



Highlights from the IG Living Teleconference, May 7, 2015

Topic: Advances in Gene Therapy for Primary Immunodeficiencies

Guest Speaker: Dr. Caroline Kuo, clinical instructor of allergy and immunology at the UCLA School of Medicine, Los Angeles, Calif., who specializes in gene therapy research for primary immunodeficiencies

A basic definition of gene therapy is to use genes to treat or prevent disease. Gene therapy started in the 1960s, when scientists discovered they could extract DNA from almost any organism and manipulate it in the lab. Knowing we were able to take a target sequence of DNA, like a gene, that may be a cause of disease and potentially fix it in the lab, we started to think of ways in which we could give that gene back to people who had disease and potentially cure them. Then, the next question became: How can we transfer this DNA to cells and then give it back to patients? As the revolution of gene therapy continued, scientists began thinking about the way in which viruses survive in the body. They survive by incorporating little parts of their DNA into the body's DNA, which allows them to escape the body's immune system. So, as scientists, we thought that if we could utilize the virus's ability to integrate little pieces of DNA, we could potentially reengineer viruses to incorporate the DNA that we *want* them to incorporate into cells. This, then, began the field of gene therapy and the idea that it was possible to treat disease with DNA, or with genes.

There are three major factors that are necessary for gene therapy. The first and foremost thing is to know what the defective gene is. Unfortunately, a lot of times, we don't know exactly which gene is causing disease. But, now, with improved DNA sequencing from DNA of patients, we *are* able to identify which specific genes are causing clinical disease. The second thing we need to know is 1) whether the gene that is defective needs to be very highly regulated, meaning it would have to put it back into its right place, or 2) whether it is just missing and the cell needs some expression of that gene, in which case we could randomly integrate the gene into a patient to hopefully deliver enough either normal protein or normal expression. The third thing we need to know is, once we're able to deliver genes, how do we get those cells that are fixed back into the patient, and how do we make sure those cells stay there and go to the right place. Due to the need for these three factors, the initial gene therapy studies focused on blood diseases or diseases that come from the bone marrow. The reason for this is that we know that with bone marrow transplants, we are able to extract bone marrow from a patient, manipulate those cells and give them back. Therefore, we knew that we could potentially use gene therapy to fix cells that are affected in the bone marrow by certain diseases.

One of the first studies looking at gene therapy was in a disease called severe combined immunodeficiency (SCID) caused by adenosine deaminase (ADA) deficiency, also known as bubble boy disease. Babies born with ADA-SCID have no immune system, and almost all of them will die by the age of 1 if they don't get a bone marrow transplant. The first trial, conducted by the National Heart, Lung and Blood Institute and the National Cancer Institute, was in a 4-year-old girl. In the study, they used gene therapy to put ADA, the missing enzyme, back into her white blood cells. When they did that, she was able to produce the ADA enzyme again, which "rescued" her immune system. However, it wasn't long-lived; it was a temporary effect. But the importance of this trial was that it was determined that gene therapy can be done safely; it wasn't harmful. And, it established that DNA can be used as a form of treatment.

Today, there are over 1,800 gene therapy clinical trials going on worldwide. The majority of those, about 60 percent, focus on treating cancer. There are many ways for doing that. One of the main ways is taking cancer cells and introducing genes that make them die. Every person has natural genes that regulate apoptosis, or cell death. In cancer, these apoptotic genes go awry and the cells don't die. Instead, the cancer cells keep proliferating. One way to use gene therapy for cancer is by putting back into the body the genes that cause cells to die. Another way is to put tumor suppressor genes into the body so that cancer cells that are treated can be suppressed. We've also used ways in which we can harness a person's immune system by taking the cancer patient's immune cells and modifying them so they can actively seek out cancer cells and kill them. Cancer gene therapy is a growing field right now.

On the heels of cancer gene therapy is gene therapy for monogenic diseases. Monogenic diseases are those in which we know what the gene defect is, and they're typically caused by a defect in one gene. Currently, there are over 160 trials for gene therapy monogenic diseases. The goal is to take the affected gene in the lab, fix it and then deliver it to stem cells. For primary immunodeficiencies (PI), stem cells reside in the bone marrow, they are long-lived and they can continue to divide and differentiate into cell types that can serve various functions in the body. In the case of SCID, in which the gene defect arises from a stem cell in the bone marrow, if we can fix that bone marrow, then that bone marrow stem cell will produce all the lineages that are now corrected and that are long-lasting in a patient.

Currently, the gene therapy trials that are going on for PI include different types of SCID, chronic granulomatous disease, Wiskott-Aldrich syndrome and leukocyte adhesion deficiency. The reason that gene therapy works well for PI is that, typically in PI, the cells that are defective are the leukocytes, which include T cells, B cells, K cells, neutrophils and antigen-presenting cells, and they're all derived from the bone marrow stem cells. So if we can fix those bone marrow stem cells, we can cure the patient. Before gene therapy was available, there was only one treatment that we're still using today: allogeneic bone marrow transplant. With allogeneic bone marrow transplant, we know that the bone marrow cells are diseased, so we can search for a match and find someone who does not have the disease and conduct a bone marrow transplant. However, there are risks with

allogeneic bone marrow transplant, including graft versus host disease or reaction to foreign cells. But, with gene therapy, which takes a person's own cells, fixes them and gives them back, there's no risk.

The classic method for conducting gene therapy is to take a gene that we know is missing or defective and put it in a virus that has been modified so that it no longer has its infectious properties. The common virus that is used today is a lentivirus. It's derived from an HIV, but it's significantly modified so that it's no longer infective and it no longer can integrate itself into a patient's genes. All we're using is the kind of noninfective parts of the virus to deliver the gene we want into patients' cells. The traditional forms of gene therapy have focused on delivering these functional genes to diseased cells. And now that this field is advancing so quickly, we're focusing more on targeted gene therapy. So not only are we delivering the correct gene into cells, we're also targeting them to their right location in the genome. What that means is we can start to correct defects in genes that should be under tight control, and we can do it more of a precise manner.

Safety of gene therapy is always a big concern. While we're utilizing these viruses to transfer modified DNA, and we've modified them so they're no longer infective, there's still always the chance they may become infective. A lot of work is spent before a clinical trial to make sure that does not happen with the virus. The other concern is that viruses typically insert DNA randomly. So, we might get the gene that we want into a person's genome, but when that gene is actually inverted near an enhancer, a certain part of the gene causes it to become more activated. When that happens, called insertional oncogenesis, there may be abnormal growth of cells resulting in leukemia and lymphoma. This is something that has happened in previous clinical trials for Wiskott-Aldrich syndrome and X-linked SCID. Significant research has to be conducted to prevent these things from occurring before starting another trial. We are continuing to modify these viruses, so not only are they becoming better at helping us deliver the gene we want, but they are becoming safer. And, in some cases, we are finding no instances in which the gene is integrating near a dangerous location. Finally, gene therapy is tightly regulated by the U.S. Food and Drug Administration and the Recombinant DNA Advisory Committee. These organizations look at every trial that goes into effect, and they focus on patient safety.

Overall, we know that gene therapy holds a lot of promise and is a quickly growing field. It offers the possibility of cure for those with very serious illnesses who either can't tolerate a bone marrow transplant from a foreign donor or who are too sick to undergo the chemotherapy, or conditioning, that is necessary for a bone marrow transplant. And what we are seeing is, that at some point, this may be the standard of care for certain diseases.

Click on the link below for an article on gene therapy written by Dr. Caroline Kuo and Dr. Roger Kobayashi – http://www.igliving.com/magazine/articles/IGL_2014-10_AR_Gene-Therapy-The-Cutting-Edge-of-PI-Therapy.pdf