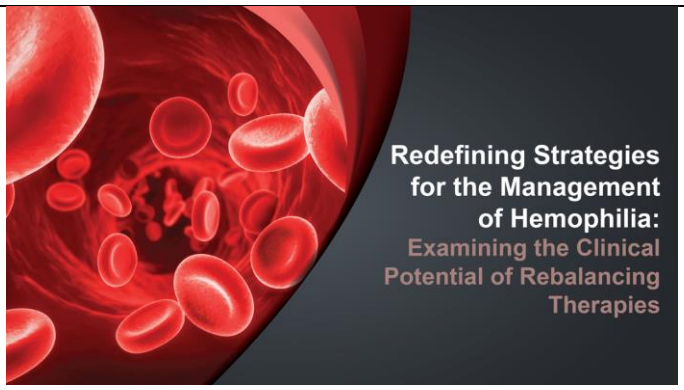
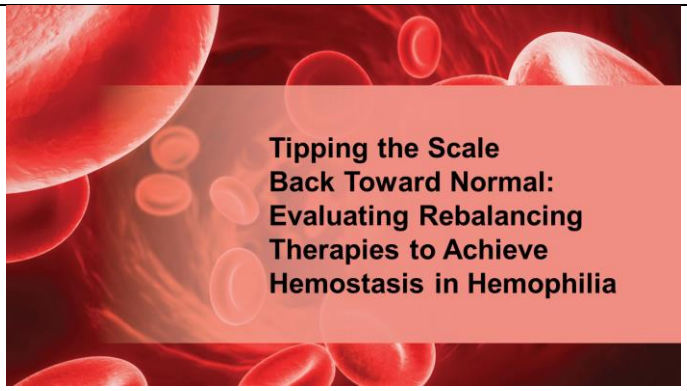
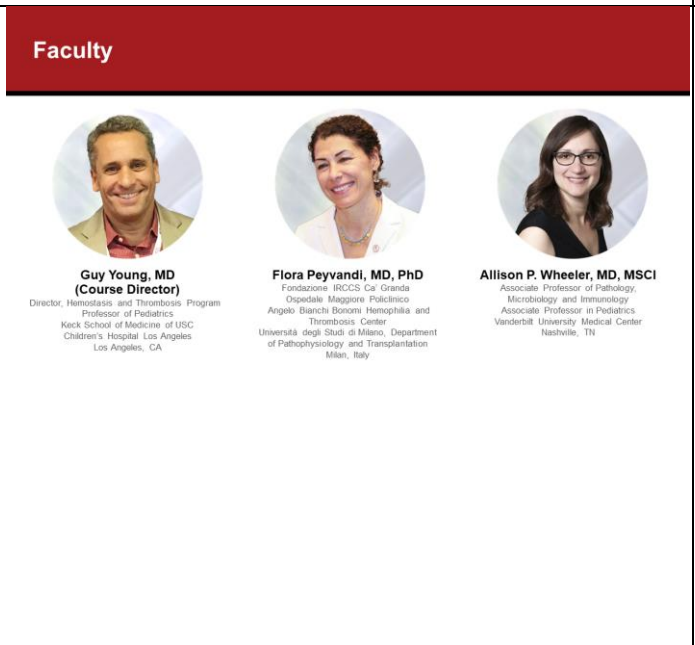
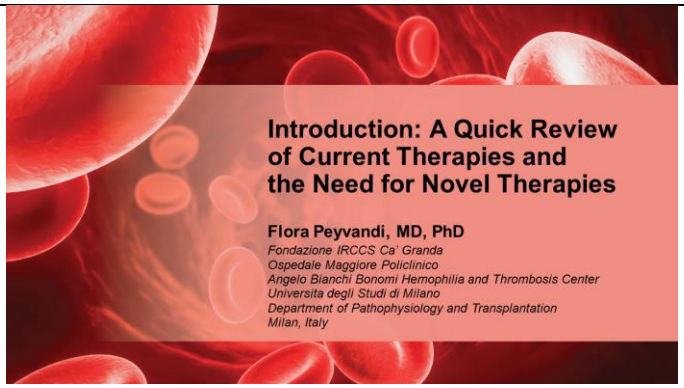
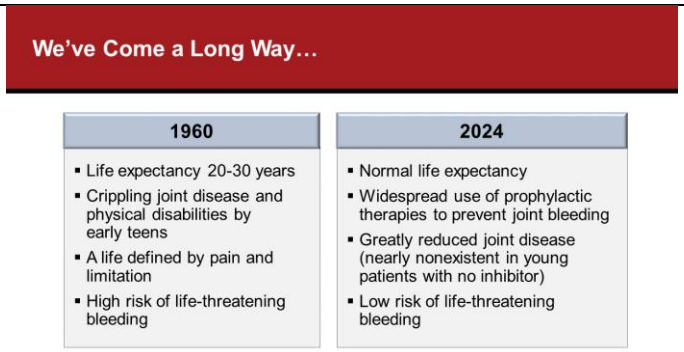
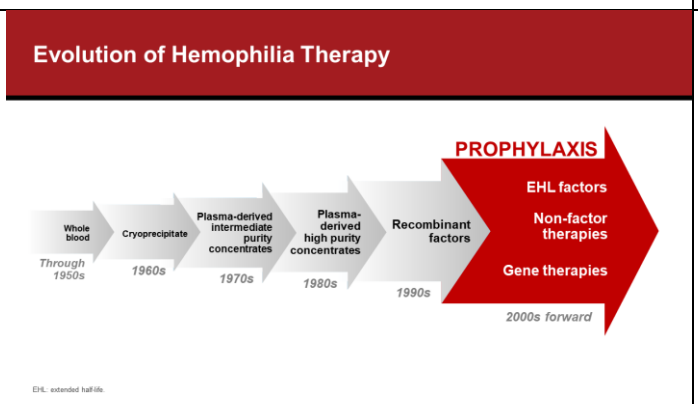
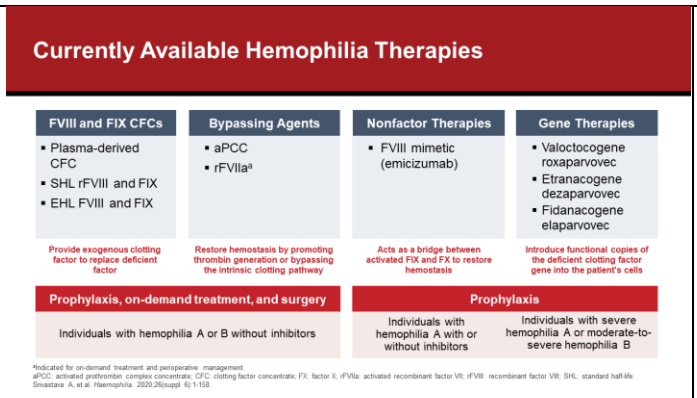
1.		***[Guy Young, MD]*** Hello, everybody. My name is Guy Young from Children's Hospital Los Angeles, University of Southern California. And I'm really glad that you're here with us to be discussing "Redefining Strategies for the Management of Hemophilia." We're going to be, in particular, you can see in the subheading, examining the clinical potential for rebalancing therapies.
2.		Another way to think about it is tipping the scale back toward normal. We're going to evaluate rebalancing therapies to achieve hemostasis in hemophilia. That's really the first part of this talk.
3.		We have really excellent faculty. There's me, I'm the course director. I've already introduced myself. We have the excellent Flora Peyvandi. Flora is an exceptionally experienced physician in adult hematology and an expert in hemophilia. And you can see that she's from the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center at the University of Milan in Milan, Italy. And then we have Dr. Allison Wheeler. Dr. Wheeler is an associate professor of pathology, microbiology, and immunology and associate professor of pediatrics at Vanderbilt University. And I'll be sharing this discussion and these talks with them.

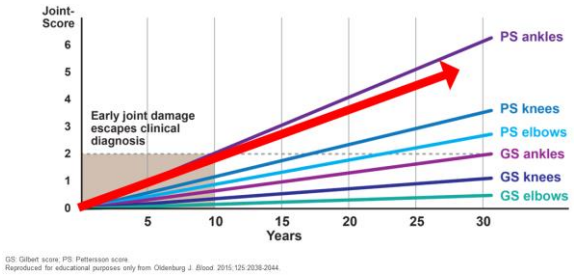
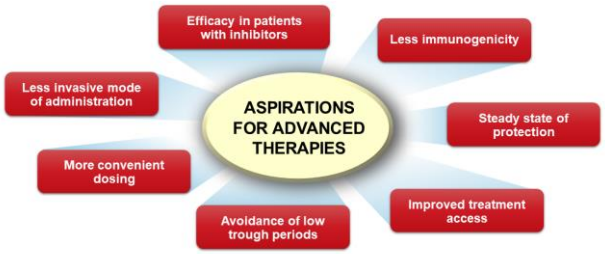
4.	**Learning Objectives** ▪ **Explain** the latest clinical understanding of the secondary hemostasis cascade under physiological conditions, hemophilic conditions, and thrombosis conditions ▪ **Describe** the mechanism of action and downstream clinical effects on hemostasis of non-factor rebalancing therapies under investigation for the management of hemophilia ▪ **Evaluate** clinical data on emerging rebalancing therapies targeting anti-thrombin and other coagulation inhibitors considering varying outcomes, including PK/PD, joint bleeding, spontaneous bleeding, annual bleeding, and safety/tolerability PK/PD: pharmacokinetic/pharmacodynamic	So, our agenda: first, Dr. Peyvandi is going to take us on a quick review of current therapies and the need for novel therapies. That will serve as the introduction to our session. I will then discuss exploring new mechanisms to restore hemostasis—rebalancing coagulation. And then Dr. Wheeler is going to talk about attaining new goals: is a functional cure possible with emerging therapies that rebalance coagulation?
5.		***[Flora Peyvandi, MD, PhD]*** Thank you very much, Dr. Young, for this kind introduction. I'm going to do the session on hemophilia education and what are the novel strategies for treatment in these types of rare disorders?
6.	**Overview of Hemophilia A and B** **Hemophilia A** **Prevalence:** 1:5000 males **Mode of inheritance:** X-linked recessive **Clinical symptoms:** Joint bleeding, muscle hematoma, soft tissue bleeding **Characteristics of missing clotting factor (FVIII):** ▪ **Function:** Cofactor ▪ **Molecular weight:** 280 kDa ▪ **Normal plasma concentration:** 0.1-0.25 µg/mL  **Hemophilia B** **Prevalence:** 1:25,000 males **Mode of inheritance:** X-linked recessive **Clinical symptoms:** Joint bleeding, muscle hematoma, soft tissue bleeding **Characteristics of missing clotting factor (FIX):** ▪ **Function:** Enzyme ▪ **Molecular weight:** 55 kDa ▪ **Normal plasma concentration:** 3-5 µg/mL FVIII/IX: factor VIII/IX Image adapted for educational purposes from Castaman G, Matino D. *Haematologica*. 2019;104:1702-1709.	First of all, we have to bring to your attention: this kind of rare disorder could be due to deficiency of factor VIII, which is called hemophilia A, or due to deficiency of factor IX, which is called hemophilia B. And the prevalence of these 2 types of disorders is changing. Hemophilia B is much rarer, and hemophilia A is 1 case in every 5000 males. Both of them are X-linked recessive disorders, and clinical manifestations are very similar, mainly with joint bleeding, muscle hematoma, and soft tissue bleeding. There are some data in the literature that report that the severity of the bleeding in patients with hemophilia B might be lower than hemophilia A, but I would say there are not enough data to confirm such a conclusion. In terms of the protein: for factor VIII, you can see that the role of factor VIII in hemostasis is a cofactor. And for factor IX, an enzyme. The

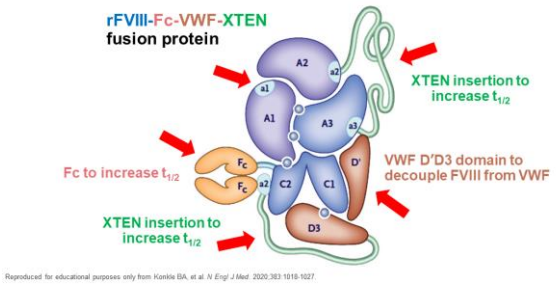
		molecular size of factor VIII is almost 5 times higher than factor IX, so it is much more complex. And the concentration of this protein is also much lower, about 0.1 to 0.25 µg/mL. That concentration is significantly higher, in fact, for factor IX, about 3 to 5 µg/mL.
7.		Here we can see how the life expectancy of these patients who are missing factor VIII and factor IX, or who have hemophilia A and B, has changed in the last 50 years. The life expectancy in the '60s was about 20 to 30 years. However, patients with hemophilia now have a normal life expectancy. In the '60s to '70s, we had patients with severe joint damage, significant physical limitations, and poor quality of life due to pain and a high risk of life-threatening bleeding. In 2024, we now have several novel drugs, which are improving the quality of life of our patients with easier prophylactic treatment, greater clinical improvement, and also a low risk of life-threatening bleeding. During the last 5 decades, a lot has been happening for diagnosis and for treatment.
8.		In the '50s, patients were treated with whole blood. In the 80's, after the cloning of factor VIII in 1984 and factor IX in 1989, recombinant products were developed. And then following that, recently in the last 2 decades, we have seen extended half-life products, nonreplacement therapies, and gene therapy. What I'm going to do now is discuss a little bit about these achievements and how the treatment of patients has changed.

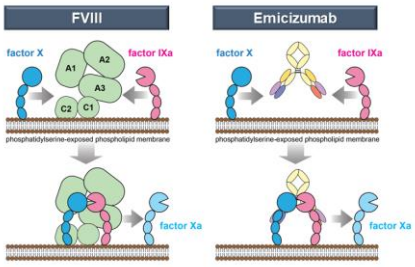
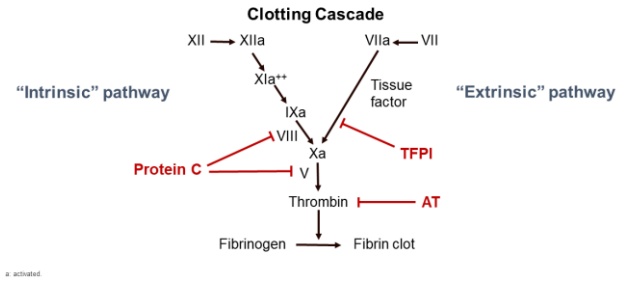
9.		Right now, we have availability of several products, depending on where you are living in the world. We have factor VIII and factor IX, plasma-derived products, a standard. We have, for recombinant products, both standard half-life products and extended half-life products. For those patients who have inhibitors, there is availability of bypassing agents, which could be recombinant factor VIIa with a shorter half-life, and we have activated prothrombin complex concentrates, which have a longer half-life. And both of them have been very, very important in the treatment of the patient with inhibitors, until the arrival of the factor VIII mimetic, emicizumab. This nonfactor therapy was really revolutionary and changed the lives of our patients with hemophilia, particularly those with inhibitors or neutralizing antibodies against factor VIII and factor IX. And finally, we have gene therapy. There are 3 available gene therapy products. And gene therapies, along with nonfactor therapies, are useful for prophylaxis. The level of gene expression from gene therapy allows it to be used not only for prophylaxis but also for minor surgeries, and possibly even for regular surgeries. So, I would say that there is no doubt that all these products could help us better treat our patients in both conditions of on-demand and prophylaxis, and also during surgery. We have to keep in mind that nonfactor therapies are only for prophylaxis and usually are subcutaneous.

10.	**Prophylaxis With Factor Replacement** ▪ Regular replacement of FVIII or FIX to **prevent** bleeding —Original goal of prophylaxis was to maintain factor levels >1%-2% ▪ Hemophilia A —FVIII $t_{1/2}$ 12 hours —FVIII 3 times weekly (sometimes every other day) ▪ Hemophilia B (FIX) —FIX $t_{1/2}$ 18-24 hours —FIX twice weekly $t_{1/2}$: half-life	Let's start with prophylaxis using factor replacement. This was conceptually the initial treatment for the patient—regularly replacing the missing factors, both factor VIII and factor IX, to prevent bleeding. The original treatment was mainly based on keeping factor levels over 1% or 2%, because the half-life of the product for factor VIII was about 8 to 12 hours, and that required 3-times-weekly intravenous infusion. For hemophilia B, the half-life was about 18 to 24 hours, which means twice weekly. So, that short half-life, or standard half-life, was not allowing us to keep a trough level higher than 1% or 2%. So, the protection of our patients was much higher in the first 24 hours, but then it was getting reduced in the following days.
11.	**EHL Factor (First-Generation)** **FVIII** ▪ FVIII attached to Fc or PEG (single-chain FVIII) ▪ $t_{1/2}$ extended 1.5 times —~18 hours ▪ Given twice weekly or every 4-5 days (more often to maintain higher trough levels) ▪ Trough levels ~5% (variable) **FIX** ▪ FIX attached to Fc, albumin, or PEG ▪ $t_{1/2}$ extended 4-5 times —~4-5 days ▪ Given once every 7-14 days ▪ Trough levels >10%-15% PEG: polyethylene glycol	With the introduction of the first generation of extended half-life products, using different strategies—mainly Fc-fusion or PEGylated product—there was a big change in terms of the half-life, particularly for factor IX. This allowed for a substantial reduction in the number of infusions, shifting from twice a week to every 7 days or even every 14 days. And the trough level is much higher, over 10% to 15%. And that was due to the extension of the half-life of the product by about 4 to 5 times. With factor VIII, the same result has not been achieved. And the reason is the half-life of von Willebrand factor. As you know, factor VIII travels with von Willebrand factor. You can extend the half-life of factor VIII, but because of the limitation of von Willebrand factor's half-life, the half life of factor VIII is limited to about 17 to 18 hours. And that's the reason why, maximum, you could increase the interval

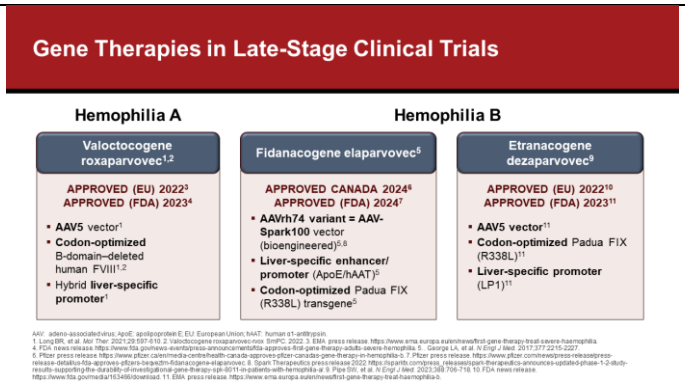
		from every 2 to 3 days to every 4 to 5 days. And the trough level was increased up to 5%.
12.	**Current Factor Therapy** **Pros** ▪ Replacing what is missing ▪ Long history of use ▪ Safe (except inhibitor risk) ▪ Peak levels are in normal range ▪ Can give extra doses ▪ Same product to treat bleeds **Cons** ▪ Must be given as an IV ▪ 2-4 times/wk for FVIII ▪ 1 per week/2 weeks for FIX ▪ Difficult to adhere ▪ Many kids need ports ▪ Factor levels fluctuate ▪ Trough levels lead to bleed risk IV: intravenous.	So, what are the pros of this factor replacement therapy? Replacing means introducing what is missing. And with that type of a strategy, you can plan which level of protection you need, and you can set a goal to achieve that level. There is a long history, as I said, almost 30 to 50 years, of using these drugs. They are safe, except the development of neutralizing anti-factor VIII (particularly), and to some extent, also factor IX (much less) antibodies, during the first 20 to 50 exposure days. But after that period of time, the development of inhibitors is very, very rare. The peak level is in the normal range. After 15 to 30 minutes, we can have a completely normal level of factor and administer extra doses when needed for surgery, or for acute bleeding. The same product can be used to treat bleeds. Yes, that means you can have 1 single product, and that 1 single product could be measured with the same assay and managed every time for both prophylaxis and for acute bleeding. What are the cons? The cons are the method of infusion, which is intravenous. And in kids, that was a big issue, 2 to 4 times per week for factor VIII and almost 2 times per week for factor IX. In adolescence, there are difficulties in adherence. Many kids need ports to maintain accessibility of the veins (easy access for intravenous administration). And also, factor levels fluctuate and affect physical ability. This means that levels are changing, both over time and interindividually, and the trough level leads to

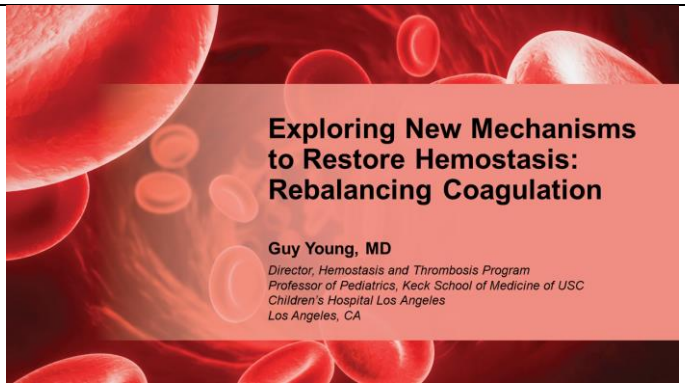
		bleeding risk, when the level gets lower and the risk of bleeding increases.
13.	**Joint Scores Worsen Despite Intensive Prophylaxis**  GS: Gilbert score; PS: Pettersson score. Reproduced for educational purposes only from Oldenburg J. Blood. 2015;125:2038-2044.	Now, what is the condition and situation of the joints? We know that all joints of patients could be affected. However, you can see as the age of the patients increases, the damage to some joints is higher, and ankles are a big issue for the patients. And, particularly, this is after 20 years. However, you can see the conservation of the joints in the first 5 to 10 years is very, very important. So, we can see ankles, knees, elbows—these are the type of joint that we have to work on preserving and try to avoid any kind of damage by using prophylaxis. However, despite the usage of these products and intensive prophylaxis, you can see worsening of these joints with increasing age in our patients.
14.	**Beyond Standard Factor Replacement** 	So, what are the aspirations now for advanced therapy? There are several. For the reasons that we have explained, they are more convenient, have less invasive modes of administration, show efficacy in patients with inhibitors, have less immunogenicity, provide a steady state of protection, improve access to treatment, and avoid low trough periods. So, for all those reasons, having extended half-life products has been a really great advantage.
15.	**Transformative Therapies** ▪ **FVIII modification: Efanesoctocog alfa (rFVIII-VWF D'D3-XTEN)** ▪ FVIII mimetics: eg, emicizumab ▪ Re-balancers of hemostasis —siRNA • siRNA-AT for all patients with hemophilia —Inhibitors of inhibitors • Activated protein C inhibitor for all patients with hemophilia • Anti-TFPI for all patients with hemophilia ▪ Cure or near-cure —Gene therapy for hemophilia A and hemophilia B AT: antithrombin; VWF: von Willebrand factor; siRNA: small-interfering RNA; TFPI: tissue factor pathway inhibitor.	But let's see the second generation of extended half-life products, or those that are achieving almost normalization of the factor levels. The novel product, which is called efa (efanesoctocog alfa), is a combination of recombinant von Willebrand factor—adding to it a fragment of von Willebrand factor, which is D'D3 —and Fc plus XTEN. The combination of all these different components

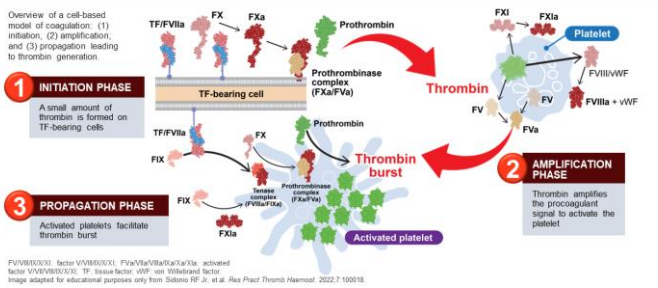
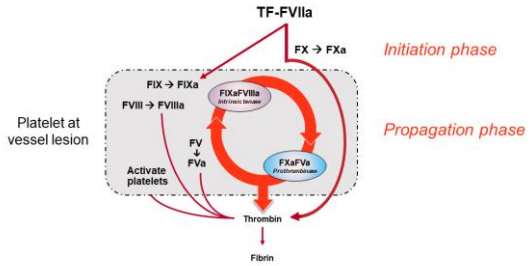
		makes the half-life of this product significantly higher. And we're going to see that. Then, factor mimetics and rebalancing agents represent a very new concept in hemostasis. We will see what that means and how that works. And finally, we are heading towards a near cure. We are not yet at a cure, but maybe in the future. For now, we are looking at long-term responses with gene therapy for both hemophilia A and B.
16.	**FVIII Replacement Therapy: Efanesoctocog Alfa (BIVV001) Fusion Protein**  Reproduced for educational purposes only from Konkle BA, et al. *N Engl J Med*. 2020;383:1018-1027.	Let's start with the first component, which is efanesoctocog alfa or BIVV001. This product, as I said, consists of recombinant Fc/von Willebrand/factor VIII/XTEN and is a very interesting and fascinating product, which reduces significantly the number of bleeding episodes in our patients with the increase of the half-life of factor VIII and also less degradation of the product. With this molecule, you are able to actually have protection of about 40% for the first 3 days and about 10% to 15% after a week.
17.	**Transformative Therapies** ▪ FVIII modification: Efanesoctocog alfa (rFVIII-VWF D'D3-XTEN) ▪ **FVIII mimetics: eg, emicizumab** ▪ Re-balancers of hemostasis —siRNA • siRNA-AT for all patients with hemophilia —Inhibitors of inhibitors • Activated protein C inhibitor for all patients with hemophilia • Anti-TFPI for all patients with hemophilia ▪ Cure or near-cure —Gene therapy for hemophilia A and hemophilia B	What about the factor VIII mimetics?

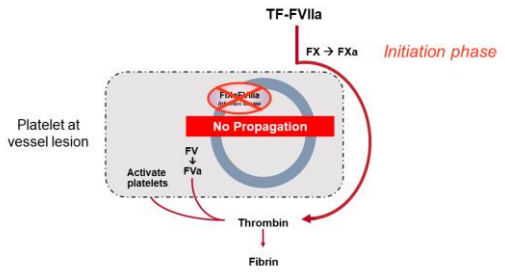
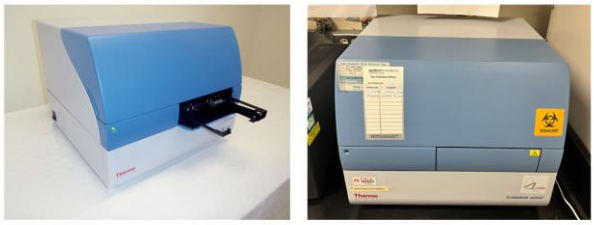
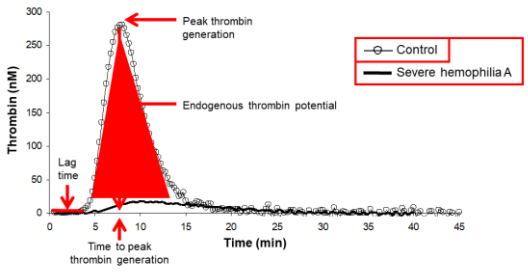
18.	**Emicizumab: FVIII Mimetic** ▪ Humanized bispecific antibody ▪ **Exerts FVIII mimetic activity** ▪ Not affected by FVIII inhibitors ▪ Good subcutaneous absorption ▪ Long $t_{1/2}$ (4-5 weeks)  Shima M, et al. *N Engl J Med*. 2016;374:2044-2053.	For example, emicizumab. That molecule, which, as I said, was revolutionary, changing the history of treatment of patients. It is a humanized bispecific antibody mimicking factor VIII activity by using 2 antibody arms. One binds to factor IX and the other one to factor X, activating factor X to factor Xa. It is not affected by factor VIII inhibitors. That means it could be used in both hemophilia A with or without inhibitors. You cannot use it in hemophilia B. The infusion is subcutaneous, and the half-life is about 4 to 5 weeks. There were several clinical trials. And also, now we have a large body of real-world evidence to show a very high efficacy of this product and good safety.
19.	**Transformative Therapies** ▪ FVIII modification: Efanesoctocog alfa (rFVIII-VWF D'D3-XTEN) ▪ FVIII mimetics: eg, emicizumab ▪ **Re-balancers of hemostasis** —**siRNA** • **siRNA-AT for all patients with hemophilia** —**Inhibitors of inhibitors** • **Activated protein C inhibitor for all patients with hemophilia** • **Anti-TFPI for all patients with hemophilia** ▪ Cure or near-cure —Gene therapy for hemophilia A and hemophilia B	What about rebalancing agents? This is, again, a different and new concept of treating hemophilia. How does it work?
20.	**Rebalancing Hemostasis** 	Here you can see the cascade of coagulation with almost 12 procoagulant proteins. And you can also see in the red part the 3 natural anticoagulants: antithrombin, tissue factor pathway inhibitor (TFPI), and protein C. The new concept of this therapeutic approach is, instead of increasing the procoagulant activity of each single factor by its administration, to reduce the activity of these natural anticoagulants.

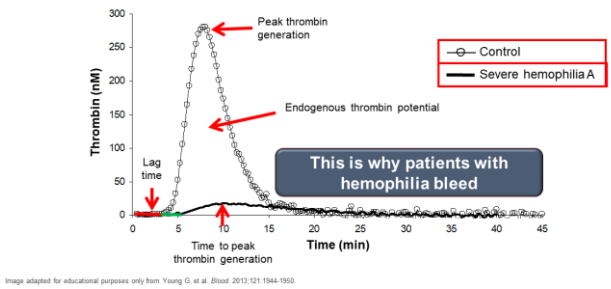
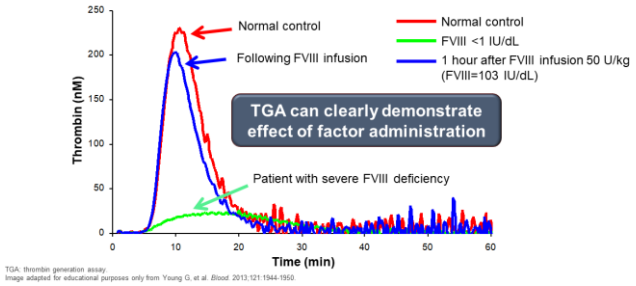
21.	**Transformative Therapies** ▪ FVIII modification: Efanesoctocog alfa (rFVIII-VWF D'D3-XTEN) ▪ FVIII mimetics: eg, emicizumab ▪ **Re-balancers of hemostasis** —**siRNA** • **siRNA-AT for all patients with hemophilia** —**Inhibitors of inhibitors** • **Activated protein C inhibitor for all patients with hemophilia** • **Anti-TFPI for all patients with hemophilia** ▪ Cure or near-cure —Gene therapy for hemophilia A and hemophilia B	And with this approach, you can use a different therapeutic strategy, like silencing the transcription of RNA of antithrombin in the liver. By reducing antithrombin, you are increasing levels of procoagulant activity, and that reduction leads to antithrombin levels of 15% to 35%, which has been shown to be very efficacious in the reduction of the number of bleeding episodes. Other strategies include an activated protein C inhibitor, which could be used for all patients with hemophilia A and hemophilia B with and without inhibitors. And also, the use of anti-TFPI for all patients with hemophilia is another novel strategy, and can be used for both patients with hemophilia A and B with and without inhibitors.
22.	**Emerging Rebalancing Therapies Mostly Target Natural Anticoagulants** ▪ Antithrombin —Fitusiran ▪ TFPI —Concizumab —Marstacimab —Befovacimab ▪ Protein C —SerpinPC —SR604 ▪ Subcutaneous administration ▪ Long half-lives, stable PK ▪ Hemophilia A and B ▪ Also patients with inhibitors **But:** ▪ How to measure? ▪ Thrombosis risk ▪ Antidrug antibodies PK: pharmacokinetic	The first one, which was the reduction of activity of antithrombin—the molecule is called fitusiran. And for anti-TFPI, there are 3 molecules—concizumab, marstacimab, and befovacimab—that are in clinical trials. Concizumab has been approved for hemophilia B with inhibitors in Canada. And SerpinPC and SR604 work on protein C. All of these molecules are administered by subcutaneous infusion. They have a long half-life and stable pharmacokinetics. They could be used for hemophilia A and B with or without inhibitors. The difference in treatment here compared with replacement therapy is that you don't have the peak and trough, and the protection of the patients are stable over time. And that stability should help the patient to have more safety, stability, and security and to be more productive. However, there are some difficulties due to the novelty of these therapies, and that requires more work in the future. We need to

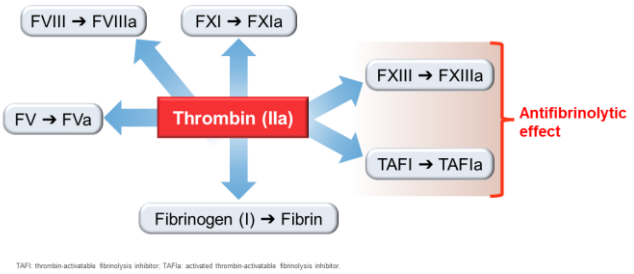
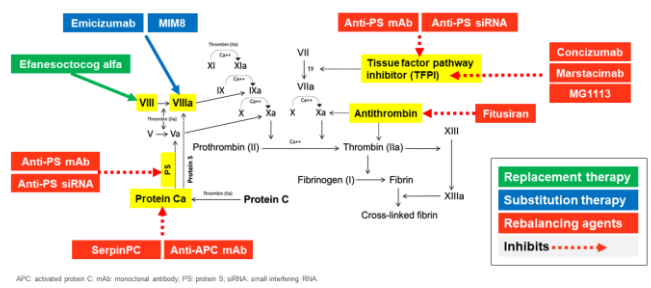
		learn how to measure them. Thrombin generation has been used but still is not standardized. Thrombin generation assays are used in research but not in the clinical activity of [hemophilia] centers, for the management of patients. There could be a thrombosis risk because if you're increasing the level of the protection in these patients, they are becoming more and more normal and they are very similar to the general population. And the general population could have thrombosis. That's the reason why we need to learn from each single product. And the presence of antidrug antibodies, which could be present in different categories. However, what is important to understand is the neutralizing antibody, which fortunately seems to be low with these agents.
23.		And the last part is gene therapy. Several phase 3 clinical trials have used adeno-associated virus (AAV) vectors. And here you can see that 2 of them have been approved by the United States Food and Drug Administration and the European Medicines Agency, and 1 was approved in 2024 for factor IX deficiency. What has been used? The first gene therapy (valoctocogene roxaparvovec), is approved for hemophilia A and uses an AAV vector by codon optimization and B-domain–deleted factor VIII. And the promoter is a liver-specific promoter. The second gene therapy, fidanacogene elaparvovec, is a recombinant, adeno-associated, liver-specific enhancer/promoter using codon optimization and also a factor IX Padua mutation, which significantly increases the level of factor IX expression. The third gene therapy,

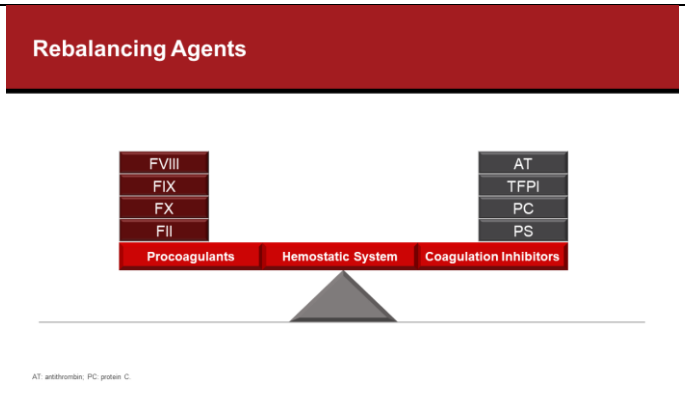
		etranacogene dezaparvovec, is approved for hemophilia B, and also uses an AAV5 vector with codon optimization, including factor IX with Padua mutation, with a liver-specific promoter. Several data from the clinical trials have been published, showing the safety and efficacy of each of these products. Now, the work for all of us—clinicians, scientists, and patient organizations—is to understand the safety and efficacy of each single product. We need to harmonize communication and data to make it available and transparent for clinicians and patients, so that in the future, we can understand the power, potency, and benefits of each product and determine which type of product is suitable for which type of patient. So, individualization would be the future. And there is not 1 single product that could be suitable for all patients. We have, fortunately, several products that could be used for different patients. Thanks very much for your attention.
24.		***[Guy Young, MD]*** All right. Well, thank you, Dr. Peyvandi, for that really excellent introduction to this session. And I'm going to take over now and talk about exploring these new mechanisms to restore hemostasis, basically these rebalancing mechanisms.

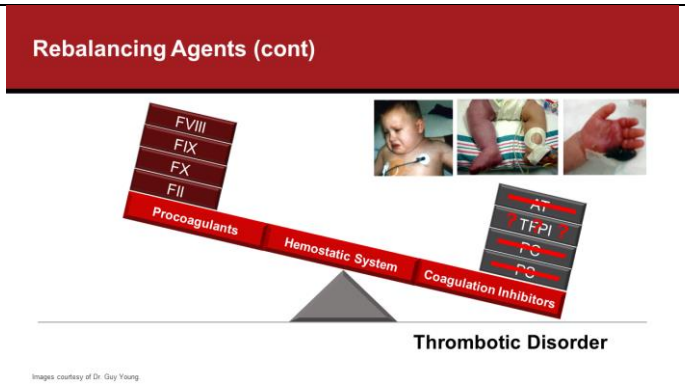
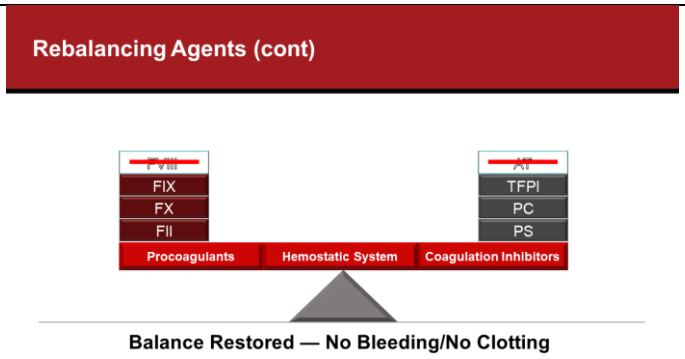
25.	**Hemophilia Is a Bleeding Disorder Caused by Defective Thrombin Generation** 	So, let's take a look at the role of thrombin generation in the coagulation cascade. And the coagulation system is really not a cascade. But it involves 3 steps: initiation, amplification, and propagation. In the initiation phase, small amounts of thrombin are formed on tissue factor–bearing cells via the tissue factor pathway. Now, this amount of thrombin—or, this basically small amount of thrombin that is formed—has multiple functions. And you can see here its functions are to activate factor V to Va, factor VIII to VIIIa, and also factor XI to XIa. This happens on the surface of the platelet. With activated factor VIII and activated factor V, and, if needed, activated factor XI, we then get into the thrombin burst. And this is really the amplification to propagation phase. Because we really need a burst of thrombin to generate fibrin in its proper form, as well as what's not on the slide, factor XIII and thrombin-activatable fibrinolysis inhibitor, which ultimately help to form a stable fibrin clot.
26.	**Healthy Hemostasis** 	Another simpler way to look at it is like this. Again, the initiation phase started by tissue factor. When tissue factor is exposed to the subendothelium via VIIa and Xa, a small amount of thrombin activates platelets, and on the surface of these activated platelets, factor VIII gets activated and factor V gets activated. And factor VIII, the cofactor for factor IX, and factor V, the cofactor for factor X, then result in this propagation phase, where we generate a large amount of thrombin. And that large amount of thrombin

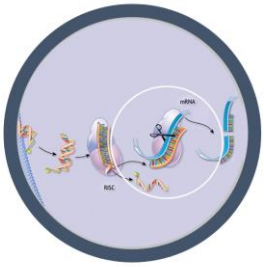
		will then generate a large amount of fibrin to make a clot.
27.	**Hemostatic System Without FVIII or FIX** 	So, what is the hemostatic system like without factor VIII or factor IX? That is, if you have hemophilia A or hemophilia B. The initiation phase works. You have generated a small amount of thrombin. And this is important because as we talk about some of these coagulation inhibitors and the inhibitors of those coagulation inhibitors, in other words, the rebalancing agent, it's important to understand that hemophilia patients can generate small amounts of thrombin. The problem is they cannot get to the propagation phase, and they cannot generate large bursts of thrombin. And that's where any drug to treat hemophilia must have a way to overcome this.
28.	**Thrombin Generation Device**  Images used for educational purposes only from Riley P. *Genet Eng Biotechnol News*. 2012;32:52 (left) and courtesy of Guy Young, MD (right).	One way to assess thrombin generation is through a thrombin generation device, which you can see here. This is the one that's in my lab on the right side. And there's a picture from a journal article on the left side where you see the chamber that's open.
29.	**Thrombin Generation Curve**  Image adapted for educational purposes only from Young G, et al. *Blood*. 2013;121:1944-1950.	Let's walk through a normal or controlled thrombin generation curve and one with severe hemophilia. So, the y-axis is the amount of thrombin formed, and the x-axis is time. Initially, there's a bit of a lag. And this is normal in every situation to have a lag time, a few minutes before thrombin starts to be formed. Then we have peak thrombin generation, the highest point the curve gets to. We have the time to the peak. And, importantly, then this area under the curve.

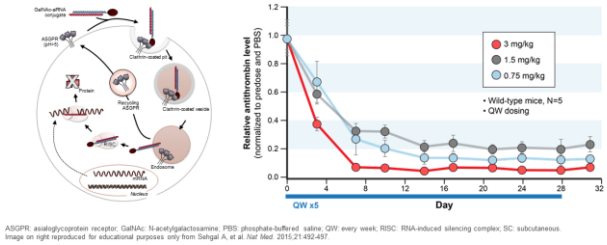
		This area under the curve is called the endogenous thrombin potential.
30.	**Thrombin Generation Curve (cont)**  Image adapted for educational purposes only from Young G, et al. *Blood*. 2013;121:1944-1950.	So, this is the normal curve. Look at a patient with severe hemophilia. The lag phase is more or less the same, but slightly longer. But here's the big difference: although the time to peak thrombin might be similar, look at the difference in the peak thrombin, where we're up well over 250 nm here, and we're barely blipping to 10 or 20 nm here. That's the difference. Hemophilia patients cannot generate thrombin. Of course, the endogenous thrombin potential, the area under the curve. You don't need calculus to know that that area there is much larger than the little bit of area under this curve. And this is why hemophilia patients bleed. They cannot generate thrombin.
31.	**Thrombin Generation Curve (cont)**  TGA: thrombin generation assay. Image adapted for educational purposes only from Young G, et al. *Blood*. 2013;121:1944-1950.	So, what if we took a hemophilia patient and gave them factor VIII, for example? Well, there's the normal curve. There's the hemophilia curve—severe factor VIII deficiency. And if we give a patient 50 units/kg of factor VIII—correct their factor VIII basically to normal—they get a normal thrombin generation curve. So, this is a good experiment that shows you that we can see the difference with treatments for hemophilia, particularly those that generate thrombin such as factor VIII.

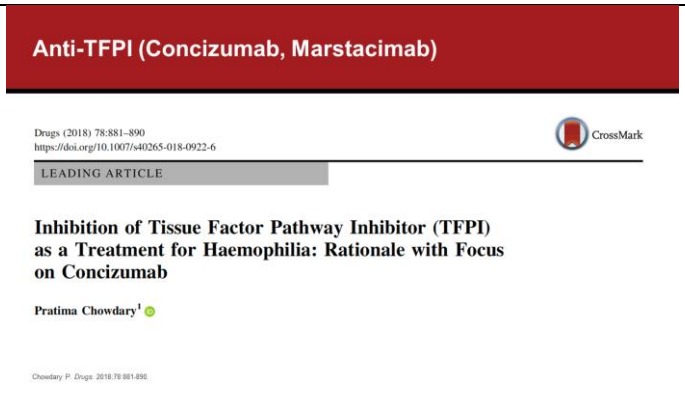
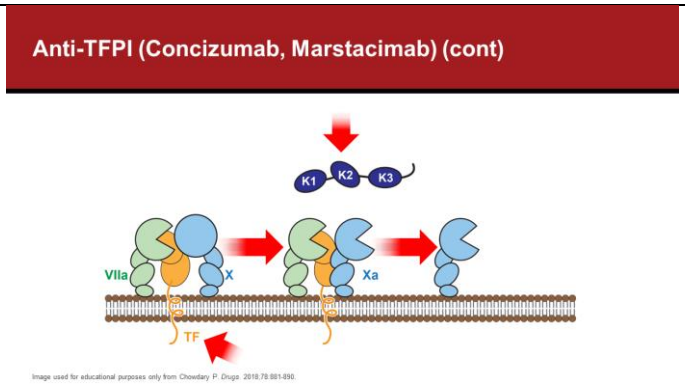
32.	**Procoagulant/Antifibrinolytic Effects of Thrombin on Coagulation Factors**  TAFI: thrombin-activatable fibrinolysis inhibitor; TAFIa: activated thrombin-activatable fibrinolysis inhibitor	Now, thrombin has lots of different roles. We did start to show it to you on one slide, but I want to make sure we capture all of them here. You already mentioned activating factor V and VIII, the cofactors of the coagulation cascade. Also activating factor XI. Factor XI typically is only needed under situations of surgery or stress or hemostatic stress. And this is why patients with factor XI deficiency don't generally bleed unless they have hemostatic stress. Thrombin, of course, converts fibrinogen to fibrin as a key function to help make the clot, and the fibrin is the actual material of the protein that makes up the clot. But also, thrombin activates 2 other proteins: factor XIII and the factor XIIIa. The factor XIII cross-links fibrin clots, makes them much stronger, and TAFI or thrombin-activatable fibrinolysis inhibitor. The name says it all. It's activated by thrombin, and it inhibits fibrinolysis, so it's another protein that helps to make and increase the strength of the clot and the resilience of the clot. So, those 2 together is the antifibrinolytic effect of thrombin, whereas the rest is the procoagulant effect of thrombin.
33.	**Mechanisms of Action of Novel Therapeutics**  APC: activated protein C; mAb: monoclonal antibody; PS: protein S; siRNA: small interfering RNA.	So, let's take a look at the mechanisms of action of novel therapeutics. We have in green replacement therapies, in blue substitution therapies, and in red the rebalancing agents. So, efanesoctocog alfa, a relatively new factor VIII replacement therapy. It functions right there at factor VIII. We have emicizumab. And at some point in the future, Mim8. These are bispecific antibodies that essentially substitute for the function of activated factor VIII, bringing factor IX and X into the proper alignment to generate factor

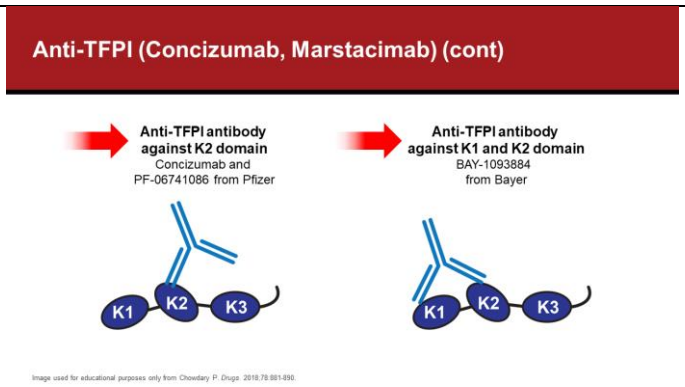
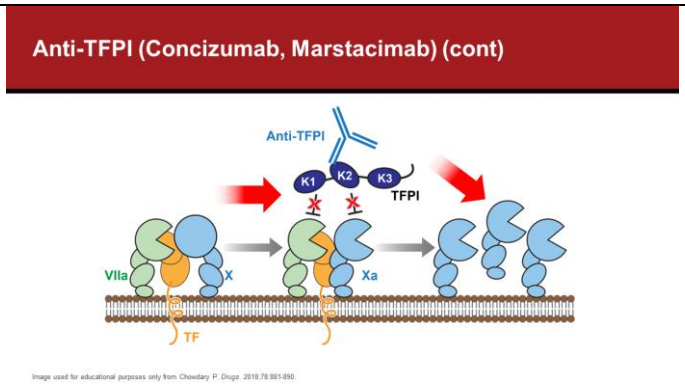
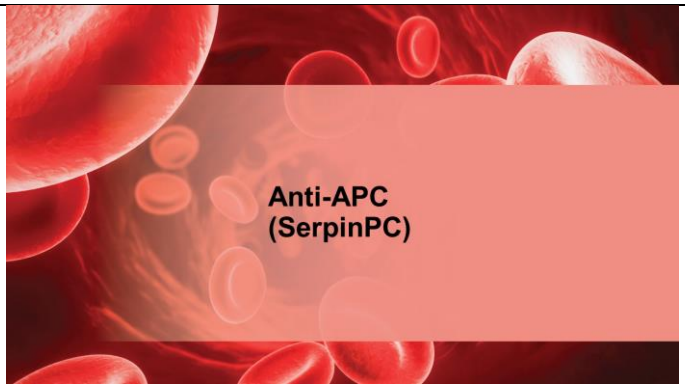
		Xa. And then we have the rebalancing agents. So, the dashed line means inhibits. Fitusiran inhibits antithrombin, which, as its name implies, inhibits thrombin. And it also inhibits factor Xa and actually other proteins in the cascade. But mostly, the main effect is the inhibition of thrombin, as the name implies. We have *S*erpinPC and also an anti-activated protein C monoclonal antibody, both in clinical development, that inhibit activated protein C. And then we have drugs that inhibit TFPI, concizumab and marstacimab. You've probably heard of them—been in clinical trials for quite a while. And then MG1113, which is a Korean product that's also in clinical trials. And then finally, there are companies working on anti-protein S, monoclonal antibodies as well as anti-protein S small interfering RNAs (siRNAs). And these work, of course, to inhibit protein S, and protein S is the cofactor for protein C. Protein S also serves as a cofactor for TFPI.
34.		So, what do we mean by rebalancing agents? Well, the coagulation system is normally in a hemostatic balance. You can see this balance on a seesaw here.

35.		If we're missing a protein on the procoagulant side, we have a bleeding disorder. Here're some pictures of patients of mine with different types of bleeding.
36.		If we're missing a protein on the other side, typically antithrombin protein C, protein S deficiency, we know that that's a thrombotic disorder. The deficiency of TFPI has question marks on it, because we're not really clear that a deficiency of TFPI leads to increased thrombosis. There really isn't strong evidence for that.
37.		Nevertheless, if we are missing, for example, factor VIII as you see there on the left side, and we also then inhibit antithrombin, then we can rebalance the coagulation system without adding factor VIII back. So, it's a way to rebalance the system by working on the other side, the coagulation inhibitor side of the coagulation cascade. And the same could happen if we had factor IX or TFPI blocked off, for example. So, the balance is restored, and the goal is no bleeding and no clotting.
38.		So, can we get the balance right?

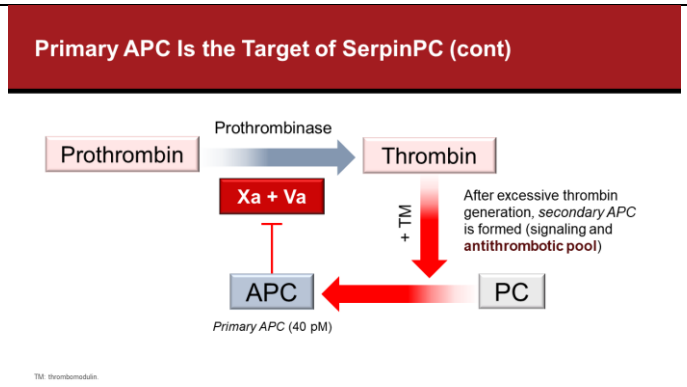
39.	**Rebalancing Agents (cont)** Poor bleed control, no thrombosis Bleed Control / Hemostatic System / No Thrombosis	Well, if we don't get the balance fully corrected this way, we can have poor bleed control but are not likely to have thrombosis.
40.	**Rebalancing Agents (cont)** Good bleed control, thrombotic events Bleed Control / Hemostatic System / No Thrombosis	If we tilt the balance too far, we may have really good bleed control, but we may end up with thrombotic events. So, we really need to get this balance exactly right.
41.	**Rebalancing Agents (cont)** LETTERS nature medicine An RNAi therapeutic targeting antithrombin to rebalance the coagulation system and promote hemostasis in hemophilia Alfica Sehgal[1], Scott Barros[1], Lacramioara Ivanciu[2], Brian Cooley[3], June Qin[1], Tim Racie[1], Julia Hettinger[1], Mary Carioto[1], Yongfeng Jiang[1], Josh Brodsky[1], Harsha Prabhala[1], Xuemei Zhang[1], Husain Attarwala[1], Renta Hutabarat[1], Don Foster[1], Stuart Milstein[1], Klaus Charisse[1], Satya Kuchimanchi[1], Martin A Maier[1], Lubo Nechev[1], Pachamuthu Kandasamy[1], Alexander V Kel'in[1], Jayaprakash K Nair[1], Kallanthottathil G Rajeev[1], Muthiah Manoharan[1], Rachel Meyers[1], Benny Sorensen[1], Amy R Simon[1], Yesim Dargaud[4], Claude Negrier[4], Rodney M Camire[2] & Akin Akinc[1] Sehgal A, et al. *Nat Med.* 2015;21:492-497.	So, with that, I want to start talking about some of the rebalancing agents and their mechanisms of action. This is a paper that is very comprehensive published in *Nature Medicine*, which basically goes through the preclinical development program for fitusiran, which is an RNA interference therapeutic. We now call that siRNA.
42.	**RNAi Therapeutics** A New Class of Innovative Medicines ▪ Harness natural pathway —Catalytic mechanism —Mediated by small interfering RNA or "siRNA" ▪ Therapeutic gene silencing —Any gene in genome —Distinct mechanism of action vs. other drug classes —Unique opportunities for innovative medicines ▪ Clinically validated platform —Human POC in multiple programs  Image reproduced for educational purposes only courtesy of Alnylam.	So essentially, how does this work? Well, siRNAs are a novel class of innovative medicines. There are already several of these products that are on the market for uses outside of hemophilia. And in this case, we have an siRNA that is working against antithrombin. The general mechanism of siRNA, as you see here, you basically have a sequence of RNA that is essentially a gene-silencing mechanism. So, this small sequence of this siRNA will bind to its

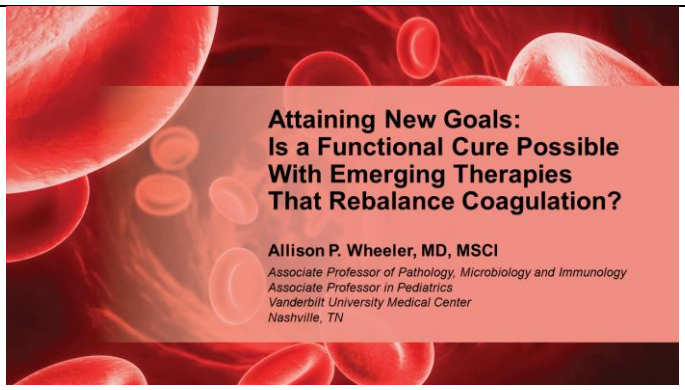
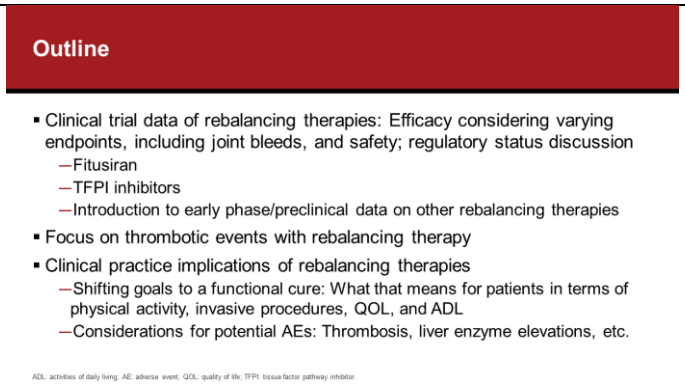
		complementary messenger RNA. The messenger RNA of whatever protein you want to reduce the quantity of in the body. It then gets the silencing mechanism going and basically reduces the production of that specific protein. There's a lot more detail into the way that this works that you can find in that article.
43.	**Fitusiran** SC-administered GalNAc-conjugated siRNA-targeting antithrombin  ASGPR: asialoglycoprotein receptor; GalNAc: N-acetylgalactosamine; PBS: phosphate-buffered saline; QW: every week; RISC: RNA-induced silencing complex; SC: subcutaneous Image on right reproduced for educational purposes only from Sehgal A, et al. *Nat Med*. 2015;21:492-497.	So, here we have fitusiran. This is an siRNA, as I mentioned already, that blocks antithrombin. It has a conjugate that brings it essentially to the liver. You can see the cellular process in a slightly different cartoon on the left, where it gets incorporated into the RNA-induced silencing complex (RISC) and then blocks the messenger RNA from getting transcribed into the protein. On the right side are the animal experiments from that paper, which show you that it takes a little bit of time, a few weeks. But after a few weeks, you can basically knock down the production of antithrombin. On the y-axis is the relative antithrombin level. You can really knock it down pretty close to 0, and you can see that you could do that in a dose-dependent manner.
44.	**Fitusiran Mechanism of Action** 	So, let's have a look at this RNA cartoon. Fitusiran is an siRNA therapy that acts as a rebalancing agent to block antithrombin production and restore thrombin levels, resulting in a rebalance between procoagulation and anticoagulation.

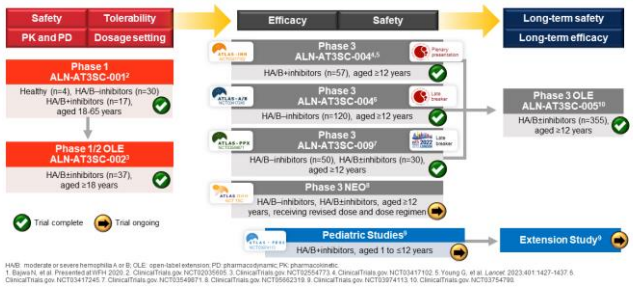
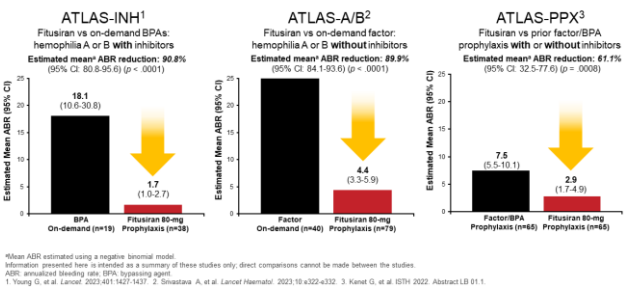
45.		I hope you learned something from that animation of the function or the mechanism of action of fitusiran. So, now we're going to talk about anti-TFPI molecules. And we'll focus on concizumab and marstacimab. They're the 2 that are furthest along in clinical trials.
46.		This paper here has a very nice figure, which I'm going to use. It says "focus on concizumab." But essentially, this is the mechanism of action for both concizumab and marstacimab.
47.		Well, first let's take a look at tissue factor. Tissue factor is a transmembrane protein that mostly sits in the subendothelium. When the endothelium ruptures, then you have activation, and it helps to bring factor VIIa or activates factor VIIa, which, along with factor X, generates a factor Xa that you can see here. TFPI is a Kunitz domain type of protein inhibitor. And basically, you can see K1, K2, K3, or the different Kunitz domains. And its job, as its name implies, is to inhibit the tissue factor pathway.

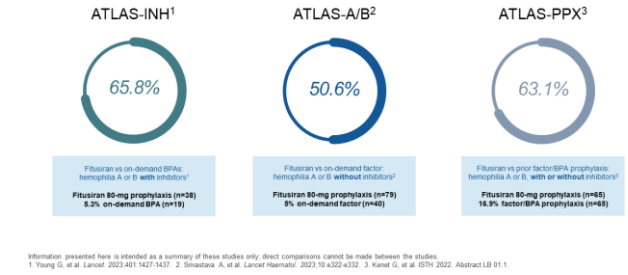
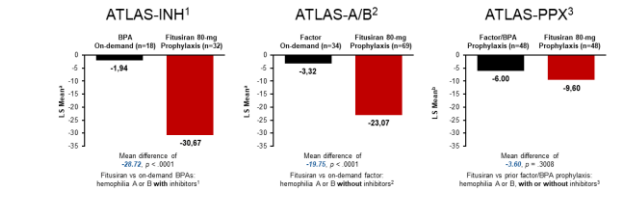
48.		And so, it basically sits here on the factor VIIa/tissue factor/factor Xa complex or factor X complex and inhibits it from generating factor Xa. So, if we inhibit TFPI, then we can essentially restore the factor Xa. Production will basically go back to the state that we were before, where the tissue factor pathway is functioning properly and generating factor Xa. Concizumab and marstacimab. So that PF with long numbers after it from Pfizer is now called [concizumab]. They are single-domain inhibitors. They inhibit the K2 domain of TFPI. On the right side was a product that was being developed by Bayer—it is no longer being developed—that inhibited 2 domains, the K1 and K2 domains. This product, the Bayer product, led to a few unusual thrombotic events and therefore its development was discontinued.
49.		So, this is essentially the function. If you have an anti-TFPI, you're blocking TFPI. That allows the tissue factor/factor VIIa/Xa complex to function and to release additional factor Xa. Then that Xa, of, course generates thrombin along with factor Va. And that's where the inhibition happens. And that's where the extra factor Xa generation happens.
50.		So, let's turn to another molecule called anti-activated protein C. That's the mechanism. The molecule is called SerpinPC.

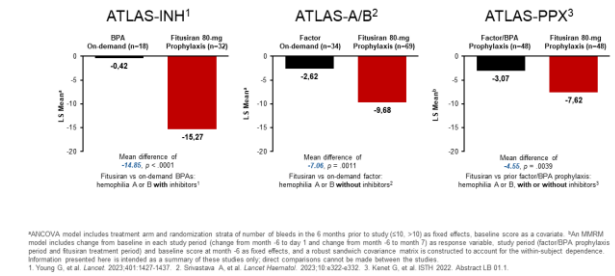
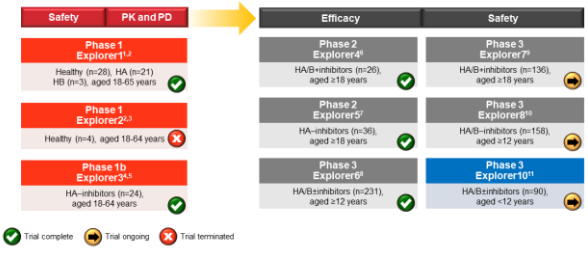
51.	**Anti-APC (SerpinPC)** But, can we get the balance right? SerpinPC was designed to restore thrombin generation without increasing the risk for thrombosis  	Now, this molecule learned a bit from the other ones because we did see thrombotic events in the early trials. So, with the Bayer anti-TFPI molecule, it wasn't developed further because of thrombotic events. There have been thrombotic events with concizumab. There have been thrombotic events with fitusiran. None yet with marstacimab, but with the other drugs, yes. So, in this case, there's a rational design here. SerpinPC was designed to restore thrombin generation without increasing the risk of thrombosis. You can think of it like breaking the seesaw so you get good bleed control but no increase in thrombosis.
52.	**Anti-APC (SerpinPC) (cont)**  blood THROMBOSIS AND HEMOSTASIS **Design and characterization of an APC-specific serpin for the treatment of hemophilia** Stéphanie G. I. Polderdijk,[1] Ty E. Adams,[1] Lacramioara Ivanciu,[2] Rodney M. Camire,[2] Trevor P. Baglin,[3] and James A. Huntington[1] [1]Department of Haematology, University of Cambridge, Cambridge Institute for Medical Research, Cambridge, United Kingdom; [2]Division of Hematology, The Children's Hospital of Philadelphia, Philadelphia, PA; and [3]Department of Haematology, Addenbrooke's Hospital, Cambridge University Hospitals National Health Service Trust, Cambridge, United Kingdom Polderdijk SGI, et al. *Blood*. 2017;129:105-113.	And so, this was the preclinical development of this molecule that the inventors called SerpinPC.
53.	**Primary APC Is the Target of SerpinPC** ▪ APC shuts down prothrombinase ▪ Primary APC refers to the APC that is circulating ▪ Secondary APC is generated only after thrombin generation ▪ Inhibition of primary APC allows early prothrombinase (initiation stage) more time to make thrombin ▪ Efficacy is achieved from inhibition of primary APC ▪ No further bleeding reduction by inhibiting secondary APC ▪ Secondary APC is important in preventing thrombosis NHP, nonhuman primates. Polderdijk SGI, et al. *Blood*. 2017;129:105-113.	Basically, the primate target is primary activated protein C, which is different than protein C itself. This is the activated form of protein C, and activated protein C basically shuts down the prothrombinase complex, which is factor Va and X. And, actually, also inhibits the intrinsic tenase factor VIII. So, it basically inhibits factor Va and factor VIIIa. And primary activated protein C is referring to the activated protein C that is circulating. Secondary activated protein C or secondary formation happens only after thrombin generation, and the goal of that is to prevent

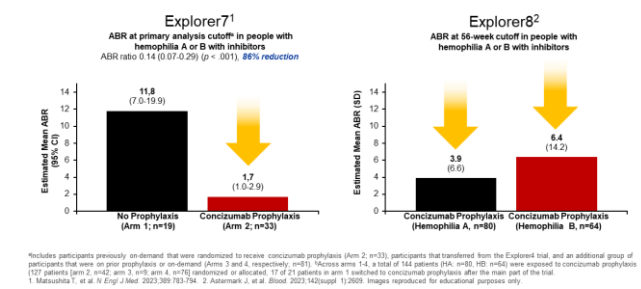
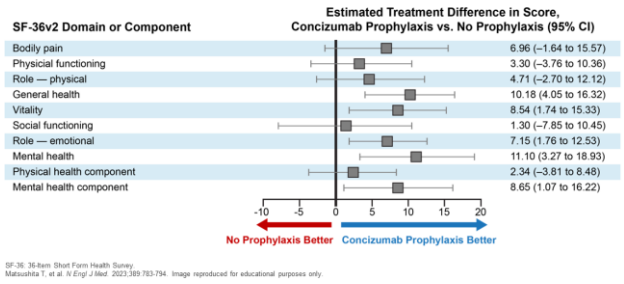
		clot formation from being too exuberant to prevent the thrombin around the area of the clot from causing thrombosis intravascularly. Inhibition of primary activated protein C allows early prothrombinase initiation stage more time to make thrombin, and this is how it can generate more thrombin. But because we're only inhibiting activated protein C and not protein C, the secondary pool of activated protein C, which is then generated via thrombin, is still available to prevent thrombosis. So, that's the idea behind this molecule, which is essentially, let's improve thrombin generation, but without increasing the risk of thrombosis, and in this case by preserving the secondary activated protein C pool.
54.		So, it kind of looks like this. The prothrombinase is factor Xa and factor Va, and prothrombin is then converted to thrombin by the prothrombinase complex. Once you have thrombin generation formed, thrombomodulin will then bind to thrombin and convert protein C to activated protein C, which then inhibits the prothrombinase complex. So, SerpinPC is here to essentially block the primary activated protein C pool that is already circulating. But the secondary pool that is generated after thrombin generation is preserved.

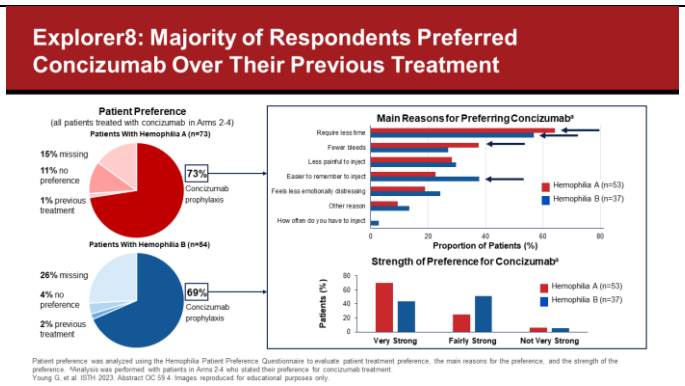
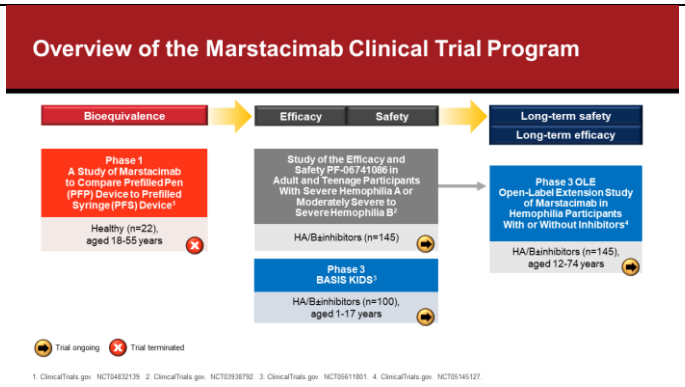
55.		And so, with that, I'm going to stop and I'm going to pass the baton over to Dr. Wheeler. And Dr. Wheeler is going to take a look at some of these molecules and ask the question: is a functional cure possible with emerging therapies that rebalance coagulation? And so, Allison, take it away. ***[Allison P. Wheeler, MD, MSCI]*** Thank you, Dr. Young. So, I'm Allison Wheeler, and we are going to talk a little bit about attaining new goals. Is a functional cure possible with emerging therapies that rebalance coagulation?
56.		So, the outline for this part of the program is to discuss the clinical trial data of rebalancing therapies. We're going to talk about efficacy considering a number of different endpoints, including joint bleeds and safety. We're going to talk about this in the context of fitusiran, an anti-antithrombin molecule, as well as 2 TFPIs. And then, I'm going to briefly introduce early phase and preclinical data on other rebalancing therapies. We're going to talk about adverse events with a focus on thrombotic events. And then, we're going to talk a little bit about clinical practice implications and how to think about rebalancing therapies in the context of our patients.

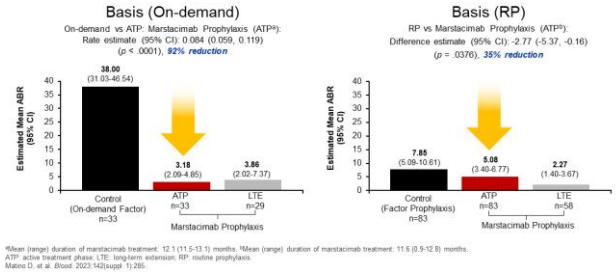
57.	**Overview of Fitusiran Clinical Program[1]** 	So, just to jump into it, I'm first going to just very briefly review the fitusiran clinical trial program. We're really going to focus on the efficacy trials, the three phase 3 pivotal trials that were performed for this drug: the ATLAS-inhibitor trial looking at patients with hemophilia A or B with inhibitors who are greater than or equal to 12 years old; the ATLAS-A/B trial looking at the same patient population greater than or equal to 12 years old, the patients without inhibitors; and then the ATLAS-prophylaxis trial comparing patients with hemophilia A or B with or without inhibitors who are taking standard factor or bypassing agent prophylaxis compared with fitusiran prophylaxis, again greater than or equal to 12 years old. Prior to these phase 3 trials, there was a phase 1 trial program that looked at safety, tolerability, pharmacokinetics, and dynamics and set the dose for the phase 3 trials. But we're not going to go into those data today. Of note, there are ongoing pediatric studies for this drug, as well as long-term safety and efficacy trials for all 3 of the phase 3 clinical trial studies that I mentioned a moment ago.
58.	**Fitusiran Phase 3 Efficacy Data** 	So, looking at the efficacy data again: ATLAS-inhibitor trial, ATLAS-A/B trial, and ATLAS-prophylaxis trial. Right here we're looking at the estimated mean annualized bleeding rate (ABR) for each of these comparison populations. You can see here that fitusiran prophylaxis demonstrated a statistically significant decrease in this mean ABR throughout the course of the study. And in this patient population, patients with inhibitors, comparing on-demand patients with fitusiran prophylaxis, there was a 90.8% reduction in the estimated mean ABR. For the

		ATLAS-A/B trial, patients without inhibitors, that reduction was 89.9%. Both of those trials demonstrated fairly significant statistical differences between these 2 groups. For the ATLAS-prophylaxis trial, we see a smaller difference between the 2 groups. Patients taking factor or bypassing agent prophylaxis had an ABR of 7.5, and those getting fitusiran prophylaxis had 2.9. This was a 61.1% reduction and statistically significant. But just to point out again, this is comparing 2 prophylaxis groups as opposed to the other 2 studies comparing on-demand versus prophylaxis.
59.	**Fitusiran: Percentage of Participants With Zero Bleeds in the Efficacy Period**  Information presented here is intended as a summary of these studies only; direct comparisons cannot be made between the studies. 1. Young G, et al. *Lancet*. 2023;401:1427-1437. 2. Srivastava A, et al. *Lancet Haematol*. 2023;10:e322-e332. 3. Kenet G, et al. ISTH 2022. Abstract LB 01.1.	Another way to look at efficacy is to look at the percentage of participants with 0 bleeds in the efficacy period. And you can see here the percentages of the patients who are receiving fitusiran prophylaxis in this clinical trial program who had 0 bleeds: 65.8% in the inhibitor trial, 50.6% in the A/B trial, and 63.1% in the prophylaxis trial. This was compared with much smaller numbers in the patients who were not receiving prophylaxis: 5.3% of patients receiving on-demand bypassing agents, 5% of patients receiving on-demand factor, and 16.9% receiving factor prophylaxis.
60.	**Fitusiran Prophylaxis Improved HRQOL as Measured by Haem-A-QoL Physical Health Domain**  ¹ANCOVA model includes treatment arm and randomization strata of number of bleeds in the 6 months prior to study (≤10, >10) as fixed effects, baseline score as a covariate. ²An MMRM model includes change from baseline in each study period (change from month -6 to day 1 and change from month -6 to month 7) as response variable, study period (factor/BPA prophylaxis period and fitusiran treatment period) and baseline score at month -6 as fixed effects, and a robust sandwich covariance matrix is constructed to account for the within-subject dependence. Information presented here is intended as a summary of these studies only; direct comparisons cannot be made between the studies. ANCOVA: analysis of covariance; Haem-A-QoL: Haemophilia Quality of Life Questionnaire for Adults; HRQOL: health-related quality of life; MMRM: mixed model for repeated measure. 1. Young G, et al. *Lancet*. 2023;401:1427-1437. 2. Srivastava A, et al. *Lancet Haematol*. 2023;10:e322-e332. 3. Kenet G, et al. ISTH 2022. Abstract LB 01.1.	Another way to look at efficacy for new drugs is to look at quality of life. So, for the fitusiran program, they used the Haemophilia Quality-of-Life Questionnaire for Adults (Haem-A-QoL), which is a hemophilia-specific questionnaire. Of note, lower numbers in this quality-of-life questionnaire, or more negative numbers, are the beneficial measurement that we're looking for. So, these tables demonstrate the physical health domain of the Haem-A-QoL

		questionnaire. And as you can see in the ATLAS-inhibitor and ATLAS-A/B trials, there was a statistically significant difference between patients receiving on-demand therapy—either bypassing agent or factor—and patients receiving fitusiran prophylaxis, with an improvement in quality of life for patients on fitusiran prophylaxis. For the ATLAS-prophylaxis trial, you can see that there was a leaning toward a lower score in the fitusiran prophylaxis group, but this number was not statistically significant. And so, in comparing patients on one type of prophylaxis versus another, we saw less of an impact than comparing those receiving on-demand therapy with fitusiran prophylaxis.
61.	**Fitusiran Prophylaxis Improved HRQOL as Measured by Haem-A-QoL Total Score**  *ANCOVA model includes treatment arm and randomization strata of number of bleeds in the 6 months prior to study (≤10, >10) as fixed effects, baseline score as a covariate. †An MMRM model includes change from baseline in each study period (change from month -6 to day 1 and change from month -6 to month 7) as response variable, study period (factor/BPA prophylaxis period and fitusiran treatment period) and baseline score at month -6 as fixed effects, and a robust sandwich covariance matrix is constructed to account for the within-subject dependence. Information presented here is intended as a summary of these studies only; direct comparisons cannot be made between the studies. 1. Young G, et al. *Lancet*. 2023;401:1427-1437. 2. Srivastava A, et al. *Lancet Haematol*. 2023;10:e322-e332. 3. Kenet G, et al. ISTH 2022. Abstract LB 01.1.	Using that same quality-of-life questionnaire but looking at the Haem-A-QoL total score, you can see similar patterns with more dramatic improvements in the quality of life in the patients receiving fitusiran prophylaxis compared with either bypassing agent or factor on-demand therapy. But in this measurement, you can actually see that there was a statistically significant difference between the 2 different types of prophylaxis in the ATLAS-prophylaxis study.
62.	**Overview of the Concizumab Clinical Trial Program**  1. ClinicalTrials.gov. NCT01228669. 2. Pasca S. *J Blood Med*. 2022;13:191-199. 3. ClinicalTrials.gov. NCT01631942. 4. ClinicalTrials.gov. NCT02490787. 5. Eichler H, et al. *J Thromb Haemost*. 2018;16:2184-2195. 6. ClinicalTrials.gov. NCT03196284. 7. ClinicalTrials.gov. NCT03196297. 8. ClinicalTrials.gov. NCT03741881. 9. ClinicalTrials.gov. NCT04083781. 10. ClinicalTrials.gov. NCT04082429. 11. ClinicalTrials.gov. NCT05135559.	Moving on to the concizumab clinical trial program, you can see a similar pattern to what we saw in the fitusiran study program. Patients with hemophilia A or B with inhibitors were studied in the phase 2 explorer4 trial and then this phase 3 explorer7 trial. Patients without inhibitors were studied in the phase 2 explorer5 trial and then phase 3 explorer8 trial. And you can see there's also an ongoing phase 3 pediatric trial for patients with or without inhibitors. Again,

		this phase 3 and phase 2 clinical trial program was preceded by phase 1 studies looking at safety, pharmacokinetics, and pharmacodynamics. And again, there are long-term extension trials for all of these phase 3 programs.
63.	**Explorer7 and Explorer8: Phase 3 Efficacy Data for Concizumab** 	So, looking at the efficacy data—again, estimated mean ABR—for the explorer programs. The explorer7 trial compared patients without prophylaxis, so on-demand therapy compared with patients with concizumab prophylaxis. And you can see that there was a significant decrease in the mean estimated ABR in this patient population: an 86% reduction. When looking at the explorer8 clinical trial data, we're just looking at patients who are on concizumab prophylaxis who had hemophilia A—an estimated mean ABR of 3.9. And patients with hemophilia B, an estimated mean ABR of 6.4 (without that comparison group that we saw previously).
64.	**Explorer7: HRQOL Was Improved With Concizumab** 	Looking at the health-related quality-of-life studies that were done for the concizumab program, the 36-item Short Form (SF-36) version 2 was chosen. And this is a very general quality-of-life questionnaire looking at various domains or aspects of life, but not specifically focusing on one disease entity. You can see in this forest plot that there was a trend for most, if not all, of those domains or components to be leaning toward concizumab prophylaxis being better. But there were also a number of them where there were statistically significant differences and improvements in the concizumab prophylaxis group (general health, vitality, mental health, emotional improvement).

65.		For the explorer8 trial, there was also a questionnaire looking at whether or not patients preferred their previous treatment over their concizumab treatment for prophylaxis. And as you can see in both patients with hemophilia A in red and patients with hemophilia B in blue, the majority of patients preferred concizumab prophylaxis to their previous prophylactic regimen. These patients were then asked why they preferred the concizumab prophylaxis. And you can see a number of reasons here. The main reasons why patients with either hemophilia A or B preferred concizumab prophylaxis were requiring less time to receive their prophylaxis and having fewer bleeds. And this was present for both hemophilia A and hemophilia B. But patients with hemophilia B also felt that it was easier to remember to inject than it was to perform their previous prophylactic program. When looking at the strength of preference for concizumab, most of the patients felt very strongly or fairly strongly that they preferred the concizumab prophylaxis to their previous prophylactic regimen.
66.		Finally, looking at the marstacimab clinical trial program, we can see that the BASIS trial combined all of the patients in their single trials—looking at patients with hemophilia A or B with or without inhibitors—in their phase 3 trial for both adults and pediatric patients. These phase 3 trials are still ongoing, as well as the long-term extension trial. And again, we're preceded by a phase 1 trial looking at the pharmacokinetics of the drug.

67.	**Marstacimab: Phase 3 Efficacy Data**  [a]Mean (range) duration of marstacimab treatment: 12.1 (11.5-13.1) months. [b]Mean (range) duration of marstacimab treatment: 11.5 (0.9-12.8) months. ATP: active treatment phase; LTE: long-term extension; RP: routine prophylaxis Matino D, et al. *Blood*. 2023;142(suppl 1):285.	For the marstacimab clinical trial program, we've really just received efficacy data on the estimated mean ABR. But you can see here on the left-side table for patients receiving on-demand factor only, there was a statistically significant difference or 92% reduction between the on-demand group and the active treatment phase group of the phase 3 trial. And you can also see that active treatment phase data persisted into the long-term extension group. On the right side, you can see patients who were receiving factor prophylaxis, comparing them with patients in the active treatment phase receiving marstacimab prophylaxis. And you can see a 36% reduction in that estimated mean ABR. And again, continuing into that long-term extension phase.
68.	**Overall Efficacy: Key Takeaways** ▪ Bleeding rates in phase 3 clinical trials were higher than ideal (ABR 1.3-6.4). ▪ QOL is comparable to on-demand in most instances; however, demonstrates improvement/benefit toward rebalancing agents	So, what's the overall takeaway from all of these efficacy data? We see that bleeding rates in these phase 3 clinical trial programs did show improvements compared with on-demand and also compared with factor prophylaxis in the studies that looked at that. But the ABRs were a little bit higher than maybe we would have expected, with the top number being an ABR of 6.4 bleeds per year. So, something to think about and something to consider when thinking about these drugs for our patients. We also do see that although quality of life was comparable to other prophylactic regimens, there did seem to be a significant improvement or benefit toward rebalancing agents, especially when comparing with on-demand therapy.

69.

Fitusiran and Concizumab: Thromboembolic Risks

Both fitusiran and concizumab were safe and well-tolerated in phase 3 clinical trials, but carry a potential risk of thromboembolic events

Agent	Clinical Trial	Thromboembolic Events
Fitusiran	ATLAS-INH[1]	4 TEAEs of special interest, suspected/confirmed VTE, in 2 (5%) patients: • DVT (non-serious), subclavian vein thrombosis (serious), superficial thrombophlebitis (non-serious) • AT activity before onset: 11.9%, 7.8%-11.6%
	ATLAS-A/B[2]	No suspected/confirmed thromboembolism
	ATLAS-PPX[3]	2 suspected/confirmed thromboembolic events in 2 (3%) patients • Cerebrovascular accident and thrombosis (suspected thrombosis on papilla of left eye) • After treatment restart, no thromboembolic events were reported
Concizumab	Explorer7[4]	During "on-treatment" period[a]: • Groups 1-4: 1 event in 1 (1%) patient (renal infarction; non-fatal) During "on-treatment, without data on initial regimen" period[b]: 0 events
	Explorer8[5]	4 thromboembolic events in 2 (1.3%) patients • DVT, pulmonary embolism, superficial vein thrombosis in 1 patient; acute myocardial infarction in 1 patient; all non-fatal

[a]The period during which patients were exposed to OD treatment with BPAs or concizumab treatment. [b]The period during which patients were exposed to OD treatment with BPAs or concizumab treatment, with the exclusion of the data on the initial concizumab regimen.
DVT: deep vein thrombosis; TEAE: treatment-emergent adverse event; VTE: venous thromboembolism.
1. Young G, et al. *Lancet*. 2023;401:1427-1437. 2. Srivastava A, et al. *Lancet Haematol*. 2023;10:e322-e332. 3. Kenet G, et al. ISTH 2022. Abstract LB 01.1. 4. Matsushita T, et al. *N Engl J Med*. 2023;389:783-794. 5. Astermark J, et al. *Blood*. 2023;142(suppl 1):2609.

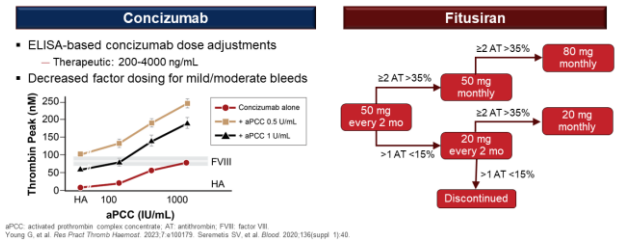
But what about risks or adverse events with these drugs? So, the marstacimab clinical trial program has not identified any thromboembolic risks or any patients who've experienced thrombosis. But both fitusiran and concizumab clinical trial programs did have patients who experienced thrombosis during their phase 3 studies. You can see the outline here of the various studies in which thromboembolic events occurred.

The ATLAS-inhibitor study: there were 4 treatment-associated adverse events of special interest, specifically suspected or confirmed venous thromboembolism, and this occurred in 2 patients. The ATLAS-A/B trial: there were no thromboembolic events that were noted, but the ATLAS prophylaxis trial did have 2 suspected or confirmed thromboembolic events in 2 patients. Additionally, the concizumab clinical trial program, the explorer7 program, had 1 patient who experienced a thromboembolic event, and the explorer8 program had 2 patients who experienced thromboembolic events.

70.

Fitusiran and Concizumab: Safety and Risk Mitigation

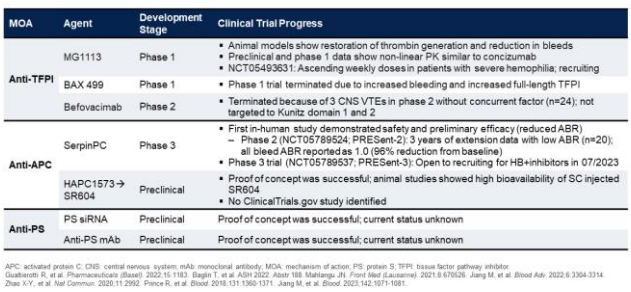
Fitusiran and concizumab: In both trial programs, patients experienced thrombosis, resulting in clinical and laboratory evaluation and subsequent risk mitigation



aPCC: activated prothrombin complex concentrate; AT: antithrombin; FVIII: factor VIII.
Young G, et al. *Res Pract Thromb Haemost*. 2023;7:e100179. Seremetis SV, et al. *Blood*. 2020;136(suppl 1):40.

So, what happened with these thromboembolic events? They caused pauses in the clinical trial programs and caused the companies investigating these drugs to look into why these patients experienced thrombosis and what risk mitigation could be done to prevent those. For the concizumab trial, subsequent to the risk mitigation strategy being implemented, patients have an enzyme-linked immunosorbent assay (ELISA)-based concizumab dose adjustment. Patients have their concizumab level tested after 4 weeks of treatment and then dose adjusted to hit a

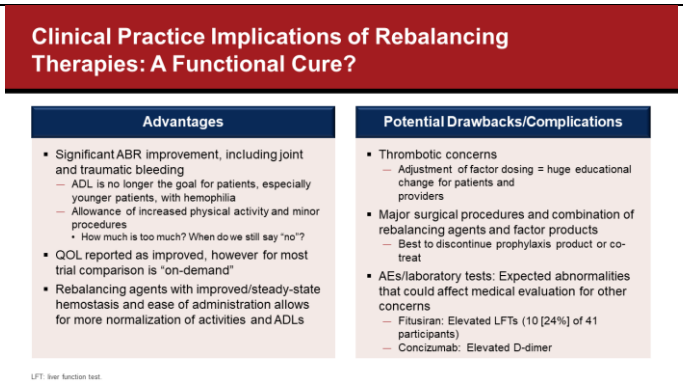
		therapeutic range of 200 to 4000 ng/mL. In addition to that, based on thrombin generation studies, there were decreased recommendations for factor dosing for patients with mild and moderate bleeds. In the fitusiran clinical trial program, there was a more specific dose adjustment that was recommended based on antithrombin activity. And as you can see in the figure on the right side of this slide, there are multiple dose-adjustment options for these patients based on their antithrombin activity. If the antithrombin is greater than 35%, then dose increases are recommended, and if the antithrombin is less than 15%, then dose decreases are recommended with the potential to discontinue the drug if the antithrombin level can't reach that specific range. In addition to that, and not noted on this slide, there is also decreased factor dosing for mild-to-moderate bleeds that is recommended to be taken concurrently with fitusiran.
71.	**Fitusiran, Concizumab, and Marstacimab: Overall Favorable Safety Profiles in Phase 3 Trials** **Fitusiran (ATLAS-INH,[1] ATLAS-A/B,[2] ATLAS-PPX[3])** • **Common AEs across trials:** Liver enzyme elevations, URTI, headache, nasopharyngitis, and abdominal pain • **Special AEs of interest:** Elevated liver enzymes (>3x ULN), cholecystitis, cholelithiasis, and thromboembolic events (rare) • **Reported TEAEs leading to discontinuation:** Spinal vascular disorder and suspected spinal vessel thrombosis in 1 patient (ATLAS-INH), cholecystitis in 1 patient; increased alanine aminotransferase concentrations in 1 patient (ATLAS-A/B) • **No treatment-related deaths reported** **Concizumab (Explorer7[4] and Explorer8[5])** • **Common AEs:** Arthralgia, injection-site erythema, URTI, and elevation of prothrombin fragments 1 and 2 • **Special AEs of interest:** Thromboembolic events (rare) • **Explorer7:** 1 death related to COVID-19 respiratory complications; patient had ceased concizumab treatment 10 days prior and had additional risk factors (obesity and hypertension) • **Explorer8:** 1 serious AE resulting in fatal intra-abdominal hemorrhage; 6 patients withdrew due to AEs **Marstacimab (Basis[6])** • **Most common AEs (reported in phase 2[7]):** Hemarthrosis, injection-site reactions, arthralgia, and hematoma • **Special AEs of interest:** COVID-19, hemorrhages, hypersensitivity, hypertension, injection-site reactions, gastrointestinal varices, and hepatic disorders • **No discontinuations due to AEs, no deaths or thromboembolic events reported** ULN: upper limit of normal; URTI: upper respiratory tract infection. 1. Young G, et al. *Lancet*. 2023;401:1427-1437. 2. Srivastava A, et al. *Lancet Haematol*. 2023;10:e322-e332. 3. Kenet G, et al. ISTH 2022. Abstract LB 01.1. 4. Matsushita T, et al. *N Engl J Med*. 2023;389:783-794. 5. Astermark J, et al. *Blood*. 2023;142(suppl 1):2609. 6. Matino D, et al. *Blood*. 2023;142(suppl 1):285. 7. Mahlangu J, et al. *Br J Haematol*. 2023;200:240-248.	When we look at the additional safety concerns for each of these clinical trial programs, they're actually very reassuring and overall have a very favorable safety profile. And the fitusiran trial, one thing to note in terms of adverse events was increases in liver enzymes. In some patients, these liver enzyme increases were greater than 3 times the upper limit of normal. And so, something to think about when considering this drug for patients. In the concizumab trial, there were also noted to be laboratory changes, specifically increases in D-dimer and elevation of prothrombin fragments 1 and 2. And then in the marstacimab trial, we can see that there were no specific adverse events attributed to the drug itself. But in all 3, we can see

		increases in injection-site reactions and then expected adverse events that we would anticipate with the general population (eg, upper respiratory tract infections, headaches, abdominal pain), and then specific things associated with the hemophilia population (hemarthrosis or arthralgias) that are associated with long-standing hemophilia.
72.	**Other Hemostatic Rebalancing Agents** 	When looking at other rebalancing agents, we can see a number of them are coming through the clinical trial pipeline. There have been 3 anti-TFPI agents that are being looked at. The MG1113 agent has completed a phase 1 clinical trial and is recruiting for the next phase, showing similar preclinical and phase 1 data to other anti-TFPI molecules. However, there have been 2 previous anti-TFPI molecules where this study had been terminated due to either increased bleeding or increased venous thromboembolic events. Looking at anti-activated protein C molecules, the SerpinPC clinical trial program is the most advanced, currently in phase 3 clinical trials. The PRESent-3 clinical trials are open to recruiting patients at this time. And then we can see there're a number of preclinical studies: one anti-activated protein C molecule and 2 anti-protein S molecules that are currently being investigated.

MOA	Agent	Development Stage	Clinical Trial Progress
Anti-TFPI	MG1113	Phase 1	• Animal models show restoration of thrombin generation and reduction in bleeds • Preclinical and phase 1 data show non-linear PK similar to concizumab • NCT05493631: Ascending weekly doses in patients with severe hemophilia; recruiting
	BAX 499	Phase 1	• Phase 1 trial terminated due to increased bleeding and increased full-length TFPI
	Befovacimab	Phase 2	• Terminated because of 3 CNS VTEs in phase 2 without concurrent factor (n=24); not targeted to Kunitz domain 1 and 2
Anti-APC	SerpinPC	Phase 3	• First in-human study demonstrated safety and preliminary efficacy (reduced ABR) – Phase 2 (NCT05789524; PRESent-2): 3 years of extension data with low ABR (n=20); all bleed ABR reported as 1.0 (96% reduction from baseline) • Phase 3 trial (NCT05789537; PRESent-3): Open to recruiting for HB+inhibitors in 07/2023
	HAPC1573→ SR604	Preclinical	• Proof of concept was successful; animal studies showed high bioavailability of SC injected SR604 • No ClinicalTrials.gov study identified
Anti-PS	PS siRNA	Preclinical	Proof of concept was successful; current status unknown
	Anti-PS mAb	Preclinical	Proof of concept was successful; current status unknown

APC: activated protein C; CNS: central nervous system; mAb: monoclonal antibody; MOA: mechanism of action; PS: protein S; TFPI: tissue factor pathway inhibitor.
Gualtierotti R, et al. *Pharmaceuticals (Basel)*. 2022;15:1183. Baglin T, et al. *ASH 2022. Abstr 188*. Mahlangu JN. *Front Med (Lausanne)*. 2021;8:670526. Jiang M, et al. *Blood Adv*. 2022;6:3304-3314. Zhao X-Y, et al. *Nat Commun*. 2020;11:2992. Prince R, et al. *Blood*. 2018;131:1360-1371. Jiang M, et al. *Blood*. 2023;142:1071-1081.

73.



So, what does this mean for our patients? In my mind, it means things are getting a little bit more complicated. But also, it means that we have a lot more options. So, thinking specifically about the rebalancing agents and the advantages of these agents. There is an improvement in ABR. So, the question is: as our patients have decreases in their ABRs, can their activities of daily living be a little bit easier for them? Can they have more experiences, such as increases in physical activity and participation in sports, than they did previously? Can minor procedures be performed with minimal increase in prophylactic treatment or additional treatment after the procedures? These are all things that are possible with these medications. As we learn more about them, we're going to be able to learn where that line is drawn. How much is too much? What can we allow our patients to do, and when should we still say no to our patients?

As we see improvements in quality of life, we start seeing more health equity in these patients, and we're able to provide them with more opportunities. And rebalancing agents do have the potential to show improvements in all of these aspects of life, especially with the steady-state hemostasis and the ease of administration that they all allow. However, there are some drawbacks. The thrombotic concerns that I mentioned are not to be ignored and not to be discounted. And even the risk mitigation strategies of requiring lower factor doses for mild and moderate bleeds is something that's going to require a lot of education for both our patients and other providers who might be seeing our patients in emergency or

		urgent settings. We're really going to need to start thinking more about major surgical procedures and how we're going to treat our patients in these circumstances. Are we going to have the time to discontinue these rebalancing prophylactic treatments, or are we going to have to co-treat with the rebalancing agent and factor products? Again, thinking about those dose adjustments depending on the circumstances of the surgical procedure. And we're also going to have to watch for laboratory abnormalities that are going to be expected with these medications. The elevation in liver function testing that we see with fitusiran, the elevated D-dimers that we've seen with concizumab—these are things that we're going to have to educate our patients on and consider in the context of their total health. And whether or not we should consider one drug versus another because of those other health concerns or complications of other health concerns for each individual patient.