A stem cell approach. Two Stanford researchers—dermatologist Anthony Oro, MD, PhD, the Eugene and Gloria Bauer Professor, and stem cell scientist Marius Wernig, MD, PhD—are leading another project involving use of stem cells as a vehicle to correct the defective gene. They begin by taking skin cells from a patient and reprogramming them to become pluripotent stem cells, which have the potential to grow into any cell type in the body. They then insert the corrected gene into these all-purpose stem cells, which can be produced in unlimited quantities. These are then converted back into skin cells that are now carrying the correct gene. The modified skin cells can be grafted in sheets onto the patients' wounds. The advantage to this approach is its potential to be permanent. The researchers hope to begin clinical trials soon.

Protein therapy. Dr. Tang is leading a trial involving injections of a purified form of the type VII collagen directly into the patient's bloodstream. The goal is to get enough of the protein into the skin to enable it to heal. Patients receive the protein for a few months, then are given a placebo for another two months; the investigators don't know which treatment the patients are receiving. So far, five patients have been treated with the drug, with the wounds monitored by the researchers. Another five patients are expected to participate in the trial.

Topical collagen VII treatment. Dr. Marinkovich's group also has developed a gel that contains purified type VII collagen protein which can be placed directly on wounds. His lab has spent several years refining a purification process for the protein. The researchers have tested it in animal models and found it works very well, he said. "This could be the simplest approach of all," he said. "You don't have to transfer the gene. You just transfer the protein in a cream onto the wound." Moreover, it could be applied to the eyes, mouth, and other affected areas of the body that might not be reached through other therapies. He has patented the drug and hopes to begin clinical trials soon.

EASING SUFFERING, IMPROVING LIVES, FINDING SOLUTIONS

Dr. Khavari, who joined Stanford in 1993, said the dramatic progress he's seen in EB research over the decades makes him optimistic that viable therapies for patients are close at hand.

"In 30 years, we have moved from having virtually no treatment options for patients to having a wide range of potential therapies in various stages of testing," said Dr. Khavari. "We are motivated every day by our patients and their families, who continue to inspire us with their remarkable spirit in the face of enormous challenge. We are dedicated to finding a treatment to help ease their pain and suffering."

The department is building a translational research hub at Stanford's Redwood City campus, which will serve as a focal point for quickly moving discoveries from the lab into the clinic to directly benefit patients. The lab is not yet complete, with resources needed for facilities, including space for patient blood draws and storing of patient samples, as well as for the hiring of additional staff to coordinate clinical trials. Your gift will help enable completion of the lab, which is essential to moving trials forward.

There is also still much research to be done to further understand the disease and its varying impacts on patients. For instance, some develop an aggressive cancer known as squamous cell cancer, which is a common cause of death among EB patients. But it's not understood how wound healing contributes to the cancer and why some patients develop it but others do not. Stanford scientists are also continually searching for new therapeutic targets for the disease while nurturing the next generation of clinician-scientists who can carry on Stanford's tradition of leadership in the field. Philanthropic support could help move these initiatives more quickly from discovery to the clinic.

By making a gift, you can participate in advancing the intensive research under way, both in the laboratory and in the clinic, dramatically improving the lives of patients with this devastating condition. Your contribution could help relieve the suffering of those like young Theo and their families by expanding treatment options and offering hope for a better future.

COVER: Butterfly superimposed on an image of human skin fibroblast cells under fluorescent microscope. DNA in blue; Actin fibers in green.

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Epidermolysis Bullosa

Theo Fulgencio is a 10-year-old boy from Brazil who loves his scooter and his iPhone, both of which help distract him from the skin disease that threatens his life. Theo has a severe form of epidermolysis bullosa, or EB, which has left a large swath of his chest and abdomen covered with red, blistering wounds that don't heal.

That is because Theo has a form of EB in which he lacks the gene for a key protein, type VII collagen, which serves as a kind of glue that holds the skin together. Without it, the skin becomes so fragile that it can easily rub off. The slightest touch can cause pain, blistering, and the formation of wounds that leave patients vulnerable to infection and to a potentially lethal form of skin cancer. The disease affects as many as 50,000 Americans. Those with the more severe forms often die before reaching their 20s. In some circles, these patients are referred to as "butterfly children", likening the fragility of their skin to that of a butterfly's wing.

In years past, there's been little hope for children like Theo, as there is no approved therapy for EB. Patients receive only palliative treatment, in which caregivers—often parents—must bind their wounds with oily dressings every other day in an excruciatingly painful process. But Theo's parents have taken heart in a new clinical trial of topical gene therapy at Stanford. It is one of a group of promising approaches being tested at the School of Medicine to treat the devastating condition.

"We are in the United States just for this trial. We are hoping, hoping," said Theo's mom, Clara Colker. "They have to do something. I'm optimistic. Of course, I am. I have to be."

STANFORD LEADING THE WAY IN EB RESEARCH

For more than 30 years, Stanford has been a global leader in the field of EB research and care. It began in 1988 when Eugene Bauer, MD, an EB specialist, became chair of the Department of Dermatology. Dr. Bauer, who would later be appointed Dean of the School of Medicine, established the first EB clinic and began recruiting other experts in the field to build what has become the nation's most formidable EB program, drawing patients from around the world.

"I remember Gene Bauer saying, 'If there were one disease we could fix, it would be EB,' because it causes such an immense amount of suffering," said Jean Tang, MD, PhD, a Stanford professor of dermatology who conducts clinical trials in the disease.

Dr. Bauer made Stanford one of the main sites in the National EB Registry and recruited a team of dermatologist researchers, including Paul Khavari, MD, PhD, Al Lane, MD, and Peter Marinkovich, MD, to develop and test molecular therapies for EB. Later, Anthony Oro, MD, PhD, and Jean Tang, MD, PhD, joined the EB team, collectively expanding the focus of research on EB in exciting new directions.

"The opportunity to advance the clinical care of EB patients in the EB Registry and other clinics and the chance to bring therapies for EB from the lab into the clinic—that was why I came to Stanford many years ago and Gene Bauer helped make it all happen," said Dr. Marinkovich, an associate professor of dermatology. "From the start it's been a highly synergistic experience of many talented faculty working together for the EB cause, which still exists to this day."

The Stanford clinic is multidisciplinary, as EB patients have wide-ranging needs. It includes social workers, physical and occupational therapists, nutritionists, and wound nurses specifically trained to work with these patients. It also incorporates hand specialists to help with patients' hand deformities, hematologists to help control their chronic anemia, gastroenterologists to manage their digestive problems, and anesthesiologists who can work in their fragile airways.

"I think EB clinics are one of the most important things," said Theo's mom. "This is the real 'cure' when you have the condition. As a mother, I think about his lifetime. He needs his iron levels to be good. He needs to eat. He needs to be able to walk. He needs to keep use of his hands. It is the whole body. So you need a multidisciplinary team. When you don't have a cure, it's having a team that supports you. And having a family that supports you."

Though there are many EB patients in Brazil, she said, there are no clinics specifically dedicated to the disease. Theo's parents and two young siblings have made a commitment to move to the United States temporarily so Theo can get the care he needs and participate in the Stanford trial.

THE FIRST LANDMARK GENE THERAPY CLINICAL TRIAL

Stanford's research program has progressed dramatically over the years, culminating in a phase 3 gene therapy trial that could yield the first federally approved therapy for the condition. The trial had its roots in the late 1980s and early 1990s, when Dr. Marinkovich's mentor, Robert Burgeson, PhD, discovered type VII collagen, the protein deficient in dystrophic EB and along with Dr. Marinkovich discovered laminin-332, the protein lacking in most cases of junctional EB, a particularly severe form of the disease. They showed that these proteins were critical to helping cement the skin and prevent it from blistering. The discoveries led to cloning of the genes that code for these proteins and the identification of EB-associated mutations within these genes.

With those discoveries in hand, Paul Khavari, MD, PhD, professor and chair of the Department of Dermatology, began experimenting with a form of gene therapy in a mouse model of the disease. He and his colleagues were able to successfully insert the missing collagen gene into sheets of skin that they grafted onto the animals. These modified skin cells helped heal the animals' wounds. That work paved the way for human trials that began in 2013.

So far, seven patients have received the therapy, and all have benefited. In the trial, the scientists took a small piece of the patients' skin, then inserted the corrected gene for type VII collagen into the cells. Then they grafted these gene-corrected skin cells in sheets to the wounds of patients while they were under general anesthesia.

The research team treated 36 of the patients' wounds with the gene-corrected skin. In nearly all of the wounds, patients experienced healing of 50 percent or more. The patients felt less pain and itching, and the healing persisted for up to a year, the researchers reported recently in a medical journal.

The federal Food and Drug Administration has designated the treatment as Breakthrough Therapy, which will help speed up the process of getting it to patients. Stanford has developed partnerships with industry for this and other trials to help move the research forward. A phase 3 trial of the treatment is now planned as a prelude to possible FDA approval of the technology.

"If it really does improve wound healing, it could be the first drug approved for EB and the first gene therapy of a skin disease," said Tang, who called it a "super-exciting time to be doing EB research."

NEW CLINICAL TRIALS AND SOLUTIONS

Stanford scientists are pursuing a number of other ongoing research investigations to correct EB. Many are already in clinical trials and involve simpler ways to treat the disease, like topical gels or creams as well as injectable therapies. Some therapies are being studied in the lab for clinical use in the near future, including a treatment that uses a method of engineering stem cells to incorporate the missing gene.

"Our overarching goal is to develop increasingly effective and safer molecular corrective therapies which are easier for EB patients to use," Dr. Marinkovich said.

Gene therapy in a topical cream. In one of the trials, Dr. Marinkovich and his team are using a herpes simplex virus—the same virus that causes cold sores. The researchers have removed the part of the virus that causes cold sores and instead have turned it into a vehicle to deliver the type VII collagen gene directly to patients' wounds. He and colleagues initially tested this approach in mice. In clinical trials they have shown that when the cream is applied directly to patient skin, it can safely restore collagen VII production and promote wound healing.

Dr. Marinkovich has treated eight patients so far, including Theo. During a clinic visit, as Theo lies back on a treatment table, Marinkovich gently applies the cream over the boy's torso and then covers the area with a dressing. During the process, Theo is busy playing games on his iPhone and says he feels no pain. He and his family returned every other day for two weeks of treatment with the experimental cream.

In the patients treated thus far, Dr. Marinkovich said the results have been good, with the wounds healed and free of blisters. None of the patients have had negative reactions to the virus. He said this topical therapy has advantages in that it is less expensive and less labor-intensive than some of the other treatments.

"I'm really excited about it because I think it has the potential to reach a lot more patients around the world," he said. "The risk is less. You don't have put patients under general anesthesia. The costs are less than having to do cell manufacturing for every patient."

Gene therapy via injection. In another gene-therapy trial, researchers are inserting the type VII collagen gene into fibroblasts, cells found in the inner layer of the skin. In this trial, researchers take a small sample of the patient's skin, extract some of the fibroblasts, then engineer the cells to incorporate the collagen gene. They then inject the modified cells directly into the patient's wounds. The therapy is done under general anesthesia or conscious sedation, as the cells are injected as many as 60 times. Patients go home the same day of treatment.

Dr. Marinkovich said the therapy has been tested in phase 1 and 2 trials in adults only. All have tolerated the treatment well, with some short-term wound healing, he said. Phase 3, which is expected to start in late 2019, will include up to 15 patients, both adults and children.

Dr. Peter Marinkovich, Dr. Jean Tang, and Dr. Paul Khavari (*left to right*) with patient Garrett Spaulding, who like Theo, has Epidermolysis bullosa. He has been a patient at Stanford since his birth in 1997.

PHOTO: Max Aguilera-Hellweg

"We are motivated every day by our patients and their families, who continue to inspire us with their remarkable spirit in the face of enormous challenge. We are dedicated to finding a treatment to help ease their pain and suffering."

— **PAUL KHAVARI, MD, PHD**
Carl J. Herzog Professor in Dermatology in the School of Medicine