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REVIEW

RNAi for the Treatment of People with Hemophilia: Current Evidence and Patient Selection

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Abstract: Severe hemophilia is associated with spontaneous, prolonged and recurrent bleeding. Inadequate prevention and treatment of bleeding can lead to serious morbidity and mortality. Due to the limitations of intravenous clotting factor replacement, including the risk of inhibitory antibodies, innovative novel therapies have been developed that have dramatically changed the landscape of hemophilia therapy. Ribonucleic acid interference (RNAi) has brought the opportunity for multiple strategies to manipulate the hemostatic system and ameliorate the bleeding phenotype in severe bleeding disorders. Fitusiran is a RNAi therapeutic that inhibits the expression of the natural anticoagulant serpin antithrombin. Reduction in antithrombin is known to cause thrombosis if coagulation parameters are otherwise normal and can rebalance hemostasis in severe hemophilia. Reports from late stage clinical trials of fitusiran in hemophilia A and B participants, with and without inhibitory antibodies to exogenous clotting factor, have demonstrated efficacy in preventing bleeding events showing promise for a future "universal" prophylactic treatment of individuals with moderate-severe hemophilia.

Keywords: hemophilia, RNAi, siRNA, fitusiran, inhibitor, ribonucleic acid interference, small integral RNA

Introduction

Hemophilia A and B are inherited X-linked recessive bleeding disorders caused by mutations in coagulation factor VIII (hemophilia A) and IX (hemophilia B). These are essential proteins of the blood coagulation system that involves a cascade of zymogen activation that leads to the production of the serine protease thrombin, and ultimately cross-linked fibrin to form a stable blood clot.[1] Functional deficiency in coagulation factors VIII and IX results in abnormal hemostasis and excessive bleeding. In untreated severe hemophilia, where factor levels are <1% of normal, spontaneous bleeding occurs that frequently affects joints and can involve critical organs. A similar bleeding phenotype, with less frequent spontaneous bleeding, can occur in moderate hemophilia where factor levels are 2–5%. Onset of bleeding often begins in infancy, and from early childhood recurrent spontaneous joint bleeding can develop with consequential chronic painful and disabling joint arthropathy.[2] The current standard of treatment is replacement of the deficient factor VIII or IX with recombinant or plasma-derived factors. Clotting factor replacement is effective at preventing and treating the complications of hemophilia,[3] but usually requires regular intravenous infusions 2–4 times a week with standard half-life factors. Extended half-life factors reduce the frequency of infusions, but individuals with severe hemophilia A still require intravenous prophylaxis 2–3 times a week.[4,5] Frequent intravenous treatment is inconvenient and even distressing, particularly for infants, young children and their parents/caregivers where central venous access is often required that carries risk of infection.[6] Lack of compliance comes hand-in-hand with difficulties of administering treatment and the complications of bleeding ensue.[7] Additionally there is a risk of alloantibodies against the factor treatment, termed inhibitors, that occurs in approximately 30% of people with severe hemophilia A and 10% in severe hemophilia B.[8,9] Inhibitors render standard treatment ineffective making patients more prone to severe and disabling bleeding, and treatment to eliminate inhibitors is costly,

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invasive and has a high failure rate of approximately 20–40% in hemophilia A[10] and 70% in hemophilia B.[11] Apart from immune tolerance therapy to eliminate inhibitors conventional treatment has been to administer bypassing agents, recombinant factor VIIa or activated prothrombin complex concentrate (aPCC) either on demand (OD) for bleeding, or as prophylaxis[12] that, again, is costly and invasive, requiring good venous access.

Due to the limitations of traditional treatments for hemophilia A and B these disorders have become a target for innovative strategies to overcome these challenges. The explosion of novel treatments that can transform the lives of people with hemophilia (PwH) has made this a compelling area of medicine over recent years. A major breakthrough in the management of haemophilia A and inhibitors arrived with the development of a bispecific antibody that partially mimics factor VIII activity, emicizumab (F.Hoffman-La Roche manufactured by Genentech).[13] This treatment is the first licensed subcutaneous treatment for hemophilia and has become an established prophylactic treatment in inhibitor and non-inhibitor patients.[14,15]

Making PwH free of regular prophylactic treatment altogether is now an attainable goal with gene therapy. As hemophilia is a monogenic disorder it has always been an attractive target for gene therapy and immense progress has been made in its clinical development.[16] Though gene therapy has demonstrated great promise as a potential cure for hemophilia, immunological responses to the viral vector can lead to treatment failure and emerging long-term data has shown responses may not be sustained in hemophilia A.[17] Additionally there are concerns regarding long-term safety of gene therapy, particularly viral integration into the host genome and oncogenesis.[18] Gene therapy may be an opportunity to overcome the barriers to successful treatment of inhibitors and the efficacy of gene therapy in this patient population is being studied (ClinicalTrials.gov Identifier: NCT03734588).[19,20]

Emicizumab has had great success due to the convenience of administration and stable pharmacological effect, which has fueled the demand for other treatments administered in a similar manner that can encompass a broader patient population. This can be achieved by targeting the natural inhibitors of coagulation. Normal hemostasis relies on an intricate balance of the generation of thrombin though the activation of coagulation proteins and their inhibition with regulatory proteins.[21] Deficiency of the key regulatory anticoagulant proteins are known to increase the risk of venous thrombosis in a person with otherwise normal coagulation proteins.[22] In hemophilia these deficiencies have been shown to ameliorate the hemophilia bleeding phenotype.[23] Manipulating natural inhibitors of coagulation has been demonstrated to effectively restore functional hemostasis using antibody neutralization of tissue factor pathway inhibitor[24] and protease nexin 1,[25] activated protein C specific serpin inhibition[26] and antithrombin inhibition by nanobodies.[27] Another strategy to rebalance hemostasis in hemophilia is to prevent the expression of the coagulation-inhibitory serpins is by utilizing ribonucleic acid interference (RNAi).

RNA Interference

RNAi, otherwise known as post-transcriptional gene silencing, is a highly conserved pathway whereby expression of a particular gene is inhibited through the targeting and destruction of an mRNA molecule by an RNA molecule.[28] This ancient process protects eukaryotic cells against invasion by exogenous genes, such as viral DNA or double-stranded RNA, and regulates expression of protein-coding genes.[29] Gene expression is inhibited when short RNA molecules, either micro RNAs (miRNA) or small integral RNAs (siRNA) bind to and functionally inactivate endogenous mRNA.

The concept of RNAi arose when, after attempts to overexpress the chalcone synthase gene in Petunia plants, expression of pigment was inhibited resulting in white flowers.[30] The term RNAi was coined by biotechnologist Craig Mello and Andrew Fire in 1998 with the discovery that double-stranded RNAs were the cause of post-translational gene silencing in the nematode worm Caenorhabditis elegans.[31] This RNAi phenomenon led to the discovery of siRNA, typically double-stranded RNA molecules 20–25 nucleotides in length, that could induce RNAi silencing in mammalian cells.[32,33] It was recognized siRNAs can potentially suppress any disease-related gene of interest and it has been a long and complex process to harness the widespread therapeutic possibilities. Unmodified siRNA is rapidly degraded and will not accumulate into the intended tissue.[29] In early clinical studies off-target effects were demonstrated with unmodified or slightly modified siRNA-targeting vascular endothelial growth factor A and R1 (VEGFA and VEGFR1) for local administration in age related macular degeneration.[34] Targeted siRNA against VEGFA or VEGFR1 to suppress choroidal neovascularization from age related macular degeneration via Toll-like receptor 3 and its adaptor molecule TRIF induced

secretion of proinflammatory cytokines IL-12 and IFNy.[34,35] Further barriers against efficacy and safety include enzymatic degradation by endogenous nucleases, rapid clearance, immune recognition, and egress from the bloodstream into the desired tissue.[29,36] Some of these barriers have been overcome with chemical modification of siRNAs that can reduce off-target toxicity and enhance specificity and activity without compromising the siRNA activity. Phosphonate and ribose modification prevents siRNA degradation and are utilized in the Alnylam Pharmaceuticals Standard Template Chemistry universal modification pattern.[37] These modifications have made the theory of siRNA a clinically applicable reality and revolutionized therapeutics by circumventing the challenges of small molecules or antibodies having to recognize complex protein conformations. This technology was successfully applied with the first FDA approved (August 2018) siRNA, patisiran (ONPATTRO) to deliver chemically modified anti-transthyretin siRNA to treat hereditary amyloidogenic transthyretin amyloidosis with polyneuropathy.[38] Patisiran employs lipids to protect entrapped siRNA from nuclease attack and renal clearance and transport to target tissue.[29] After intravenous administration of patisiran ionizable lipid nanoparticles associate with apolipoproteins facilitating endocytosis into liver.[39] During this period of siRNA development therapeutic applications in hemostasis had been explored. Lipid-based reagents had similarly allowed successful delivery of siRNAs to mouse livers in the first synthetic siRNA study on modifying the expression of liver coagulation proteins via hepatocyte nuclear factor 4a and CCAAT/enhancer binding protein 2 transcription factors.[40] Using the same mode of delivery siRNA silencing of the genes for the serpins protein C and antithrombin led to severe coagulopathy and macrothrombosis in multiple tissues in mice.[41]

Another method to surmount the evolutionary defenses to keep invading RNA outside of the cell was the design of N-acetylgalactosamine (Ga1NAc)-siRNA conjugates.[42] Suppressing the expression of proteins produced by hepatocytes requires facilitating the entry of siRNA circulating in the blood stream after parenteral administration into the cytoplasm of hepatocytes; GA1NAc was utilized for this purpose as it avidly binds to the Asiaglycoprotein, which is highly expressed on hepatocytes and endocytosed. GA1NAc-siRNA conjugates can thus enter the hepatocyte and target cellular RNA and influence protein expression. This was successfully utilized in givosiran, the second FDA approved siRNA to treat acute hepatic porphyrias.[43]

Fitusiran

Fitusiran (ALN-AT3, Alnylam/Sanofi) is a RNAi therapeutic developed for prophylaxis of bleeding in hemophilia A and B. This synthetic siRNA is covalently linked to a triantennary Ga1NAc ligand. The Ga1NAc-siRNA conjugate specifically targets antithrombin messenger RNA (mRNA) to lower antithrombin production (Figure 1). Antithrombin, encoded by the SERPINC1 gene, is a serpin produced by the liver that has an essential role in maintaining the balance of hemostasis. It functions as a natural anticoagulant serpin by potent inhibition of thrombin, and to other serine proteases including factors Xa and IXa. Antithrombin has a half-life of 2–3 days, normal plasma concentrations are 112–140 µg/mL and the standard reference range is 80–120% of normal.[44] Individuals with inherited deficiency of antithrombin usually have levels of 40–60%;this carries a lifetime risk of venous thrombosis and is well known to cause a more severe thrombotic tendency than other inherited thrombophilias.[45,46] Homozygous antithrombin deficiency usually causes in utero death, but rare cases have been reported.[44] Examination of the small number of reported cases of individuals with severe hemophilia and co-inherited thrombophilia has indicated reduction in the severity of their bleeding phenotype.[23,47,48] Murine studies showed subcutaneous injection of fitusiran caused potent and durable reduction of antithrombin in both wild-type and hemophilia A mice, with an associated increased in thrombin generation and enhanced hemostasis.[49] In the Phase I dose escalation study to evaluate the safety and tolerability of subcutaneous fitusiran in healthy volunteers and non-inhibitor moderate-severe adult hemophilia A and B participants antithrombin levels were reduced by approximately 50% at doses between 0.015mg/kg and 1.8mg/kg (Table 1).[50] Peak fitusiran plasma levels occurred 2–6 hours after administration and rapidly decreased with a mean elimination half-life of 2.6–5.3 hours. Plasma levels of fitusiran increased and antithrombin decreased in a dose-dependent manner in the hemophilia patients (Table 1). Lowering of antithrombin was associated with increased thrombin generation at similar levels in hemophilia A and B patients. When antithrombin levels was reduced >75% from baseline peak thrombin generation values were at the lower end of the range observed in the healthy participants and thrombin generation values were similar to those described in mild hemophilia.[51] There was a prolonged pharmacological effect with AT recovery at a median slope of 10–15% per month after fitusiran discontinuation indicating this treatment offers consistent hemostasis over time. Further exploratory analysis suggested this translates as fewer bleeding

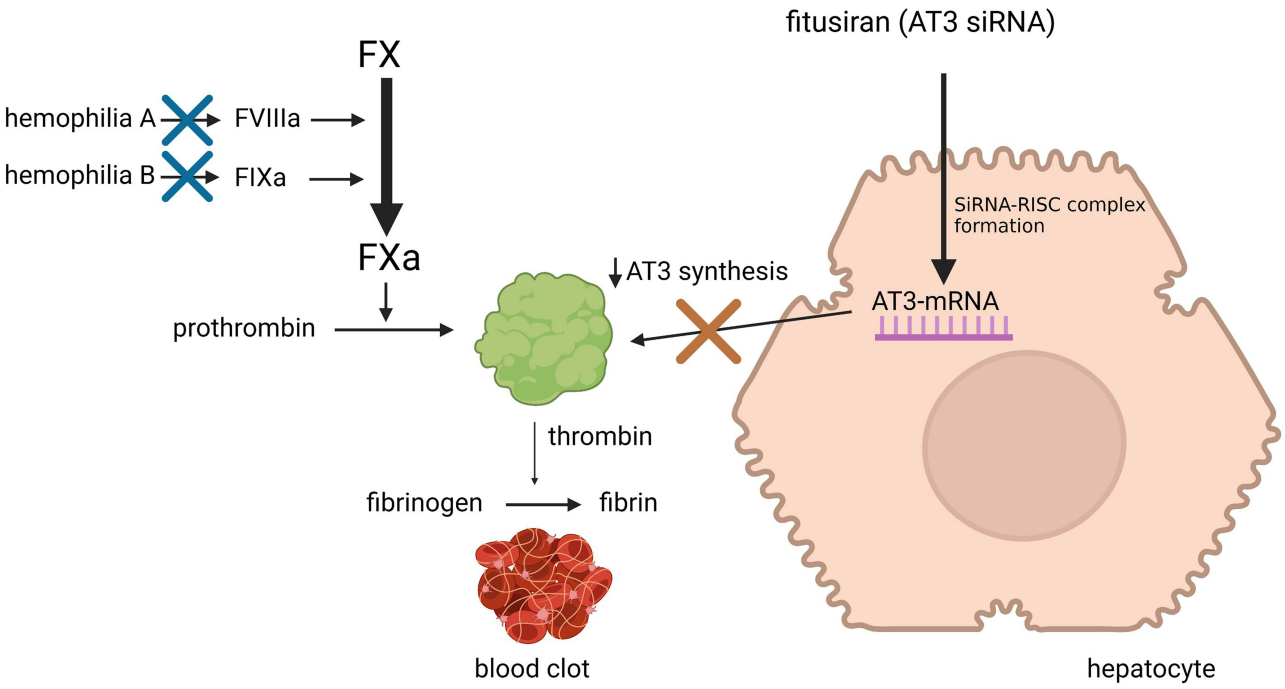

Figure 1 Diagram of the mechanism of action of fitusiran. After entering the hepatocyte the Ga1NAc- siRNA conjugate binds with a ribonucleoprotein forming a RNA-induced silencing complex (RISC). It targets AT3 mRNA and silences the gene, thus inhibiting antithrombin production. Consequently FIXa, Xa and thrombin lack inhibition and there is increased generation of thrombin to form blood clots. Created with BioRender.com.

episodes in patients, and reported episodes were successfully treated with clotting factor replacement. Fitusiran was well tolerated with injection site reactions and arthralgia being the most reported adverse events. Transient increases in in liver aminotransferase was observed in 36% of participants and one participant had reactivation of hepatitis C infection that was thought unrelated to the study drug. No patients developed anti-fitusiran antibodies.

Due to the small sample size the phase I study was extended to evaluate the long-term safety and tolerability. In this extended study fatal cerebral venous sinus thrombosis occurred after factor VIII concentrate was repeatedly administered after an initial misdiagnosis of subarachnoid hemorrhage and all fitusiran trials were temporarily halted.[52] The US Food and Drug Administration allowed the studies to continue after the trial protocol was amended to protect participants from the risk of thrombosis. For bleeding episodes lower doses of factor must be used; factor VIII 10–20 IU/kg or factor IX 20–30 IU/kg.[53] In vitro data on thrombin generation of bypassing agents and antithrombin reduction means it is likely lower doses are required[54] so aPCC 30–50 units/kg (standard dose 50–100 units/kg) or Factor VIIa < 45 mg/kg (standard

Table 1 Comparison of Median Nadir Lowering of at with Varying Fitusiran Dosing Regimens

Fitusiran Dose	Frequency	Mean Nadir Reduction AT	Median Peak Thrombin Height with > 75% AT Reduction
0.075mg/kg	Weekly	61%	67–68nM
0.225mg/kg	Monthly	70%	
1.8mg/kg	Monthly	89%	
50mg	Monthly	82%	
80mg	Monthly	87%	

Notes: There was a mean AT reduction from baseline of 87+ 1%[49] and 87.4%[54] in the two phase 1 studies. The median peak thrombin height range for healthy volunteers was 65–210nM; thrombin generation at > 75% reduction of AT from baseline by fitusiran was similar to lower healthy range.

dose 90mg/kg) should be given for bleeding in participants with inhibitors. Should a thrombotic episode occur in a PwH receiving fitusiran AT concentrate should be administered aiming for AT levels of 80–120%, in combination with anticoagulation and clotting factor or BPA replacement.

The trial program was extended to an open label Phase I, part D, study of efficacy in hemophilia participants with inhibitors.[55] Participants were treated with once monthly fixed subcutaneous doses of fitusiran at 50mg or 80mg. Again, the most common adverse events were mild and transient injection site reactions and increases in liver aminotransferases that were all assessed as mild to moderate reactions that did not require fitusiran suspension. The hepatotoxicity of Ga1NAc- siRNA has been shown as mainly due to RNAi-mediated off target gene silencing.[56] Due to the proportion of PwH with a history of hepatitis C infection, with 12/17 participants affected, this is thought to contribute to the risk of transaminitis related to this liver-specific therapeutic. Elevated d-dimer was measured as a potential predictor of thrombosis and was observed in 47%, more with the 80mg dose (55%) compared to the 50mg dose (33%). As d-dimer is also an acute phase reactant the clinical significance of this is unknown. Peak plasma levels were similar to the phase I study at 4 hours. The mean nadir AT level was 18% in the 50mg dose group and 12.5% in the 80mg dose group corresponding as 82% and 87.4% reduction from baseline (Table 1). As demonstrated previously reduced AT correlated with increased thrombin generation. Overall bleeding episodes were reduced with an improvement in quality of life; 65% participants reported no bleeding episodes (Table 2). The median aPCC dose used was 28.6 U/kg and no thrombotic events occurred. This study suggested a fixed monthly fitusiran dose is safe and efficacious for long-term treatment of inhibitor and non -inhibitor hemophilia patients.

The ATLAS trial program continued to expand (Table 3) and recently the findings of the Phase III ATLAS-A/B (ALN-AT3SC-004) study of the safety and efficiency of fitusiran in hemophilia A and B participants aged >12 years without inhibitors and previously receiving standard OD factor treatment were presented.[57] Participants were randomized to receive their usual OD treatment or monthly fitusiran 80mg s/c. Of the 79 participants that received fitusiran 50.6% did not have any bleeding episodes requiring factor treatment compared with 5% in the OD arm. The median annualized bleeding rate (ABR) was 0.0, with a clinically meaningful and statistically significant 89.9% reduction in ABR compared to the OD arm. No thrombotic events occurred during the study.

The ATLAS-INH (ALN-AT3SC-003) study evaluated the safety and efficacy of fitusiran in inhibitor patients >12 years and randomized participants to receive either 80mg fitusiran monthly or OD bypassing treatment.[58] Again, there was a significantly statistical reduction in ABR of 90.8% and 65.8% and fitusiran-receiving participants had zero bleeds compared with 5.8% in the OD arm. Seven patients in the fitusiran arm had treatment emergent serious adverse effects, including subclavian vein thrombosis and thrombosis. One patient with a spinal vascular disorder and thrombosis had study treatment discontinuation.

Table 2 Dosing Regimens and Bleeding Events in Published Fitusiran Studies

Study		Dosing Regimen	Bleeding Events Reported by Participant	Median ABR
Pasi et al 2017[50]	Phase I dose escalation	Part B Once weekly 0.015, 0.045 or 0.075mg/kg weekly		
		Part C Once monthly 0.225, 0.45, 0.9 or 1.8mg/kg or Fixed dose 80mg monthly	67% reported no spontaneous bleeds 56% no bleeding events	0
Pasi et al 2020[55]	Phase I part D	Once monthly 50mg or 80mg	65% no bleeding events	0
Young et al 2021[58] (ATLAS-INH)	Phase 3 open label	Once monthly 80mg	65.8% zero treated bleeding events	0
Srivastava et al 2021[57] (ATLAS-A/B)	Phase 3 open label	Once monthly 80mg	50.6% no bleeds that required treatment	0

Table 3 A Summary of the ATLAS program Fitusiran Studies

Trial	Phase	Subjects	Age	Inhibitors	Status
ALN-AT3SC-001	1	3 healthy subjects 42 Hemophilia A/B	≥ 18 years	Non-inhibitor	Complete
ALN-AT3SC-002	2	Hemophilia A/B Completed and tolerated study drug dosing in ALN-AT3SC-001	≥ 18 years	Non-inhibitor	Ongoing
ALN-AT3SC-003	3	Hemophilia A/B On demand bypassing agents	≥ 12 years	Inhibitors	Ongoing
ALN-AT3SC-004	3	Hemophilia A/B On demand factor replacement	≥ 12 years	Non-inhibitor	Ongoing
ALN-AT3SC-009	3	Hemophilia A/B with previous factor or bypassing agent prophylaxis	≥ 12 years	Inhibitor and non- inhibitor	Ongoing
ALN-AT3SC 005	3	Hemophilia A/B Previous participation in ALN-AT3SC-003 or ALN-AT3SC-004 or ALN-AT3SC-009	≥ 12 years	Inhibitor and non- inhibitor	Ongoing
ATLAS-PEDS	2/3	Hemophilia A/B	1-12 years	Inhibitor	Ongoing

Notes: Three global Phase 3 studies are ongoing, including 1) ALN-AT3SC-003 (ClinicalTrials.gov: NCT03417102) adolescents and adults ≥ 12 years with hemophilia and factor VIII or IX inhibitors previously treated with on-demand bypassing agents (BPA) 2) ALN-AT3SC-004 (ClinicalTrials.gov: NCT03417245): adolescents and adults with hemophilia without inhibitory antibodies previously treated with on-demand factor replacement therapies; 3) ALN-AT3SC-009 (*ClinicalTrials.gov identifier* NCT03549871): a single-arm, one way crossover study for adolescents and adults with hemophilia with or without inhibitors, and 4)ATLAS-PEDS (*ClinicalTrials.gov identifier* : NCT03974113) children ages 1–12 years with hemophilia and inhibitors. Bypassing agents (BPA) are recombinant factor VIIa or factor eight inhibitor bypassing agent (FEIBA)

Fitusiran in Other Rare Inherited Bleeding Disorders

As fitusiran does not target a specific coagulation factor it should theoretically prevent bleeding in other rare bleeding disorders. Addition of fitusiran to the plasma of patients severely deficient in factor V, VII or X has demonstrated improvement in thrombin generation.[59] AT-targeting RNAi may therefore be a therapeutic option for patients with rare bleeding disorders requiring prophylaxis where there is limited or no availability of clotting factor concentrate.

Other Strategies for RNAi Therapeutics in Hemophilia

Protein S

Protein S (PS) is a natural anticoagulant serpin encoded by the PROS1 gene. It regulates the generation of thrombin by acting as a cofactor for activated protein C in the inactivation of coagulation factors Va and factor VIIIa.[60] It is also a cofactor for tissue factor pathway inhibitor that inhibits factor Xa.[61] Homozygous PS deficiency can cause purpura fulminans and fatal disseminated intravascular coagulation (DIC).[62] Heterozygous PS deficiency is associated with venous thromboembolic events.[22] Like AT this regulator of hemostasis is an attractive target for RNAi therapeutics in the prevention of hemophilia-associated bleeding. Murine PS siRNA administered to hemophilia A ($F8^{-/-}$) and hemophilia B ($F9^{-/-}$) mice reduced bleeding in tail-bleeding assays, prevented intraarticular bleeding and PS expression was reduced in both the plasma and synovium.[63] High expression of PS and TFPI by synovial cells was found indicating this PS as a RNAi therapeutic target may further protect the joints in individuals prone to hemophilic arthropathy. Further studies to evaluate the safety of Ga1NAc conjugated to PS siRNA showed it was well tolerated mice with no evidence of DIC making further progress towards Phase 1 trials.[64]

Heparin Cofactor II

Heparin cofactor II (HCII) is a serpin secreted by the liver and encoded by the SERPIND1 gene.[65] Like AT, HCII has an essential role in regulating hemostasis through inhibition of thrombin.[66] HCII inactivates thrombin less efficiently than AT but glycosaminoglycans, such as heparin and dermatan sulfate enhance its effect. Dermatan sulfate is found mainly in the walls of blood vessels and has a particularly potent catalytic effect catalyzing HCII. This indicates HCII primarily exerts its effect in connective tissue. Like the better described inherited thrombophilias, deficiency of HCII has been associated with clinical thrombosis,[67] but other studies have shown the frequency of HCII deficiency in people with thrombosis is similar to healthy individuals.[68] The effect of RNAi on HCII has been studied in mice by conjugating siRNA-HCII with Ga1NAc.[69] This study suggested that by inhibition of HCII thrombin generation could be upregulated and hemostatic ability improved thus making HCII a potential target for hemophilia treatment.

Selection of Hemophilia Patients for RNAi Therapy

The development of plasma fractionation in the 1970s made the quality of life and life expectancy of PwH begin to increase. Transmission of hepatitis B and C and HIV through contaminated blood products to treat hemophilia tragically caused the loss of many lives. Now with recombinant clotting factors and successful antiviral therapies a significant rise in life expectancy is being seen.[70] As the hemophilia population ages, with associated co-morbidities, there are added complexities to their care. The ATLAS trial participants do not represent the entire hemophilia population as they were carefully selected by their clinicians and trial criteria, with key exclusion of inherited thrombophilia and thrombotic history. Fitusiran, therefore, cannot be presumed to be a "one size fits all" prophylactic treatment for severe hemophilia. As Phase IV and real-world data are accumulated clinicians are likely to select patients in line with the ATLAS program enrollment criteria. As inherited thrombophilia affects over 7% of certain populations some PwH will not be able to receive fitusiran based on thrombophilia testing alone.[71,72] Data is needed to examine if the least prothrombotic and more frequent factor V Leiden and prothrombin G20210A mutations in conjunction with siRNA antithrombin suppression translates as a clinical thrombotic risk. The risk of thrombosis demonstrated in the ATLAS trials can be minimized through screening patients for thrombotic risk factors, modification of cardiovascular risk factors and cautious treatment of bleeding episodes. As well as the need to have a normal baseline AT prior to commencing fitusiran the AT response should be monitored in clinical practice to select which patients can safely continue fitusiran. Evaluation of the

thrombotic events related to fitusiran suggest there is association with AT levels of <10%. Therefore, as with ongoing trials, patients with AT levels of <15% should have fitusiran withdrawn. It has been reported to achieve the desirable ABR while minimizing adverse thrombotic events there should be an upper AT threshold with target levels of 35.[73] Future studies aim to further mitigate the risk of thrombosis by starting with 50mg dosing 2 monthly, and adjusting the dose and frequency based on the AT levels. The outcome of this study will guide clinicians on whether lower initial doses and tight control of AT target levels will minimize thrombosis risk enough to encompass a broader population of hemophilia patients, particularly those with cardiovascular risk factors, including rising age.

The lifestyle of a PwH may impact their selection for treatment. Though it has been established the risk of spontaneous bleeding with fitusiran is low, in clinical practice we are so far unable to precisely quantity the risk of bleeding from activities with high-risk physical impact. PwH who frequently play sports that carry a significant risk of head injury may be advised to use clotting factors prior to activity so there is confidence of a normal level of hemostasis, e.g., factor VIII or IX levels measurable at >50%. The same may apply to a PWH with an occupation that puts them at risk of major injury and bleeding.

It also needs to be considered increased bleeding risk can emerge in the ageing population, such as hemorrhagic stroke, bowel angiodysplasia or malignancy.[74] These co-morbidities alongside hemophilia may require the need for prophylaxis for the first time in the life of a PwH, or frequency of prophylaxis may need to be increased. Venous access is often challenging in the elderly, and vein fragility along with restricted joint movements due to osteoarthritis or hemophilic arthropathy may render self-treatment with intravenous infusions unfeasible and makes subcutaneous fitusiran, every 1–2 months, as attractive and convenient option. In these cases the bleeding and thrombotic risks would need to be carefully weighed against each other before a treatment decision is made.

Difficulties with venous access is not restricted to the elderly and is a major challenge in the early years of life. Traditional treatment of inhibitors, that most commonly affects children, requires high frequency intravenous infusions of clotting factor and/or BPAs. Recent data has reported the frequency of inhibitors in hemophilia B to be higher than previous findings;[9] emicizumab cannot be used in this population and it is therefore likely they will be prioritized for fitusiran due to the unmet need and the high potential to turnaround the quality of life of the PwH and their caregivers.

PwH in the ATLAS trial programme were under the care of hemophilia centres and able to receive high quality medical care. This does not reflect global hemophilia care and access is a worldwide problem. Only 30% have access to clotting factor replacement therapy[75] and up to 10% of children with severe hemophilia in the developing world sustain intracranial hemorrhage.[76] Fitusiran could benefit PwH in developing countries with limited availability of medical care as the infrequent dosing and ease of administration has the potential to mitigate some of the difficulties with refrigerated storage and access to hospitals for instance with mobile monthly dosing clinics.

Conclusion

The goal of hemophilia therapy is to produce steady state hemostasis through the restoration of thrombin. With unprecedented progress in hemophilia treatments there has been a paradigm shift with expectations rising to aim for comparable quality of life to people without hemophilia.[12] RNAi therapeutics have been a major contributor to this change in landscape with numerous possibilities of rebalancing hemostasis through targeted suppression of serpin mRNA. This not only provides opportunities in the improving the care of PwH, but other people with rare severe bleeding disorders that have limited treatment options. Fitusiran is in late stages of clinical development and has been studied in over 200 participants in the international ATLAS clinical program. It has demonstrable efficacy in preventing bleeds through targeted reduction in antithrombin and restoration of thrombin generation in adolescents and adults with severe-moderate hemophilia A and B. Fitusiran differs from hemophilia treatments licensed to date as it is not only effective if inhibitors are present but also in both hemophilia A and B. If fitusiran is shown to be effective and safe in children, as is currently being assessed in Phase II and III studies, it has the potential to be an almost universal prophylactic treatment for hemophilia, with some caveats in patient selection. As the effective dosing regimens of 50mg and 80mg given subcutaneously every 1–2 months are much less frequent than standard prophylaxis with clotting factor infusions it could to transform the quality of life of PwH, particularly for those with inhibitors. Long-term

morbidity from hemophilic arthropathy may be prevented with the greatest benefit offered to those with severe hemophilia managed with OD clotting factor treatment. Infrequent injections with sustained pharmacological effects has the additional benefit of granting 'hemophilia-free days', where the PwH is relieved of the psychological burden of their disease.[77]

Overall fitusiran may transform lives of PwH across the world but should be prescribed after careful consideration, with particular attention to thrombotic risk factors, and with ongoing caution until there is enough data to enable clinicians to confidently select patients appropriately.

Disclosure

Dr Sara Boyce reports grants from Sangamo Therapeutics Ltd, personal fees, advisory board and speaker fees from CSL Behring, outside the submitted work. Dr Savita Rangarajan reports being a principal investigator in ongoing clinical Trials from Sanofi, during the conduct of the study; consultant from Reliance Life Sciences, advisory board from Pfizer, Sigilon, and Takeda, outside the submitted work. The authors report no other conflicts of interest in this work.

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