# (Gene Therapy)$^2$

BY IDELLE DAVIDSON

**A husband-and-wife team of scientists targets glioblastoma multiforme, the world's most deadly primary brain tumor. With novel gene therapies in hand, a cure may be just a few clinical trials away.**


THEIR OFFICES SIT A FEW FEET APART, A NECESSITY SINCE THEY CONSULT FREQUENTLY THROUGHOUT THE DAY. She is outgoing and he is quieter, but both speak with a creator's pride about their work. For 14 years, Maria Castro, PhD and Pedro Lowenstein, MD, PhD, have worked side by side, pursuing the most important puzzle of their careers. They hope to crack the mystery of glioblastoma mutiforme (GBM), the most common and deadly type of primary brain tumors, of which 17,000 are diagnosed each year in the U.S. Success may be close at hand.

Glioblastomas are malignant tumors that develop from abnormal, star-shaped brain cells, called astrocytes. The tumors spread like fingers from glial, or supportive tissues of the brain. Most cases occur after age 50, but there are reports of glioblastomas in children and teenagers as well. Unfortunately, the prognosis is dim. Only one in four patients survives for two years. Usually they succumb within six months to one year.

"This is a cancer that has seen very few treatment advances during the last five decades," says Dr. Castro. Even when neurosurgeons remove the tumor, there are always unseen cells that infiltrate the brain and cannot be removed surgically. The cells continue to divide and grow new tumors. "Despite breakthroughs in neurosurgery, radiotherapy and chemotherapy, the tumor ultimately kills the patient, no matter how aggressively you treat it," she says.

Drs. Castro and Lowenstein hope to reverse this desperate situation. When Cedars-Sinai recruited the couple five years ago, their work had already shown great promise through the newly evolving science of gene therapy *{see sidebar page 17}*. At the time, they both served as professors of molecular medicine at the University of Manchester in the United Kingdom. Then, in 1999, one of their papers was published in the highly respected peer-reviewed medical journal, *Nature Medicine*. It catapulted their careers to the top of the international scientific community. "We were able to reveal the side effects of gene therapy to treat brain tumors, making it much more effective and safe," says Dr. Lowenstein.

Now Dr. Lowenstein directs the Board of Governors Gene Therapeutics Research Institute at Cedars-Sinai and holds the Bram and Elaine Goldsmith Endowed Chair in Gene Therapeutics. Dr. Castro, who co-directs the Institute with her husband, was named chairholder of the Medallion Endowed Chair in Gene Therapeutics Research.

As Dr. Lowenstein stands at the blackboard in his office, he attempts to explain the principles of their work. Dr. Castro watches from the table, which is cluttered with stacks of notebooks and neuroscience journals. Dr. Lowenstein absentmindedly runs his fingers through his beard and then draws circles and arrows—representing tumors and gene delivery systems—to illustrate his points. He speaks cautiously, tempering his enthusiasm.

"What you can do with gene therapy which you cannot do with regular classical pharmacology, is that you can put things into the body that are not there," says Dr. Lowenstein. "You can use genes to cure disease."

A large portion of their work involves engineering disabled viruses that serve as vectors, or vehicles, to unload "suicide" or "homicide" genes into the brain tumor. For the vectors to act, patients must also receive an injection of Ganciclovir, a common antiviral medication. It is an intricate dance: The Ganciclovir reaches the tumor, then the suicide gene transforms the Ganciclovir into a compound that inhibits cell division.

"The healthy cells in the brain don't divide, so the suicide genes just kill tumor cells," says Dr. Castro. "The approach is safe."

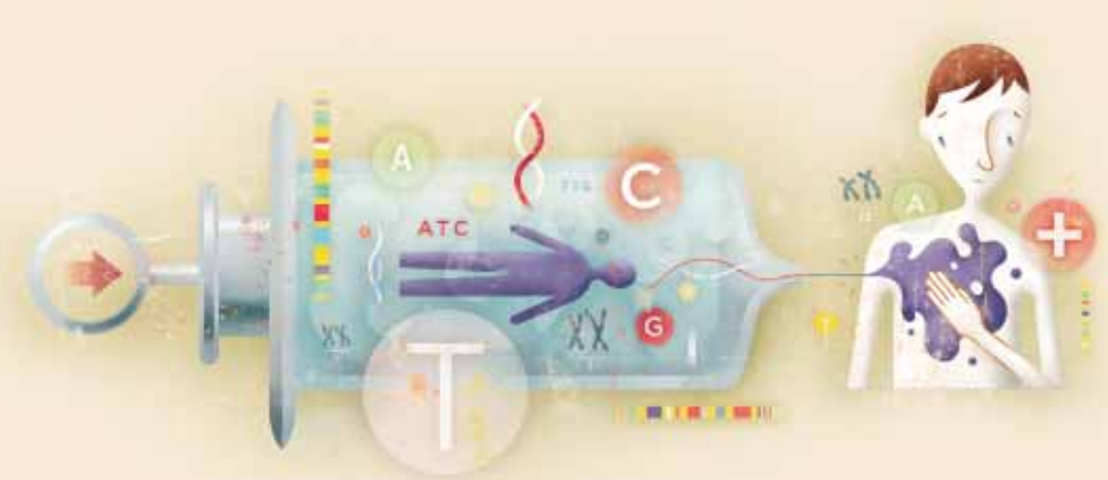
# What is Gene Therapy?

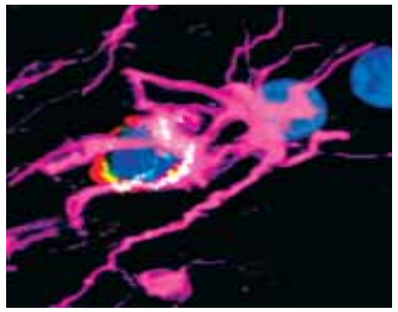
**We resemble our mothers or fathers, grandmothers or grandfathers, because of the genes we inherit. These genes are carried on chromosomes, thread-like structures found in the nucleus of all cells. Genes are composed of deoxyribonucleic acid, or DNA, which encodes the information needed to make proteins. These proteins perform most of our cellular functions. When DNA is damaged, proteins cannot function properly, resulting in disease.**

**Gene therapy is a technique that uses genes as medicines, and can also directly correct defective genes. Researchers isolate normal DNA and then pack it into a vector—usually a disabled virus. The vector delivers its healthy genetic cargo into the target cell, which rebuilds the missing protein and transforms the cell to normal.**

**Scientists at the Cedars-Sinai Gene Therapeutics Research Institute hope to develop genetic cures for Parkinson's and Alzheimer's diseases, a wide-range of cancers, multiple sclerosis, heart disease, hemophilia, diabetes, and other disorders.**

**—I.D.**



A killer T cell (red) engaging a target infected brain cell (purple). GTRI scientists have recently discovered how T cells kill infected brain cells, opening up new ways of enhancing immune-mediated brain tumor killing. With gene therapy, these basic science advances are being translated into new therapies—in this case, for the treatment of brain tumors

So far, their gene therapy approach has worked in rodent models. The scientists are assisted by a laboratory staff of about 25 researchers, students, and volunteers. "We have shrunk and completely eliminated very large brain tumors in animals," says Dr. Castro. "We have also trained the animals' immune systems to develop memory. Those systems kick in and destroy any tumors that return."

That training involves cytotoxic lymphocytes, a type of white blood cell that attacks tumor cells and kills them. "The lymphocytes are like sentinels of the body," says Dr. Castro. "They circulate throughout." Hopefully, those lymphocytes will find the tumor cells that the neurosurgeons or the radiotherapists cannot detect.

"It has taken us 10 years to get to this point," says Dr. Lowenstein. "We are taking this step by step. These are patients' lives. We don't want to give false hope."

Dr. Castro looks at her husband and smiles at his hesitancy. "We have a therapy that has worked in clinical models fantastically well, and it is now going to clinical trials. I do think we have something quite exciting!"

They plan to begin Phase 1—the safety portion of the trial—within the next couple of years. They will ask the National Institutes of Health to support 12 to 20 patients to participate initially. If results are as expected, the trial will expand to other medical centers.

Drs. Castro and Lowenstein are pursuing other gene therapy approaches as well. One is the bullet-like use of a bacterium toxin called pseudoma aeruginosa. The toxin inhibits overall protein synthesis in malignant cells, causing them to die. Because the therapy is specifically targeted to the tumor cells, surrounding healthy tissues remain unharmed.

As reported in a recent issue of *Cancer Research*, Drs. Castro and Lowenstein also tested a genetically engineered virus to deliver two proteins to animal models harboring brain tumors. The first, Ad-TK, kills cancer cells. The other, Ad-Flt3L, stimulates dendritic or immune cells. When injecting both therapies in combination, 70 percent of animals survived and their large tumors disappeared or shrank dramatically.

Although Drs. Castro and Lowenstein were both born in Argentina, they didn't meet until years later while working in the U.S. Castro earned her PhD in biochemistry from the National University of La Plata, Argentina. Lowenstein received his MD followed by a PhD in medicine from the University of Buenos Aires, Argentina.

They come from unique backgrounds. Dr. Lowenstein is the son of German Jews who escaped the Holocaust and ended up in Argentina. "We had many family members who were killed in the camps," he says. "Neither of my parents finished high school because they escaped from the Nazis." Dr. Lowenstein realized early on that education is important in a world that is sometimes politically unstable. "My mother used to tell me, 'If you have to move, all you can take with you is what you have in your head.'"

Dr. Castro's father is an engineer and her mother is a pharmacist. "I was exposed to a very intellectual environment. We were interested in politics, humanities and science," she says.

When Drs. Castro and Lowenstein met, the study of gene therapy was just on the scientific horizon. Few researchers had applied the concept to brain diseases. That is when the two joined forces.

Today, they work as a team but with clearly defined roles. Dr. Castro focuses on the brain tumor program. Dr. Lowenstein takes charge of brain immunology and autoimmune diseases, such as multiple sclerosis, in which the immune system attacks the brain. "At times it can get a bit intense because we don't switch off, even at home," says Castro.

"We are very good friends and we talk a lot," adds Dr. Lowenstein. "But as scientists we have to be very critical of every protocol, everything that we do. Maria can be as critical of my work as she needs to be, and I can be the same with her."

"That's true," says Dr. Castro. "The secret is that we always have each other's best interest at heart."