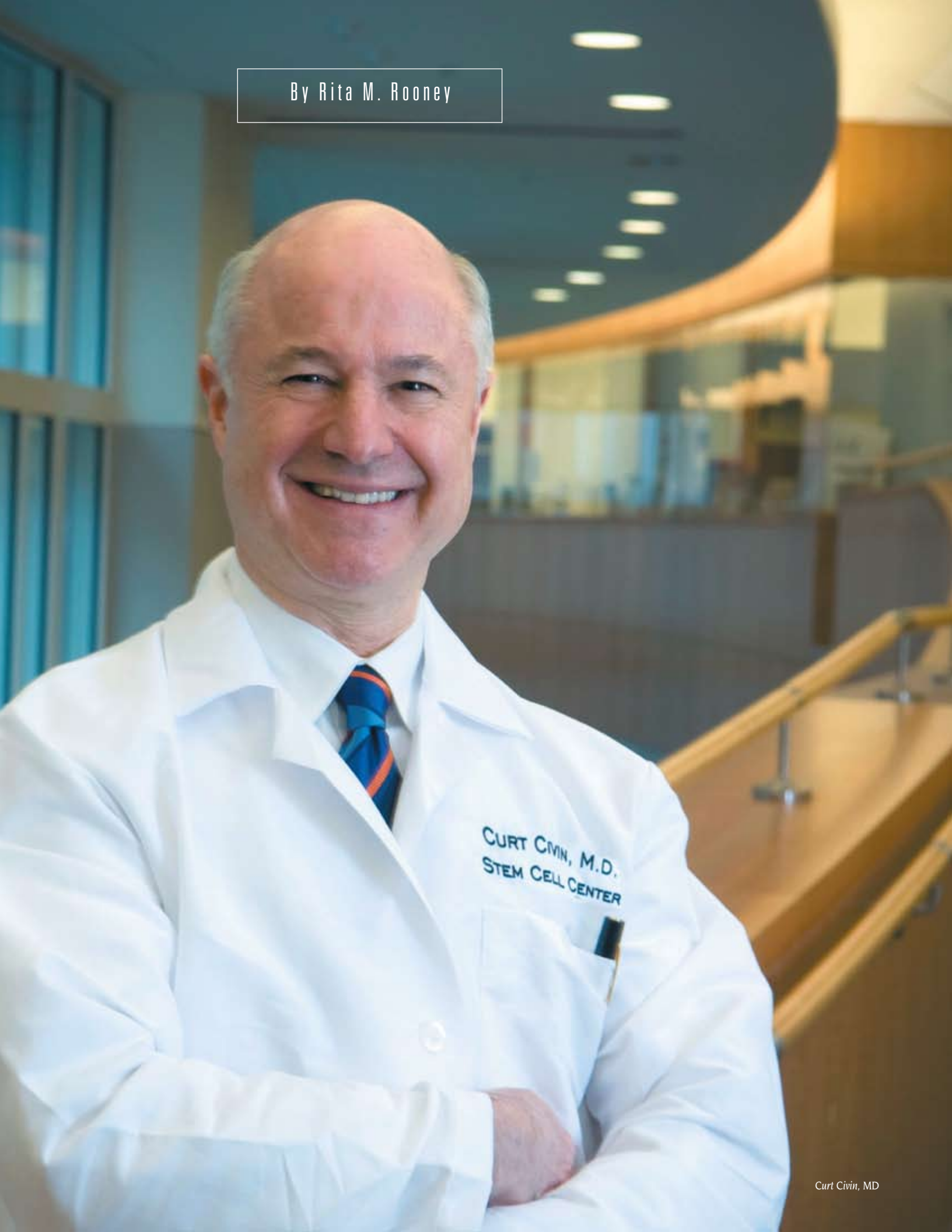
By Rita M. Rooney



Curt Civin, MD

Stem Cells:
Beyond Transplantation

Just a few years ago, Curt Civin, MD, professor of pediatrics and associate dean for research, was called to the emergency department by a pediatrician concerning a 15-year-old boy with undiagnosed symptoms. After a thorough examination and battery of tests, doctors still hadn't reached any conclusion about the source of the boy's illness. Civin looked into the microscope and was quite certain he had the answer. He ordered a molecular test that quickly pinpointed a mutation in a specific enzyme, and he was able to immediately diagnose chronic myeloid leukemia, a cancer for which a new revolutionary drug had recently been developed.

"I told the young man I had both good and bad news for him," Civin says. "The good news was that I could cure his life-threatening disease. The bad news—he would have to go to school the next morning."

Civin, who is internationally recognized for his stem cell discoveries, now serves as director of the University of Maryland Center for Stem Cell Biology and Regenerative Medicine. At the time he was asked to help diagnose the boy's condition, stem cells had only recently been used in development of the "miracle" drug, Glevac, which he administered to the patient. It was as simple as telling the young man to take a pill and call him in the morning. There would be no hair loss and only minimal side effects. A year earlier, therapy would have taken a much different course. A bone marrow transplant might have been performed, but that procedure is highly toxic and not always successful. In this case, bone marrow transplantation could be held in reserve for the patient if his disease developed a resistance to Glevac. Instead, life for this teenager returned to normal after a good night's sleep.

In that one extraordinary healing episode lies the essence of stem cell research. It isn't just about transplantation. The cells today are being effectively explored for diagnosis and treatment, and tomorrow—prevention.

For three days in September, 2009, the global stem cell community gathered in Baltimore for the World Stem Cell Summit, presented by the Genetics Policy Institute and co-hosted by the University of Maryland and Johns Hopkins. More than 1,200 world class scientists gathered to focus on the science, business, policy, law and ethics of stem cell research. The setting was an appropriate one in that Maryland, with one of the largest biotech components in the country, is indisputably among those states taking the lead.

Civin reports one of the highlights of the summit was a collaborative agreement between Maryland and Califor-

Photos by Richard Lippenholz

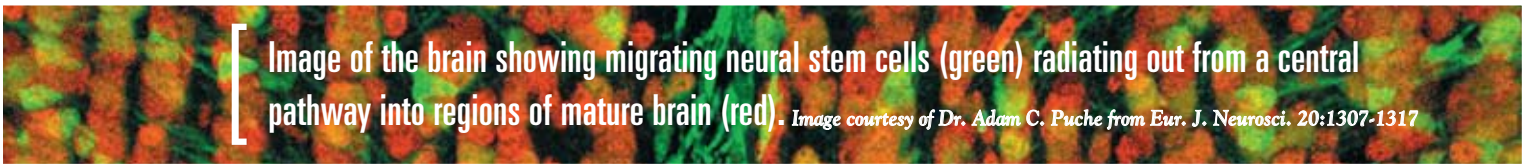


Image of the brain showing migrating neural stem cells (green) radiating out from a central pathway into regions of mature brain (red). *Image courtesy of Dr. Adam C. Puche from Eur. J. Neurosci. 20:1307-1317*

nia, formed by the Maryland Technology Development Corporation and the California Institute for Regenerative Medicine.

"This amounts to an exciting commitment between two states at the forefront of stem cell work," he reports. "It establishes a protocol in which we will join together and pool resources to help scientists in both states share their talents and discoveries."

Advancing from Bench to Bedside

Meanwhile, Maryland's new center is driven by a single imperative—working quickly beyond bench science to the actual use of stem cells to transform medicine. In 1984, Civin propelled the success of transplantation research and clinical applications with his discovery of a way to isolate and purify the cells. This milestone opened the door to improved treatment for bone marrow transplantation. Patients who earlier had to be transplanted with a donor's whole bone marrow could benefit from the transplantation of purified hematopoietic or blood-forming stem cells.

Civin is a visionary, and it shows in the manner in which he discusses his work. While talking about ongoing research in medical school laboratories, he will suddenly switch to a "what if" perspective. On the one hand, he is the pragmatic scientist outlining ongoing studies with definitive strategies. But then he becomes animated as he skips ahead to what the next decade may bring, and the listener is treated to a rare glimpse of advances barely imagined a few years ago.

"A lot of our work involves the use of stem cells as models," he says. "Right now, when we want to test a new drug, we test it on animals. But is a mouse the perfect model? We can often cure cancer in the mouse and not in the human."

He goes on to say the availability of models for all human organs and systems would enable studies that better approximate patients, and adds that models for toxicity would increase the safety of clinical trials in that relying on animal models can lead to inappropriate conclusions.

"Imagine a situation in which we want to use a specific drug for a pregnant woman but are worried that it might harm the fetus," he says. "At this point, we test it on a pregnant animal to see if it crosses the placenta. If it does, ordinarily we wouldn't use it. There might be a situation, however, in which the doctor considers the risk necessary. How much better it would be if we had information from stem cell models to find out if the drug is harmful to the developing organs."

One of 30 founding members of the center, Laure Aurelian, PhD, professor of pharmacology and experimental therapeutics, is a molecular virologist interested in gene therapy. Her work involves putting genes in cells, and is aimed at treating diseases from brain cancer to alcoholism.

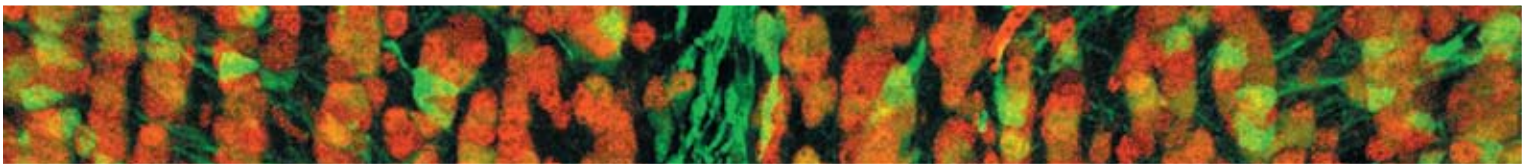
"I became interested in stem cells in 2004 when my lab discovered they are involved in a certain skin disease called Herpes associated erythema multiforme," she reports. "We showed that this skin lesion is caused by infection of the blood forming stem cells that are also given to bone marrow transplant patients with Herpes simplex virus. We reasoned that the virus infection of these cells is a major cause of morbidity and mortality for transplant patients."

It seems Herpes is fond of stem cells. When they see these cells, they attack, resulting in a disease known as graft versus host disease (GVHD) in bone marrow transplant recipients. Aurelian, who has been NIH funded since the beginning of her professional life, got a three-year $200,000 a year grant from the Maryland Stem Cell Research Fund to prove, through a clinical trial, her conviction that the disease is caused by Herpes infected stem cells, and that it is Herpes, not GVHD as currently defined, that is the culprit.

"Presently, we wait three days after transplantation, and if Herpes is activated, the patient is given an anti-viral

Her work involves putting genes in cells, and is aimed at treating diseases from brain cancer to alcoholism.

Laure Aurelian, PhD



medication," she says. But there are questions, she maintains, about the incidence of Herpes involvement, and the attendant wisdom of waiting to medicate.

Twenty patients have been tested in the trial so far, and 13 of the results have been analyzed. Aurelian hopes ultimately to have a total of 40 trial patients and expects to complete her studies in little more than a year.

The conclusions to date are about what she expected. Five patients did not develop the lesion. Of the eight who did, she found Herpes in the skin of all, and the severity of the lesions correlated with the number of Herpes-infected circulating stem cells and virus infection of the skin, not the stage of GVHD as described by pathology. She is submitting another grant application to study effective treatment modalities once final results have been evaluated.

"Decisions will have to be made regarding dosage, selected drugs, whether the drug should be administered intravenously, and possibly earlier than it is now," she says.

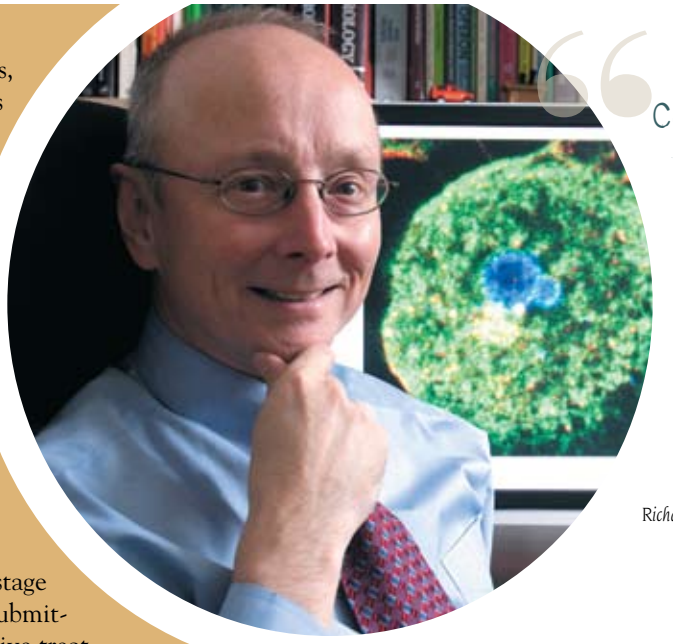
Perhaps the most significant aspect of Aurelian's discovery is its implication for other clinical uses of stem cells since Herpes infects these cells without exception, and any application of stem cell therapy is subject to this invasion.

Probing still another challenge, Aurelian reports she is working on ways to make better stem cells. "There are two problems associated with these cells," she says. "They don't travel well, and though they are effective in replacing bad cells once they reach the site, they are not always effective in treating the disease. This may be because not enough of them get to the site, or because they are not that rugged."

She has become one of many researchers bent on creating so-called "super stem cells." Her method is different from most however. Instead of taking other human genes and putting them into stem cells, she is using a gene she has been working with all her life—her friend and arch enemy, the Herpes virus gene. She says clinical trials have shown that, through manipulation, the gene can protect the brain from neuro-degeneration. In collaboration with the department of neurology, she is now putting the gene into stem cells to see if this will alter their ability to survive, go to a site and eliminate a lesion. "It's what I call my exciting expedition," Aurelian says hopefully.

In commenting on the strength and diversity of research within the center, Civin says the work being done by founding members and many others at the university strikes at the core of a biomedical mind set that is measured, not by published papers, but by benefits to patients and community health.

He looks ahead to the first clinical stem cell trials for spinal cord paralysis authorized by the Federal Drug Administration's limited clearance of Geron's investigational drug, GRNOPCI1. When those trials are finally underway, some of the first patients likely will be treated in the Shock Trauma Center.



> "Cells from the skin can be readily grown and expanded; so they can be manipulated and made into stem cells."
>
> *Richard L. Eckert, PhD*

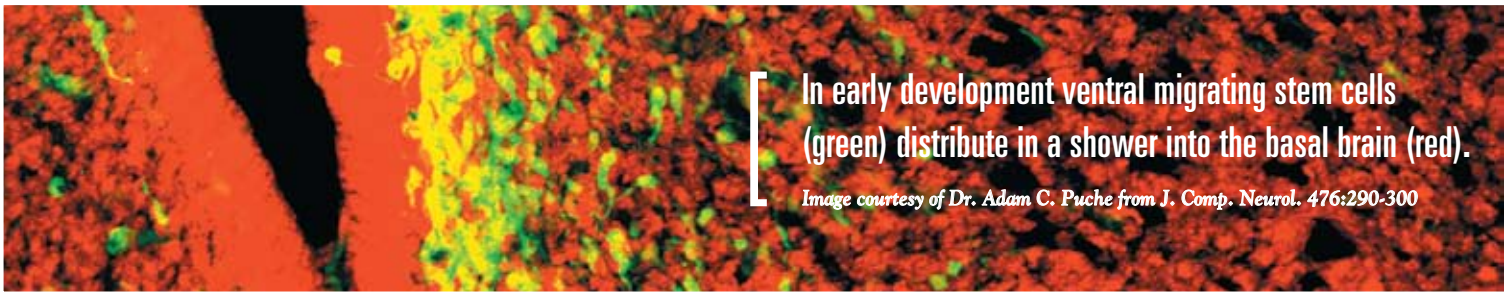
Looking only Skin Deep

From the point of view of a stem cell scientist, skin is a wonderful source, according to Richard L. Eckert, PhD, professor and chair of biochemistry and molecular biology.

"It's accessible and plentiful," he says. "Cells from the skin can be readily grown and expanded; so they can be manipulated and made into stem cells."

Eckert says the fact that the skin has stem cells has been known for some time, but the ability to do something with them has only emerged in the last two years. His lab, in collaboration with colleagues at the University of Iowa, put a stem cell maintenance protein, OCT-4, into mouse cells, and by treating them with a medium, turned them into neurons. The researchers now are working to transfer their methodology to human cells. The hope is that the cells will become multi-potent, capable of becoming several different types of cells.

"We're in the final stages now," he explains. "We're actually doing demonstration experiments, changing the lineage from skin cells to neurons.



In early development ventral migrating stem cells (green) distribute in a shower into the basal brain (red).

Image courtesy of Dr. Adam C. Puche from J. Comp. Neurol. 476:290-300

Eckert, also a founding member of the center with a major grant from the Maryland Stem Cell Research Fund, has been continuously funded as a principal investigator since 1989, and currently is PI on several NIH grants. He holds two US patents.

In essence, he is taking a cell that wants to be a keratinocyte or skin cell, and forcing it, with the protein OCT-4, to become a multipotent cell. He then "tricks" it into becoming a neuron.

"Neurons are only the first step," Eckert says. "The major thrust is to be able to make other cell types. We've already been able to make blood cells. Our hope is to make cells that can be therapeutic, such as those that might be used for replacement in neurodegenerative disease and other illnesses in which a properly functioning cell is necessary."

Still another application of Eckert's research is the derivation of lines from patients with certain diseases; so that the genome, which will be changed in the diseased cell, can then be studied.

Eckert's recent work is yielding considerable attention as few people realized until recently that epidermal cells could be manipulated to make stem cells. However, he has been using skin cells scientifically for 20 years. When he was at Harvard, the lab in which he trained was the first group to take a small piece of skin from the body and expand it to other body surfaces in about three weeks. The process was used to treat a group of children with third-degree burns all over their bodies. Skin was taken from under the arm where it hadn't been damaged, and was expanded as sheets of skin to cover the burned surfaces.

"That science has launched this next phase of the technology, and has led us into transforming stem cells into other cell types," he says. "It's a big move."

Possibly the greatest misconception people have about stem cell research is that its sole purpose is transplantation. Civin says, "The truth is that, even if we never transplanted a cell, there would be unlimited benefits to be gained from our research." He adds that, by studying normal blood development, genes that are mutated in cancer are identified, and these genes become targets for therapy. So the cells themselves, even if they never are used explicitly for treatment, are valuable in discovering the means to treatment.

"For instance, after we first purified blood-forming cells, we wanted to find out what made them tick," he explains. "We identified an important molecule that is mutated in leukemia. Then we found once again that cancer is smart and, in many cases of acute leukemia, corrupted this key process by mutating or turning the gene on at high levels. This made the stem cells divide and survive better, and led to the conversion of normal stem cells into leukemia cells. With this information, it was possible to develop drugs targeted directly to the molecular problem, the mutation of this gene in leukemia."

Civin argues the best way to treat any disease is through prevention. He also agrees that prevention is one of the most difficult of all scientific objectives to achieve. However, he perceives that stem cells will play a major role in prevention in the not-too-distant future.

"We know that a mutation in a certain part of a gene becomes part of the mechanism by which cancer happens," he says. "Now we can look for agents in the environment that cause those precise mutations. These kinds of studies are extremely active here at our center, and are among the most exciting, perhaps the most challenging, and certainly among the most important to the future of medicine."

Founders of the University of Maryland Center for Stem Cell Biology and Regenerative Medicine

School of Medicine Faculty

Gorgun Akpek, MD
Aergei Atamas, MD, PhD
Laure Aurelian, PhD
Alexey Belkin, PhD
Angela Brodie, PhD
Richard Eckert, PhD
Shengyn Fang, PhD
Ricardo Feldman, PhD
Paul Fishman, MD, PhD
Nancy Fossett, PhD
Da-Wei Gong, MD, PhD
Anne Hamburger, PhD
Feng Jiang, MD, PhD
David Litwack, PhD
Zhenqiu Liu, PhD
Stuart Martin, PhD
Margaret McCarthy, PhD
Mervyn Montiero, PhD
Sarah Netzel-Arnett, PhD
Elizabeth Powell, PhD
Adam Puche, PhD
Yun Qiu, PhD
Jean-Pierre Raufman, MD
David Scott, PhD
Huakun Xu, PhD
Steven Zhan, PhD
Li Zhang, PhD

University of Maryland College Park, Department of Bio-Engineering

John Fisher, PhD

University of Maryland Biotechnology Institute

Mariusz Karbowski, PhD