Global Hemophilia Researchers Gather to Share Findings


Hemophilia researchers from across the globe gathered at the National Hemophilia Foundation’s Ninth Workshop on Novel and Gene Transfer for Hemophilia to discuss research findings, ask questions and learn about the field’s latest findings. Biochemists had the chance to see progress on a clinical level, while physicians received a glimpse of the basic science discoveries.

As gene therapy continues to emerge as a potentially viable treatment for hemophilia, researchers presented a wide range of findings on the subject. These included mechanisms using viruses as transporters, or “vectors,” for therapeutic gene delivery; viral vector composition; immune responses to vectors; vector delivery systems; bioengineered stem cells and nonviral methods for gene delivery.

“There’s even more diversity of presentations at this meeting compared to the last two or three. Rather than focus on a number of types of methodologies, we’ve actually increased the diversity of approaches. That tells us that this is more difficult than we anticipated,” said Glenn Pierce, MD, PhD, co-organizer of this and all of the previous workshops, from Bayer HealthCare, Berkeley, California. Dr. Pierce and the other organizers presented a number of questions to the research community. Some of those were answered in the presentations; others will drive the research of the scientists returning to their laboratories.

The Goal

In theory, it’s a simple idea: overcome a defective gene by plugging in a healthy one. The cells with the new gene then make enough error-free copies of the gene to produce a desired function, like production of clotting factors. Gene transfer therapy seems to offer the perfect solution for diseases like hemophilia that are caused by a mutation on a single gene—the gene that codes for factor VIII in hemophilia A and factor IX in hemophilia B.

Further, the severity of hemophilia is measured on a gradient scale; the less clotting factor present, the more serious the disease. If the amount of intact factor VIII-producing cells increases by even a small percentage, the outcome could be substantial in terms of daily life—less bleeding into joints, less frequent prophylactic injections and less worry. “We know from many years of experience that a small amount of clotting factor—factor VIII or factor IX—will prevent the acute and chronic consequences of factor deficiency,” said David Lillicrap, MD, of Queen’s University in Kingston, Canada, a workshop organizer and one of many hemophilia experts attending the meeting.

Rather than subjecting people with severe hemophilia to regular injections of bioengineered clotting factor, it would be advantageous to push—and sustain—factor levels above the threshold. While this has been achieved through gene therapy in hemophilic dogs, human patients have yet to experience equal success.

The Research

Viral Vectors

Though hemophilia is caused by a defect in a single gene, its simplicity stops there. The gene that codes for factor VIII is large and cumbersome. It requires a cleverly developed vehicle to transport it into cells. By engineering a virus to “infect” cells with a healthy factor VIII gene, researchers have been relatively successful. Dirk Grimm, PhD, of the University of Heidelberg in Germany, is working with an adeno-associated viral (AAV) vector as a carrier for the
factor VIII gene. As a small, nondisease-causing virus capable of infecting many cell types, AAV is an attractive vector candidate. “We're basically trying to take natural evolution one step further, modifying the natural virus for therapeutic purposes,” Dr. Grimm said. He is altering an AAV gene by borrowing genetic material from its relatives, attempting to create an AAV gene that infects liver cells that produce factor VIII.

The use of viral vectors, however, has potential problems. If the virus can integrate itself into the genome, becoming a permanent presence in the body, it could be dangerous. “The biggest safety problem in the past has been that these vectors can cause cancer,” said Basil Golding, MD, of the U.S. Food and Drug Administration. Though rarely reported in studies using integrating viral vectors, it can happen. The virus’s hitchhiking DNA inserts itself into the target cell genome, potentially causing a mutation if inserted where it doesn’t belong. However, most of the DNA that the AAV delivers does not integrate into the recipient’s genome. On the other hand, Thierry VandenDriessche, PhD, University of Leuven in Belgium and workshop co-organizer, has worked with both integrating and nonintegrating vectors to deliver coagulation factor genes. He has found benefits to both DNA delivery systems, but indicated that it is too early to identify one approach as the best.

The other barrier facing viral vectors is the wrath of the immune system. Even if an adeno-associated virus does not cause disease, the body still considers it foreign and the immune system mounts an attack. “The delivery of DNA itself does not seem to be the problem anymore. Safety seems to have become a bigger issue than efficacy, which used to be a big issue in the past couple of years,” said Katherine A. High, MD, The Children’s Hospital of Philadelphia, a workshop organizer and pioneer in understanding this immune response. While his lab leaves the immune system challenges to other scientists, Grimm’s research will certainly be strengthened by researchers focusing on disguising the virus from the immune system.

Nonviral Vectors
Instead of using viruses to insert the factor VIII gene into cells, Michele Calos, PhD, of Stanford University in California, injects DNA directly into a vein near the liver of hemophilic mice. An enzyme helps sneak the DNA into the body’s genome, not randomly but in consistent locations. This induces the activity of the gene, while reducing the carcinogenic risk. “Simplicity is an advantage when you’re doing something like this. We have a good chance to not encounter some really difficult biological roadblocks,” said Dr. Calos. In an experiment on mice with hemophilia B who were injected with factor IX, 20% of their liver cells made the missing factor protein. “These are definitely therapeutic levels,” Dr. Calos reported.

Since Dr. Calos has effectively demonstrated that her nonviral methodology works in small animals, her lab is currently running experiments on pigs to see if results are similar. Soon, the experiments will be conducted on monkeys, the final model before trying the process on humans. Although the DNA embeds itself safely into the genome, the physical act of injecting DNA into the liver vein is being assessed for safety. There is concern that the method could potentially encourage cancerous growth, since the cells around the injection site become agitated and respond by dividing rapidly. Although tumor growth as a result of hydrodynamic delivery has never been observed in the mice models, Dr. Calos’ team will closely monitor the process in larger animals.

Stem Cells
Another version of gene therapy now in development uses stem cells. Rather than injecting viral vectors into the body, this method first plugs the gene into hematopoetic stem cells (those that have differentiated enough to become bone marrow cells), which can then morph into various blood cell types. Next, researchers insert the cells into the body, like a miniature version of organ transplantation.

“We’ve got a two-pronged approach: modifying the factor VIII to optimize its expression properties and at the same time, combining it with a technology that has been proven to work clinically to cure a genetic disease,” said Christopher Doering, PhD, of Emory University School of Medicine in Atlanta, Georgia. Dr. Doering’s lab found that a genetically modified version of a pig factor VIII gene is much more effective at secreting the protein than its human counterpart. His research team plugged this gene into mouse stem cells, transplanted them into mice, and found consistently high levels of factor VIII in the blood. A year later, the mice were still producing their own factor VIII at normal levels.

With this type of transplantation, the immune system must first be weakened. Otherwise, it will do its job of recognizing the new cells as foreign invaders that need to be destroyed. Altering the immune system, even temporarily, has its risks though. Dr. Doering’s lab continues working to find ways to transplant the modified stem cells with the least amount of immunosuppression.

Bioengineered Clotting Factors
As many scientists focus on the future therapies for hemophilia, others are perfecting what already works—therapeutic injections of the missing clotting factor. The aim of this research is to make clotting factors with longer half-lives, decreasing the frequency of injections.

Therapeutic factor VIII is no longer derived mainly from transfused blood. Instead, scientists use cultured cells to make copies of the protein. This is achieved by introducing the gene that codes for factor VIII into well-characterized animal cells cultured in the laboratory. Within one day, a cell will pick up the DNA and integrate it into its own genome. After a few billion cells are made, they are transferred to fermentation vats where functional factor VIII or factor IX is produced, ready for clinical use after protein purification. As a natural process of the body’s regulation of the clotting cycle, enzymes known as proteases constantly gobble up factor VIII, making its lifespan in the bloodstream short. That’s where the University of Michigan’s Randal Kaufman, PhD, and colleagues come in. They’re altering factor VIII’s genetic sequence to make it last longer in the bloodstream. To do this, Dr. Kaufman’s lab is examining the pathway by which factor VIII is regulated and then destroyed.

Dr. Kaufman’s lab found that stressed-out cells don’t fold the large and clunky factor VIII protein very well. Misfolded factor VIII causes a mess in the endoplasmic reticulum (ER), the cell organelle that handles protein folding. Dr. Kaufman and his colleagues have found a way to reduce intracellular stress by feeding the cells antioxidants. “Antioxidants help protein folding in the ER,” Dr. Kaufman said of his results. As the amount of properly folded factor VIII increases, more of it is able to exit the cell, where it can be used for protein replacement or potentially for gene therapy.

Numerous other presentations focused on altering factors VII, VIII and IX to improve upon the normally existing proteins. These modified proteins have features that can be used for improved protein therapy.
and, in some cases, gene transfer. The excitement generated by the molecular biologists and protein biochemists could be felt, as some of the most promising protein versions are on the pathway toward clinical testing in patients.

Clinical Trials
Clinical trials are pushing forward, slowly and deliberately. They begin with a few human subjects receiving a low dose of gene vector. The subjects are monitored very closely, often one at a time. Amit Nathwani, MD, PhD, of University College London, is preparing for a clinical trial for hemophilia B that will assess the safety of a vector designed by colleagues at St. Jude Children’s Research Hospital in Memphis, Tennessee. In his large animal study done in macaques, he said, “Expression in these three animals is between eight percent and 13 percent at 18 months after delivery.” These results are similar to those obtained a few years ago by Pierce, High and colleagues at Avigen and The Children's Hospital of Philadelphia. The prospect of duplicating the results in patients who will undergo the therapy is promising. Maintaining 18 months of factor VIII expression in a human patient would signify a major breakthrough in clinical hemophilia research. High’s group has initiated another clinical trial using a slightly different AAV vector than Nathwani’s. It will assess the use of transient immunosuppression to obtain multi-year cures, as shown in mice, dogs and macaques.

Pathways to Improved Treatment
As the clinical scientists get closer to improved treatment with each experiment, teams of researchers are working behind the scenes on a smaller scale. The immunologists are figuring out how a single component of the immune system works to reject cells that contain viral vectors, the biochemists are elucidating the exact pathway by which factor is expressed and the geneticists are studying how rebuilt genes work. While this research is less showy and more technical, it is crucial to the ultimate understanding of hemophilia as a disease.

“Although the concept of gene transfer and some of these other modifications are relatively straightforward, the pragmatics and the biology are anything other than straightforward,” Dr. Lillicrap said. “We need to focus our understanding on the basic mechanisms. Unless we can do that, we’re not going to be able to move ahead with confidence and success.”

The pairing of a comprehensive understanding of each component of hemophilia with macro-level research like clinical trials could ultimately be the key to a cure. Although there are no clear-cut answers yet, the field is expanding as different methodologies for gene therapy are tried and tested.

“There is now increasing diversity,” added Dr. Pierce. “For the individual with hemophilia, it’s a very exciting time. The current treatments, which are very safe and effective, will get even better through research on modified clotting factor proteins and improved transfer of clotting factor genes.”
Speakers (continued)

Peter Turecek, PhD
Baxter BioScience
Vienna, Austria

Luk Vandenberghe, PhD
University of Leuven
Leuven, Belgium

Sam Wadsworth, PhD
Genzyme
Framingham, Massachusetts

Alisa Velberg, PhD
University of North Carolina
at Chapel Hill
Chapel Hill, North Carolina

J. Fraser Wright, PhD
The Children’s Hospital of Philadelphia
Philadelphia, Pennsylvania

Discussants

Valder Arruda, MD, PhD
The Children’s Hospital of Philadelphia
Philadelphia, Pennsylvania

Mark Brooker
World Federation of Hemophilia
Montreal, Quebec, Canada

Alessio Cantore, MSc
University Vita-Salute San Raffaele
Milan, Italy

Hengjun Chao, MD
Mount Sinai School of Medicine
New York, New York

Andrew M. Daviddoff, MD
St. Jude Children’s Research Hospital
Memphis, Tennessee

Donna Ditchele, MD
New York Well Cornell Center
New York, New York

Hildegard Erfl, MD
Wioer Institute
Philadelphia, Pennsylvania

Gary E. Gilbert, MD
Harvard Medical School
Boston, Massachusetts

Basil Golding M.D.
FDA
Bethesda, Maryland

W. Craig Hooper, PhD
Centers for Disease Control and Prevention
Atlanta, Georgia

Yasubko Ikeda, PhD, DVM
Mayo Clinic
Rochester, Minnesota

Hayan Jiang, PhD
Bayer
Richmond, California

Carl June, MD
University of Pennsylvania
Philadelphia, Pennsylvania

Kevin Kelley
New England Biolabs, Inc.
Ipswich, Massachusetts

Barbara A. Konkle, MD
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania

Rebecca Link, PhD
NIH
Bethesda, Maryland

Susan Low, PhD
Syntox Pharmaceuticals
Waltham, Massachusetts

Richard Metz, MD
NIH Board of Directors
Los Angeles, California

Robert Montgomery, MD
Medical College of Wisconsin
Milwaukee, Wisconsin

Timothy Nichols, MD
University of North Carolina
at Chapel Hill
Chapel Hill, North Carolina

David Page
World Federation of Hemophilia
Montreal, Quebec, Canada

Junliang Pan, PhD
Bayer
Richmond, California

Martin Pince, MD
The Children’s Hospital of Philadelphia
Philadelphia, Pennsylvania

Margaret Kagry, MD
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania

R. Jude Samulski, PhD
University of North Carolina
at Chapel Hill
Chapel Hill, North Carolina

Mark Skinner
World Federation of Hemophilia
Washington, DC

Junfeng Sun, MD
University of North Carolina
at Chapel Hill
Chapel Hill, North Carolina

Arthur Thompson, MD, PhD
Puget Sound Blood Center
Seattle, Washington

Karen Tubridy, PharmD
Syntox Pharmaceuticals
Waltham, Massachusetts

Wing-Yen Wong, MD
Baxter
Glendale, California

Supporting sponsors:

Sponsors:
Coalition for Hemophilia B
NIH Office of Rare Diseases
National Heart, Lung, and Blood Institute
World Federation of Hemophilia

Friend
Maxygen

The National Hemophilia Foundation is dedicated to finding better treatments and cures for bleeding and clotting disorders and to preventing the complications of these disorders through education, advocacy and research. Its programs and initiatives are made possible through the generosity of individuals, corporations and foundations as well as through a cooperative agreement with the Centers for Disease Control and Prevention (CDC).