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# Product type and other environmental risk factors for inhibitor development in severe hemophilia A

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## Abstract

The development of FVIII inhibitory antibodies is currently the most challenging complication of treatment, affecting ~30% of severe hemophilia A patients. These inhibitors inactivate FVIII, rendering the treatment ineffective, causing disability and increasing morbidity and mortality. Inhibitor development results from a complex multicausal immune response involving both genetic and environmental risk factors. One of the most important modifiable risk factors is the source of FVIII products, eg, plasma-derived or recombinant FVIII. Other environmental risk factors, such as age at first treatment, regimen, and intensity of treatment, could contribute to inhibitor development. Severe bleeds, surgery, concomitant infections, or vaccinations may all be events initiating danger signaling resulting in an immune reaction towards administered FVIII. All in all, the etiology of inhibitor development still remains unclear. The risk factors have been stratified into genetic and environmental, but there are no definitive data to determine the impact of each of them.

**KEYWORDS**
hemophilia A, inhibitor, plasma-derived, recombinant products, risk factors

**Essentials**

- Treatment complications in hemophilia A: inhibitors development.
- Type of FVIII products: the latest updates on plasma-derived or recombinant FVIII immunogenicity.
- SIPPET study, the first randomized study contrasting plasma-derived FVIII and recombinant FVIII products.
- The impact of other environmental risk factors in the etiology of inhibitor formation, such as age at first treatment, regimen and intensity of treatment, severe bleeds, surgery, concomitant infections, or vaccinations.

## 1 | INTRODUCTION

Hemophilia A is an inherited bleeding disorder caused by the plasma deficiency of coagulation factor VIII (FVIII). The clinical picture of patients with hemophilia A is characterized by bleeding episodes that occur spontaneously or at the time of trauma.[1] The earliest and most serious bleeding in neonates is intracranial hemorrhage, which occurs in 1%-4% of severely affected cases.[2] With age, the hemorrhagic diathesis affects soft tissue, joints, and muscles, leading to arthropathy, deformity and disability.[3,4] To prevent these

complications, patients are treated by means of regular infusions of plasma-derived or recombinant FVIII concentrates. There have been many advances to optimize and ensure the safety and effectiveness of FVIII concentrates during the last 30 years, after the successful control of complications caused by such bloodborne pathogens as HIV and hepatitis C.[5] However, these improvements are darkened by a serious complication, ie, inhibitor development, recorded in more than 30% of severely affected patients within their initial 20-30 exposure days (EDs) to administered FVIII. The risk of inhibitor development is greatest in previously untreated patients (PUPs) and is strongly associated with the severity of the disease. FVIII inhibitors consist of a polyclonal population of antibodies, mainly IgG4, IgG1, and IgG2 subclasses that target multiple antigenic sites within the A2, A3, and C2 domains of the FVIII protein.[6,7] The appearance of these alloantibodies neutralizes the hemostatic effect of FVIII concentrates, rendering the management of bleeding episodes difficult.

The immunology of inhibitor development is complex and not completely understood. The etiology of inhibitor development is the result of a multistep process that involves a cascade of interactions between genetic and environmental determinants. Several predisposing genetic risk factors (ie, inhibitor family history, ethnicity, *F8* gene mutations, and variants in a number of genes involved in the immune response) have been reported to have an effect on inhibitor development. The type of *F8* gene mutation has been clearly associated with inhibitor risk, and null mutations are associated with the highest risk in patients with severe hemophilia A.[8] A large number of environmental factors may contribute to the risk of inhibitor, such as the type of FVIII product, age at first treatment, intensity of treatment, and danger signals such as surgery, severe bleeds, vaccinations, and infections.[9–11] Identifying the environmental factors implicated in inhibitor development might permit anticipation of its onset and possibly lead to the potential to intervene, and thereby change patient treatment and improve outcomes.

This article gives an overview on the debated role of the source of FVIII concentrate and on the impact of other environmental risk factors in the etiology of inhibitors. Our review includes recent studies presented during the International Society on Thrombosis and Haemostasis (ISTH) 2017 meeting on the (i) timing and severity of inhibitor development, and (ii) the impact of danger signals.

## 2 | HISTORICAL OVERVIEW

A circulating inhibitor, called "anticoagulant," was identified for the first time in the 1940s in a patient affected with hemophilia.[12,13] Three hours after the blood transfusions, the patient began to bleed continuously and the coagulation time remained markedly prolonged.[12,13] Later, this inhibitor was shown to be a γ-globulin and to appear only after repeated transfusions of whole blood.[14,15] Thus, the anticoagulant was suggested to be the result of an immunization of patients to the deficient factor. Only in the late 1960s to early 1970s was it recognized that circulating anticoagulants were antibodies which developed in patients with hemophilia in response

to replacement therapy.[16] At that time, determinants of the occurrence of circulating anticoagulants in hemophilia were unknown. In addition, it was not clearly established whether the development of circulating anticoagulants was influenced by the quantity, type, or frequency of replacement therapy.[17] During these years hemophilic patients were receiving much more treatment than in previous early years due to the development of technologies able to separate FVIII from large pools of donor plasma that resulted in the availability of freeze-dried, lyophilized FVIII concentrates. However, there was no evidence that one type of FVIII concentrate was more prone than others to produce inhibitor. In the 1980s and 1990s, there was a considerable amount of controversy and debate on the risk of inhibitor formation associated with the type of plasma-derived FVIII products with different purity. In those early days, inhibitor incidence was low, corresponding to 6%-7% in patients treated exclusively with plasma cryoprecipitate and remained relatively low, around 9%-10%, in patients treated with intermediate-purity FVIII concentrates.[18–22] The introduction of viral inactivation steps to produce new plasma-derived products improved the safety by minimizing the potential of bloodborne pathogen transmission. However, these additional steps in the manufacturing process probably made plasma-derived products more immunogenic with a higher risk of inhibitors which was estimated up to 20%-25%.[23–27] For instance, the introduction of a pasteurized version of a previously dry-heated FVIII concentrate in order to obtain a higher purity concentrate (CPS-P) was associated with an outbreak of inhibitors in multi-transfused patients in the Netherlands and Belgium.[28,29] The heat-treated product was associated with a rate of new inhibitor development of 4.4/1000 patient-years, which increased to 20.1/1000 patient-years with the new pasteurized product.[28,29] The authors concluded that the complex process of FVIII purification and viral inactivation, such as pasteurization and solvent-detergent treatment, may have altered the molecule, provoking inhibitor development.[28,29]

## 3 | IMPACT OF RECOMBINANT PRODUCTS: FROM OBSERVATIONAL TO RANDOMIZED STUDIES

The subsequent novel availability of recombinant FVIII concentrates in the early 1990s has added another variable to the issue of inhibitor risk. These preparations, derived from nonhuman mammalian cell lines, are associated with changes in the posttranslational processing or tertiary structure of FVIII that may lead to the formation of neoantigens and make these preparations more immunogenic than plasma-derived FVIII products.

The safety and efficacy of recombinant FVIII products were primarily tested in previously treated patients (PTPs, ie, patients having at least 150 EDs) for bleeding episodes, as requested by the guidelines for marketing authorization. These studies also evaluated the risk of inhibitor formation and indicated that in PTPs recombinant FVIII products were no more immunogenic than plasma-derived FVIII.[30,31] However, PTPs are at a much lower risk of inhibitor

development than PUPs.[30–33] In the first few studies designed to evaluate safety, efficacy, and inhibitor risk of recombinant FVIII concentrates in PUPs, all the investigated products were well tolerated and not associated with significant adverse events, and the responses to treatment were excellent. However, the cumulative incidence of inhibitors was high, varying between 25% and 30%.[30–33]

In the next few years, some evidence emerged that different FVIII preparations had different degrees of immunogenicity, and a number of reports showed a lower rate of inhibitors in PUPs treated with plasma-derived products than with recombinant products. The cumulative risk of inhibitor development ranged from 20% to 33% in PUPs treated with various plasma-derived products.[34,35] In contrast, patients treated with a single plasmatic concentrate had a cumulative incidence ranging from 10% to 12.4%,[19,23] whereas inhibitor development in patients treated with a single recombinant product was much higher, ranging from 27.6% to 36%.[32,36–39]

Strong clinical support to the view that plasmatic FVIII concentrates are less immunogenic than recombinant FVIII was provided in 2003 by a systematic review on the epidemiology of FVIII inhibitors.[40] In an analysis of 801 PUPs in the frame of 13 observational studies originally carried out to evaluate the efficacy and safety of an array of FVIII products, the risk of inhibitor development was more than double in patients treated with recombinant FVIII as opposed to plasma-derived products.[41] However, two subsequent multicenter observational studies, the CANAL (Concerted Action on Neutralizing Antibodies in severe haemophiLia A) study (316 PUPs)[42] and the larger RODIN (Research Of Determinants of INhibitor development) cohort study (574 PUPs)[43] showed no difference in immunogenicity between plasma-derived and recombinant FVIII products. Unexpectedly the RODIN study also reported that second-generation full-length recombinant products were associated with an increased risk of inhibitor as compared with third-generation products (adjusted hazard ratio 1.60; 95% CI, 1.08-2.37). Subsequently, this higher immunogenicity observed for second-generation full-length recombinant products was confirmed in a cohort of patients included in the Rèseau FranceCoag and in the UK children registered in the UKHCDO National Haemophilia Database (NHD) and most recently in the FranceCoag PUP cohort.[44–46] Two additional studies, the EUHASS study[47] and a Canadian cohort study,[48] who also investigated the higher immunogenicity of the second-generation recombinant products, did not confirm the RODIN data, but were small or did not inform adjustment for confounding. Subsequently, an attempt was made to reconcile these contrasting results. Some meta-analysis and systematic reviews, based also on data from individual observational studies, reported a higher risk of inhibitor development with recombinant FVIII than with plasma-derived FVIII, although the difference were attenuated after adjustment for confounders.[49–51] It must be kept in mind that all observational studies may suffer from confounding by indication, ie, when the choice of product class was made on the basis of treaters' perception of inhibitor risk.

To sum up, observational studies, meta-analysis, and systematic reviews suggested the existence of a difference between plasma-derived and recombinant FVIII products and that plasma-derived products determine a lower incidence of inhibitors, but due to confounding these results were not conclusive. For instance, in a debate article on the question whether the rate of inhibitors was higher with recombinant or plasma-derived products, the author stated that "it is unlikely that in the near future we will have sufficient prospective randomized studies to resolve definitively the dilemma of inhibitor induction."[52] This statement highlights the complexity to carry out a randomized clinical trial but also its need in order to obtain final and conclusive results on the major current complication of hemophilia therapy.

With this background and gaps of knowledge, the first randomized study contrasting plasma-derived and recombinant FVIII products, the Study on Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) trial was started in 2010 and published in 2016.[53] The study included 251 previously untreated hemophilia A boys (<6 years) randomly assigned to receive a VWF-containing plasma-derived FVIII product or a recombinant FVIII product and followed for inhibitor development for 50 EDs. Patient enrollment took place in 42 centers from 14 countries in the world. The SIPPET study provided clear evidence of a differential immunogenicity risk associated with recombinant FVIII products. The cumulative incidence of all inhibitors was 26.8% (95% CI, 18.4-35.2) with plasma-derived FVIII and 44.5% (95% CI, 34.7-54.3) with recombinant FVIII,[52] showing that the class of recombinant FVIII products was associated with an 87% higher incidence of inhibitor than plasma-derived FVIII products.[53] These data created a debate within the hemophilia treatment community on the clinical consequences of the outcome of SIPPET.[54] In addition, a few post hoc analyses of SIPPET provided additional data. For instance, the rate of inhibitor incidence was evaluated over time every 5 EDs (from 0 to 50 EDs) in hemophilia patients treated with different classes of FVIII products.[55,56] The highest rates of inhibitor occurrence were developed in the first 10 EDs, with a great contrast between recombinant and plasma-derived FVIII during the first 5 EDs: hazard ratio 3.14 (95% CI, 1.01-9.74) for all inhibitors and 4.19 (95% CI, 1.18-14.8) for high-titer inhibitors. For patients treated with plasma-derived FVIII, the peak of inhibitor development occurred later (6-10 EDs) and was of shorter duration. These results emphasize once again the high immunologic vulnerability of patients during the earliest exposure to FVIII but also the strongest response to recombinant FVIII products.[56] Finally, it must be mentioned that most recently Calvez et al.[46] reported data from a French national cohort study concordant with findings from the SIPPET randomized trial, ie, a higher risk of inhibitor development in patients treated with recombinant than with plasma-derived products (1.64; 95% CI, 0.82-3.25).

## 4 | ENVIRONMENTAL RISK FACTORS

### 4.1 | Age, treatment regimen, and intensity of treatment

The possible role played by other environmental risk factors in inhibitor development, such as age at first treatment, type of treatment

regimen, and intensity of treatment, has been extensively discussed. Additional risk factors such as severity of bleeds, surgery, concomitant infections, or vaccinations have also been implicated in the context of concurrent immunological danger signals resulting in immune reactions in association with FVIII administration (Figure 1).[9–11]

The age at which to initiate therapy is a debated matter. In the early 2000s, Lorenzo et al. showed that the incidence of inhibitors was higher in patients initiating therapy before the age of 6 months than for older pediatric patients.[34] In keeping with this study, van der Bom et al. confirmed the role of first treatment at an early age in the development of inhibitors, with a cumulative incidence at 100 EDs of 34% (95% CI 7%-61%) in patients starting therapy before the age of 6 months and 13% (0%-27%) in those starting between 1 and 1.5 years.[57] These observations were consistent with subsequent findings by Santagostino et al. in patients who had their first treatment before the age of 11 months, an effect, however, that was attenuated after adjustment for FVIII genetic risk factors.[58] Subsequently, a multivariate analysis, conducted in the frame of a French cohort of patients with severe hemophilia, showed a threefold difference in inhibitor risk between children treated for the first time before 6 months of age compared to those treated after 12 months of age.[59] Furthermore, the data published in the CANAL study demonstrated that the rate of inhibitor was associated with age at first treatment, diminishing from 41% for patients treated within the first months of age to 18% in those treated after 18 months, but this association largely vanished after adjustment for Confounders.[42] In a report by Chalmers et al.,[60] the exposure to FVIII throughout the neonatal period showed no association with a higher incidence of inhibitors than in patients treated later during the first year of life. Two additional studies published most recently found no association between age of therapy initiation and inhibitor formation during the first years of life.[46,61] To sum up, the data reported in the literature are discordant, in all likelihood owing to the observational nature of the studies, and thus fail to support firmly the hypothesis that a first replacement therapy at an early age increases the risk of inhibitor formation.

The role of prophylaxis has also been examined as a potential risk factor in the development of inhibitors. In the frame of the CANAL study, Gouw et al. showed tha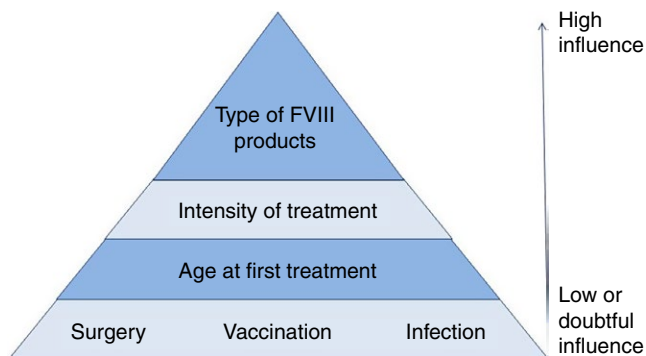t prophylaxis was associated with a decreased inhibitor risk as compared with on-demand treatment,[42] but the subsequent case-control study of the UKHCDO did not confirm the protective role of prophylactic regimens.[62] In a pilot study conducted in 2010, Kurnik et al. analyzed whether a low-dose prophylaxis regimen for the first 20 to 50 EDs did induce tolerance to the administered FVIII and minimize inhibitor development[63] and found that the cumulative inhibitor incidence in the low-dose group was strikingly reduced compared with the control group on a standard protocol (OR: 0.048, 95% CI, 0.001-0.372). The authors concluded that early beginning of prophylaxis decreased immunological signals during the first 20 EDs with FVIII and proposed this therapeutic strategy in order to reduce the risk of inhibitor formation in patients with severe hemophilia A.[63] Given these results, a low-dose prophylaxis regimen was tested in a clinical trial, called Early Prophylaxis Immunologic Challenge (EPIC) Study (NCT01376700, clinicaltrials. gov), which had to be terminated prematurely because of an excess of inhibitor development.[64] Furthermore, the RODIN study demonstrated that prophylactic FVIII treatment decreased more the inhibitor risk in patients with low-risk *F8* mutations (small deletions and insertions, missense mutations, and splice site mutations) than those with high-risk mutations (large gene deletions, nonsense mutations, and intron 1 and 22 inversions) (aHR, 0.61, 95% CI, 0.19-2.0 and aHR, 0.85, 95% CI, 0.51-1.4, respectively).[65] In these studies, a low inhibitor incidence during prophylaxis was explained as an immune tolerance upon exposure to infused FVIII. Cumulatively, the data of the literature on the issue of the protective effect of prophylaxis on inhibitor formation appear still inconclusive.

The intensity of FVIII treatment covers a spectrum of determinants including peak treatment moments of intense exposure, defined as ≥5 consecutive days with treatment as first exposure or anytime in the first 50 EDs. In the multicenter CANAL cohort study, peak treatment moments at the time of first treatment was associated with a 3.3-fold (95% CI, 2.1-5.3) higher risk of inhibitor development than in patients who received treatment with more days elapsing between each treatment.[42] In keeping with the CANAL study, a multicenter cohort study showed that patients who had a major peak treatment moment at the time of the first treatment had a 2.1 (95% CI, 1.0-4.5) times higher risk of inhibitor onset than those who only had a treatment on a single day or two consecutive days.[66] In addition, the effect of peak treatment moments was also estimated at any exposure day during the first 50 EDs. Peak treatment moments of three or five consecutive days at any exposure day again increased the risk of inhibitor development, but less markedly (RR 1.6; CI, 1.0-2.6).[67] Maclean et al.,[62] in a multicenter case-control study of the UK Haemophilia Centre Doctors' Organization (UKHCDO), reported that peak treatment moments major than five consecutive days at any time during early exposure had the effect to increase the inhibitor risk (OR: 2.7, CI, 1.4-5.4). This finding was confirmed in patients with peak treatment moments of 10 EDs or more (OR: 5.5, CI, 1.5-20), whereas peaks of 3 EDs were not associated with an increased risk (OR: 0.9, CI, 0.5-1.8).[62]

In a systematic review by Eckhardt et al.,[67] the findings of the three aforementioned studies were pooled. Peak treatment



**FIGURE 1** Environmental risk factors for development of inhibitors in severe hemophilia A

moments at the first treatment were associated with a higher risk of inhibitor development. Consecutive treatments on at least 3 days was associated with a higher risk of inhibitor development (crude OR: 2.1; 95% CI, 1.2-3.7) compared with <3 EDs. This association was more marked when an intensive treatment of 5 EDs was compared with one of <3 EDs (crude OR, 4.1; CI, 2.6-6.5).[67] Recently, Calvez et al. confirmed that peak treatment moments greater than 5 EDs were associated with an increased inhibitor risk (OR: 1.99, CI, 1.37-2.91), most pronounced in those patients with peaks of 10 EDs or more (OR: 3.51, CI, 2.20-5.61).[46]

To sum up, there is agreement between the available studies that peak treatment moments are associated with an increased risk of inhibitor development and that this effect is stronger when the peak treatment occurred at first exposure.

## 4.2 | Immune system and danger signal

Over the last few years, a commonly suggested explanation for the environment-related inhibitor risk is the danger signal effect.[9–11] The danger theory, which appeared in 1994, proposes that the immune system is more worried about damage than foreignness, and is brought into action by alarm signals from injured tissues rather than by the recognition of nonself.[68] The danger model asserts that the immune system is activated by danger signals from injured cells, such as those exposed to pathogens, toxins, and mechanical damage.[69] In hemophilia, the danger signal effect implies that endogenous or exogenous danger or damage signals present at the time of FVIII infusion stimulate the immune response. The danger theory describes how alarm signals from stressed, injured, or dying cells may stimulate an immune reaction, with no implications of foreign antigens. Therefore, severe bleeds, trauma, surgery, or concomitant infections may all be events starting danger signaling in hemophilia patients, resulting in an immune reaction towards administered FVIII. It is hypothesized that if the initial meeting between FVIII and CD4+T cells occurs in the presence of "danger" signals (ie, severe bleeds, trauma, or surgery with major tissue injury) the innate immune response becomes activated, with up-regulation of a response to FVIII.[70] Thus, tissue injury may clarify, partially, the increased inhibitor incidence after periods with intensive treatment.

## 4.3 | Surgery

Surgery is characterized by tissue damage with the related release of endogenous danger signals that could potentially promote inhibitor development. A case-control study by Santagostino et al. in 2005 found no association between surgery and risk of inhibitor development, but the study was too small to lead to definitive conclusions.[58] In contrast, the CANAL study reported that surgical procedures carried an increased inhibitor risk[42] and a subsequent meta-analysis of four studies by Eckhardt et al.[67] showed that the risk of inhibitor development after surgery at first treatment with FVIII was evidently associated with a heightened risk, with a pooled OR of 4.1 (95% CI, 2.0-8.4) compared with FVIII first treatment for other reasons (ie, bleeding or prophylaxis).[67]

Nevertheless, the RODIN study showed that surgery at first treatment does not cause an increased inhibitor risk (adjusted RR, 1.2; 95% CI, 0.54-2.6). In summary, surgery has been associated in some studies with inhibitor formation but heterogeneity in type of surgery, and duration and doses of FVIII treatments might justify the differences between some of them.

## 4.4 | Vaccinations

The impact of vaccinations concurrent with FVIII infusion has been suggested as a potential exogenous trigger in inhibitor development. A recent observational study evaluated data from 375 PUPs on the effect of vaccinations given close to FVIII exposure (48 hours before to 24 hours after), during the first 75 EDs.[71] More than half of the patients had received a vaccination concomitantly with FVIII treatment, but no association was found between inhibitor development and vaccinations. Vaccination is a broad term, and there may be differences depending on the type of vaccine and route of administration. A recent publication by Lai et al.[72] conducted in a hemophilic mouse model suggests that intramuscular influenza vaccinations have a protective effect on inhibitor formation, whereas intravenous vaccination might have a danger signal effect.[72] The research on the impact of vaccination on inhibitor development is still preliminary, and no definitive conclusions can be drawn.

## 4.5 | Infections

Few studies have investigated the effect of infections on inhibitor development, and thus conclusions are often difficult to draw.

In recent years, there has been substantial progress in the understanding of the complexity of the host–microbiota relationship and its effects on human health.[73,74] Microbiome-wide studies have revealed important associations between specific microbiomes and a range of ailments including autoimmune disease, cancer, as well as metabolic, neurodegenerative, and inflammatory diseases. Expanding evidence suggests that a dynamic interaction exists between microbes and environmental cues (including diet and drugs) within the microbial organ that shapes mucosal and systemic immunity. An interesting study, presented at ISTH 2017 meeting by Tarrant et al.,[75] reported that targeted modification of the gut microbiome in hemophilic mice influences inhibitor incidence following administration of recombinant FVIII products. Those mice with a manipulated microbiome developed anti-FVIII IgG antibodies after a therapeutic regimen with injections of FVIII. This study highlights that the gut microbiome may have a crucial role in modulating the immune response to the therapy with FVIII products,[75] but these data are still preliminary.

## 5 | CONCLUSION

The most important modifiable risk factor for inhibitor development in PUPs is the treatment with a FVIII concentrate. Therefore, the

choice of the least immunogenic FVIII product during the first 10 EDs is crucial.[55,56] Furthermore, physicians should plan the least intense but effective regimens in PUPs in order to avoid exposure to danger signals such as bleeds, trauma, surgery, or concomitant infection concurrent with FVIII infusion and perhaps to reduce the risk of FVIII inhibitor in PUPs. The impact of multiple environmental risk factors is varied and since these factors are often interrelated, it is difficult to identify the relative contribution of each single risk factor.

## 6 | FUTURE PERSPECTIVES

The reasons of inhibitor development are still not fully understood and several studies in recent years have revealed the broad complexity of this issue. The risk of developing FVIII antibodies is strongly related to genetic factors, in particular to nonmodifiable *F8* gene mutation, as reported by two presentations at the ISTH 2017 meeting.[76,77] One of the most influential and potentially modifiable risk factors is the type of FVIII concentrate, however many novel alternative products for hemophilia A treatment are entering the clinic with exciting results, as reported at the ISTH 2017 meeting.[78–80]

The research is currently looking at whether danger signals are triggering inhibitor development and also trying to better understand the immunology of inhibitors. Several research studies are exploring the immune response at the level of the antigen presenting cells (APC), T and B cells, and plasma cells and the potential translational strategies for treating hemophilia patients. New research areas are interested in identifying potential early biomarkers which might indicate the onset of inhibitors in advance.

## RELATIONSHIP DISCLOSURE

F.P. is a member of the scientific Advisory board of Ablynx, Shire, and F. Hoffmann-La Roche. She is a consultant of Kedrion Biopharma and Octapharma. I.G. has no relevant disclosures to declare.

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