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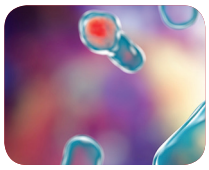

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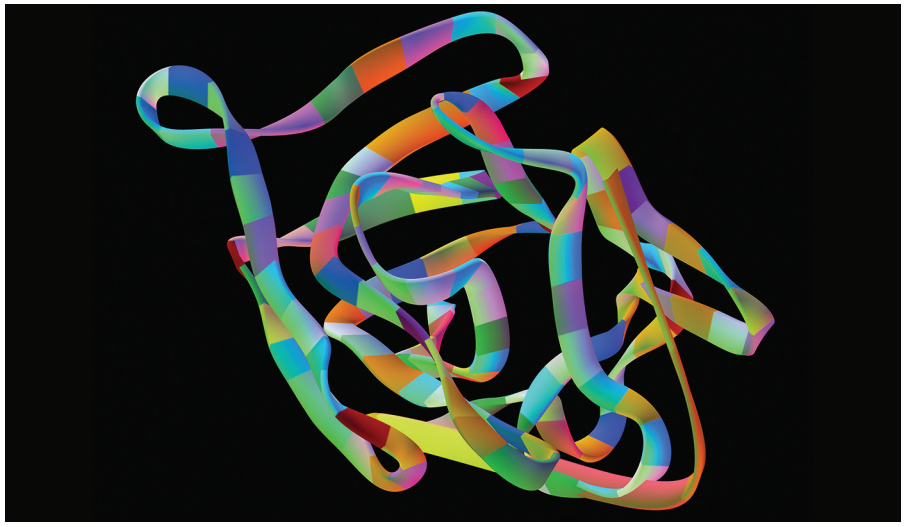
Early clinical data raise the bar for hemophilia gene therapies

Several gene therapy companies reported promising early clinical data in July showing that they could restore healthy blood clotting factor levels in people with severe hemophilia. BioMarin Pharmaceutical presented interim results from a phase 1/2 trial in patients with hemophilia A at the World Federation of Hemophilia 2016 World Congress, demonstrating its gene therapy could increase factor VIII activity levels to at least 10% of normal in all seven subjects who received the highest dose, with two patients producing more than double the normal levels several months after treatment. Spark Therapeutics and uniQure also provided encouraging updates from their own phase 1/2 trials for gene therapies for hemophilia B at the same Orlando meeting.

"We raised the bar," says Barrie Carter, head of vector biology at BioMarin. Before these trials, the expectation was that gene therapies might elevate clotting factor levels enough to transform severe hemophilia into a moderate or mild (~10% of normal) form of the disease. Now, it seems it may be possible to restore patients' natural clotting to eliminate the disease altogether—and, in fact, at the upper activity levels observed in the BioMarin trial the worry shifts from uncontrolled bleeding episodes to excessive clotting in the blood vessels. "It's a nice problem to have," says Amit Nathwani, a hematologist at the University College London (UCL), who did much of the preclinical work on the gene therapy before licensing the product to the San Rafael, California–based BioMarin.

Some onlookers, however, warn against jumping to conclusions based on initial findings drawn from small patient populations followed for only a short period of time. "You've got to be leery of early data in this space," says Allan Haberman, principal of Haberman Associates, a biotech consultancy in Wayland, Massachusetts. "Because the issue is: How long is this effect going to last?"

The first attempts to restore normal coagulation by gene therapy were directed at hemophilia B, caused by a mutated factor IX gene. Although hemophilia B doesn't affect that many people—only roughly 25,000 worldwide (mostly men, as it is an X-chromosome linked condition) compared with the 125,000 afflicted by hemophilia A—scientists considered the genetic deficiency in hemophilia B more straightforward to tackle. This is because the gene coding for factor IX is smaller and easier to insert into many viral vectors with robust expression than the factor VIII gene mutated in hemophilia A.



Alfred Pasieka / Science Source

A gene therapy approach has the potential to obviate the need for regular intravenous injections of bioengineered coagulation factor VIII (pictured) to prevent bleeds in patients with severe hemophilia A.

But early trials of gene therapies in hemophilia B between 1998 and 2001 yielded modest and temporary benefits for patients, with some major safety concerns. The first real success came only in 2010 when a team led by Nathwani and Andrew Davidoff from the St. Jude Children's Research Hospital in Memphis, Tennessee, began to treat ten patients with severe hemophilia B with a single intravenous infusion of adenovirus-associated virus (AAV) vector expressing a codon-optimized human factor IX transgene behind a liver-specific promoter (*N. Engl. J. Med.* **365**, 2357–2365, 2011).

Research performed a decade earlier by Katherine High, a hematologist then at the Children's Hospital of Philadelphia, had a profound influence on their choice of AAV vector. High had found that the AAV serotype 2 vector—the most common AAV strain found in the human population—shortened the response to two months because most recipients had pre-existing neutralizing antibodies to the virus. To overcome the immunity hurdle, the St. Jude–UCL collaborators placed the AAV2 sequences in a capsid from the less prevalent AAV8 strain. A single intravenous infusion of this vector restored factor IX expression in patients to sustained levels in the range of 1–6% of normal values with no toxic effects after three years, on average (*N. Engl. J. Med.* **371**,1994–2004, 2014). At the highest dose, four out of six patients initially experienced a transient increase in liver transaminase levels, a marker of liver damage, but this was managed successfully with corticosteroid treatment. Nathwani is continuing to follow the patients from this landmark academic trial (which was supported by grants from the US and UK governments along with various charitable organizations), while also preparing to launch another phase 1/2 trial for hemophilia B with a next-generation AAV vector through his London-based gene therapy startup, Freeline Therapeutics.

Also in phase 1/2 testing with therapies for hemophilia B are Spark, uniQure and Dimension Therapeutics; Sangamo BioSciences expects to join them before the

Table 1 Commercial gene therapy products in clinical development for hemophilia

	Company	Product	Vector	Therapeutic gene	Manufacturing platform	Year in which first patients dosed in phase 1/2 trial
Hemophilia B	Shire	BAX 335	AAV8	Padua mutant factor IX	HEK293 cells	2013
	Spark Therapeutics/ Pfizer	SPK-9001	Engineered AAV	Padua mutant factor IX	HEK293 cells	2015
	uniQure	AMT-060	AAV5	Wild-type factor IX	Baculovirus	2015
	Dimension Therapeutics	DTX101	AAVrh10	Wild-type factor IX	HEK293 cells	2016
	Sangamo Biosciences	SB-FIX	AAV6	Zinc-finger-nuclease-mediated integration of wild-type factor IX into the albumin locus in hepatocytes	Baculovirus	Expected 2016
	Freeline Therapeutics	FLT-180	Engineered AAV	Undisclosed	HEK293 cells	Expected 2017
	Bioverativ	Undisclosed	Lentivirus	Padua mutant factor IX	HEK293 cells	Expected 2018
Hemophilia A	BioMarin	BMN 270	AAV5	B-domain deleted factor VIII	Baculovirus	2015
	Spark Therapeutics	SPK-8011	Engineered AAV	B-domain deleted factor VIII	HEK293 cells	Expected 2016
	Dimension Therapeutics/ Bayer	DTX-201	Undisclosed	B-domain deleted factor VIII	HeLa cells	Expected 2017
	Shire	BAX-888	AAV8	B-domain deleted factor VIII	HEK293 cells	Expected 2017
	Sangamo Biosciences	SB-525	AAV6	B-domain deleted factor VIII	Baculovirus	Expected 2017

end of the year (**Table 1**). All of these companies, save for Sangamo, have followed variations on the basic St. Jude–UCL vector design with improvements including changes to the AAV capsids, the therapeutic gene constructs, the promoter sequences and other design elements (*Nat. Biotechnol.* **34**, 791–793, 2016).

The torchbearer in this space, however, is Baxter. In 2013, the company initiated the first commercial gene therapy trial for hemophilia B—two years before any other company. Baxter employed a vector developed by R. Jude Samulski, director of the University of North Carolina (UNC) Gene Therapy Center, at his previous startup, Chatham Therapeutics. Samulski is currently on leave from UNC to serve as CSO of Bamboo Therapeutics, a gene therapy company focused on neuromuscular conditions, now owned by Pfizer.

The Baxter product was similar to the one tested two years earlier by the St. Jude–UCL group, but it used a naturally occurring, hyperfunctional version of the coagulation factor IX known as the Padua mutant, which differs by just one amino acid and yields up to 15-fold higher expression levels. One individual treated with the Baxter gene therapy, BAX 335 (which moved in 2015 to Baxter's pharmaceuticals spinoff Baxalta, a company now wholly owned by Shire), achieved sustained factor IX expression of 20–25% of normal, which is considered curative. But immune reactions against the AAV8 capsid occurred in the highest dose cohort, and factor IX levels dropped dramatically, despite intervention with prednisone.

Although the trial testing BAX 335 continues, Shire recently announced plans to advance a different gene therapy for hemophilia B. Fritz Scheiflinger, head of hematology research at Dublin-based Shire, declined to discuss specific modifications the company is making, but he did state that "we are not changing the basic setup. It is AAV8. It is going to be fully based on 335."

Spark also achieved curative factor IX levels with the Padua mutant version using an engineered AAV capsid selected for its liver tropism—a strategy that according to High, who is Spark's co-founder, president and CSO, allows vector doses that are low enough to avoid triggering an immune response while still achieving elevated expression levels. After three to seven months of follow-up, the Philadelphia-based company (which is developing its gene therapy with New York–based Pfizer) observed factor IX activity levels in the range of 20–44% of normal in four subjects with no one developing elevated liver transaminases.

By comparison, uniQure—the Amsterdam-based company credited with gaining the first approval for a gene therapy in the Western world (*Nat. Biotechnol.* **30**, 1153, 2012)—relies on an AAV5 capsid. The initial cohort received ten times the dose as was administered in the Spark trial and yet achieved factor IX levels of only 3–7% of normal for up to nine months in five subjects, with one patient needing steroid intervention.

Despite the lower expression levels, uniQure's chief medical officer, Christian Meyer, expects that the choice of AAV will help carve out a commercial niche as different patients will have different neutralizing antibodies. "There is going to be room for several serotypes," he says. Annalisa Jenkins, CEO of Cambridge, Massachusetts–based Dimension, points to another benefit of going against the grain on AAV selection. "It affords us IP [intellectual property] protection and freedom to operate," she says. Dimension is using AAVrh10 technology, a clade E AAV similar to AAV8, isolated from natural sources, that it licensed from Regenxbio, a Rockville, Maryland–based company cofounded by gene therapy pioneer James Wilson from the University of Pennsylvania.

Of note, both uniQure and Dimension are using codon-optimized wild-type sequences of factor IX, not the Padua mutant—"the conservative approach in terms of immunogenicity," says Roland Herzog, a gene therapy researcher at the University of Florida in Gainesville. That's the form included in factor IX replacement therapies and it's conceivable that the body could recognize a mutant as foreign and develop neutralizing antibodies against it. However, no patient treated to date with either BAX 335 or Spark's SPK-9001 has developed such an immune response.

Only BioMarin has, so far, successfully steered a factor VIII gene therapy for hemophilia A into the clinic. Researchers find the factor VIII gene challenging to work with because the full-length cDNA encoding the protein is several thousand nucleotides longer than the 5,000 base-pair size limit imposed

Box 1 Roche's game-changing bispecific factor VIII mimic

Roche has revealed follow-up results from a first-of-its-kind approach to tackle factor VIII deficiency in individuals with hemophilia A. Weekly treatment with emicizumab improved clotting and nearly eliminated all forms of bleeds in patients followed for 33 months on average. Before the intervention, these same patients taking part in the phase 1/2 study experienced between one and three bleeds on average per month. The benefit was observed in patients with and without inhibitors to factor VIII, with no major safety problems linked to the antibody.

The data reported in July build on earlier results from the same 18 patients published two months earlier in the *New England Journal of Medicine* (**374**, 2044–2053, 2016), prompting onlookers to predict blockbuster potential for the antibody in this space. "The science looks very exciting and quite elegant," says Ronny Gal, a senior analyst at Sanford Bernstein in New York. "If validated, I think that, over time, a lot of patients would seek to use this product."

Emicizumab is a humanized bispecific monoclonal antibody engineered to mimic the function of factor VIII. With one antibody arm, it binds clotting factor IXa, and with the other factor X to generate the active form of factor X needed for coagulation. It originated at Chugai Pharmaceutical, now majority owned by Basel, Switzerland–based Roche and being co-developed by the company's S. San Francisco, California–based subsidiary Genentech.

Recombinant factor VIII is the standard of care for people with hemophilia A, but after months and years of repeated infusions, around 30% of patients develop inhibitory antibodies to the recombinant protein. Treatments to induce tolerance and eliminate inhibitors are available, but these work only in approximately three-quarters of cases. The rest of the patients need to resort to so-called bypassing agents—drugs that offer a workaround for the missing factor VIII, but have short half-lives and need to be administered daily or every other day for prophylaxis. Even then, the average patient continues to have around one bleeding episode per month.

In contrast, with its long half-life of 4–5 weeks, emicizumab can be taken less often—Roche is testing dosing weekly, biweekly and once every four weeks—and as it is delivered subcutaneously, rather than with intravenous infusions as all other products, this will likely enhance compliance—in people with and without inhibitors. Emicizumab is "easier, simpler and faster," says Guy Young, a pediatric hematologist at Children's Hospital Los Angeles and trial investigator. "I think [emicizumab] has the potential to be transformational for patients—really, truly, life-altering." *ED*

by AAV vectors. BioMarin, with help from its licensee Nathwani, got around this problem by omitting much of factor VIII's B-domain, which is not needed for blood clotting, and then squishing in a small but active synthetic, liver-specific promoter. (All the other companies hot on BioMarin's heels are pursuing a similar approach.) The resulting 4,970 base-pair, single-stranded DNA is packaged into an AAV5 capsid and manufactured using a baculovirus–insect cell expression system. Traditionally, gene therapies have been manufactured in human embryonic kidney cells; Dimension is developing a platform that uses HeLa cells.

The baculovirus system offers higher yields with an easier scale-up, but it seems to reduce potency, pushing up the doses needed to obtain a response. That could explain in part why uniQure, which also uses the baculovirus platform, needed higher doses in its trial than Spark did. Likewise, the BioMarin trial required doses 120 times higher than those administered by Spark, and the therapy led to increased liver enzymes in the high-dose group. Although the immune responses were mild and successfully controlled with steroid treatment, Carter says that BioMarin "may look at refining the dose a little bit" when it launches a phase 2b trial next year.

Another company using the baculovirus production system for its gene therapy is Sangamo BioSciences, which is making three AAV6 vectors—one containing a corrective copy of factor IX, and two containing genome-editing zinc-finger nucleases—to stably insert factor IX into the albumin gene locus that is highly expressed in hepatocytes. "By having these genetically modified cells, we can maintain a durable response," says Michael Holmes, head of research at the Richmond, California–based firm, which expects to begin clinical trials later this year with what would be the first *in vivo* genome-editing therapy in human testing.

Sangamo's approach should help get around one of the main limitations of most AAV-based gene therapies: they tend not to integrate into tissues and, instead, persist in the body as episomes. This could be a disadvantage when treating children with hemophilia as the gene therapy's successful expression will necessarily dwindle over time. "Growing liver tissue will dilute out a non-integrating vector," explains Luigi Naldini, director of the San Raffaele Telethon Institute for Gene Therapy in Milan.

Naldini is currently working with Bioverativ, a new hemophilia-focused spinoff from Cambridge, Massachusetts–based Biogen, to develop a gene therapy that will permanently insert the Padua factor IX mutant gene into liver cells with an integrating lentiviral vector. An added benefit of this approach, notes Naldini, is that humans tend not to have pre-existing immunity against lentiviruses because the vector's envelope glycoprotein comes from a vesicular stomatitis virus that mostly infects livestock. Olivier Danos, Biogen's head of gene therapy, says the program is currently being evaluated in monkeys and remains at least two years away from the clinic.

Every company in clinical testing, for now, is enrolling patients with no inhibitory antibodies against factor replacement therapies. Some preclinical research in dogs and mice suggests that gene therapies can have a tolerizing effect to eradicate these antibodies that neutralize coagulation factors, but companies first want to demonstrate that their products work for populations without this added complication. People with inhibitors against factor VIII might have another option in an innovative new antibody therapy from Roche that mimics the function of the missing clotting factor (**Box 1**). People with inhibitors against factor IX, unfortunately, may just have to wait.

Elie Dolgin *Somerville, Massachusetts*

> **"When we realized I had a nuclear bomb in my body, we were very scared. And when we learned that I was cancer-free, all I could think was, 'How blessed am I?'"** Leukemia patient Karen Koehler, who had to contend with several adverse events from CAR-T therapy before becoming cancer free. (*STAT*, 23 August 2016)
>
> **"We believe that this effort [against Mylan's price hikes on EpiPens] likely will follow the same playbook that lawmakers used to shame Gilead, Valeant and others. Congressional hearings and a press onslaught, but no substantive legislative action."** Spencer Perlman, analyst at Height Securities of Washington, DC, is despondent, yet when Hillary Clinton called the price increases outrageous, on August 24, biotech stocks went tumbling. (*USA Today*, 25 August 2016)