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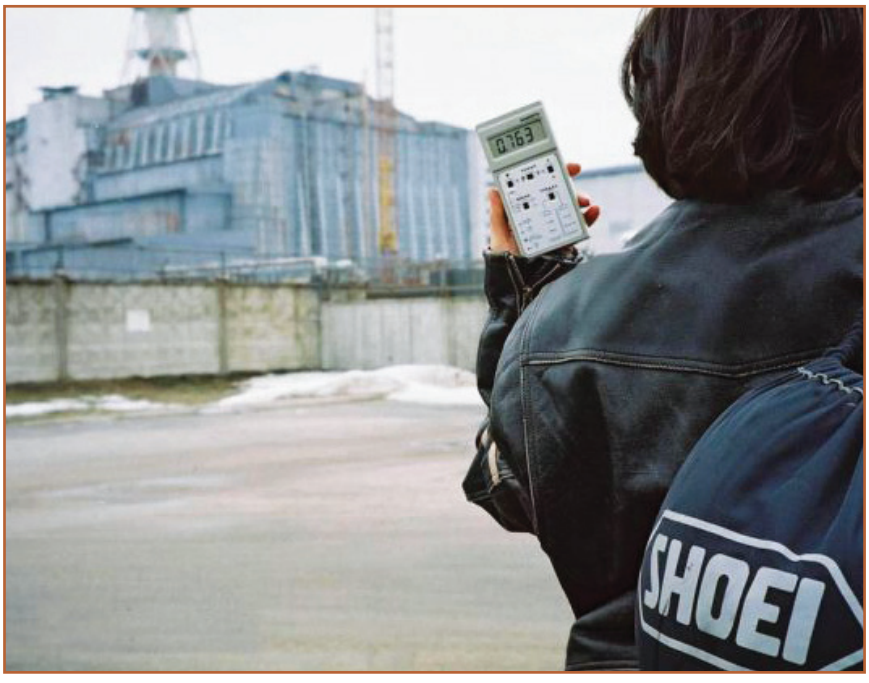
Chapter 4
Crests and Falls

Chernobyl

In an event that has highlighted for the world the dangers of nuclear power, on the morning of April 26, 1986, the Chernobyl Nuclear Power Plant experienced a catastrophic accident. An explosion at reactor Number 4 released a significant amount of radioactive material into the air. Plant workers were immediately exposed to high doses of radiation, resulting in textbook cases of acute radiation syndrome (ARS). More than 30 separate fires broke out; some of these fires threatened



A view of the Chernobyl Nuclear Power Plant. The Geiger counter shows even after 15 years had passed (date of the image) there were still detectable levels of radiation.

Courtesy of Elena Filatova.

to consume the other three nuclear reactors, an event that could have caused subsequent explosions.

Firemen, knowing full well the possible deadly consequences of radiation poisoning, rushed to the scene.[1] Russian analyst, Robert Ebel, explains that these heroes put themselves in harm's way to limit the effect of the blast:

> *The danger that the fire would travel along the roof of the turbine hall meant that the initial fire-fighting efforts were concentrated on the roof. It was during this action that many of the firefighters received what were eventually fatal doses of radiation… The firemen lacked proper protective gear, the roof was built of highly flammable materials, and the fire-fighting trucks were not equipped to attack fires on buildings of this height, in some places reaching 235 feet. The chance of survival for those firemen who made it to the rooftop was minimal; it was for many a sentence of death.*[1]

Firefighters, plant workers, doctors and even passersby—those who were near the power plant that day received fatal doses of radiation. While the Pripyat citizenry were evacuated, the ARS victims needed immediate medical attention. Eighty-four patients were treated at hospitals in nearby Kiev, Ukraine. Another 115 patients were sent to Moscow. In fact, almost all of the most seriously ill patients went to Moscow. The very worst cases were sent to one hospital in particular, Moscow Hospital 6.

Moscow Hospital 6 was primarily known as a hematology-oncology ward. But it had an unusual specialty: treating radiation-afflicted patients. The evening of April 26, deputy director, Dr. Angelina Guskova (MD, PhD), was connected to Chernobyl medical officials via a direct hotline. Dr. Guskova was assisted by American transplant surgeon, Dr. Robert Gale, who had offered his services to the Soviets upon learning of the nuclear disaster. Gale and Guskova were looking at individuals who had taken on an incredible amount of radiation. Twenty of the patients had suffered from Level IV exposure—between 6 and 16 Grays (Gy) of radiation. The chance of survival from ARS becomes negligible when exposure exceeds 6 Gy. For others who had experienced 4-6 Gy, the chances of survival were less than 50%.

Facing such dire medical circumstances, the doctors decided to perform bone marrow transplants on several of the patients, hoping that new stem cells would reverse bone marrow failure and give

these patients a chance at life. They chose only the patients who were suffering from "an extremely severe and irreversible degree of myelodepression."[2] (Myelodepression is the clinical term for compromised bone marrow activity.) Thirteen patients, all suffering from Levels III and IV doses of radiation, received stem cell injections.

Unfortunately, for most of the patients, it was too little, too late. The patients had all suffered severe radiation burns. Seven of the thirteen patients died from injuries to the skin, gastrointestinal tract, and lungs, while four others died from viral-bacterial infections. While the bone marrow transplants may have been able to help restore the patients' bone marrow function, they proved unable to save the patients' lives.

Of the thirteen patients who received bone marrow transplants in the aftermath of Chernobyl, two survived. These two were distinctly fortunate; they both had sisters whose bone marrow could be used for donation. Even then, the bone marrow transplants were only a temporary measure; both patients rejected the donated cells after several weeks, but by then, they had begun producing their own stem cells.[2]

Another six patients received fetal liver cell transplants, with the hope that this source, rich in stem cells, would be less likely to trigger graft-versus-host disease and that they would be accepted by the body. Unfortunately, all but one patient who received these transplants died from skin and intestinal injuries. The last patient, who had received between 8-10 Gy of radiation, clung to life for several days after her transplant, before finally passing away. Her autopsy revealed that her own cells had begun the process of myelopoiesis (production of bone marrow, bone marrow cells, or blood cells in bone marrow); her body had begun forming new bone marrow. Given the complexity of the situation, doctors were unable to determine whether the results were from the transplant.

For the two patients who survived, it is impossible to know whether the transplants were the reason for their recovery, or whether they would have survived without the stem cell injection. Dr. Guskova decided that "in an emergency situation like the one described, the group of persons for whom bone marrow transplants would be indicated with a reasonable prospect of success is extremely limited."[2]

Comparison of the BMT-patients with the non-treated control group leads to few conclusions. In both groups, receiving over 9 Gy

Beyond Chernobyl

After Chernobyl, Western doctors as well as analysts and policymakers asked themselves: how did the staff of Moscow Hospital 6 know so much about radiation exposure?[1] Dr. Gale admits that he was exposed to information that had not previously traveled outside the confines of the Soviet Union, saying, "The value of having me accurately report the accident results, in the eyes of [Soviet Premier] Gorbachev and the Politburu, was far greater than the value of withholding prior information from me."[A]

It was partly through scrutiny of the actions of Moscow Hospital 6 that the world became more aware of the fact that the Soviet Union had amassed a significant body of knowledge pertaining to radiation exposure, a fact that further indicated that the Soviets had been less than open about previous nuclear incidents within their borders.

was an automatic death sentence. For those who received between 6.5-9 Gy, one patient from each group survived. But for those receiving less than 6.5 Gy, survival rates were better for those who did not receive a bone marrow transplant. All five patients in this category, without the transplant, survived. In contrast, out of four patients who received BMT, only one lived.[2]

It seems that in several cases, the stem cell transplants were rejected, resulting in graft-versus-host disease (GVHD), which further weakened the body. This disease, combined with infection, was established as the cause of death for several patients. Dr. Gale explained in a report published soon after the accident that these patients might have developed graft-versus-host disease precisely because they had not received higher doses of radiation. As Gale describes, the higher probability that the lower dose of radiation did not completely ablate the patients' bone marrow made it more likely that there would be enough cells remaining to provoke an immune reaction from the donated bone marrow.

The more irradiated the bone marrow is, the less likely it is to reject bone marrow transplants from non-matching donors. It is imperative to use stem cells that are less likely to be rejected by the body. Dr. Gale had hoped that transplantation with fetal liver cells could be effective in the Chernobyl patients, explaining that, "Since the immune system is not fully developed at this time, histoincompatible fetal liver cells are less likely to cause graft-versus-host disease than comparable mismatched adult bone marrow cells." Whether they would have worked on the Chernobyl patients is impossible to know; the patients all died from unrelated causes. Dr. Gale was reluctantly forced to conclude that this may always be the case:

> *Regardless of the interpretation of these cases, it is certain that bone marrow transplantation can only save a small proportion of victims of radiation accidents; irreversible damage to other organs is likely to limit the success of this approach.*[3]

Further, the transplant surgeon was compelled to acknowledge, "Although transplantation of hematopoietic stem cells can facilitate bone marrow recovery, this procedure is associated with such complications as graft-versus-host disease, interstitial pneumonitis, and iatrogenic immune suppression."[3] In other words, it was still a dangerous treatment option that exposed the patient to GVHD and potential infection.

It would be disingenuous to call Chernobyl a success in the advancement of stem cell treatment, but it was still an important event for stem cell research. It was one of the first times that stem cell treatments were performed on more than a few individuals, and it provided both Soviet and Western doctors an opportunity to refine their procedures. It also demonstrated how much researchers had yet to understand before stem cell-based therapy offered a legitimate treatment option.

Chernobyl was just one event within the wider backdrop of the Cold War, which spurred stem cell research on both sides of the Atlantic. Tragically, the atmosphere of mutual mistrust meant that many scientists who could have worked together as colleagues instead kept the full extent of their discoveries classified. Still, there were significant moments of cooperation, breakthroughs in stem cell research, and an early exploration of the concept of the stem cell as a treatment option for serious disease. As the Iron Curtain fell, however, there were still major hurdles to overcome. It would take another generation of medical scientists to develop stem cell treatments that would forever relegate these early results to the annals of history.

The Fall of the Iron Curtain

With the fall of Soviet Russia, there began a new era of stem cell research. Reminiscent of Russia in 1917, the collapse of the Soviet Union and subsequent political upheaval meant that funding for scientific research disappeared, practically overnight.[4] Dr. Vadim Repin (PhD), a former Soviet researcher and current correspondent member of the Russian Academy of Medical Sciences, acknowledges that with the transition, "It was very difficult to live in Russia without a decent base of funding and support."[B] Repin is the author of two excellent stem cell books published in 2003 and 2010. Repin transitioned into a private company in Russia, but for many of his colleagues, there was no pressing reason to continue research at home when the facilities of the entire world were available. Soviet researchers suddenly had the freedom to travel, and with the technological expertise they had, the world was at their fingertips. The Soviet diaspora provided an impetus for burgeoning stem cell research programs around the world.

Removed from the context of the Cold War, there was no longer any urgent reason to continue stem cell research. But scientists had already had a glimpse at the tantalizing prospects of stem cells, and they knew that there was more to accomplish.

The next decade was marked by a dazzling series of scientific breakthroughs that awakened widespread interest and brought stem cells to the forefront of public discourse. From a research standpoint, these breakthroughs have been tremendous, and they represent some of the most fascinating discoveries in all of science.

From a therapeutic standpoint, however, their effect has been less pronounced. Several of the most exciting breakthroughs involve the isolation of a new source of stem cells, an event that inevitably presaged a wave of optimism and excitement. Later, as researchers turned a more skeptical eye to the new cell source, they would realize that it was unsuitable for clinical applications, or useful for treating only a small collection of rare diseases, at best.

This roller-coaster of expectations has characterized the world's understanding of stem cell research since the late 1990s. The event which was perhaps most responsible for expanding public knowledge of stem cells was the isolation of embryonic stem cells (ESCs).

Embryonic Stem Cells

Stem cell research has a long and storied history that stretches over a hundred years. However, it was only in 1998 that stem cells exploded onto the front page of the newspapers and the forefront of public consciousness. It was then that University of Wisconsin professor, Dr. James Thomson (PhD, VMD) isolated cells from the inner cell mass of human blastocysts, or early-stage embryos. Using these cells, sometimes referred to as the embryoblast, Thomson created a line of embryonic stem cells. These cells had the advantages of differentiating into almost any other cell type; they were pluripotent.

That same year, Dr. John Gearhart (MD), professor and director of Pediatric Urology at Johns Hopkins University, discovered another source of pluripotent stem cells, derived from cells in fetal human tissue.[5] More specifically, these cells came from the germ cells of fetal reproductive tissue.[6] Using different cells, both Thomson and Gearheart's teams had created a new source of pluripotent stem cells that offered a tantalizing possibility of future scientific breakthroughs. It was the end of the twentieth century, Dolly the sheep had been cloned only two years earlier, and suddenly the future seemed more like a utopian science fiction novel, thanks to stem cells. A 1999 issue of *Science* magazine declared pluripotent stem cell research to be the scientific breakthrough of the year.[6] Embryonic stem cell research captured the public's imagination, and, as we know, also set off a firestorm of controversy that rages to this day.

Thomson, when interviewed by MSNBC in June of 2005 regarding his legacy, admitted that "embryonic cells would play a more important role in fundamental research than in transplantation therapies."[7] He also acknowledged that different stem cell lines were needed for breakthroughs in translational medicine, stating that the stem cell lines at the time were not adequately suited for such applications.[7] Thomson believed that ESCs (which at that time he called ESs) would be a useful platform for testing experimental drugs. In fact, he went on to found a biotechnology company in order to follow this line of inquiry. Thomson explained that:

> *[Embryonic stem cells] will be a pervasive research tool that anybody interested in understanding the human body will use. And that will lead to knowledge, for the development of new drugs or whatever, that has absolutely nothing to do with transplantation. This will change human medicine in ways that don't make the front pages. And people will not even realize it's happened. My prediction is that that will be the long-term legacy of these cells.*[7]

It is Thomson himself, who is considered the founder of the embryonic stem cell concept, who argues, along with many others in the scientific community, that the role of ESCs is in research, not translational medicine. There are a few clinical trials in the United States that are using ESCs or ESC derived products. So from what avenue can we find breakthroughs in stem cells' ability to offer medicinal benefits? The answer to that question appears to lie with adult stem cells.

Dr. Darwin J. Prockop (MD, PhD), Director of Texas A&M's Institute for Regenerative Medicine, explained in 2006 that:

> *...adult stem cells may be more useful for repairing damage to tissues by trauma, disease, or perhaps uncomplicated aging. Recent observations are providing increasing evidence for the concept that adult stem cells are part of a natural system for tissue repair.*[8]

Despite the fact that embryonic stem cells are more poised for breakthroughs in the field of scientific inquiry than medical advancement, the furor regarding ESCs was responsible for a renewed surge of academic interest in the field. The events of 1998 caused scientists to take another look at the possibilities that stem cells offered. A 2002 special report on stem cells in the *Journal of Pathology* summates:

It is Thomson himself, who is considered the founder of the embryonic stem cell concept, who argues, along with many others in the scientific community, that the role of ESCs is in research, not translational medicine.

Induced pluripotent stem cell (iPS): adult stem cells that are genetically altered into becoming embryonic stem cells, and expressing the traits that are inherent to embryonic stem cells.

> *Ever since the first reports 4 years ago that pluripotential embryonic stem cells can be extracted and successfully propagated from early human blastocysts and aborted fetuses, both the general public and the scientific community have been gripped by the potential promise of stem cell-based therapies for the treatment of a wide variety of diseases that affect our vital organs.*[9]

It is ironic to think that the discovery of human embryonic stem cells has helped fuel further research into the clinical uses of human adult stem cells, but it also demonstrates the extent to which the academic community has been focused on this field in the last couple decades.

Induced Pluripotent Stem Cells

In 2006, Dr. Shinya Yamanaka (MD, PhD), Professor at the Institute for Frontier Medical Sciences at Kyoto University, declared that he had discovered a way to regress fully formed adult mice cells into a stem cell-like state. The next year, he announced that he had performed a similar procedure with human cells, transforming adult fibroblasts into stem cells. His announcement was followed soon after by Professor Thomson's, who again made headlines by announcing that he had created a similar stem cell population. The resulting stem cells, termed **induced pluripotent stem cells** (iPS cells), had the potency of embryonic cells, without being derived from human embryos.

The media quickly announced that a "truce" had arrived in the "stem cell war" between those who favored ESC research and those who opposed it. With iPS cells, there was no need to destroy human embryos in order to access pluripotent stem cells.

Scientific enthusiasm, however, faded somewhat after a research study by biologist, Dr. Robert Lanza (MD), suggested that iPS cells were more senescent than embryonic stem cells. Lanza's research also indicated that iPS cells had less proliferative capacity, and were more likely to commit apoptosis (cellular death), than their embryonic counterparts.[10] Lanza declared soon after his study was completed that, "There was a 1,000 to 5,000-fold difference" between the proliferative ability of these two cell types.[11]

While iPSs allowed scientists to sidestep the ethical debate surrounding stem cells, their reduced proliferative ability hinted that they were not an effective therapeutic option.

Furthermore, iPS cells are even more prone to safety concerns than their embryonic counterparts. A Japanese research team, which included Dr. Yamanaka, acknowledged in the *Proceedings of the National Academy of Sciences* that "iPS cells are likely to carry a higher risk of tumorigenicity than ES [embryonic stem] cells, due to the inappropriate reprogramming of these somatic cells, the activation of exogenous transcription factors, or other reasons."[12]

The process of reverting an adult cell into a pluripotent stem cell is a complicated one; the cell is essentially being coaxed to do something against its nature. This is not the case for cancer cells that revert backwards from a differentiated state to a nondifferentiated one. The cell is artificially aged as it goes through different cell culturing procedures which forces it to drastically alter its behavior. Some or all of these factors may contribute to the iPS cell's greater likelihood of malformation.

Researchers continue to test different iPS cell lines on animal models, and so far there are certain lines that have not shown any tendency to turn tumorigenic.[12] It seems possible that, if the iPS cells are cultivated differently, they may become more suitable for potential therapeutic uses.

Umbilical Cord Blood and Placenta Banking

Concomitant with embryonic stem cell research, scientists began wondering if the stem cells within **umbilical cord blood** (CB) could be used to treat serious disorders. Umbilical cord blood, it was revealed in 1978, contained its own source of hematopoietic stem cells. Using this knowledge, a medical team led by Dr. Elaine Gluckman (MD, PhD), of the Hematology Hospital Saint Louise in Paris, used umbilical cord blood-derived stem cells to treat a young boy with Fanconi's anemia.[13] The stem cells came from the boy's HLA-identical sister, making the first umbilical cord stem cell treatment an allogeneic one.

After a baby is delivered, the mother's body releases the placenta, the temporary organ that transfers oxygen and nutrients to the baby while in utero. Until recently, in most cases the umbilical cord and placenta were discarded after birth without a second thought. But during the 1970s, researchers discovered that umbilical cord blood could supply the same kinds of blood-forming hematopoietic and mesenchymal stem cells as a bone marrow donor. With umbilical cord stem cells, it is possible to perform either autologous or allogeneic treatments: families can bank their child's cord blood and use it

Umbilical cord blood (CB–cord blood): blood that is contained in the umbilical cord, which connects mother to fetus throughout pregnancy. This blood is rich in stem cells, and is either stored for later use or discarded after birth.

The debate still rages as to whether umbilical cord blood contains enough stem cells to affect a therapeutic result.

if the child becomes ill, or turn to a registry of HLA-matched donors otherwise. And so, umbilical cord blood began to be collected and stored.

A group of Cleveland researchers, led by Dr. Mary J. Laughlin (MD), helped bring this cell source closer to a clinical translation in 2001, when they used CB from an allogeneic source to treat leukemia and aplastic anemia in adults. Before then, medical science had already established umbilical cord blood as a treatment option for children with blood disorders. But as Dr. Laughlin explained, "Researchers have wondered whether the small amount of stem cells in cord blood can create a whole new immune system in fully grown adults, who are also more likely than children to reject a less-than-perfect transplant."[14]

As Laughlin describes, the concerns of the scientific community were manifold. But one of the major concerns had to do with incompatibility. If the stem cells were allogeneic, would the host's body just reject the transplant? This concern was definitively addressed by Laughlin's study, which demonstrated that "just two ounces of blood harvested from an umbilical cord can create a new blood-producing system, and we do not even need a perfect match for a successful transplant because of the immature nature of cord blood stem cells."[14] Laughlin's study showed 90% of the treated patients growing new, healthy blood cells after the transplant.[C] The incidence of GVHD, a significant concern in stem cell transplants, was also lower than expected.

Cord Blood Stem Cells: The Limitations

Today, there have been over 20,000 cord-blood transplants performed to treat a variety of disorders.[15] However, it is worth noting that cord-blood transplantations are most often performed in lieu of bone-marrow transplantations; that is to say, their use may be restricted to certain types of blood disorders. In fact, umbilical cord blood and placental stem cells are considered most valuable for treating chronic blood-related disorders, and scientists note that their ability to differentiate into non-blood-related cells is "far from proven in a clinical sense."[15, 16]

The debate still rages as to whether umbilical cord blood contains enough stem cells to effect a therapeutic result. While Laughlin et al.'s study showed clinical benefit for adult patients, scientists, like Indiana University, School of Medicine's professor, Dr. Hal Broxmeyer (PhD), still worry that CB contains a "low, and sometimes limiting, number

of cells collected in single donor units which can be less than optimal for engraftment of many adults and higher–weight children."[15]

Despite these difficulties, successful cultivating techniques may be able to produce enough cord blood-derived stem cells to create a therapeutic benefit. But for now, it seems that umbilical cord blood and placenta-derived stem cells are useful mainly for treating blood disorders.

The truth is that a breakthrough in one area of stem cell research advances the knowledge base of the entire field.

Pushing the Field Forward

In a 2010 forum on stem cell research and its potential affect on the healthcare industry, Dr. George Daley (MD, PhD), the Samuel E. Lux IV Chair in Hematology at Harvard Medical School, was asked to determine whether embryonic or adult stem cell research was more important to the stem cell field. His answer: "Scientists don't engage in those debates. Scientists see all this research as very fertile space, which all needs to be pushed forward."[D]

Politicians and media figures are quick to describe researchers of different stem cell types as opposed to each other's work. Metaphors like the "stem cell war" and the "stem cell race" depict the main dynamic among researchers and clinicians as antagonistic. The truth is that a breakthrough in one area of stem cell research advances the knowledge base of the entire field.

Embryonic stem cells and induced pluripotent stem cells may not be the right choice for therapeutic applications, while umbilical cord-derived stem cells may be limited to blood-borne disorders. But the discovery of these cell types has led to an explosion of interest in stem cell research, which has ultimately brought us closer to stem cell-derived treatment applications. The research that has been done with these cells, and the research that is continuing to be performed, offers the scientific community a collection of unique insights that help all researchers perform their jobs more effectively. Each stem cell discovery provides inspiration to other researchers, and creates kinetic energy which pushes the field inexorably forward.

Adult stem cell researchers have also created their own kinetic energy, advancing their knowledge base at a dizzying rate. Since the days of Chernobyl, the field of adult stem cells has made a quantum leap. A new generation of hematologists, neuroscientists, pathologists and others have discovered new adult stem cell types and made clinical breakthroughs that make allogeneic stem cell therapy possible today.

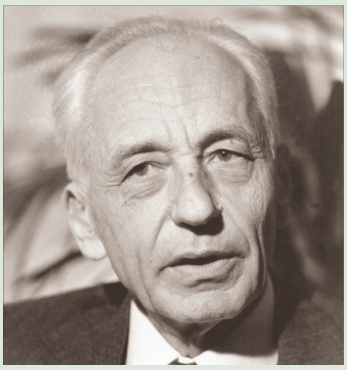
Chapter 5
A Quantum Leap

Advancing to Allogeneic Treatment

Drawing from the lessons of bone marrow transplants, stem cell researchers who hoped to use adult stem cell treatments in a clinical setting realized two things. First, a source of healthy allogeneic stem cells could be useful in a medical emergency. Second, these allogeneic cells would only be useful if they were accepted by the body and, in the case of bone marrow-derived stem cells, if they refrained from initiating graft-versus-host disease.

With bone marrow transplantations, the risk of rejection is reduced through the use of immunosuppressant drugs and serotype matching. Unfortunately, immunosuppressants can leave transplant patients vulnerable to infections. Similarly, patients must wait until an organ that matches their serotype becomes available, an event that often occurs too late.

For allogeneic stem cell treatment, the ultimate goal has been to develop a master cell bank that can be used to treat anyone, without



Although research into the function and behavior of HLAs advanced notably with the advent of genomic mapping, scientists have been aware of leukocyte antigens for over 50 years. It was in 1958 that Dr. Jean Dausset (MD), who was the head of the Immuno-haematology Laboratory at the National Blood Transfusion Centre of France, discovered the first leukocyte antigen, HLA-A2. Seven years later, he would discover that these antigens were part of larger genomic groups, which he dubbed the Major Histocompatibility Complexes (MHC). These and other discoveries earned Dr. Dausset the Nobel Prize in 1980.

danger of rejection and without the need for serotype matching or immunosuppressants.

A better understanding of how to accomplish this goal requires a better understanding of the problem. How does the body know which cells are its own, and which cells are foreign? The answer can be summed up in three letters: HLA.

Human Leukocyte Antigens (HLAs): The Body's Secret Passwords

In the 1990s, private and public groups began work on mapping the human genome. The decoding of the genome, completed at the turn of the millennium, was a massive project, the ramifications of which affected scientists of various disciplines. One result of these genome sequencing projects was an advanced understanding of nucleotide sequencing. More specifically for stem cell researchers, these scientific advancements helped them better identify the different HLA proteins which act as identification signals to the body. HLAs, or human leukocyte antigens, are still not completely understood today, but research has allowed us to reach certain conclusions.[1]

HLA are like the body's secret passwords: the human leukocytes encode proteins that appear on the outer portion of the body's cells. Killer T-cells, the white blood cells tasked with ridding the body of foreign pathogens, interpret these proteins as signals. The right signal leads to acceptance; the wrong signal causes the T cell to attack. In a transplant, the HLA that are of the same type as the patient will be accepted by the body. HLA that encode a different protein will send off the wrong signal. Conversely, in GVHD, if the body does not have the right type of HLA proteins as the bone marrow transplant, the donated T-cells will attack.

But if T-cells only react if the wrong signal is given, what happens if there is a poor or no signal at all? T-cells are engineered to react only when they recognize an HLA-encoded protein. But stem cells, if cultivated early enough and with the proper methodology, will not develop much HLA. During Chernobyl, the Russian clinical team used fetal liver cells on several patients, partly because, "During the second trimester of gestation, [the] fetal liver is a rich source of hematopoietic stem cells. Since the immune system is not fully developed at this time, histoincompatible fetal liver cells are less likely to cause graft-vs-host disease than comparably mis-

matched adult bone marrow cells."[2] But, these fetal tissue transplants represented a cross-section of cells that may have already been in more advanced stages of tissue development.

How does the body know which cells are its own, and which cells are foreign? The answer can be summed up in three letters: HLA.

With a better understanding of how HLA proteins work, scientists were able to better understand why previous transplant procedures failed. Drawing from this, stem cell researchers have since worked to determine the optimal parameters for cultivating immune-privileged fetal-derived stem cells, ones that would remain multipotent and controllable without inducing an immune response from the patient's body. By doing so, some researchers have found a solution to transplant rejection, simply by circumventing the HLA proteins that make other transplant procedures risky.

A good understanding of how fetal stem cells work has also required a better understanding of fetal cell biology. Dr. Vadim Repin, the former Soviet researcher who has published several reviews on fetal cell therapy, points out that "fetal cell biology is a very specific area that combines areas of knowledge from genetics, embryology, biochemistry, modern cell biology, and medicine."[A]

With such an interdisciplinary focus required, it was not an easy task to determine the suitability of fetal stem cell transplant procedures. Today, however, we know that fetal stem cells—when harvested at the proper time and under the appropriate procedures—are not only immune privileged, but also have more proliferative capacity and less senescent tendencies than their counterparts, qualities that make them ideal for stem cell treatment. Dr. Repin stated, "If you have the possibility of receiving a treatment from an available bank of fetal [stem cell] tissue, that would be the primary echelon of treatment." But not all stem cells come from fetal origins. Bone marrow-derived stem cells, for example, usually come from adults. In the United States, treatments with fetal-derived stem cells are not currently available; so, could clinicians develop a therapeutic adult stem cell regimen that would not rely on fetal tissue?

It turns out that they can. There is one stem cell type that can be used in the treatment of a broad variety of diseases, and does not need to be cultivated from fetal sources in order to bypass the body's immune response. In the 1980s and 1990s, several teams of researchers would reveal the surprising attributes of mesenchymal stem cells, one of the most valuable stem cell type for clinical use.

Mesenchymal Stem Cells and Continued Research

After Friedenstein's groundbreaking discoveries in the 1960s and 1970s, a biophysicist from the United Kingdom took up the baton and considerably advanced our knowledge of mesenchymal stem cells. Dr. Maureen Owen (PhD), of the Department of Orthopaedic Surgery at the Nuffield Orthopaedic Centre in Oxford, England, was already a well-recognized expert in the field of bone tissue research when she started investigating MSCs. Poring over Friedenstein's research, she quickly realized the full potential of these cells.

Alone and in collaboration with Friedenstein, Owen started looking more closely at the possibility of modifying mesenchymal stem cell behavior by modifying their *in vitro* conditions.[3] For example, Owen would use epidermal growth factor to increase the colony size of cell populations, or use hydrocortisone to prime the cells to differentiate into osteocytes, the cells which comprise bone.[4] Her research created a solid understanding of how to work with MSCs and prepare them for eventual *in vivo* use.

Another researcher, Dr. Arnold Caplan (PhD) of Case Western Reserve University, ushered in a new era for mesenchymal stem cells by using them to perform clinical trials in the United States. During the early '90s, Caplan's focus on mesenchymal stem cells was so exhaustive that other scientists began referring to MSCs as "Caplan cells."[5] Recognition of Caplan's efforts provided these cells with a nickname, but it was Caplan himself who gave them the name "mesenchymal stem cells" in a 1994 paper published in the *Clinics in Plastic Surgery* journal.[6] Friedenstein had used the term bone marrow-derived multipotent marrow cells, while Owen preferred the name "stromal stem cells."[3] But it was Caplan's name that eventually stuck, giving us the nomenclature we use today, and explaining the use of the acronym MSC.

In 1995, Caplan led the Phase I clinical trial which demonstrated to the world that mesenchymal progenitor cells (MPCs) could be isolated, expanded and placed *in vivo*, thus establishing a potential protocol for stem cell-based treatments. Twenty-three patients received autologous MPCs which were cultivated and then re-injected into their bone marrow. Caplan's team proudly concluded in a *Bone Marrow Transplantation* paper that "no adverse reactions were observed with the infusion of the MPCs. MPCs obtained from can-

cer patients can be collected, expanded *in vitro* and infused intravenously without toxicity."[7] Caplan's results demonstrated that MSCs were ready to begin the process of transitioning into the clinic.

The MSC as a Peacemaker

Caplan's work put Case Western Reserve University on the cutting edge of mesenchymal stem cell research, and more clinical trials began. In 2000, a Case Western Reserve University team began a clinical trial to study the effects of MSCs in breast cancer patients who had received high doses of chemotherapy.[8] The researchers, led by Drs. Omer Koç (MD) and Hillard Lazarus (MD), again used autologous MSC transplantation on a group of patients. The doctors were hoping that the MSCs might assist in "hematopoietic stem cell rescue," bringing HSC levels up. And it worked; hematopoietic stem cells recovered rapidly, and platelet and neutrophil (a type of white blood cell) levels rose.[9] The researchers could not safely attribute their results to the mesenchymal cells, however, as the patients had received the MSCs as part of an ongoing treatment regimen.[9] With the patients receiving other blood cells as well, there were too many variables for scientists to reach any firm conclusions.

But this and other studies helped open the floodgates. A 2001 article pointed out "the pivotal role of MSCs in the bone marrow microenvironment and their ability to support hematopoiesis first sparked *Bone Marrow Transplantation* physicians' interest in these cells."[9] Physicians wondered: How can the insertion of MSCs into the bloodstream activate hematopoietic stem cells, an entirely different type of stem cell?

Part of the answer has to do with an unexpected property of MSCs. These cells are immunomodulators; they have the potential to lessen the immune system's response to a perceived threat.[10] With this ability, MSCs can play the role of the peacemaker in a transplant operation, preventing host tissue and donated tissue from going to war with each other.

In contrast to a full bone marrow transplant, which contains a collection of different cells with varying levels of HLA, a mesenchymal stem cell transplant will not trigger immune rejection or GVHD. The implications of this discovery were significant; if MSCs were able to accomplish some of the benefits of a bone marrow transplant without any of the drawbacks, then they could lend

Dr. Koç and others explained that these miraculous cells offered "potential therapy to enhance allogeneic hematopoietic engraftment and prevent graft-versus-host disease."

themselves to therapeutic regimens for dozens of diseases. Koç and Lazarus pointed out back in 2001 that "the number of MSCs is estimated to be too few in an average bone marrow graft," suggesting that additional injections of just MSCs could be useful for BMT patients.[9] In a subsequent paper, Koç and others explained that these miraculous cells offered "potential therapy to enhance allogeneic hematopoietic engraftment and prevent graft-versus-host disease."[11]

A Treatment for Acute Radiation Syndrome (ARS)

This new generation of studies had revealed a useful treatment for acute radiation syndrome: using MSCs in conjunction with, or instead of, bone marrow transplants. Repin, who has written several books on stem cells, explained that:

> *During the Chernobyl tragedy, we didn't have the necessary knowledge regarding the real potency of stromal cells during irradiation damage. Without this knowledge, there was not much the doctors in Moscow could do to help, especially because the radiation was so high; the patients received mega-doses of radiation; it was Armageddon.*
>
> *It was only later, maybe 15 years later, that we learned the role of mesenchymal stem cells. Now we have the knowledge to apply mesenchymal stem cells in the case of acute radiation syndrome.*[B]

Studies from Case Western Reserve University and other institutions have demonstrated that MSCs have four mechanisms of action which can be used to combat acute radiation syndrome:

1. *Anti-inflammatory effects:* Following ARS, the immune system is damaged and fails to provide regular immunomonitoring functions. This in turn leads to the increased incidence of infection and an inflammatory response. MSCs are proven to produce anti-inflammatory factors such as IL-10 (interleukin 10), which may alleviate pathological consequences and symptoms.
2. *Support for hematopoiesis:* As a result of acute radiation, the blood cells are damaged and need to be restored. Bone marrow-derived MSCs can accelerate new blood cell production by either direct interaction with hematopoietic stem cells in the bone marrow or by production of chemical factors such as G-CSF/GM-CSF (colony stimulating factors.)

3. *Mobilization of the patient's own stem cells:* Injected MSCs have a capability to migrate inside damaged organs and attract the patient's own stem cells to the site of injury by producing factors such as SDF-1 (stromal cell-derived factor 1). This may significantly enhance post-radiation regeneration.
4. *Anti-apoptotic effect:* Radiation can induce cell death via apoptosis. MSCs can rescue the damaged cells from apoptotic death by forming a gap junction with these cells and delivering anti-apoptotic factors such as SDF-1 and G-CSF.

These four mechanisms of action are beneficial in many conditions, not just the treatment of ARS. For example, brain trauma often causes a hypoxic (oxygen deprived) environment and results in cell apoptosis. Anemia of chronic disease can lead to a depletion of blood cells. Congenital conditions are often marked by a malfunction of the body's own, endogenous, stem cells.

Originally, research into MSCs was motivated by a desire for cellular protectors against ARS. Today, a collection of leading clinicians believe that allogeneic MSCs can be viewed not just as protectors, but as cellular repairmen, working to undo the damage caused by trauma or degeneration.

Allogeneic MSCs can be viewed not just as protectors, but as "cellular repairmen."

Regulating the Microenvironment

Yet another advantage of MSCs is that they appear to have a beneficial role in forming and regulating the stem cell microenvironment, or niche. Ever since Schofield and Chertkov's exploration of the idea of the niche, scientists have continued to investigate how a stem cell's interaction with an environment can help shape the eventual role the cell will have in the body.

The microenvironment is one of the main mechanisms through which stem cells receive their marching orders, responding appropriately to the needs of a certain area of the body. As a *Transfusion Medicine and Hemotherapy* paper explains:

> *Various intrinsic programs and pathways facilitate the unique characteristics of stem cells, and these programs, in charge of everything from maintenance, to proliferation, to eventual differentiation of the stem cells, have to be tightly controlled by the microenvironment.*[12]

Various intrinsic programs and pathways facilitate the unique characteristics of stem cells, and these programs have to be tightly controlled by the microenvironment.

But if the microenvironment is damaged or altered, the stem cells will not be as effective.

Osteoblasts: originating from fibroblasts, these cells, when mature, aid in bone production.

Dr. Kharazi, who spent eight years as chief scientist of the Immunotherapy Laboratory at St. Vincent Medical Center in Los Angeles, explains the concept of a faulty microenvironment-stem cell interaction with a simple metaphor:

> *Just as a social environment might affect a child, a microenvironment affects a stem cell. If you take well-behaved children and suddenly transplant them into a terrible school or a bad neighborhood, social science has taught us that this can have an effect. These environmental factors can stunt their full potential.*[C]

Kharazi continues the metaphor by elaborating:

> *You need a good environment, you need good influences, in order to ensure that these kids reach their potential. In stem cell science, there's a similar idea as to the effect the microenvironment has on stem cells. If the environmental factors aren't right, the stem cells will be less effective.*

Reciprocally, mesenchymal stem cells have the potential to bring a stem cell niche to full operating strength. Just as a good neighborhood requires good teachers, police officers, and paved roads, so does a microenvironment have several different types of cells that serve as regulatory components for stem cells. For example, hematopoietic stem cells have their primary niche in the bone marrow. The hematopietic stem cell niche, researchers have discovered, has several different components which affect the role that HSC cells will play in the body.[12] These components include **osteoblasts** and stromal cells, which result from MSC differentiation, illustrating how MSCs contribute to a positive microenvironment for hematopoietic stem cells.[13]

The ability of MSCs to work with HSCs is evidence of the mesenchymal stem cell's remarkable ability to work with other cells to help the body heal. It is evidence that stem cells are not just biological superstars; they are also team players.

Expanding the Field

While some scientists continued to explore the uses of MSCs and HSCs, others turned their attention to newer arrivals to the stem cell field, in the form of newly discovered stem cell types.

Exploring the potential of these new stem cell populations was not always a simple task. In several cases, a fundamental conceptual

shift had to occur before scientists were able to realize these discoveries. This was certainly the case when it came to the discovery, isolation, and subsequent use of neural stem cells, the stem cells that are responsible for forming neurons. In the same way that Maximov would not have been able to conceive of hematopoietic stem cells without first becoming an adherent of the unitarian theory of hematopoiesis, researchers would probably not have considered using neural stem cells for clinical applications, if it were not for significant breakthroughs in the theory of neurogenesis.

Adult Neurogenesis

Can neurons in the brain regenerate themselves? Until the end of the last century, the answer was presumed to be "no." It was widely believed that we entered the world with approximately 100 billion neurons, and that the number simply dwindled from there, without any generation of new, functional neurons. Previous scientific research had suggested that this might not be true for all animals; Dr. Joseph Altman (PhD) of Massachusetts Institute of Technology (MIT) discovered neuron generation (**neurogenesis**) in rats in 1962.[14] Altman went on to hypothesize that the adult brain was capable of creating new neurons, a process called adult neurogenesis. Working with fellow scientist Dr. Gopal Das (PhD), Altman went on to discover adult neurogenesis in other species, further cementing his hypotheses.[15] In a drama familiar to students of medical history, his results were mostly dismissed by the scientific community. Many scientists refused to accept the idea of adult neurogenesis, which meant that very few research institutions were interested in studying the proliferative capacity of different brain cells.

This institutional complacency was shaken again in the 1980s, when Dr. Fernando Nottebohm (PhD), a professor at Rockefeller University in New York, found similar evidence of adult neurogenesis in birds. He admitted to *The New Yorker* journalist Michael Specter that his findings were "a real shock, because we had all been taught that an adult brain was supposed to stay the same size, with the same cells, forever."[16]

With the body of research amassed by Nottebohm, scientists were forced to revise one of their most commonly held assumptions; that the adult brain was incapable of regeneration. Altman, whose own studies were initially marginalized by the scientific community, was later seen as a scientific visionary when a new

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Neurogenesis: the development of the nervous system, including nerves and nervous tissue.

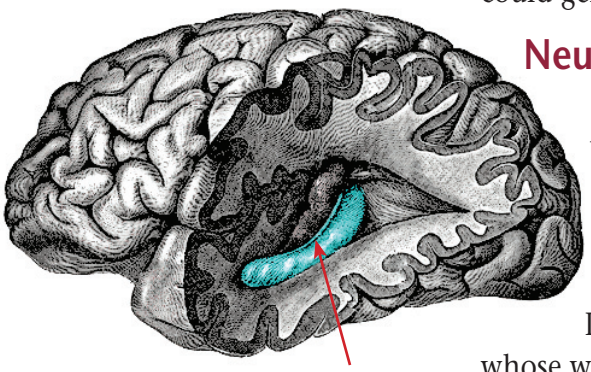
generation of neurologists acquainted themselves with his work. But many argued that Altman and Nottebohm's results could not be applied to humans. Dr. Charles Gross (PhD), a Princeton professor, acknowledges, "People basically said, 'Even if this is true, big deal. It's just birds. All they do is fly around.'"[16] In contrast to the brain of a small animal, the human brain was viewed as far too complex to go through the process of adult neurogenesis.

Neurobiologist Dr. Fred Gage (PhD), of the Salk Institute for Biological Studies, elaborated in an interview the reasons why scientists held to these beliefs, even without evidence to support their theories:

> *First of all, neurons are very complex cells—long branches, receiving hundreds of thousands of connections. The idea that confused people is how something as complex as a neuron could undergo cell division. This idea was not well integrated with the emerging notion that maybe some primitive cells remained and that those were doing the dividing… The other roadblock was that there were several prominent statements in the literature contending that adult neurogenesis couldn't happen, because the brain and structures like the hippocampus need to be stable for memory to be stable. If new brain cells were added, that would make it hard to store long-term memories. It was a loose statement, but it resonated with many people.*[17]

Gage was instrumental in proving these beliefs wrong. In 1998, Gage, with Swedish neuroscientist Dr. Peter Eriksson (PhD), published a paper in the scientific journal *Nature Medicine*. The paper, entitled "Neurogenesis in the Adult Human Hippocampus," established that the human hippocampus (the part of the brain responsible for much of our memory, as well as our spatial orientation) could generate new neurons.[18]

The hippocampus is the part of the brain responsible for memory and spacial orientation.



Neural Stem Cells

As the concept of adult neurogenesis evolved, researchers began further experimenting with an exciting new multipotent line of stem cells which had been discovered in the brain. Dr. Evan Snyder (MD, PhD), Director of the Program in Stem Cell and Regenerative Biology at the Burnham Institute for Medical Research, was one of the researchers whose work in the late 1980s and early 1990s was seminal in establishing the properties of these neural stem cells (NSCs).[19, 20]

Snyder explains that his early experiments with these cells compelled him to accept the newly-developing theory of adult neurogenesis:

> *Initially, I was simply trying to build a brain; it was my original intention merely to place various cell types within a dish, and then mix-and-match them to see if I could build an* in vitro *brain structure for research purposes. But I never got to that point, because I would start with one cell that would differentiate into other cell types.*[D]

Because the theory of adult neurogenesis had not been fully sketched out, Snyder and his colleagues were originally skeptical that they were watching a neural stem cell in action.

> *My colleagues believed that I had used a tissue culture artifact, that my results were a byproduct of trying to grow cells in a dish. After a while, I said, 'I don't think this is a mistake. I actually think this is how the brain is put together.'*

Snyder's realization represented a serious breakthrough for stem cell research, but his were not the only results that boded well for the potential of neural stem cells. Other pioneers include Drs. Carlos Lois (MD, PhD) and Arturo Alvarez-Buylla (PhD), who tracked the migration of neural progenitor cells to different areas of the adult brain.[21] Lois and Alvarez-Buylla, who are now professors at Massachusetts Institute of Technology and University of California, San Francisco, respectively, acknowledged, "The generation, migration, and differentiation of neurons are generally thought to end soon after birth."[22] But their research demonstrated that neuronal cells may have the ability to migrate from one area of the brain to another well into adulthood.

Considering NSCs for Treatment

Before the new theory of adult neurogenesis had been articulated, the dominant paradigm was that the brain could not heal itself after an injury. The studies that demonstrated that the brain was able to produce new neurons during adulthood, as well as studies that demonstrated that neuronal precursors were capable of migration, had researchers asking, "Since it is now evident that new neurons are constantly being generated in the adult, why does the mature brain display only a very limited capacity to repair?"[21]

Scientists attempting to answer this very question quickly learned that there is a proliferation of neural precursors after an injury to

With every clinical study and bench-top breakthrough, the vision of stem cell treatment becomes more closely aligned with reality. More and more, people are beginning to accept that, with stem cells, we have the tools to counter an epidemic of degenerative and traumatic conditions.

the body's central nervous system. It is believed that the reaction of these precursors, which include neural stem cells as well as neural cells in their very first stage of differentiation, represent the body's attempt to repair the damage.

But an adult nervous system has significantly fewer stem cells than the nervous system of, say, a young child. Thus, "the small population of endogenous neural stem cells seem unable to reconstitute fully and restore function after damage."[21] There are not enough NSCs in place to make the repairs; more are needed.

With this realization came another important question: could introducing new NSCs into the brain affect brain injury, or even injury to the nervous system in general? Preliminary studies with fetal neural cells, and their effect on Parkinson's disease, left "little doubt that under appropriate conditions, cellular grafts can integrate and function in the brain."[21, 23] Scientists began conceiving of potential neural stem cell therapy treatments for a host of neurological conditions. This fueled the massive amount of pre-clinical and clinical research from which stem cell clinicians draw upon today. Later research revealed that the central nervous system (CNS), consisting of the brain and the spinal cord, possesses a level of immune-privilege: the cells of the immune system will not cross the blood brain barrier (BBB) that separates the brain from the rest of the body. This characteristic makes it possible to use allogeneic stem cell treatments for neurodegenerative diseases.[24] Admittedly, this situation becomes more complicated in the case of trauma to the CNS, which can rupture the BBB, a fact which argues for the use of fetal neural stem cells for treatments of traumatic injury to the central nervous system.

Although other organs do not share the brain's inherent immune-privileged status, many contain their own population of stem cells, which can be isolated and cultivated. Scientists have found stem cells for the lining of the nose, hair follicles, reproductive organs, the cardiac system, the skin, even for mammary glands. It stands to reason that these stem cells can be used to repair damage to the appropriate tissue. And if a body can use its own stem cells, it also has the potential to use donated stem cells of the same type, as long as those cells exhibit immune-privileged characteristics.

In science, a major discovery is often accompanied by a shift in thinking. Maximov's discovery of hematopoietic stem cells went

hand in hand with the nascent unitarian theory of hematopoiesis; the Russian aristocrat's theory put him on the front lines of an intellectual debate which took decades to resolve. Similarly, the theory of adult neurogenesis represented a shift in scientific dogma without which clinicians would not have been able to grasp the implications of neural stem cell therapy. More generally, the idea that the human body contains many distinct populations of stem cells required scientists and doctors to re-evaluate several basic biological concepts. But today, what was once a far-out theory has become fact; stem cells have transitioned from a hypothetical concept to a biological truth.

A conceptual shift is occurring as policymakers and medical experts increasingly realize the potential of stem cell-based clinical treatment. With every clinical study and bench-top breakthrough, the vision of stem cell treatment becomes more closely aligned with reality. More and more, people are beginning to accept that, with stem cells, we may have the tools to counter an epidemic of degenerative and traumatic conditions.

The metaphor of stem cells as tools is, in fact, an apt one. If there is a problem with the heart, it stands to reason that cardiac stem cells will be the appropriate tool for the job. But if we advance our understanding of stem cells, we will see that, just as a complicated task may require several different tools, seriously compromised tissue may require several different stem cells in order to heal. This concept may sound both simple and logical, however, it also represents a shift in our understanding of how to use stem cells to achieve optimal clinical results.

But if we advance our understanding of stem cells, we will see that, just as a complicated task may require several different tools, seriously compromised tissue may require several different stem cells in order to heal.

Multi-cell Treatment

Consider the human brain as the body's engine. Composed of hundreds of billions of neurons, the brain requires intricate chemical and biological processes to maintain its full power. Like an engine, it is meant to be handled with the utmost care. So what if something goes wrong with your engine? If you use just a screwdriver, you can perhaps fix some of the problem. But you'll need several different tools, perhaps even a whole toolbox, in order to restore the engine's function.

The same concept applies to multi-cell treatment. If the brain suffers an injury, neural cells will most likely be required. Proponents

of multi-cell therapy argue, however, that neural stem cells are not enough. They point to mesenchymal stem cells, which can restore the microenvironment, and hematopoietic stem cells, which enrich the blood supply, as examples of other stem cell types that could play a useful supporting role in effecting repair for a neurological injury. Astroglia may also provide neuron support.

Previous studies have demonstrated that MSCs have an incredible ability to regulate and strengthen the stem cell niche, so that other stem cells can be used to their optimal ability. In his interview, Kharazi further explains this concept:

Take the situation of a stroke. In a stroke, significant numbers of neurons have died off. Logically, we need to apply neural stem cells to the area. These NSCs will differentiate into neurons, to replace the neurons that have died off. But if these neurons don't have the appropriate environment to proliferate and differentiate in, the overall effect will be diminished. The theory is that MSCs can create that kind of environment. The microenvironment of the brain is shifted to accommodate neural stem cells and promote differentiation.

People need food, clothes and shelter to operate. Stem cells, similarly, need a proper environment. They cannot differentiate in a vacuum.

The inter-relationship between different stem cells can grow beyond just creating optimal environmental factors. Research has shown that the relationship between two sets of stem cells is far more complicated than a simple linear connection. Instead, it is more like a dense, inter-connected web, where thousands of connections work synergistically.

Cells can produce growth factors, inhibitors, and stimulatory factors, proteins and enzymes. Kharazi gives one example:

Mesenchymal stem cells often produce VEGF, or vascular endothelial growth factor. The main purpose of VEGF is to stimulate the growth of new blood vessels. Mesenchymal stem cells can both produce and respond to VEGF. When one MSC emits this signal, other MSCs modulate their behavior upon detecting this growth factor. Now, there are 22 sub-populations of VEGF, different variations on the same signal. And some of these sub-populations are also expressed by other stem cells. So, if MSCs are emitting a certain VEGF-upregulating signal, other stem cells might respond.

And this, Kharazi continues, is just one of the growth factors that MSCs can stimulate. Currently, medical science has not yet fully mapped out the complicated inter-relationships between different cell factors. But Kharazi notes:

We know that cells can work not just by themselves, but synergistically with each other. One cell may produce some factor, a growth factor for example, to which another cell responds. So if you're just introducing one type of stem cell into the body, and you don't have that second cell type to produce that growth factor, you won't trigger the necessary response from the first cell type. You have to introduce both cells to the body in a conjunctive treatment regimen, so that one cell promotes the working mechanism of another.

The Realization of Dr. Nikolay Mironov

This basic idea, that different stem cells can constitute a whole that is more than the sum of the parts, is what originally drove Dr. Nikolay Mironov (MD, PhD), currently an associate professor of Neurology at the University of Post Graduation Education of the Kremlin's Presidents Hospital in Moscow, to begin crafting the concept of multi-cell treatment. Mironov began his involvement in the stem cell field in the 1970s, studying the ways that stem cells could be used to treat a range of disorders. Primarily working with neurological disorders such as stroke, Parkinson's and trauma to the spine, Mironov sought and eventually received permission to start treating patients approximately 20 years ago. His first patient was an American baseball player, whom Mironov treated for a neurological condition. Later, Mironov went on to treat prominent members of the Soviet establishment.[E] Provided with regulatory approval for small-scale clinical studies in Moscow, Mironov has continued to advance his clinical protocols, the most important of which is his pioneering use of multi-cell treatment.

For patients with CNS disorders, Mironov and his colleagues began by first using neural transplantation techniques. As his protocols evolved and as new developments occurred in the field, Mironov was able to use more sophisticated culturing techniques. His patients responded to neural stem cell treatment alone, but Mironov felt he could achieve better results. One problem, he realized, was that:

The administration of neural cells alone does not address the endothelialization that needs to occur in order to support the

Fate and Trophic Factors

What is the fate of the mesenchymal cells that are injected into the blood stream? Dr. Jeffrey Karp (PhD), of the Harvard Stem Cell Institute, and James Ankrum, a graduate student at Harvard-MIT Division of Health Sciences and Technology, note that only a small percentage of the cells reach the target tissue. Most are entrapped in the capillaries within the liver, spleen and lungs. The MSCs exert their primary action based on immunomodulatory properties as opposed to the ability to engraft and differentiate. This effect occurs through the secretion of many trophic factors. For example, micro-embolisms in the lungs create a local injury that activates the MSCs to secrete TSG-6, a powerful anti-inflammatory protein. The researchers note that the secretion of this protein from MSCs is 120-fold greater than that of fibroblasts obtained from the same donor.[27]

endogenous and transplanted cells with a blood supply. What is needed therefore is a treatment therapy that addresses both the regeneration of damaged or lost neurons, and the regeneration of the endothelial framework for the damaged area.[26]

In other words, the neural stem cells were reaching the afflicted area, but they did not have enough blood to operate. The neural stem cells were, as Kharazi put it, good kids suddenly thrust into a bad neighborhood.

Mironov realized that in order to effect more significant repair of neurological injuries or conditions, he also needed to boost the endothelial framework that would allow the injected NSC cells a chance to thrive. Realizing that mesenchymal stem cells had the ability to differentiate into connective tissue and thus re-energize the vascular system, Mironov tried a new procedure; he inserted NSCs into the central nervous system, as he had before, but this time he also administered MSCs through the patient's circulatory system. The results, he found, were better than when he had used neural stem cells alone.

Neurological disease is just one possible application of multi-cell therapy. Another example includes the treatment of diabetic retinopathy. While the obvious choice of cells for treating eye diseases would be retinal pigment epithelium (RPE) cells, mesenchymal stem cells can also be used to great effect. Dr. Paul Tornambe (MD), former president of the American Society of Retina Specialists, explains, "Diabetes is a disease of small blood vessels, and diabetics go blind because of poor profusion in the eye."[F] The clinical hypothesis is that the MSCs may be able to strengthen the underlying blood vessels.

In fact, Mironov's multi-cell treatment concept lends itself to many different therapies. When treating traumatic injury, it is almost always beneficial to clean up the microenvironment of the injured area and increase blood flow. In instances of organ failure such as heart failure, different populations of stem cells have been shown to achieve benefit.

Today, clinical studies involving multiple stem cell treatment are being performed outside the United States. Results of selected case studies can be found in the chapters ahead. In the United States, however, the FDA's policy has been to start with single-cell use for consideration in clinical trials. The FDA's position is to first fully understand all the properties of individual stem cells, before study-

ing their properties in conjunction. But when these administrative hurdles are cleared, multi-cell therapy will offer a treatment option that would bring even greater results to those who have been afflicted by serious conditions.

From the Past to the Present

The development of multi-cell therapy, in conjunction with many other scientific breakthroughs, has brought us to where we are today. We are on the threshold of a medical revolution, one that stands to help millions in the future. In the next few chapters, we will offer you a scientific explanation of how and why stem cell treatments are able to achieve results, demonstrating these improvements with a collection of clinical case studies. In conjunction with our explanation of the science behind stem cell treatments, these patients' stories will bear powerful testament to this life-changing therapy.

These pages will reveal not only their stories, but the stories of their doctors, their caretakers, their family members and friends, revealing an emotional aspect of stem cell research that cannot be shown through facts and figures. The patients who have benefitted from stem cell therapy stand today not just as a representation of the power of science, but of the power of the human will to overcome significant challenges and to never accept "no" as an answer.

Placebos and Primary Endpoints

One of the confounding factors in understanding the results of both investigator-initiated clinical studies and more rigorously designed clinical trials is the placebo factor. It is well-known, though not well understood, that the control group often improves, to a degree, without treatment. Individual patients may also improve with treatments that do no harm, but have no effect.

With clinical trials there is also the challenge of study design and the selection of the primary endpoints, or outcome measures. It is possible for patients to have clinical improvement in a variety of areas, however, if this improvement is not the primary endpoint, then the trial is deemed unsuccessful. Careful inclusion criteria must also be applied to clinical trials. These factors complicate our understanding of stem cell treatment efficacy.