THE 12th WFH GLOBAL FORUM

On Research and Treatment Products For Bleeding Disorders

NOVEMBER 4-5, 2021 • VIRTUAL

SUMMARY

WFH
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Summary of the 12th WFH Global Forum on Research and Treatment Products for Bleeding Disorders

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EXECUTIVE SUMMARY

The 12th WFH Global Forum on Research and Treatment Products held November 4-5, 2021, brought to the forefront scientific developments in treatment of hemophilia, von Willebrand disease (VWD) and other bleeding disorders, and current challenges related to safety and access. Sessions focused on scientific, socioeconomic, and global aspects of high interest to the bleeding disorders community and enabled interdisciplinary discussions on current issues in the field.

This year's global forum looked at the need to increase global plasma collection and supply to meet increasing global demand for immunoglobulin products and other plasma-derived medicinal products. It is important to differentiate blood components from plasma for fractionation, define separate blood and plasma policies, invest in plasmapheresis programs, establish science-based donor policies, and minimize current waste of recovered plasma.

The meeting also focused on new types of therapy and risk of thrombosis in hemophilia patients being treated with non-replacement therapy (emicizumab) and in patients treated with rebalancing therapies (bispecific antibody, antithrombin siRNA, anti-TFPI, activated protein C inhibitor). Thrombosis in hemophilia is rare but can be life-threatening. The risk in patients on clotting factor replacement therapy is estimated to be 1 per 1,000 persons per year. The rate of thrombotic events in patients on emicizumab has been relatively low so far but expected to rise as more patients receive this therapy. Some non-replacement and rebalancing therapies in clinical development currently have shown higher thrombotic risk.

Experts also presented the latest developments in von Willebrand disease, including advances in diagnostic testing, new treatment modalities, and new guidelines on diagnosis and management of people with VWD. Treatment for VWD has lagged behind advances in the hemophilia field; only recently has the first recombinant von Willebrand factor therapy become available. There are unmet clinical needs for treatment and long-term prophylaxis for patients with VWD, which would reduce significant morbidity and improve quality of life. Collection of clinical data on treatment and the use of prophylaxis in people with VWD is a key priority.

The session on hemophilia gene therapy addressed a number of safety issues. Oncogenesis remains a preclinical observation largely limited to murine models but it is necessary to remain vigilant. Transaminitis elevation is a real and immediate problem affecting many patients who receive gene therapy, especially those with hemophilia A, and more investigation is needed both on the mechanism and ways to mitigate the adverse effect. The session also highlighted the risk of hepatocellular carcinoma and the importance of monitoring liver health post-HCV treatment, and monitoring for liver toxicity in gene therapy clinical trials.

The bleeding disorders community is seeing unprecedented changes in treatment which hold a lot of promise but are accompanied by new challenges. There are also existing challenges such as inhibitors which continue to require further investigation and more effective interventions. Access to treatment in low-resource settings and low- and middle-income countries remains a pressing priority and focus of the WFH Humanitarian Aid Program. Aggregation and comparison of real-world data from sources such as the WFH Annual Global Survey and World Bleeding Disorders Registry help identify target areas for improvement of treatment and care, and help provide a strong basis to leverage support and resources for better care.

PLASMA AND ACCESS

Geopolitical access to treatment and COVID-19 impact

Against a backdrop of increasing global need for plasma-derived medicinal products (PDMPs), the COVID-19 pandemic has had significant impacts on plasma collection and supply globally, stated Brian O'Mahony, CEO of the Irish Haemophilia Society. Unpublished data on per capita use of immunoglobulin (Ig) products in selected countries show overall global use increased 8% annually from 2010 to 2019, led by substantial increases in the United States, Australia, and Canada, and significant increases in many European countries. During the pandemic, source plasma collections in the United States, which collects most of the plasma for the production of PDMPs used globally, decreased by 19% in 2020 compared to 2019. Shortages of Ig products were reported in Spain, Italy, Germany, and Belgium. There were no reported shortages of clotting factor concentrates (CFCs).

While plasma industry aims were to restore plasma collection levels to pre-pandemic 2019 levels by the end of 2021 and collect 20-30% more plasma in 2022, demand continues to increase, and there are also new indications for Ig therapies including for COVID-19. Projections indicate that by 2026, an additional 30 million litres of plasma will be needed for global requirements. Ig use in Europe currently greatly exceeds the amount of plasma collected in European countries; they would need to ramp up from the current collection of 5.5 million litres to 11.6 million litres to meet their Ig use. The public sector shortfall in 2025 is estimated to be 7.7 million litres.[1] A new EU Blood Directive is being developed. Key needs are to separately define blood components and plasma for fractionation, invest in plasmapheresis programs with plasma collection from paid and unpaid plasma donors, and establish donor policies, Mr. O'Mahony said.

Donor recruitment, deferral policies and plasma collection in the UK

Dr. Thomas Kreil, Head of Global Pathogen Safety/Global Quality at Takeda, discussed how advances in virus testing and elimination compel revisiting whether donor policies implemented in the 1980s and 1990s still make sense today, such as donor deferrals to minimize risk of HIV, HBV, and HCV transmission associated with risky sexual practices and with tattoos/piercings; and deferral of UK plasma from fractionation due

to risk of variant Creutzfeldt-Jakob disease (vCJD) transmission, now that prion removal by plasma protein manufacturing processes is confirmed.[2] Pathogen reduction steps lead to a risk reduction in the range of 1 millionfold or higher per dedicated virus reduction step.[3]

The ability to test directly for virus genomes using nucleic acid amplification testing (NAT) and polymerase chain reaction (PCR) tests has also helped further enhance plasma safety. The processes also work successfully for different types of viruses, including unknown or emerging viruses of concern over the last 20 years, such as the West Nile,[4] H5N1,[5] Chikungunya,[6] Zika,[7] and hepatitis E (HEV)[8] viruses. Plasma for fractionation is not tested for these viruses; however, in all cases, tests were done to verify that inactivation and removal processes had dealt with the virus. Highly sensitive testing has confirmed the absence of residual virus. It is important to differentiate and have differentiated policies for the two product classes: blood components for transfusion, which do not have manufacturing processes for virus inactivation and removal, and plasma for fractionation into plasma derivatives, which do.[3]

Plasma safety and residual pathogen risk with plasma-derived clotting factors

Due to improvements in the screening, testing, and surveillance of the mandatory viral infections (HIV-1, HIV-2, HBV, HCV) in blood collection services, the residual risk of transmissible diseases can be considered very low. However, there continue to be infectious diseases which emerge and re-emerge and threaten plasma supply despite being non-transfusion transmissible, said Dr. Svetoslav Slavov of the Hemocentro Ribeirão Preto, São Paulo, Brazil.

Residual risk is shaped mainly by emerging viruses which can contaminate plasma and plasma derivatives. Viral metagenomics can screen virtually all types of plasma (donor plasma, pooled plasma , etc.) to identify the whole viral composition and specific viruses present.[9,10] A small Brazilian study of 4 hemophilia patients characterized their virome composition and detected many commensal viruses, including infectious HCV in 3 patients.[10] Metagenomic sequence identification detected a rare type of HCV for Brazil, HCV genotype 4a. Metagenomic surveillance can be useful for evaluating residual risk of viruses in pooled plasma for product manufacturing; plasma-derived CFCs; and cell cultures for producing of recombinant factors.

DDAVP intranasal shortage and alternatives

Desmopressin acetate (DDAVP) is a key treatment for a large cohort of patients with a range of non-severe inherited bleeding disorders. The global recall of intranasal DDAVP in July 2020 and ongoing halt of manufacturing have had significant impacts on access to treatment in many countries around the world. Three leading clinicians shared regional perspectives and responses to the shortage of supply. They highlighted the need for more education and awareness of the use and benefits of DDAVP treatment for people with von Willebrand disease (VWD), mild or moderate hemophilia A, platelet disorders, and other bleeding conditions.

United States

Dr. Steven Pipe, Professor of Pediatrics and Pathology at the University of Michigan, United States, described the collaboration between NHF and the Hemophilia Alliance, which comprises federally funded HTCs in the United States, to provide an alternative DDAVP nasal spray for patients during the prolonged interruption of supply. At the NHF's request, the U.S. Food and Drug Administration (FDA) added the DDAVP nasal spray (sold as Stimate in the United States) to the drug shortage list. The Hemophilia Alliance undertook the upfront development costs and work with STAQ Pharma as compounding partner started in April 2021.

The recall notices from manufacturer Ferring Pharmaceuticals stated that potential evaporation out of the container and resulting super potent doses were the key issues for the Stimate DDAVP nasal spray. STAQ consulted with stakeholders from the Hemophilia Alliance and it was decided that a 6-dose vial with room temperature storage and a 90-day after opening expiration date would be safe and appropriate for patients and reduce the risk of evaporation being an issue. As of October 2021, the drug was approved and licensed in 39 states and patients were responding to the alternative DDAVP nasal spray appropriate to expectations and with no adverse events.

United Kingdom

Dr. Dan Hart, Consultant Hematologist at the Royal London Hospital Haemophilia Centre, UK, noted that although DDAVP use is "on demand" (at the times of need), it can be life-impacting treatment (e.g., for heavy menstrual bleeding; for avoidance of inhibitor risk in mild/moderate hemophilia A). However, data on the use of DDAVP is poorly reported across Europe relative to data on clotting factor concentrates, and access is seemingly under prioritized in some settings. A search of the UK National Haemophilia Database showed that 17,035 of 34,835 registered patients (48.9%) could theoretically benefit from DDAVP. Patients with mild hemophilia A (FVIII:C ≥10 IU/dL), VWD (RiCoF >10 IU/dL) excluding type 2B, or qualitative platelet disorder (excluding Bernard Soulier or Glanzmanns) and between ages 2 to 70 were deemed theoretically eligible for DDAVP. The eligible patients consisted of 51.3% of women and 46.2% of men in the database. Extrapolation of the UK data suggests that there may be substantial numbers of patients with bleeding disorders eligible for DDAVP in each country.

Current challenges for the community are the variations in access to the subcutaneous DDAVP preparation and in its availability in correct strength (15mcg/mL) in countries across Europe. The absence of the subcutaneous preparation in some countries augments the impact of the intranasal DDAVP recall. It is not clear why the subcutaneous preparation has not been prioritized to be widely available across the continent given benefits such as reduced infusion time, reduced side effect profile, and potential for broader community use.

Middle East

Dr. Magdy El-Ekiaby, Senior Consultant in Hematology at the Shabrawishi Hospital Hemophilia Centre, Cairo, Egypt, described results from a Middle East survey on the use and availability of DDAVP in Saudi Arabia, Lebanon, Tunisia, and Egypt. The survey looked at whether injectable DDAVP is registered in the country; whether intranasal DDAVP (150mcg/spray) is registered; whether there was a current shortage of the intranasal DDAVP; and which bleeding disorders are being treated with DDAVP (mild or moderate hemophilia, type 1 VWD, and/or inherited platelet disorders[11]). The survey was sent to 20 contacts within the Hemophilia Eastern Mediterranean Network (HEMNET). There were 7 respondents.

All reported that most forms of DDAVP are not available on a regular basis. DDAVP formulations for the management of bleeding disorders are registered in two countries; injectable DDAVP is registered in both countries, whereas intranasal DDAVP for hematological indications is registered in one. Both countries reported occasional use based on personal import of DDAVP by the patients themselves and/or their families. The lack of laboratory testing for evaluation of DDAVP efficacy in the countries surveyed makes it unpopular, and the preference is to use CFCs. The burdens of DDAVP efficacy assessment (e.g., travel for testing) makes it unpopular among patients and/or their families especially with respect to the safety and efficacy benefits of CFCs.

SAFETY

Reporting of safety issues: roles and responsibilities

Dr. Leonard Valentino, CEO of the U.S. National Hemophilia Foundation, discussed roles and responsibilities in safety surveillance. Good pharmacovigilance requires that all stakeholders understand their responsibilities for monitoring the effects of medical drugs, detecting adverse events and safety issues, and warning of potential risks. Of crucial importance, there must be a clearly established safety reporting pathway from the manufacturer/drug sponsor and regulatory authority to pharmacies, healthcare professionals, and patient organizations who should all receive safety information.

The stakeholder responsibilities, in brief, are as follows:

- Manufacturers are responsible for post-marketing safety alerts, periodic adverse experience reports, and letters to healthcare professionals.
- Regulators are responsible for overseeing voluntary and mandatory adverse event reporting and databases as well as drug safety communication.
- Healthcare professionals are responsible for surveillance and reporting, and informing and counselling patients.
- Patients are responsibility for monitoring and reporting.

Patients are both the stakeholders most impacted by safety information and the primary sources of information; all other stakeholders have an obligation to recognize that patients are at the centre of pharmacovigilance. Patients considering or receiving

treatment with investigational drugs must understand the basics of clinical trials, informed consent, drug safety reporting, and novel therapies in the pipeline. Patients must also understand the importance of participating in registries (e.g., World Bleeding Disorders Registry, WFH Gene Therapy Registry) to support information gathering and safety signal monitoring around existing and new therapies.

Non-replacement therapies and risk of thrombosis

Dr. Mike Makris, Professor at the University of Sheffield, UK, discussed risk of thrombosis in hemophilia patients treated with non-replacement and rebalancing therapies (bispecific antibody, antithrombin siRNA, anti-TFPI, activated protein C inhibitor). A 2020 study of over 6,100 patients on emicizumab identified 20 thrombotic events in total (including 4 thrombotic microangiopathy events); 6 cases were associated with the use of activated prothrombin complex concentrate (aPCC) along with emicizumab.[12] The number of thrombotic events reported are expected to continue to rise as more patients are treated with emicizumab.

Preliminary data from ongoing studies show that some non-replacement therapies currently have a higher thrombotic risk. Fitusiran, a siRNA agent, has shown higher mean antithrombin activity relative to baseline at 10-20 days after the first dose.[13] Following the occurrence of 5 thrombotic events (cerebral, spinal, atrial, cerebral venous sinus thromboses) in several patients, measures are being taken to reduce thrombotic risk: education of patients and clinicians, reduction of concentrate dose for breakthrough bleeds, and higher target antithrombin levels above 20%. Among anti-TFPI clinical trials, the concizumab trial has had 5 thrombotic events in 3 of 129 patients. The marstacimab trial has had no thromboses in 58 patients to date. The activated protein C inhibitor trial has had no thromboses in 34 patients to date. Given that clinical trials are performed on selected low-risk patients under basal conditions in which risk of thrombosis is low, data is needed on stress situations where risk of thrombosis is higher, such as surgery, infection, inflammation, and cancer.

Post hepatitis C monitoring and long-term follow-up

Hepatitis C is a common bloodborne viral infection that affects millions of people worldwide. Chronic HCV infection is a leading cause of liver cancer. HCV treatments and success rates have improved over the past 20 years. Today, oral HCV antiviral therapy can cure HCV infection with very high success rates (96%) in all 6 major HCV genotypes. There is no evidence that people with hemophilia have different success rates from the general population. Dr. Bruce Luxon, Chair of the Department of Medicine at Georgetown University in Washington, DC, described post-HCV monitoring and long-term follow-up.

Liver biopsy was initially the "gold standard" to assess fibrosis and quantify liver health; pre- and post-treatment biopsy data have since shown fibrosis reversal in the majority of cases.[14,15] Liver biopsy is not usually done in patients with bleeding disorders. Many

non-invasive techniques are now available to assess fibrosis including innovative combinations of standard tests (APRI), specialized proprietary tests (Fibrosure, Fibrotest), and elastography (ultrasound or MRI).

Treatment of HCV is now very routine; there are multiple oral regimens with nearly universal cure. However, there is wide geographic variability in access to treatment. Curing the HCV infection does not cure the liver disease. Patients can still have complications such as ascites, variceal bleeding, or liver cancer. Confirmation of the extent of liver fibrosis can be made by non-invasive measures such as specialized blood tests (Fibrosure, APRI) and elastography (intervals of 12-18 months). Patients with advanced fibrosis (F3 or F4) or cirrhosis are at risk of complications. For risk of hepatocellular carcinoma or cirrhosis, ultrasound screening should be done every 6 months to determine if fibrosis has improved.[16] For risk of variceal bleeding, endoscopic screening by EGD esophagoscopy should be done every 1-2 years. Assessment of liver fibrosis and risk of complications should be made with the aid of a hepatologist, especially if a gene therapy research protocol is an option.

Long-term follow-up of non-factor replacement therapies

Michael Recht, Chief Science Officer, American Thrombosis and Hemostasis Network (ATHN), described ATHN 7, a 4-year natural history study of 500 people with hemophilia with or without inhibitors receiving a FDA-approved therapy, i.e., non-factor, bypassing agent, or clotting factor replacement product. The primary objective is to determine the safety of the products being used. It collects adverse events data including: allergic or other acute events, treatment-emergent side effects, transfusion-transmitted infections, inhibitor development, thrombosis and cardiovascular events, malignancies, neurological events, and deaths. ATHN 7 also collects data on adverse events of special interest that have arisen in clinical trials of non-factor replacement therapies: thrombotic microangiopathies, potential drug-induced liver injury, development of anti-drug antibodies, severe and unanticipated bleeding, and hospitalization.

Adverse events included allergic and skin reactions in a patient on factor concentrate therapy; a severe bleeding event due to trauma in a patient on emicizumab. The death of a patient on emicizumab thought by the local investigator to be unrelated to the therapy. Assessment of quality of life using the EQ–5D–5L questionnaire showed that the majority of patients had no problems with mobility, self-care, usual activities, and anxiety/depression. In addition, 47.5% of patients reported having no pain/discomfort, whereas 28.7% of patients had slight problems, 16.6% had moderate problems, and 5.7% had severe problems. Despite these issues, patients rated the same as age-matched individuals in the United States without chronic conditions. The data showed overall health state of 83.9%, which compares favourably to 80.4% for the US population in general.

TREATMENT QUESTIONS

Extravascular distribution of factor IX

Darrel Stafford, Professor of Biology at the University of North Carolina, United States, gave an overview of the extravascular reservoir and distribution of FIX. FIX binding to collagen IV in the extravasculature appears to be physiologically important for hemostasis, with 3-4 times more FIX in the extravasculature than in the plasma.[17] The extravascular reservoir of FIX is readily accessible to the intravascular space and functionally contributes to hemostasis. Some EHL FIX products have failed to deliver their promise that "higher plasma factor IX coagulation provides longer protection." Breakthrough bleeds have been reported in phase 4 EHL FIX trials. A survey of select US and Canadian hemophilia centres found a high percentage of breakthrough bleeds in patients on rFIX-PEGylated/Rebinyn (50%) and recombinant FIX-albumin/rFIX-FP/Idelvion (62%); none were reported in patients on rFIX-Fc/Alprolix.[18] A case series study reported 3 patients on rFIX-FP with breakthrough bleeding while having plasma FIX activity levels of 45%, 46% and 82%, which were most likely related to decreased extravascular distribution.[19]

The extravascular pool and distribution of FIX and expression of cross-reactive material positive (CRM+) or negative CRM-) FIX variant also contribute to hemostasis. Preclinical data suggest no further coagulation benefit beyond 150 IU/kg in CRM- hemophilia B mouse model. FIX variants with increased recovery and half-life may not be the hoped-for panacea. Patients with endogenous defective FIX require much different treatment than CRM- patients. Higher dose and short dosing interval are likely required to maintain adequate prophylaxis for CRM+ patients due to ongoing competition from the endogenous defective FIX.

Extravascular pool of factor IX: clinical implications for EHLs

Dr. Robert Sidonio, Pediatric Hematologist/Oncologist at the Aflac Cancer and Blood Disorders, Atlanta, United States, discussed extravascular FIX and understanding of its role in hemostasis and presented clinical data on several extended half-life FIX products. Development of EHL FIX has been based on the hypothesis that FIX in the extravascular space bound to collagen IV is the relevant FIX rather than the FIX in the bloodstream; and the hypothesis that higher dose of FIX (150 IU/kg/dose) is needed to saturate collagen IV receptors for optimal bleed control and protection. (Note: This dose is not approved in any country.) EHL products achieve different FIX trough levels and different pharmacokinetics due to their paths of distribution. Excessive bleeding has been reported in some patients treated with EHL FIX despite high trough levels.[19]

In a retrospective survey of the use and performance of EHL FIX productts (rFIX, rFIXFc, rFIX-FP, N9-GP) in clinical practice at 6 US and Canada-based hemophilia treatment centres, poorly controlled bleeding events requiring more frequent/higher doses of EHL FIX than anticipated were reported in 62% of patients of rFIX-FP, and 50% of

patients on N9-GP.[17] Post-approval phase 4 clinical trials show variations in annual bleed rates, target joint resolution, and joint pain/arthralgia with different EHL FIX products: 8 of 59 patients (13.6%) on rFIX:FP developed a target joint; target joint resolution was achieved in 6 of the patients.[20] No patients on rFIX:Fc developed target joints but 6 of 116 patients (5%) had arthralgia. Arthralgia was reported in 2 of 44 patients (4.7%) on rFIX at 50 IU/kg/dose twice per week, and in 4 patients (9.1%) at 100 IU/kg/dose once per week.[21] A nonacog alfa rFIX phase 4 trial comparing prophylaxis vs. on-demand treatment in moderately severe to severe hemophilia patients (100 IU/kg/dose once per week) reported arthralgia in 5 of 25 (20.0%) in each cohort.[22]

In ongoing clinical trials, N9-GP has resolved most but not all target joints. Among 13 patients on 40 IU/kg/dose, 18 of 20 target joints (90%) were resolved as of >350 days.[23] Comparison of N9-GP at 10 IU/kg/dose and 40 IU/kg/dose showed likelihood to respond to a single dose for a bleed was higher in the 40 IU/kg/dose cohort (99% vs. 84%) and resolution of target joint success was much higher in the higher dose cohort (67% vs. 7.7%).[24] Spontaneous bleed rate was higher than anticipated, particularly in the 10 IU/kg/dose cohort (70% vs. 50%).

Starting emicizumab in very young pediatric patients

Dr. Gil Kenet of the Israel National Hemophilia Center and Thrombosis Institute, Ramat Gan, Israel, described the use of non-replacement therapy (emicizumab) in infants and young children to date. Standard prophylaxis with clotting factor replacement has been the cornerstone of joint disease prevention in hemophilia treatment. For children with hemophilia, early initiation of prophylaxis is crucial to prevent life-threatening bleeds, particularly intracranial hemorrhage. Emicizumab adds to the options for prophylaxis. However, the decision-making process of when to start treatment, with what agent, and how to establish prophylaxis is becoming more complex. Key questions include: How should FVIII tolerance be acquired and maintained in previously untreated patients on emicizumab? How should prophylaxis with emicizumab be monitored? How will the use of emicizumab affect the incidence and occurrence of FVIII inhibitor?

Brief experience and limited data with emicizumab in young children imply that starting emicizumab prophylaxis requires careful consideration, despite the more convenient subcutaneous route of administration.[25, 26] For children newly diagnosed with hemophilia, it is recommended that healthcare providers have a discussion with the parents about the risk of inhibitor development in relation to exposure to clotting factor concentrates and unknowns in the use of emicizumab before completion of 50 exposures. The product should be chosen to minimize the risk of inhibitor development. Prophylaxis with emicizumab is expected to delay the patient's exposure to factor concentrates, therefore, it may take many years to reach 50 exposures, the main period of risk for inhibitor development. Regular exposure to FVIII in a prophylaxis regimen appears protective, whereas sporadic and high-dose FVIII

exposure only during on-demand treatment of bleeds is more likely to induce inhibitors.[25,27,28]

Real-world data on emicizumab use in patients with hemophilia with or without inhibitors at 3 US hemophilia treatment centres showed that all patients experienced a decrease in annual bleeding rates and treated bleeds and joint bleeds.[29] No thrombotic events, thrombotic microangiopathies, or deaths have occurred. No anti-drug inhibitor or loss of efficacy has been reported (testing for anti-drug antibodies has not been routinely performed). Data from the longitudinal Israeli study of 113 severe hemophilia A patients (including 31 with inhibitors) on emicizumab followed for up to 2.5 years showed ABRs were significantly reduced and there were no bleeds in 49% of patients. Emicizumab steady state levels and thrombin parameters were well maintained in both children and adults.[30,31]

A key question is at what age should emicizumab be started. Based on current experience,
emicizumab should be started before 9 months of age in cases of very early bleeding requiring long-term prophylaxis when IV therapy is impossible or extremely difficult or in cases of parental anxiety regarding risk for intracranial hemorrhage. Emicizumab should be started after 9 months of age when the goal is avoidance of central venous access devices or if there is parental preference over factor concentrates. There is currently limited data on emicizumab and how to address the first 50 exposures to factor, when inhibitor risk is highest. It is unclear whether emicizumab alone may delay, increase, or reduce inhibitor formation. If emicizumab is started, consideration might be given to the addition of FVIII once or twice weekly to induce FVIII tolerance until 50 exposures are reached. Until more results are available, it remains unknown whether to start emicizumab alone or with FVIII for the first 50 exposures.

Subcutaneous delivery of factor

Dr. Johnny Mahlangu, Professor at the University of the Witwatersrand, Johannesburg, South Africa, discussed the benefits of subcutaneous delivery of clotting factors and their potential to address some of the challenges and unmet needs with intravenous factor replacement therapy: immunogenicity, intravenous burden and adherence, and maintaining of steady state factor levels. The rationale for subcutaneously administered clotting factor is based on the slow absorption of drug protein in the plasma using this route with time to maximum concentration of up to 8 days; slow subcutis convection and diffusion to lymphatic and venous capillaries resulting in absorption rate-limited PK and prolonged systemic exposure; and prolonged half-life due to the drug depot returning to circulation via the lymphatic and venous systems.

Subcutaneously administered clotting factors offer several advantages: higher and more stable factor levels, continuous protection, convenience, and easier administration by quick and simple injection. They are ideal for pediatric patients. Immunogenicity of subcutaneous rFVIIIa has been high. In phase 1/2 studies,

subcutaneous rFVIIIa (N8-GP) was associated with a high incidence of antibodies in previously treated patients with severe hemophilia A resulting in a decline in FVIII and increased bleeding events. Further clinical development of subcutaneous N8-GP was suspended. Phase 1/2 studies of another subcutaneous rFVIIIa (OCTA101) have been completed; the program remains active but is no longer recruiting. Immunogenicity of subcutaneous rFVIIa (eptacog alpha; marzeptacog alfa/MarAA) and subcutaneous rFIX (dalcinonacog alfa) appear to be low, therefore, these programs are progressing to phase 2 and 3 of development. It is important to note that immunogenicity in the early phases of clinical development is not always predictive of future immunogenicity.

VON WILLEBRAND DISEASE

Novel treatment modalities for VWD

Dr. Cécile Denis of France's National Institute of Health and Medical Research (INSERM), discussed options to improve the management of von Willebrand disease (VWD), novel treatment modalities for specific VWD types and subtypes, and genetic approaches using gene therapy, gene editing, transcriptional silencing. Management of VWD over the past 30 years has consisted of von Willebrand factor (VWF) and DDAVP. Despite the heterogeneity of the disease, similar therapeutic approaches are used for the different VWD subtypes.

Previous studies have shown reduced VWF levels are also associated with reduced FVIII levels. In type 3 VWD patients, it is unclear which part of the bleeding is caused by the absence of VWF and which part is caused by the absence of FVIII. Insights from recombinant FVIII treatment in VWD patients suggests rFVIII provides hemostatic relief under some conditions in type 3 VWD patients, however, the half-life of rFVIII in type 3 VWD patients is too short to consider it as a prophylaxis option. Very long-acting FVIII could be considered, such as BIVV001, which has a half-life of more than 40 hours and circulates independently of VWF.

Emicizumab may have potential clinical application in VWD. Three reported cases have shown a markedly reduced bleeding tendency in type 3 VWD patients upon treatment with emicizumab. *Ex vivo* perfusion studies using blood from VWD patients suggest improved thrombus formation upon the addition of emicizumab. These data suggest that compensating FVIII deficiency in VWD using emicizumab could provide a clinical benefit, but long-term follow up and additional clinical evaluation is needed.

Current hurdles for the genetic approaches include the need to specifically target endothelial cells (most systems are targeting hepatocytes) and determine how many endothelial cells need to be targeted in order to obtain a clinically relevant effect. Another challenge is how to optimize VWF expression. In addition, gene therapy approaches may be limited by the presence of dominant-negative mutations. With siRNA therapy, the key issue is how to specifically attack missense mutations at the mutated allele.

VWD prophylaxis

Dr. James O'Donnell, Professor of Vascular Biology at the Royal College of Surgeons, Ireland, discussed the rationale for long-term prophylaxis in VWD and current unmet clinical and research needs. Spontaneous joint bleeds occur in an estimated 50% of type 3 VWD patients and 10% of types 1 and 2 VWD patients.[32] Prophylaxis may be useful to prevent joint bleeds and future arthropathy, control recurrent refractory mucosal bleeds (epistaxis, heavy menstrual bleeding, and gastrointestinal bleeding), and treat recurrent iron deficiency.[32]

The biological functions of VWF are more complex than FVIII. VWF serves as a carrier for FVIII, and stabilizes and protects FVIII against proteolysis and clearance.[33] VWF influences primary platelet plug formation and secondary thrombin generation.[34] Bleeding phenotype in VWD does not correlate with plasma VWF levels. Data from the Zimmerman Program showed similar abnormal bleeding scores in patients with different VWF levels in the 10-50% range.[35] Studies have shown that "normalization" of plasma VWF levels with ageing or pregnancy is not necessarily correction of bleeding phenotype.[36,37] This shows that treatment/optimal therapeutic target must be tailored for each individual patient based on personalized VWF pharmacokinetics.

Important unanswered questions include: selection of patients, optimal prophylaxis treatment regimen, target peak and trough VWF and FVIII levels, and relative importance of VWF in contrast to FVIII in different types of bleeding: joint bleeds, gastrointestinal bleeding, heavy menstrual bleeding, and inflammation and angiogenesis. A systematic review of long-term prophylaxis in VWD found that VWF prophylaxis reduced risk of bleeding episodes, heavy menstrual bleeding, and hospitalization.[38] There is a critical need for adequately powered clinical trials to augment the evidence and address unanswered questions.

Limitations of the VWD guidelines

Dr. Paula James, Clinical Co-Chair of the VWD Diagnosis Guidelines Development Panel and Professor of Hematology at Queen's University, Kingston, Canada, discussed the development and limitations of the VWD Diagnosis and Management Guidelines published in 2021.[38, 39] Guidelines development followed the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Methodology.[40] Priorities were determined by an international scoping survey. There were 601 respondents from 71 countries, 82% of which were high-income countries and 18% of which were low/middle-income countries.

The limitations of the guidelines are inherent in the low or very low certainty in the evidence identified for many of the questions. The biggest limitation was lack of high-quality evidence; only 3 of 23 recommendations were deemed by the panel as strong. The guidelines took the perspective of high-resource settings. Some

recommendations may not be fully achievable in low- and middle-income settings. VWD is a heterogeneous disorder, therefore, it is a challenge to make recommendations that are broadly applicable. There are also challenges related to the many different healthcare settings, different payers and insurers, and other factors that can have impacts on patient ability to access care.

Feedback following publication has been very positive. One criticism was wrong classification of some recommendations as being strong or conditional. For clinicians, strong recommendations indicate most individuals should receive the intervention and conditional recommendations suggest that different choices and interventions will be appropriate for different patients depending on their values and preferences. Classifications were limited by the lack of scientific data. Shared decision-making on individual patient management is essential. Another criticism was he diagnostic threshold of type 1 VWD as VWF level <0.30 IU/mL regardless of bleeding and VWF level of <0.50 IU/mL to confirm diagnosis of type 1 VWD in patients with abnormal bleeding. This is a strong recommendation based on low certainty in the evidence of effects. It prioritizes assigning a clear diagnosis to patients who bleed. It has been well received by US treaters, however, it does not account for differing pathophysiology.

The recommendation on genetic testing suggests using either the VWF:FVIII binding (VWF:FVIIIB) assay or targeted genetic testing (when available) for patients with suspected type 2N VWD in need of additional testing, given the serious bias with both assays used as the reference standard, novel variants identified with unknown phenotype, and the importance of genetic testing for family counselling. The recommendation was deemed as conditional based on low certainty in the evidence from diagnostic accuracy studies. However, the feedback suggests that genetic testing should have been prioritized. While the VWF:FVIIIB assay is not widely available, plasma samples can be shipped.

The recommendation on using long-term prophylaxis rather than no prophylaxis in patients with VWD with a history of severe and frequent bleeds is conditional based on low certainty in the evidence of effects and the low-quality evidence. Additional data is a key priority for future VWD research, and will make the recommendation a strong one. Gaps in research and understanding were identified; with new evidence, recommendations could and should be updated. There are active discussions regarding low- and middle-income country adaptations.

New initiatives in VWD diagnostic tests

Dr. Angela Weyand, Assistant Professor of Pediatrics at the University of Michigan, United States, gave an overview of VWD testing and diagnosis including new diagnostic assays. All patients suspected to have VWD should be tested; patients without personal or family history of bleeding do not need testing.[41] The International Society on Thrombosis and Haemostasis Bleeding Assessment Tool (ISTH BAT) is used to identify patients with positive/abnormal bleeding scores and patients with

negative/normal bleeding scores. As VWD is prone to pre-analytical errors and VWD levels are affected by extraneous forces leading to significant numbers of false positives and false negatives,[42] patients should then have blood tests to measure complete blood count (CBC), prothrombin time (PT), and partial thromboplastin time (PTT); and optional fibrinogen and thrombin time (TT) testing. Those with normal results are deemed to not have VWD. Those with abnormal results should have specialized laboratory blood tests to measure FVIII level, VWF antigen level (VWF:Ag), and VWF/ristocetin cofactor activity (VWF:RCo) to confirm VWD diagnosis and determine its severity.

The majority of centres perform repeat testing if there is high suspicion of VWD, using a cutoff of >100 IU/dL for VWF antigen or activity yields to rule out a diagnosis of VWD.[43,44,45] As VWF:RCo assay results show higher coefficient of variation and the assay has difficulty assessing VWF levels <20 IU/dL leading to false positive results, newer assays such as the VWF glycoprotein Ib binding assay (VWF:GPIbM) and VWF binding to wild-type recombinant glycoprotein Ibα receptor assay (VWF:GPIbR) should be used where available; these assays have not yet been approved in the United States.

There are several new assays. Automated multimer analysis measures platelet-dependent VWF activity and VWF:Ag ratio. However, this assay is limited in availability and by its turnaround time. Hydragel automated multimer analysis has shown 74% concordance with in-house multimer analysis but with discrepancies in acquired VWD and type 2 VWD; 3 patients with type 2A VWD were not identified.[46] The ELISA-based multiplex activity assay simultaneously analyzes GPIb, collagen III and FVIII binding, in addition to VWF:Ag and VWF propeptide (VWFpp). Overall, the assay is able to correctly identify 72.9% of diagnoses.[47]

REAL WORLD DATA

WFH hemophilia treatment guidelines: setting the standards of care

Donna Coffin, WFH Director of Research and Education, described the new WFH Guidelines for the Management of Hemophilia published in 2020, dissemination of the guidelines through training and education initiatives, and implementation of the practical recommendations and standards of care in clinical practice. The guidelines were developed with the aim of global applicability in high-resource and low-resource countries. Chapters were updated following review of new evidence, led by a panel of experts including a minimum of 25% membership of people with hemophilia or caregivers. New chapters address the topics of principles of care, prophylaxis, genetic assessment, inhibitors, and outcome assessment.

The level of hemophilia care around the world varies considerably. Key goals are to reduce variations in clinical care globally. Using a stepwise approach, the WFH is now focusing on implementation of the guidelines through training and education of healthcare professionals, and patients, towards influencing clinical practice and

improving health outcomes for patients globally. With funding support from the Hemophilia Alliance, the WFH has developed an online Resource Hub with slide sets, narrated videos, key message documents, and recommendations. Spanish, French, Russian, Arabic translations are in progress.

Humanitarian Aid initiatives based upon the WFH treatment guidelines

Since 1996, WFH humanitarian aid initiatives have reached 110 countries and over 1 billion units of clotting factor concentrates and other treatment products from industry partners have been distributed worldwide. The WFH is currently working with 81 countries. Dr. Jay Martin, Development and Training Manager of the Humanitarian Aid Program, described activities to provide educational support and access to scientific information and to train treaters on the use of treatment products. The goals are to assist treatment centres and national health systems to achieve best-practice standards of care in alignment with the WFH treatment guidelines.

During the COVID-19 pandemic, many activities have shifted to virtual platforms, which have expanded their reach and participation. Activities included: scientific sessions on EHL factor concentrates and novel therapies in inhibitor management, case conferences and bedside rounds on hemophilia diagnosis and management for front-line doctors and second-line treaters, and Training the Trainers workshops. In 2021, over 1,000 people in 66 countries participated in the virtual events. Activities in 2022 include scientific sessions on prophylaxis, surgeries, and immune tolerance induction; and laboratory workshops on diagnosis of hemophilia and inhibitors in Africa, South and East Asia, and Southeast Asia and West Pacific.

The WFH uses the Knowledge, Attitude and Behaviour (KAB) Model for measuring and assessing the benefits of its educational initiatives. Level of knowledge is evaluated through assessments before, during, and after the scientific sessions, anonymous poll questions, discussions during question and answer periods, and open forum exchanges. Questions are integrated within post-session evaluations and surveys to assess whether participants feel they have gained new knowledge. Follow-up surveys are used to assess whether there have been changes in clinical practice, particularly in terms of increased diagnosis of hemophilia and other bleeding disorders, adoption of best practices, and implementation of prophylaxis regimens.

Measuring outcomes with WBDR and GTR

Donna Coffin, WFH Director of Research and Education, gave an overview of the WFH's data collection platforms and sources for measuring outcomes in people with hemophilia. The Annual Global Survey (AGS) collects and compiles NMO-reported population-level data on an annual basis from reporting countries. The World Bleeding Disorders Registry (WBDR)launched in 2018 is a registry of clinician-reported, patient-level data such as number of bleeds, type of treatment, and hospitalization. The Gene Therapy Registry (GTR) to launch in 2022 will collect clinician-reported, patient-level

data on gene therapy safety and efficacy endpoints. Each registry also has a patient app (myWBDR and myGTR) that allows patients to report their own data directly and includes quality-of-life measures using the Patient Reported Outcomes Burdens and Experiences (PROBE) questionnaire. These sources together provide a 360-degree view of patient data.

The AGS collects NMO-reported data and provides population-level aggregate data such as percentage of people with severe hemophilia on prophylaxis. Such data can be useful for many purposes such as resource allocation by WFH and for advocacy, but do not provide data on patient outcomes such as number of bleeds or quality of life. The WBDR collects patient-level data from the patient app myWBDR, which includes treatment and quality of life data. The WBDR also collects clinician-reported individual patient data such as number of bleeds, hospitalization rate, and joint status. Ultimately, the goal is to help bring about changes in clinical practice that lead to improvements in key patient outcomes to reduce ABR and hospitalization rates, and improved quality of life. As of November 2021, WBDR enrollment consisted of 8,891 people with hemophilia from 96 hemophilia treatment centres in 46 countries.

Annual Global Survey: capturing new products

Jeff Stonebraker, Associate Professor at North Carolina State University, United States, discussed the challenge of how to measure the treatment of hemophilia care globally and over time with new products that have different modalities. Factor usage (IU per capita or population) has been used since the 1970s for comparisons, advocacy, and resource allocation decisions. The availability of extended half-life factor concentrates and emicizumab in recent years has added new classes and types of treatment to the product mix, which has made it difficult to measure trends and compare usage and cost-effectiveness towards benchmarking hemophilia care. He described the undertaking to develop a method for converting international units (IU) of standard or extended half-life FVIII and milligrams of emicizumab into a single metric (equivalent unit) and for deriving an equivalent conversion unit for SHL and EHL FIX.

SHL/EHL conversion ratios were derived based on the weighted-average annual factor utilization (IU/kg/patient) and number of patients using SHL and EHL products in real-world studies and data from the Canadian Bleeding Disorders Registry (CBDR) and Web-Accessible Population Pharmacokinetics (WAPPS) compared to prescribing information. The conversion ratios were then applied to WFH Annual Global Survey data from Canada, Ireland, UK, and US (49 of 147 countries provided EHL and emicizumab usage data to the WFH AGS, but only Canada, Ireland, UK, and US were 100% complete.) The prescribing information in the United States and Europe is marginally different. The SHL/EHL conversion factors are higher when calculated based on the prescribing information than on real-world studies, which are considered more representative of clinical practice. FVIII and emicizumab usage were converted into a single metric for hemophilia A; and SHL and EHL FIX usage were converted into a

single metric for hemophilia B. These conversions generated equivalent units for SHL and EHL factor concentrates and emicizumab:

- hemophilia A: 1 IU EHL FVIII = 1.04 IU SHL FVIII; 1 mg emicizumab = 70 IU SHL FVIII
- hemophilia B: 1 IU EHL FIX = 1.87 IU SHL FIX

Usage of a single, harmonized metric will facilitate benchmarking across different countries or longitudinally, irrespective of the case-mix of treatment options.

How patient-reported level data can be used to compare therapies before and after switching

Dr. Martin Scott, Consultant Hematologist with the UK National Haemophilia Database (NHD), described the NHD and Haemtrack patient-reported home treatment system, and how the data can be used for within-patient analysis and comparison of therapies before and after switching. All patients with bleeding disorders in the UK are registered in the database. Haemtrack enables people with hemophilia and other bleeding disorders to record and report their treatment using an Android or iPhone app, or web-based or paper reporting. Patients report all treatments (dose, brand, batch), the reason for treatment (prophylaxis, new bleed, follow-up treatment, ITI), and bleed details (cause, position, dose interval, pain score, effect on function).

The NHD collects bleed and treatment data from large sample sizes, with high-level data from an unselected group. It has the ability to identify a specific study population (e.g., patients taking a specific product, patients reporting prophylaxis, patients reporting prophylaxis compliantly [≥ 75% of issued treatment]) and make meaningful analyses of treatment practices and outcomes, which are useful in administrative, regulatory and research applications. The NHD is a unique source of real-world data in terms of sample size and granularity of data. Its dataset consists of demographics, diagnostic data, inhibitor status/history, quarterly treatment returns, product, volume, patient weight, adverse events, hemophilia joint health score (HJHS), genetics, and cause of death. The data also enable within-patient comparisons of treatment practices and outcomes such as pre- and post-product switch tracking of ABR, AJBR, infusion frequency, factor consumption, and HJHS; and longitudinal analysis of patients on new types of therapy.

GENE THERAPY

Importance of integration as a potential safety risk or unknown

Adeno-associated viral (AAV) vectors are used in hemophilia gene therapy to deliver FVIII or FIX to the liver, which is the primary site of FVIII and FIX synthesis and the chosen tissue for transgene expression for hemophilia gene therapy. Dr. Paul Batty of the Department of Pathology and Molecular Medicine at Queen's University, Kingston, Canada, discussed AAV integration, potential safety risks, and persistence of transgene expression in hemophilia gene therapy preclinical and clinical trials. Ongoing AAV-

based hemophilia gene clinical trials have shown persistent clinically relevant expression for over 5-10 years, lower bleed rates and lower factor concentrate requirement.[48] However, there is variable expression among patients and long-term duration of transgene expression is not yet known. The main adverse events seen have been infusion reactions and transaminitis but long-term safety risks are unknown.

Preclinical studies have shown different findings. Recurrent integration was seen in mice only in the Rian locus (Chr12) in Mir341, however, these integration findings in mice have not been seen in large animals or clinical studies. Long-term preclinical studies in canines have seen AAV integration at low frequency throughout canine genome,[49] and increase in factor expression with clonal expansion of cells harboring integrated AAV vectors.[50] Some studies in mice have seen an association with hepatocellular carcinoma. However, no HCC or chronic liver disease was seen in pre-clinical studies in canines. The translational relevance of pre-clinical model findings is currently unclear. No genotoxicity has been seen in clinical AAV gene therapy studies. Further studies are needed to evaluate questions surrounding integration in clinical studies.

Duration of steroid therapy and its complications in gene therapy

Transient hepatotoxicity is the most frequent adverse event reported in AAV-mediated liver-directed gene therapy. Dr. Margareth Ozelo, Professor at the University of Campinas, Brazil, described immune response to AAV vector and results on the use of corticosteroids on-demand and prophylactically in AAV-based hemophilia gene therapy clinical trials. In recent hemophilia B gene therapy clinical trials, transient alanine aminotransferase (ALT) elevations have occurred in 16% of patients at 2e13 vg/kg dose (UniQure) and 20% of patients at 5e11 vg/kg dose (Spark/Pfizer**)**, all of whom then received on-demand oral corticosteroids; and in 50% of patients at 4.5e11 vg/kg to 1.5e12 vg/kg dose (UCL-Freeline), all receiving oral corticosteroids and methylprednisolone prophylactically.

In AAV hemophilia A gene therapy clinical trials, higher ALT elevations are seen at various stages and doses: 33% in patients at 2e12 vg/kg dose (Spark/Roche); 75% in patients at 2e13 vg/kg dose (Bayer); 79% in the patients at 6e13 vg/kg dose (BioMarin); and 80% in the 3e13 vg/kg dose (Pfizer/Sangamo). The Spark/Roche trial began with on-demand steroids then switched to prophylaxis. In the three other trials, 65-80% of patients were treated with oral corticosteroids beginning 1-3 months post-gene therapy. The immunogenicity data suggest that low-dose AAV has lower transduction. Higher doses of AAV vector may improve transduction efficiency but may also augment AAV capsid-specific T-cell response.[51]

Assessment of T-cell-mediated immune response to AAV capsid is performed using the IFN-γ ELISpot (enzyme-linked immunospot) assay and the CD137 surface marker expressed on antigen activated T-cells and NK cells.[52] Good correlation between AAV capsid-specific immune response (IFN-γ ELISpot results) and ALT levels and/or factor

expression was shown in some GT clinical trials,[53,54] but other GT clinical trials observed no correlation.[55] Other cellular markers are unknown.

Transient hepatoxicity is well controlled in patients responsive to corticosteroids. The most frequent adverse event observed following systemic administration of AAV vector in liver-direct gene therapy trials occurs during a period of 4-16 weeks after gene transfer and is AAV vector dose-dependent. Potential mechanisms for transaminitis include: immune response (cellular immune response to AAV capsid or AAV genome), cellular stress (unfolded protein response, programmed cell death) and endoplasmic reticulum stress (GRP78 may be used as a biomarker), and vector contaminants. Corticosteroids helped in most cases to resolve the transient hepatotoxicity and to preserve transgene expression. A phase 3 BioMarin trial (BMN 270-301) found no significant changes in FVIII expression after cessation of corticosteroids, generally after 52 weeks of treatment. Patients with low ALT levels at baseline appear to use corticosteroids more frequently and for longer periods of time. Key questions include whether corticosteroids_should be used prophylactically, the best time to start, the optimal dose and duration, and whether or not to combine corticosteroids with other immunosuppressants.

Development of hepatocellular carcinoma

Dr. Heiner Wedemeyer, Professor in the Department of Gastroenterology, Hepatology and Endocrinology at Hannover Medical School, Germany, discussed risk of hepatocellular carcinoma, including in patients with controlled or cured viral hepatitis, and how to screen patients for HCC. HCC development is an emerging risk in AAV-mediated hemophilia gene therapy trials. A case report from UniQure's phase 3 gene therapy trial in adults with hemophilia B described HCC in a 69-year-old patient confirmed HCV positive in 2003, who showed no significant fibrosis (Fibroscan 5.7 kPa) when later evaluated for HCV eradication therapy and successfully treated as shown by sustained virologic response.[56,57] The patient had a social history of prior smoking and alcohol consumption and a family history of cancer.

Control of HCC is associated with tumour-antigen-specific T-cell responses.[58] HCC after HCV cure is associated with systemic inflammation.[59] There are changes over time and various factors which may contribute to HCC risk and the production of liver cancer (hepatogenesis): viral hepatitis, inflammation, fibrosis, and cirrhosis. Screening is important because treatment options have increased and improved, and outcomes are excellent if HCC is detected early. Comorbidities associated with increased HCC risk include: metabolic conditions such as metabolic/non-alcoholic fatty liver disease and non-alcoholic steatohepatitis, specific genetic diseases such as hemochromatosis, vascular conditions, and some autoimmune liver disorders such as autoimmune hepatitis and viral hepatitis.[60] Other risk factors include inactive lifestyle, body weight, and drug, tobacco, or alcohol consumption.[61] A recent study found Aspirin (acetylsalicylic acid) reduces HCC risk[62]; however, Aspirin is contraindicated in people with bleeding disorders. The use of statins in patients with HBV is also associated with

reduced HCC risk.[63] Recent genetic analysis has identified new HBV biomarkers (HBV DNA, HBsAg, and HBV core-related antigens) to be associated with increased HCC risk.[64]

Review on measurement of FVIII and IX post gene therapy

Dr. Annette Bowyer, Senior Biomedical Scientist at Sheffield Teaching Hospitals NHS, UK, described measurement of FVIII and IX post-gene therapy and assay differences reported between one-stage reagents, chromogenic reagents, and one-stage and chromogenic assays. Assay discrepancies have been reported by FVIII and FIX gene therapy clinical programs. Laboratory data are sparse; not all methodologies and not all reagents have been disclosed. All FVIII gene therapy approaches use AAV and B-domain deleted rFVIII. Gene therapy trials of B-domain-deleted FVIII have shown one-stage assay results 1.6 fold higher than chromogenic assay results.[65,66] Possible causes of discrepancy include reagent effects, cell line, kinetic studies of one-stage and chromogenic FVIII:C, and accelerated activation of FX and thrombin generation.[65] FIX gene therapy trials have been ongoing for almost a decade. Three rFIX molecules are used: wild-type, FIX Padua, and codon-optimized FIX. FIX Padua transgene discrepancies have been seen in inter-laboratory variability and intra-laboratory variability.[67]

FDA regulatory requirements for monitoring gene therapy recommends that to better interpret factor activity, sponsors also consider performing in vitro studies using samples containing the transgene product from animal plasma or ex vivo-transduced cells to compare the performance of one-stage and chromogenic assays.[68] Both assays should be calibrated in IUs of factor activity and should use a reference standard analogous to the expressed transgene, if available. The FDA recommends using various clinical factor activity laboratory assays in preclinical animal studies and, where feasible, assays intended for human use. The FDA also recommends that sponsors perform analytical studies to clarify the biochemical root causes for any discrepancies observed, addressing methodology (one-stage vs. chromogenic), reagents (phospholipids, activators, chromogenic substrates), conditions (incubation times, temperature), and choice of reference standards. Measurement of transgene expression will be necessary.

ABSTRACT PRESENTATIONS

Roshni Kulkarni, Director of the Centers for Bleeding and Clotting Disorders and Professor at Michigan State University, United States, described the clinical development of efanesoctocog slfa (BIVV001), a new class of FVIII replacement therapy.

Johnny Mahlangu, Professor at University of the Witwatersrand and NHLS, Johannesburg, South Africa, discussed long-term safety and efficacy of the anti-TFPI monoclonal antibody marstacimab in patients with severe hemophilia A or B based on results from a phase 2 long-term treatment study.

Ulrike Reiss, Associate Member of St. Jude Children's Research Hospital In Memphis, United States, discussed the protocol for gene therapy clinical trials in lower-middle income families.

Baisong Mei, Senior Global Project Head of Rare Diseases and Rare Blood Disorder Clinical Development at Sanofi in Cambridge, United States, gave an update on the status of fitusiran.

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