Promising Research Paves the Way for Future Clinical Trials

While five groups have brought their treatment to patients, many more continue to refine their work in the laboratory. Numerous talks at NHF's Gene Therapy Workshop discussed modifying viral vectors to improve delivery while reducing side effects. Others looked at alternatives to the viral system. Following is a snapshot of a few ongoing studies along with a preview of novel strategies presented at this year's workshop.

(l-r) Michael Coyne, MD; Gilbert C. White II, MD; Yubin Kang, MD; Bin Zhang, PhD; Lingfei Xu, MD, PhD; Roland W. Herzog, PhD; Anja Ehrhardt, PhD; Steven Wesley Pipe, MD; Cathryn Sanghae Mah, PhD; Hengjun Chao, MD; Tal Kafri, MD, PhD; W. Keith Hoots, MD; Jeanne M. Lusher, MD

(l-r) Researchers Polly Matzinger, Robert Hebbel and David Lillicrap

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Avigen Inc. is developing many different ways to deliver adeno-associated virus (AAV). First, the company successfully created an AAV vector to transport the gene for factor IX (FIX), though it has been a greater challenge to fit the bulky gene that codes for factor VIII (FVIII) into the small AAV shell. Linda Couto presented results from animal studies in mice and dogs that showed that Avigen has met this challenge. The vector appears to be safe and the hemophilic dogs are able to produce small amounts of the needed factor in their blood.

Researchers also continue to manipulate adenoviral vectors in the laboratory. David Lillicrap of Queen's University in Ontario, Canada, has tested his FVIII vector in three hemophilic dogs with varying success. One dog has maintained production of FVIII for three months. While Lillicrap's group has achieved success in the dog model, the group is not jumping into clinical trials. "I personally think it is premature to be using AV (adenovirus) in human studies for hemophilia with what we know at the moment," Lillicrap said. Adenovirus is attractive because it infects cells efficiently—a quality that isn't as true with the alternative vectors—AAV or lentivirus. Adenovirus also holds potential because scientists have the tools for producing ▶

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it in large quantities. But one obstacle persists. When animals and humans are infected with adenovirus, they invariably mount an immune response to the vector. Lillicrap observed this in his dogs. During the first few days after infection, liver enzymes increased, a sign the organ is being assaulted, and platelets—required in the blood clotting process—decreased. This toxicity passed after a few days but is disturbing nonetheless. "We need to find ways of delivering the virus in a more stealth-like fashion," Lillicrap said. If the body doesn't recognize the virus, it would hopefully not react to it.

Viral vectors provide advantages for gene therapy. They are inherently designed to cross the cell membrane, offering safe transport for the delicate genetic material. But they also bring drawbacks, namely the immune response elicited by these foreign proteins. Robert Hebbel from the University of Minnesota is working on a viral-free system. His team has grown cells obtained from blood and infected these cells with DNA coding for FVIII. The blood cells, now functioning as a mini factory for FVIII synthesis, were then injected into mice. The researchers were able to show that FVIII continued to be produced inside the mice and at a level sufficient to correct a bleeding disorder.

Alternative modes of delivery

The question isn't merely what to give—but also how to give it. Will the final gene therapy be administered through a vein, a muscle or even an artery with a direct line to the liver? Or should we hope for the simplest and easiest approach—provide the new genes in a pill or nasal spray?

Unfortunately, the most convenient methods, either oral or nasal delivery, come with a host of challenges. Progress is slow but the potential benefits spur the effort. Kam Leong and his colleagues at Johns Hopkins University have encapsulated naked DNA in a material derived from crab shells. They disguised

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their concoction in Jello™ and fed it to mice. The gene successfully crossed the gut wall into the blood and the scientists were able to detect the gene product in the mice. Assem Ziady from Case Western Reserve University takes a different tack. He and his team have condensed the gene into particles and delivered these particles into mice through their airway. In a promising result, the "inhaling mice" showed synthesized factor in the blood stream.

Veering from the gene therapy theme, Polly Matzinger and Oral Alpan of the National Institute of Allergy and Infectious Diseases, in collaboration with William Velander from the Virginia Polytechnic Institute and State University are working with FVIII and FIX in its active protein form. The original goal of their studies was to devise a method of protecting individuals with hemophilia from developing inhibitors, the antibodies that can destroy administered factor. In immunology jargon, preventing this immune response is referred to as "tolerance." Matzinger and Alpan wanted to show that mice could develop tolerance to the factor protein if they were first exposed to it in their diet. They have now tested this theory by feeding mice milk containing FVIII or FIX. The milk was produced by a pig, genetically engineered by Velander to secrete factor into its milk. The concept was based on previous research in sheep. Researchers had shown that sheep raised on their own milk were tolerant of the proteins found in sheep's milk, while sheep raised on cow's milk developed inhibitory reactions to the proteins in sheep's milk.

The results have been encouraging on two fronts. First, Matzinger and Alpan have shown that mice fed factor mixed into milk do not develop inhibitors when additional factor is infused in the blood, whereas mice that have not been exposed to the special milk do develop inhibitors.

Serendipitously, the pair observed a secondary benefit to this "special milk." To their surprise, a small percentage of the factor in the milk survived the harsh conditions of the gut and passed intact into the bloodstream of the mice. Not only did the protein make its way into the blood stream, but it remained active. In other words, hemophilic mice drinking this special milk no longer bled uncontrollably.

Excited by these unexpected results, the team plans to carry out similar studies in dogs. As a note of caution Matzinger pointed out that the intestines of mice are different from humans and dogs, thus proteins ingested by humans may behave differently than in mice. But those concerns don't dampen Matzinger's enthusiasm. She's anxious to see if this simple mode of administration can one day translate into an efficacious treatment.

When taken in sum, the field of research resembles a pyramid. At the base, a multitude of approaches are analyzed in the laboratory. Over time, successful strategies advance and are tested in higher animals while numerous ideas are abandoned. Eventually, a still smaller group make it to clinical trials with just a select few reaching the apex—the cure. Only time will tell which will survive these rigorous elimination rounds and ultimately provide the long-awaited cure.