Hello, greetings to everyone. Glad that you're participating today. Welcome to this program on *Latest Advances in Gene Therapy for Hemophilia*, with a focus on an integrated care model to improve outcomes.

I'm Dr. Steven Pipe. I'm in Pediatric Hematology and Oncology at the University of Michigan. I've been leading the pediatric program here in our Hemophilia and Coagulation Disorders Program for almost 30 years and I also direct the Special Coagulation Laboratory. And in recent years, I've been able to be part of multiple clinical trials involved in gene therapy. And that is going to be part of our discussion today.

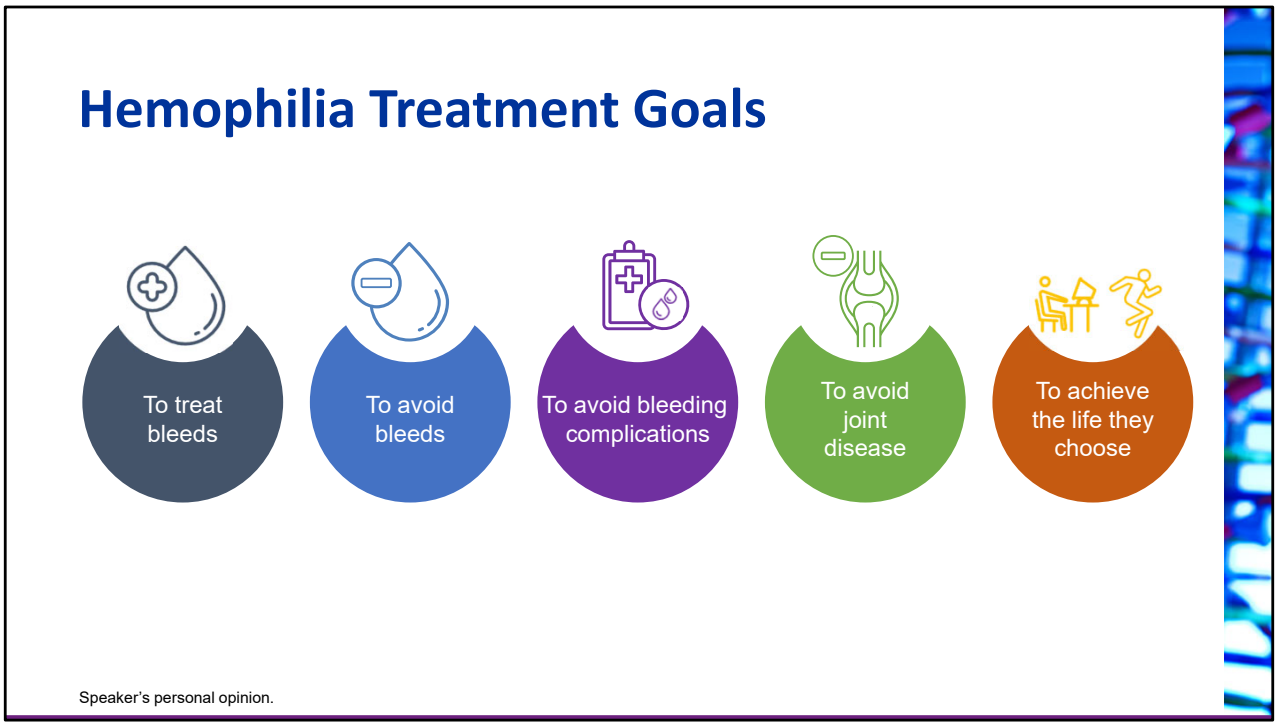
This program is being provided by MediCom Worldwide, and it is supported by an educational grant from BioMarin.

Faculty Disclosure – Steven W. Pipe, MD

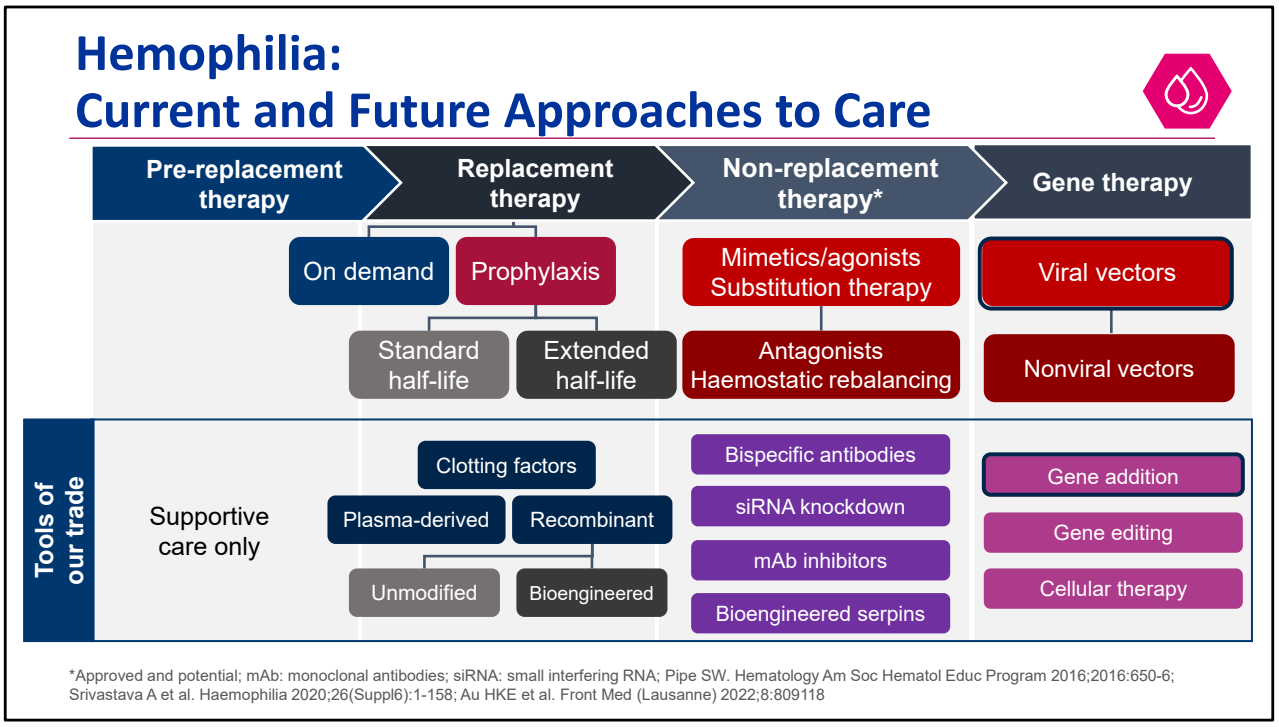
- **Consulting:** ApcinteX, ASC Therapeutics, Bayer AG, BioMarin Pharmaceutical Inc., Catalyst Biosciences, CSL Behring, Freeline, Genentech – A Member of the Roche Group, GeneVentiv Therapeutics, HEMA Biologics, LLC, Intellia Therapeutics, Inc., Novo Nordisk A/S, Pfizer Inc., Regeneron Pharmaceuticals, Inc., Sangamo Therapeutics, Sanofi, Spark Therapeutics, Inc., Takeda Oncology, and uniQure N.V.
- **Contracted Research:** Siemens
- **Scientific Advisory Board:** GeneVentiv Therapeutics

All relevant conflicts of interest have been mitigated prior to this activity.

These are my disclosures. Just so you know that I have participated in a lot of the clinical trials that we are going to talk about today, and I've also provided consultation to companies that are developing many of these therapies. But I have no vested interest in the outcomes of any of these trials.



So as we're talking about the rationale for gene therapy, we have to keep in mind what the key hemophilia treatment goals are for our patients. We want to have therapies that can effectively treat bleeds, therapies that can help avoid bleeding, and avoid the complications of bleeding. And we definitely want to have therapies that are going to avoid the long-term complication of joint disease. Then also we want to help patients achieve the life that they choose to live, and this is all part of achieving health equity as it pertains to hemophilia.

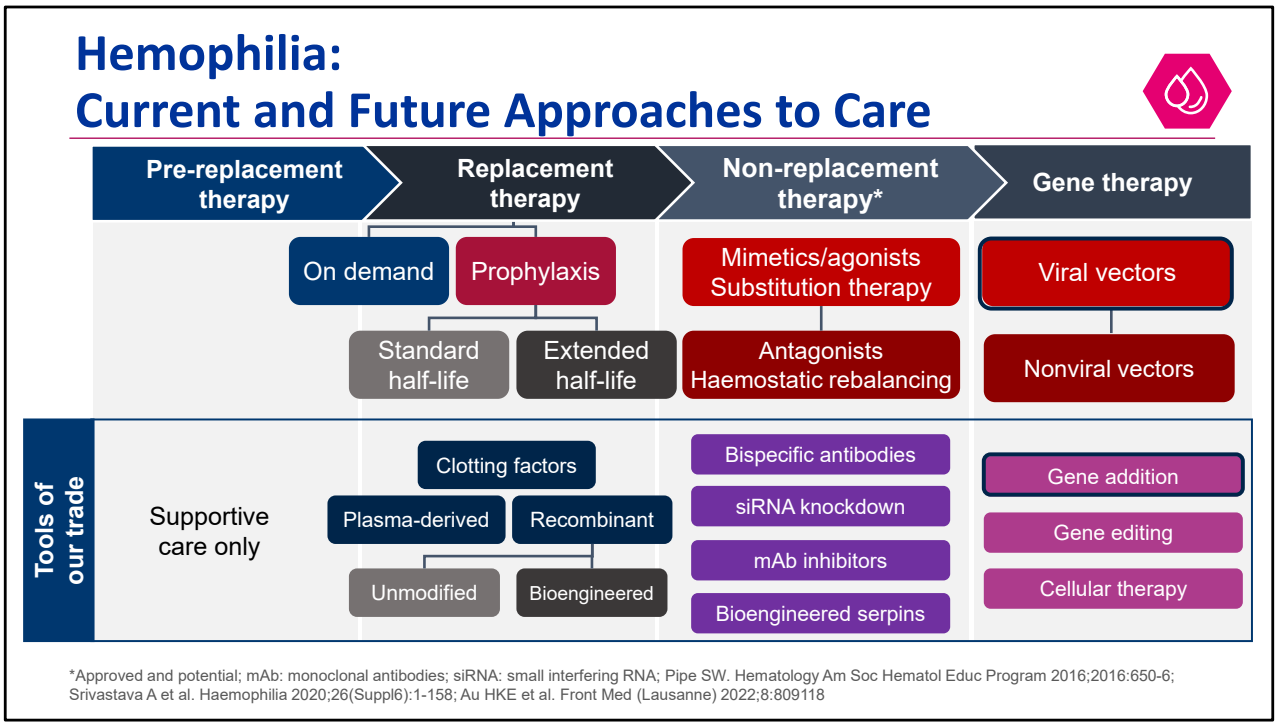


The replacement therapy era began when we were able to get purified clotting factors out of plasma and that's how it really started in the late 1960s and early seventies. However, even back then, we recognized that treating on demand really was not addressing the fundamental problem of hemophilia as repeated bleedings into joint were going to still lead to advancement of joint disease. So the prophylactic era is what I have worked in and maybe all of you have worked in as well. And we have used prophylactic clotting factors in recent years, primarily recombinant versions, to try to prevent bleeding and hopefully to have an impact on joint outcomes.

Now there have been some innovations on the recombinant platform because various bioengineering strategies can alter the properties of some of these clotting factors to enhance outcomes for patients.

The most effective bioengineering strategies have extended the half-life of the factor. So now we have extended half-life versions of Factor VIII and Factor IX, many of which have dominated the replacement therapy era. We're now just a few years in, however, to at least one approved non-replacement therapy and of course that is emicizumab. This is a memetic that substitutes for the role of factor VIII in blood clotting.

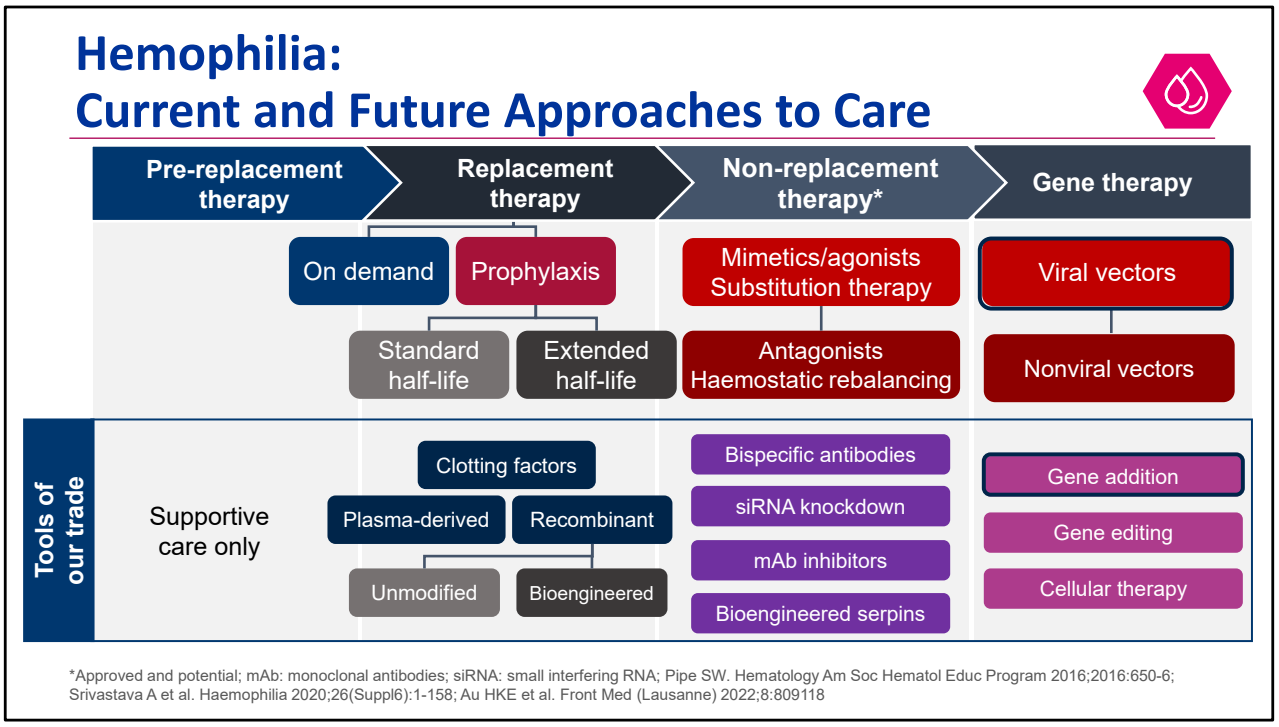
And, you know, some of the advantages of this bispecific antibody in that it can be given subcutaneously, it has a very long duration of action. We have treatment regimens where we can treat either weekly, or every two weeks, or every four weeks.



And the studies have been pretty convincing that Hemlibra prophylaxis has advantages even in outcomes for patients over replacement therapy, primarily with respect to improvements in annualized bleed rate and also the proportion of patients who are not having any bleeding in any given 6-month or 12-month interval. Now unfortunately, emicizumab is limited to patients with hemophilia A since it substitutes for factor VIII's function.

So we don't right now have an approved therapy for hemophilia B patients. There are a number of additional non-replacement therapies that are under clinical development. And these all share a common feature that they are aiming to rebalance the hemostatic system. If you think of a balance between your procoagulants like factor VIII or factor IX and the natural anticoagulants, which primarily are tissue factor pathway inhibitor, antithrombin and then activated protein C.

Part of the problem with hemophilia is that when you have a deficiency of factor VIII or factor IX, this shifts the balance because you still have the full weight or the still full function of these natural anticoagulants. So we rebalance that either through replacing factor VIII or factor IX, or using a substitution therapy like emicizumab for hemophilia A, but it's also true that we could rebalance hemostasis by just counteracting the functions of these natural anticoagulants. And this was really an, you know, a light bulb moment for me to realize that you could see this kind of rebalancing. Because what

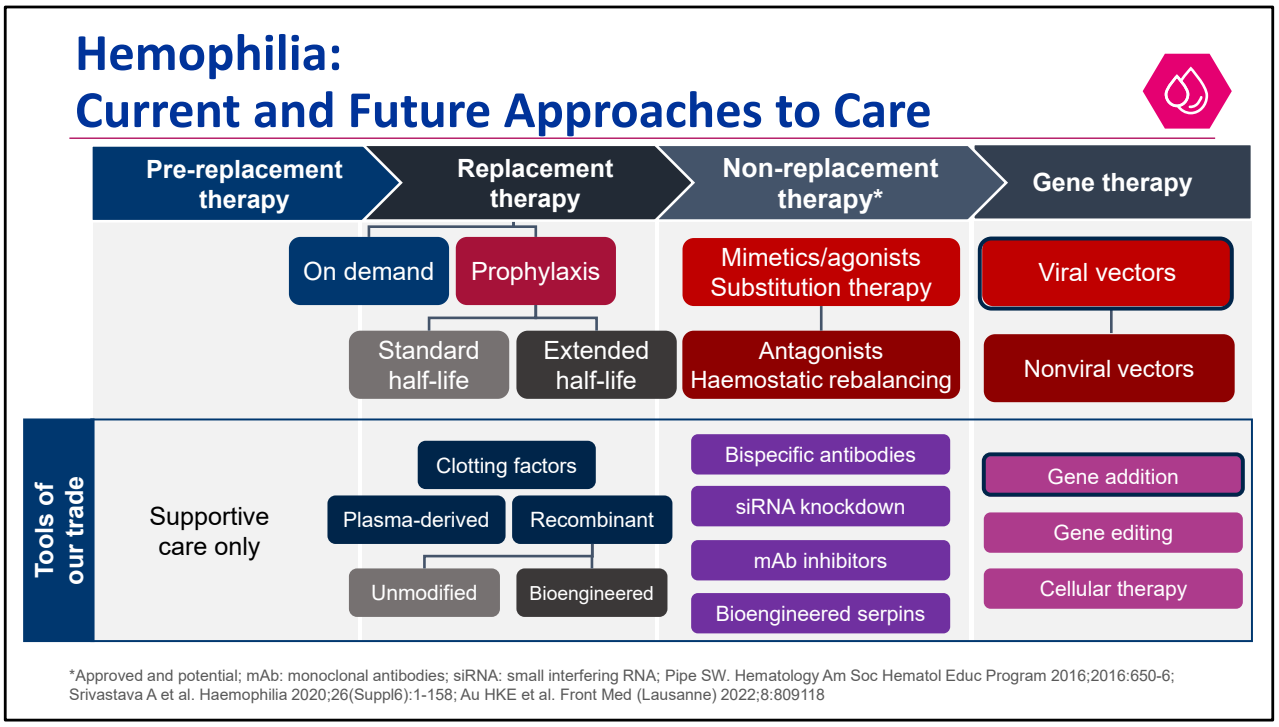


happens is, if you knock down the functions or the amounts of these natural anticoagulants, you improve thrombin generation. And that's really what we're seeking to do with replacement therapy. So they really shouldn't be thought of too differently.

So there's three active programs that are well into clinical trial development. Fitusiran is a small interfering RNA, which knocks down antithrombin levels, and that's now late in phase 3 development. There are a couple of monoclonal antibodies that target tissue factor pathway inhibitor, concizumab, and marstacimab. And these are also well into their phase 3 programs.

And then the newest one is a bioengineered serine protease inhibitor or serpin as it's called, SerpinPC, which targets activated protein C. This just had its first readout in December at the ASH meeting, and also is showing that it can provide effective prophylaxis in patients.

And what's neat about all of these platforms of therapy is that they are cross-platform, meaning they will, because you're targeting the natural anticoagulant side, they'll work in hemophilia A, hemophilia B, with or without inhibitors. For the first time, we might have these non-factor therapies that can be used in all these clinical contexts.

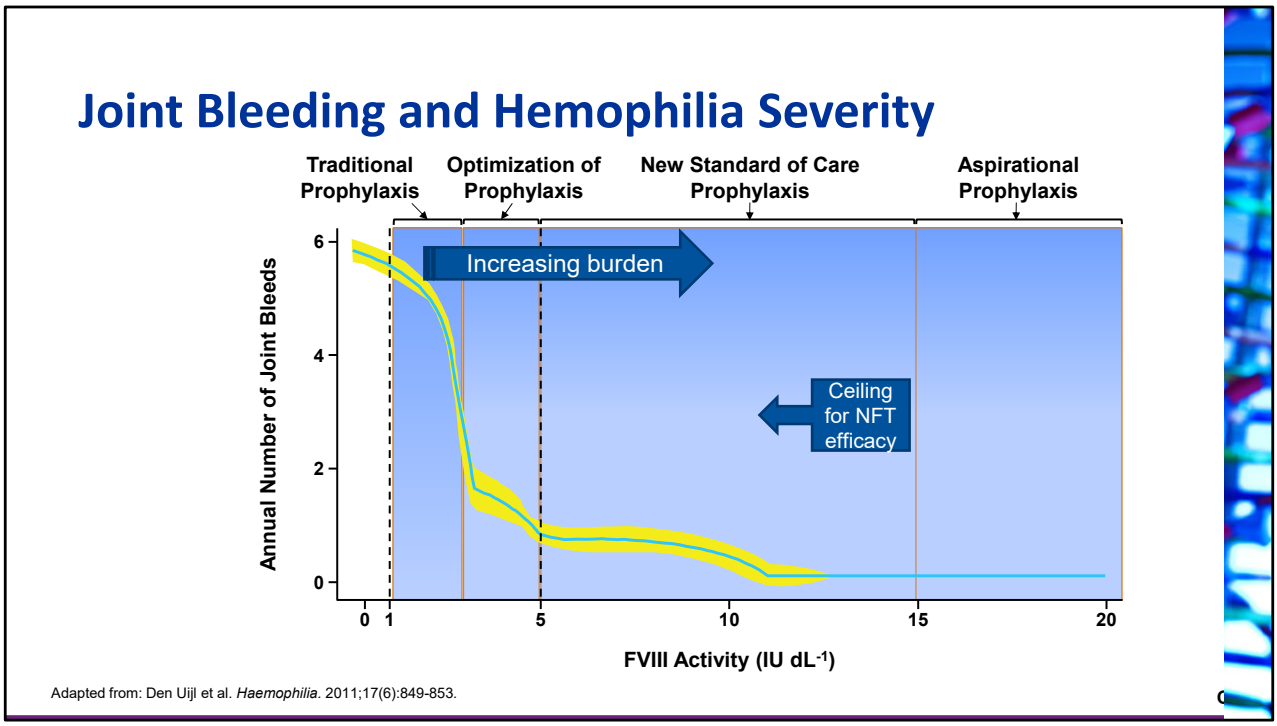


*Approved and potential; mAb: monoclonal antibodies; siRNA: small interfering RNA; Pipe SW. Hematology Am Soc Hematol Educ Program 2016;2016:650-6; Srivastava A et al. Haemophilia 2020;26(Suppl6):1-158; Au HKE et al. Front Med (Lausanne) 2022;8:809118

On that background of where the field has been moving, our focus today is to talk about, well, what does gene therapy have to offer over some of these other developments? Now gene therapy is a big umbrella. We can think about gene therapy as gene addition, just replacing a missing gene. So for factor VIII or factor IX, replacing the missing gene. Gene editing would be either inserting a gene in a specific location within the genome, or it could mean going in and actually correcting a mutation within a gene.

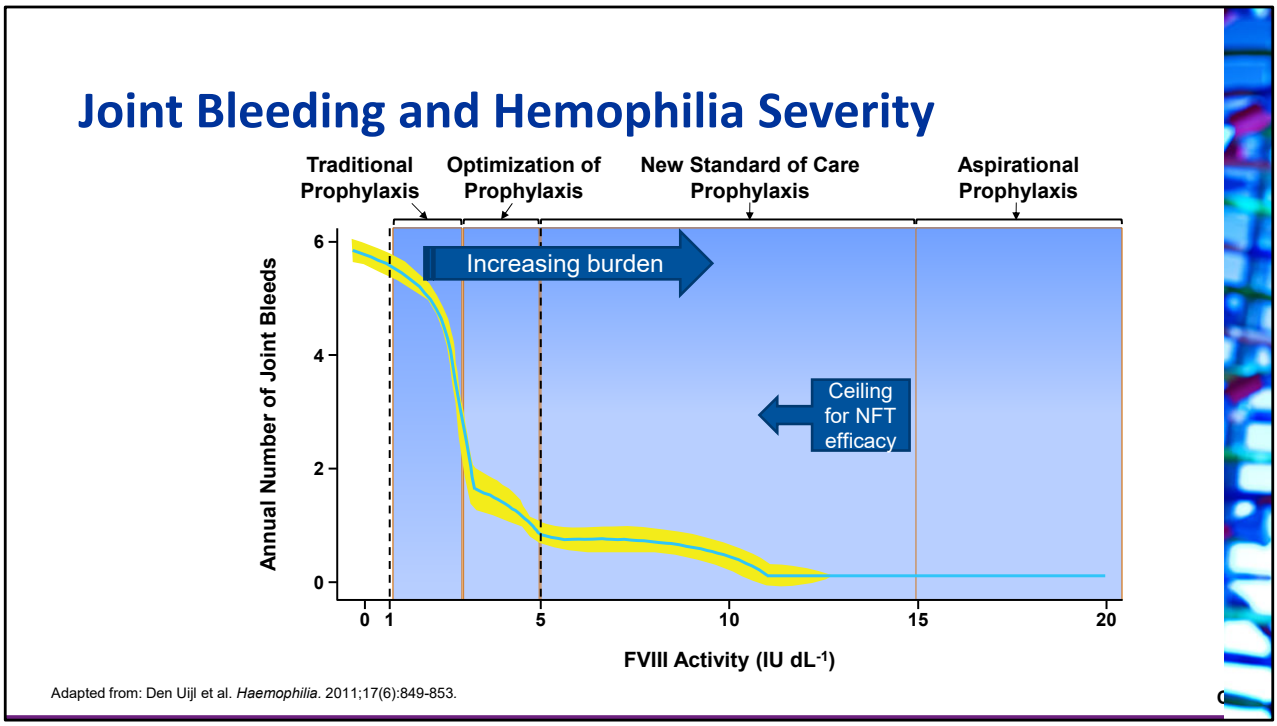
And then there's also cellular therapies, which can be thought of as gene therapies. In this case, what you're doing is you're taking some cells out of the body, you're modifying them in the laboratory, and then you're giving them back to the patient in their modified form. The classic example of this would be CAR T-cell therapy that's used in malignant applications.

All of these gene strategies require getting the gene into the patient and into the target cells. And we can think of non-viral and viral approaches. Right now, the viral approaches by far have the greatest efficiency, at least for in vivo application. And so, the platform of therapy that we're primarily talking about for hemophilia are using viral vectors to do gene addition.

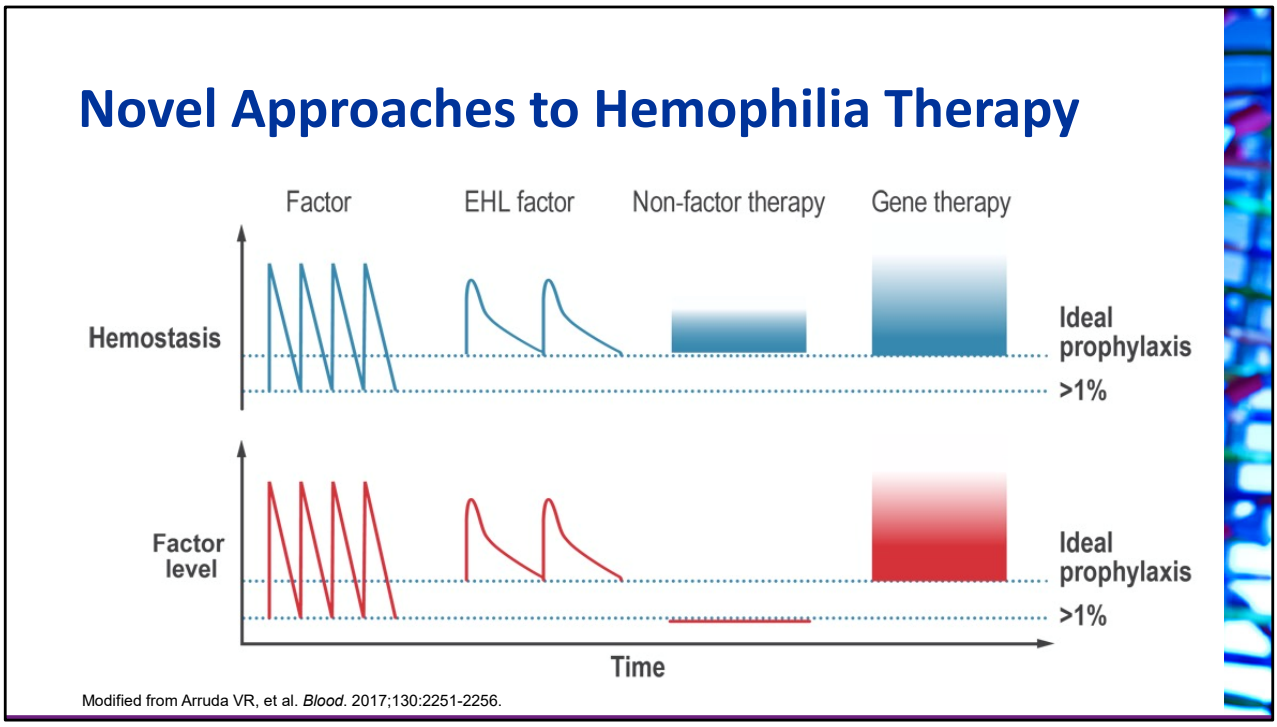


Now to set up the rationale for why we should be embracing gene therapy, we have to go back to sort of first principles of what are some of the thresholds for breakthrough bleeding in hemophilia. This is extrapolated data from a study by Den Uijl from the Netherlands, where they looked at the natural history of patients across all the different severities in their clinic. So what you have across the bottom is the basal factor levels of those patients. And then in the vertical axis, we have the average number of annual joint bleeds that they would see in those patient populations. For less than 1%, these of course have the highest risk for bleeding, but you can see that even if you have a basal level of anywhere from two to 3% you have a substantial improvement in risk of breakthrough joint bleeding.

That further goes down if you get down to levels that are now above 5%. And at some point, somewhere around 12 to 15%, the risk of having joint bleeding goes almost to zero. Now, knowledge of this natural history has actually informed our approach to prophylaxis. If we think of traditional prophylaxis, what we are aiming is to shift the phenotype from severe hemophilia to what was deemed to be sort of a moderate phenotype. So that meant keeping trough levels that were not lower than say 1 to 3%. But with the advent of some of the extended half-life factors that we talked about earlier, we can now optimize prophylaxis and particularly with different tools that we have available, such as not just the extended half-life, but also population pharmaco-kinetic tools where we can individualize, and dose optimize for patients. We can now more consistently get patients into trough ranges that don't fall lower than 3 to 5%, and that has been associated with improved outcomes.



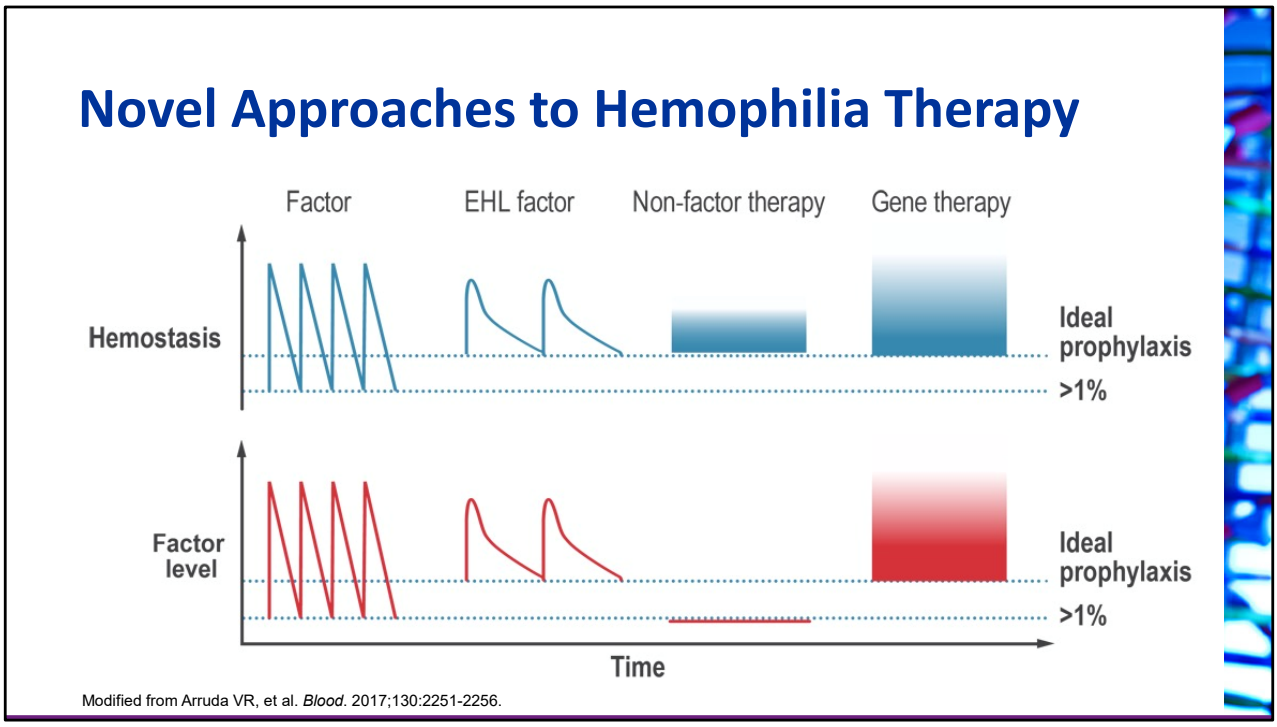
However, Hemlibra has sort of opened up a new vision of what the new standard of care prophylaxis is. And if we look at how aggressive does your factor replacement have to get to get patients into a range that's consistently above 5%? The burden of IV therapies particularly with factor VIII, is just too high. We really can't maintain patients consistently with trough levels that are well above 10%. The non-factor therapies like emicizumab also have a ceiling. You can't keep increasing the dose of emicizumab and getting more and more efficacy. And so, we think the clinical efficacy of, if you want to talk about the sort of factor VIII equivalency or factor VIII-like activity of emicizumab, most studies would suggest that it's probably in the 10 to 15% or maybe 10 to 20% range. If we really want to have an impact on overall, particularly joint bleeding and abrogating all joint bleeding in a patient, we really want to be aiming what I would call aspirational prophylaxis, which is consistently having levels that are above 15 to 20%.



Now this graphic also highlights some important principles about all these therapies we've talked about so far. Across the bottom you have a readout of the factor levels that you can measure from the laboratory. And what's being depicted on the left is typical factor replacement with a standard half-life, factor VIII or factor IX, and you get the familiar peaks and troughs after each prophylactic dose.

And correspondingly in the upper graph is you have the expected hemostatic effect of those levels. So, you have a direct correlation between a factor level and the hemostatic effect. And what we're trying to do here, as we talked about, is keep the trough levels above 1%, traditional prophylaxis.

Well, the extended half-life factors have accomplished a couple of things. They've allowed us to stretch out the interval of the dosing, but they've also allowed us to raise those trough levels and optimize it for patients. In the third column there, we have non-factor therapy like emicizumab. Now here we have a paradigm shift because we have a hemostatic effect, but there's no corresponding factor level. What we've had to get used to is just giving a product like emicizumab and getting an expected effect without actually having a measurable level that is clearly a correlate of the efficacy of the drug.



But the main benefit of the emicizumab comes from this steady state hemostatic effect where we don't have these peaks and troughs. What we're achieving with gene therapy, I think is kind of the best of both worlds, because what's going to happen is you're going to get an expression of factor VIII or factor IX in the plasma, it's being expressed endogenously, no more IV infusions.

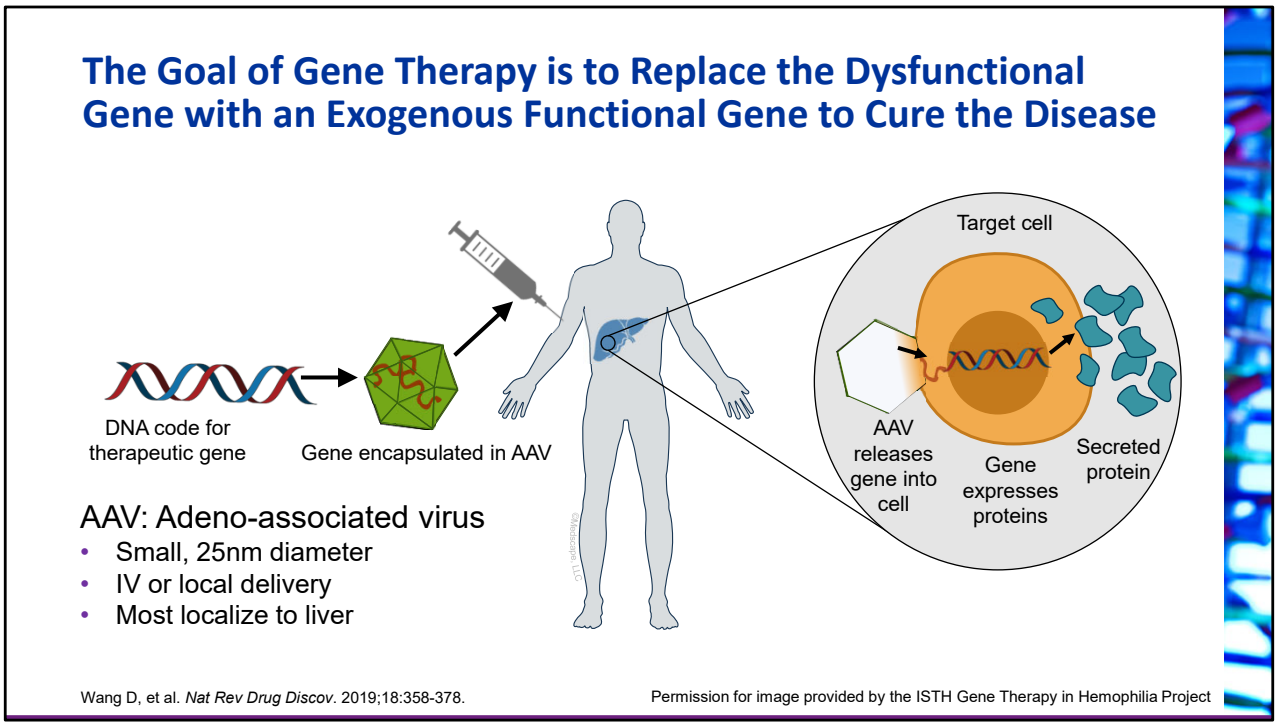
So you're going to get a steady state factor level, which you can measure from a blood test. And there's going to be a corresponding hemostatic effect that we're used to. If a patient has a steady state level of 30%, we sort of know what the expected hemostatic efficacy will be with that.

Gene Therapy in Hemophilia

- Is a choice in the field of hemophilia treatment for hemophilia B and is likely to become a choice for hemophilia A
- Clinical trials have demonstrated that **one single intravenous infusion** of adeno-associated virus (**AAV) vector** containing *F8* or *F9* cDNA can achieve:
  - High protein expression levels
  - Durable factor expression for years
  - Marked reduction in bleeds even compared to factor prophylaxis
  - Cessation of prophylaxis regimens

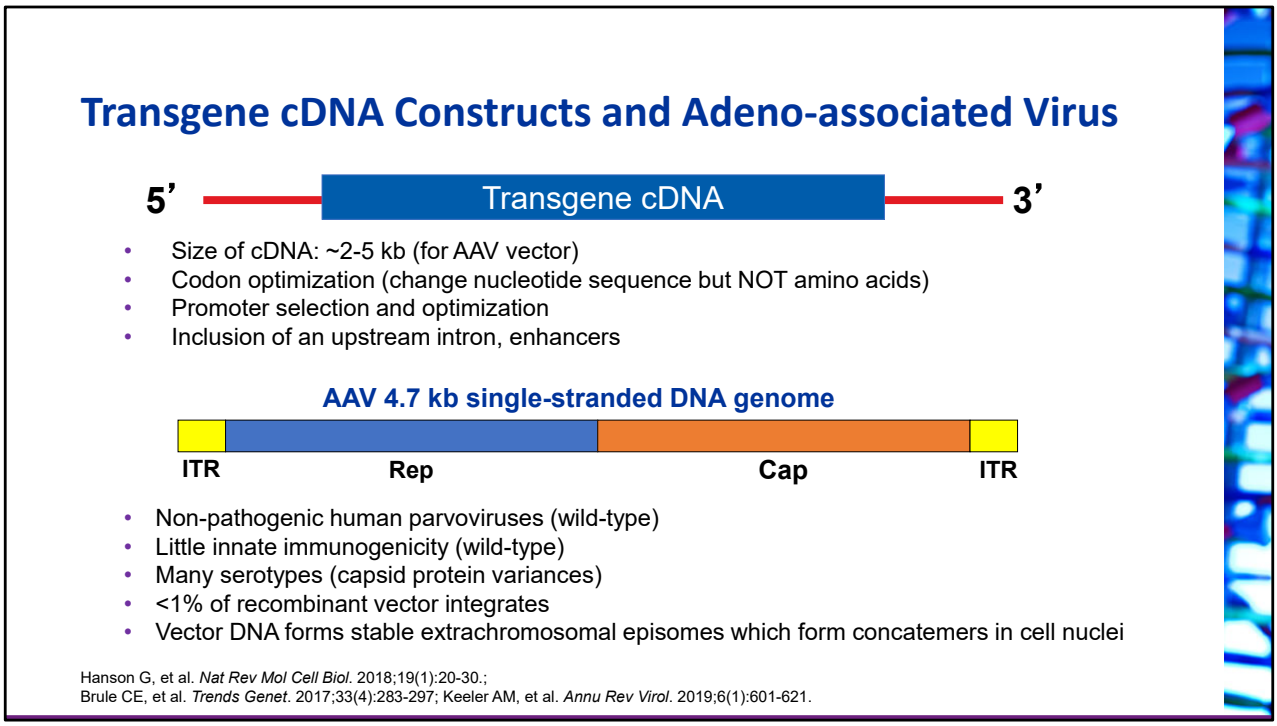
Gene therapy is now a choice in the field of hemophilia treatment of hemophilia B based on the approval of HEMGENIX, just a couple of months back and it's likely to become a choice for hemophilia A.

ROCTAVIAN is under review currently with at least an adjudication date somewhere towards the end of June. The clinical trials have demonstrated that with a single intravenous infusion of an adeno-associated virus vector that contains either factor VIII or factor IX, that we can achieve high protein expression levels, durable factor expression for years, marked reduction in bleeds, even when compared to factor prophylaxis. And the majority of individuals can stop their prophylactic regimens. This is the platform we're going to talk about and then we're going to get into some of the granularity of the results.



I mentioned that the real breakthrough in gene therapy was how to deliver a functional copy of the transgene in factor VIII or factor IX into the body and have it express at a level that would be beneficial for patients. It was deemed that the liver is a useful target because it's capable of making factor VIII or factor IX, and the viral vectors that were selected for this, these adeno-associated viruses naturally home to the liver. We call that the tropism of the vector, and that tropism is determined by this protein capsule that encapsulates the transgene. Adeno-associated viruses are small viruses. They can be given intravenously. Most of them show tropism to the liver. And the nice thing is that they are not associated with any known pathology in humans.

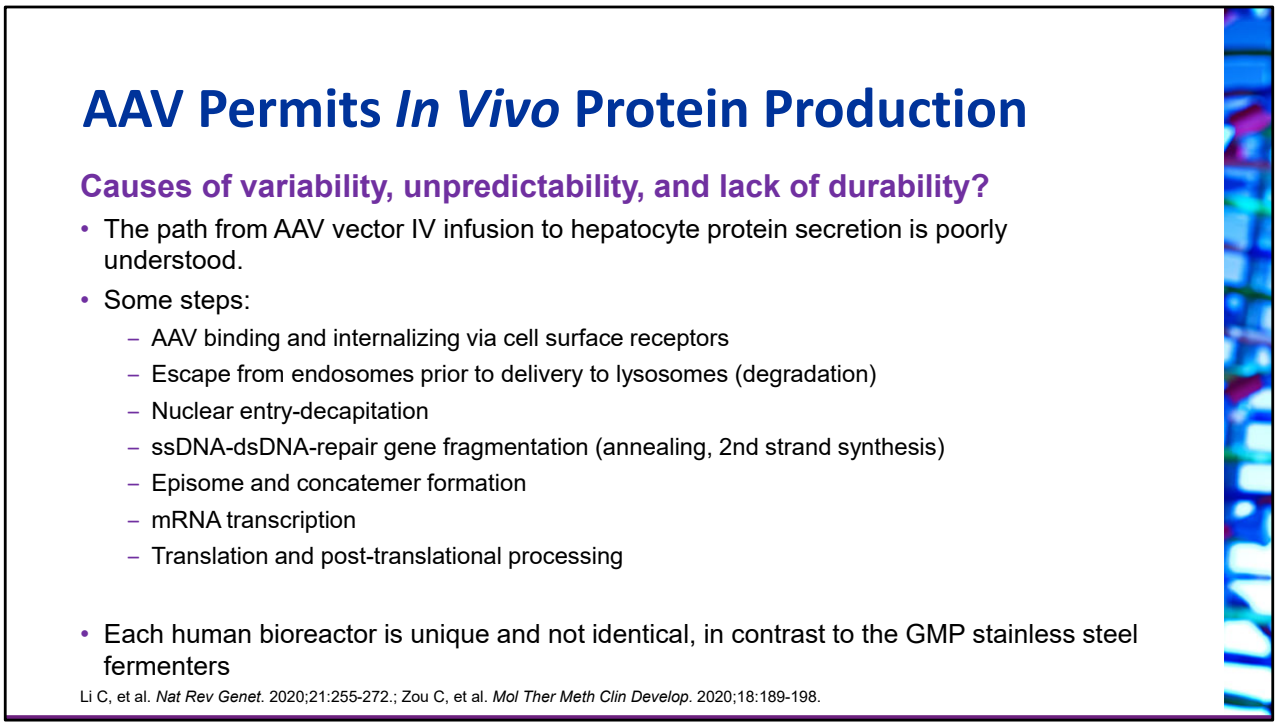
We encounter them naturally in the environment, but they don't induce any disease. So, by packaging the DNA for factor VIII or factor IX, into this capsid envelope that is the vector that's delivered through that single IV infusion. It, homes to the liver. It gets taken up into the cell. The AAV escapes from the endosome, and it shuttles to the nucleus where it then delivers the payload, the factor VIII or factor IX transgene into the nucleus. And there it's going to take advantage of the natural mechanisms that are going to read the code off the DNA and produce a message. And then ultimately a protein, and that protein is going to be made in the normal synthetic pathway. And then ultimately, you'll start getting secreted protein, a factor VIII or factor IX, which will then reach a steady state based on the clearance of the protein. And that's what we measure in the laboratory.



When we are talking about the genes that we're delivering here, there's two components. The AAV has its own genome, but this is what allows it to replicate inside of a human. And we're not aiming to do that. These interior genes called the Rep and Cap genes don't exist in these AAV vectors. What we're going to do is we're going to take that transgene cDNA, which codes for factor VIII or factor IX, and we're going to replace that, where those viral genes used to be.

Some of the good things about these AAV vectors, I mentioned that they're nonpathogenic. They have little innate immunogenicity. So, you don't mount a real violent immune response to these. They come in many serotypes and the serotypes are the proteins that make up that capsid envelope. And that's where some of the number variants I'll talk to you about, come from.

The other thing is that these can deliver a transgene and have it expressed in a cell without it integrating into the patient's own DNA. It remains outside of the chromosomes. Now, there is some degree of integration, but it is at a very low rate. Much, much less than than 1% of the vector integrates.



We have to embrace with this technology a great degree of variability, unpredictability, and individual outcomes and also some concerns about the durability of how long you are going to continue to get expression from the transgene and it's poorly understood what is responsible for this. If you look at some of those steps that I talked to you about, the AAV having to bind and then get internalized by the cell surface receptors, then it has to escape from the endosomes so that it doesn't get degraded and can actually deliver the transgene to the nucleus. Once it gets into the nucleus, there's some additional steps where it has to duplicate the DNA to make a full double stranded DNA. Then it forms these circular mini chromosomes or what are called episomes, and some of them will even have multiple copies of the transgene that get assembled end to end into what are called concatemers. Then there's all the regulation at the cellular level for transcription and translation and the post translational processing. So that all taken together, it's maybe not surprising that we have a lot of individual variability between patients.

You know what we're used to with a recombinant production facility is we can optimize all of those steps and get a very consistent expression from those production runs, but a human bioreactor is completely unique. Everybody is an individual and so we do have to embrace some of this variability.

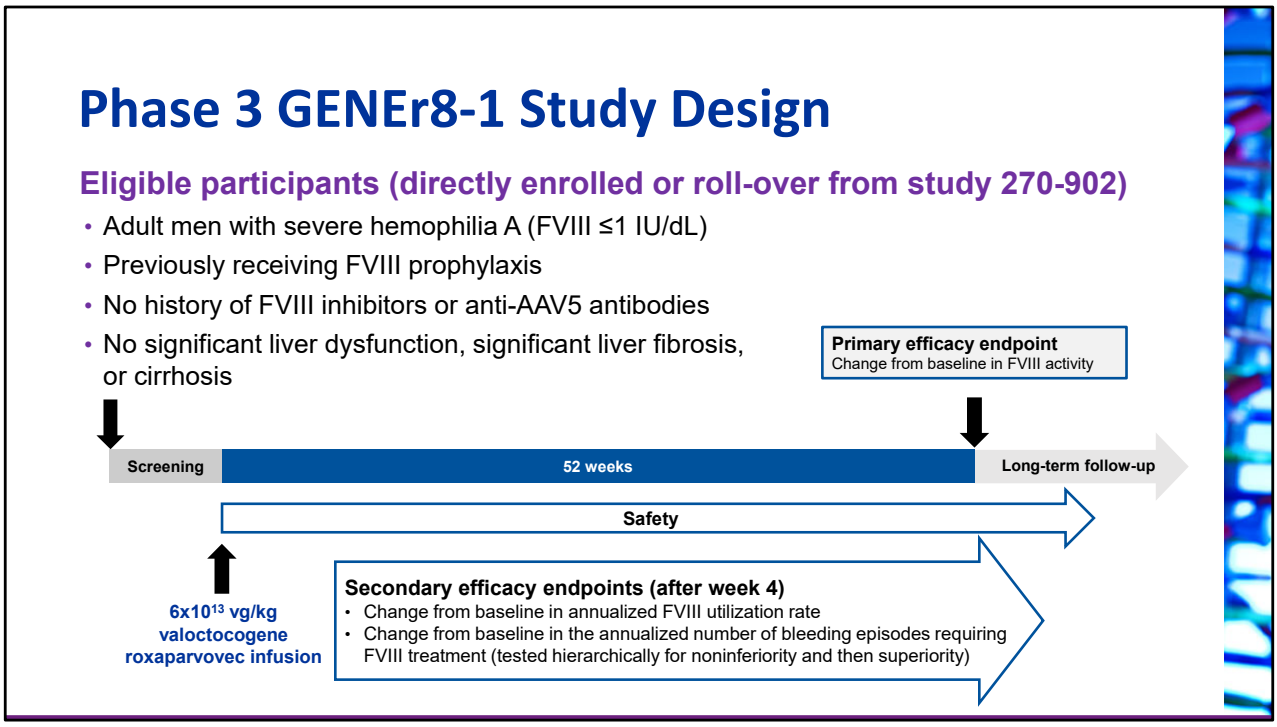
Adverse Effects

- **Elevation in liver transaminase** is the main toxicity observed
- Majority of these events were managed with **corticosteroids**, but duration of steroids can range from 22 days to over 500 days
- Some cases have been associated with partial or complete loss of transgene expression
- The pathophysiological mechanism for the liver toxicity remains unclear
- Capsid-specific cytotoxic T-cell responses against the vector-transduced cells is one explanation of this liver toxicity
- Other causes may include intrinsic hepatocyte dysfunction and/or the innate immune response

Manno C, et al. *Nat Med*. 2006;12(3):342-347.; Mingozzi F, et al. *Nat Med*. 2007;13:419-422.; Ertl HCJ, et al. *Hum Gene Ther*. 2017;28:328-337.

At a general level, we should be aware of some of the key adverse effects related to gene therapy. The one you need to remember is what's been observed, which are elevations in liver transaminases as the main toxicity observed. Liver transaminases are the ALT and AST assays that we typically measure. The reason we pay attention to these is some of the cases of patients who've had these liver toxicity have been associated with either a partial or a complete loss of the transgene expression. The patient was starting to express after delivery, and then they got this transaminase elevation, and then the factor VIII and factor IX levels just kind of fall off a cliff. And once they're lost, they're lost. And so, the whole procedure then could be for naught.

It is important to recognize when this is happening. The majority of these events are managed with corticosteroids, and the reason is because it's thought that this is a manifestation of a cytotoxic T-cell response directed against the capsid that was delivered into the cell. And so, the immune system is sort of targeting those cells where the capsid is being broken down by the cellular machinery and then presented to the immune system. The full pathophysiologic mechanism here I think is still a little bit unclear, but when you see the responses that are being used in the trials, which are primarily immunosuppression, it's because of the belief that these are at least rooted in this immune response.

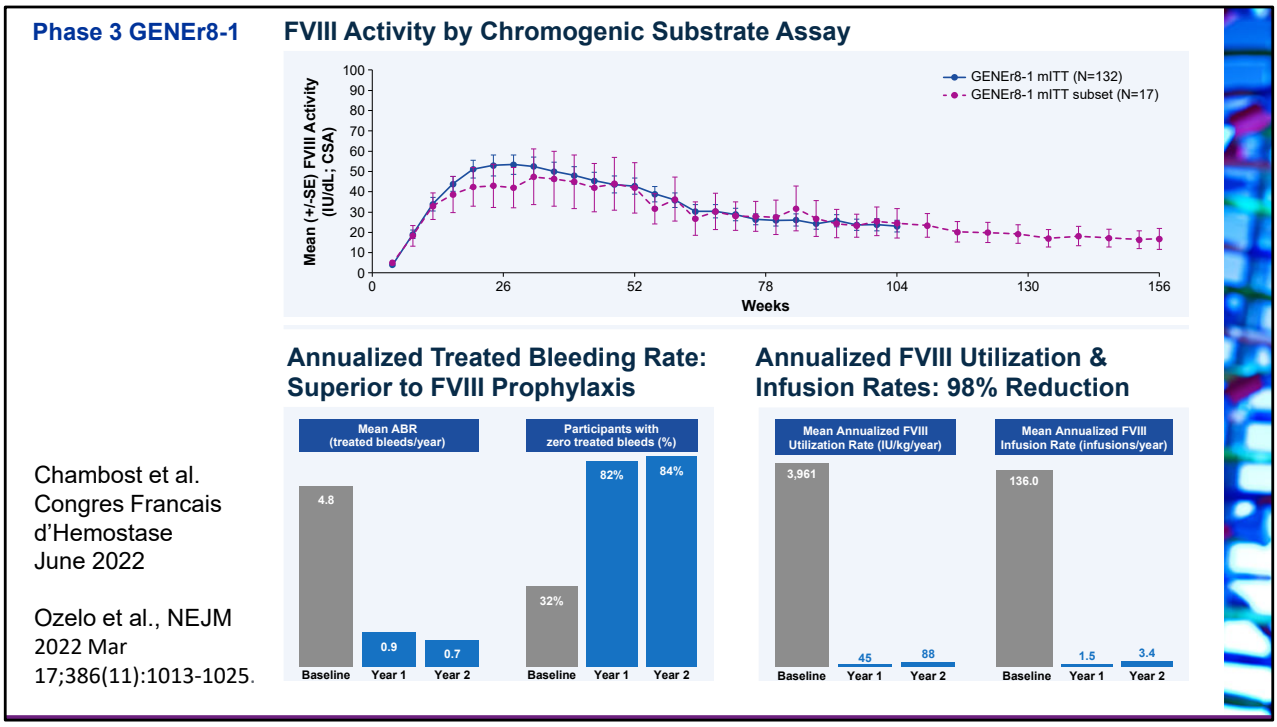


So, let's look at the clinical trial data that have supported these late phase trial results. We're going to first talk about the phase 3 GENEr8-1 study from BioMarin. The eligible participants were adult men with severe hemophilia. They had previously been on factor VIII prophylaxis, so they were all well experienced in receiving factor VIII.

None of them had even a history of factor VIII inhibitors, and they also had to have no evidence of pre-existing antibodies directed against the vector, which is an AAV5 capsid. Now, why did we have to worry about this? Old studies had demonstrated that because of exposure to wild-type AAV in the environment, our bodies can mount antibodies, and these can cross-react against these AAV vectors. So even though we wouldn't naturally have encountered an AAV5 likely, the antibodies we have generated in other contexts could neutralize the vector and block its ability to transduce the liver. For this study, all those patients were excluded. They also had to have good liver health, and that makes sense because we're delivering the factor VIII to the liver. They had to have no evidence of liver dysfunction. They couldn't have any evidence of liver fibrosis or cirrhosis. That's an important point because this age of population, a good proportion of them are still dealing with the legacy issues related to hepatitis C.

The clinical trial designed for this was both direct enrollment as well as a rollover from a trial where patients were on their standard of care prophylaxis with factor VIII. And that was important because we were collecting their factor utilization and their bleed data before they received the gene therapy vector. And then during the evaluation phase we could see what happens when they stop prophylaxis, not only what their factor VIII expression level was, but what would impact on their factor VIII utilization and on their annualized bleed rate.

Latest Advances in Gene Therapy for Hemophilia: An Integrated Care Model to Improve Outcomes



Chambost et al. Congres Francais d'Hemostase June 2022

Ozelo et al., NEJM 2022 Mar 17;386(11):1013-1025.

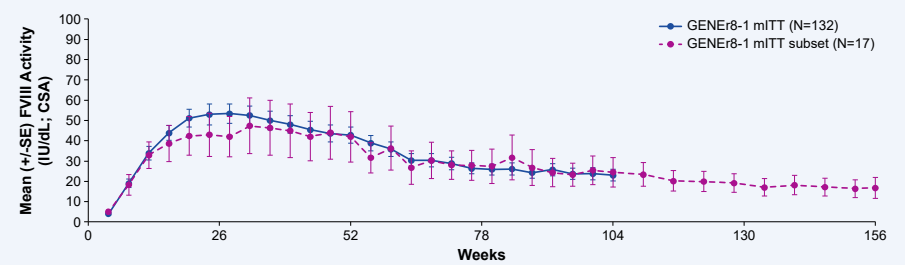
This is the top line data. And the reason you see two graphs of this is the factor levels across the top is because there were 17 patients who were directly enrolled in the study. They have about a one year advance. And so, they have about three years of follow up. And then the rollover patients, there's 132 of them. You can see that their factor VIII expressions exactly overlap each other. I think we can extrapolate for the whole group that this is the destiny of their factor expressions. So, things looked really good at sort of the six month point with mean levels here that were well within the normal range, but there's been a consistent year on year decline in the factor levels.

Majority of patients are still expressing at levels that have allowed them to remain off prophylaxis, but the durability of that expression has been of concern. However, when you look at the impact clinically for these patients, it's been profound. Remember, in the rollover population, we collected their annualized bleed rate while they were on standard of care prophylaxis, and you can see that their annualized bleed rates here at baseline was about 4.8 bleeds per year.

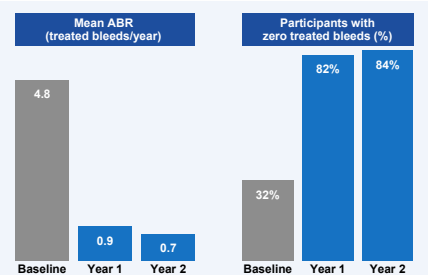
Latest Advances in Gene Therapy for Hemophilia: An Integrated Care Model to Improve Outcomes

Phase 3 GENEr8-1

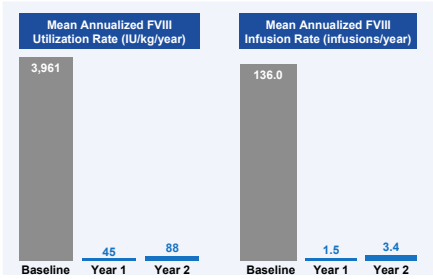
FVIII Activity by Chromogenic Substrate Assay



Annualized Treated Bleeding Rate: Superior to FVIII Prophylaxis



Annualized FVIII Utilization & Infusion Rates: 98% Reduction



Chambost et al. Congres Francais d'Hemostase June 2022

Ozelo et al., NEJM 2022 Mar 17;386(11):1013-1025.

Those bleeds essentially went down to zero despite coming off prophylaxis and relying on the endogenous factor VIII production. Also, it was a minority of participants on standard care factor prophylaxis who experienced zero bleeds in a given one year window. However, now we see the majority of individuals post gene therapy 82-84% who are experiencing zero bleeding events.

And when you're no longer doing prophylaxis, your mean annualized factor VIII utilization also goes down dramatically. And you can see the impressive reductions in factor VIII usage as well as the mean number of infusions. Imagine going from having 136 infusions, IV infusions a year to essentially, just a handful.

Ongoing Hemophilia A Gene Therapy Trials

Sponsor (program)	Vector (cell line)	Transgene	Dose* (vg/kg)	Status	Required steroids	FVIII: C activity (FU years)
BioMarin (BMN 270, GENEr8-1)[1]	rAAV5 (insect)	coBDD-FVIII	6E12 2 or 4E13 6E13	Phase 1/2 concluded (N = 15) Phase 3 ongoing (N = 134)	7/7 (6E13)	60 IU/dL (year 1) 16.4 IU/Dl (year 4)
Spark (SPK 8011)[2]	rAAV-Spark200 (mammalian)	coBDD-FVIII	5E11 or 1 or 2E12	Phase 1/2 concluded (N = 14) Phase 2 recruiting	7/9 (2E12)	>10% (2-3 years) (N = 9: 2E12)
Sangamo-Pfizer (SB-525)[3]	AAV6 (insect)	coBDD-FVIII	9E11 or 2E12 1 or 3E13	Phase 1/2 concluded (N = 11) Phase 3 recruiting	4/5 (3E13)	64.2% (~1 year) (N = 1: 3E13)
UCL-St. Jude (GO-8)[4]	AAV8 (mammalian)	coFVIII-V3	6E11 2-6E12	Phase 1/2 ongoing (N = 7)	2/3 (2E12)	7-69% <1 year (N = 3: 2E12)
Bayer (BAY2599023)[5]	rAAVhu37 (mammalian)	coBDD-FVIII	1 or 2E13	Phase 1/2 ongoing (N = 6)	3/6	8-40% (<1 year) (28 weeks 1E13)
Spark (SPK 8016)[6]	rAAV-Spark200	coBDD-FVIII	5E11	Phase 1/2 concluded (N = 4)	3/4	6-22% at 1 year

[1] Pasi KJ, et al. *N Engl J Med*. 2020;382:29-40. [2] George L, et al. ISTH Virtual Congress. 2020. [3] Pfizer R&D day September 15, 2020.
[4] Nathwani AC, et al. *Blood*. 2018;132:489. [5] Pipe SW, et al. *Res Pract Thromb Haemost*. 2020;4(Suppl 1). [6]Sullivan SK, et al. Presented at EAHAD Virtual Congress; February 2-5, 2021.

So that is the trial that has gone the furthest and is currently under a regulatory review. But there's a number of other ongoing trials in Hemophilia A. Some of the differentiators that you can pay attention to are different vector capsids are being used. Why would you be exploring other capsids? Well, they could have different advantages, a little bit more potency, better able to transduce the liver. Some of them might open up broader eligibility because perhaps using a different capsid, a patient who's ineligible for one vector capsid might be eligible for another. They all use a truncated form of factor VIII, the so-called B domain deleted factor VIII.

Now we've been using B domain deleted factor VIII for years and years clinically in the recombinant proteins. But the reason this is needed is the AAV capacity is just too big to stuff in the factor VIII, even the B domain deleted just barely fits into these AAV capsids. The factor IX transgene is considerably smaller, and so it easily fits inside the capsid.

Some of the newer sort of second generation investigations that are ongoing are using modified versions of the factor VIII transgene to try to enhance its secretion efficiency.

Ongoing Hemophilia A Gene Therapy Trials

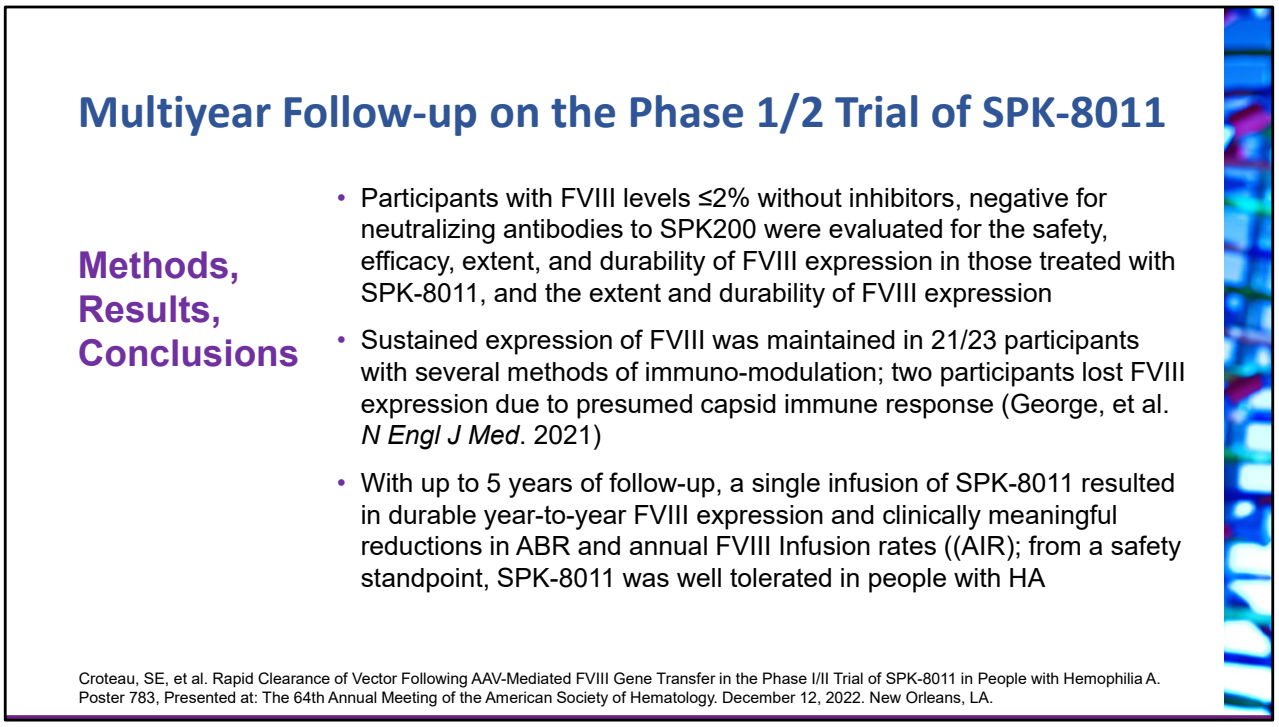
Sponsor (program)	Vector (cell line)	Transgene	Dose* (vg/kg)	Status	Required steroids	FVIII: C activity (FU years)
BioMarin (BMN 270, GENEr8-1)[1]	rAAV5 (insect)	coBDD-FVIII	6E12 2 or 4E13 6E13	Phase 1/2 concluded (N = 15) Phase 3 ongoing (N = 134)	7/7 (6E13)	60 IU/dL (year 1) 16.4 IU/Dl (year 4)
Spark (SPK 8011)[2]	rAAV-Spark200 (mammalian)	coBDD-FVIII	5E11 or 1 or 2E12	Phase 1/2 concluded (N = 14) Phase 2 recruiting	7/9 (2E12)	>10% (2-3 years) (N = 9: 2E12)
Sangamo-Pfizer (SB-525)[3]	AAV6 (insect)	coBDD-FVIII	9E11 or 2E12 1 or 3E13	Phase 1/2 concluded (N = 11) Phase 3 recruiting	4/5 (3E13)	64.2% (~1 year) (N = 1: 3E13)
UCL-St. Jude (GO-8)[4]	AAV8 (mammalian)	coFVIII-V3	6E11 2-6E12	Phase 1/2 ongoing (N = 7)	2/3 (2E12)	7-69% <1 year (N = 3: 2E12)
Bayer (BAY2599023)[5]	rAAVhu37 (mammalian)	coBDD-FVIII	1 or 2E13	Phase 1/2 ongoing (N = 6)	3/6	8-40% (<1 year) (28 weeks 1E13)
Spark (SPK 8016)[6]	rAAV-Spark200	coBDD-FVIII	5E11	Phase 1/2 concluded (N = 4)	3/4	6-22% at 1 year

[1] Pasi KJ, et al. *N Engl J Med*. 2020;382:29-40. [2] George L, et al. ISTH Virtual Congress. 2020. [3] Pfizer R&D day September 15, 2020.
[4] Nathwani AC, et al. *Blood*. 2018;132:489. [5] Pipe SW, et al. *Res Pract Thromb Haemost*. 2020;4(Suppl 1). [6]Sullivan SK, et al. Presented at EAHAD Virtual Congress; February 2-5, 2021.

You will see quite a broad range of differences between the doses that are being used from 10 11 vector particles per kilogram well up into 1013. So, with a 100 to 200 fold variation, you would say, well, you know, what do we take from that? It's hard to know.

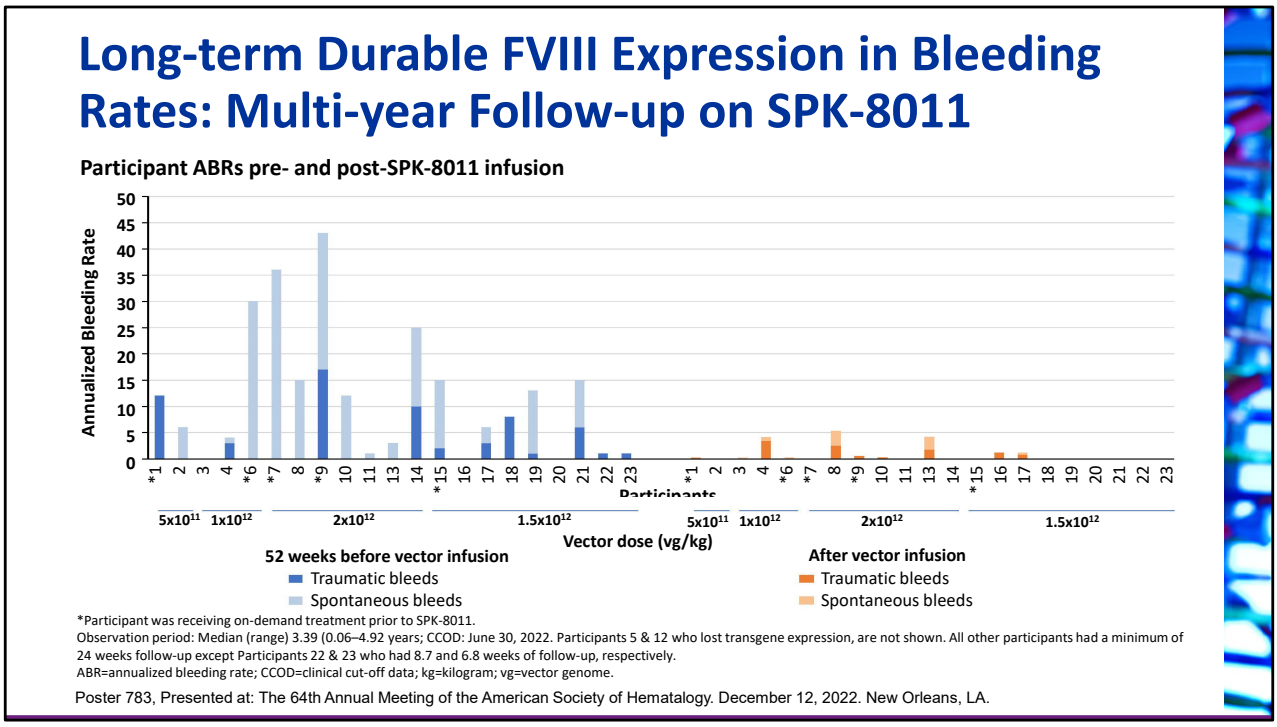
You would think that some side effects would be lessened by using a lower dose, but on the other hand, the reason they're using these lower doses is because the potency of transducing the liver is so much higher. So, it's not clear whether lower dose is always correlated with a better safety outcome.

Some of these are either in phase three or have completed and just you know, waiting data readouts. But a number of them are still early phase one, phase two. What's common to all of these is that all of these have had transaminase elevations occur in the majority of participants, which means the majority of the patients have had to go on a period of immunosuppression with steroids. And there's considerable variability in the readout of the factor levels.



We did get a recent update on one of the other trials. This is the SPK-8011 from Spark. Here participants had factor VIII levels up to 2%, again without inhibitors, and they also had to have no neutralizing antibodies to this modified AAV vector called SPK200.

And what they have reported from this trial, at least in the phase I/II, is that they saw sustained expression of factor VIII in 21 of 23 participants. However, two participants did lose factor VIII expression, presumably due to that immune response I mentioned. And with up to five years of follow up it looks like a single infusion is able to give durable year on year expression and clinically meaningful reductions in annualized bleed rates as well as annual factor VIII infusion rates with a safety profile pretty similar to the other protocols.



This looks at the individual 23 patients there, and what you can see because they collected data again on prophylaxis and then rolled over into the dosing. You can see the range of annualized bleed rates there in the blue bars, differentiating between spontaneous in light blue and traumatic bleeds in dark blue. And then look what happened after vector infusion, that for majority of the participants, spontaneous bleeds essentially disappeared for most individuals. You just see a smattering of occasional traumatic bleeds. So really impressive bleed control overall.

Alta Study Updated Results: Follow-up at 156 Weeks of Giroctocogene Fitelparvovec

Table. Factor VIII Activity Levels by 1-Stage and Chromogenic Assay for the Giroctocogene Fitelparvovec 3e13-vg/kg Cohort

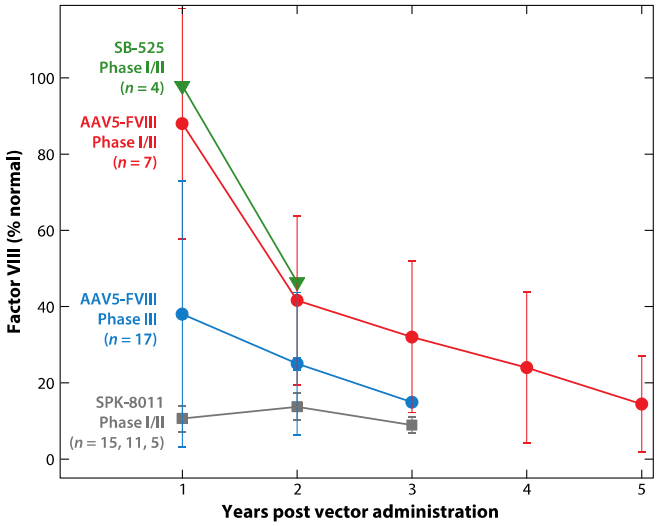
Factor VIII Activity, % Normal, Median (min, max)	Study Week						
Assay	**Week 12**	**Week 24**	**Week 52**	**Week 78**	**Week 104**	**Week 130**	**Week 156**
1-stage clotting	93.7	104.8	31.1	57.5	27.5	23.3	22.9
	(82.7, 167.7)	(30.5, 212.6)	(12.0, 191.3)	(3.8, 144.2)	(4.1, 99.1)	(5.4, 164.5)	(22.6, 129.0)
Chromogenic	62.1	70.1	20.1	40.1	16.3	12.3	12.5
	(51.8, 109.5)	(20.4, 123.8)	(7.8, 122.3)	(0.9, 114.7)	(0.9, 71.6)	(0.9, 113.2)	(11.8, 91.1)
Patients, n	5	5	4[a]	4[a]	5	4[a]	3[b]

[a] There was 1 patient each who was unable to attend visits at Weeks 52, 78, and 130.
[b] Two patients had not yet reached Week 156 at the time of the data cut.
min, max=minimum, maximum

Poster 3461, presented at the 64th Annual Meeting of the American Hematology Society.

This is another study, this used a different vector capsid and AAV6 capsid. This was conducted by Pfizer and Sangamo. What is being shown here is the factor activity levels over time. You're seeing two different ways of measuring the factor levels, the traditional clotting factor, one stage activity, and then what's called the chromogenic assay. All the trials show this difference between these two assays. It just has to do with the mechanism of the readout of the assay and how affected by these transgenes. So in general, the one stage activity is about 1.5 to 1.7 times higher than the chromogenic. But what you can see is early on in the trial, we're seeing essentially normalization of the factor VIII levels. But over time, again, we're seeing that year on year decline that still is not fully understood, but it brings into question the durability of this strategy.

Multiyear FVIII Activity After AAV Gene Therapy



Samelson-Jones BJ, and George LA. *Annu Rev Med.* 2023;74:231-247.

This is looking at a composite from a recent review of all of the trials that have reported to date. The AAV5 is the BioMarin studies. In red is the phase one, two, and then in blue is the phase three study. And then layered on here is the Sangamo and Pfizer in green. Again, showing this year-on-year decline that has been pretty steady. The SPK-8011 is expressing at a significantly lower level compared to the other trials, but not showing perhaps the same degree of decline over time. Are these studies all going to sort of converge down to the same level and then maybe maintain a low level of expression long term? We'll have to wait and see.

Ongoing Hemophilia B Gene Therapy Trials

Sponsor (program)	Vector (cell line)	Transgene	Dose* ($x10^{11}$vg/kg)	Status	Required steroids	FIX:C activity (FU years)
CSL/uniQure[1]	AAV5	ssFIX-R338L	200	Phase III, Drug approved 11/22/22	9/54	41.5% (1 year) 52/54 discontinued prophy
Pfizer/Spark (SPK 9011)[2]	SPK100	ssFIX-R338L	5	Phase III	28/45	25% (2 year)
Freeline[3]	AAVS3	scFIX-R338L	7.5–9.5	Phase I/II	4/10	2-60% (~1 year)

[1]George LA, et al. *N Engl J Med*. 2017;377(23):2215-2227.; [2]https://www.pfizer.com/news/press-release/press-release-detail/pfizer-announces-positive-top-line-results-phase-3-study. [3]Chowdary P, et al. *Res Pract Thromb Haemost*. 2020;4(suppl 2):17.

Okay. Now let's shift gears to hemophilia B. So, the one that has been approved is etranacogene desaparvovec, known as HEMGENIX. This was just approved at the end of November, that's from CSL and uniQure. This uses an AAV5 capsid similar to the BioMarin ROCTAVIAN product. However, what I want you to take from here is that although different vector capsids are being explored in some of the other trials, what they all have in common is they're all using a modified version of factor IX called the Factor IX Padua. This is a single mutation that was derived from a family in Padua, Italy who had sky high factor IX levels, and it was determined that they all had this point mutation. This has been incorporated into all of the ongoing hem b trials because it boosts the activity of factor IX by about six-to-eight fold. So now you're able to shift the efficacy up substantially and improve outcomes for patients.

In general, we can say that the transaminase elevations have been less frequent in the heme b trials, that means less people being on corticosteroids and also spending less time overall on corticosteroids. We do still see considerable interpatient variability, but overall, we're achieving levels that are meaningful for patients, which I'll show you.

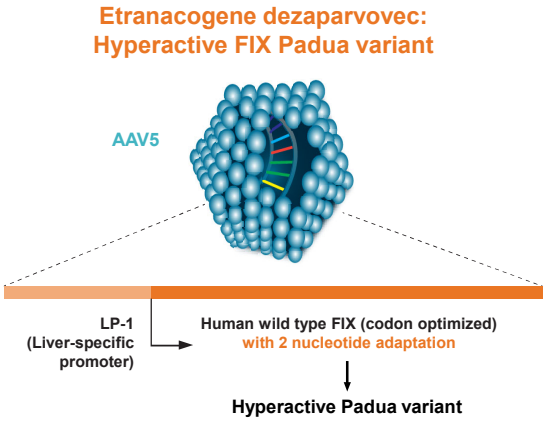
Etranacogene Dezaparvovec

Etranacogene dezaparvovec (AAV5-Padua hFIX):

- Developed by combining the AAV5 vector from AMT-060 (AAV5-WT hFIX) with the naturally occurring Padua FIX variant
- Enhanced version of AMT-060, which demonstrated efficacy and safety in a Phase 1/2 trial (N=10)[1]
  - Stable expression of WT FIX at 4.5-5 years[2]
  - No late-emergent safety signals[2]

A Phase 2b study long-term follow-up is ongoing[3,4]

- Mean FIX activity at 2 years was 44.2% with no new treatment-related AEs



AE, adverse event; WT, wild type; AAV, adeno-associated virus; FIX, Factor IX; wt, wildtype
1. Miesbach W, et al. *Blood*. 2018;131:1022-1031. 2. Miesbach W, et al, ASH 2020; Poster #33724. 3. Von Drygalski A, et al. *Blood Adv.* 2019;3:3241-3247.
4. Von Drygalski A, et al, ASH 2020; Oral presentation #672.

So, just a little bit background on etranacogene desaparvovec. This was developed by taking an AAV5 vector from a previously explored vector called AMT-060. That was only different in that it was a wild-type factor IX. And there was longer term data from that prior vector in 10 individuals with severe hemophilia B who've now had stable expression of wild-type factor IX for more than five years with no late emergent safety signals.

Now what's important there is that their level of expression is two different dose cohorts and in the highest dose cohort they were just barely getting into the mild range, but with substitution of the Padua it was anticipated that you would get all the safety and expression benefits of AMT-060, but now with the enhancement of the highly active factor IX-Padua. We did a phase 2b study in just three individuals, in order to test this revised vector which was called AMT-061. These three gentlemen have had mean factor IX expression well beyond now three years actually, and they are maintaining near normal, to normal expression of factor IX. So that's that multiplication effect from the Padua variant.

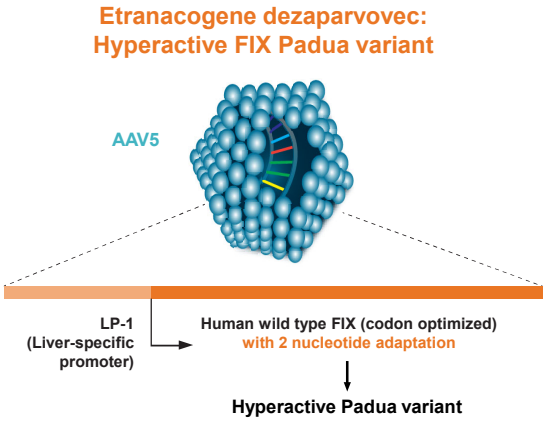
Etranacogene Dezaparvovec

Etranacogene dezaparvovec (AAV5-Padua hFIX):

- Developed by combining the AAV5 vector from AMT-060 (AAV5-WT hFIX) with the naturally occurring Padua FIX variant
- Enhanced version of AMT-060, which demonstrated efficacy and safety in a Phase 1/2 trial (N=10)[1]
  - Stable expression of WT FIX at 4.5-5 years[2]
  - No late-emergent safety signals[2]

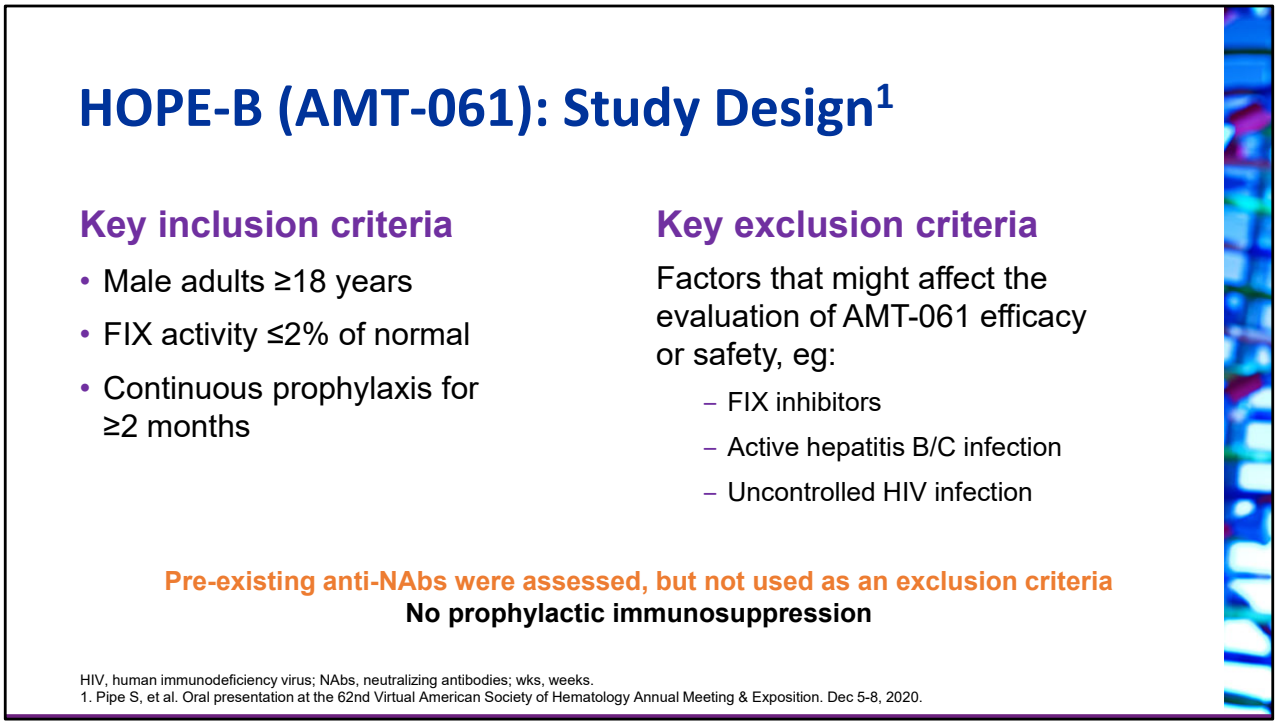
A Phase 2b study long-term follow-up is ongoing[3,4]

- Mean FIX activity at 2 years was 44.2% with no new treatment-related AEs



AE, adverse event; WT, wild type; AAV, adeno-associated virus; FIX, Factor IX; wt, wildtype
1. Miesbach W, et al. *Blood*. 2018;131:1022-1031. 2. Miesbach W, et al, ASH 2020; Poster #33724. 3. Von Drygalski A, et al. *Blood Adv.* 2019;3:3241-3247.
4. Von Drygalski A, et al, ASH 2020; Oral presentation #672.

Now, what's also important before we go into the data on the phase 3 trial is patients have been dosed in these trials who had neutralizing antibodies to the AAV5. They developed a more sensitive antibody assay to determine the titers of the preexisting immunity. And when they back tested against patients who got the vector with AMT 060, they found that there really was no difference in the expression pattern in those patients. For the phase 2b study we did not screen out anybody if they had neutralizing antibodies. We took all comers, and it just happened that the three gentlemen who got dosed, all had significant titers against AAV5. So the decision was made to go into the phase 3 trial that we would screen all the patients for AAV5 preexisting immunity, but we would dose all of them anyways.



These were the inclusion criteria. Again, it was adults who were at least 18 years of age. They had to have factor IX activity in the severe or moderately severe range. They all had to have previously been on continuous prophylaxis for at least a few months before they entered into the lead-in phase. And then just as the same design as the other trials, we followed them for at least six months on standard of care prophylaxis with their factor IX products. Then they received dosing with the AMT-061.

We did exclude patients who had history of inhibitors, anybody who had active hepatitis, and had uncontrolled HIV. Just to emphasize again, pre-existing anti-neutralizing antibodies were assessed, but they were not used as an exclusion criteria, and no prophylactic immunosuppression was given. If patients had transaminase elevation, we responded with a course of immunosuppression with corticosteroids.

The HOPE-B Phase 3 Clinical Trial: 24-month Follow-up

Methods

- Adult male participants with severe or moderately severe hemophilia B (FIX ≤2%), with or without pre-existing AAV5 neutralizing antibodies (NAbs), were infused with a single dose of etranacogene dezaparvovec ($2x10^{13}$ gc/kg), following a ≥6-month lead-in period receiving FIX prophylaxis
- FIX activity, annualized bleed rate (ABR), and FIX infusions were assessed frequently

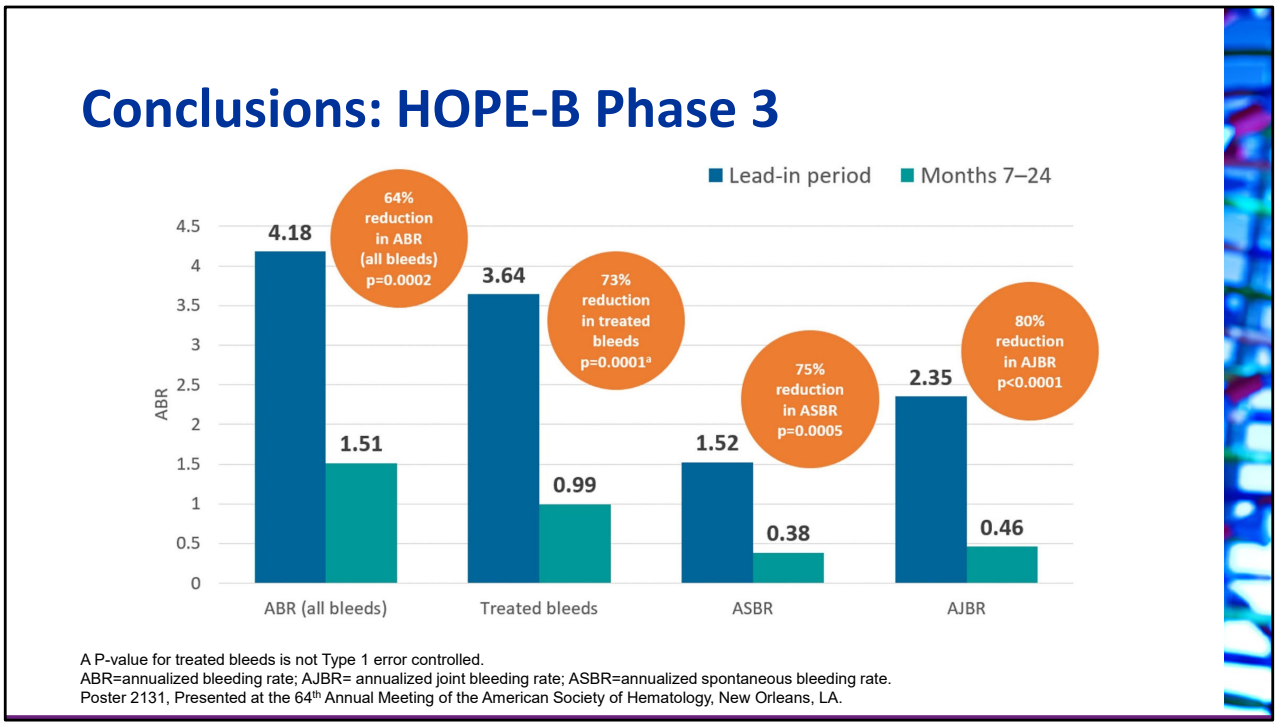
Results

- Of the 54 participants, 52 (96.3%) discontinued and remained free of continuous FIX prophylaxis from Day 21 to Month 24, including 20 participants with baseline AAV5 NAb titers up to 1:700
- Mean ABR for all bleeds during Months 7–24 post-treatment was significantly reduced by 64%
  - (mean ABR 1.51; *P*=0.0002)
- There was an overall 96% reduction in mean unadjusted annualized FIX consumption from the lead-in period

Poster 2141, Presented at: 64th Annual Meeting of the American Society of Hematology; December 10, 2022, New Orleans, LA.

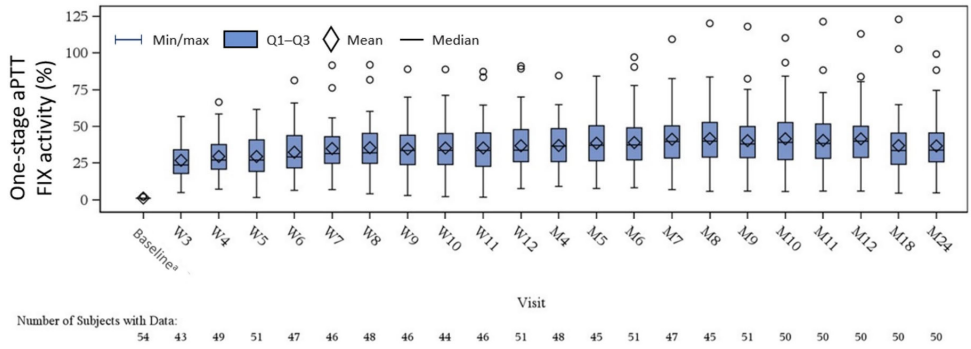
So the dosing for this is 2x1013 gc/kg. And we collected factor IX activity, annualized bleed rate, and then the number of factor IX utilization. The top line results is of 54 participants, 52 have discontinued and remained free of continuous factor IX prophylaxis from as early as day 21 all the way through now two years of follow up. This included 20 participants who at baseline had AAV5 neutralizing antibody titers up to as high as 1:700.

The mean annualized bleed rate for all bleeds collected from month 7 to 24 was significantly reduced by 64% compared to their standard of care prophylaxis. This endogenous expression of factor IX at these levels is clearly superior to what we're achieving with factor IX prophylaxis. And overall, because they came off prophylaxis, there was a 96% reduction in their annualized factor IX consumption compared to the lead-in period.



This looks at the impact comparing the lead-in to that follow-up phase of month 7 to 24. The reason that was chosen is most patients achieved their steady state expression by about six months, and it also helped to account for any patients who might have been still getting treated with corticosteroids, related to a transaminase elevation. So that's why the window was collected from month 7 to 24. But here you can see the significant reduction in all bleed readouts, all bleeds, treated bleeds, spontaneous bleeds, as well as joint bleeds.

FIX Activity Level Over 24 Months in the HOPE B Phase 3 Trial



[a] Baseline FIX was imputed based on participant's historical hemophilia B severity documented on the CRF. If the participant had documented severe FIX deficiency (FIX<1%), their baseline FIX activity level was input as 1%. If the participant had documented moderately severe FIX deficiency (FIX≥1% and ≤2%), their baseline FIX activity level was input as 2%. The standard error was not provided at baseline.

aPTT, activated partial thromboplastin time; CRF, case report form; FIX, factor IX; M, Month; W, Week.

Poster 2141, Presented at: 64th Annual Meeting of the American Society of Hematology; December 10, 2022, New Orleans, LA.

Here's the level of expression. This is all readouts with the one stage factor IX clotting. And what you can see is in contrast to the factor VIII trials is we see relatively, steady expression that is continuing through two years. And if we extrapolate what we've seen with this vector in the previous form that I mentioned to you earlier, you know, those gentlemen are still having stable expression well beyond five years.

And there are trials with hem B using AAV delivery that participants have been followed for more than 12 years, also showing consistent stable expression. What we're expecting from the longer-term outcomes in hem B is that we should be able to maintain steady expression at these levels. And so, these are all just sort of in the near normal range. If you haven't seen these, what are called box and whisker plots before, the box is the 25th to 75th percentiles of expression. The whiskers are showing the mins and the max. And then the diamond in the middle is the mean, and then the dash across the middle is the median.

Optimistic Results About Neutralizing Antibodies to AAV: Secondary Results from HOPE-B

Summary of FIX activity[a] (%) by baseline NAb status (FAS)

AAV5 NAb-	n	Baseline 33	6 months 33	12 months 32	18 months 33	24 months 33
	Median (min-max)	1.0 (1.0–2.0)	37.30 (8.4–97.1)	38.65 (5.9–113.0)	35.0 (4.5–122.9)	35.40 (4.7–99.2)
	Mean (SD)	1.15 (0.36)	40.61 (18.64)	44.82 (23.21)	39.87 (24.08)	38.55 (19.19)
AAV5 NAb+	**n**	**21**	**18**	**18**	**17**	**17**
	Median (min-max)	1.0 (1.0–2.0)	35.60 (8.2–90.4)	39.95 (8.5–73.6)	32.00 (10.3–57.9)	33.50 (9.1–88.3)
	Mean (SD)	1.24 (0.44)	35.91 (19.02)	35.54 (17.84)	31.14 (13.75)	32.98 (18.51)

[a]One-stage FIX activity assays. Only samples uncontaminated with exogenous FIX were included in analysis. LS mean from repeated measures linear mixed model with visit as a categorical covariate.
AAV5=adeno-associated virus serotype 5, FAS=Full Analysis Set, FIX=factor IX, LS=least squares, max=maximum, min=minimum, Nab=neutralizing antibody, SD=standard deviation.

Poster 2139, presented at the 64th Annual Meeting of the American Society of Hematology, New Orleans, LA.

What's important is, well, did the neutralizing antibodies have any impact on the overall factor IX activity and what this graph is showing neutralizing antibody negative on the top, neutralizing antibody, positive across the bottom. There's nominal numerical difference in the expression levels, but nothing that's clinically significant.
We don't see any real significant adverse impact, at least up to a titer of 1:700. Now, the reason I say that, there was one individual who had a sky-high neutralizing antibody titer of over 3,200, and he did not have a clinical response. So, it does appear that there might be some threshold in which a neutralizing antibody is just too much
to overcome.

Optimistic Results About Neutralizing Antibodies to AAV: Secondary Results from HOPE-B (continued)

HOPE-B primary endpoint (ABR) by baseline Nab status

Endpoint	≥6-month lead-in period	Months 7–18 post-treatment period			Months 7–24 post-treatment period		
	Adjusted ABR (95% CI)[a]	Adjusted ABR (95% CI)[a]	Rate ratio (post-treatment/lead-in) (95% CI)[a,b]	p-value[c]	Adjusted ABR (95% CI)[a]	Rate ratio (post-treatment/lead-in) (95% CI)[a,b]	p-value[c]
All bleeding episodes (FAS, n=54)	4.18 (3.21–5.44)	1.51 (0.81–2.82)	0.36 (0.20–0.64)	0.0002	1.51 (0.83–2.76)	0.36 (0.21–0.63)	0.0002
All bleeding episodes (FAS, baseline AAV5 Nab<1:700) (n=53)	3.86 (2.89–5.17)	1.07 (0.63–1.81)	0.27 (0.17–0.43)	<0.0001*	1.09 (0.67–1.79)	0.28 (0.17–0.46)	<0.0001*
All bleeding episodes (baseline AAV5 Nab-) (n=33)	3.80 (2.56–5.65)	0.93 (0.44–1.98)	0.25 (0.14–0.43)	<0.0001**	0.80 (0.39–1.67)	0.21 (0.12–0.37)	<0.0001**
All bleeding episodes (baseline AAV5 Nab+ <1:700) (FAS, n=20)	4.29 (3.06–6.01)	1.30 (0.63–2.70)	0.30 (0.15–0.61)	0.0005*	1.65 (0.84–3.26)	0.39 (0.18–0.82)	0.0065*

[a]Adjusted ABR and comparison of ABR between the lead-in and post-treatment periods was estimated from a repeated measures generalized estimating equations negative binomial regression model accounting for the paired design of the study with an offset parameter to account for the differential collection periods. Treatment period was included as a categorical covariate.
[b]One-sided p-value ≤0.025 for post-treatment/lead-in <1 was regarded as statistically significant.
[c]The upper limit of the CI of the rate ratio was compared to the non-inferiority margin of 1.8. If the upper limit was <1.8, then non-inferiority was declared.
*Post-hoc analysis not controlled for Type 1 error. **Subgroup analysis not controlled for Type 1 error.
AAV5=adeno-associated virus serotype 5, FAS=Full Analysis Set, FIX=factor IX, LS=least squares, max=maximum, min=minimum, Nab=neutralizing antibody, SD=standard deviation.

Poster 2139, presented at the 64th Annual Meeting of the American Society of Hematology, New Orleans, LA.

This is also looking at, so no impact on the, significantly on the factor IX expression, but also no meaningful impact on any of the clinical readouts. Whether you look at any of the bleed rates, there doesn't appear to be any significant impact of neutralizing antibody positivity. If you look at the approval for HEMGENIX, you will see that there's really nothing identified about excluding patients who have neutralizing antibodies. However, given that there, this outcomes were for patients who have this titer up to 1:700, it is advised that you obtain a neutralizing antibody titer before you dose a patient. And it's being encouraged that we continue to collect data on patients
going forward.

BAX 335 Hemophilia B Gene Therapy Phase 1/2 Clinical Trial: Long-term Safety and Efficacy Follow-up

- The longest ongoing clinical gene therapy trial using FIX R338L transgene
- Of eight participants, only one demonstrated durable transgene expression – requiring FIX replacement to resume
- Root-cause analysis identified CpG (Cytosine-phosphate-Guanine) content in the vector may have triggered immune response with loss of gene expression in 7/8 recipients
- **The one participant with durable FIX had a missense variant in the IL6R gene**

Escobar M, et al. Poster will be presented at: The 64th Annual Meeting of the American Society of Hematology. December 12, 2022. New Orleans, LA.

This is just to highlight to you, an older trial in hemophilia B. This was the first trial to use the Padua transgene. It was done by Baxalta at the time. There were only eight participants and only one subject demonstrated durable transgene expression. The root cause was that all of the other patients, this vector seemed to trigger a pretty significant immune response and they all lost their gene expression.

But there was one participant who did have durable factor IX expression, and they've analyzed this patient further and it turns out he has a missense variant in his IL6 receptor gene. And it's possible that this missense variant somehow altered the immune response and allowed him to have durable expression. So it just sort of ties in again, this idea about these vectors inducing an immune response and the risk, related to getting that immune response or not responding appropriately.

Clearance of AAV-5 Vector Genome in Hemophilia A Patients

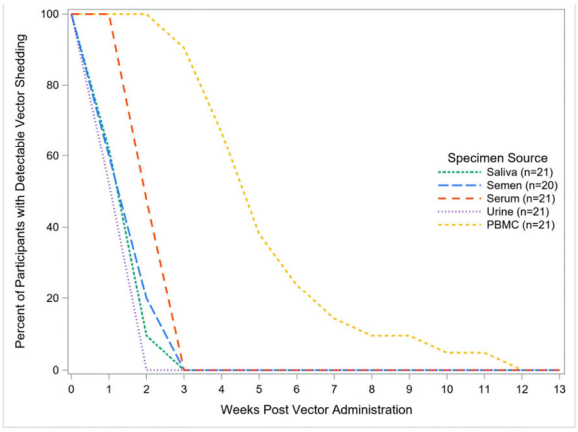


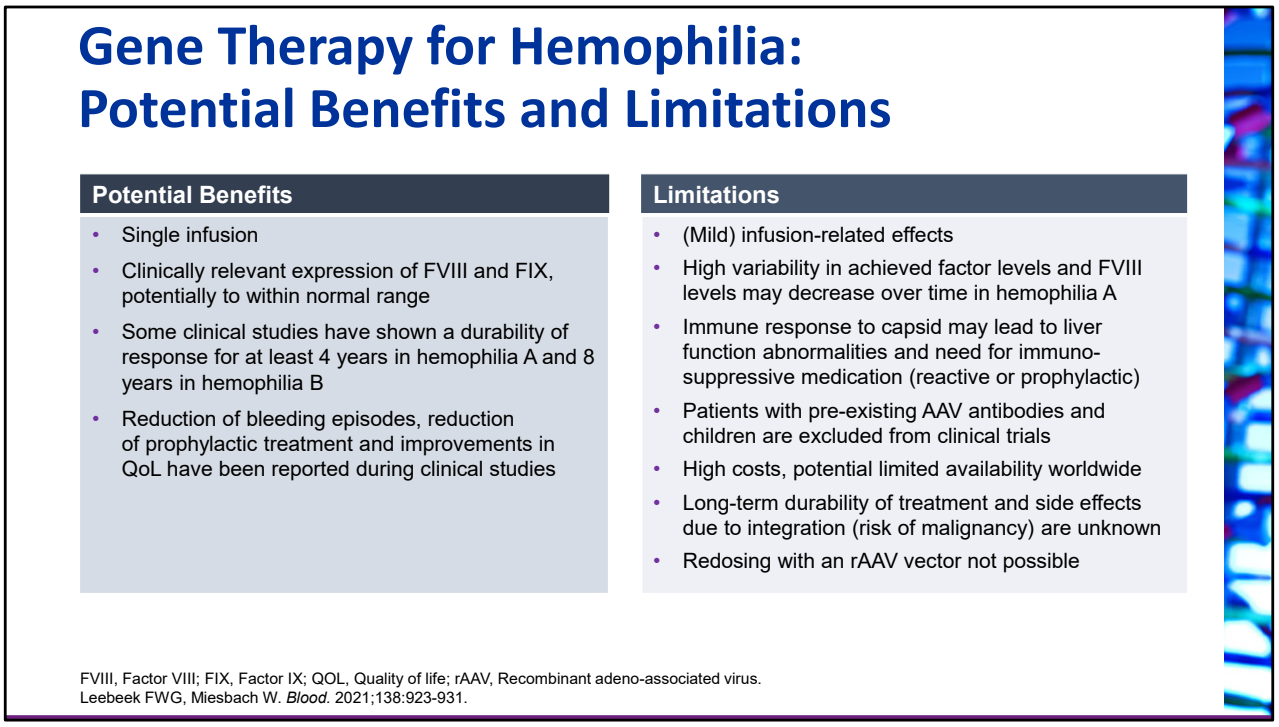
Figure: Percentage of participants with detectable vector shedding.

Table: Time to vector clearance, weeks

	Saliva	Semen	Serum	Urine	PBMCs
n	21	20	21	21	21
Mean (SD)	1.7 (0.64)	1.8 (0.77)	2.5 (0.51)	1.5 (0.51)	5.6 (2.22)
Median (IQR)	2 (1–2)	2 (1–2)	2 (2–3)	2 (1–2)	5 (4–6)
Min, max	1, 3	1, 3	2, 3	1, 2	3, 12

Poster 4783, will be presented at: The 64th Annual Meeting of the American Society of Hematology. December 12, 2022. New Orrleans, LA.

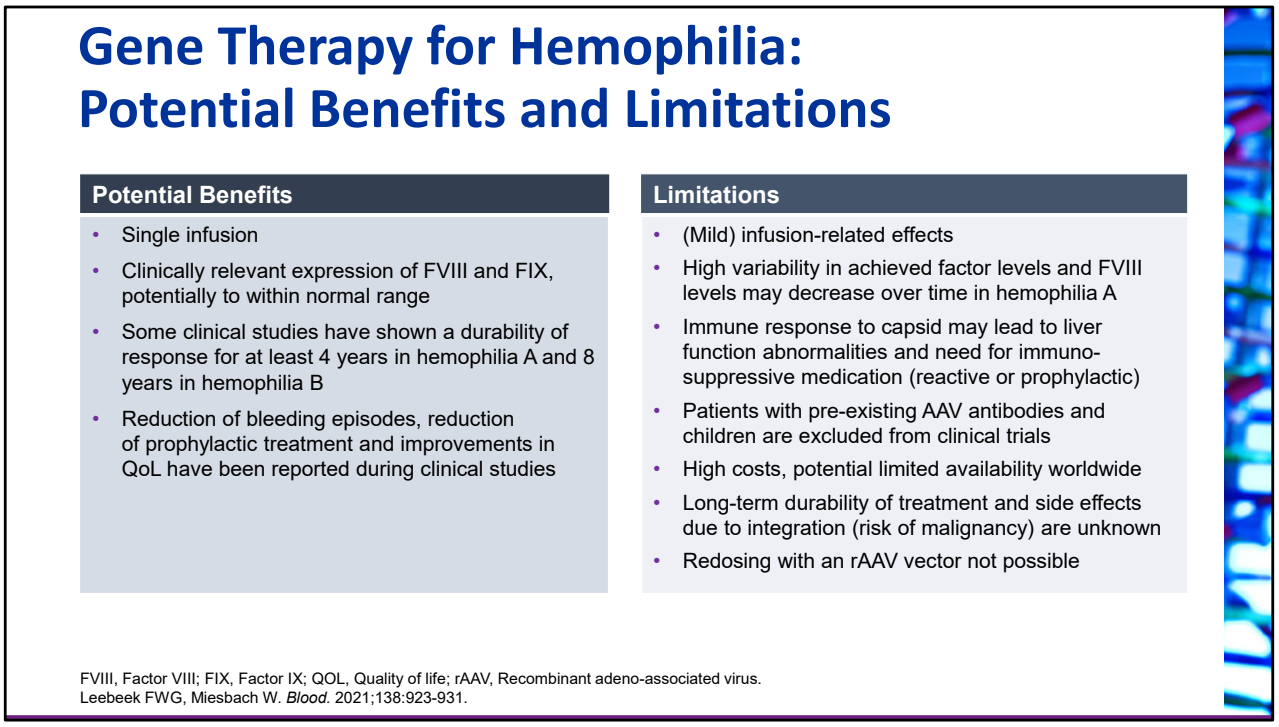
Many clinicians have questions about, well, how long does it take this vector to clear out of the different body fluids? This was recent data presented at ASH just a couple months back, and this is looking at a number of different body tissues here, saliva, serum, semen, urine. You can see that it actually clears very quickly that the vector particle elements are no longer seen in these body fluids. Where it's the most longer lived is actually in long-lived white cells, pBMCs. And you can see that that extends out longer. It gives me confidence when I see this graph, that if you want to promote some barrier contraception just to deal with the presence in the semen, it's probably a reasonable recommendation. But for sure over several months, it's fully cleared at least related to vector particles.



In sum up here, if we look at all the data collectively, the potential benefits of gene therapy is that a single infusion will lead to clinically relevant expression of factor VIII and factor IX, potentially within the normal range.

In some, clinical studies have shown a durability of response for at least four years in hem A, eight years or longer in hem B. We see clear improvements in a reduction of bleeding episodes, reduction of the need for prophylactic treatment, and what I haven't shown you here is some evidence of quality-of-life measures that are clearly improved.

Some of the limitations. There are mild infusion related effects, nothing that we can't handle in the outpatient setting for the majority of patients, but high variability in achieved factor levels, particularly with factor VIII, and then this concern of decreasing over time. I mentioned the immune response to the capsid and the need for a timely immunosuppressive course of therapy, timed with elevations of the transaminase. Patients with pre-existing AAV antibodies are an exclusion criteria for almost every protocol except for the one I showed you with the etranacogene desaparvovec.



Children have been excluded from these trials. It's not just a safety issue, it's because we're delivering a gene addition that is remaining episomal, so outside of the DNA of the patient. And so in a developing liver with rapid cellular division, the transgene would be lost over time if it was if it was given to a child. A different platform of therapy is going to have to be used to treat pediatric patients. Costs are going to be high for this, and this is going to limit its availability worldwide. And we don't know the long-term durability of treatment or if there's any side effects from rare integration events. There have been malignancies observed in the trial, but they've been analyzed extensively with genomic analysis. And to date, none have shown to have been related in any way to the vector integration events. But we have to tell all patients, at least under current technology, there's no possibility of re-dosing at least with another recombinant AAV vector. This has to be a one-time treatment.

Conclusions

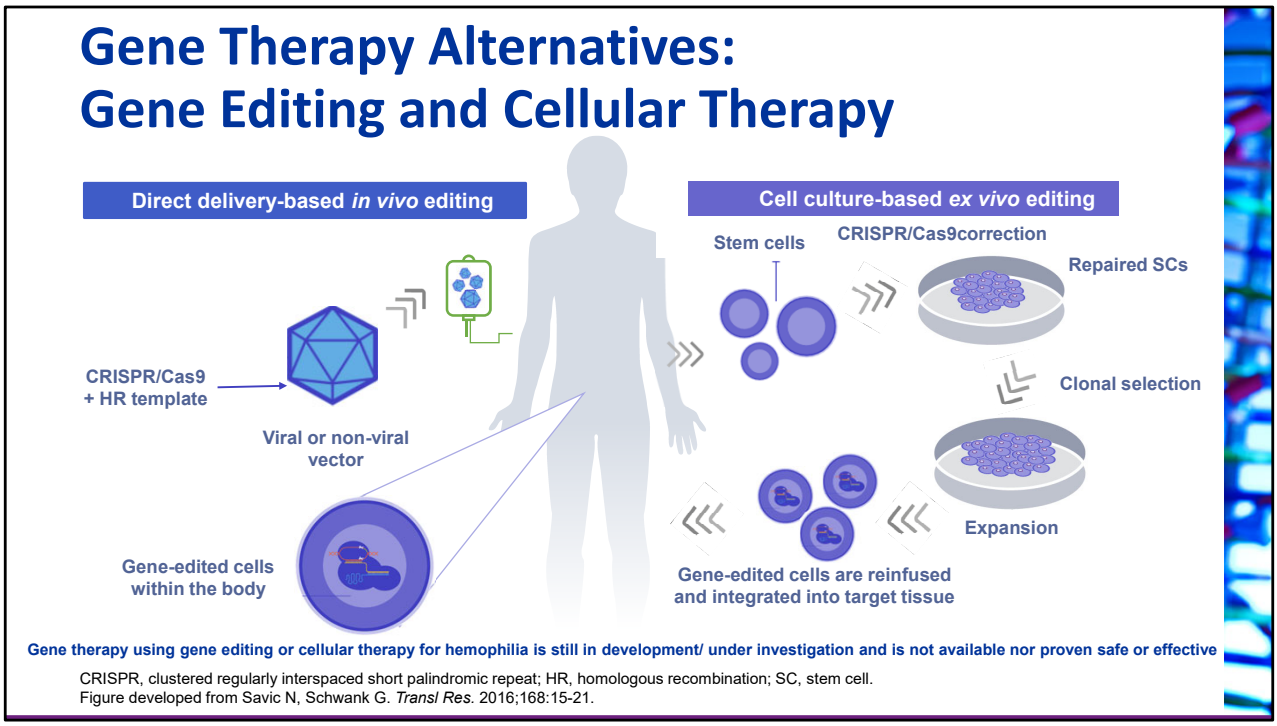
- **Gene therapy will become a powerful approach in the management of hemophilia**
  - Clinical trials of gene therapy have reported long-term therapeutic factor expression with FIX and FVIII in up to near-normal ranges
  - Extraordinary reduction of bleeding events
  - Most prophylaxis free

I think this is going to become a powerful approach in managing hemophilia. We've seen long-term therapeutic benefit from expression of both factor VIII and factor IX. Extraordinary reduction of bleeding events, and most patients have been able to remain prophylaxis free.

Critical Issues

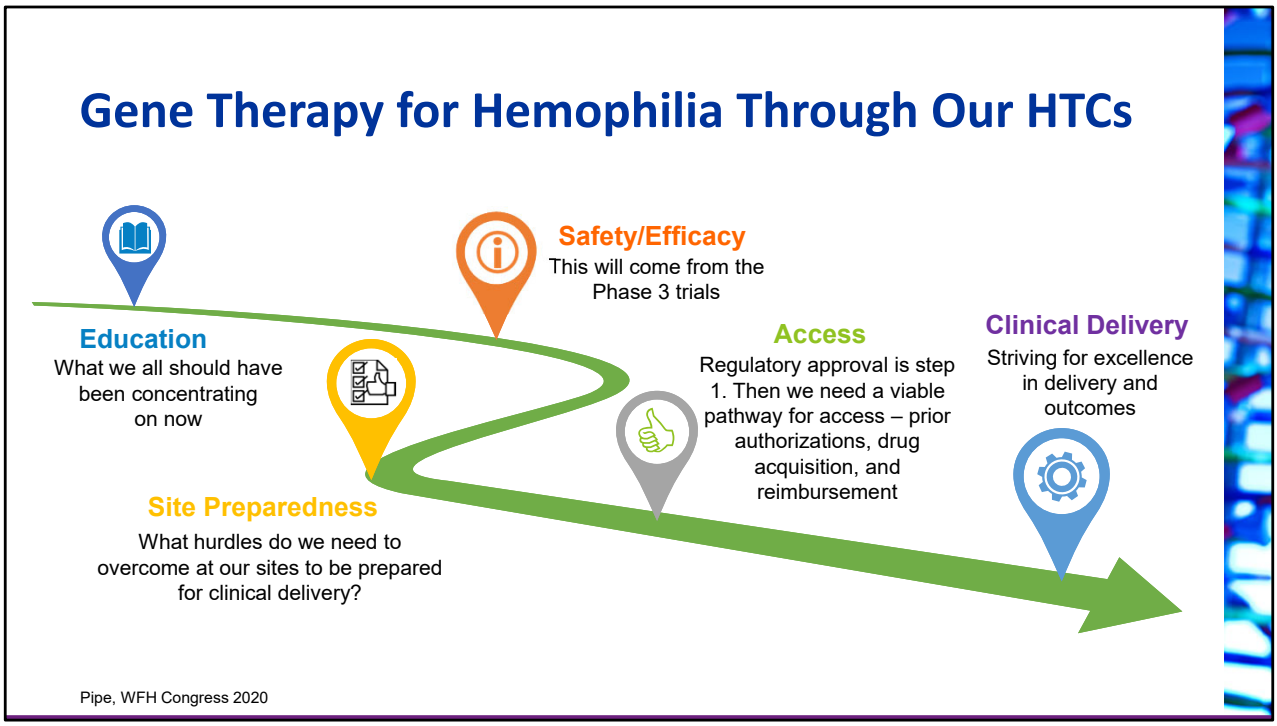
- The increase in liver enzymes
- Requirement for steroid use
- Variability of response and durability of achieved factor levels
- The safety profile of different AAV serotypes
- The effect of the vector manufacturing process
- The potential genotoxicity derived from integration events
- The discrepancy in FVIII and FIX clot-based vs chromogenic activity assays

Some of the critical issues: liver enzyme elevation, the need for steroids, the variability of the response, questions about durability of the factor levels. There's questions about, you know, are there any safety differences between any of these AAV serotypes? There's a lack of standardization as it relates to the vector manufacturing. We have questions about any potential genotoxicity derived from integration events. And then I mentioned to you this discrepancy in factor VIII and factor IX activity readouts, although I really don't think this is going to manifest in any significant way for clinical care for these patients.



There are alternatives that are coming. Gene editing strategies can be done both in vivo as well as in, ex vivo and cell culture. And actually, a couple of protocols are already launching. We're going to be conducting our first gene in vivo gene editing with a CRISPR/Cas9 strategy, in which case the factor IX is going to be integrated directly into a targeted site in the patient's genome.

This, of course, does open up the possibility of potentially treating pediatric patients sometime down the line. Look for some data from these trials coming out in months and years to come.



For the last few minutes, I just want to briefly focus on some of the principles of integrating this into our comprehensive care management.

What we've been doing for the last few years is education along the lines of what we're doing today. We're getting the safety and efficacy results from the phase three trials, and some of them have gone through a regulatory review. However, getting regulatory approval is just kind of step one. Then we need to see what the prior authorizations look like. What's drug acquisition and reimbursement going to look like? And so, what we're trying to work on is preparing the sites to get them ready to actually delivering this therapy through the hemophilia treatment centers with the goal of striving for excellence in that delivery and outcomes as we do with other platforms of therapy.

Four Universal Principles for the Introduction of Gene Therapy to People with Haemophilia[1]

- The person with hemophilia (PWH) should be at the center of decision-making
- All PWH should have an equal opportunity to access gene therapy
- The safe introduction of commercial gene therapy with lifelong follow-up is paramount to ensuring long-term success
- The integrated comprehensive care model currently employed for the treatment of hemophilia improves outcomes and is best placed to support the introduction and long-term follow-up of gene therapy

[1]Miesbach W, et al. *Haemophilia*. 2021;27(4):511-514.

There are some core principles that I think are important. The person with hemophilia should be at the center of the decision making. This is a big deal to do a one-time treatment about this. They should have a full opportunity for shared decision making and walking through all the data and determining if this is really for them. We want to make sure that all persons with hemophilia should have an equal opportunity. We don't want to have different geographies or different, you know, socioeconomic impacts creating discrepancy on who can actually get this therapy.

We think that the safe introduction of commercial gene therapy with lifelong follow up is really paramount to ensuring long-term success. We want to make sure the patients understand this is a one-time treatment, but you're going to continue to be engaged in comprehensive care through your lifespan afterwards.

And we think that it's this integrated comprehensive care model which we employ in HTCs, that really is the best place to introduce this new platform of therapy and report out on the long-term outcomes.

EAHAD-EHC Statement on Gene Therapies Hub-and-Spoke Model

"One and Done"

does NOT mean

"Get and Forget"

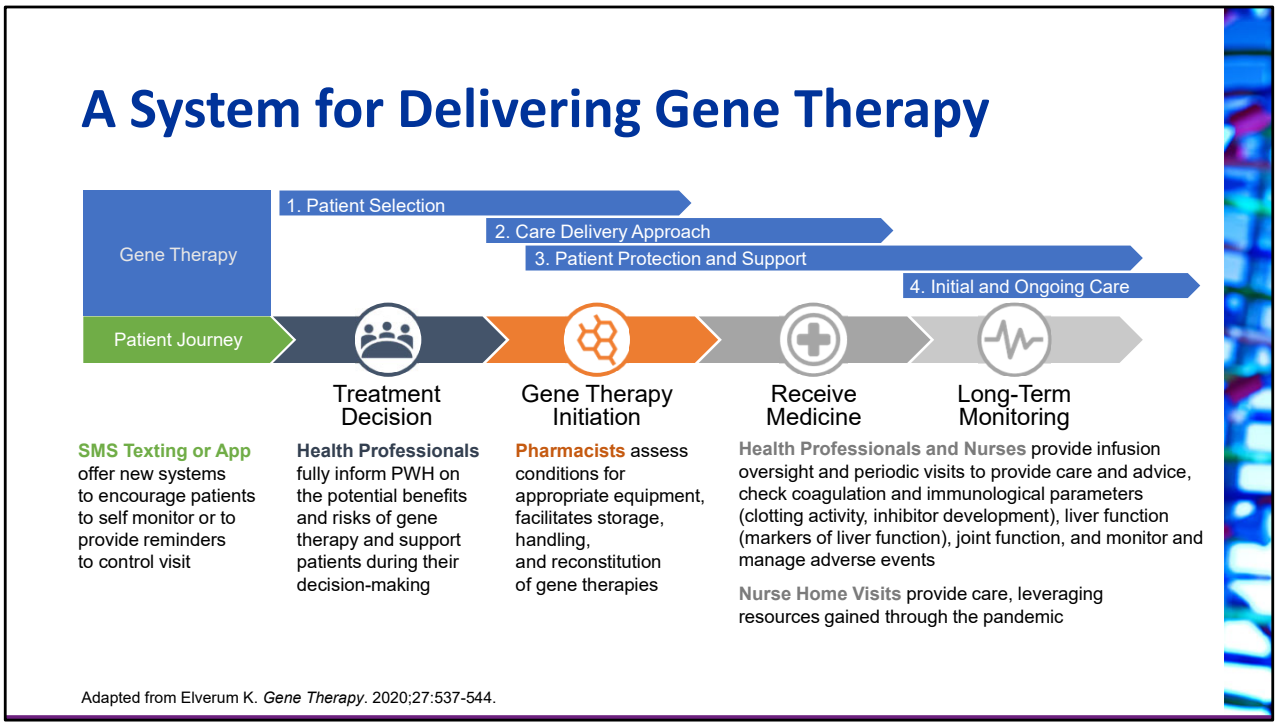
To ensure the safe introduction, use, monitoring and optimal learning regarding the delivery of gene therapies over time, EAHAD and EHC jointly call for all first-generation gene therapies to be managed using a hub-and-spoke model, as follows:

- Prescribed and managed exclusively by expert hemophilia comprehensive care centers (as the national hubs), *and*
- Monitored, by hemophilia treatment centers in close communication with the primary expert hub (as spokes linking into that hub)

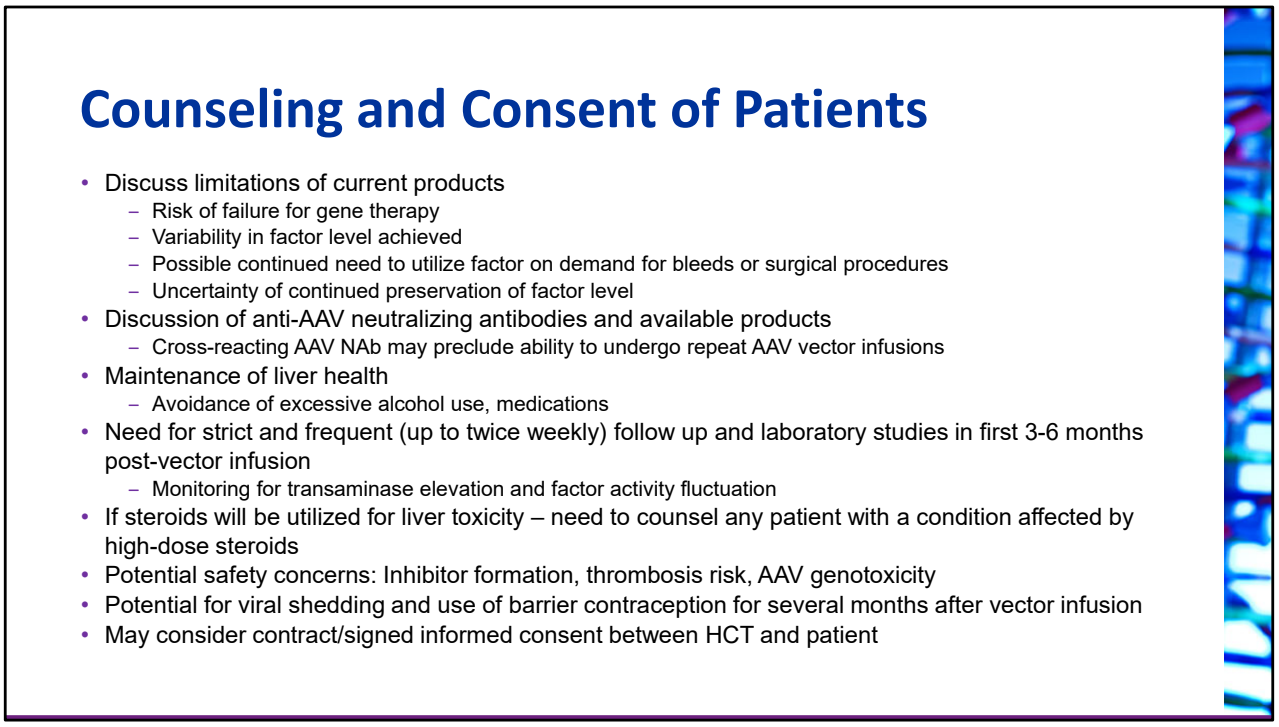
Now, does every center have to do gene therapy or be prepared to do this? Well, different groups have had thoughts about this. The Europeans have actually promoted a hub and spoke model, in which case certain centers with the expertise and capacity would be the ones that would do the dosing. And then the other centers would function as sort of referral and or, follow-up centers.

They are moving forward with that model. I think that model could work clinically in the US as well. If I think how things have gone in our region here. There's just a couple of centers that have actually been involved in the gene therapy trials, but yet I've had referrals from multiple of my colleagues at other centers. And I think that model could continue to work even with commercial delivery.

We have to continue to reiterate to patients and also our treatment teams that yes, this is a potentially a one and done therapy, but it doesn't mean get and forget. There's a lot of follow-up principles that are here.



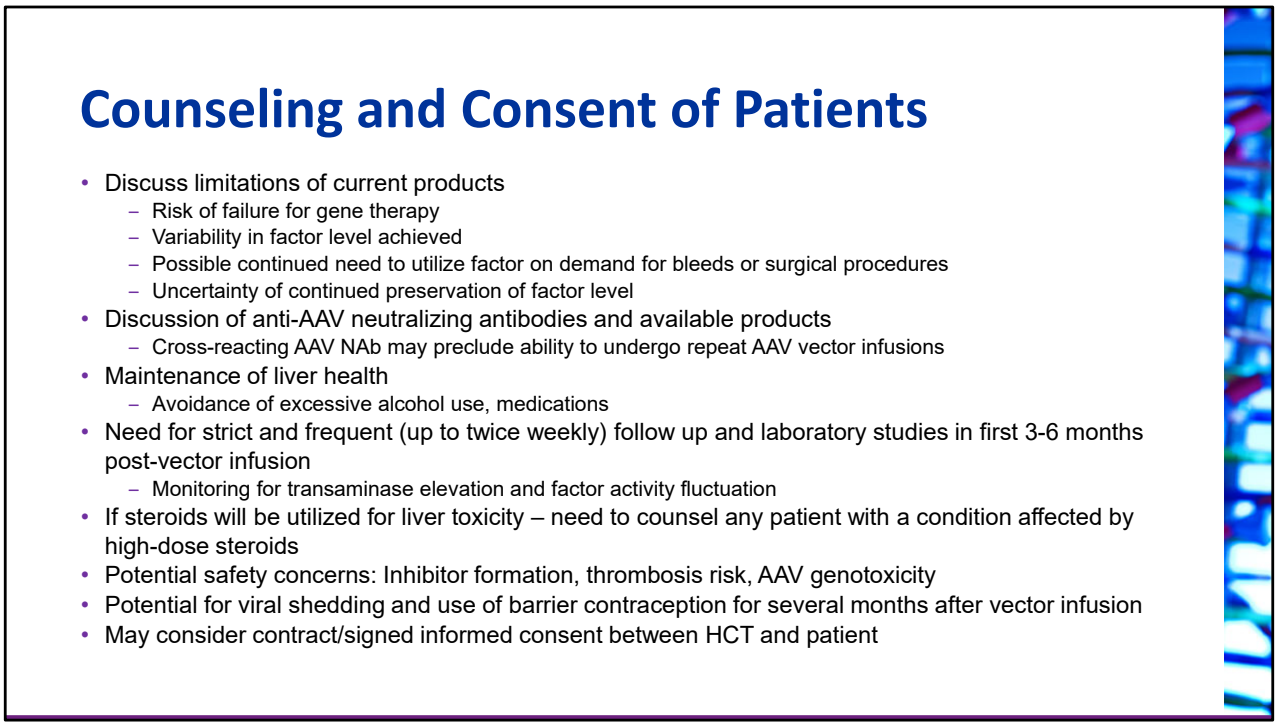
So, what this is laying out here is sort of, some of the, if you like the patient journey from, you know, identifying a patient, the treatment decision, actually getting the gene therapy delivered, and then the long-term monitoring. And there are different phases of the care delivery where we have to be vigilant and we're going to be engaging different components of our multidisciplinary team.



Some of the important principles in the initial phase is, how to properly counsel and consent of patient for this treatment. We should be able to discuss the limitations of their current products. I showed you the rationale that we have not reached the finish line for what our current platforms of therapy can achieve as far as preventing all bleeds and preserving joints.

And so, gene therapy has something to offer over our existing therapies and we have to discuss the impact of neutralizing antibodies and help patients understand why they may not be eligible for certain treatments. This is a great opportunity to put more effort and liver health discussions, including in our pediatric population. So paying attention to how we counsel on exercise, diet, excessive alcohol use, concomitant medications. All of these are going to potentially have implications on whether they can actually receive gene therapy in the future.

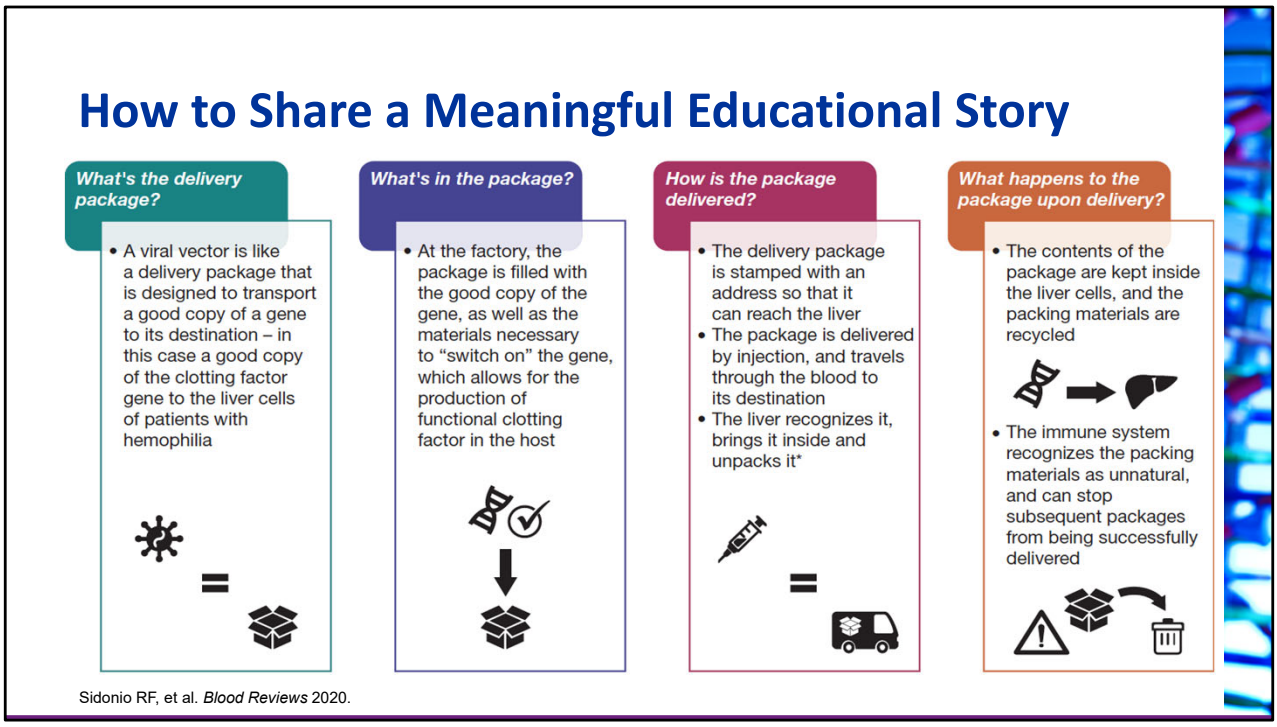
Before you launch into getting this therapy, I want a patient to understand that he's going to have to commit to probably up to twice weekly lab draws, at least for the first three to six months, so that we can monitor for those transaminase elevations and introduce the immunosuppression as quickly as possible and avoid loss of any factor expression.



Since patients may need corticosteroids, we have to counsel them about, you know, what the potential side effects could happen with high dose steroids.

Some of the potential safety concerns. We've never seen any inhibitors induced by gene therapy to date. Thrombosis risk has been seen in patients who achieve super physiologic levels, but from those curves I've shown you, that appears to be a transient risk and it's unknown whether the genotoxicity is an issue over the long term yet.

I mentioned the viral shedding, may be a good commonplace recommendation for at least barrier contraception for several months after vector infusion. And some centers have actually talked about maybe having a patient actually sign a contract or informed consent to show that they've walked through all these different issues.



I'm not going to go through this now, but this is a really useful review in which we put in how to share a meaningful educational story. And this uses the analogy of receiving an Amazon delivery box as a model for a gene therapy delivery. And I think being able to explain it to a patient and walk them through all these different steps will help you hit all the key educational points to help them understand the gene therapy. So, hopefully have a chance to review that if you haven't seen it before.

Logistical Needs: Pre-Infusion

Biological

- There are federal regulations regarding product handling
- Most institutions have a biosafety committee that must approve use of GMO on campus – you should check this in your specific situation
- Institutional preparedness for product handling and administration
  - Infection control committee
  - Nursing handling/infusion
  - Patient/staff precautions
- Pharmacy preparedness
  - Product receipt, handling, storage

GMO, genetically modified organism

Some of the other items here. This is a genetically modified material we're giving. There are some biosafety issues that come up on different campuses and hospitals and, institutional preparedness for how you handle and administer the product. You might have to go through different committees to get it approved. I don't think it's a barrier, but you do have to go through some pharmacy preparedness to make sure they can receive, store, and reconstitute this for infusion.

Pre-Infusion Screening

- Liver health
- Neutralizing antibody assay (companion device central lab sends out)
- Obtain baseline transaminase results at planned post-infusion monitoring site
- Reimbursement approvals/authorization
  - Drug acquisition

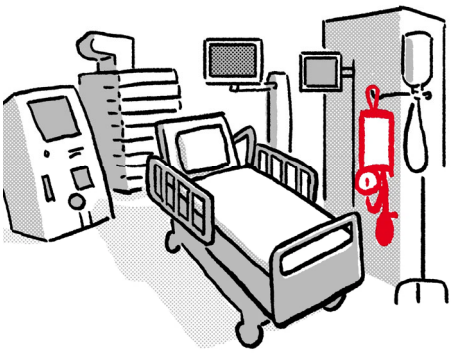
Part of pre-infusion screening is a liver health assessment that's not just lab assays, but might involve some of these functional tests for fibrosis like liver elastography. Also, the neutralizing antibody assays are going to be a key screening point for almost all participants, who are getting this either in clinical trials or commercially. And even with HEMGENIX, it's going to be recommended that you get a neutralizing antibody assay. There's actually a mechanism by which you can order this for your patients.

Reimbursement, and prior authorizations are still being worked out at different levels. And so, we'll have to wait that different payers, may have different rules related to that, but hopefully they mostly follow the label.

Logistical Needs: Peri-Infusion

Infrastructure and staff

- Pharmacist who is willing/able/trained to handle the product
  - Reconstitution – thaw time and containment needs
- Trained skilled nurses for infusion
- Physicians available during the infusion
  - In case of a reaction
- Safe area to conduct infusion
  - Appropriate containment – eg, infusion room
  - Suitable to respond to infusion reactions
    - Crash cart available
    - Proximity to emergency room if needed
  - Plan for infusion modification if needed
    - Infusion rate change, supportive therapeutics

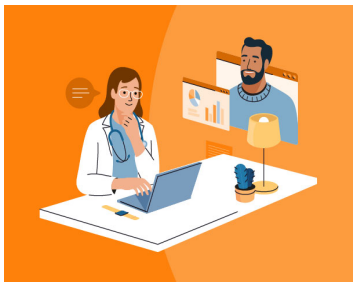


Peri infusion. You're going to have to deliver this in a setting where you have the appropriate infrastructure and staff to supervise a biologic administration. But you absolutely can do this in your outpatient areas. You just have to be able to handle typical biologic reactions which can still be done in the outpatient setting.

Logistical Needs: Post-infusion

Infrastructure and staff

- Clinical staff knowledgeable in post-infusion needs
  - Includes laboratory testing and interpretation and management of immune reactions/ elevated transaminases/immunosuppression
- Clinical laboratory that can process samples safely/properly



Monitoring

- Schedule of assays – transaminases and factor levels
- Immunomodulation plan and appropriate prescriptions
- Communication plan between patient, lab, and follow-up center

Post-infusion gets interesting because you're going to have to have a clear sense of what clinical staff are going to be responsible for, making sure the patient goes for their testing, who's going to see the results, who's going to, determine whether this meets criteria for triggering the immune response and making sure the patient gets the appropriate corticosteroid dosing initiated.

So having some sort of a schedule of assays and you're going over with the patient and then some team member who's going to be sort of tracking that in the outpatient setting. And then if you're going to do this in partnership with another center, having a good communication plan for how you're all going to work together.

Interaction Between the Dosing Center and Follow-up Center/Home HTC

- Regular laboratory measurements before gene therapy
- Travel between dosing and management centers
- Regular laboratory measurements after gene therapy

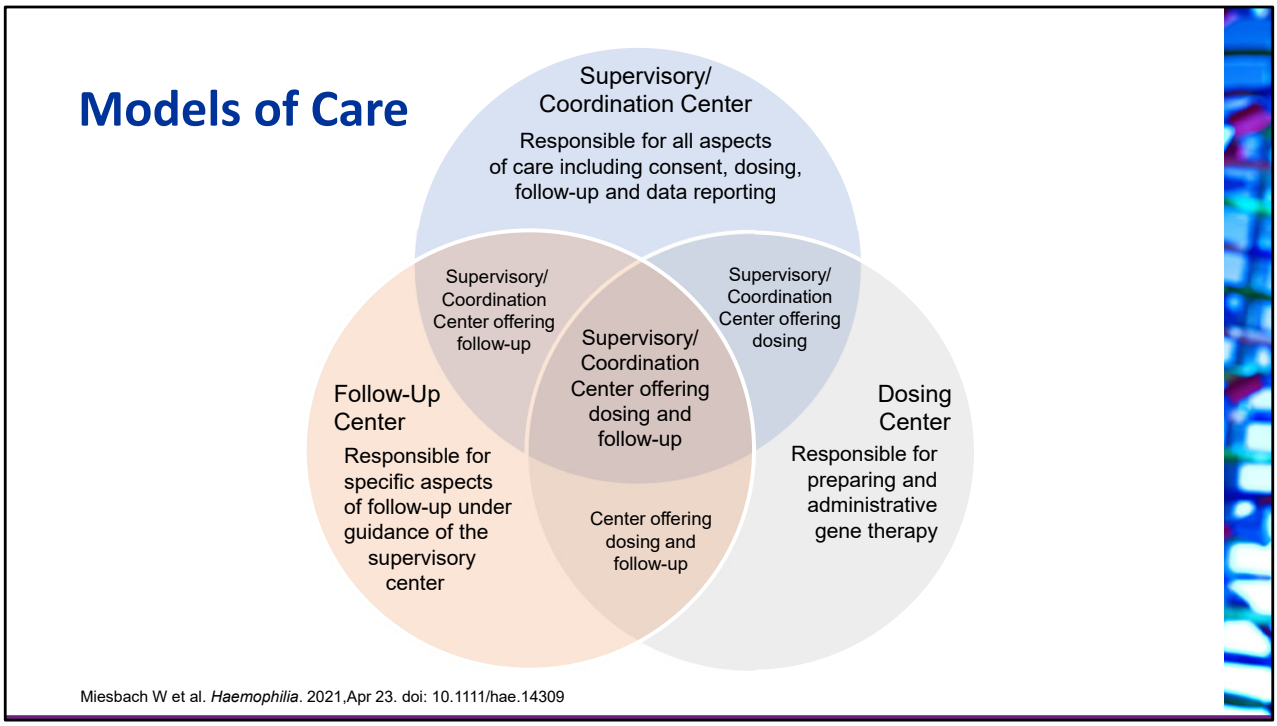
When to stop regular factor replacement?

Early detection of ALT elevation

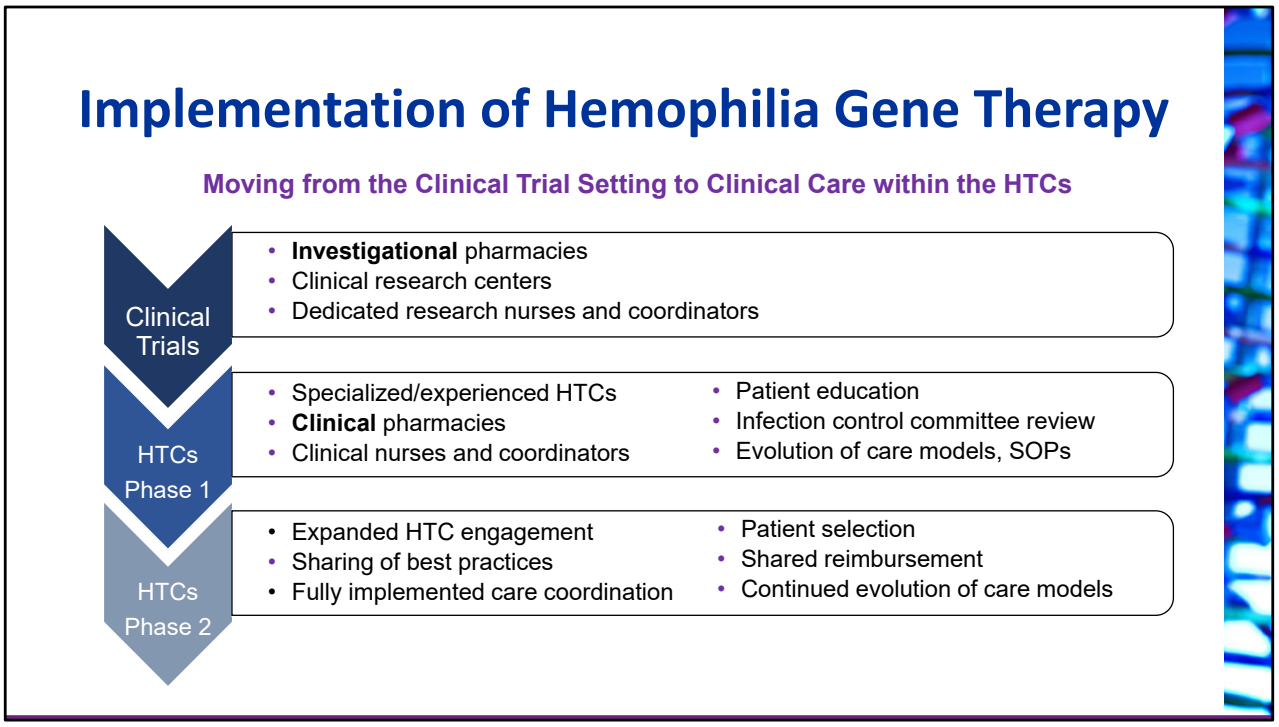
Potential immunosuppressive treatment

And the three sort of key questions that I think when more than one center is involved is, what's the criteria for when you want to stop factor replacement? How are you going to be able to detect the LT elevations as quickly as possible? And who is going to be charged with supervising the immunosuppressive treatment?

Latest Advances in Gene Therapy for Hemophilia: An Integrated Care Model to Improve Outcomes

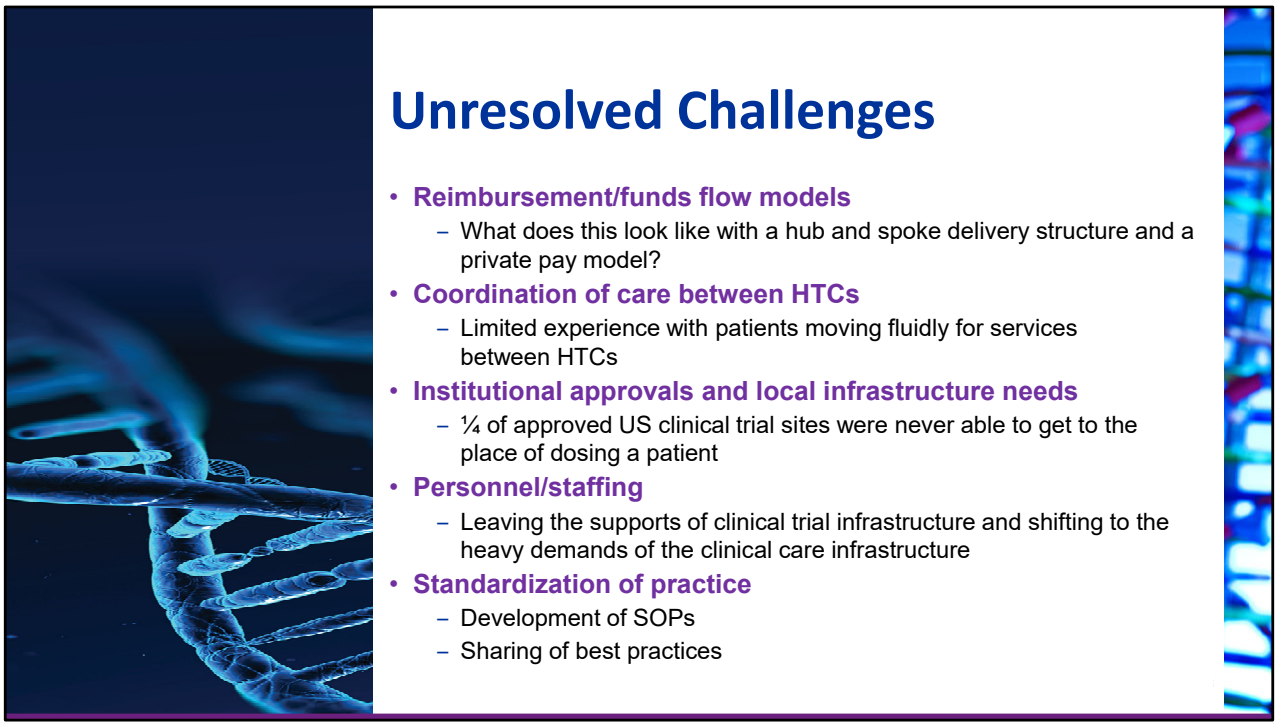


There's a role for each center, I think in participating in the care of the patient, whether it's the initial referral or screening of the patient, the dosing, or the follow-up or even one center sort of coordinating the care across two or more at HTCs. I don't think there's going to be a certification process for gene therapy, but I do think centers are going to self-select based on how they see they can participate in these different roles.

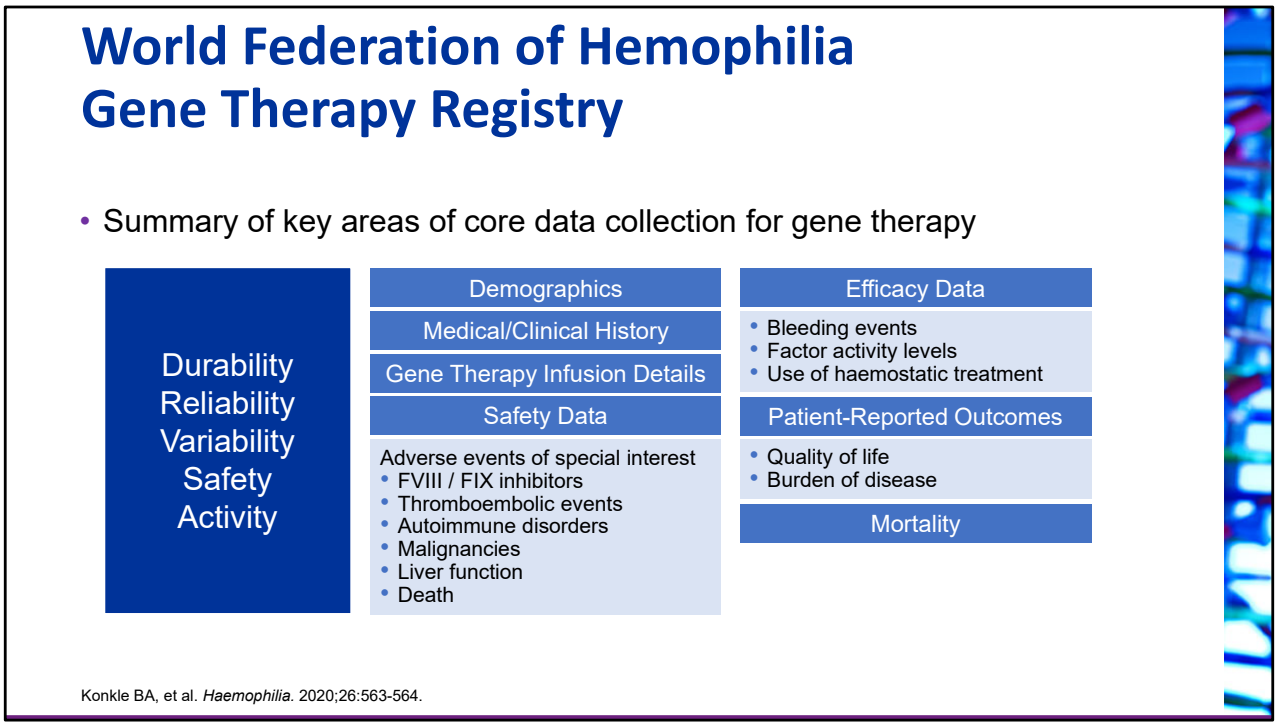


As we move from clinical trials to the clinical implementation, of course we are going to be leaving the confines of our investigational pharmacies and our clinical research centers with dedicated research nurses and coordinators, and now we're going to be moving into using our clinical pharmacy, our clinical nurses and coordinators.

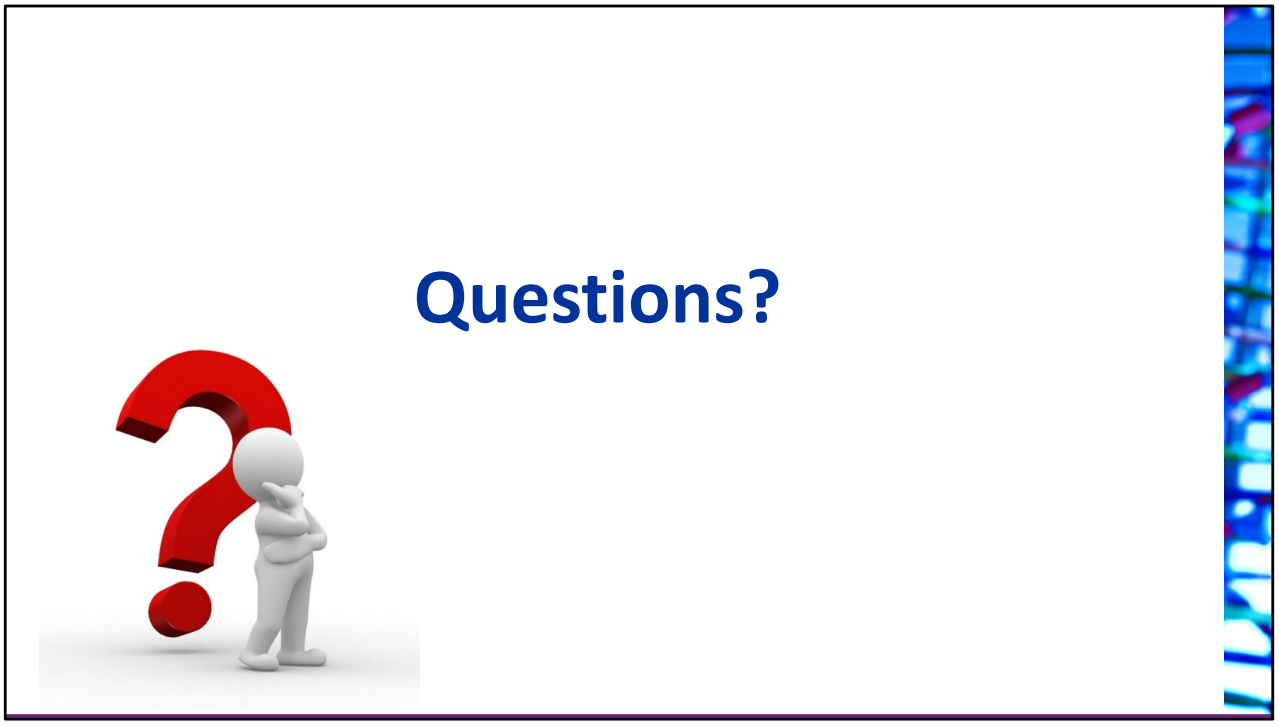
We're going to have to develop some new care models standard of standard operating procedures, which can be shared amongst the HTCs. So, I think in the first phase of the rollout of this, the experienced centers who've been involved in the clinical trials are likely to be doing the early dosing, but we can work cooperatively to get other centers up and running as quickly as possible.



And then long-term follow up is going to be an important issue. There are some other unresolved challenges about what the reimbursement and funds flow look like, particularly when we're sharing patients between centers, but I think this is something we can overcome, maybe something we can discuss during the question answer period.



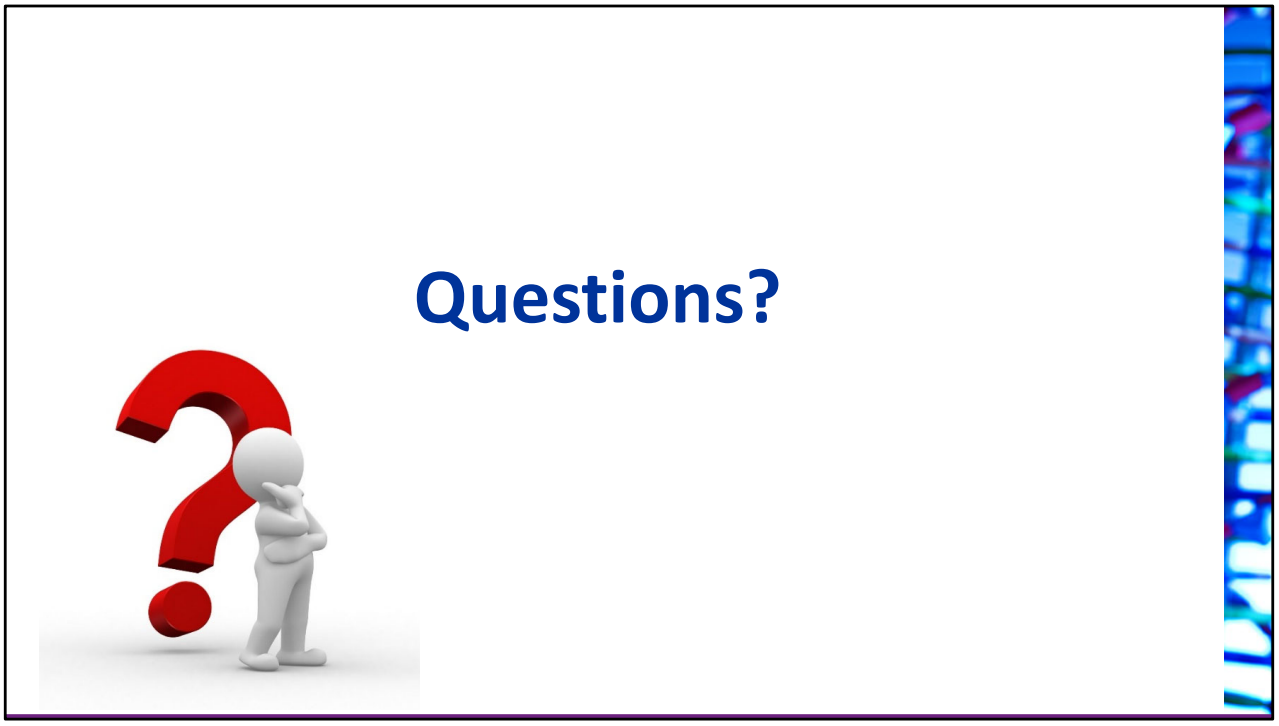
This is the platform that's been laid out globally for collecting long-term data and the gene therapy registry from the World Federation Hemophilia is up and running. Any center can participate in this. Athen also will have a long-term follow up and they're also going to potentially share data for that long-term data collection.



Okay. So that was a lot of information, but I did want to hit some important points related to a q and a. Some of these had actually come in, in advance.

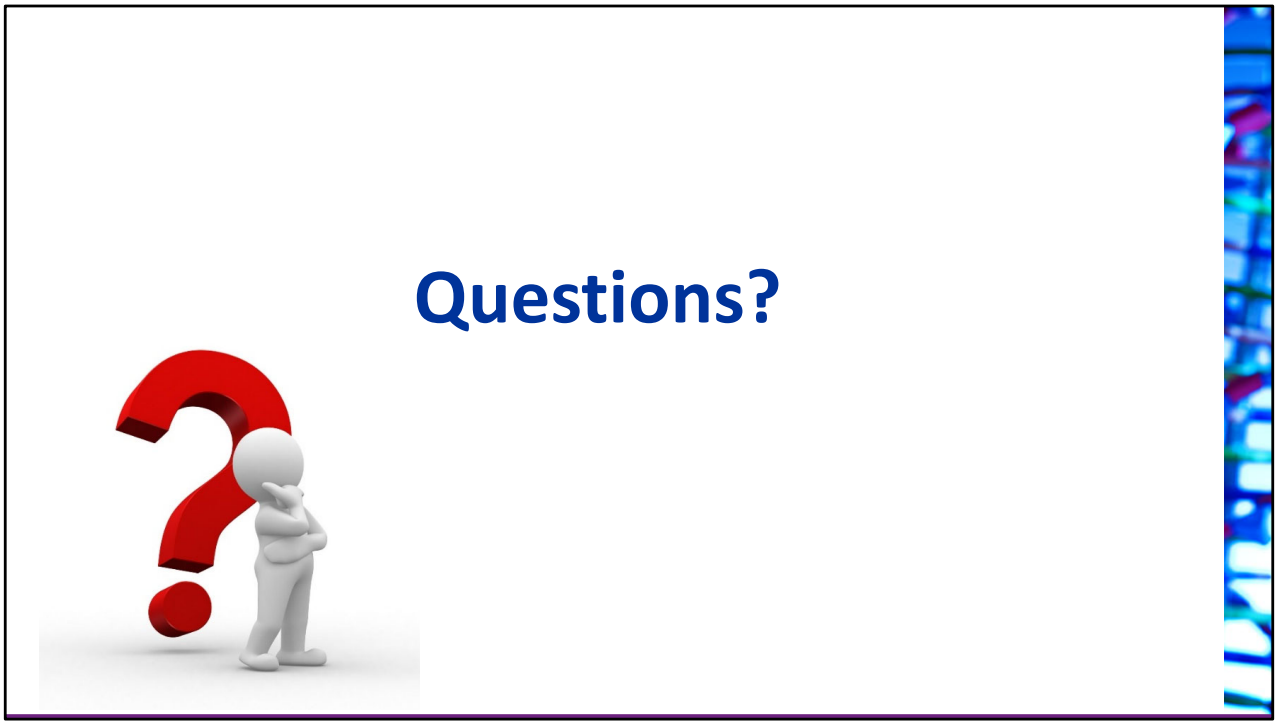
And so, first question here is, in the HEMGENIX trial, for patients who were positive for neutralizing antibodies, were they at any higher risk for the immune response? So, the transaminase elevation or how much corticosteroids, and actually the answer is no. The neutralizing antibodies did not impact that and so, that doesn't appear to be a safety issue. The patients who did have the immune response overall did tend to have somewhat lower factor IX expression overall. But all of the patients on the HEMGENIX trial, there was only nine of the 54 that triggered the corticosteroid treatment. And although they had somewhat lower overall factor IX expression, didn't impact their clinical bleed control, and all of their factor levels stabilized.

Question on any insight regarding the financial logistics of delivering gene therapy through the HTCs. Still remains to be seen, there is 340b pricing available and I believe what we've heard is that there's going to be contracting through the alliance which means any center who's an alliance partner will be able to get access to that 340b pricing.



I think we have experience in those models of resource sharing even between centers potentially. I think we can develop regional models of care here so that we can ensure that everybody's getting compensated for their role in the process, role in the screening and identifying patients, the counseling, role in the dosing and what's needed there. and then also in the follow-up period. I think we can ensure that we can do this regionally and keep everybody whole from a financial perspective.

Someone asked what's likely to be the biggest barrier to patients entering treatment and or physicians referring them? Neutralizing antibodies are going to be the biggest screen note, at least for all of the hem A trials, most of the hem B trials outside of HEMGENIX, but I think the biggest barrier is this is a new platform of therapy. And though I think it's a game changer for individuals who've had a good outcome, some of the variability and the durability concerns are still going to give people pause. You know this is not a one-time decision, it's a one-time treatment, but I don't think it's a one-time decision. Meaning you can introduce the patient to this platform now, maybe six months later, a year later, there'll be more data to share, and you can continue to bring them up to speed. I think we'll see greater adoption over a period of time.



One other question that came up that I think is really intriguing is: if a patient has a full correction after gene therapy will they no longer be eligible for things like special healthcare services, which is what we have in Michigan. So basically, you know, Medicaid support, will they be deemed no longer having the condition? And I would argue no because this is not physiologic restoration of factor VIII or factor IX. They may not need to be on prophylaxis, but there's no guarantee they don't need factor for trauma, or they don't need factor for surgical management. And we don't have any data to support whether there is durability over decades and decades.

So, I think it would be wrong to disqualify them just because they went through hemophilia treatment. I think this is different from, say, a von Willebrand patient who may have been misdiagnosed as a child and then gets a reassessment and determine that they no longer have the condition. To my assessment, no, I don't think gene therapy should disqualify anybody.

Hopefully this, information will build up your confidence about the data, about how you're going to be thinking about implementing this at your center. And we'll stimulate some more discussions at a regional level. So that's all the time we have for today. I really thank you for your attention. Thanks for joining us and hope y'all have a great day.

