dish culture.

The problem for the researchers was that most of the time it was extremely difficult to tell a germ cell from a somatic cell. "You could probably tell the difference between the cells via electron microscopy," said Garbers. "But for the normal lab routine, you can't take days and days to do electron microscopy, and of course one does not want to kill the cells."

Then, in 2002, Dr. Garbers caught a rather lucky break while using a special gene promoter called ROSA on his samples. A gene promoter is the section of DNA that controls the initiation of RNA transcription as a product of that gene. "This particular promoter was one that had been used by many people, because when you put what's called a reporter gene like green fluorescent protein behind this promoter, you can see the green color in every cell."

Garbers then related his Fleming moment. "What happened was this promoter landed, completely by chance, somewhere in the genome of one rat line so that the expression of the gene only took

place in the male or female germ line of the female or male. So now all of our germ line cells are green and no other cells are green. It's as easy as can be to identify the germ cells now.

"Since our germ cells are marked as green, if we transfer into the testis of a nongreen male rat, we can follow the development of our cells," Garbers added with satisfaction. "These stem cells should colonize and then form differentiated germ cells. And that's the ultimate proof that you have stem cells in your culture . . . you put them back into an animal and they self-renew, appear immortal, and form the cells of the lineage they're supposed to form."

At the end of our talk, Dr. Garbers reiterated that he strongly thought one of the advantages of the technologies he is involved in is that they are much less controversial than the use of other stem cell types. "One could be collecting cells from an adult, so that an embryo would never enter the picture. People might say 'Yeah, there was the potential for an embryo with those cells you took, but it never even became an embryo, and so therefore it was okay to make stem cells out of them.' Trust me, we'll never call what I work with 'embryonic' stem cells."

CHAPTER 15

NEEDLES AND HAYSTACKS

Imagine for a moment that you're on a manhunt for a group of fugitives. You're in luck, because the fugitives have been trapped in a large open-air stadium in Pasadena. As a matter of fact, they're so engrossed with watching this year's Rose Bowl that they're not going anywhere. They're not even trying to hide. While sitting in a helicopter that is hovering over the stadium, you dig out a pair of high-power binoculars and start scanning the crowd for their faces.

There are only six fugitives at large. How long do you think it would take you to find them if they are randomly scattered through the crowd? There are, after all, 98,636 seats in the stadium, and for the Rose Bowl, they're sold out. As you zoom in with your binoculars to look at each face, your odds of spotting even one of the fugitives are greater than 1 in 15,000. It's an interesting dilemma: Can you find a single one before the helicopter runs out of fuel in mid-air? How does one find the proverbial needle in a haystack?

HARD SHELL, SOFT CORE

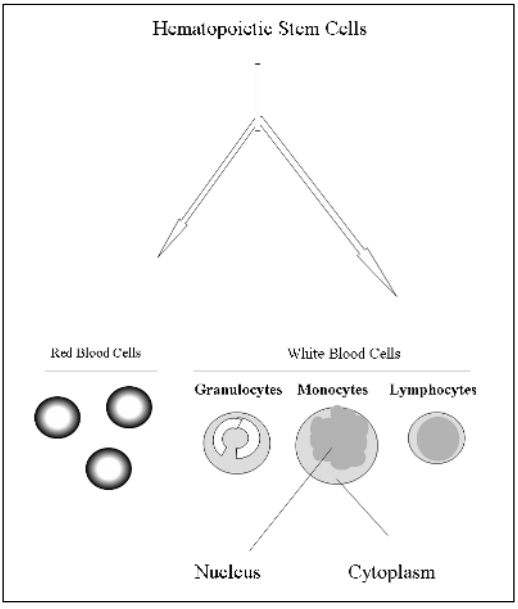
These are the same daunting odds that stem cell researchers used to face when locating stem cells hidden in the bloodstream or bone marrow. The theory that some unknown organ or chemical signal constantly replenished the supply of cells in our bloodstream dates back to the early 1900s. This was confirmed half a century later via experiments with cellular tissue and bone marrow.

Today, we know the brief lives that most of our blood cells have. Their lifespan ranges from a few days for some white blood cells up to four months for the 25 *trillion* red blood cells that pass oxygen around the body like an unimaginably complex bucket line. The millions upon millions of replacement cells that must be churned out are created by *hematopoietic* stem cells.

These cells reside in the body's bone marrow, a sort of sticky, jellylike paste that is found in the hollow core of certain bones. On occasion, they can be found in the bloodstream proper. These stem cells work more efficiently than an auto-production line, releasing all types of blood cells into the blood stream via the thin tissue and capillaries that surround each living bone in a glistening, gelatinous envelope.

FIGURE 15-1

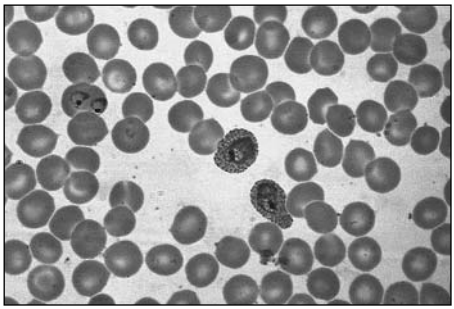
Hematopoietic stem cells and their resulting products.



Hematopoietic stem cells are similar to embryonic stem cells in that they can become different cell types, but their differentiation is limited to blood cells, such as white or red blood cells and platelets.

They resemble certain kinds of white blood cells in that they are (to use the medical terminology) "morphologically nonadherant, possess a rounded nucleus, and contain a low cytoplasm to nucleus ratio."

FIGURE 15-2
A typical slide of almost identical looking cells from the bloodstream. A hematopoietic stem cell is very difficult to pick out visually.



In other words, in a random blood sample, it is practically impossible to identify these cells under a microscope. The beleaguered researcher, tracking down a single type of cell in a huge sample would spend hours scanning through the sample under the microscope, hoping that one feature or another would allow him or her to pick the cell out from the general background of 14,999 other very similar looking cells.

SPIN CYCLE

Since hematopoietic stem cells are the building blocks of blood, bone marrow or stem cell transplants are necessary when the bone marrow becomes diseased or damaged. Treatments have already become available that use the patient's own hematopoietic stem cells to treat certain cancers and blood diseases. These treatments are known as *autologous stem cell therapies*.

In one form of treatment for patients with lymphoma, called an autologous stem cell transplant, stem cells from the patient's own marrow are removed and stored. First, growth factors or other treatments are administered to artificially boost the number of stem cells in the marrow. Since it is easier to get to the stem cells via a fluid medium such as a pint of blood, a second treatment is administered to encourage some of the hematopoietic stem cells to vacate the bone marrow and enter the bloodstream.

The cells are then harvested during a process called *apheresis*. Apheresis, which comes from the Greek word "to take away,"

entails removal of whole blood from a patient or donor. The collected fluid is placed in a machine that is a variation of a centrifuge, a kind of chamber that spins at high velocity. This forces the blood to separate into its pure liquid (plasma) and semisolid (cellular) components. The separated portion containing the cells is then withdrawn, and the remaining components are transfused back into the patient.

The patient then receives high doses of chemotherapy and/or radiation therapy to wipe out or "condition" the immune system and bone marrow in preparation for the replacement of the stem cells previously harvested. Sometimes, the portion of marrow that has been removed from the patient is also treated to purge it of cancer cells before being returned, or "engrafted" to the patient.

In this case, the hematopoietic stem cells are not used for treating the patient. Instead, they counter the worst effects of the chemo- and radiation therapy, providing the clinician with a few key advantages.

Since the stem cells are available to "re-populate" the areas where cells have been destroyed by the chemicals or rads of energy, higher doses can be used to kill more of the cancerous cells without fear of complete destroying the patient's own marrow. The key benefit of this kind of procedure over regular bone marrow transplants is that the cells, if taken from the patient, are known to be 100 percent compatible and won't ever be rejected.

MIGRATION ON CUE

Recently, there have been significant advances in hematopoietic stem cell growth, migration, and collection, which will greatly increase the effectiveness of treatments. In August 2005, scientists at the University of Cincinnati and the Cincinnati Children's Hospital Medical Center gained fresh insight into how the blood-regenerating hematopoietic stem cells travel from bone marrow to the bloodstream.

The researchers had been studying the migration of stem cells in lab mice, as they needed a mammalian model that closely mimicked the human system. If they could isolate the key factor that made the

stem cells pack up and leave their spongy home inside our bones' hollow spaces, then they could potentially synthesize the compound or group of compounds. If such a substance existed, then they would be able to guide mass numbers of stem cells into the bloodstream as efficiently as an orchestra leader conducting a symphony, allowing the precious cells to be more easily collected for use in transplants or apheresis.

The research was successful. It was determined that a group of proteins called the RAC GTPase family played the major role in regulating the location and movement of stem cells from bone marrow to bloodstream and back again. "Our findings demonstrate that RAC GTPase proteins are essential for injected stem cells to migrate into the correct location in the bone marrow," senior study author Dr. David Williams, head of experimental hematology at Cincinnati Children's Hospital Medical Center, said in a prepared statement.

Williams' team determined that a drug called NSC23766, also developed at Cincinnati Children's Hospital, significantly increased RAC GTPase activity. This allowed the scientists to give a direct chemical command to the mouse hematopoietic stem cells, telling them to emigrate from bone marrow into the bloodstream, where they could be more easily harvested. The next step in this research, which is to replicate the result in human patients, will lead to much more efficient ways to harvest these critical stem cells.

CARDIAC REJUVENATOR

The ability to harvest stem cells in the blood was still on my mind when I spoke with Andrea Hunt, the vice president of cellular therapy at Baxter Healthcare Corporation. Around 2002, the company was actively looking at the use of adult stem cells in the cardiac area. What resulted was the development of an adult stem cell therapy for treating chronic myocardial ischemia, which is a severe form of coronary artery disease.

According to Hunt, Baxter was well placed to enter the field, since the company already carried products for the collection and

storage of bone marrow and stem cells. "The first is the Amicus device, which is a device that's out there in the field that actually has the ability to collect a mononuclear cell preparation." In sum, it is a state-of-the-art version of the centrifuge used in the apheresis process. "This collection of mononuclear cells contains a variety of stem cells, but we're actually looking for the *CD34 positive cell*," Hunt told me.

In humans and most other mammalian species, CD34 is a molecule that is expressed on the surface of primitive hematopoietic cells. Research has repeatedly shown that, for reasons that are still under debate and investigation, CD34 positive cells can be used to reconstitute blood vessels.

The CD34 positive cell that Baxter Healthcare was looking for, in other words, would have the right molecular structure on its surface to mark it as a "builder" of blood vessels. Baxter's groundbreaking therapy would involve concentrating this type of cell into the millions and placing them into the parts of the heart suffering from chronic myocardial ischemia.

"CD34 cells are the most studied stem cell. They have the capacity to turn into different types of blood cells, and what's been proven preclinically is that new blood vessels are formed as a result of the injection of these cells," Hunt noted. "That was associated with greater blood flow to this part of the heart in preclinical studies. It's believed that potentially cytokines or other substances are released by the cells, which allow the formation of vessels, even through cardiac muscle tissue."

RED BEAD HARVEST

The problem facing the scientists developing the therapy was how to separate out the CD34 positive stem cells. The Amicus device could concentrate the mix into a sort of wet, shapeless slurry of cells, but did nothing to help sort the cellular wheat from the chaff.

At this point, Hunt mentioned almost casually that "the cell mixture is put onto the Isolex magnetic cell separator. And that device actually pulls out the CD34 positive cells, so you get a more purified cell population."

It turned out that the Isolex was a newly developed magnetic cell separation system that used extremely small "magnetic" particles, called Dynabeads. The little red beads were the brainchild of Professor John Ugelstad at the University of Trondheim, Norway and are manufactured by Dynal Biotech.[1]

Since then, the company's amazing product has become the method of choice for the isolation and manipulation of biological material. In appearance, a small mound of the beads looks like an impossibly fine pile of lumpfish caviar. But because the beads are so tiny, the biological materials that they can help manipulate can include cells, nucleic acids, proteins, and pathogenic microorganisms.

A quantity of the beads is slathered with an antibody that will stick to the CD34 cells, then introduced into the cellular mixture the way one would toss grains of barley into a cooking pot of soup. Once the CD34 cells have stuck to the magnetic beads, the Isolex is powered on to retrieve the beads, now coated with the CD34 cells which have glommed on to their surfaces. "A peptide is then added to the solution that releases the 34 cell from the antibody-coated bead," said Hunt. "You end up with just the CD34 positive cells."

As Hunt's team continues to pursue the unique blood-vessel rejuvenation therapy, it's interesting to see how the problem of isolating this rare stem cell has been solved. Coincidentally, it also answers the question posed at the start of this chapter. Pulling needles out of haystacks isn't a difficult task at all—as long as you have the right kind of magnet.

1. In 2005, Dynal Biotech was acquired by Invitrogen Corporation.

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CHAPTER 16

BANKING ON CORD BLOOD

> What was a discard has become valuable—indeed priceless—to many children with leukemia, and perhaps in the future to children with AIDS and autoimmune diseases, such as diabetes and rheumatoid arthritis.
>
> —*Science*, Volume 268, May 12, 1995

Fanconi anemia (FA) is a rare genetic disease where the patient's bone marrow fails to properly produce blood cells. Aside from bone marrow failure, FA is characterized by abnormally short stature, increased incidence of solid tumors, and skeletal anomalies—predominantly in the hands and forearm. Many children who are born with FA either have no thumbs, or are left with only marginally useful stumps on the edge of the hand. In short, it is a serious disease and many of those afflicted do not reach adulthood.

In 1988, the first successful cord blood transplant to treat FA was given to a five-year-old boy in France. The infusion of cord blood cells revitalized his blood production system and today, that boy is well and healthy. The next transplant was performed in 1991

on a child with chronic myelogenous leukemia, a type of cancer of the myeloid line of blood cells. It too was successful in reversing the course of the disease.

While both transplants were properly hailed in the press as opening new doors to treating disease, they did not garner anywhere near the media attention that stem cell research has in the last few years. And because over 5,000 cord blood transplants have been completed as of this writing, the idea of transplants hardly seems earth-shattering today. But make no mistake, the concept of transplanting cord blood is a crucial part of the stem cell story and offers great promise for the future.

GOLD IN THE MINE

Cord blood is the blood remaining in the umbilical cord and placenta after birth. Much like bone marrow, cord blood is one of the richest sources of stem cells that can ever be collected. It is removed from the umbilical cord and the placenta right after the cord has been cut.[1]

This blood used to be discarded as part of the heap of tissues known as the "afterbirth." But with increasing knowledge and awareness of the benefits of this blood today, people are saving or donating this blood to a cord blood bank. Yet again, the common theme in the development of cellular therapy is the conversion of tissues thought to be "medical waste" into medical gold mines.

The hematopoietic stem cells found in abundance in cord blood are the building blocks of a person's red blood cells, platelets, and the immune system. Since stem cells have the ability to become many other types of cells, they are used by the body in the repair and maintenance of many other damaged cells in the body.

Transplants of cord blood also have a unique advantage over bone marrow transplants. The problem with the older technology

1. Since cord blood can also be found in the placenta, it sometimes appears in news articles as "placenta blood" or "placental blood." On occasion this is misreported as a second type of blood product when it is actually the same substance as cord blood.

was that many patients were unable to find a suitable donor for the bone marrow. Every year, an estimated 14,000 people in the United States alone require a bone marrow transplant for life-threatening disease.

In the past, the comparative shortage of donors—or the inability to find a compatible donor—created a bottleneck of people who should have been cured, but were unable to take the final step of undergoing a transplant. Today, by using a transfusion of stem cells in cord blood from a healthy donor, a new blood and immune system can be generated, giving patients a better chance of making a full recovery.

Cord blood cells also have an advantage over the stem cells residing in the bone marrow in that they are less "mature." Because of this characteristic, cord blood cells are not as fully integrated into a patient's immune system and hence are less likely to attack cells they perceive as "foreign."

As a result, patients who receive stem cells from cord blood as opposed to marrow are far less likely to reject the transfusion. For all these reasons, cord blood transplantations are today more and more commonly used in situations where traditional bone marrow transplantation had been the norm.[2]

SCARLET INSURANCE POLICY

The benefits of cord blood and its future potential has not been lost on concerned parents, particularly ones who know they have a history of genetic illness in one or both branches of the family. In order to ensure that their child can survive some of the most deadly diseases and debilitating conditions, an entire industry has grown up around the "banking" of blood from the baby's umbilical cord.

Banking a child's cord blood is becoming a more frequently requested and common-sense hedge against disease or illness in the future. It is also an investment in the possibility of taking part in

2. Bone marrow stem cells are still very useful in other areas, including therapies mentioned in this book. They are the most commonly harvested and utilized source of adult stem cells today.

any future benefits that are discovered in the field of stem cell research, particular adult stem cell research. As it stands today, cord blood technology can be used to fight more than seventy types of genetic illnesses, from a mere 50- to 75-milliliter sample of cord blood—which is only about enough blood to fill a large shot glass.

An additional benefit is that the entire family may benefit from the banking procedure. Cord blood stem cells are matched to the child's family lineage and are more likely than bone marrow to be a suitable match for family members. There is a 25 percent chance, for example, that cord blood will be a perfect match for a sibling.

This is important because some studies have shown that survival rates double when a patient receives a stem cell transplant from a relative. And of course, families who have their babies' stem cells banked have, with relative ease, positioned themselves to take advantage of the medical breakthroughs that will eventually come from stem cell research.

GENETIC PURGE

One area in cord-blood cell banking, where a great deal of disagreement remains, is whether a child's stored blood could be used to treat his or her own genetic disease. It is thought that because the cord-blood stem cells in the mixture carry the same genetic anomaly, transfusing the sample back into the patient would be the biological equivalent of putting dirty oil back into an automobile during an oil change.

At least one article on the subject boldly claims that "most transplant physicians would not use a child's own cord blood to treat leukemia." This is for two reasons. In the first place, the child's cord-blood stem cells harbors the same defect. Therefore, all the transplant does is to put more pre-leukemic cells back into the patient. Second, in a child with leukemia, the immune system has *already* failed to prevent leukemia. Since cord blood from the same child reestablishes the child's own immune system, it does not help inoculate the body against a recurrence of the disease.

Some private cord-blood banks like to tout that they can "purge" the blood sample of cancerous leukemia cells. However,

there is no hard evidence that the cord-blood cells that carry the leukemic mutation actually express the surface markers or antigens needed to remove them. Thus, purging of cord blood for leukemic or pre-leukemic cells would be considered speculative by many scientists and clinical physicians.

Other publications just as boldly claim the opposite conclusion. "Thousands of autologous stem cell transplants (using one's own cells) are performed every year for diseases such as leukemia, lymphoma, myeloma, and many solid tumors." Backing up this point of view are citations from the *Journal of Clinical Oncology*.

The *Journal's* reports indicate that even with early-onset variations of leukemia, the child's stem cells are still recommended for use in transplantation.[3] Two physicians are quoted as stating that the " . . . stem cells in cord blood should be normal and free of malignancy, giving them a potential advantage over autologous cells collected during hematologic remission from a patient with malignancy." The issue promises to be clarified as more medium and long-term studies are completed.

BLOOD SUSPENSION

At present, there are two methods for collecting cord-blood stem cells: *in-utero* and *ex-utero*. For the in-utero collection, cord blood is collected while the obstetrician is waiting for the placenta to be delivered naturally. Typically, there is a period of seven to ten minutes between the time that the umbilical cord is cut to deliver the baby and when the placenta is delivered. (Hence the archaic term for the tissue, the "afterbirth.") This is ample time to collect the cord blood. If an ex-utero collection is performed, the placenta is delivered and then placed in a sterile supporting structure with the umbilical cord hanging through the support.

After the collection, the cord blood unit is shipped to the lab. A small portion of the sample is extracted so that it can undergo viral testing, including tests for HIV and Hepatitis B and C, and tissue typing. Next, the blood is processed and then cryopreserved.

3. "Early onset" of a disease in this context means "within twelve months of birth."

Cryopreservation is the process whereby tissue is stored by cooling it to extremely low temperatures. Depending on the processing method used by the bank, the red blood cells may be separated out and removed. Whichever way the unit is processed, a *cryopreservant* is added to the cord blood to allow the cells to survive the cyrogenic process.

There are many types of cryopreservant fluids but all serve the same purpose: they retard the formation of ice crystals. The main problem with freezing cells is that the expansion and formation of ice can puncture cell membranes, rendering the cells useless and detrimentally affecting the tissue. This is one of the main barriers to achieving routine cryogenic suspension of people. (It's also what causes the "freezer burn" that can develop on meat left too long in the kitchen freezer.)

The sample is slowly cooled to minus 90° Celsius. Again, this is to retard the formation of ice crystals. It can then be added to a liquid nitrogen tank that will keep the cord blood unit frozen at minus 196° Celsius. By bringing molecular movement down to almost zero, the unit can be stored for years.

CONCERNS OVER PUBLIC/PRIVATE BANKING

Both commercial and nonprofit cord-blood banks are available to collect and store cord blood. Most of the commercial facilities charge fees, typically ranging from $1,500 to $2,500, not including annual fees, to collect and store cord blood for a family's own private use, in the event it is needed by the donor infant or a matched family member at a later time. Public banks, on the other hand, accept donations to be used for anyone in need, though there are very strict regulations that public banks need to follow in order to add the donated units to a registry.[4]

It should be noted that cord-blood banking is still in its developmental stages. As with any new field, particularly one involving

4. The National Marrow Donor Program has a list of public cord blood banks on their website, located at www.marrow.org.

human medicine, there will be issues that need to be resolved before it becomes widely established practice.

The main concern of cord-blood banking, private or public, is that the long-term viability of cryogenically frozen cord blood has yet to be firmly established. Again, this may very well come about empirically—as the blood that has already been stored ages, the upper time limit to developing effective treatments will eventually be known.

A major concern that exists solely with public cord-blood banking is how to ensure that the collected cord blood is contaminant and disease-free. Because of privacy concerns, most ethics review boards have concluded that blood donated to a public bank cannot be permanently linked to the donor.

Cord blood that is donated to a public bank goes through a series of tests for potentially harmful genetic disorders and viruses. But some genetic disorders, such as congenital anemias or immunodeficiencies, might not become apparent in the donor for months or years. By that time, it is almost certain that all the identifying information will have been lost or purposely removed. This remains an important, unsolved concern, as it is possible that the recipient of the blood could also develop these disorders later in life.

Storage at private banks has also proven somewhat controversial, though the objections are based on different grounds from those raised about the public banks. Privately run cord-blood banks have come under fire when no specific family member has been identified as needing a transplant. Because the need for a future transplant is purely speculative, private cord-blood banks have been criticized for preying upon the insecurity of new parents.

EVEN ODDS

At the heart of the criticism is the contention is that the chances of a child needing a transfusion of their own cord blood are very small, in comparison to the high storage fees at private banks. Either side of the argument is difficult to evaluate. The cause of many cancers is simply not well known. Added to this mystery factor is the diffi-

culty in accurately calculating the odds that a family will use the cord blood or benefit from new treatments.

One medical study cited claims that the odds that a child will someday need to use his or her own cord stem cells are 1 in 400. Furthermore, the odds that a newborn or a family member may benefit from banked cord blood are estimated at 1 in 200. These odds do not include the emerging and potential use of stem cells to treat other ailments, such as spinal cord injuries.

Assume for argument's sake that the odds cited for the likelihood of your child needing their own stem cells are accurate. Balance that against the spending of, say, $1,500 for the initial storage and $100 per year thereafter for the next two decades—a total of $3,500—for what may be the upper limit as to how long the cells can be minimally useful.

To some, any price within their means is worth paying to make their child safer from disease. Others might come to the conclusion that a 1 in 400 chance is too remote a possibility. Rather than spend money where there is a relatively low—albeit deadly—form of harm, one might take the $3,500 to spend on safety-proofing their home and buying the best child seat on the market to install in the family car.

CHAPTER 17

SO CLOSE AND YET SO FAR

Progress in stem cell research is at times optimistic, exhilarating, and frustrating. Oftentimes the work seems to take two tiny steps forward, then one large one back. This has nothing to do with the politics, the moral arguments, or even the amount and type of funding. It is simply the result of piling hope upon hope that there is a key buried in the core of what becomes a human embryo. The long-held dream is that this key can unlock the mystery of why so many of us must suffer what Shakespeare called "the heart-ache and the thousand natural shocks that flesh is heir to."

For proof, we need look no further than the experiments being done with stem cells out "on the fringe" of what medicine considers treatable. Even as recently as a century ago, without antibiotics or a real understanding of surgical hygiene, deep puncture wounds were beyond medical help, and it was accepted that one-quarter of women would die of infections related to childbirth.

But medical researchers continue to try and push back the darkness of what lies out at the edge of "the treatable." And there is one condition that meets all the criteria for the ongoing stem cell drama:

spinal cord repairs. It combines great hope, a desire to combat suffering, crushing failure, and no small amount of celebrity.

SUPERMAN FALLS

In the spring of 1995, actor Christopher Reeve was competing in the cross-country riding event at the Commonwealth Dressage and Combined Training Association finals in Culpeper, Virginia. A careful rider, Reeve had followed all safety procedures properly and was wearing a rider's standard helmet and a protective vest. Reeve was approaching one of the jump obstacles—a triple-bar about 3½ feet high—when his horse abruptly stopped, refusing to complete the jump. Reeve was thrown and landed on his head.

The injury shattered the first two cervical vertebra. It severed the brain stem right below where it emerged from the brain and became the spinal cord. Immediately after the injury, Reeve's body and head were not held together except by the fleshy connection of neck muscles.

The spinal cord injury rendered Christopher Reeve a quadriplegic and confined the athletic, 6-foot 4-inch actor to a wheelchair for the remainder of his life. Yet there remained some hope for improvement. Reeve's second cervical vertebra was separated from the third, but by a distance of only 20 millimeters. Those 20 millimeters made Reeve a prime candidate for recovery if the doctors and scientists could unlock the secret of nerve regeneration.

The study of nerve regeneration itself is a contentious field. It is known that different parts of the nervous system seem to regenerate at different rates. For a long time, it was assumed that the peripheral nervous system was able to regenerate, while the central nervous system—the brain and spinal cord—typically did not regenerate at all.

Peripheral nerves are known to grow along the path of the severed nerves. The supporting cells, also known as Schwaan cells, of the peripheral nerves are known to release growth factors that support the development of nerve cells to rebridge a gap.

How the Schwaan cells sense the size of the gap and know when to stop releasing growth factor is as much a mystery as the exact chemical components in the "growth factor." The reason nerve

cells do not usually regenerate quickly is that scar tissue forms at the injury site. Glial cells, which surround damaged nerves, multiply to form a dense protective scar. For some unknown reason, they also secrete barrier molecules that strictly limit the ability of nerve cells to grow.

BRIDGING THE GAP

It is theorized that the chemical limitations placed on nerve regrowth, particularly in the spinal column and skull, is a result of the trial-and-error of evolution. The central nervous system is one of the core systems without which the body cannot function. It is so valuable that it is the only system the body armor-plates by encasing it in solid bone.

The bone armor in turn puts an absolute limit on the size of the tissue so enclosed. This is why an injury leading to a swelling of the brain is so devastating—any compression of nerve tissue on the bone causes damage and pain. In order to avoid this fate, the nerves in these areas may have been programmed to sharply limit growth—with the unfortunate side effect that injuries in these areas simply do not recover quickly, if at all.

Reversing this effect may fall to Dr. Hans Keirstead, a biologist and rising star at the University of California at Irvine. He could possibly be the scientist who ushers in the new era of spinal cord therapy. Over five years, he has patiently coaxed embryonic stem cells to transform into a specific type of nervous system cell that carries the nerve impulses along the spinal cord.

In March 2006, he demonstrated the amazing advances he had made on a highly publicized episode of the television show *60 Minutes*. Using laboratory rats with injured spinal cords, Keirstead had injected his test subjects with 1 to 2 million human embryonic stem cells directly into the injury site.

"This is a video of an injured rat. The signal that's going from the brain down to the spinal cord controlling all of the muscles of the body are interrupted by a spinal cord injury," Keirstead explained. The cells, following their mysterious chemical protocols, glommed on to the injury site and re-established the nerve hookups that allowed impulses to run along the spine. The end result? Within

two months, the partially paralyzed rats had regained their ability to walk.

Buoyed by his success, Keirstead plans to start clinical trials with humans in 2007, likely in collaboration with Geron. "I think we could call this a dazzling success if we saw the smallest improvement in the ability of a human to do anything that they could not do," Dr. Keirstead said in the interview. He admitted that he was concerned about the side effects to the human patients, but was optimistic that a winning therapy would result. "I'll be losing sleep, no doubt, when this first gets into humans . . . this is a risky endeavor, like any clinical trial."

A ONE-TIME MIRACLE

One indicator of how high the risk could be is the case of Hwang Mi Soon. Two decades ago, Hwang was involved in a terrible accident where she had fallen off a bridge. As a result, she had been rendered paraplegic, completely paralyzed from the hips down.

Now in her mid-thirties, she appears in news articles about her life as an attractive, fine-boned woman with a will of iron. Her fame is a consequence of the amazing triumphs and setbacks in the field of stem cell research.

Hwang is from South Korea. Though she is not related to Hwang Woo Suk of the cloned dog "Snuppy" fame, there is a tenuous tie. Both the charming scientist and the determined patient reached the pinnacle of their potential at roughly the same time. To the country's fledgling scientific community, it must have seemed for a while that literally anything was possible if they were willing to roll up their sleeves and start working in their labs to create yet more miracles.

In the fall of 2004, medical researchers attempted to regrow Hwang's spinal cord tissue by injecting umbilical "cord blood" stem cells into her damaged spine. By the twenty-fifth of November, she had made dramatic progress, with the ability to rise out of the wheelchair that had confined her for so long and shuffle a few steps with the aid of a metal walker.

Hwang's steps may have been small, but the leaps that the news took were anything but tiny. Papers around the world high-

lighted the amazing advancement. The *New York Post* titled one article "Stem-Cell Gal's Miracle Steps," while a New Zealand paper followed up the same theme, calling it "The Miracle Cure for Paralysis."

Supporters of stem cell research using only adult stem cells—of which umbilical cord cells are a subcategory—felt vindicated. Cord-blood stem cells were free of the controversy surrounding embryonic stem cells. Furthermore, they would be much easier to obtain, thanks to efforts of public as well as private "cord-blood" banks, where the tissues containing rich amounts of the vital cells were donated and frozen for future use in containers surrounded by wisps of liquid nitrogen.

It was no wonder that religious conservatives were more than a little giddy. Tony Perkins, president of the Family Research Council, summed it up when he wrote: "The pro-life community can say to supporters of embryonic stem cell research, we told you so."

Wesley J. Smith, a senior fellow at the Discovery Institute, wrote up the incident in detail. Citing a study by the periodical *Cytotherapy*, he reported how the South Korean scientists had used umbilical, not embryonic cord-blood stem cells to restore feeling and mobility to Hwang. The article written in *Cytotherapy* read in part:

> The patient could move her hips and feel her hip skin on day 15 after transplantation. On day 25 after transplantation her feet responded to stimulation. On post-operative day (POD) 7, motor activity was noticed and improved gradually in her lumbar paravertebral and hip muscles. She could maintain an upright position by herself on POD 13. From POD 15 she began to elevate both lower legs about 1 cm, and hip flexor muscle activity gradually improved until POD 41.

Smith noted that research using adult stem cells for patients with spinal-cord injuries had also been reported elsewhere. For example, he wrote that "Similar results for patients with spinal-cord injuries have been reported in human trials in Portugal using the patients' own olfactory (nasal) stem cells. These studies have not yet been published in a peer-reviewed journal, though the very promising results in the first American patients have been testified

to in a Senate subcommittee hearing and featured on the PBS television series *Innovation*."

POST HOC ERGO PROPTER HOC

Sadly, these proclamations were all a little too self-congratulatory, and much too quick on the trigger. It turned out that Hwang's treatment had only fleeting benefits that soon wore off. After a few weeks, Hwang found that she could no longer adequately control her legs, and was once again wheelchair-bound.

The researchers scrambled to find out what possibly could have happened. The stem cells they had injected had not turned cancerous, nor had they been rejected. To this day, what happened to Hwang Mi Soon remains a mystery.

One of the few possible explanations of what happened is quite plausible—that what took place was a heartbreaking example of a "post hoc" fallacy.[1] Post hoc, also known as "coincidental correlation," is a logical fallacy which assumes that if one event happens before another, then the first must be the cause of the second.

The fallacy lies in coming to a conclusion based only on the order of events, which is not an accurate indicator. That is to say, Hwang was injected with the cord blood cells, then a little later on she was able to walk, albeit temporarily. Since the injection took place before she was able to walk, therefore it was the injection that had to be the *cause* of her ability to rise from the wheelchair.

But was it really? Medical investigators noted that the injection of stem cells wasn't the only variable that had been introduced into the situation. At the same time Hwang had received the stem cells, she had also undergone spinal surgery to remove the pressure of bone pressing on the spinal cord.

This surgery is called a *laminectomy*, and it could have provided her a similar benefit. Compression that takes place when bone presses against the spinal cord can be directly responsible for neurological deficits.

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1. "Post hoc" is a shortened, modern version of the Latin platitude "*Post hoc ergo propter hoc,*" meaning "After this, therefore because of this."

Therefore, the decompression of the spinal cord could result in the type of functional improvements that had been attributed to the stem cells. Hwang's recovery may have been a result of this decompression instead of the umbilical-cord-blood stem cell transplant, or even an unstable combination of both factors.

On the other hand, in the stem cell's favor, it appeared that 41 days after the stem cell transplantation, testing showed that new growth had occurred at the injury site, which also showed signs of spinal cord regeneration. Thus it was argued that "more of the same" treatment would continue the regenerative process.

THE SECOND ATTEMPT

In April 2005, a Hanyang University medical team led by Professor Kim Jae-min, decided to make a second attempt at a successful stem cell therapy to cure Hwang's persistent paralysis. Once again, Kim's team transplanted stem cells into Hwang's spine, which were harvested from cord blood retrieved in the vein of an umbilical cord after the delivery of a baby.

This time, the research team wisely tried to dampen expectations. Kim cautioned against any hasty celebration. "We believe this clinical test will be safe," Kim said, "but nobody can secure 100 percent safety in the long term, and we should be cautious in making any predictions." The initial results were encouraging. "Even though the development pace has slowed down, I feel my spine keeps improving," said Hwang. "Now I can raise my feet myself to an extent that people can recognize it."

But in the end, the results again turned into disappointment. Not only did the hoped-for improvements—being able to completely rise from the wheelchair—fail to appear, Hwang suffered a nasty infection at the injection site. It is unclear what role the stem cells played, but the end of the experiment left her in constant pain.

Hwang's current physician, neurosurgeon Jeong Yeong Seon at Bundang Cha Hospital, theorized that injecting stem cells into his patient's spine probably relieved some of the pressure temporarily but did not lead to any meaningful improvement. In other words, it is possible that the injections themselves, not the cells in the sy-

ringe, were the key element that allowed Hwang to walk again, no matter how briefly. Saddened by Hwang's result, Jeong said, "There should have been more clinical testing on animals before they tried these procedures on people."

HOPE AND ETERNITY

One cannot help but read the advances of stem cell research in this area without an occasional feeling of frustration. Embryonic stem cells are showing great results—but so far, only in animals. And the adult stem cells have proven to have short-lived effects if any at all. But hope, to coin an old, well-worn phrase, does really spring eternal. After his own riding injury, Christopher Reeve and his wife Dana became the leading lights in bringing public attention to spinal injuries and the use of stem cells to potentially heal them.

Chris and Dana chaired the Christopher Reeve Paralysis Foundation, which funds research on paralysis and works to improve the lives of the disabled. As of this writing, the Foundation has awarded over $60 million in research grants and millions more in quality-of-life grants for those suffering from disabling spinal injury.

Reeve was also active in the lobbying effort to expand the use of federal funds in embryonic stem cell research. It is due to much of his effort that the issue remains a dominant one in the political arena, and the human face he put on the suffering that could be avoided is a difficult one to counter.

On October 10, 2004, at the age of 52, Reeve died of heart failure brought on by an infection. Much like his iconic turn on the screen as Superman, he had created a new legend of how one person could triumph over suffering and put it to earnest, compassionate purpose.

Upon Reeve's passing, a great number of political cartoons were drawn to commemorate his life and work. My personal favorite is one that depicts Reeve not as Superman, but as an angel. Gabriel is offering Reeve a pair of angel wings. Reeve declines with a smile and says, "*No thanks. I'd rather walk.*"

CHAPTER 18

THE MITOCHONDRIAL BARRIER

It is the dog days of summer in Southern California, and the sunshine is bright and merciless. Lying south of the greater Los Angeles area—and seamlessly blending their borders together in a sprawl of suburbs—is Orange County. The area is an interesting mix of ethnicities and beliefs. There are vast expanses of terra-cotta colored homes, often covered with winding vines of bougainvillea; so many have the reddish Spanish tile on the roof that one suspects a local ordinance requiring it. It is simultaneously home to the birthplace of President Richard Nixon, the animatronic wonders of Disneyland, and the skyscraper-proportioned Crystal Cathedral, a church with modernistic architecture, a gigantic electronic organ, and over 70,000 windows.

Orange County is commonly known as a conservative stronghold in a predominantly liberal area (a local joke is that Mickey Mouse wears a "Ronald Reagan" watch), so its bid for a large stake in the area of stem cell research is taken very seriously. The "O.C.," as it is called on prime-time television, carries a great deal of economic muscle. The county contains around 1 percent of the

U.S. population, but it is home to 3 percent of the companies featured in the Fortune 500.

The University of California system has a campus here as well, in the core city of Irvine. While not as well known as its larger campuses (UCLA and USC steal most of the headlines with their cross-town rivalry to the north), the campus has quietly positioned itself as an "up and comer"—especially in the fields of medicine and engineering. The newly founded Center for Molecular and Mitochondrial Medicine and Genetics, lead by Director Doug Wallace, is a prime example of this intent to compete with the bigger, better-known, and better-endowed campuses on fields outside of the football stadium.

A SECOND SET OF BLUEPRINTS

Professor Wallace is a self-admitted evangelist when it comes to the research of mitochondrial DNA, also known as mtDNA. He firmly believes that the genes inside a mitochondrion are the real predictors of disease. Whether a person is predisposed to develop Alzheimer's disease or Parkinson's or other age-related ailments will depend not on the state of their cellular nuclei but the condition of their mitochondria. "I think the world is on the wrong track," he said in a prior interview.

Doug Wallace is an energetic, charming academician, whose glasses give him the look of an absentminded professor. In person, he proves to be a charismatic personality and an eloquent, forceful speaker, although he does have his quirks. The dashboard of his Volvo S70 is home to a pair of yellow and blue plastic nudibranchs—a kind of ocean-going mollusk that looks like a slug after a team of Pixar animators decided to color it in shades of bright neon. Wallace laughingly refers to the pair as "Slugworthy One and Two."

Though I met him on a blazing summer day, he wore a heavy gray, tweedy suit and a green tie swirled with organic paisley patterns. Organic patterns are definitely his personal taste; his office is littered with trilobite fossils, amethyst geodes, and Mayan tribal masks. His mind is as wide-ranging as the office would hint, and he makes connections that few people would initially think of. One of

these insights involved the intersection of stem cells and mitochondria structures inside the cells, and could raise a big "red flag" on the development of therapeutic cloning.

Wallace began studying the mitochondria in the early 1970s as a graduate student at Yale. Although the latest advances had been in the field of nucleic DNA, his interests lay elsewhere—in the cell structures that actually performed the functions of keeping the cell alive. He began to study the DNA inside the cell's mitochondria and that decision turned out to be prescient.

Dr. Sue Bryant, the dean of UC Irvine's school of biological sciences, helped recruit Wallace from Emory University three years ago. She describes him as "one of a kind" and explains, "We're just coming out of that place where everything is focused . . . on the nucleus and on nuclear genes." Doug Wallace was ahead of the curve by three decades. While his current theories are still considered controversial, many of his past assertions are now becoming widely accepted.

For example, Wallace and his colleagues found that mitochondrial DNA can mutate, just as nucleic DNA does. More disturbingly, they found that certain mutations could cause diabetes, heart disease, and Kearn-Sayre syndrome, where the afflicted person's body loses the ability to control heart, eyes, and most major muscle movement.

Many people know from high-school biology that each cell in their bodies contains a biochemical "blueprint." They know that this blueprint is stored in coils of double-helixed DNA, deep in the core of the cell's nucleus. It is less well known that each cell also contains a second kind of DNA. It is this DNA that could very well hold the key to the future of much stem cell research.

These second sets of blueprints exist in the cell structures called the *mitochondria*. Mitochondria, which derive their name from Greek *mitos* "thread" and *khondrion*, "granule," are cellular structures that convert organic materials into energy via the process of *oxidative phosphorylation*. The number of mitochondria in a cell is directly related to the level of the cell's average metabolic activity. Logically, more mitochondria are present with higher levels of activity. In some cases, the mitochondria can take up over one-quarter of the cell's internal volume.

TWO SEPARATE ORGANISMS INTO ONE

One of the first topics that Professor Wallace wants to discuss is the evolutionary development of these mysterious structures. Why is it that the mitochondria alone, of all the other structures in the cell besides the nucleus, have their own DNA? The existence of this separate type DNA suggests to many scientists that at one point, mitochondria were separate entities from their current host cells.

"Three billion years ago there were two totally different organisms," Wallace asserts. "One organism ultimately gave rise to the structures that we call the nucleus. . . . The other was a bacterium that had developed the ability to make energy very efficiently by oxidizing carbohydrates and fats that it got out of the environment with the oxygen that was made by the plants to generate energy."

This is the core of what evolutionary cellular biologists refer to as the *endosymbiotic* theory. Prior to the development of the nucleus, cells lacked a membrane-bound packet to contain their genetic material. Instead, these so-called *prokaryotic* cells contained a single circular chromosome within a general region called the nucleoid, or a smattering of small circular pieces of DNA, called plasmids, spread randomly throughout the cell. Despite the lack of a central clearinghouse for their genetic material, these types of cells do quite well for extremely simple organisms. For example, most bacteria are prokaryotes.

The theory is that *eukaryotic* cells (cells containing, among other things, nuclei) first appeared when one prokaryotic cell was absorbed into another without being digested. These two cells are thought to have then entered into a symbiotic relationship. One cell—the absorber—generated the nucleus and the cytosol, a sort of highly organized molecular "soup" that comprises the internal cell fluid. The "absorbee" retained its energy-generating abilities and became the mitochondria that super-charged the new cell's existence.

In Professor Wallace's view, the prokaryotic cell simply did not make enough energy to allow it to form multicellular plants and animals. It was too energy-poor. Had the eukaryotic cell not arisen, quite possibly life would not have evolved beyond the stages where it could be observed in the petri dish. "But because of this symbiotic

event, the two organisms joined together," Wallace explains. "By putting their two different lifestyles together, they were much better than the sum of their parts. It's because of that symbiosis that this new cell with the mitochondria could then go ahead and evolve into most of your plants and animals."

It's easy to see how the absorber cell got the benefit of a cellular turbo charging. But what did the cell that became the mitochondria ever get out of the deal? Wallace's answer is that the absorbing cell's innards—the *cytosol*—provided a new niche for a bacterium to exploit. Nature is endlessly inventive, and life generally evolves into any available niche, where there is no competition, no predators, and an abundance of food.

"The mitochondrion got all the carbohydrates and fats it wanted from the cytosol, so it didn't have to swim around and find it," explains Wallace, "So, that one-cell organism could survive in that niche. Then it would grow and replicate and pass its genes on to the next generation more successfully then it would have done on its own."

SMOKE AND FLAME

The pairing of the two organisms was, to put it mildly, extremely successful. So much so that the vast majority of multicellular life, from comb jellies to chimpanzees, is made up of eukaryotic cells. Each cell has a distinct nucleus for passing on physically expressed genetic traits and specific organelles, including the power-producing mitochondria.

Since the mitochondrion acts as the cell's power source, think of it as a factory or coal-burning power plant. Like any power plant, there are inputs and output. The inputs for the mitochondria include sugars (usually in the form of broken-down carbohydrates) and fatty acids. The mitochondria turn this fuel into energy. To continue with the power plant analogy, the "burning" of the lumps of coal produces inevitable output. The intra-cellular output is a combination of adenosine triphosphate (ATP), water, heat, and molecules called "free radicals."

ATP is an extremely special molecule in that it is able to store

and transport chemical energy within cells—consider it the electrical current that is generated by the power plant. (ATP is also known by biochemists as the "molecular currency" of intracellular energy transfer.) The free radical molecules could be thought of as the "smoke" that escapes the plant as the fuel is burned.[1]

In chemistry, free radicals are atomic or molecular species with unpaired electrons or an otherwise open-shell configuration, making them especially likely to take part in unwanted chemical reactions. Because of this, the free radicals that are produced can, over time, seriously damage the DNA of the mitochondria. This damage limits the lifespan of the cell, and in turn the lifespan of the entire organism.

Doug Wallace made the point very clearly over lunch that day. "From my point of view, biology is information. Information is encoded in information-rich molecules. Oxygen radicals—reactive oxygen species—will oxidize any kind of released compound. But if you damage a protein, you can remake it. As long as you have the information you can make another one just like it. What really matters is when [a free radical] damages the information."

Wallace noted that with all of the cells that were constantly being replaced at the end of their lifespan and those that were being created anew, the "Michael" sitting before me shares almost no atoms with the "Michael" that was born. All human beings are constantly undergoing the same simultaneous buildup and teardown. From a biological viewpoint, there was nothing inherent about the physical structure of a person. What was important was the information that was specific to creating you.

By that token, if oxygen radicals damaged the mitochondrial DNA and this information is needed to maintain the power plant, then serious long-term consequences would take place. "You can damage a power plant, say damage the wall, but as long as you have the blueprints to repair the wall, that's no problem. But if you destroy the blueprints, then you damage the wall, the wall stays damaged, and *that's* what causes aging."

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1. Ironically, given the nature of the analogy being used, the most commonly observed "free radical" reaction is combustion.

While admitting that his argument as to what truly controls the biological clock is still highly speculative, Wallace is emphatic in his belief that he sees the key to what drives the clock's mechanism. "It's the accumulation of the damage to the information in the mitochondrial DNA that erodes the ability of the system to repair itself. Once it can't repair itself, it will go off line, and come to a stop."

In a prior interview with *The Orange County Register*, Wallace makes this point even more vividly. If, as he hypothesizes, the mitochondria act as cellular power plants, then they allow the city to run. "If the blueprints are sound and the contractors built them well, all will be fine. But if the contractors were lazy or sloppy, the power plants would spew smoke into the air, sputter, and fail. The city would go dark, and all would collapse into chaos."

LIVING OR DYING BY THE CLOCK

This potential for cellular functions to come to a stop—in effect, succumbing to their own life spans as their mitochondria begin to fail—is one of two key tie-ins that Doug Wallace sees between his research and the use of stem cells. If his suspicions are indeed correct, then the biological age of the cell's mitochondria is a serious barrier to the use of stem cells in therapeutic cloning.

"What worries me is that nobody's worrying about where the mitochondrial information is coming and going," Wallace says. "They're putting old mitochondrial information from the somatic cell . . . to make this therapeutically cloned tissue, but the mitochondrial DNA of that old cell has already been subjected to damage [from oxygen radicals], so it already has the faulty information. Therefore, you're in effect pre-aging the stem cells," Wallace concludes emphatically.

Consistent with this hypothesis would be the "premature" death of the first cloned sheep, Dolly. If Professor Wallace's ideas are conclusively proven, her death actually would not have been premature. In fact, it would have been right "on time," given that cells from a six-year old sheep were used from the start. In essence, instead of creating a newborn lamb, the researchers were creating an animal that had been "pre-aged" by six years. Dolly's lifespan ran its as-

signed biological time because her clock had *already* run down substantially by the time they cloned her.

The negative implications for the idea of therapeutically cloning organs are staggering. To take just one example, consider that stem cell researchers are trying to help an 80-year old patient whose kidneys are failing. They decide to take a sample of the patient's cells to be used with a line of existing stem cells to grow, in vitro, a brand new pair of kidneys.

But the kidneys aren't brand new, if the barrier thrown up by the mtDNA exists. Eight decades of free radical damage have done their work on the mitochondria's blueprints and reduced their operating efficiency. What has been created—and would be implanted into the body at a great deal of post-operative stress—are a new pair of eighty-year old kidneys, which are just as prone to failure as before. In a single stroke, the luster could be taken off of one of the great promises of stem cell therapies.

Wallace also raises the question about how this pre-aging of the tissues or organs created from stem cells could affect the human body. In another hoped-for scenario, therapeutic cloning could, in effect, be a fail-safe measure against many types of cancer. Pancreatic cancer, for example, is almost a guaranteed death sentence today. Creating a new pancreas, even if it were advanced in age and prone to failure, would be a net benefit because it does not contain cancerous cells.

Wallace allows for this, but then suggests that preliminary research has turned up disturbing possibilities even in this area. His group's data suggests that a major predictor of cancer itself is damage to the mitochondrial DNA, not the nucleic DNA. It is still a radical notion, and one that is under much debate in the scientific community to this day.

Yet one of Wallace's prime concerns is that not only could preaged cells be less healthy by virtue of their accumulated mtDNA damage, but they could also be more prone to being cancerous. "You take old mitochondrial DNA, you put it into a stem cell that you're now going to make into a pancreatic cell, but if that mitochondrial DNA has now increased its risk of becoming transformed or making new cancer, you have gone back to increasing the cancer risk in the person that you're trying to treat."

MITOCHONDRIAL EVE

The second key tie-in that Wallace sees between his research and the use of stem cells comes from his study of the matrilineal heritage of mitochondrial DNA. The passing on of mitochondrial genes led Wallace into some of his group's best-known work, in the field of human ancestry. The mitochondrion's DNA has a couple of characteristics that distinguish it from its better-known nuclei cousin.

First, mitochondrial DNA in human beings is set out in a series of rings that carry 16,569 base pairs on 37 genes. By comparison, the nucleic DNA contains more than 1.5 billion base pairs. (Of course, 99 percent of these pairs are identical in every human being.) If put in terms where a base pair equaled a single person, then one could think of the number of mitochondrial base-pairs as the equivalent of the population of Laramie, Wyoming. The number of pairs in the nuclear DNA, on the other hand, is equivalent to the entire populations of China and Japan put together.

The second unique characteristic is that mtDNA is typically passed on only from the offspring's mother. This special trait of mitochondrial genetics means that there is exceedingly little change in the mtDNA from one generation to the next. Nucleic DNA is altered by 50 percent in every generation, which is why babies will typically combine physical traits from both parents (the mother's eyes, the father's chin, and so on). By comparison, the rings of mitochondrial DNA are practically clones of the mother's and stay so for generations.

This slow pace of change allows the rate of mutation to be easily tracked and measured. The ability to follow the changes in turn is a powerful tool for tracking matrilineage. The pace of "genetic drift" in mitochondrial DNA has been utilized in the tracking of many species thousands of generations into the past. When this technique was applied to humans, the result was the discovery of one of the first human lines of mitochondrial DNA, the so-called *Mitochondrial Eve*. She is thought to have been a woman who lived around 150,000 years ago.

Professor Wallace is quick to give credit where it is due. He notes that "Allen Wilson of Berkeley coined the term 'Mitochondrial Eve,' but we were the first to show that all mitochondrial DNAs coalesce

back to a single DNA. We have spent the last twenty-five years defining all the mitochondrial DNA sequence lineages."

Perhaps equally as fascinating as the age of "Eve" is the ability to trace back the geographical spread of humanity. Surprisingly enough, the genetic code validates the hypothesis that humans evolved in Africa. Wallace says with confidence, "They came out of Africa, then colonized Eurasia and then they crossed the Bering land bridge and colonized the Americas. We worked all that out. We know all those mutations, all the lines, and all the sequences."

Despite the romantic name with the Biblical overtones, it is worth mentioning that "Eve" was not the only living human female in her era, as presented in the Old Testament's story of Adam and his spouse. Rather, she would have been one of a large group of women alive in 150,000 B.C. The distinguishing characteristic that makes her case so compelling is that all the other matrilineal lineages were broken when a woman had only sons, or no children at all. By contrast, Mitochondrial Eve produced an unbroken line of daughters all the way across *fifteen thousand centuries*.

CREATING THE CYBRID

There is more than a little irony in the idea that this one area of Professor Wallace's studies—the study of mitochondrial lineage—could well be the key to handling the barrier of aged mtDNA in therapeutic cloning. Apparently, there is a logical way to beat the biological clock when creating a therapeutically cloned organ. It could, in theory, smash the barrier that free radical damage has done to the mitochondrial information.

"We don't have answers to all these problems," admits Wallace, who leans forward to provide emphasis, "But as part of my very early work in this area, we developed a method by which we could transfer mitochondria from one cell to another. We call it the cytoplasmic hybrid, or *cybrid*."

The cybrid's creation begins where the nucleus is removed from a cell. According to Wallace, this can be done physically, or using a compound called Citaglazin B. This compound alters the density gradient of the cell's cytoskeleton. In essence, it allows a researcher

to pull a nucleus right out of the cell the way one might pull the hard pit out of a soft, ripe cherry.

The remaining cytoplasm, which contains the remaining cell organs such as the mitochondria, can be fused with another cell. This would allow scientists to actually transfer mitochondria from one cell to another with relatively little difficulty. Wallace thinks that this and related technologies might be able to allow us to develop ways where we could actually manipulate the mitochondria inside the stem cells.

The ability to create optimal combinations of nucleic and mitochondrial DNA would allow us to create tissues that would be rejuvenated and at a low cancer risk. To return to one of the prior examples, take the eighty-year-old patient who needs new kidneys. Stem cell researchers could take the nuclear DNA from the patient, but transfer in mtDNA from someone who is only twenty years old, in effect turning the clock back for the newly created organ.

In this hypothetical example of a cybrid, any organ (liver, pancreas, kidney, and so on) could be grown with your genetic material in it. But wouldn't there be a conflict if it was implanted in your body? Since there's somebody else's mitochondrial DNA utilized, wouldn't it be sensed as a foreign organ?

This is the same problem that has faced transplant surgeries from the start—the possibility of having the organ decisively rejected. The body's immune system, exquisitely designed to sense foreign invaders or tissue that does not belong, would attack the new organ as if it were a foreign object and treat it the same way as a raging infection.

Wallace thinks this could happen—but only if the mitochondrial DNA was foreign enough to trigger the body's alarms.

The content of the mitochondrial DNA results in different protein antigens being present on the cell's surface. In other words, they express upon the surface of the cell certain proteins, which the immune system uses to recognize a foreign body or an invading bacteria. To the immune system, the wrong proteins on the surface of a cellular body is the equivalent of an intruder trying to get through a secure building using the wrong ID card. It sets off an

alarm directing the white blood cells to the exact location where the anomaly was sensed.

"If the oocyte that the somatic cell is fused to has mitochondrial DNA that is foreign enough, then when they put those stem cells back into that individual, he or she will reject the stem cells from the mitochondrial antigens," says Wallace. "But we have spent the last tweny-five years defining *all* the mitochondrial DNA sequence lineages. . . . We worked all that out. We know all those mutations, all of the lines, and every one of the sequences."

The research done has already begun to identify and label each mitochondrial lineage. The exact number of lineages depends on the level of genetic resolution one is looking for. There are, for instance, nine major European lineages, with the total number of very close lineages in the order of about thirty.

"The point is that we could do a mitochondrial DNA genotype match between the nucleus and the donor oocyte and make sure that they are compatible," explains Wallace. "This is a whole other area that nobody's even thinking about, and we spent a lot of time working those lineages."

It is a startling thought, but one that is well within the realm of possibility. Donor compatibility at the cellular level has been established, as we already do similar testing for the compatibility of bone marrow transplants.

GENETIC CARTOGRAPHY

The idea of being able to map out mitochondrial lineage in order to find the best mtDNA donors for therapeutic cloning is a heady one. UC Irvine's Center for Molecular and Mitochondrial Medicine and Genetics has spent the last three decades mapping out the genetics of the mitochondrial DNA. In essence, it's acted as a sort of a Human Genome Project for mtDNA.

The underlying direction of the research is to determine the causes of many of the common age-related degenerative diseases, which could be alleviated by therapies developed from stem cell technology. Up until now, these causes have been extremely difficult to resolve.

Wallace is adamant that this difficulty is simply the result of the

fact that researchers were only looking at the DNA of a cell's nucleus. They did not, or not sufficiently, look at the contents of the mitochondrial DNA, he insists. "Since the mitochondrial DNA has a totally different inheritance system, a totally different interaction because it makes thousands of copies, and because it has a key function of providing energy, they may have missed the very factor that they were looking for."

Wallace's views may again be simply ahead of the curve. The evidence in his favor is slowly accumulating. One of his latest studies, which was published by the National Academy of Sciences, shows a distinct correlation between mutations in a cell's mitochondrial DNA mutations and the development of prostate cancer.

Despite this development, there is a strange reluctance in the wider scientific community to pursue this line of research further. For all the effort spent on genetic research, it is strange that this second set of cellular blueprints is relatively ignored as candidates for possible cures—or causes—of serious illness.

Doug Wallace attributes the lack of interest to a phenomenon discussed in the book *Structure of Scientific Revolutions* by Thomas Kuhn. In it, Kuhn argues that scientific advancement is *not* evolutionary, but rather is a "series of peaceful interludes punctuated by intellectually violent revolutions." In each revolution, one conceptual world view is simply replaced by another. In astronomy, it could be argued that the transition took place when the scientific community broke from the Aristotelian way of looking at things to the Copernican view. In biology, it was the acceptance of the germ theory as the cause of disease and infection. And in physics, it was the shift from Newton to Einstein.

Additionally, Kuhn's book makes the point that people are predisposed to developing paradigms as a way of structuring information, even though the paradigms are potentially incomplete. Unfortunately, once enough people have agreed upon a paradigm and it has predictive value, they come to believe that it is, in fact, complete. It becomes exceedingly difficult to ask a question outside the accepted framework.

In the case of genetic research, the prevailing paradigm is that all important information is in the nucleus. However, should stem cells yield the potential for creating new tissues and organs to allow

spinal-cord patients to walk again or to replace failing kidneys, then the mitochondrial barrier—and how to surmount it—will take center stage. Bill Parker, dean of graduate studies at UC Irvine, puts it very aptly. "Should that field at some point be considered for a Nobel Prize, [Doug Wallace] will be right in the center of it."

IT'S ALL ABOUT TIME

I concluded my talk with Professor Wallace by asking where he thought stem cell technology could lead, especially if the obstacle presented by the mitochondria was conclusively resolved. He replies, "I think we're back to the same issues that are currently being discussed about stem cells, which is, 'Can you get them to differentiate?' 'Can you get rid of the potential to grow uncontrolled and hence form tumors?' 'Will they enter into the cell and form the right connections and do the right jobs?' "

Wallace thinks that these technical challenges are resolvable—but that it will take a lot more time. It is in the nature of experiments with human systems. "They're slow," he explains. "You have to grow the cells, you have to have model systems to test the things, and all of these things take time. So, the idea that we will be able to have therapeutic solutions to complex degenerative diseases in a very short period of time, I think, is extremely optimistic. These solutions will come, but they will come in the matter of years, not months."

It all comes back to the subject of time, whether as part of the stem cell research timetable or the life clock of a cell. And yet Wallace's speculation as to the nature of a "barrier" set up by the cell's mitochondria—assuming it can be overcome—leads one to a startling conclusion. If it is possible to "turn back the clock" of biology by replacing, say, a dying organ with one that is in the prime of health, then how long, in theory, could the human body's workable lifespan be extended? It could be a substantial amount.

If that is the case, the ultimate irony would be that the mitochondrial "barrier" is actually a gateway—one that provides a glimpse of clinical immortality.

CHAPTER 19

CAUTIONARY TALES: THE COMING STEM CELL DECADE

Ultimately, what people think about stem cell research is rather incidental to what is actually taking place. It's much like thinking about the weather, the Internet, or the proliferation of nuclear weapons. Regardless of what one believes, from the staunchest opponent to the most radical proponent, the stem cell field and the results from its research are here to stay.

This sense of absolute certainty is evident in those who work with these tiny engines of creation and chaos. Much like the team in Trinity, New Mexico, upon exploding the first atomic bomb in 1945, these researchers realize that the game has completely changed. They know that there is no way—short of a complete collapse of civilization—that the genie can but put back in the bottle.

TALENT FOR DISRUPTION

In the best of circumstances, forecasting the future of any field is a challenge. Over the years, predictions in many different areas have left more than a few experts with a mass of egg on their faces. Legendary director D.W. Griffiths claimed that the coming of the motion picture would lead to world peace. One particularly hapless talent agent turned down representation of the Beatles on the grounds that "guitar bands were going out of style." And the specter of worldwide cooling leading inexorably to the next Ice Age from the 1970s gave way to the opposite fear in the 1990s of runaway global warming and the future melting of the ice caps.

The advances that may flow from the study of stem cells are even more difficult to predict. The reasons are twofold. First, stem cell study by its very nature is entwined in the stickiest issues that overlap the fields of science, culture, religion, and politics.

The same threads of thought are wound tightly through the abortion issue, human cloning, eugenics, human evolution, and questions of religious faith versus secular bioethics. This implies that a strong tug from any one of these areas could open new doors—or close off entire areas of potential research. An example of such a "tug" could be an intense lobbying effort to legitimize human cloning, or a Supreme Court case that returns decisions about abortion rights back to the states. Pressures of this sort can shunt the line of research and discoveries off the original track in completely different directions.

There's a second, even more intractable reason that stem cell predictions come with an extra helping of murk. Stem cell research is a classic example of *disruptive technology*. Coined during the height of the dot-com era, a technology is "disruptive" when it ends up providing a radically new technological innovation, product, or service that overturns the existing dominant one.[1] It's why stem cells truly are engines of creation and chaos—any change they make may

1. The term "disruptive technology" was coined by Clayton Christensen in his 1997 book, *The Innovator's Dilemma*. Other examples of disruptive technology include nuclear weaponry, the automobile, and of course, the Internet itself.

affect the very nature of humanity, and will make us all ultimately question who—or what—we are becoming.

A CHILL IN THE AIR

The disruptive nature of the discoveries involving stem cells also cannot help but be compared with the last modern-day "gold rush"—the dot-com decade of the 1990s. The fact that stem cells could be the next new thing that transforms the world from top to bottom has not been lost on many investors or venture capitalists. This book has discussed the federal, state, and city machinations to capture as much of the start-up market as possible.

But even with the heady mixture of hype and hope, one has to ask: How robust is the field? Could a well-publicized accident throw a chill into the entire industry that puts it into a tailspin? And can we predict how the entire stem cell industry is likely to develop?

Let's take a hard look at the possibility that the support for stem cell research could be much like the skin of an apple—bright and polished, but very thin. As of this writing, anyone questioning the future popularity of this field is treated with skepticism. After all, while the field of cold fusion was derailed by a couple of overzealous researchers, the amount of money flowing into stem cell companies did not take a serious plunge after the revelations involving Dr. Hwang Woo Suk's misdeeds.

To which I reply: consider the case of Jesse Gelsinger. Jesse was an 18-year-old young man who suffered from a rare liver disorder called ornithine transcarbamylase disorder, or OTC. This genetically based disorder interferes with the body's ability to get rid of ammonia, which then accumulates in the blood, leading to the onset of a coma, brain damage, and death.

While OTC is a rare disorder (1 out of every 40,000 births), it is an extremely lethal mutation. Fifty percent of those afflicted die in the first month, and half of the remaining survivors perish by the age of five.

Jesse had already beaten the odds and was living a normal life with the help of regular dosages of medications to ensure that ammonia did not build up to toxic levels in his body. In 1999, he

was approached by researchers at the University of Pennsylvania to participate in a cutting-edge experiment in gene therapy.

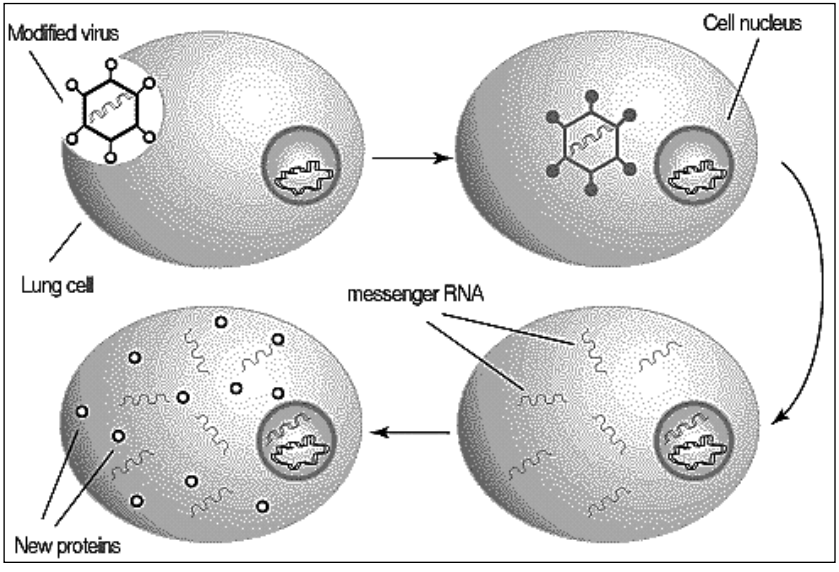
The idea was to use a special virus designed to carry healthy copies of a gene into his body, theoretically curing him of his affliction. According to his father, Paul Gelsinger, "[Jesse] believed, after discussions with representatives from Penn, that the worst that could happen in the trial would be that he would have flu-like symptoms for a week. He was excited to help."

Reassured by the researchers, Jesse took the brave step of volunteering for the research. Rather than approach the university scientists desperately looking for a cure for himself, he deliberately placed himself at risk in the clinical trial specifically to help other sufferers of the disease.

Jesse was injected on September 13, 1999 with special adenoviruses carrying a repair gene. Other patients had been injected in the same way prior to Jesse's treatment, and had suffered few if any significant side effects. However, Jesse was injected with a higher amount of the virus that only one other person had ever been given, and complications began within the first day.

FIGURE 19-1

How gene therapy using adenoviruses as transport "vectors" works.



The researchers were completely confounded when the virus began viciously attacking Jesse's internal organs. He lapsed into a coma as the virus ignored its mission of delivering its genetic payload and sent his system into shock. Four days after receiving the injection, on the 17th of September, 1999, Jesse died as a direct result of the gene therapy experiment.

NO PLACE FOR MISTAKES

The subsequent investigation revealed that the scientists involved in the trial broke several rules of conduct. First, it was determined that the study was never as risk-free as the researchers had claimed. Second, the head researcher, James Wilson, was found to have a financial stake in the therapy's success.

As a result of the investigation, Wilson stepped down from his post at the University of Pennsylvania. Jesse's parents eventually settled the case with the university for an undisclosed sum. And as of last report, James Wilson has been banned by the FDA from continuing in any research involving human beings.

Needless to say, the Gelsinger case was a severe setback to the promising field of gene therapy. Shortly after the initial investigation, the National Institutes of Health disclosed that they had received reports stating that serious side effects had been found in 691 other gene therapy trials—and of these, only 39 had been reported to federal agencies, as was required. Significantly, these trials utilized gene therapy techniques similar to the one used on Jesse, and they had been under way since 1993.

Members of Congress who participated in the investigation were equally shocked. Senator and physician Bill Frist (whom we have already bumped into more than once in these pages) was particularly prominent in these hearings. He came down especially hard on those who seemed to be more interested in pushing the envelope of science over patient welfare, stating: "If we ask patients to participate in moving science forward, then we must be assured that gene therapy clinical trails are safe. . . . There is absolutely no room, no place for mistakes that compromise patient safety."

Some changes were made to the approval process for proceeding with human trials; among them was the establishment of the

Association for the Accreditation of Human Research Protection Programs. The purpose of this association is to give an additional level of review to experiments regarding the treatment of human subjects. Additional legislation to increase the level of government review of human clinical trials was also proposed by the senior Democratic senator from Massachusetts, Ted Kennedy.

The Republican-controlled Congress came under fire for not fully supporting these changes. However, many Republicans felt that each of the additional steps would slow down the research and testing process for gene-based therapies. Ironically, it is the Republicans who could be accused—because of the Bush restrictions on stem cell lines—of "slowing down" the research process and of callously allowing precious time to be squandered before therapies for the sick can be developed.

The two situations actually do have some significant differences. The additional restrictions on gene therapy were aimed at protecting human beings. On the other hand, depending on one's point of view, stem cell restrictions protect no one but an anonymous cell that, in certain circumstances, has the potential to differentiate and grow into a human being.

And of course, the other key difference is that stem cell trials haven't resulted in a fatal clinical misfire. At least not yet.

LOOKING LEFT AS WELL AS RIGHT

Just as one can't be completely confident in the rise of stem cell research and its applications, it's too simplistic to write the field off as too fragile to survive a shock or two. Some argue that clinical mishaps could seriously impact the field of stem cell research. But critics of that argument correctly point out that even with the tragedy of Jesse Gelsinger, gene therapy is alive and well. In fact, as of late 2005, there are still several hundred gene therapy trials in progress in the United States alone, many of which will undoubtedly lead to the cure of otherwise fatal genetic disorders.

Tragedies in dramatically new types of medicine are often balanced out by equally dramatic successes. In the case of gene therapy, the first experiment—conducted in January 1999—used similar

means to Jesse Gelsinger's: a viral payload to cure a genetic disease. The recipient, Ashi DeSilva, was cured of a crippling immune deficiency that affected the white blood cells. Recognizing the overall promise of gene therapy, the people who watched the field were willing to continue on in the face of setbacks.

The rise of specific pressure groups to stand in the doorway of further stem cell research is an easy prediction to make. Not because stem cells are unique or disruptive in their own right. Rather, it's because in the hyper-pluralistic world we live in today, groups to support or oppose ideas ranging from the use of antibiotics to the sale of fast food seem to spontaneously generate out of thin air.

And it would be naïve to assume that these opponents will come solely from the "religious right." Although many people on the religious-conservative side have expressed serious reservations about the research, the qualms rapidly diminish once *embryonic* stem cells are removed from the picture. The fact that adult stem cells could someday be pushed back into that near-magical totipotent stage did not cause concern—perhaps because the cell's ability to develop into an embryo would be the result of artificial human interference as opposed to the natural act of conception.

The "human interference" in meddling with stem cells might also come under serious question from pressure groups on the opposite side of the fence. In fact, there are many cases involving genetic tampering that spawned protests on the left, whereas the religious right raised nary a voice in protest. These include experiments on gene splicing to produce mold-resistant strawberries or to create square tomatoes.

Perhaps the best-known example would be the well-organized protests against Monsanto, a U.S.-based vendor of genetically engineered agricultural products. In recent years, Greenpeace and related organizations have protested and blocked the growing and use of Monsanto's Bt corn variety MON 810 in places as close to home as Iowa and as far abroad as India.

In part, these groups have sought the effective enforcement of the Cartagena Protocol on Biosafety, which restricts the free entry of genetically modified organisms (GMOs) to a signatory country.

According to the protocol, a government must be informed before any GMOs are imported into the country by a private firm.

The claims against Bt corn are varied. Bt is supposed to pose a threat to consumers of either the direct product or meat produced by animals raised on a diet of the genetically modified corn. The pest-resistance of the corn is detrimental to monarch butterflies among other insects, threatening the natural biodiversity. Finally, there is evidence that "gene flow," the process whereby genes transfer from one population to another, does take place between Bt MON 810 and regular corn.

In 2003, Greenpeace led a huge protest in the Philippines against Monsanto and the World Trade Organization, which underwrites many of the agribusiness projects in the third world. A huge yellow banner was unfurled by three activists, who rappelled down to the seventh floor of a 13-story building housing the local conference. The banner read: *Monsanto + WTO = Environmental Destruction*.

There is no reason to think that stem cell research, as it unlocks our ability to clone, create, and modify ourselves and the world around us, will be immune to similar criticism.

WAKE ME WHEN IT'S 2015

It's highly likely that the sheer force of demand will overwhelm any opposition to the process—or the results—of stem cell research. Once commercially feasible therapies are available for relatively widespread disorders, say for sickle-cell anemia, those demanding access to the cure would be hard to stop, regardless of what a specific interest group says. But this in turn begs the question: When will commercially feasibly therapies start to arrive on the market?

While it would be wonderful to predict that within the next few years we will have unlocked the secret to replacing any organ in the body, a review of past data leads me to the opposite conclusion. That is, the number of commercially available therapies developed from stem cell technologies in the next eight to ten years will be extremely *small* in number and *limited* in scope.

In a nutshell, there is no "Moore's Law" in biology. Moore's Law states that at the current rate of technological development, the

complexity of an integrated circuit—and the commensurate rise in computing power—will double every eighteen months. This is why the computer that you purchase in January is often close to being hopelessly outmoded by the next Christmas.

But historically, advances in biology—particularly cellular biology—are done in years or even decades, not financial quarters. In fact, one of the cautionary factors in how "robust" the stem cell field of study really is comes from the fact that investors might lose patience in the relatively slow-moving biotech industry.

Those on the stem cell bandwagon will surely mention that Moore's Law does have an impact on biotech, in that our superior computing power has lead to faster research and more breakthroughs. And they'll predict that as more money and political power comes to bear on the field, more talented minds and ever-better equipment will make all the difference.

These are valid points. However, until the biotech companies are given carte blanche to market their products to the consumer market without years of testing by the FDA, the 8- to 10-year timeframe of "limited and small" numbers of remedies will be the more likely outcome. But let's move a little bit further, past the years 2015 to 2020, and the horizon starts looking a lot more promising.

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CHAPTER 20

EPILOGUE: THE COMING STEM CELL CENTURY

At first glance, it seems unlikely that a 1990s-era biotech company founded in the heart of the rags-to-riches "dot-com triangle" would have a lot in common with a Scottish animal research firm that dates back to World War I. And yet with the phenomena of easy capital transfer, corporate acquisition, and global transmission of data, the partnership works. It may very well be "ground zero" for any world-changing technology resulting from stem cell research.

Their common goal is to mass-produce transplantable cells that will repair or replace damaged body parts or organs. The cells will be tissue matched so as to avoid triggering rejection by the patient's immune system. Plans are to use these cells to treat ailments ranging from heart disease, stroke, Parkinson's, Alzheimer's, spinal cord injury, diabetes, osteoarthritis, bone marrow failure, and second or third-degree burns. And this is not a "fly by night" startup: Both companies bring a long history of success in their fields. In essence,

this partnership is one of the best bets in town so far as guessing where the next major stem cell advance might arrive on the scene.

SCOTLAND AND SILICON VALLEY

The Scottish portion of the twosome is the Roslin Institute, a research center housed in a low-slung, modernistic building in Edinburgh. Named after a local highland village, the company was established in 1993. However, the company counts as part of its "bloodline" its initial founding as the Institute of Animal Genetics (IAG) in 1919.

Originally begun under the auspices of the University of Edinburgh, in the 1990s, this otherwise unremarkable veterinary institute became a major deal in scientific circles with the cloning adventures of its commercial subsidiary, Roslin Bio-Med. Roslin came to international attention due to the talents of Dr. Ian Wilmut, who led the research team that cloned Dolly the sheep. Since then, Roslin has become a notable pioneer in the efficient and targeted production of transgenic animals.

Geron, the other half of the unlikely pair, is one of the biotechnology companies discussed earlier in the book. It is worthy of additional mention. Geron was founded in 1990 by Dr. Michael West. West is lauded as a science visionary, who was one of the first to discover that inserting a person's own DNA into an unfertilized egg cell could create tissue-specific embryonic stem cells.

The Menlo Park, California-based company focuses on creating medicines based on telomere (the DNA at the end of a chromosome) and stem cell research that can provide cures to disease or extend the human lifespan. With over sixty employees dedicated to West's vision—and with a slew of backers who have patiently waited for the company to someday turn a profit with their products—Geron is an ideally placed resource.

Several years ago, Geron acquired Roslin with the express intention of integrating their two strongest core accomplishments. Geron brought its premiere human stem cell and telomerase technologies to the table. Roslin's techniques in facilitating nuclear transfer were a perfect match to improve on Geron's existing research. The

combined technologies are expected to eventually help in regenerating failing human organs and to allow the commercial sales of genetically modified cloned animals.

West's two ventures—Geron/Roslin and Advanced Cell Technology Corporation, where he is the chief scientific officer—deserve special mention because they are physical manifestations of Dr. West's determination to conquer the aging process. West is an unabashed genius who, according to his own writings, has grown in the course of his studies from a creationist to a scientist who openly seeks to affect the course of human evolution. He is no stranger to controversy, and was once asked by a reporter, "Just what does it mean to play God?"[1]

In the context of stem cell research, "playing God" means that one can take a great step forward to where diseases that are "slate wipers"—i.e., they kill most all of their victims—are eliminated. For example, if stem cell research can identify the process of aging itself, we could learn how to make cells either instantly expire or last for generations. A drug that could increase cellular life spans would mean that the entire organism could live far longer. Another drug that stops the life cycle at the cellular level would be the instant death knell for all 400 known types of cancer.

What it means for researchers to "play God," perhaps, is to be allowed to take us toward an earthly paradise where a great deal of human suffering simply does not exist. Let's take a quick look at where we might be headed.

TWENTY YEARS OUT: LIVING IN A STATE OF PUNCTUATED EQUILIBRIUM

Chapter 19 proposed that over the next decade great strides will be made in the field of stem cell research. The future looked bright as long as the field could overcome the potential pitfalls of patient

1. One of Dr. West's many talents is that he is an engaging writer. In his 2003 book, *The Immortal Cell: One Scientist's Quest to Solve the Mystery of Human Aging*, he documents his personal quest to find—and alter or shut down—the biological machinery that relentlessly works to limit our lifespan beyond what is theoretically feasible.

safety and the pressure of special interest groups from all sides wishing to control and channel the research. However, we also hypothesized that in the absence of a biological version of Moore's Law—where the research could lead to a doubling of the improvements every eighteen months—commercially feasible technologies would be relatively rare in the first decade.

The second decade out promises to be more amazing as the companies working in the field of stem cell research build on the knowledge base acquired at such a painfully slow pace. These firms—Geron, Genentech, ATC, and others not yet founded—will begin to focus on production and bringing the cost of these therapies down to where patients from all social strata can use them. Finally, products resulting from the more difficult-to-handle embryonic stem cells will be entering the marketplace to compete against the more established offshoots from cord blood and bone marrow cells.

The state of the market will likely be one familiar to evolutionary biologists, as it will be one of *punctuated equilibrium*. This means that when a stem cell breakthrough occurs, it will do so in a sporadic, relatively quick manner. Uniform and steady growth of the field is unlikely, as any advance in the field—say a new, cheaper method to grow tissues for implantation—is likely to act as a disruptive technology.

Site-specific injections of stem cells will be one of the first widespread methods of administering these therapies. This has already been shown to work, both in cases of blood vessel repair and nerve cord regeneration (in animal trials). The way that the injected cells know what to grow into—and when to stop growing—will be a much more tightly controlled process. These methods promise to rejuvenate coronary arteries—thereby reducing the effects of heart disease. This will also be the way to repair damaged nerve tissue, eliminating the tortuous recovery period of those who suffer spinal cord or other injuries to the nervous system.

The use of tissue cultures will be another widely-used initial approach to applying stem cell technology. For example, where organ damage is too extensive for injections of cellular solutions to be effective, thin sheets or patches of tissue can be used. To continue

with the example of heart disease—which is, after all, one of the greatest killers in our country—one can easily see how useful a sheet of tissue that has been grown into cells that line blood vessels could be. To replace or repair a torn or blocked artery, the tissue could be rolled up like a little scarlet cigarillo and used as a transplant that the body would never even notice, let alone try to attack.

FORTY YEARS OUT: REPLACEMENT PARTS AND CELL BANKING COME OF AGE

A chief limitation on the use of cellular solutions or even tissue cultures that are no more than three cells thick is the time factor. In Chapter 1, we saw how stem cell technology was used to save a young woman's life after she had suffered a catastrophic injury in a car wreck. The choice of the hypothetical event was no accident.

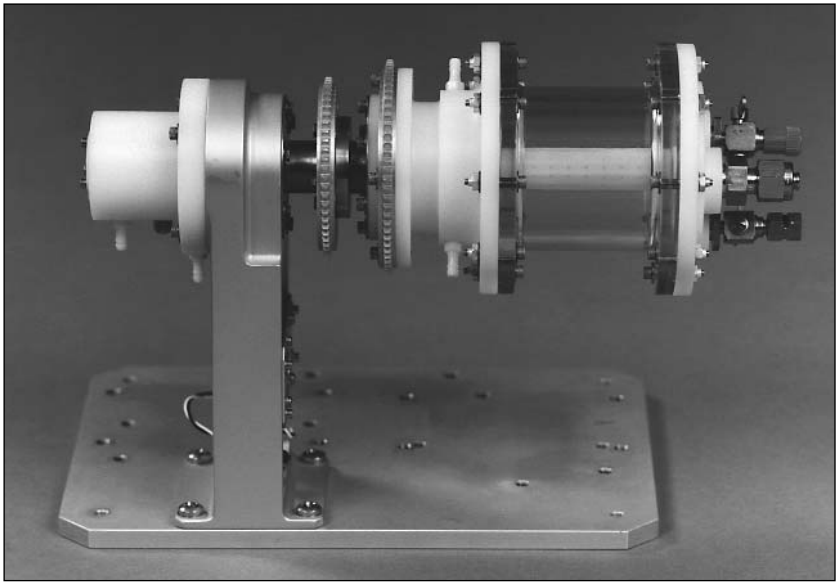
It is one thing to develop cures and tissue replacements for a long-term, chronic disease. Suffering is prevented and life extended, which are both excellent results, but therapies that can help cure a disease that slowly kills or cripples over decades will not help the person dying of organ failure in the ICU. Speedy regeneration and complete organ replacement will again change the face of medicine and how critical injuries are treated in the hospital.

For example, time has been the main obstacle to the development of the relatively simple process of creating skin tissue using a burn victim's own cellular DNA. As discussed in earlier chapters, the technology works wonderfully even today—but the treatments are frustratingly limited. Two to three-inch squares of skin tissues, which are as fragile as an eggshell in cellular thickness, have often not been enough to help the patients most in need of it.

Therefore, the next big advancement of this period will be in the related field of bioreactors. A bioreactor is a device meant to grow cells or tissues for cell cultures. Today, these devices are most commonly cylindrical vessels, ranging in size from a large bleach bottle to a Chevy Suburban. In 1997, NASA announced that it had developed a new type of bioreactor that could artificially grow heart tissue, skeletal tissue, ligaments, and other types of tissue for

FIGURE 20-1

A picture of the NASA bioreactor's key component, the rotating wall vessel.



study. Originally invented by NASA to model the effects of microgravity on cells, the key to the new technology's success is the rotating wall vessel bioreactor. The rotating unit spins a fluid medium filled with cells, encouraging cells to grow naturally into more complex structures than simple triple-cell sheets.

The bioreactors of the future will not only be able to grow complex organ structures and entire body parts, they should also be able to speed up the growth process of the human cell. Where time is of the essence, technology—which has been successful in speeding up almost any existing process, from growing tons of flu vaccine to processing cacao beans—will be utilized to cut the waiting time of days or weeks down to hours.

It's likely that patients will be able to contribute their own pluripotent cell material. While we may continue to advance in our ability to re-forge any cell in the body so that it becomes pluripotent, it is more probable that we'll be able to pull our own genetically malleable material from a storage bank with no more difficulty than making a withdrawal from a bank safe deposit box.

The withdrawal would be made from an institution described in Chapter 16, whose concept already has been around for over a decade now. Both privately held and public cord blood banks were founded in the 1990s in response to the success of umbilical cord blood transplants in treating types of anemia and leukemia. Cord blood, part of the "afterbirth" that is still commonly discarded, contains hematopoietic stem cells that can form red or white blood cells and platelets. Even now, cord blood is recognized as useful in treating blood and immune system diseases.

Private banking allows families to preserve their children's blood for their own possible use later in life. As of this writing, for-profit private banks charge a fee of around $2,000 to preserve cord blood. As the potential health benefits emerge from the use of the stem cells found in this precious resource, one can expect the price of the service to drop as massive numbers of people take the first step to give their child an extra piece of "health insurance" for the future.

SIXTY YEARS OUT: I HAVE SEEN THE FUTURE, AND IT'S BARRY BONDS

Barry Lamar Bonds is justly considered to be one of the greatest players the sport of baseball has ever seen. He has won a record seven MVP awards, eight Gold Glove Awards, and is the only player in history to have hit 500 home runs and steal 500 bases. And yet, this towering superstar of an athlete has been one of several top players who have made fans ask an uncomfortable question: To what degree, if any, does the use of chemical enhancements account for his accomplishments?

Bonds became mired in scandal in late 2003 when a federal grand jury indicted Greg Anderson, a member of the Bay Area Laboratory Co-Operative (BALCO). Anderson had also been Bond's trainer for the past three years. BALCO is a controversial sports nutrition center located south of San Francisco and has achieved a certain amount of notoriety due to allegations that the lab provided banned performance enhancing drugs to athletes. It is also alleged to have provided a designer anabolic steroid with the jaw-cracking name of tetrahydrogestrinone, or THG.

According to Bonds' testimony, Anderson had given him a rubbing balm and a liquid substance. Anderson called the balm "the cream" and the liquid "clear." But one of BALCO's founders identified the "clear" as THG, and prosecutors contended that the "the cream" was some sort of muscle-enhancing testosterone ointment. Bonds would later claim in his defense that he "unwillingly" used steroid compounds, thinking that they were "flaxseed oil."

Major League Baseball (MLB), rightly sensing the fans' outrage over the scandal, has toughened its steroid-testing policy. MLB has instituted a system of more frequent tests, even in the off-season, and stiffer penalties, starting with a ten-game suspension for first-time offenders. Year-round, random testing is a big step forward in prevention and enforcement because it won't be as easy for steroid-users to circumvent the system as before.

The case of Barry Bonds and the specter of artificial enhancement leads to an interesting dilemma that I suspect is not far off in the future. How could an athlete who was seeking to gain an "edge" on his or her competition do so without testing positive for chemical enhancements? The answer is obvious: Enhance by using cellular means.

A drug test by its very nature looks for suspicious chemical imbalances. By contrast, consider an injection of customized stem cells, say to build extra muscle mass. The enhancement would never show up on any test—because after all, the cells would indeed be the athlete's very own genetic structure, right down to each individual gene.

But it won't just be athletes who are "customizing" their bodies. Given a few more decades, the technologies involved in stem cell research will have moved beyond the purely therapeutic stage and into issues of body enhancement and customization. Growing extra muscle, removing fat cells, even reshaping one's facial bone structure might all be possible with a visit to the doctor. Just as with many medical advances that originally started out to save lives—witness the change of use of the laser or reconstructive "plastic" surgery—stem cell technologies will over time be used for cosmetic alterations to the human body.

EIGHTY YEARS OUT: OPEN SOURCE MAGIC

One trend that has consistently shown up in over time in all sorts of technology has been the phenomenon of increasing *mutuality*. In this case, mutuality refers to interactions involving close physical contact or a high degree of comfort and familiarity with the process or result of technology.

An excellent example of the slow but steady growth of mutuality is in the area of firearms. The earliest drawings of gunpowder weapons show church bells that were pressed into service to make primitive cannons. The fuse is lit using a long taper while the gunners hide behind a nearby barrier in case the bombard blew up!

Fast-forward a few decades and you'll see gunners standing confidently by their artillery units with less fear of going up along with the weapon. Move a few decades further on and the first muskets appear—as strange-looking handheld weapons which are propped up like a one-legged table on a single prong in order to steady the weapon's aim. A whole century would go by until soldiers were confident enough in the weapon's safety to start putting the butt of the gun against the shoulder. Comfort and confidence in the technology grows steadily as the technology improves.

Similarly, in the ensuing decades, stem cell technologies could lose their mystique and become commonplace. The general public might be able to experiment to their heart's content in areas where only multimillion dollar labs can tread today. There is precedent for this happening, and it dates back to the heady days of the dot-com revolution.

Computer programming was once restricted to a select few experts. Eventually, however, the field was opened up to anybody who wished to learn how to "instruct" the new machines on how to do everything from sending information to the printer to "talking" with other computers hundreds of miles away. Over time, one of the niche operating systems, called Linux, began to be developed by its own user communities and grew organically depending on the needs of its users. In other words, Linux became the premiere example of the *open source* mentality. Open source denotes

a purely independent, collaborative effort, similar to the way in which the Internet began opening up beyond the military and educational areas.

There is no reason to think that the same thing won't happen with stem cell research. Once the Human Genome Project publishes all of its findings online, where anyone can view which marker genes control the growth of specific kinds of cells, open source magic should begin to spread. Perhaps gene splicing and stem cell growth kits will be no more exotic to the people of 2085 to 2090 than children's chemistry sets are to us today.

100 YEARS AND ON: DEATH SPIRALS AND DEMOGRAPHY

Stem cell technology is bound at the hip to concepts such as cloning. In fact, one technology will often abet the other by complementing it. Within a century, it's not difficult to imagine that simple and relatively cheap methods of organ replacement will be available, easily extending a person's reproductive lifespan. Even without the ability to clone an entire person from a single cell, stem cell technologies could fundamentally alter the power of entire countries and cultures.

The reasoning behind this idea boils down to the simple fact that "demography is destiny." Journalist and commentator Mark Steyn, in a February 2006 column for the daily *Australian*, gives a compelling example of how a technical advance altered demography—and in doing so, completely changed the world.

> Demography doesn't explain everything but it accounts for a good 90 per cent. . . . Why is this newspaper published in the language of a tiny island on the other side of the earth? Why does Australia have an English Queen, English common law, English institutions? Because England was the first nation to conquer infant mortality.
>
> By 1820 medical progress had so transformed British life that half the population was under the age of 15. Britain had the manpower to take, hold, settle, and administer huge chunks

of real estate around the planet. Had, say, China or Russia been first to overcome childhood mortality, the modern world would be very different.

If one accepts the idea that demographically a society needs a fertility rate of 2.1 births per woman to maintain a population-steady state, then many countries of today would stand to benefit from a demographic "shot in the arm." Australia comes in slightly below the replacement mark at 1.77, while several developed nations in Europe such as Italy, Spain, and Germany are flirting with the dangerously low birthrate of 1.3 per woman. Why dangerous? Because once rates fall below 1.3, the society in question enters a sort of "death spiral" where the population will free-fall until it reaches a new, lower equilibrium.

Fancy mathematics are not needed in order to make the politicians of these countries aware of how a major population shortfall would affect everything from military power to consumption trends and the ability to pay for social welfare systems. Some ideas to reverse this have already been proposed and acted upon—for example, Italy's extremely generous leave policy in regard to pregnant women.

This kind of strong legislative medicine has been tried with mixed success. If it does not turn the growth rate around, then it is easy to see how tempting it would be for a country in such straits to embrace stem cell work as a way to keep their population around longer, and perhaps even rely on wholesale reproductive cloning to ensure that they are not overwhelmed by a faster growing neighbor.

To us today, it seems bizarre to predict that a country will want to add even more members in what is proving to be a crowded, resource-hungry world. Yet this could be one of the many "ripples" caused by the appearance of stem cell innovations. Ripples that no one can see coming, but only cope with once they occur.

Given the talent that these little cells have for both creation and chaos, we have little choice but to rely upon the wisdom of the next few generations to handle the cellular genie that has been released

from its T-flask bottle. To paraphrase historian Paul Kennedy, it may be that the countries and individuals living in the century of the stem cell will be able neither to create nor direct the resulting events—and a great deal will depend on the skill with which they manage to sail on the stream of time.

APPENDIX A

PROTOCOLS AND NUTRIENT MIXES FOR CULTURING HUMAN CELLS

EMBRYONIC STEM CELL THAWING AND PRESERVATION MAINTAINING[1]

If you are embarking in growing embryonic stem cells, be prepared to refeed them daily. All procedures should be carried out using sterile techniques.

The growth and maintenance media for embryonic stem cells is M15: DMEM (no pyruvate, high glucose) 15 percent FBS, 1X GPS, 1XBME.

Handling Embryonic Stem Cells

Growth, maintenance, passing, freezing, and thawing is conducted in a manner to protect and maintain the quality of the cells and keep them in a pluripotent state. Serum quality is critical for successful

1. From Baylor College of Medicine, Houston, Texas.

growth of embryonic stem cells and especially true for blastocysts. The quality of the feeders is very instrumental. Remember also that in passing, freezing, and electroporating embryonic stem cells, it is best that the cells are still at exponential growth (80 percent confluence) for optimal results.

Quick Thawing Procedure

1. Remove cells from the freezer and quickly thaw in a 37° Celsius waterbath.
2. Transfer the cell suspension to a sterile 15 mL tube. Add 10 to 12 mL of M15 media to 1 mL of cell suspension.
3. Gently mix and pellet the cells by centrifuging @ 1000 rpm for 7 minutes.
4. Aspirate off supernatant and resuspend cells into 6 mL of M15, and plate out cells in a 6-cm feeder plate.
5. Refeed cells daily with fresh M15. Upon 80 to 85 percent confluence, cells need to be passaged or frozen. (M15 media: DMEM, 15 percent FBS, 1X GPS, 1XBME)

Passage of Embryonic Stem Cells

Embryonic stem cells typically should be passaged every 2 to 4 days (apart from colonies under selection). If passaging is neglected the cells will differentiate and you will select for variants that might have lost totipotency.

Cells must be fed when media begins to turn orange.

Yellow media (acid pH) is very bad for embryonic stem cells and should be avoided at all costs. If you are planning to passage and believe that the cells might turn yellow overnight, feed last thing in the evening and again the next morning before passaging. DO NOT PASSAGE CELLS WHEN MEDIA IS YELLOW.

1. Check cells under the microscope for 80 to 85 percent confluence.
2. Refeed cells 3 to 4 hours before passaging them.

3. Aspirate media off. Wash one time with PBS. Add 500 µl of trypsin to a 6-cm plate, or 1 to 1.5 mL of trypsin to a 10-cm plate.
4. Incubate @ 37° Celsius for 15 minutes.
5. Add media, M15 to inactivate the trypsin. About 2 mL to 1 × 6-cm dish or 4 to 5 mL to 1 × 10-cm dish.
6. With a transfer pipette, pipet up and down several times to separate the cells and break any colonies.
7. Determine the number of feeder plates you need, depending upon the passage you are doing. Add fresh media, M15 to the feeder plates (to 1 × 6-cm feeder dish, add 6 mL of media; to 1 × 10-cm feeder dish, add 12 mL of media). Split ratios for embryonic stem cells can vary from 1:1 to 1:10. Do not exceed 1:10.

The area relationships for the various dishes are as follows:

Dish	Media	Trypsin	Area (cm2)	Diameter (actual)
(6-mm) 96 well	200 µl/well	30–50 µl	0.3	0.6 cm
(10-mm) 24 well	1.0 mL	200 µl	1.8	1.5 cm
(30-mm) 6-well plate	3–4 mL	400 µl	9.6	3.5 cm
(6-cm) dish	6 mL	0.6 mL	21.2	5.2 cm
(10-cm) dish	12 mL	1.5 mL	60	8.7 cm
(15-cm) dish	30 mL	2.5 mL	154	14 cm

Some typical passaging ratios:

1:6 = 1 × 60 mm to 2 × 90 mm

1:6 = 1 × 30 mm to 1 × 90 mm

1:4 = 1 × 30 mm to 2 × 60 mm

1:5 = 1 × 24 well to 1 × 30 mm (6-well plate)

1:6 = 1 × 96 well to 1 × 24 well

8. Aliquot the cell suspension into plates in the volume specified for each plate. Remember to use Feeder plates. Always check the feeders before using them. They should be confluent, no gaps, not contaminated, and not dividing. Use feeders that are older (1 to 2 weeks old), the advantages are many: Any contamination is assessed, also any dividing run-away cells can be detected, and the passage will be earlier. Also, older feeders have settled nicely and flattened.
9. Mix to have a uniform cell distribution. Return plates to the TC 37° Celsius incubator.

Slow Freezing Procedure

1. Check cells under the microscope for 80 to 85 percent confluence.
2. Refeed cells 3 to 4 hours before passaging them.
3. Aspirate media off. Wash one time with PBS. Add 500 µl of trypsin to a 6-cm plate, or 1 to 1.5 mL of trypsin to one 10-cm plate.
4. Incubate @ 37° Celsius for 15 minutes.
5. Add media, M15 (M15 media: DMEM, 15 percent FBS, 1XGPS, 1XBME) to inactivate the trypsin. About 2 mL to 1 × 6-cm dish or 4 to 5 mL to 1 × 10-cm dish.
6. With a transfer pipette, pipet up and down several times to separate the cells and break any colonies. Collect cell suspension in a centrifuge tube and add more media to count.
7. Count a 200 µl aliquot and calculate the total cell number. From this, calculate the volume of media required to give a final density of 3.0 × 107 cells/mL. This density is very important, do not deviate from it.

8. Pellet cells by centrifuging @ 1000 rpm for 7 minutes.
9. Aspirate off supernatant and resuspend the pellet in ½ the volume calculated in Step 7 above, with M15 media.
10. Add ½ the volume with 2X Freezing Media (60 percent DMEM, 20 percent FBS, 20 percent DMSO, freshly prepared); the cell suspension is diluted as a result: 10 percent DMSO is the final concentration. Add the freezing media drop-wise, mixing well after each addition.
11. Aliquot the suspension into sterile freezing vials, pre-labeled with the cell type (AB2.2, AB1, etc.), clone number, passage number, and date. A typical aliquot would have 0.3 mL to 0.4 mL of embryonic stem cells (@ a density = 3.0 × 107 cells/mL). This is about 9 × 106 cells to 12 × 106 cells total/vial.
12. Place vials into a freezing container. It is critical that the freezing rate is not faster than 1¡C/minute. Do not use any untested styrofoam container since freezing rates vary greatly and this will most likely result in death of most of your cells. Freeze cells overnight at –70° Celsius (24 hours).
13. Next day, transfer cells to the Liquid Nitrogen freezer (or –135° Celsius freezer).

HAM'S CELL GROWTH MEDIUM

This list includes:

- All 20 of the amino acids from which proteins are synthesized
- A purine (hypoxanthine) and a pyrimidine (thymidine) for the synthesis of nucleotides, and their polymers DNA and RNA
- Two precursors (choline and inositol) needed to synthesize some of the phospholipids in the cell

- Eight vitamins, all of which serve as parts of coenzymes
- The coenzyme lipoic acid
- Glucose as a source of energy and carbon atoms
- The inorganic ions Na+, K+, Ca2+, Cu2+, Zn2+, and CO2+ (E. coli may need some of these as well but in such tiny amounts that it can acquire them as impurities in the other ingredients of its medium.)

Even when all these ingredients have been mixed together, most mammalian cells still fail to grow unless some blood serum (e.g., from a human or a calf) is added. Just what metabolic need is met by this supplement is uncertain, but trace amounts of hormones in the serum are probably important.

Why does a mammalian cell require such a complex broth? It is the price of multicellularity. A mammal is made up of hundreds of different cell types, each specialized to perform one or a few functions.

All the many other functions of life—including the synthesis of many of the organic molecules it needs—a mammalian cell delegates to other cells. The extracellular fluid, derived from the blood, supplies it with these cells. Ham's tissue culture medium is an attempt to recreate this extracellular fluid.

Ham's Tissue Culture Medium for Mammalian Cells
(amounts dissolved in 1 liter of triple distilled water)

L-Arginine	211 mg	Biotin	0.024 mg
L-Histidine	21 mg	Calcium pantothenate	0.7 mg
L-Lysine	29.3 mg	Choline chloride	0.69 mg
L-Methionine	4.48 mg	i-inositol	0.54 mg
L-Phenylalanine	4.96 mg	Niacinamide	0.6 mg
L-Tryptophan	0.6 mg	Pyridoxine hydrochloride	0.2 mg
L-Tyrosine	1.81 mg	Riboflavin	0.37 mg
L-Alanine	8.91 mg	Thymidine	0.7 mg
Glycine	7.51 mg	Cyanocobalamin	1.3 mg
L-Serine	10.5g	Sodium pyruvate	110 mg
L-Threonine	3.57 mg	Lipoic acid	0.2 mg
L-Glutamic acid	14.7 mg	$MgSO_47H_20$	153 mg
L-Asparagine	15 mg	Glucose	1.1g
L-Glutamine	146.2 mg	NaCl	7.4 g
L-Isoleucine	2.6 mg	KCl	285 mg
L-Leucine	13.1 mg	Na_2HPO_4	290
L-Proline	11.5 mg	KH_2PO_4	83 mg
L-Valine	3.5 mg	Phenol red	1.2 mg
L-Cysteine	31.5 mg	$FeSO_4$	0.83 mg
Thiamine hydrichloride	1 mg	$CuSO_4S_2O$	0.0025 mg
Hypoxanthine	4 mg	$ZnSO_4.7H_2O$	0.028 mg
Folic acid	1.3 mg	NaHCO3	1.2 g

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APPENDIX B

THE CALIFORNIA STEM CELL RESEARCH AND CURES INITIATIVE: SELECTED TEXT FROM CALIFORNIA PROPOSITION 71

PROPOSAL

The measure authorizes the state to sell $3 billion in general obligation bonds to provide funding for stem cell research and research facilities in California. A new state medical research institute would be established to use the bond funds to award grants and loans for stem cell research and research facilities, and to manage stem cell research activities funded by this measure within California. The major provisions of the measure are discussed below.

New State Institute Created

This measure would establish the California Institute for Regenerative Medicine to award grants and loans for stem cell research and

research facilities. The institute would also be responsible for establishing regulatory standards for stem cell research funded by the grants and loans and managing such research and the development of related facilities. The institute could have a staff of up to fifty employees who, under the measure, would be exempt from state civil service requirements.

The institute would be governed by a 29-member Independent Citizen's Oversight Committee (ICOC), comprised of representatives of specified UC campuses, another public or private California university, nonprofit academic and medical research institutions, companies with expertise in developing medical therapies, and disease research advocacy groups. The governor, lieutenant governor, treasurer, controller, speaker of the assembly, president pro tempore of the senate, and certain UC campus chancellors would make the appointments to the ICOC.

General Obligation Bond Funding

The measure would authorize the state to sell $3 billion in general obligation bonds, and limit bond sales to no more than $350 million per year. The measure states its intent, but does not require in statute, that the bonds be sold during a ten-year period.

For at least the first five years after the measure took effect, the repayment of the principal would be postponed and the interest on the debt would be repaid using bond proceeds rather than the General Fund. Subsequent interest and principal payments after that five-year period would come from the General Fund. The proceeds from the bond sales would be placed in a new California Stem Cell Research and Cures Fund and used primarily to fund the various activities of the institute. The funds authorized for the institute would be continuously appropriated without regard to fiscal year.

Once the measure took effect, the institute would receive a $3 million start-up loan from the state General Fund for initial administrative and implementation costs. The institute would later repay the General Fund loan using the proceeds from the sale of bonds authorized under this measure.

How Funding Would Be Spent

Under the measure, any funding needed for various bond-related costs (for example, the cost of administering the bond sales) would be deducted before bond proceeds were spent for other purposes.

The institute would be able to use up to 3 percent of the remaining bond proceeds for general administrative costs and up to an additional 3 percent for administrative costs associated with grant-making activities. The remaining funds would be used for the grants and loans for research and research facilities.

Priority for research grant funding would be given to stem cell research that met the institute's criteria and was unlikely to receive federal funding. In some cases, funding could also be provided for other types of research that were determined to cure or provide new types of treatment of diseases and injuries. The institute would not be allowed to fund research on human reproductive cloning.

Up to 10 percent of the funds available for grants and loans could be used to develop scientific and medical research facilities for nonprofit entities within the first five years of the implementation of the measure.

Benefits from Royalties and Patents

The ICOC would establish standards requiring that all grants and loans be subject to agreements allowing the state to financially benefit from patents, royalties, and licenses resulting from the research activities funded under the measure.

Right to Conduct Stem Cell Research

Consistent with current statute, this measure would make conducting stem cell research a state constitutional right.

FISCAL EFFECTS

Borrowing Costs

As noted earlier, this measure provides that no General Fund payments for the bonds would occur in the first five years after it took effect. The costs to the state after that would depend on the interest

rates obtained when the bonds were sold and the length of time it took to repay the debt. If the $3 billion in bonds authorized by this measure were repaid over a 30-year period at an average interest rate of 5.25 percent, the cost to the General Fund would be approximately $6 billion to pay off both the principal ($3 billion) and interest ($3 billion). The average payment for principal and interest would be approximately $200 million per year.

Institute Operating Costs

As noted earlier, this measure would limit the amount of bond funding available that the institute could use for its administrative activities. The measure does not specify what would happen if the institute's administrative costs were greater than the amount of available bond funding. The amount of additional General Fund support that would be required, if any, is unknown, but would be unlikely to exceed a few million dollars annually.

Loan Repayment Revenues

If the institute awards loans in addition to grants for stem cell research and facilities, the institute would eventually receive revenues from the repayment of those loans. The measure specifies that any such loan repayment revenues would be used either to provide additional grants and loans or to pay ongoing costs for the administration of the bonds.

State Revenues from Research

As noted earlier, this measure would allow the state to receive payments from patents, royalties, and licenses resulting from the research funded by the institute. The amount of revenues the state would receive from those types of arrangements is unknown but could be significant. The amount of revenue from this source would depend on the nature of the research funded by the institute and the exact terms of any agreements for sharing of revenues resulting from that research.

Effects on University System

To the extent that the UC system receives a share of the grants awarded by the institute, it could attract additional federal or pri-

vate research funding for this same purpose. The UC system could also eventually receive significant revenues from patents, royalties, and licenses.

Other Potential Fiscal Effects

If the measure were to result in economic and other benefits that would not otherwise have occurred, it could produce unknown indirect state and local revenue gains and cost savings. Such effects could result, for example, if the added research activity and associated investments due to the measure generate net gains in jobs and taxable income, or if funded projects reduce the costs of health care to government employees and recipients of state services. The likelihood and magnitude of these and other potential indirect fiscal effects are unknown.

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Appendix A

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Appendix B

Proposition 71: Stem Cell Research Funding—State of California. From smartvoter.org/2004/11/02/ca/state/prop/71

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GLOSSARY OF TERMS

Adult stem cell: An undifferentiated cell found in a differentiated tissue that can renew itself and (with certain limitations) differentiate to yield all the specialized cell types of the tissue from which it originated.

Allogeneic: Two or more individuals (or cell lines) are stated to be allogeneic to one another when the genes at one or more loci are not identical in sequence in each organism.

Amnion: The innermost intrauterine membrane around the fetus and the amniotic fluid.

Antibody: A Y-shaped protein secreted by B cells in response to an antigen. An antibody binds specifically to the antigen that induced its production. Antibodies directed against antigens on the surface of infectious organisms help eliminate those organisms from the body.

Antigen: A substance (often a protein) that induces the formation of an antibody. Antigens are commonly found on the surface of infectious organisms, transfused blood cells, and transplanted organs.

Apheresis: From the Greek, "to take away." A medical technology in which the blood of a donor or patient is passed through an apparatus that separates out one particular constituent and returns the remainder to the circulation.

Apoptosis: Genetically programmed cell death.

ASCs: Acronym for "Adult Stem Cells."

Assisted reproductive technology: Fertility treatments that involve a laboratory handling eggs or embryos, such as in vitro fertilization (IVF).

Astrocyte: One of the large neuroglia cells of nervous tissue.

Autoimmune disease: A condition that results from T cells and/or antibodies that attack the cells or tissues of an individual's own body.

Autologous transplant: Transplanted tissue derived from the intended recipient of the transplant. Such a transplant helps avoid complications of immune rejection.

Blastocoel: The cavity in the blastula of the developing embryo.

Blastocyst: A preimplantation embryo of about 150 cells. The blastocyst consists of a sphere made up of an outer layer of cells (the trophectoderm), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass, or "ICM").

Blastula: An early stage in the development of an ovum consisting of a hollow sphere of cells enclosing a cavity called the blastocoel.

Bone marrow: The soft, living tissue that fills most bone cavities and contains hematopoietic stem cells, from which all red and white blood cells evolve. The bone marrow also contains mesenchymal stem cells that a number of cell types come from, including chondrocytes, which produce cartilage.

Bone marrow stromal cells: A stem cell found in bone marrow that generates bone, cartilage, fat, and fibrous connective tissue.

Cell-based therapies: treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or depleted adult cell populations or tissues.

Cell culture: Growth of cells in vitro on an artificial medium for experimental research.

Cell division: Method by which a single cell divides to create two cells. This continuous process allows a population of cells to increase in number or maintain its numbers.

Clone: From the Greek word meaning "twig." A line of cells that is genetically identical to the originating cell—in this case, a stem cell.

Culture medium: The broth that covers cells in a culture dish, which contains nutrients to feed the cells as well as other growth factors that may be added to direct desired changes in the cells.

Cytoplasm: The contents of a cell other than the nucleus. Cytoplasm consists of a fluid containing numerous structures, known as organelles, which carry out essential cell functions.

Dendrite: Extension of a nerve cell, typically branched and relatively short, that receives stimuli from other nerve cells.

Differentiation: The process by which early unspecified cells acquire the features of specific cells, such as heart tissue, liver, or muscle.

Directed differentiation: Manipulating stem cell culture conditions to induce differentiation into a particular cell type.

DNA: Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions for making all the structures and materials the body needs to function.

Ectoderm: The upper, outermost of the three primitive germ layers of the embryo. It gives rise to skin, nerves, and brain.

Embryo: In humans, the developing organism from the time of fertilization until the end of the eighth week of gestation, when it becomes known as a fetus.

Embryoid bodies (EBs): Clumps of cellular structures that arise when embryonic stem cells are cultured. Embryoid bodies contain tissue from all three of the germ layers: endoderm, mesoderm, and ectoderm. Embryoid bodies are not part of normal development and occur only in in-vitro conditions.

Embryonic germ (EG) cells: Cells found in a specific part of the embryo/fetus called the gonadal ridge that normally develop into mature gametes.

Embryonic stem (ES) cells: Primitive (undifferentiated) cells from the embryo that have the potential to become a wide variety of specialized cell types.

Endoderm: Lower layer of a group of cells derived from the inner cell mass of the blastocyst; it later becomes the lungs and digestive organs.

Erythroid cell: Red blood cells.

ESCs: Acronym for "embryonic stem cells."

Ex vivo: Outside the living body.

Feeder cell layer: Cells that are utilized in co-culture to maintain pluripotent stem cells. Cells usually consist of mouse embryonic fibroblasts.

Fertilization: The process whereby male and female gametes unite.

Fetal calf serum: A type of culture medium often used in the culture of stem cells. It provides a number of growth factors.

Fetus: A developing human from usually eight weeks after conception to birth.

Fibroblast: Cells that give rise to connective tissue.

Fluorescence-activated cell sorting (FACS): A technique that can separate and analyze cells, which are labeled with fluorochrome-conjugated antibody, by their fluorescence and light scattering patterns.

Gene: A functional unit of heredity that is a segment of DNA located in a specific site on a chromosome. A gene directs the formation of an enzyme of other protein.

Genital Ridge: Formation of a genital ridge requires at least two genes, WT-1, which is also important in early kidney formation, and SF-1, required for the development of both the gonads and adrenal glands.

Genome: The complete genetic material of an organism.

Germ cell: A sperm or egg, or a cell that can become a sperm or egg. All other body cells are called somatic cells.

Gestation: The period of development of an organism from fertilization of the ovum until birth.

Gonad: The embryonic sex gland before it becomes a definitive testis or ovary.

Gonadal ridge: Anatomic site in the early fetus where primordial germ cells (PGCs) are formed.

Granulocyte: A type of white blood cell filled with microscopic granules that are little sacs containing enzymes, compounds that digest microorganisms.

Green fluorescent protein (GFP): Fluorescent-protein dye used to tag and trace particular genes and cells of interest.

Hematopoiesis: Generation of blood cells, mainly in the bone marrow.

Hematopoietic stem cell (HSC): A stem cell from which all red and white blood cells evolve.

HES cell: Human embryonic stem cell, a type of pluripotent stem cell.

HSC markers: Cell-surface molecules that are used to identify hematopoietic stem cells.

Hypoblast: The inner cell layer, or endoderm, which develops during the formation of the embryonic germ layers.

Identical twinning: Process in which genetically identical organisms arise from symmetrical division and separation of totipotent cells.

Immune-function assay: A general term for a number of tests based on an immune cell's ability to carry out a particular immune function.

Immune system cells: White blood cells or leukocytes that originate from the bone marrow. They include antigen-presenting cells, such as dendritic cells, T and B lymphocytes, and neutrophils, among many others.

Immunocompromised mice: These genetically altered mice are used for transplantation experiments because they usually do not reject the transplanted tissue.

Immunofluorescence: The detection of antibodies by using special proteins labeled with fluorescein. When present, the specific organism or antibody is observed as a fluorescent material when examined microscopically while illuminated with a fluorescent light source.

Immunogenic: Relating to or producing an immune response.

Immunohistology: Examination of tissues through specific immunostaining techniques.

Immunophenotyping: Identification of various types of immune cells by sorting them according to their cell-surface markers.

Inner cell mass: The cluster of cells inside the blastocyst. These cells give rise to the embryonic disk of the later embryo and, ultimately, the fetus.

In utero: In the uterus.

In vitro: Literally, "in glass," meaning in a laboratory dish or test tube; in an artificial environment.

In vitro fertilization (IVF): An assisted reproduction technique in which fertilization is accomplished outside the body.

In vivo: In the living subject; in a natural environment.

Irradiate: Application of radiation from a source (heat, light, x-rays) to a structure or organism.

Karyotype: The full set of chromosomes of a cell arranged with respect to size, shape, and number.

Keratinocytes: Cells that synthesize keratin and are found in the skin, hair, and nails. A fibrous protein is produced by keratinocytes and may be hard or soft. The hard keratin is found in hair and nails. The soft keratin is found in the epidermis of the skin in the form of flattened non-nucleated scales that slough continually.

Lacunae: The spaces occupied by cells (e.g., chondrocytes and osteocytes) of calcified tissues.

Leukemia inhibitory factor (LIF): A growth factor necessary for maintaining mouse embryonic stem cells in a proliferative, undifferentiated state.

Leukocyte: A white blood cell or corpuscle. Leukocytes are formed from undifferentiated stem cells that give rise to all blood cells.

Lymph nodes: Widely distributed lymphoid organs within the lymphatic system where many immune cells are concentrated.

Lymphatic system: A network of lymph vessels and nodes that drain and filter antigens from tissue fluids before returning lymphocytes to the blood.

Marker: See Surface marker.

Mast cell: A large tissue cell that does not circulate in the blood. They are also important in producing the signs and symptoms of hypersensitivity reaction, such as those of an insect sting, and certain forms of asthma.

Maternal gene product: A product in the male organism of a gene from the X chromosome.

Meiosis: A process where two successive cells divide and produce cells, eggs, or sperm that contain half the number of chromosomes in the somatic cells. During fertilization, the nuclei of the sperm and ovum fuse and produce a zygote with the full chromosome complements.

Melanocyte: A cell that produces the dark pigment melanin; responsible for the pigmentation of skin and hair.

Memory: The ability of antigen-specific T or B cells to "recall" prior exposure to an antigen and respond quickly without the need to be activated again by CD4 helper T cells.

Memory cells: A subset of antigen-specific T or B cells that "recall" prior exposure to an antigen and respond quickly without the need to be activated again by CD4 helper T cells.

Mesoderm: The middle layer of the embryonic disk, which consists of a group of cells derived from the inner cell mass of the blastocyst. This middle germ layer is known as gastrulation and is the precursor to bone, muscle, and connective tissue.

Monoclonal: From a single cell.

Monoclonal antibody (MoAb): An exceptionally pure and specific antibody derived from hybridoma cells. Because each of the clones is derived from a single B cell, all of the antibody molecules it makes are identical.

Monocyte: A white blood cell derived from myeloid stem cells.

Morphology: The shape and structural makeup of a cell, tissue, or organism.

Mouse embryonic fibroblast (MEF): Cells that are used as feeder cells when culturing pluripotent stem cells.

Multipotent stem cells: Stem cells that have the capability of developing cells of multiple germ layers.

Myelin: A fatty sheath that covers axons of nerve cells. It is produced by oligodendrocytes and provides an insulation for nerve conduction through the axons.

Myelin sheath: Insulating layer of specialized cell membrane wrapped around vertebrate axons. This sheath is produced by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system.

Neural crest: A band of cells that extend lengthwise along the neural tube of an embryo and give rise to cells that form the cranial, spinal, and autonomic ganglia, as well as becoming odontoblasts, which form the calcified part of the teeth.

Neural plate: A thickened band of ectoderm along the dorsal surface of an embryo. The nervous system develops from this tissue.

Neural stem cell (NSC): A stem cell found in adult neural tissue that can give rise to neurons, astrocytes, and oligodendrocytes.

Neural tube: The embryological forerunner of the central nervous system.

Neurofilament (NF): A type of intermediate filament found in nerve cells.

Neuron: A nerve cell, the structural and functional unit of the nervous system. A neuron consists of a cell body and its processes, an axon, and one or more dendrites. Neurons function by the initiation and conduction of impulses and transmit impulses to other neurons or cells by releasing neurotransmitters at synapses.

Node: A knot or knob; a protrusion or swelling; a constricted region; a small, rounded organ or structure.

Oligodendrocyte: Cell that provides insulation to nerve cells by forming a myelin sheath around axons.

Oocyte: Developing egg; usually a large and immobile cell.

Ovarian follicle: An external, fluid-filled portion of the ovary in which oocytes mature before ovulation.

Oviduct: The passage through which the ova travel from the ovary into the uterus.

Passage: A round of cell growth and proliferation in culture.

Placenta: The oval or discoid spongy structure in the uterus from which the fetus derives its nourishment and oxygen.

Plasticity: The ability of stem cells from one adult tissue to generate the differentiated types of another tissue.

Pluripotent stem cell (PSC): A single stem cell that has the capability of developing cells of all germ layers (endoderm, ectoderm, and mesoderm).

Polarity: The presence of an axial, nonsymmetric gradient along a cell or tissue.

Population doublings: A doubling in the number of cells when grown in culture.

Precursor cells: In fetal or adult tissues, these are partly differentiated cells that divide and give rise to differentiated cells. Also known as progenitor cells.

Pre-implantation embryo: The very early, free-floating embryo, from the time the egg is fertilized until implantation in the mother's womb is complete.

Primary germ layers: The three initial embryonic germ layers—endoderm, mesoderm, and ectoderm—from which all other somatic tissue-types develop.

Primitive streak: The initial band of cells from which the embryo begins to develop. The primitive streak establishes and reveals the embryo's head-tail and left-right orientations.

Radioimmunoassay: A sensitive method of determining the concentration of a substance, particularly a protein-bound hormone, in blood plasma.

Ribonucleic acid (RNA): A chemical that is similar in structure to DNA. One of its main functions is to translate the genetic code of DNA into structural proteins.

Ribosome: Any of the RNA- and protein-rich cytoplasmic organelles that are sites of protein synthesis.

Somatic cell nuclear transfer: The transfer of a cell nucleus from a somatic cell into an egg from which the nucleus has been removed.

Somatic cells: Any cell of a plant or animal other than a germ cell or germ cell precursor.

Stem cell: A cell that has the ability to divide for indefinite periods in culture and to give rise to specialized cells.

Stromal cell: A nonblood cell that is derived from blood organs, such as bone marrow or fetal liver, which is capable of supporting growth of blood cells in vitro. Stromal cells that make this matrix within the bone marrow are also derived from mesenchymal stem cells.

Surface marker: Surface proteins that are unique to certain cell types capable of detection by antibodies or other detection methods.

T cells: A type of white blood cell that is of crucial importance to the immune system. Immature T cells migrate to the thymus gland in the upper chest cavity, where they mature and differentiate into various types of mature T cells and become active in the immune system in response to a hormone called thymosin and other factors. T-cells that are potentially activated against the body's own tissues are normally killed or changed ("down-regulated") during this maturation process.

Telomerase: An enzyme that is composed of a catalytic protein component and an RNA template and that synthesizes DNA at the ends of chromosomes and confers replicative immortality to cells.

Telomere: The end of a chromosome, associated with a characteristic DNA sequence that is replicated in a special way. A telomere counteracts the tendency of the chromosome to shorten with each round of replication.

Teratocarcinoma: A tumor that occurs mostly in the testis.

Teratoma: A tumor composed of tissues from the three embryonic germ layers. Usually found in ovary and testis. Produced experimentally in animals by injecting pluripotent stem cells, in order to determine the stem cells' abilities to differentiate into various types of tissues.

Thymus: A lymphoid organ located in the upper chest cavity. Maturing T cells go directly to the thymus, where they are "educated" to discriminate between self and foreign proteins. (See Tolerance.)

Tissue culture: Growth of tissue in vitro on an artificial medium for experimental research.

Tolerance: A state of specific immunologic unreponsiveness. Individuals are normally tolerant to their own cells and tissues. Autoimmune diseases occur when tolerance fails.

Totipotent: Having unlimited capability. The totipotent cells of the very early embryo have the capacity to differentiate into extra embryonic membranes and tissues, the embryo, and all postembryonic tissues and organs.

Transcription: Making an RNA copy from a sequence of DNA (a gene). Transcription is the first step in gene expression.

Transcription factor: Molecules that bind to RNA polymerase III and aid in transcription.

Transgene: A gene that has been incorporated from one cell or organism and passed on to successive generations.

Translation: The process of forming a protein molecule at a ribosomal site of protein synthesis from information contained in messenger RNA.

Trophoblast: The extraembryonic tissue responsible for negotiating implantation, developing into the placenta, and controlling the exchange of oxygen and metabolites between mother and embryo.

Trypsin: An enzyme that digests proteins. Often used to separate cells.

Undifferentiated: Not having changed to become a specialized cell type.

Unipotent: Refers to a cell that can only develop in a specific way to produce a certain end result.

Vascular: Composed of, or having to do with, blood vessels.

White blood cell (WBC): The primary effector cells against infection and tissue damage. WBCs are formed from the undifferentiated stem cell that can give rise to all blood cells. Also known as a leukocyte.

X inactivation: The normal inactivation of one of the two X chromosomes in females.

Y chromosome: The chromosome that determines male gender.

Zona pellucida: A thick, transparent noncellular layer that surrounds and protects the oocyte.

Zygote: A cell formed by the union of male and female germ cells (sperm and egg, respectively).

INDEX

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