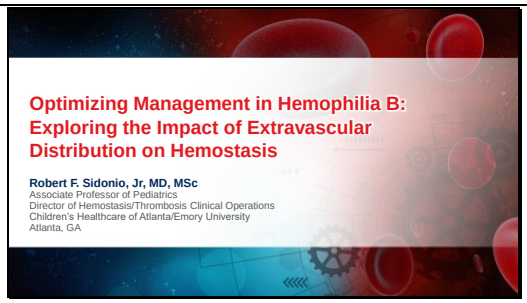
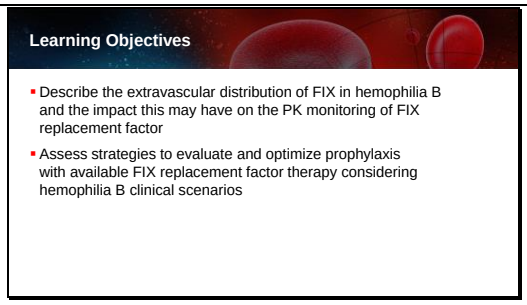
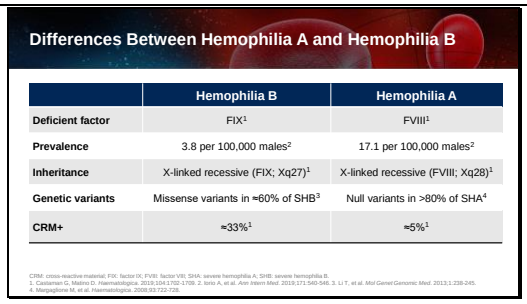
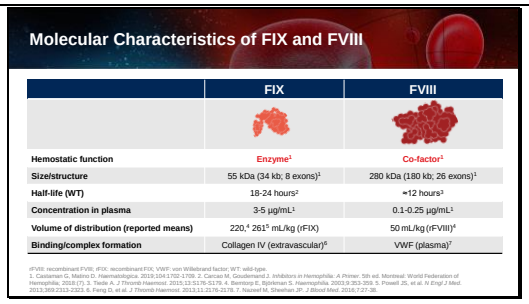
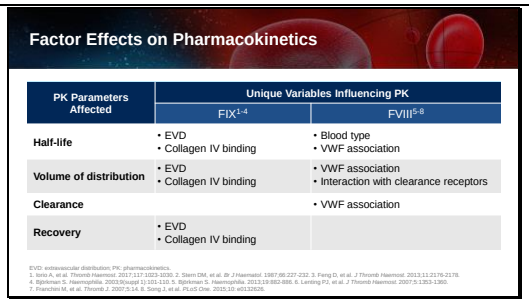
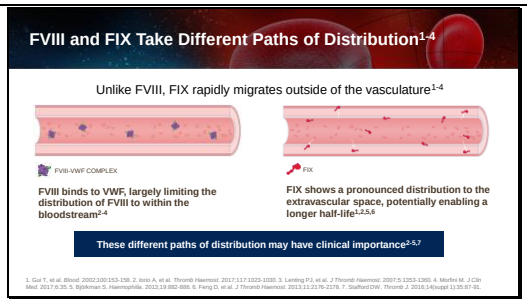
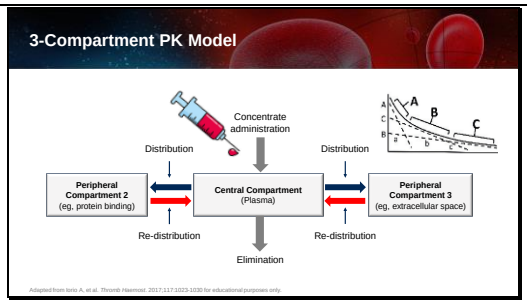
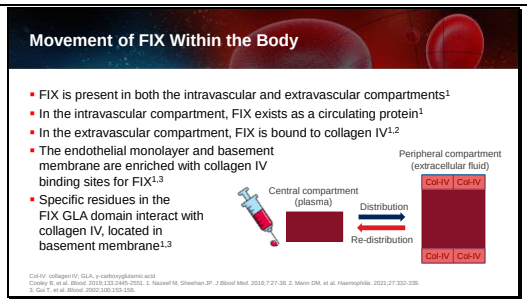
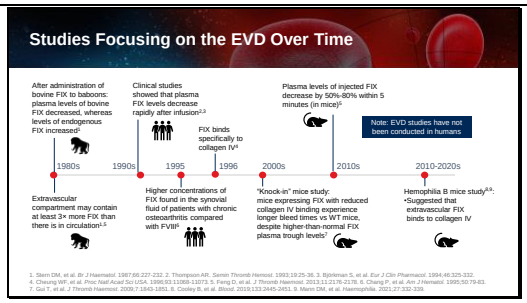
Shifting Goals in Hemophilia A and B: Targeting the Intrinsic Mechanism of Disease Pathology with New Treatments

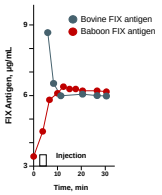
Optimizing Management in Hemophilia B: Exploring the Impact of Extravascular Distribution on Hemostasis

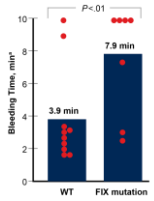
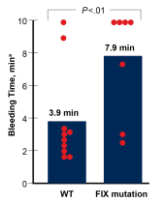
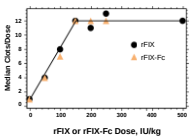
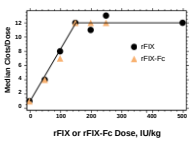
1		Thank you very much for participating in this session. The title of the presentation is, "Optimizing Management in Hemophilia B: Exploring the Impact of Extravascular Distribution on Hemostasis." I'm Robert Sidonio, Jr. I'm an associate professor of pediatrics and director of clinical operations at my institution, which is Children's Healthcare of Atlanta at Emory University, in Atlanta, Georgia. Let's go ahead and get started.
2		These are the quick learning objectives, and they're fairly straightforward: We're going to describe the extravascular distribution of FIX, its impact on monitoring. We're going to talk about strategies to evaluate and optimize prophylaxis. And we're going to do this in the context of evolving new factor products on the market, and talk a little bit about why hemophilia B is different than hemophilia A. So let's go ahead and get started.
3		So I think it's really important, and oftentimes we're taught that hemophilia A and B are fairly similar; they're consecutively only one difference. But there is a pretty significant difference. As you can see, the prevalence is significantly lower for hemophilia B. They're both X-linked recessive disorders. But probably one of the key differences is, outside of a few rare situations, missense mutations make up the largest amount of the severe hemophilia B. And that's in contrast to null variants being more common as the genetic variants in severe hemophilia A. And because of this, there's a difference in what's called cross-reactive material positivity. So there are small amounts of FIX, albeit potentially defective, that are floating around in those with severe hemophilia B much more so than in those with

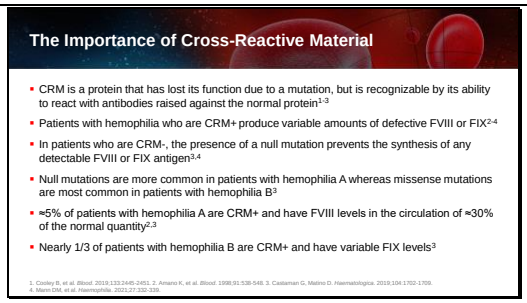
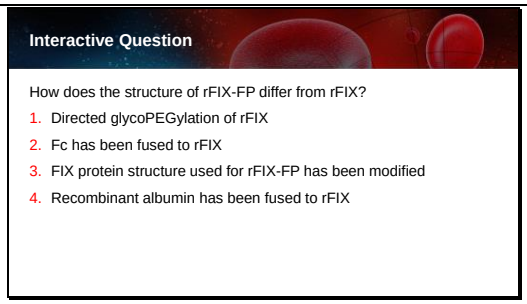
		hemophilia A. And that may explain some differences in bleeding phenotype and how patients respond to factor products. All of those are still theories.
4		When you look at the molecular characteristics of the products, FIX is an enzyme and FVIII is a cofactor. There are significant differences in size. We know this based on gene therapy studies. The half-life is much longer in hemophilia B. And the concentration of the amount of protein is much higher for FIX than in FVIII. This may explain some issues with developing complications in those with inhibitors. And probably one of the key differences is, when you look at things that it binds to, FVIII largely resides in the plasma. It's bound to von Willebrand factor. And when you look at the volume of distribution in them, FIX has interactions outside of the intravascular space and binds to something called collagen IV in the extracellular matrix, which we're going to talk a lot about in the upcoming slides.
5		We know that there are some effects on pharmacokinetics. There are some unique differences. When you look at half-life, FVIII has largely limited extension of the half-life because of its association with VWF. There are some new products trying to decouple that. The volume of distribution in FIX is significantly larger because it does have interactions outside the blood vessel. And clearance is associated with VWF and FVIII and not so much, obviously, in FIX.

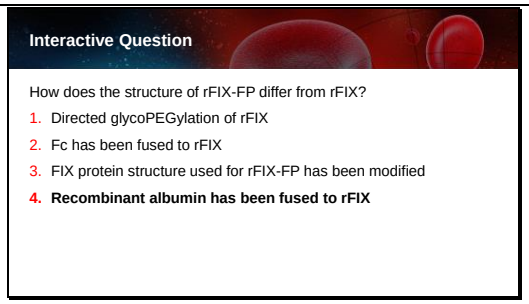
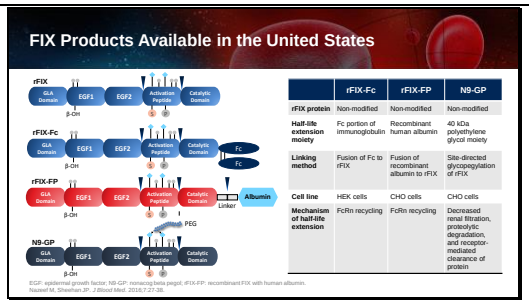
		And recovery can be affected largely by the extravascular distribution of FIX.
6		So let's look at this visually. When you look at FVIII and FIX, if you take a look on the left-hand side of the slide, FIX, as mentioned before, binds to VWF, limiting its distribution to within the bloodstream. And in comparison, and in contrast, FIX, as it enters the bloodstream from an injection, a good, significant portion of it leaves that circulation, binds the IV collagen in the extracellular matrix. And there's some redistribution that we'll talk about. So, very different paths of distribution that likely have some clinical importance. Certainly, preclinical studies have shown that there's a difference.
7		One of the important things to understand is, there are some differences between the products. Some of the products have largely been described as a 3-compartment model when we're talking about standard half-life, FIX, or native protein. So when you inject, with this cartoonishly large syringe, FIX into the central compartment, it enters it, as illustrated by the color change here. And then immediately it is distributed and redistributed into different compartments—1, the extracellular space; in the other one, it's bound to IV collagen. And that's before it's redistributed and eventually eliminated. And that's visually depicted by the curve at the top of the screen, as you see here.

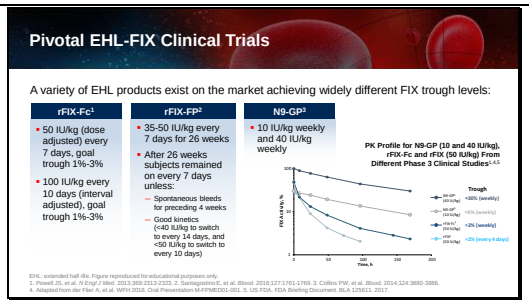
8		We know that FIX moves within the body, enters both compartments. It exists as a circulating protein, largely in the intravascular space. And mostly is bound to IV collagen in the extravascular space. And like we mentioned before, there's this basement membrane that's enriched with these collagen IV binding sites. We won't get into the discovery of this, but it was discovered many decades ago as a critical binding site for FIX. And we know that the GLA domain of FIX interacts with this IV collagen. And we know this because you can alter it and you can increase the binding and decrease the binding, which has been studied in the preclinical models in the mouse models. So again, FIX enters the central compartments. It gets distributed into the extracellular compartment. And then eventually redistributed and eliminated. Hopefully the repetition will help people understand this concept more. And again, this is not something that I've participated in understanding it. It's really more the clinical implications, which I've helped illustrate over the last few years.
9		There are a lot of great studies by lots of great physicians. A lot of this work was done at University of North Carolina. And it largely started with the main experiment in the 1980s in which they discovered and demonstrated that there was extravascular distribution, all the way until the end in which we discover that extravascular FIX binds to IV collagen. You can alter that dosing, and you can actually increase and decrease the binding. And that has an effect, as expected, on bleed control, at least in the mouse model.

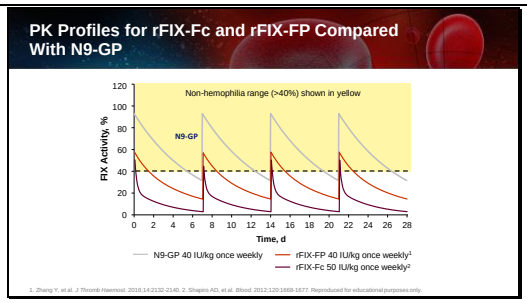
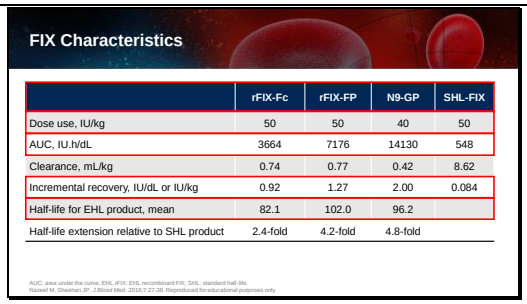
10	**Demonstration of Extravascular FIX** ▪ Injection of bovine FIX into baboons and species-specific radio-immunoassays performed ▪ Rapid dose-dependent rise in baboon FIX noted following bovine FIX injection ▪ Confirms the existence of non-circulating displaced extravascular distributed FIX  The clinical relevance of data obtained from animal models in humans is unknown. Adapted from Stern DM, et al. *Br J Haematol*. 1987;66:227-232 for educational purposes only.	The most pivotal experiments were done in 1987. They had a baboon model and they were able to measure the difference between bovine FIX and baboon FIX through the species-specific radioimmunoassays. And so what they did, they had a baboon model in which they had hemophilia B. And you can see here, on the right-hand side, when they injected, as indicated by that box there, the baboon IX was unmeasurable at time zero. And then eventually, as they injected bovine FIX, as you can see at the top of the slide, all of a sudden, in the intravascular space, you're able to measure baboon FIX that was not measurable before. And that's likely because it was displacing—the bovine IX went into circulation, displaced the baboon IX from the extravascular space, pushed it into the intravascular space. And this proved the existence of extravascular depot of FIX.
11	**Demonstration of Role of Hemostatic Extravascular FIX** ▪ FIX binds to collagen IV but clinical significance was not known ▪ Knock-in mice constructed creating K5A mutation, reducing the affinity of FIX for collagen IV The clinical relevance of data obtained from mouse models in humans is unknown. *Bleeding terminated at 10 minutes. Adapted from Gui T, et al. *J Thromb Haemost*. 2009;7:1843-1851 for educational purposes only.	Fast forwarding, other experiments that were done, they wanted to look to see, is there a clinical significance of this interaction? Because if there's no clinical significance, it's important to understand, but maybe not important for clinicians. And so, what they did is they made a knock-in mouse and they added a mutation, the *K5A* mutation in the GLA domain, which reduced the affinity of FIX for collage IV. So the theory would be that, if you decrease the interaction and nothing happens, then who cares. But if it decreases the interaction and the mouse bleeds a lot more, then that proves that that interaction is clinically significant.

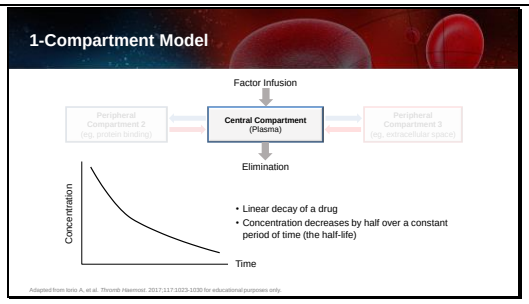
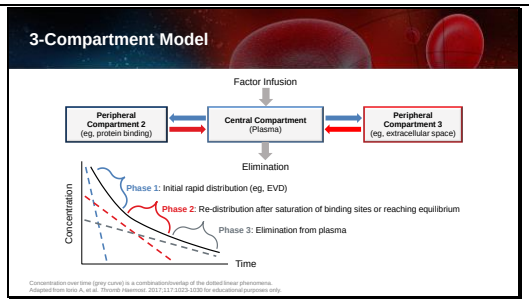
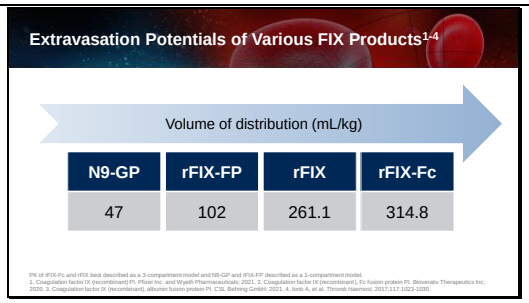
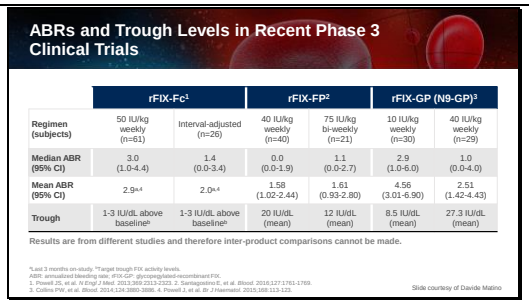
12	**Demonstration of Role of Hemostatic Extravascular FIX** ▪ FIX binds to collagen IV but clinical significance was not known ▪ Knock-in mice constructed creating K5A mutation, reducing the affinity of FIX for collagen IV ▪ Time to coagulation in mice (tail bleeding) with FIX mutation (K5A) was prolonged compared with WT FIX  The clinical relevance of data obtained from mouse models in humans is unknown. *Bleeding terminated at 10 minutes. Adapted from Gui T, et al. *J Thromb Haemost*. 2009;7:1843-1851 for educational purposes only.	And that's what happened. The tail-bleeding times, as you can see here on the right-hand side of the screen. The wild type is listed on the left-hand side. The bleeding time was 3.9 minutes. When they decreased the interaction of FIX for IV collagen, there was a lot more bleeding.
13	**Demonstration of Role of Hemostatic Extravascular FIX** ▪ FIX binds to collagen IV but clinical significance was not known ▪ Knock-in mice constructed creating K5A mutation, reducing the affinity of FIX for collagen IV ▪ Time to coagulation in mice (tail bleeding) with FIX mutation (K5A) was prolonged compared with WT FIX ▪ Preclinical research suggests a hemostatic role of extravascular FIX  The clinical relevance of data obtained from mouse models in humans is unknown. *Bleeding terminated at 10 minutes. Adapted from Gui T, et al. *J Thromb Haemost*. 2009;7:1843-1851 for educational purposes only.	So this proved that there was, at least in preclinical models, some concern that this interaction is very significant—again, suggesting a role of hemostasis of extravascular FIX.
14	**Demonstration of Role of Hemostatic Extravascular FIX (cont)** ▪ Comparison of different doses of FIX and the hemostatic effect 7 days post injection in mice with hemophilia B ▪ 50-500 IU/kg[a] of rFIX-Fc and rFIX injected ▪ Ability to maintain hemostasis evaluated using saphenous vein model  *The clinical relevance of data obtained from mouse models in humans is unknown Adapted from Cooley B, et al. *Blood*. 2016;128:286-292 for educational purposes only.	Fast forward, a couple of other experiments. What they wanted to do is they wanted to look at what the doses were. Could they actually saturate these receptors? And you can see here in the mouse model in which they were measuring the number of clots per dose, and at some point, here, you can see here, they looked at recombinant FIX-Fc and standard half-life FIX. As they increased the dose up to about 150 IU/kg, there was no improvement of hemostasis. And this was using a saphenous vein model, so different than tail-bleeding time. But largely the results have been fairly similar.
15	**Demonstration of Role of Hemostatic Extravascular FIX (cont)** ▪ Comparison of different doses of FIX and the hemostatic effect 7 days post injection in mice with hemophilia B ▪ 50-500 IU/kg[a] of rFIX-Fc and rFIX injected ▪ Ability to maintain hemostasis evaluated using saphenous vein model — Maximum efficacy achieved at 150 IU/kg  *The clinical relevance of data obtained from mouse models in humans is unknown Adapted from Cooley B, et al. *Blood*. 2016;128:286-292 for educational purposes only.	So this actually demonstrated that there's probably some saturation points. And that was about 150 IU/kg. It didn't matter how much more you gave; you didn't achieve anymore hemostasis. And likely that's because you saturated those receptors.

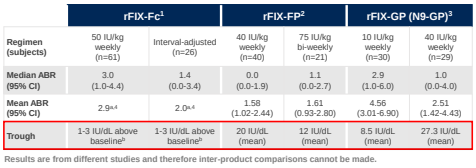
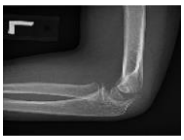
16		Going back to the discussion of cross-reactive material positivity: We mentioned before that missense mutations largely make up the severe hemophilia B patients, compared with null mutations. And what that means is, practically, that severe hemophilia B patients are much more likely to be cross-reactive material positive, compared with those with hemophilia A, and that could have clinical implication. And there have been experiments done in which mice respond better if they are CRM-negative, compared with CRM-positive. It probably matters on which type of factor products you're using as well. And certainly, there's potential for interaction of a defective circulating FIX product interfering with infused FIX products, comparing both for the same binding sites. But of course, the infused FIX is going to be much more efficacious than the defective. And so, there's some concern that some of these patients may respond better if they are CRM-negative, compared with CRM-positive. And there may be some differential between the products. So these are things that we're largely evaluating as a possibility. And more to come on this in the next few years.
17		We're at the point of the presentation now where we have an interactive question. I'm going to read the question—it's fairly straightforward—and give a few seconds to respond. The question is: How does the structure of recombinant FIX fusion protein differ from the native or recombinant FIX product? The options are: 1) Directed glycoPEGylation of FIX; so, recombinant FIX fusion protein of glycoPEGylated products

		2) Is it Fc that's been fused as a binding partner to recombinant FIX 3) Is it a recombinant FIX protein structure used that has been modified; or is it 4) Recombinant albumin that has been used as a binding protein and fused to FIX I'll give you a few seconds to let you decide on this. Hopefully you get this right. If you don't, then it's okay, we're going to go over it.
18		So recombinant FIX-FP differs in that it is a fusion product. And I'll show you on the next slide:
19		So these are all of the products available on the market. There's obviously different brand names of it for a standard half-life, but they're largely the same. Starting from the top here, the unmodified protein of FIX. You can see the GLA domain. The EGF domains. The activation peptide. And then the catalytic domain. When you look at recombinant FIX-Fc, they have the IgG portion of Fc that is bound to the catalytic domain; so that's its fusion partner, and that's what makes recombinant FIX-Fc. Recombinant FIX-FP binding partner has a cleavable linker bound to albumin, which extends the half-life. And then finally, N9-GP is glycoPEGylated specifically at the activation peptide, which is eventually removed. As you see on the right-hand side, recombinant FIX-Fc infusion protein largely have a similar half-life extension in which they're recycled through the neonatal Fc receptor.

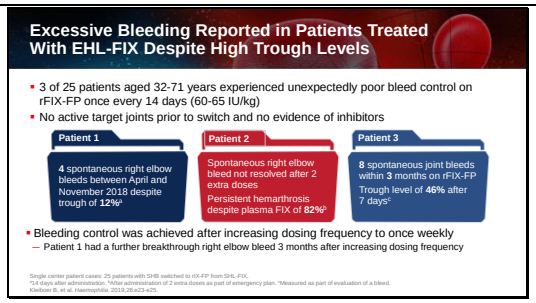
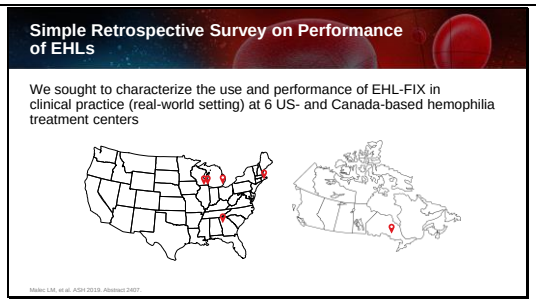
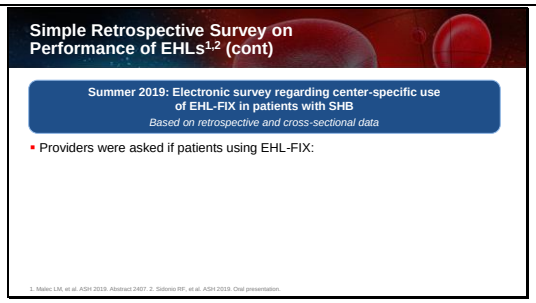
		The cell lines are different. The human cell line in the recombinant Fc versus the Chinese hamster ovary cell, which is a common cell line used for recombinant products. And that's the same cell line that's used in N9-GP, as well. We know that glycoPEGylation increases the size of the molecule, decreasing renal filtration, decreasing degradation, and reducing clearance.
20		So I think it's important when you look at what's done in a clinical trial compared with what's seen in clinical practice. If you look, first, at the PK profiles, clearly N9-GP is able to achieve the highest dosing or highest trough levels, most extension of the products. And then it goes down to fusion protein, as well as Fc. And then finally FIX standard half-life. When you look on the left-hand side, the trials for the Fc product were done as a fixed dose. One of them was dosed 15 IU/kg. There was a dose adjustment to get a goal of 1% to 3% trough. Largely most patients stayed within plus/minus 10 IU/kg. There was also an arm that looked at 100 IU/kg every 10 days. Again, adjusting to achieve a trough of 1% to 3%, which largely most of the patients were able to. When you look at the fusion protein, it was done a little bit different. Every patient got a fixed dose every 7 days for 26 weeks. And based on how they responded, their kinetics, their bleeding phenotype, they were allowed to switch to every 14 days or every 10 days. If they had poor kinetics or had any concerns from bleeding, then they stayed on the weekly dosing. And that may explain some of the differences that we'll talk about later in the real-world experience. And then, N9-GP was dosed at 10 IU/kg weekly and 40 IU/kg weekly fixed dosing. Nothing different than what was mentioned in previous trials.

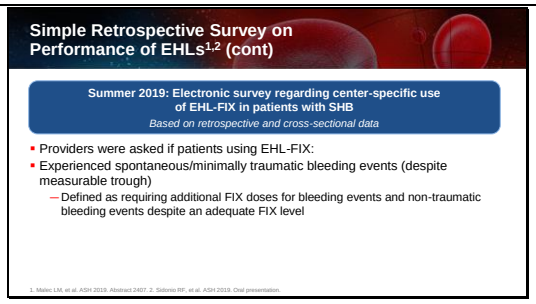
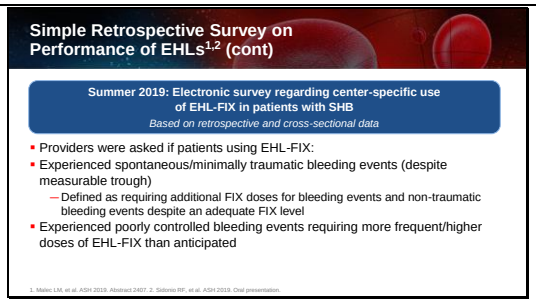
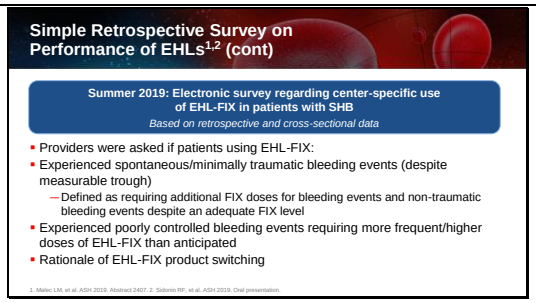
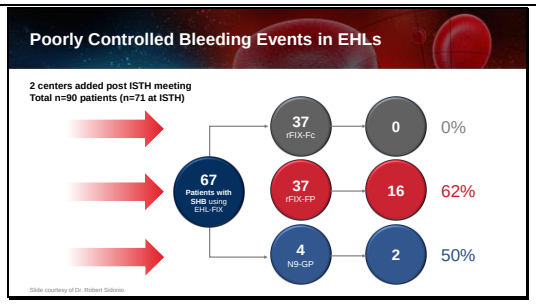
21		One of the things that's interesting to look at is when you look at the differences in the products—the gray being N9-GP; the bright red being fusion protein; and then Fc— one of the things that's intriguing about the PEGylated product is the longer time in the non-hemophilia range, using 40% of the cutoff. This is one of those things that potentially could significantly reduce the amount of bleeding. But again, I think there's going to be differences in levels between the products; that's my personal theory. And the levels aren't always indicative of bleed control. And we'll talk a little bit about that in upcoming slides.
22	 rFIX-Fc / rFIX-FP / N9-GP / SHL-FIX Dose use, IU/kg: 50 / 50 / 40 / 50 AUC, IU.h/dL: 3664 / 7176 / 14130 / 548 Clearance, mL/kg: 0.74 / 0.77 / 0.42 / 8.62 Incremental recovery, IU/dL or IU/kg: 0.92 / 1.27 / 2.00 / 0.084 Half-life for EHL product, mean: 82.1 / 102.0 / 96.2 Half-life extension relative to SHL product: 2.4-fold / 4.2-fold / 4.8-fold	One of the things I think it's interesting to note is when you look at the doses for the different products. Remember standard half-life is on the right-hand side and then the first 3 columns are extended half-life products. When you look at the dosing, they're largely similar. Areas under the curve are quite different: significantly prolonged for the Fc, longer for FP, and even longer for N9-GP. The clearances are different, as listed there. And then, one of the most interesting things is, the incremental recovery is different. What you see is that the incremental recovery is similar for Fc, as it is for standard half-life products, compared with FP. And then N9-GP has a similar recovery that you would see with a FIX product. And then finally, the half-life for the extension half-life, you can see, is obviously significantly prolonged when you looked at the means; particularly the N9-GP and the FP are a little bit longer. And you can see the prolongation in there is much longer.

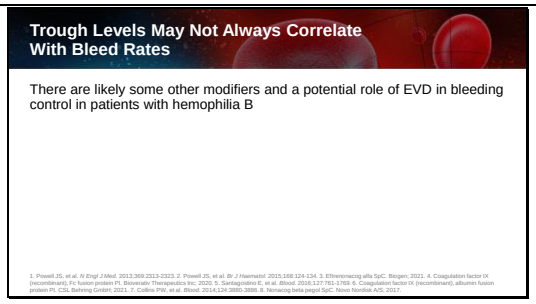
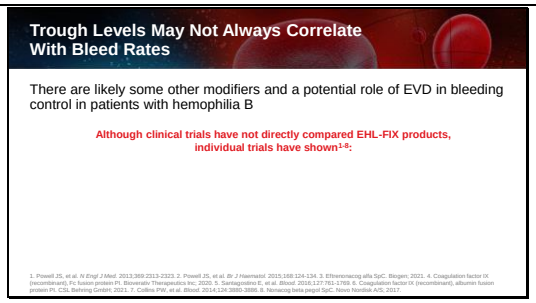
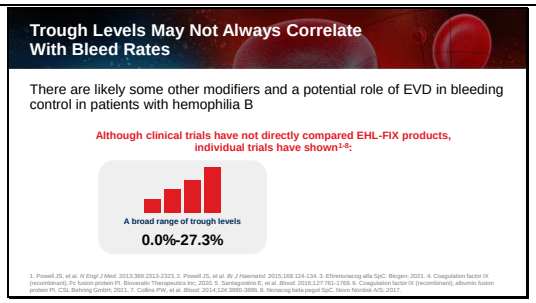
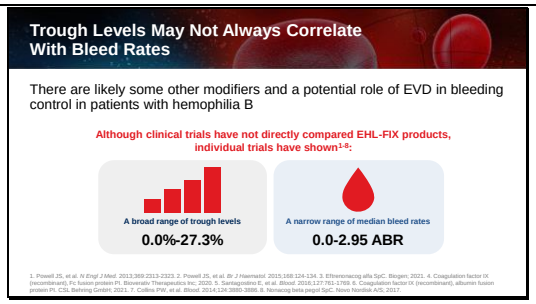
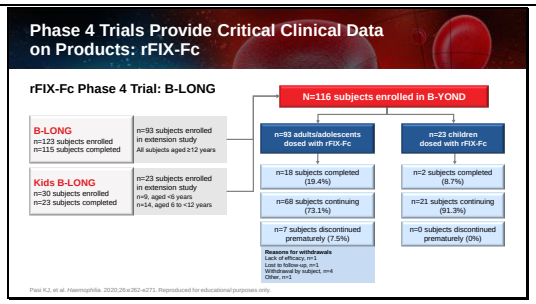
23		So we talked about the 3-compartment model. When you talk about the 1-compartment model, just as a reference, it's really simple compared with the 3-compartment model that we talked about previously.
24		There's a more complex curve. And largely Fc and standard half-life use 3-compartment models, and there's some debate about the 2-compartment model, whether it fits better for N9-GP and for FP. But obviously, still under some investigation. Again, the extravascular distribution is represented in phase 1. Phase 2, you can see that redistribution that we talked about here once it enters the central compartment. And then finally, the elimination which gives you this unique curve here.
25		As mentioned before, the volumes of distribution are different. They're about the same for recombinant FIX and Fc. And they're significantly lower for FP, and a lot lower for N9-GP. And the clinical implication of this is still under investigation. It does seem to matter in mice, and so we're trying to look at the relevancy in humans, of course.
26		Again, one of the things that I think a lot of us do is that we overly focus on half-lives and troughs, and things like that.

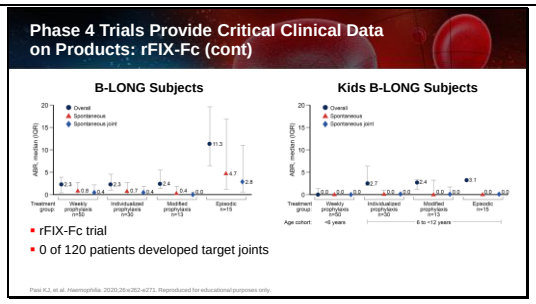
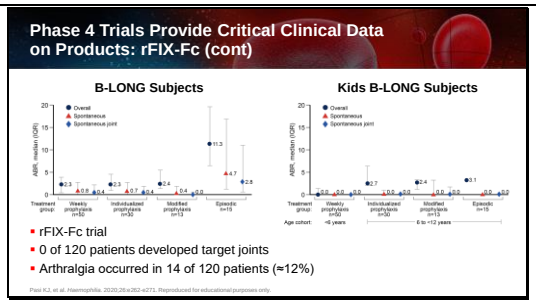
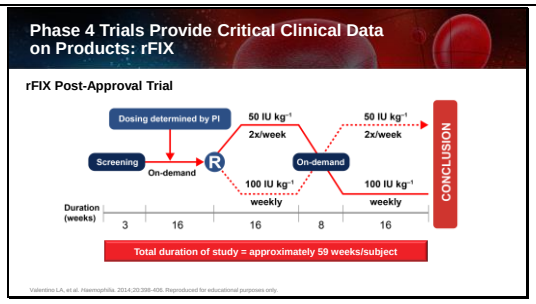
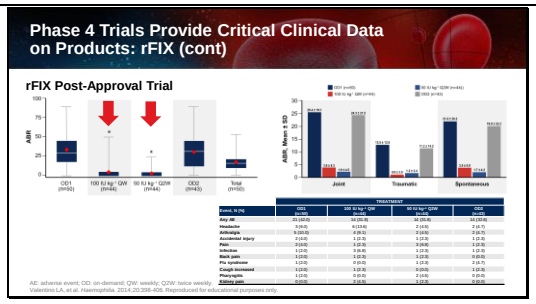
27	**ABRs and Trough Levels in Recent Phase 3 Clinical Trials**  Results are from different studies and therefore inter-product comparisons cannot be made. Slide courtesy of Davide Matino	But I think what this illustrates here is when you look at the trials here, there's significant differences in the troughs. But it's really interesting; for example, NP-GP at 10 IU/kg, trough of 8%, compared with Fc of 1%. And you actually see the ABRs appear to be slightly higher, even though the trough is more, and are similar amongst the products when you take in their pivotal dosing and what the label dosing is. So more isn't always better. Obviously, these are trials that were not done in direct comparison, but something to be aware of; that in the end, we need to worry about how people are controlling their bleeds, how confident they are, and whether they're having significant joint pain.
28	**Case Study**	So to illustrate that, and what really got me interested was this original case that I'm going to present here.
29	**Case: 7-Year-Old Boy With SHB** **Past medical history/past surgical history** ▪ Presented to the ED at age 3 years after recurrent arm swelling following a fracture from a fall; casted multiple times ▪ Plain film noted joint fluid in elbow ▪ No family history of hemophilia **Genetic testing** ▪ FIX <1% on multiple occasions (FVIII, FX, FXI, VWF normal) — c.137G>T in exon 2 of F9 gene, resulted as p.arg46Met.; missense variant  	This is a 7-year-old boy who had no family history. Presented to the emergency room with a recurrent arm swelling and some pain and fractures. And finally, over time, he was diagnosed with hemophilia B—specifically, severe hemophilia B. We did genotyping. He had an extensive workup because we didn't have the family history. And he had a missense mutation or variant.
30	**Case: 7-Year-Old Boy With SHB (cont)** **Diagnosed in setting of untreated joint bleed, likely a target joint** **Pettersson score 3 at time of diagnosis** ▪ Irregularity of the olecranon articulating surface ▪ Osteoporosis ▪ Epiphyseal enlargement **Started initially on rFIX 140 IU/kg twice a week following daily treatment for 3 days to resolve a likely target joint** ▪ Dose lowered to 60-70 IU/kg twice a week for following 2 years 	When he came in, he had a target joint because it was untreated. And he actually had a Pettersson score of 3. We don't do a lot of plain films anymore on young children, particularly those that start primary prophylaxis. But he already had damage to his joints that was seen on plain film, which obviously was upsetting, but we only saw them after diagnosis.

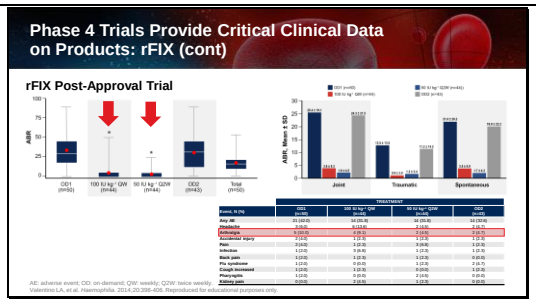
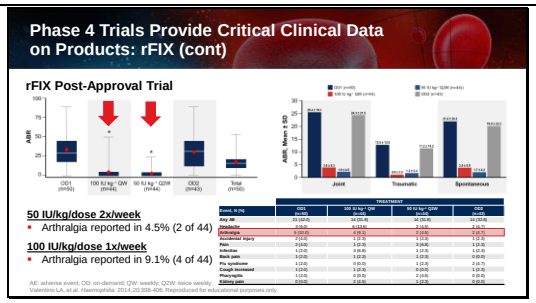
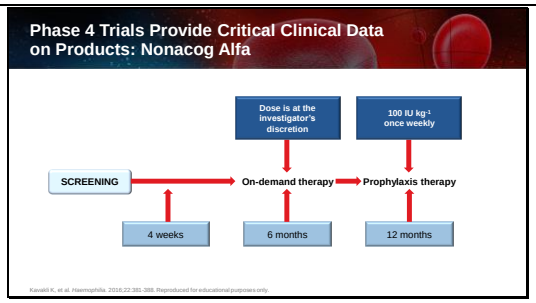
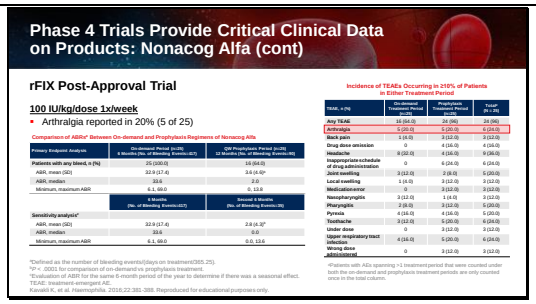
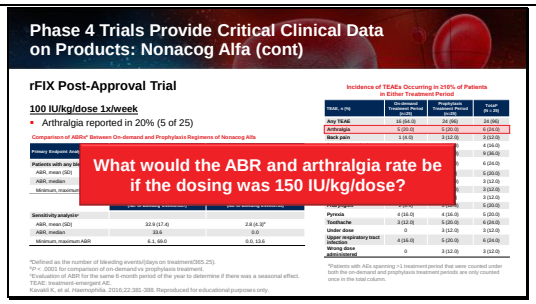
31	**Case: 7-Year-Old Boy With SHB (cont)** **Diagnosed at age 3 years in setting of untreated joint bleed** **We switched to rFIX-FP for potential for higher troughs** ▪ Started rFIX-FP at 50-IU/kg weekly with trough initially at 6% ▪ Seen in clinic due to achiness, pain in bilateral ankle and knees ▪ Because of pain and bruising, we adjusted dose to a FIX trough of >10% ▪ After 6 months and ongoing pain in ankles and left elbow, we brought in for ultrasound and plain films – Left elbow with joint fluid and worsening pathology and bilateral ankles with worsening pathology Patient case study and images provided by and reproduced with permission from Robert Sidonio.	So we treated him fairly high, dosing. Obviously, this is higher than we would typically do. And over time, once we got rid of the target joint, we lowered the dose and he remained on that for multiple years. And we initially heard of recombinant FP. We wanted to switch products. He got dosed per the label and immediately he started having achiness and pain in his ankles. We increased the dosing because he had some bruising. We got his trough over 10%. We evaluated with different assays to make sure it wasn't just our assay at our institution. And then after 6 months of going through this, we brought him in. He actually had ultrasound evidence of a joint bleed. And he actually had worse pathology in his ankles. So we thought this was quite odd because we put him on a product that had a higher area under the curve and higher troughs.
32	**Case: 7-Year-Old Boy With SHB (cont)** **Diagnosed at age 3 years in setting of untreated joint bleed** **We switched to rFIX-FP for potential for higher troughs** ▪ Started rFIX-FP at 50-IU/kg weekly with trough initially at 6% – Seen in clinic due to achiness, pain in bilateral ankle and knees – Because of pain and bruising we adjusted dose to a FIX trough of >10% ▪ After 6 months and ongoing pain in ankles and left elbow, had new joint bleeds **Started on rFIX-Fc 50-IU/kg/dose weekly with 1%-2% troughs** ▪ ABR ≈2 for last 3 years Patient case study and images provided by and reproduced with permission from Robert Sidonio.	So we didn't quite understand this. And I think one of the things that we were trying to understand is why he was having this. Then we decided to start him on recombinant Fc weekly. We talked to the mom about going back on standard half-life products, but he wanted to start Fc. And he did very well with much lower troughs and much lower bleeding. So this was a little bit odd, and we wanted to write this up, which we did. And it initiated a larger study that was led by Dr. Malec.
33	**Excessive Bleeding Reported in Patients Treated With EHL-FIX Despite High Trough Levels** ▪ 3 of 25 patients aged 32-71 years experienced unexpectedly poor bleed control on rFIX-FP once every 14 days (60-65 IU/kg) ▪ No active target joints prior to switch and no evidence of inhibitors **Patient 1**: 4 spontaneous right elbow bleeds between April and November 2018 despite trough of 12%[a] **Patient 2**: Spontaneous right elbow bleed not resolved after 2 extra doses; Persistent hemarthrosis despite plasma FIX of 82%[b] **Patient 3**: 8 spontaneous joint bleeds within 3 months on rFIX-FP; Trough level of 46% after 7 days[c] Single center patient cases: 25 patients with SHB switched to rIX-FP from SHL-FIX. [a]14 days after administration. [b]After administration of 2 extra doses as part of emergency plan. [c]Measured as part of evaluation of a bleed. Kleiboer B, et al. Haemophilia. 2019;26:e23-e25.	We weren't the only institution that saw this discrepancy. At UNC, they had a number of patients. And you can see here, Patient 1—these are noninhibitor patients and mostly adults—and this patient had 4 spontaneous bleeds, despite having a trough of 12%.

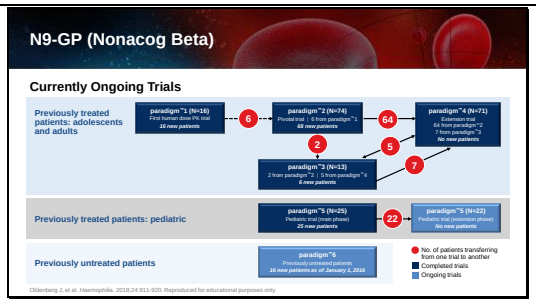
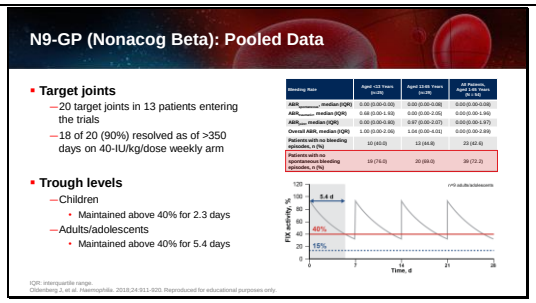
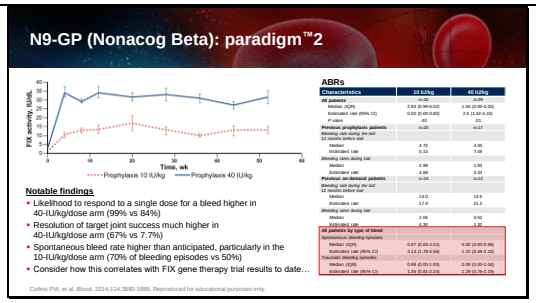
		The second patient had multiple bleeds, continued to have poor control, despite having levels outside of the non-hemophilia range. And finally, Patient 3 had 8 spontaneous joint bleeds in 3 months, despite pushing the trough levels to an almost unreasonable high level that we don't typically do because you would have to almost double the dose of product to get to that. So none of these patients had target joints. Nobody had an inhibitor. And so, clearly there's a discrepancy that doesn't make a lot of sense.
34		And they did get better once you increased the dose significantly. But obviously, that's not what we should have to do to be able to get good control.
35		So we did this institutional study. You can see the centers that were involved here. Dr. Malec was the lead for this.
36		It was a simple survey. We wanted to ask patients that were using extended half-life products:

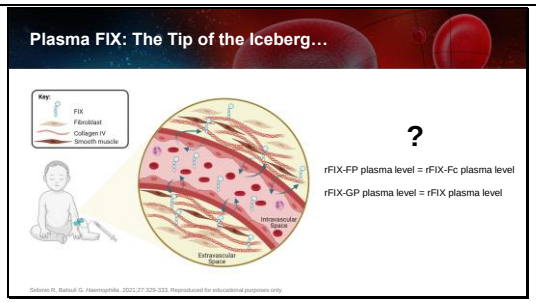
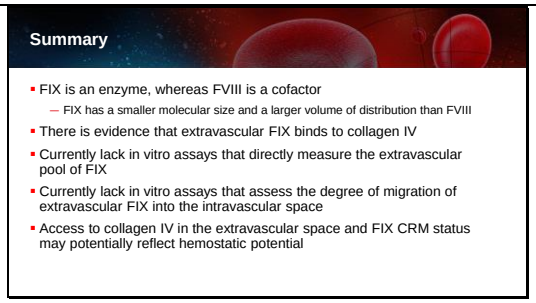
37		Were they having minimally traumatic bleeds? Spontaneous bleeds? Did they require extra doses?
38		And were they having poorly controlled bleeding events more often than anticipated?
39		And then, we wanted to ask why they switched products, of course.
40		So we only focused on extended half-life products, and we had 37 patients that switched that had no bleeding issues, no breakthrough bleeding problems, and not requiring extra doses to treat their bleeds. And that's in contrast to those that were on FP on which we had a lot of patients doing well, but 62% continued to have surprising bleeds requiring multiple doses. And then finally, we had a few patients, but obviously too early to determine, that had bleeding events on N9-GP. Probably too early to really be able to understand the difference.

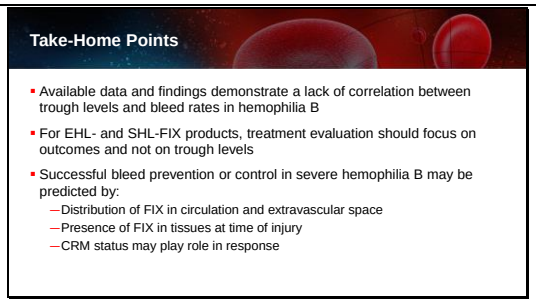
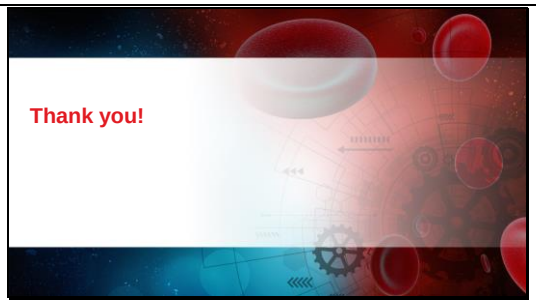
41		And so, we obviously thought there have to be some other modifiers.
42		As mentioned before, you can get trough levels up into the high 20s.
43		But where largely the ABRs have been the same.
44		It's an imperfect, obviously, measurement tool, but there clearly is more to it than just getting the trough levels up. I'm convinced if you got somebody's trough level to 27% in hemophilia A, they would essentially have a 0 ABR the majority of the time. And I think you should see the same in hemophilia B, and we're not.
45		Just a few post-approval trials before we round this up. And I'm going to go through these fairly quickly. Recombinant FIX-Fc had some post-approval trials—the B-LONG. And these were extension studies that looked at 12 and older. The KIDS B-LONG looked in children younger than 12. And you can see here that there were 116 that were enrolled. You can see a number of patients

		continued, majority; some of them discontinued for obvious reasons on long-term trials.
46		But one thing I think is really important, obviously you see episodic treatment has a much higher ABR. But in this trial,none of the patients developed target joints, which I think is really important.
47		And there were some that had arthralgia. And those patients may be some that we want to consider adjusting the doses to make sure that some of that arthralgia isn't actually breakthrough bleeds. We know that there's going to be a baseline arthralgia rate. But something I think we need to pay attention to.
48		Other trials that were done in post-approval. This was done by Dr. Valentino. This was using standard half-life FIX. It was a crossover design looking at 50 UI/kg twice a week, versus 100 UI/kg weekly. And then they would cross over to the other arm. And this was done, and the results have been shown.
49		One of the most interesting things is, we, of course, know that they're going to bleed a lot on on-demand. But look at these box-and-whisker plots, and you see the significant variability in those on 100 IU/kg weekly,compared with those on 50 IU/kg twice a week.

50		And again, I would hypothesize that some of the arthralgia, some of the variability could be explained by cross-reactive material positivity. Maybe those that did really well on 100 UI/kg weekly were not missense mutation, and those that did not respond as well were. And so, there may be some interference with defective FIX.
51		And you saw much more arthralgia in those that were on once-a-week. So there are patients that could respond really well to that and may benefit from weekly dosing.
52		And then finally, nonacog alfa and N9-GP. They looked at 100 UI/kg weekly. Sorry, this is again, standard half-life FIX, 100 UI/kg weekly. A similar study, but a single-arm study.
53		And again, to cut to the chase, you see some arthralgia issues in those that are on weekly dosing, and a big variability in response. Some patients did extremely well, and some did not do so well. Again, I would love to be able to go back and understand the genetic differences to see if that could explain why some patients did better than others.
54		It always comes back, for me, what would happen if they were on much higher doses, like in the dose that was hypothesized in the mouse model.

55		When you look at N9-GP, they had their studies. They had their extension studies as well.
56		But the most important thing is, for me, there were 20 target joints in 13 patients entering the trial. As of 350 days later, most of the patients resolved their target joints. To be honest, I don't know why all of them wouldn't; I would have expected patients that were on such high doses. The overall ABRs were quite low on this study, so you would expect. A lot of patients did very well, but it made me think why we couldn't fully resolve those target joints, particularly when you look at the kinetics of children above 40% for 2.3 days, and adults over 40% for 5 out of the 7 days of the week. And so, for me, I wonder why you wouldn't be able to resolve those target joints much quicker, and certainly by a year.
57		Again, we're going to be trying to understand this concept of time spent in the non-hemophilia range. They looked at 10-UI/kg dosing previously. And there seemed to be a lot more bleeding in that lower dose arm. So it makes me wonder, that dose of 40 UI/kg may be able to overcome some of those issues with extravascular distribution. The resolution target joints were much better with that dosing. And so, maybe getting those levels up higher may be able to overcome some of the differences that you see with collagen IV binding as well. And again, look at the gene therapy studies. Those patients had levels of 1% to 5% and had ABRs that were similar to this trial, with obviously a much lower

		trough level and overall time spent above a certain threshold.
58		Something to think about: Dr. Batsuli and I wrote a nice editorial. Hopefully you'll check it out. She did a really nice job. Beautiful illustrations. The remaining questions for me moving forward are: Do all these levels mean the same? Is the Fc level the same as an FP level? N9-GP level? A standard half-life level? I think these are all things that we should think about moving forward, and hopefully people will step up and continue to do the good research in this field.
59		In summary: We know that FVIII is a cofactor. They have a different molecular size, FIX being much smaller, being an enzyme and having a larger distribution. We're hoping that you understand that there is extravascular distribution. There is binding to IV collagen. We don't have a way to measure this. Obviously, we would love to have that ability. And there may be some differences in CRM status that may reflect the hemostatic potential that we see between products.

60		Some of the take-home points: Hopefully, we'll continue to get available data. I showed you some that demonstrates a lack of correlation between troughs and bleeds. We know that for the products, treatment evaluation should focus on outcomes, not on troughs. And then hopefully, you understand that bleed prevention and control potentially could be predicted by distribution, presence of FIX at the time of injury, and potentially CRM status may play a role in response.
61		Thank you very much for participating in this presentation and listening to me. Thank you.