Gene Therapy: What Is It? How Is It Different from CRISPR/Cas9? Why Is CRISPR/Cas9 Getting So Much Media Hype?

By Susan L. Towers, MS / Freelance writer, editor, and publication coordinator, Lewes, DE

Introduction

Cancer centers have been using genetic counseling and testing for years. Most of us are familiar with the much publicized *BRCA1* and *BRCA2* gene mutations that cause specific types of breast and ovarian cancer and with how, today, patient treatment regimens target those subtypes. Through our growing understanding of DNA sequencing, we are identifying other gene mutations that cause a multitude of diseases.

Medical researchers also are on a path to find methods to change these identified gene mutations in some way so as to eventually treat or cure the diseases they cause. This long and arduous path is *gene therapy*, and although the first clinical trials of gene therapy occurred more than 20 years ago, following years of preclinical research, gene therapy is still largely experimental. It includes such efforts as using vectors (made from virus or bacteria) to insert genes into cells, and sometimes into the genome, either at designated or nonselected sites, hoping that the new gene will override the mutated one with a corrected gene product. The National Institutes of Health (NIH) today reports more than 300 recruiting, active, and completed gene therapy clinical trials.[1] On August 30, 2017, in a monumental decision for gene technology, the US Food and Drug Administration (FDA) approved the first therapy based on gene transfer.

The FDA-approved therapy is Novartis' Kymriah™ (tisagenlecleucel) suspension for intravenous infusion, the first chimeric antigen receptor T-cell (CAR-T) approved therapy. Kymriah is indicated for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse. Unlike chemotherapies or radiation therapies, which require multiple courses over weeks or months, Kymriah is a one-time treatment that uses a patient's own genetically modified T cells to fight cancer. This form of gene therapy is performed outside the body, where the patients' own cells are engineered in cell culture to arm them with the ability to fight the cancer when reinfused back into their body.

Gene editing is a technology that medical researchers use to remove and replace mutated genes. If successful, this easily adaptable technology could transform disease treatment and possibly offer a cure. While researchers have been studying genetic editing methods in laboratories for well over a decade, the method that has landed into the national limelight is CRISPR/Cas9. This method is showing great promise in guiding the targeting machinery to the desired genetic location, to remove the faulty gene, and in some cases (though this is harder to do at this time), to replace it with a normal gene template.

Children's Hospital of Philadelphia (CHOP) is among the medical institutions at the forefront of gene therapy. Central to the work at CHOP is Beverly L. Davidson, PhD, Director of CHOP's Raymond G. Perelman Center for Cellular and Molecular Therapeutics (CCMT). Dr Davidson has received national recognition for her research in this field (see sidebar). Under her direction, CCMT is taking a multidisciplinary approach to discover new gene and cell therapies for inherited diseases. The Clinical Manufacturing Facility at CCMT was instrumental in the early development of techniques to manufacture the viral vector component that alters the patients' T cells, as well as in the advancement of other genetic and cellular research. (This research, in collaboration with Drs June, Grupp, and others, ultimately led to the development of Kymriah).

Dr Davidson was an invaluable resource in the development of this article to help members of the American Medical Writers Association understand gene therapy in general and the gene editing tool CRISPR specifically. In recent months, many stories have appeared in the national press about gene technology breakthroughs, with some articles and commentaries exaggerating accomplishments and

future predictions. While this article only touches the surface of the subject, its intent is to give an overall understanding of the technology: where it has been, where it is going, what it is, and what it is not.

Gene Therapy Brings Hope

After decades of international research and scientific collaboration, in 2003, the National Human Genome Research Institute published the complete human genome sequence. This historic occurrence opened the floodgates for medical researchers to expand their knowledge of how genes express themselves as proteins, how they interact on biological pathways, and other functional details of how diseases may occur. Understanding the part genes play meant that researchers could investigate disease at its inception.

Gene therapy represented a giant step toward establishing treatments to cure the diseases caused by these mutations by targeting and changing the expression of the gene mutation.

Testing for specific gene mutations that cause disease took a dramatic step, and so, today, people diagnosed with cancer are often immediately given genetic tests to determine if the cancer is caused by an identified gene mutation. Several cancers, including breast, ovarian, and lung cancers, already have standard treatment regimens if specific known cancer-causing mutations can be matched to mutations in specific patients. These tests also give us the opportunity to identify family members who also may be carrying the mutation and are at risk for developing the disease.

Gene therapy represented a giant step toward establishing treatments to cure the diseases caused by these mutations by targeting and changing the expression of the gene mutation. It has become a robust research environment. According to the NIH website www.clinicaltrials.gov (search: "gene therapy, active trial, recruiting, United States"), there are 129 gene therapy trials either under way or recruiting participants in the United States that are targeting a variety of cancers and blood disorders, heart and vascular problems, and neurological diseases such as Alzheimer disease. When other countries are taken into consideration, there are 187 trials in process or seeking participants.

The basic premise of gene therapy is to override the effects of a gene mutation by either providing a correct copy of the gene to the correct target tissue (essentially ignoring or overriding the bad copy) or by directly targeting the mutation and correcting its expression with editing. These new, correct genes are introduced back into cells through the use of a vector, most often engineered from a virus that has been rendered nonpathogenic by removal of the normal viral genes. Vectors have evolved over millennia to carry genetic material into cells, and this has been harnessed to transfer genes to cells in a lab dish, or into cells residing in animal model or human tissues. In the case of some cancers, such as ALL, for example, T cells are being removed from a human body, genetically altered in a laboratory, and infused back into the body. The theory is that the bioengineered T cell will bolster the person's immune system and attack the cancer. As of October 2017, the NIH showed that there were 154 clinical trials either ongoing or recruiting participants for these CAR-T therapies (www.clinicaltrials.gov [search: "CAR-T"]). Of those, 44 were in the United States. (With the majority of CAR-T clinical trials being conducted outside the United States, where will medical advances come from in the future? This is likely to be to topic of future discussion.)

Spark Therapeutics, a publicly traded gene therapy company and commercial spinoff of CHOP, has 2 active clinical trials (one for hemophilia B and one for choroideremia) and 2 trials in the participant-recruitment stage (one for hemophilia A and one for hemophilia B), according to the NIH website. Unlike the CAR-T therapies in which cells are modified outside the body, Spark infuses the virus into patients directly, where it finds its target cells and delivers its genetic payload.

"To date, CCMT has manufactured and released 40 clinical-grade products that have been used in 20 clinical studies ranging from Phase 1 through Phase 3," said Dr Davidson, a cofounder of Spark.

Dr Davidson specializes in inherited brain disorders, such as Huntington disease, and in the development of new therapies to treat these fatal diseases. She and her team are focused on translating their research into methods to treat patients with inherited neurodegenerative diseases, while others in CCMT continue to advance novel therapies for other blood disorders.

Huntington disease, she explains, is caused by a repeat expansion within the first exon of the *huntingtin* gene, and results in destruction of brain cells along with negative effects in other tissues in the body.

"Our goal is to mitigate the impact of this mutation."

Gene Editing Hones In on Specific Gene Mutations

Gene editing technology[2] is a tool medical researchers are advancing to "cut away" the mutated gene, as well as to make the manipulation of the genome easier. In short, the technology hones gene therapy. In some ways, it is a more exacting

method than most gene therapy approaches because it provides researchers with a precise way to target the problem area in specific genes. Over the years, 2 editing tools that have been used are zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs).

CRISPR, which stands for clustered regularly interspaced short palindromic repeats, is the latest of these tools and has often appeared in national news. Like the other editing methods, it too can make changes to the structure of a gene through targeting and correcting specific mutations in the DNA sequence. The difference between the CRISPR-based approaches and the other tools is the ease of its use, and in some instances, its specificity. CRISPR uses a complex of RNA and protein; the most used today is the complex CRISPR/Cas9.[3]

So far, on the research level, CRISPR has been used to edit nucleic acids in plants, microbes, and some animals, including mice, showing its potential as a tool for medical research and eventually therapy for humans. It has also been used to edit multiple genes at the same time to create small and large animal models. George Church, a leading geneticist and molecular engineer in the field, used CRISPR to splice genes from a frozen wooly mammoth into the DNA of Asian elephant skin cells in his lab at Harvard. The tissue culture represented the first time wooly mammoth genes have been functional since the animals became extinct 4000 years ago, according to a 2015 article in *Popular Science*.[4]

CCMT is using CRISPR as a workhorse tool for basic and translational studies. In an article published in the January 2017 issue of *Molecular Therapy*, Dr Davidson wrote that the technology holds promise for research into Huntington disease. According to that article, CRISPR/Cas9 "represents an exciting alternative for tackling dominantly inherited genetic disorders such as Huntington's."[5] Dr Davidson, together with the other authors, showed that CRISPR/Cas9 decreased the expression of the mutant *huntingtin* gene in lab animals and in cells taken from patients with Huntington disease.

As of August 2017, there still were no NIH-sponsored US human clinical trials using the direct application of CRISPR technology to patients. However, there were 5 trials in China listed on the clinicaltrials.gov site as recruiting participants. Four were for advanced cancers (leukemia/lymphoma, esophageal, metastatic non-small cell lung, and advanced Epstein-Barr virus-associated malignancies) and one was for HIV. Memorial Sloan-Kettering Cancer Center and Juno Therapeutics also are recruiting participants for a Phase 1 trial in which gene editing would make a CAR-T therapy for cancer more effective. The trial, targeting chronic lymphocytic leukemia, is called "A Trial of 'Armored' CAR T Cells Targeting CD19 For Patients With Relapsed CD19+ Hematologic Malignancies."

When Will Gene Therapy Translate To Treatment?

For gene therapy and its gene editing tools, the transition from laboratory models to patient care continues to be arduous and challenging. Currently, Novartis' Kymriah is the only FDA-approved genetic therapy available in the United States.*

In 2016, the European Commission approved GlaxoSmithKline's gene therapy Strimvelis to treat children with severe combined immunodeficiency due to adenosine deaminase deficiency, a rare autoimmune disease. The treatment is only available in the United States and Canada to some patients through a clinical trial at the University of California, Los Angeles.[6]

Although neither Kymriah nor Strimvelis use the CRISPR technology, CRISPR again hit the front pages in August 2017, when a research group in Oregon reported it had used CRISPR to edit the genome of a human embryo. The announcement immediately drew concern from many in the scientific community because it represents a move to change germline genes, meaning that future generations would be affected. Gene therapy research in general, and all the research and techniques discussed in this paper thus far, has avoided changes to germline genes, with its inherent ethical and long-term safety questions. The American Society of Human Genetics (ASHG) in August 2017 published a position paper recommending against germline genome editing. "While germline genome editing could theoretically be used to prevent a child being born with a genetic disease, its potential use also raises a multitude of scientific, ethical, and policy questions. These questions cannot all be answered by scientists alone, but also need to be debated by society," said Derek T. Scholes, PhD, ASHG Director of Science Policy.[7]

Conclusion

Today, we are faced with many genetic-based diseases, such as Huntington disease and other neurodegenerative diseases, hemophilia, sickle-cell anemia, blindness, cancers of all types, and autoimmune diseases, for which we have no cure. Gene therapies offer promise, although for the most part they have not reached clinical practice. Yet, researchers continue to work tirelessly to find ways to cure these diseases. Except for Kymriah, with its targeted patient group, it is difficult to predict when gene therapies will become a common tool in the physician's armamentarium. But many significant steps in the scientific path have generated excitement and hope.

"We are at the cusp of change," says Dr Davidson. "The challenge is getting from models to humans."

Susan L. Towers, MS, is a freelance writer, editor, and publication coordinator in Lewes, Delaware. She can be reached at susanltowers@gmail.com.

Meet Beverly L. Davidson, PhD

Dr Davidson is Director of the Raymond G. Perelman Center for Cellular and Molecular Therapeutics; Chief Scientific Strategy Officer and holder of the Arthur V. Meigs Chair in Pediatrics at the Children's Hospital of Philadelphia. She is also a Professor of Pathology and Laboratory Medicine at Perelman School of Medicine, University of Pennsylvania, Philadelphia.

Dr Davidson received her PhD in Biological Chemistry from the University of Michigan in 1987, and in 1994, she was recruited to the University of Iowa, where she was promoted to Associate Professor in 1999 and Professor in 2001. From 1999 to 2014, she held the Roy J. Carver Chair in Biomedical Research and was named Vice Chair for Research, Internal Medicine, from 2004 to 2014. She was named an American Association for the Advancement of Science (AAAS) Fellow in 2007, and in 2009, she received the NIH Mathilde Solowey Award and was named Member, Electorate Nominating Committee, as well as chair, Medical Sciences, AAAS. In 2011, Dr Davidson was the S.J. DeArmond Lecturer, American Association of Neuropathologists, and University of Iowa Presidential Lecturer. In 2012, she received the Carver College of Medicine Faculty Service Award and the Iowa Innovator Award. She was awarded the Leslie Gehry Brenner Prize for Innovation in Science in 2015. In April 2017, she became a member of the American Academy of Arts and Sciences.

Dr Davidson's research is focused on inherited brain disorders and the development of novel therapies to treat these fatal diseases.

She has served on numerous National Institutes of Health (NIH) Study sections, was co-chair of the Editors Panel, Transformative Award Review Committee from the Office of the Director (NIH), and currently serves on the NIH Council for National Institute of Neurological Disorders and Stroke (NINDS). She is a member of the Scientific Advisory Board of the Huntington Study Group and the Medical Research Advisory Board of the National Ataxia Foundation.

Dr Davidson is a cofounder of Spark Therapeutics, Inc and serves on the advisory boards of Sarepta Therapeutics and Intellia Therapeutics.

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*As we were going to press, the US Food and Drug Administration's Cellular, Tissue, and Gene Therapies Advisory Committee voted to recommend approval of Luxturna™ (voretigene neparvovec). Spark's lead candidate, Luxturna is indicated as a potential one-time gene therapy for the treatment of patients with vision loss due to confirmed biallelic RPE65-mediated inherited retinal dystrophies, a group of rare blinding conditions caused by one of more than 220 different genes. If approved by the FDA, Luxturna will be the first gene therapy ever authorized to treat an inherited disease in the United States.