Key issues in inhibitor management in patients with haemophilia

Keith Gomez[1], Robert Klamroth[2], Johnny Mahlangu[3], Maria E. Mancuso[4], María E. Mingot[5], Margareth Castro Ozelo[6]

[1]Katherine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free London NHS Foundation Trust, London, United Kingdom; [2]Department for Internal Medicine, Haemophilia Treatment Centre Vivantes, Klinikum im Friedrichshain, Berlin, Germany; [3]Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa; [4]"Angelo Bianchi Bonomi" Haemophilia and Thrombosis Centre, IRCCS Ca' Granda, Ospedale Maggiore Policlinico Foundation, Milan, Italy; [5]Haematology Department, Regional University Hospital "Carlos Haya", Málaga, Spain; [6]IHTC Haemophilia Unit "Cláudio Luis Pizzigatti Corrêa", INCTS Blood Centre UNICAMP, University of Campinas, São Paulo, Brazil

Introduction

Significant advances in the management of haemophilia have been achieved in the past several decades. These include the development of safe and efficacious plasma-derived and recombinant clotting factor products, use of prophylaxis as standard of care in bleeding prevention and appropriate surgical management of haemophilic arthropathy. Despite these advances, the development of high-titre anti-factor antibodies (inhibitors) remains an unresolved challenge in the management of people with haemophilia.

Inhibitors develop in 25-30% of patients with severe haemophilia A and in 1-5% of those with severe haemophilia B[1-3]. They were first described by Lawrence and Johnson in 1941[4], and in the seven decades that followed our knowledge of their pathophysiology and risk factors remains incomplete and continues to evolve. This is partly a result of the small inhibitor population with significant intra- and inter-individual variability making the conduct of studies and interpretation of results difficult. The main reason for our incomplete knowledge in this area is that inhibitor development is a multi-factorial event resulting from the variable interplay between several genetic (non-modifiable) and non-genetic (modifiable to some extent) risk factors. Despite these limitations, a number of clinical evaluations analysing treatment of inhibitor patients have been performed including randomised controlled prospective studies[5,6]. These studies provide, in part, the evidence basis for our current inhibitor management principles and practice.

Inhibitors remain a popular subject of haemostasis scientific meetings and many issues related to their pathophysiology and management are discussed and debated in the published literature. The main issues include: (i) the identification of clinically relevant risk factors for inhibitor development, (ii) the definition of the bleeding phenotype and clinical management of these patients, (iii) the usefulness and feasibility of prophylaxis with bypassing agents in the treatment of patients with inhibitors, (iv) the identification of predictive factors for inhibitor eradication, and (v) novel therapeutic approaches and molecules for the treatment and/or eradication of inhibitors. The aim of this review is to give an update on each of these issues with a focus on the current state-of-the-art knowledge and practice from the authors' personal perspective. This review focuses mainly on factor VIII inhibitors as these occur much more frequently in clinical practice.

Risk factors for inhibitor formation

The formation of inhibitors to the deficient clotting factor is the major complication of factor replacement therapy worldwide. Some of the risk factors for inhibitor formation, such as the underlying molecular defect affecting the *F8/F9* gene, are well established[7] while the importance of other factors such as the type of clotting factor concentrate remains hotly debated and poorly understood[8-11]. Increasing our understanding of these factors is critically important if we are to achieve our goal of predicting and ultimately reducing this complication of haemophilia treatment (Table I).

The marked difference in the rate of inhibitor formation between haemophilia A and B patients with the same laboratory phenotype can be attributed to two main factors: the causative genetic abnormality and differences in recognition by the immune system. Haemophilia A is unusual among monogenetic disorders in having a very high proportion of gross genetic abnormalities. These include large insertions and/or deletions and complex rearrangements which together account for about 50% of severe cases compared with 7-8% in haemophilia B[12,13]. This overrepresentation of gross abnormalities is due to two well-characterised inversions caused by recombination events between homologous sequences within intron 22 or intron 1 and

*Blood Transfus 2014; **12** Suppl 1: s319-29 DOI 10.2450/2013.0246-12*

Table I - Risk factors for inhibitor formation.

Clear evidence of increased risk of inhibitor formation	
F8 mutation type	Gross genetic abnormalities
	Mutations resulting in a null allele
	Specific missense mutations: R2150H, R2209Q, W2229C
Ethnicity	Afro-Caribbean
Young age at first treatment	Treatment before 6 months age
Treatment during co-existent inflammation	
Weaker or uncertain evidence of risk	
HLA type	
F8 polymorphisms	
Factor concentrate type	Recombinant products may represent a higher risk

their extragenic counterparts[14]. Gross abnormalities inevitably result in a null allele with little prospect of translation into peptides capable of tolerising the immune system. In comparison, alleles with missense and some nonsense mutations, which cause the vast majority of cases of severe haemophilia B[15], can sometimes be translated into peptides. Although these have no clotting factor activity and may not even be detectable as circulating antigen, they may be sufficient to tolerise the immune system to some parts of the wild-type clotting factor. The incidence of inhibitor formation is, therefore, significantly less with severe disease caused by single nucleotide abnormalities.

The molecular risk factors are not limited to the disease-causing mutation. The higher rate of inhibitor formation in Afro-Caribbeans than in Caucasians is probably due to other genetic factors[16]. For such a large gene, there are relatively few polymorphisms in *F8*[17]. Measurement of the frequencies of *F8* haplotypes showed clear differences between racial groups. In Caucasians a single haplotype predominates in 93% of the population. In contrast, three haplotypes of similar frequency (22-35%) are found in Afro-Caribbeans[16]. As the two currently available full-length recombinant protein products correspond to two of these haplotypes there is potential for a mismatch with the recipient's haplotype. This is potentially more of an issue for Afro-Caribbean patients because of their variable haplotype. However, the higher prevalence of inhibitors among haemophilia A patients of African descent in Brazil was not related to the presence of these *F8* haplotypes[18]. It may be that other genetic risk factors are implicated in the higher susceptibility to inhibitor development in haemophilia A patients of African origin.

Genetic variation in critical immune regulatory genes may also play a role. It has been suggested that polymorphisms in a variety of these genes, including those coding for interleukin-10 (IL10), tumour necrosis factor-alpha (TNFα) and cytotoxic T-lymphocyte antigen 4 (CTLA4) may be important[19]. HLA class II type, with clear differences in the incidence of specific haplotypes between races, is also a major determinant, as discussed below.

Although much of the initial research into inhibitor formation focused on cases with gross gene abnormalities, there is relatively little information about the immunogenicity of different mutations that we can learn from these defects because they are not associated with any protein production. Of potentially greater interest are the few missense mutations, some of which do not necessarily result in severe disease, that are associated with a higher rate of inhibitor formation than normal. Compared with an overall inhibitor incidence of 8%[20] for all missense mutations, Arg2150His (20%), Arg2209Gln (16%) and Trp2229Cys (29%) are associated with an unexpectedly high rate of inhibitor formation, although their phenotype is generally mild or moderate[7,20]. This suggests that there are critical differences in the epitopes presented by the mutated protein when compared with wild-type factor VIII (FVIII). The interaction between Arg2150His and the major histocompatibility complex has been investigated in one study. The findings suggested that this mutation, in combination with specific HLA class II types, could be associated with the formation of T-cell clones with specificity for wild-type FVIII[21]. Even with these mutations, inhibitors occur in a minority of patients indicating that epitopic differences interact with other mechanisms in stimulating the immune response. Unravelling these interactions is the focus of ongoing research.

The difference in the rate of inhibitor formation between patients with haemophilia A or B is not simply due to genetic factors. Factor IX (FIX) is one of several serine proteases with high conservation of tertiary protein structure. Processing of peptides derived from other members of the protein superfamily may help to tolerise the immune system to FIX even when there is a null *F9* allele. FVIII has no comparable full-length protein relatives, with only factor V (FV) sharing some similarity but with just 40% sequence homology[22]. In the circulation FVIII is closely associated with von Willebrand factor (vWF) which has a chaperone function. There is some evidence that the association with vWF reduces the immunogenicity of FVIII[23]. If this protective effect is significant, one would expect that replacement therapy with FVIII complexed with vWF might be less likely to induce inhibitor formation than pure FVIII protein. On the basis of these considerations, the most contentious issue concerning risk factors for inhibitor development in previously untreated patients with severe haemophilia A is the role of the FVIII concentrate type with conflicting data deriving from several studies comparing the rates of inhibitor

formation with recombinant or plasma-derived products. The largest meta-analysis indicated that the pooled inhibitor incidence rate was significantly higher with recombinant products (27%) than with plasma-derived products (14%)[9], although this difference was attributed to the variability in inhibitor monitoring over the last decades. This question remains unresolved and is the focus of the "Survey of Inhibitors in Plasma-Product Exposed Toddlers", the SIPPET project: an international, randomised controlled trial of plasma-derived and recombinant concentrates in previously untreated patients with severe haemophilia with development of inhibitors as the primary measured outcome (http://www.clinicaltrials.gov, NCT01064284).

There are several environmental risk factors that influence the risk of inhibitor formation such as intensive treatment in the presence of inflammation and treatment during infancy[12]. It is also well recognised that most inhibitors develop during the first 50 days of exposure[7]. The use of alternative therapies such as desmopressin or bypassing agents might lower the risk[24]. The use of immunosuppression in an attempt to reduce the inflammatory response to acute haemorrhage has been tried but appears to have little overall benefit[25]. Thus current strategies focus on avoiding other immune stimuli such as elective surgery or immunisation during, or in close proximity to, early treatment exposure[26].

The recently published multicentre RODIN study which prospectively followed up paediatric patients up to 75 exposure days over an 11-year period evaluated treatment-related risk factors for inhibitor development[27]. In this study, recombinant and plasma-derived factor VIII products conferred similar risks of inhibitor development, and neither the content of vWF in the products nor switching among products was associated with the risk of inhibitor development. Second-generation full-length recombinant products were associated with a higher risk than that associated with third-generation products[27].

Treatment strategies based on the risk of inhibitor formation

With our knowledge about risks associated with inhibitor formation in haemophilia patients, the possibility of modifying treatment strategies, especially for patients thought to be at higher risk of inhibitor formation, could be an option. From the published literature it is clear that the strongest independent risk factors for inhibitor development in previously untreated patients with severe haemophilia A are a family history of inhibitors, null *F8* mutations and intensive treatment[8]. In this light, any condition that favours intensive treatment should be avoided if possible: elective surgery including insertion of central venous access devices should be postponed and the occurrence of major bleeds should be minimised by starting prophylaxis as soon as possible. The possible protective role of early prophylaxis with a once-weekly regimen and any influence of product type on inhibitor development need to be proven before further conclusions can be drawn. Currently there are conflicting data on whether product type influences the risk of inhibitor development[9,28]. Italian national guidelines indicate recombinant products as the first choice in previously untreated patients with haemophilia, irrespectively of the severity of their disease[29]. In Brazil, until 2012, all haemophilia A patients were exclusively treated with plasma-derived FVIII[30]. The prevalence of inhibitors in all haemophilia A patients is about 9-11%, and the incidence is still to be determined[30]. However, it is not currently possible to affirm that the occurrence of inhibitors among Brazilians, often of African descent, who have not been exposed to recombinant FVIII, is lower than that in other populations[18]. Likewise, our current knowledge does not enable us to suggest one product in preference to another in situations during which patients are subject to an increased inflammatory response, such as surgery.

It is clear that the use of early prophylaxis in patients with severe haemophilia reduces the risk of joint damage[31]. However, it may also increase the risk of inhibitor formation[32], raising the question of whether patients whose disease is caused by a gross genetic abnormality or who come from high-risk ethnic groups should start prophylaxis later or use some modified regimen. At present we do not understand enough to be able to answer this question completely. Based on our current knowledge, a risk-benefit analysis, taking into account both musculoskeletal complications and inhibitor development, should guide practice and policy. Musculoskeletal complications last life-long, and they are still costly to manage with suboptimal outcomes[33], whereas inhibitor development affects only a subset of high-risk individuals[13]. Moreover, the main advantage of prophylaxis is to avoid the risk of intensive treatment. These considerations argue in favour of early prophylaxis in high-risk patients, preferably with a full regimen (3×/week or every other day), to prevent arthropathy and support the management of inhibitors if they develop. A different approach to managing high-risk patients is to initiate treatment with low-dose prophylaxis once weekly at an early stage before the first bleed. The main goal of this strategy is to avoid peaks in the treatment within this "tolerisation phase" until 50 days of exposure have been achieved and thereby prevent inhibitor development. This regimen could possibly be adapted to reflect the risk of inhibitor development. A prospective study assessing a once-weekly prophylactic regimen together with the minimisation of immunological danger signals was

initiated early in 2012 (the Early Prophylaxis Immunologic Challenge [EPIC] Study, NCT01376700), however due to the unexpectedly high incidence of inhibitor development the study has recently been put on hold.

Characterisation of the bleeding phenotype of patients with inhibitors

The question of whether the bleeding phenotype of patients with inhibitors differs from that of patients without inhibitors remains unanswered. It could be assumed that in the presence of inhibitory antibodies the bleeding tendency might be more severe; however, to our knowledge this correlation has never been clearly described in the published literature nor investigated directly. Moreover it is difficult to address this issue retrospectively since the outcomes usually applied to define the bleeding phenotype in this subgroup of patients are bleeding frequency and treatment of each bleeding episode. Indeed such features may vary greatly even in the same patient given that the clinical response to bypassing agents is difficult to predict and that these agents do not give the overall success rates obtained with factor replacement therapy in patients without inhibitors[34]. In the COCIS study, aimed at evaluating the cost of care related to the presence of long-standing high-titre inhibitors in patients with haemophilia, the average bleeding frequency reported was 0.6 events per patient per month[35], which does not differ dramatically from that observed in a series of adult patients with severe haemophilia treated on demand[36]. However this result could have been influenced by the fact that patients with an inhibitor tend to be less physically active because of the lack of preventative treatment strategies[35,37]. What is clear from published data is that the orthopaedic status of patients with inhibitors is indeed worse than that of inhibitor-free patients[37]; such observations should, however, be interpreted cautiously since they could be the result of the reluctance to perform orthopaedic surgery in this subgroup of patients due to the high bleeding risk and related direct and indirect costs rather than of the bleeding phenotype *per se*. In a surveillance report of the Centers for Disease Control and Prevention in the United States which included more than 8000 patients with haemophilia, risk factors for limitation of the range-of-motion of joints were evaluated in 4343 eligible patients[38]. The evaluation showed that irrespective of disease severity, limitation of the range-of-motion of joints increased with age and body mass index, and that in patients with severe disease the presence of inhibitors was an independent risk factor for range-of-motion limitation even at a young age[38]. Unfortunately, in this report data on bleeding frequency were only correlated with disease severity but not with the presence of inhibitors. The evaluation of the orthopaedic status as a surrogate marker of bleeding frequency and severity was the major aim of the European Study on Orthopaedic Status (ESOS), a multicentre, non-interventional, cross-sectional, case-control study in which three cohorts of patients were evaluated: two cohorts of patients with severe haemophilia A or B and inhibitors aged 14-35 or 36-65 years and one cohort of patients aged 14-35 years with severe haemophilia A or B but without any history of inhibitors[37]. Patients with inhibitors were hospitalised for orthopaedic procedures more frequently than those without inhibitors, irrespectively of age. Disability, the need for walking aids and joint pain were more frequent among the former patients. Similarly, clinical and radiological orthopaedic scores were worse in patients with inhibitors than in an age-matched group of patients without inhibitors[37]. In this study there was no difference in the annual bleeding frequency with respect to muscle bleeds across the three cohorts; however, the frequency of joint bleeds in young patients with inhibitors was similar to that in their age-matched controls but double that in the elderly, suggesting that end-stage joint damage rather than the presence of inhibitors *per se* may influence the bleeding phenotype in the long term[37].

No prospective study comparing patients with and without inhibitors with respect to bleeding phenotype has been performed and published so far. The definition of the bleeding phenotype in inhibitor patients is an important clinical issue since it may have a strong impact on treatment strategies, especially in children.

Treatment strategies based on the bleeding phenotype

Tailoring treatment of haemophilia patients with inhibitors based on predicted bleeding tendency and phenotype would be a very interesting approach. Indeed prediction of the bleeding tendency in the presence of inhibitors may guide clinicians in tailoring treatment by starting immune tolerance induction (ITI) as soon as possible, perhaps using prophylaxis with bypassing agents in order to avoid joint bleeds until having eliminated the inhibitors. In this light the use of bypassing therapy for bleeding episodes needs to be further optimised. However, up to now we are still some way from being able to reliably predict bleeding tendencies which would be a prerequisite to tailoring treatment for individual patients. And even if better predictive algorithms did exist, the approach of early initiation of ITI coupled with prophylaxis with bypassing agents is very expensive. In the light of this it is important to bear in mind that high-dose ITI can reduce the bleeding tendency even in patients with high-titre inhibitors and very low FVIII levels[39]. ITI could, therefore, be initiated without bypassing agents and

prophylaxis with recombinant activated factor VII (rFVIIa) or plasma-derived activated prothrombin complex concentrate (aPCC) introduced only when patients bleed regularly during ITI treatment.

Management of patients with inhibitors: prophylaxis

The management of haemophilia in patients with inhibitors, including prophylactic treatment, poses several difficulties: in the presence of low-titre, low-responding inhibitors (historical inhibitor peak never exceeding 5 BU/mL) FVIII/FIX concentrates can be used although at higher doses and/or shorter intervals in order to saturate inhibitors and promote haemostasis. In contrast, if high-titre, high-responding inhibitors (historical inhibitor peak that exceeded 5 BU/mL at least once) are present, FVIII/FIX concentrates cannot be given in large enough quantities to overcome the neutralising activity of the inhibitors and haemostasis should be ensured by using alternative drugs, referred to as bypassing agents[34]. Up to date, bypassing agents used in this setting are plasma-derived aPCC (FEIBA®, Baxter, Vienna, Austria), and rFVIIa (NovoSeven®, Novo Nordisk, Bagsvaerd, Denmark)[40]. Their efficacy in controlling bleeding episodes has been widely proven in patients with haemophilia and inhibitors[41-44]. However, it is often unpredictable even in the same patients, and usually lower when compared to prophylaxis using factor replacement in non-inhibitor patients. Moreover, the use of these bypassing agents according to prophylactic regimens still needs to be defined, not only in terms of efficacy, but also considering costs and the potential risk of thromboembolic events.

Recently, several case series using bypassing agents (both aPCC and rFVIIa) for prophylaxis therapy have been reported, and three meta-analyses summarise most of these cases[45-47]. These reports are mostly retrospective and there is no consensus of regimens used, bleeding phenotype and/or previous treatment history. Table II summarises the information from the case series, meta-analyses and randomised studies using bypassing agents for prophylaxis.

The first controlled, randomised study on secondary prophylaxis for inhibitor patients compared the effectiveness of two different rFVIIa doses, 90 µg/kg or 270 µg/kg per day, over a 3-month period to reduce

Table II - Summary of previous reports of prophylaxis treatment using bypassing agents for haemophilia patients with inhibitors.

Reference	Patients' data	Bypassing agent therapy	Haemostatic outcome
aPCC prophylaxis studies			
DiMichele & Negrier, 2006[48] (Post-licensure surveillance study)	14 sHA Mean age 25 y (range 3-61)	Mean dose 69 IU/kg (range 15-100)×1/d or ×1/wk or alternate days Mean period 19.5 mo (range 0.25-26)	53% mean reduction in bleed frequency (range 10-85) No thrombotic event, no AE
Valentino, 2010[49] (Meta-analysis of 6 studies)	34 sHA Mean age 10.1 y (range 3-39)	Mean dose 78.5 U/kg (range 50-100)×1/d or ×1/wk Mean period 2.3 y (range 0.1-6)	57% reduction in bleed frequency 76% reduction in joint bleeding No thrombotic event
Leissinger *et al*., 2011[6] (Randomised cross-over study)	26 sHA Median age 28.7 y (range 2.8 - 67.9)	6 mo prophylaxis with 85 U/kg ±15% 3×/week *vs* 6 mo on-demand with 85 U/kg ±15%	62% reduction in all bleeds 61% reduction in joint bleeds No thrombotic events, 1 severe allergic reaction
rFVIIa prophylaxis studies			
Brackmann *et al.*, 2000[50]	2 sHA, 2 sHB Age range 0.5-26 y	90 µg/kg bid to 2-3×/week Period 2-27 mo	Slight decrease in bleeding episodes during prophylaxis with rFVIIa compared to aPCC
Young *et al*., 2005[51]	1 sHA, 1 sHB Age 3-15 y	200 µg/kg for 6-12 h Period 12-25 mo	Decrease in bleeding episodes and hospitalisation
Morfini *et al.*, 2007[52]	12 sHA, 1 sHB Age range 2-30 y	220 µg/kg/d to 200-250 µg/kg/wk Period 4-48 mo	Decreased bleeding episodes in 12 out of 13 patients
Konkle *et al.*, 2007[5] (Randomised cross-over dose study)	21 sHA, 1 sHB Mean age 15.7 y (range 5-56)	3 mo 90 µg/kg/d *vs* 3 mo 270 µg/kg/d	45% reduction in bleed frequency with 90 µg/kg/d 59% reduction in bleed frequency with 90 µg/kg/d
Jimenez-Yuste *et al*., 2009[53]	5 sHA	90-100 µg/kg/d Median period 9 mo (range 6-22)	Reduction of median bleeding rates from 4 prior prophylaxis to 1 in the same period with prophylaxis
Young *et al.,* 2012[54] (Retrospective, observational study)	71 sHA, 15 sHB Mean age 6 y (range 0.1-52)	Median dose and frequency: Children (N =61) 139 µg/kg 7×/wk Adolescents (N =7) 165.3µg/kg 5.5×/wk Adults (N =18) 133.3 µg/kg 3/wk	46% reduction in bleed frequency for all patients; 52% reduction in bleed frequency for patients with ≥1 bleed/mo prior prophylaxis

sHA: severe haemophilia A; sHB: severe haemophilia B; y: years; d: days; wk: weeks; mo: months; AE: adverse event.

bleeding frequency[5]. After a 3-month pre-prophylaxis observation period, only patients with high rates of bleeding episodes were considered for treatment in this study. Another 3-month post-prophylaxis observation period was also considered for the assessment. Although no statistically significant difference in rates of bleeding episodes between patients give the two doses, a significant difference was found between the 3-month pre-prophylaxis period and the prophylaxis period. The study also demonstrated that the prophylactic effect of rFVIIa persisted over a 3-month post-prophylaxis period, and that the patients had an improvement in health-related quality of life. Similar results were seen with a longer period of prophylactic treatment using rFVIIa in the observational PRO-PACT study, evaluating the frequency and pattern of bleeding episodes in haemophilia patients receiving preventative treatment[54].

Recently, equivalent beneficial effects were demonstrated with aPCC in the PRO-FEIBA study[6]. This was a prospective, randomised, crossover study, comparing a 6-month period of aPCC prophylaxis with 6 months of on-demand therapy. The use of aPCC dosed at 85 U/kg ± 15% given on 3 non-consecutive days per week was associated with a 62% reduction in all bleeds and a 61% reduction in joint bleeds as compared with on-demand therapy. It is interesting to observe that for a group of patients with a good response, the overall reduction in bleeding rate was 84%. However, for another group of patients with frequent bleeding episodes the reduction during the prophylactic period was less, and in fact two of ten patients in this group had more bleeding events during prophylaxis and one patient died of a cranial haemorrhage. None of the patients enrolled in these three prospective studies using rFVIIa or aPCC showed any sign of thromboembolic events.

Generally, although studies have shown an overall reduction in joint bleeding episodes during the period of prophylaxis with bypassing agents[5,49,52,53], the benefit of bypassing agents in preventing or reducing joint deterioration in the long term is not evident. In a recent publication from the Hemophilia Treatment Centers Network in the United States, secondary prophylaxis significantly decreased the number of haemarthroses in patients with inhibitors when compared to episodic treatment in this group of patients[55]. However, prophylaxis was not shown to be associated with a significant improvement in the target joint range of motion or to prevent new target joint development.

Prophylactic treatment strategies in patients with inhibitors

Although the abovementioned studies suggest that prophylaxis with bypassing agents could be effective in patients with inhibitors, they also emphasise the large inter-individual difference in dose-response to bypassing agents and the potential role for variables other than haemostatic agents in influencing the final outcome of a therapeutic approach (e.g. bleeding phenotype, presence of target joints and/or established joint damage, undefined mechanism of action). This contributes to the difficulty in establishing a consensus on prophylactic treatment using these agents. One approach to be considered could be to initiate prophylactic treatment in inhibitor patients in whom ITI treatment has been unsuccessful. However, prophylaxis using bypassing agents for inhibitor patients is very costly and not available for all patients in all countries. In this case, short-term prophylaxis with defined short-term goals may be possible as an alternative. If prophylactic treatment is applied prior to the initiation of ITI treatment or the patient is under consideration for ITI, rFVIIa would be the preferred choice while awaiting a decline in inhibitor titres to avoid an anamnestic response as this may occur with aPCC in some patients[53,56,57].

The role of primary prophylaxis with bypassing agents in children with newly developed inhibitors is being investigated in the frame of a randomised controlled clinical trial, the ENJOIH study (http://www.clinicaltrials.gov, NCT01105546), evaluating whether daily prophylaxis with 90 µg/kg rFVIIa is superior to on-demand treatment in preventing joint bleeds and consequently joint damage in these children.

Management of patients with inhibitors: immune tolerance induction

Despite the recent achievements in treatment and prevention of bleeding events with bypassing agents the primary goal when managing inhibitor patients remains eradication of the inhibitor by ITI treatment. Successful ITI is able to normalise FVIII pharmacokinetics and improve the patient's quality of life.

The outcome of ITI is influenced by both patient- and treatment-related factors. ITI is successful in up to 80% of patients with haemophilia A and 30% of patients with haemophilia B[58]. There are different dosing regimens to achieve immune tolerance. The Bonn protocol recommends high-dose FVIII of 100-150 IU/kg body weight twice a day[59,60], the Dutch van Creveld protocol 25-50 IU FVIII/kg body weight three times a week[61]. The German Guidelines (Deutsche Ärztekammer 2008) recommend 50-100 IU/kg body weight FVIII three times a week for ITI in children with low responding inhibitors (<5 BU/mL) and 100-200 IU/kg body weight FVIII twice a day for children with high responding inhibitors (>5 BU/mL) with 76% of success[62]. The German ITI-registry (GITR)

reported full success in 78.6% of the 126 patients treated over the period from 1993 to 1999[58]. Data from the National Italian Registry on ITI (PROFIT study[63]) show that the regimen used for ITI in Italy is a median dose of 100 IU/kg body weight per day and that in 74% of cases rFVIII is used for primary ITI since in the vast majority of patients the product employed at the time of inhibitor development is further applied. In South Africa, there are currently no published guidelines regarding ITI treatment, and the Dutch protocol is usually followed. Similarly, no single protocol predominates in the UK, leading to considerable variation in which ITI regimens are used including high-dose regimens similar to the ones mentioned above based on the Malmö protocol[64] or intermediate doses based on the Dutch protocol.

It is difficult to compare different studies and registries because the patients' characteristics and definition of success of ITI-outcome vary widely. In the registries mentioned above, the outcome of ITI was poorer in patients with high historical peak titres. The first study with a selection of patients with comparable risks and a clear definition of outcome was the International Immune Tolerance Study (I-ITI)[39]. This study compared a high-dose regimen (200 IU/kg body weight per day) versus a low-dose regimen (50 IU/kg body weight three times a week) in "good risk patients" (patients with historical inhibitor titres <200 BU/mL and immediate pre-ITI titres <10 BU/mL). It was prematurely terminated because of a significantly increased number of bleeds in the low-dose arm during ITI and post-ITI prophylaxis. ITI success rates were not different in the two arms; however, ITI was more slowly achieved in the low-dose arm[39].

A retrospective analysis of the Frankfurt experience, which indicated that ITI treatment with high-purity FVIII resulted in a lower treatment success rate (29%) as compared to historically obtained results using vWF-containing agents (91%), raised the question of whether the type of FVIII product may predict ITI outcome[65]. However, due to variable use of these agents in the different registries, this observation could not be confirmed by either the International Immune Tolerance Registry (IITR) or the North American Immune Tolerance Registry (NAITR)[66-68]. Both recombinant and plasma-derived FVIII can lead to successful ITI outcomes in good and poor risk patients as a recent review by Franchini *et al.* indicates[69]. Two retrospective analyses of poor-risk cohorts in the USA and one prospectively followed series of Italian and Spanish poor-risk patients have, however, shown that the epitope specificity of the anti-factor VIII antibody may influence whether ITI with vWF-containing FVIII agents is successful[70-72].

In Germany, ITI treatment is started very early for all patients, even those with high inhibitor titres, and pursued for a relatively long time[58,59]. Data from the National Italian Registry on ITI between 1996 and 2007[63] show that the median age at initiation of ITI was 5.6 years and that the median pre-ITI inhibitor titre was 4 BU/mL, indicating that ITI is usually performed in childhood and once the inhibitor titre has dropped to below 10 BU/mL. ITI treatment in Germany and Italy is usually initiated with the factor product previously used by the patient; a switch of factors is avoided if possible. In some German centres, if ITI is not successful after 6 months, the patient is switched to a vWF-containing regimen[65]. In the absence of long-term success (after 12-24 months), in Europe, immunosuppressive agents are often considered, e.g. rituximab[73]. Rituximab is a monoclonal antibody against CD20, a protein found only on B cells, and depletes B cells in the circulation and lymphoid tissues[74]. Successful use of rituximab for ITI has been reported in case studies and small series. A review of available data lists 29 reports comprising 49 patients with congenital haemophilia A or B: 23 patients with severe haemophilia A, 16 patients with mild/moderate haemophilia A and five patients with severe haemophilia B[75]. Fifty-seven percent of these patients had previously received ITI. Eradication of inhibitors was achieved in 67% of patients with a durable result in 53% of patients. Concomitant FVIII administration was significant for a positive outcome, whereas severe disease had a negative impact[75].

Immune tolerance induction treatment strategies

Taking the current evidence into account ITI should be performed with high doses (200 IU/kg body weight per day) in patients with high-titre inhibitors with the product used before the development of the inhibitor. Consensus recommendations of an international workshop on ITI suggested high-dose regimens of ≥200 IU/kg body weight per day for poor-risk patients (patients with historical inhibitor titres >200 BU/mL and/or immediate pre-ITI titres >10 BU/mL and/or time since inhibitor diagnosis exceeding 5 years), whereas no recommendation for any particular ITI regimen was made for good-risk patients[57]. If there is no significant reduction in the inhibitor titre after 6 to 9 months, the patient could be switched from recombinant or high purity to vWF-containing FVIII concentrates. If the inhibitor titre is not reduced after 12 months, two options should be discussed: long-term prophylaxis with aPCC or rFVIIa (less effective than FVIII treatment) or combining ITI with immunosuppressive agents such as rituximab or on-demand treatment with bypassing agents.

New treatment options for patients with inhibitors

Despite their well-documented efficacy and safety profiles, aPCC and rFVIIa have pharmacokinetic and therapeutic shortcomings which could be improved. Both are known to be thrombogenic even at recommended therapeutic doses. In *ex-vivo* studies, both agents were unable to generate thrombin to the same level as seen in patients without inhibitors when treated with FVIII or FIX[76]. However, it is unclear how much improvement in thrombin generation is required to achieve clinical benefit and in some patients even a small improvement may be sufficient. Consequently, therapy with both bypassing agents is often associated with significant intra- and inter-individual variation in efficacy[77]. Both agents are widely used in the management of acute bleeding episodes with limited experience in prophylaxis.

A number of bioengineered bypassing agents are under development to improve on the shortcomings of currently available inhibitor therapies. The aims of the various development programmes are to produce rFVIIa drugs that are less thrombogenic, have a longer half-life, an increased ability to bind to platelets and increased potency with dual use in prophylaxis and the treatment of bleeds. Although a single novel rFVIIa encompassing all these features in one drug would be desirable, the strategic approach of product developers to date has been to focus on one or two of these FVII improvement features. Development approaches so far have been varied and include changes in amino acid composition, N-glycan mutations, pegylation, glyco-pegylation and albumin fusion recombinant strategies. Table III is a summary of some of the haemostatic and pharmacokinetic features of these new agents most of which are already in advanced stages of clinical development.

In preclinical studies, vatreptacog alfa showed a more rapid onset of action and formed a stronger clot which was more resistant to fibrinolysis when compared to rFVIIa[78-81]. Although a phase II trial evaluating the safety and preliminary efficacy of vatreptacog alfa showed that it was well tolerated with no signs of thrombogenicity or immunogenicity and a high efficacy rate[77], the development of this drug was discontinued following analysis of results of a phase III trial (http://www.clinicaltrials.gov, NCT01392547) after a few patients had developed antibodies to vatreptacog alfa which were able to cross-react with rFVIIa.

A phase I, randomised, placebo-controlled clinical study was conducted in 16 non-bleeding subjects to investigate safety, tolerability and pharmacokinetic properties of BAY 86-6150[82]. Pharmacokinetic analysis determined an increased half-life to a mean of 7 hours. Initial results indicated that BAY 86-6150 was well tolerated and safe in the applied doses of up to 90 µg/kg. However, recruitment to a phase II study to evaluate the safety and efficacy of this molecule in the target haemophilia population was recently discontinued due to safety concerns (http://www.clinicaltrials.gov, NCT01625390).

A well established half-life extension strategy of pegylation was coupled to N-glycan modification by Novo Nordisk to produce glycopegylated rFVIIa (N7-GP). The mechanism of prolongation of rFVIIa half-life appears to be multi-factorial with reduced renal clearance due to increased volume and reduced receptor-mediated clearance due to steric hindrance recently shown to be operative *in vitro*[83,84].

A novel approach to inhibitor treatment not related to rFVIIa is the use of recombinant B-domain deleted porcine FVIII (OBI-1). OBI-1 is a highly purified bioengineered form of porcine recombinant FVIII developed by Ipsen and Inspiration Biopharmaceuticals with the pro-coagulant and biochemical properties of porcine plasma-derived FVIII. In a preclinical mouse model of haemophilia A, OBI-1 showed significantly less immunogenicity than plasma-derived-FVIII. Furthermore, no inhibitors were detected in cynomolgous monkeys, treated with OBI-1[85]. Results of the preclinical

Table III - New bypassing agents for the treatment of haemophilia with inhibitors.

Current status	Bypassing agent (developer)	Structural properties	Haemostatic and pharmacokinetic properties	Current stage of clinical development
Discontinued studies	vatreptacog alfa (Novo Nordisk)	3 amino acid substitutions in rFVIIa molecule	High thrombin burst and rapid onset of action	Clinical development discontinued in phase III
	BAY-86-6150 (Bayer Healthcare)	rFVIIa with 4 amino acid changes and two N-glycan mutations	Increased binding to platelets and 2-3 fold increase in half-life	Clinical development discontinued in phase II/III
	N7-GP (Novo Nordisk)	40k glycoPEGlated rFVIIa	3- to 4-fold increase in half-life	Clinical development discontinued in phase II
Continuing studies	OBI-1 (Inspiration)	B-domain deleted recombinant porcine FVIII	Haemostatic activity in inhibitor patients	Phase III
	CSL 689 (CSL Behring)	Albumin fusion to rFVIIa via glycine-serine linker	6- to 9-fold increase in half-life in preclinical studies	Phase II

and clinical studies suggest that OBI-1 may be less immunogenic and safer than Hyate:C in inhibitor patients[86,87]. In an open-label clinical phase II trial conducted in patients with haemophilia A and inhibitors against human FVIII, who were experiencing a non-life or -limb threatening bleed, OBI-1 was well tolerated and did not cause any drug-related serious adverse events[85]. The current clinical programme in the development of OBI-1 is to evaluate efficacy in surgical patients who have historically demonstrated a poor response to aPCC or rFVIIa (http://www.clinicaltrials.gov, NCT01434511).

Other novel therapies currently in the early phase of clinical development include anti-tissue factor pathway inhibitor (anti-TFPI) therapy. The potential benefits of this treatment are its non-intravenous route of administration and longer duration of action which will make prophylaxis in inhibitor patients possible. No clinical data are currently available as no clinical trials with this new product have yet been completed.

Looking at the new molecules that are under development for the treatment of patients with inhibitors it is clear that the major unmet needs in this setting include the following:

- The short half-life of rFVIIa that renders therapy difficult or even unfeasible for patients with difficult venous access, in particular children. The short half-life also limits implementation of successful prophylactic programmes using these agents.
- The need for prolonged therapeutic courses in order to control bleeds in a fashion comparable with FVIII/FIX replacement therapy: for FVIII one to two infusions are enough to resolve an acute bleed in over 90% of cases if treated early, as data from several clinical trials with various recombinant FVIII concentrates have shown[1,88,89]. In contrast, in patients with inhibitors treated with rFVIIa at least three to four injections at very high doses are usually required to control approximately 90% of bleeds[43,90], and treatment with aPCC every 12 hours leads to the resolution of only 76% of joint bleeds after 36 hours[43]. Moreover, if treatment with bypassing agents is started late with respect to the occurrence of first symptoms a whole therapeutic course may last 5 to 6 days, a delay that may enable the development of difficult-to-reverse tissue damage.
- The ease of laboratory monitoring: rFVIIa or aPCC can currently be monitored by global haemostatic assays such as thrombin generation or thromboelastography. These assays are not well standardised and their availability is limited compared to that of clotting factor assays. One of the potential advantages of porcine FVIII is that it can be monitored by clotting factor assays using an appropriate standard.

Concluding remarks

Inhibitor development is a complication of haemophilia treatment that generates many challenges. These include identification of which patients are at risk, prevention and treatment of bleeding episodes and identification of predictors of successful inhibitor eradication. Current state-of-the-art knowledge and practice in these areas indicate that there has been significant progress with advances being made which will contribute to our better understanding of inhibitor risk stratification, pathophysiology and management. The rapid evolution of novel therapies should lead to increased therapeutic options with improved efficacy for haemophilia patients with inhibitors.

Authorship contribution

All Authors contributed equally to this manuscript.

Acknowledgements

The group of authors was managed by Physicians World Europe GmbH, Mannheim, Germany, who provided writing support for the preparation of the manuscript, and its activities were supported by an unrestricted educational grant from Novo Nordisk Health Care AG, Zurich, Switzerland.

Keywords: haemophilia, inhibitors, challenges, treatment.

Conflict of interest disclosure

Keith Gomez is receiving grant funding for research in the field discussed in this paper from Pfizer Ltd and Bayer Healthcare Pharmaceuticals; he is receiving honoraria for consulting or lecturing in the field discussed in this paper from Pfizer Ltd and Novo Nordisk Healthcare AG. Robert Klamroth is collaborating with Novo Nordisk, Baxter and CSL Behring for research in the field discussed in this paper; he is receiving honoraria for consulting or lecturing in the field discussed in this paper from Novo Nordisk, Baxter, Bayer, CSL Behring and Pfizer. Johnny Mahlangu is collaborating with Novo Nordisk for research in the field discussed in this paper; he is receiving honoraria for consulting or lecturing in the field discussed in this paper from Novo Nordisk, Bayer, Biogen and Inspiration industry. Maria Eva Mingot is receiving grant funding for research in the field discussed in this paper from Baxter, Novo Nordisk and Pfizer; she is receiving honoraria for consulting or lecturing in the field discussed in this paper from Pfizer and Novo Nordisk. Margareth C Ozelo is receiving honoraria for consulting or lecturing in the field discussed in this paper from Novo Nordisk and Baxter.

References

1) Bray GL, Gomperts ED, Courter S, et al. A multicenter study of recombinant factor VIII (recombinate): safety, efficacy, and inhibitor risk in previously untreated patients with hemophilia A. The Recombinate Study Group. Blood 1994; **83**: 2428-35.

2) Lusher JM, Arkin S, Abildgaard CF, Schwartz RS. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A. Safety, efficacy, and development of inhibitors. Kogenate Previously Untreated Patient Study Group. N Engl J Med 1993; **328**: 453-9.
3) Hay CR, Baglin TP, Collins PW, et al. The diagnosis and management of factor VIII and IX inhibitors: a guideline from the UK Haemophilia Centre Doctors' Organization (UKHCDO). Br J Haematol 2000; **111**: 78-90.
4) Lawrence JS, Johnson JB. The presence of a circulating anti-coagulant in a male member of a hemophiliac family. Trans Am Clin Climatol Assoc 1941; **57**: 223-31.
5) Konkle BA, Ebbesen LS, Erhardtsen E, et al. Randomized, prospective clinical trial of recombinant factor VIIa for secondary prophylaxis in hemophilia patients with inhibitors. J Thromb Haemost 2007; **5**: 1904-13.
6) Leissinger C, Gringeri A, Antmen B, et al. Anti-inhibitor coagulant complex prophylaxis in hemophilia with inhibitors. N Engl J Med 2011; **365**: 1684-92.
7) Gouw SC, van den Berg HM, Oldenburg J, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. Blood 2012; **119**: 2922-34.
8) Gouw SC, van der Bom JG, Auerswald G, et al. Recombinant versus plasma-derived factor VIII products and the development of inhibitors in previously untreated patients with severe hemophilia A: the CANAL cohort study. Blood 2007; **109**: 4693-7.
9) Iorio A, Halimeh S, Holzhauer S, et al. Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review. J Thromb Haemost 2010; **8**: 1256-65.
10) Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: a systematic review. Haemophilia 2003; **9**: 418-35.
11) Hay CR. The epidemiology of factor VIII inhibitors. Haemophilia 2006; **12** (Suppl 6): 23-8; discussion 8-9.
12) Astermark J. Basic aspects of inhibitors to factors VIII and IX and the influence of non-genetic risk factors. Haemophilia 2006; **12** (Suppl 6): 8-13.
13) Hay CR, Palmer B, Chalmers E, et al. Incidence of factor VIII inhibitors throughout life in severe hemophilia A in the United Kingdom. Blood 2011; **117**: 6367-70.
14) Wacey AI, Kemball-Cook G, Kazazian HH, et al. The haemophilia A mutation search test and resource site, home page of the factor VIII mutation database: HAMSTeRS. Nucleic Acids Res 1996; **24**: 100-2.
15) Green PM, Montandon AJ, Ljung R, et al. Haemophilia B mutations in a complete Swedish population sample: a test of new strategy for the genetic counselling of diseases with high mutational heterogeneity. Br J Haematol 1991; **78**: 390-7.
16) Viel KR, Ameri A, Abshire TC, et al. Inhibitors of factor VIII in black patients with hemophilia. N Engl J Med 2009; **360**: 1618-27.
17) Viel KR, Machiah DK, Warren DM, et al. A sequence variation scan of the coagulation factor VIII (FVIII) structural gene and associations with plasma FVIII activity levels. Blood 2007; **109**: 3713-24.
18) Santos A, Annichino-Bizzacchi JM, Ozelo MC. Inhibitors of factor VIII in hemophilia. N Engl J Med 2009; **361**: 309-10; author reply 10.
19) Oldenburg J, Pavlova A. Genetic risk factors for inhibitors to factors VIII and IX. Haemophilia 2006; **12** (Suppl 6): 15-22.
20) Goodeve AC, Peake IR. The molecular basis of hemophilia A: genotype-phenotype relationships and inhibitor development. Semin Thromb Hemost 2003; **29**: 23-30.
21) Jacquemin M, Vantomme V, Buhot C, et al. CD4+ T-cell clones specific for wild-type factor VIII: a molecular mechanism responsible for a higher incidence of inhibitor formation in mild/moderate hemophilia A. Blood 2003; **101**: 1351-8.
22) Kane WH, Davie EW. Cloning of a cDNA coding for human factor V, a blood coagulation factor homologous to factor VIII and ceruloplasmin. Proc Natl Acad Sci USA 1986; **83**: 6800-4.
23) Lacroix-Desmazes S, Repesse Y, Kaveri SV, Dasgupta S. The role of VWF in the immunogenicity of FVIII. Thromb Res 2008; **122** (Suppl 2): S3-6.
24) Rivard GE, Lillicrap D, Poon MC, et al. Can activated recombinant factor VII be used to postpone the exposure of infants to factor VIII until after 2 years of age? Haemophilia 2005; **11**: 335-9.
25) Hermans C, De Moerloose P, Fischer K, et al. Management of acute haemarthrosis in haemophilia A without inhibitors: literature review, European survey and recommendations. Haemophilia 2011; **17**: 383-92.
26) Kurnik K, Bidlingmaier C, Engl W, et al. New early prophylaxis regimen that avoids immunological danger signals can reduce FVIII inhibitor development. Haemophilia 2010; **16**: 256-62.
27) Gouw SC, van den Berg HM, Fischer K, et al. Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: the RODIN study. Blood 2013.
28) Kreuz W, Ettingshausen CE, Zyschka A, et al. Inhibitor development in previously untreated patients with hemophilia A: a prospective long-term follow-up comparing plasma-derived and recombinant products. Semin Thromb Hemost 2002; **28**: 285-90.
29) Santagostino E, Mannucci PM, Bianchi Bonomi A. Guidelines on replacement therapy for haemophilia and inherited coagulation disorders in Italy. Haemophilia 2000; **6**: 1-10.
30) Rezende SM, Pinheiro K, Caram C, et al. Registry of inherited coagulopathies in Brazil: first report. Haemophilia 2009; **15**: 142-9.
31) Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. N Engl J Med 2007; **357**: 535-44.
32) Kruse-Jarres R. Inhibitors: our greatest challenge. Can we minimize the incidence? Haemophilia 2013; **19** (Suppl 1): 2-7.
33) Lobet S, Detrembleur C, Francq B, Hermans C. Natural progression of blood-induced joint damage in patients with haemophilia: clinical relevance and reproducibility of three-dimensional gait analysis. Haemophilia 2010; **16**: 813-21.
34) Leissinger CA. Prevention of bleeds in hemophilia patients with inhibitors: emerging data and clinical direction. Am J Hematol 2004; **77**: 187-93.
35) Gringeri A, Mantovani LG, Scalone L, et al. Cost of care and quality of life for patients with hemophilia complicated by inhibitors: the COCIS Study Group. Blood 2003; **102**: 2358-63.
36) Fischer K, van der Bom JG, Molho P, et al. Prophylactic versus on-demand treatment strategies for severe haemophilia: a comparison of costs and long-term outcome. Haemophilia 2002; **8**: 745-52.
37) Morfini M, Haya S, Tagariello G, et al. European study on orthopaedic status of haemophilia patients with inhibitors. Haemophilia 2007; **13**: 606-12.
38) Soucie JM, Cianfrini C, Janco RL, et al. Joint range-of-motion limitations among young males with hemophilia: prevalence and risk factors. Blood 2004; **103**: 2467-73.
39) Hay CR, Dimichele DM. The principal results of the International Immune Tolerance Study: a randomized dose comparison. Blood 2012; **119**: 1335-44.
40) Astermark J, Donfield SM, DiMichele DM, et al. A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: the FEIBA NovoSeven Comparative (FENOC) Study. Blood 2007; **109**: 546-51.
41) Sorensen B, Dargaud Y, Kenet G, et al. On-demand treatment of bleeds in haemophilia patients with inhibitors: strategies for securing and maintaining predictable efficacy with recombinant activated factor VII. Haemophilia 2012; **18**: 255-62.
42) Kenet G, Martinowitz U. Single-dose recombinant activated factor VII therapy in hemophilia patients with inhibitors. Semin Hematol 2008; **45**: S38-41.
43) Treur MJ, McCracken F, Heeg B, et al. Efficacy of recombinant activated factor VII vs. activated prothrombin complex concentrate for patients suffering from haemophilia complicated with inhibitors: a Bayesian meta-regression. Haemophilia 2009; **15**: 420-36.
44) Knight C, Dano AM, Kennedy-Martin T. Systematic review of efficacy of rFVIIa and aPCC treatment for hemophilia patients with inhibitors. Adv Ther 2009; **26**: 68-88.
45) Valentino LA. The benefits of prophylactic treatment with APCC in patients with haemophilia and high-titre inhibitors: a retrospective case series. Haemophilia 2009; **15**: 733-42.
46) Teitel J, Berntorp E, Dolan G, et al. A consensus statement on clinical trials of bypassing agent prophylaxis in inhibitor patients. Haemophilia 2011; **17**: 516-21.
47) Young G, Auerswald G, Jimenez-Yuste V, et al. When should prophylaxis therapy in inhibitor patients be considered? Haemophilia 2011; **17**: e849-57.
48) Dimichele D, Negrier C. A retrospective postlicensure survey of FEIBA efficacy and safety. Haemophilia 2006; **12**: 352-62.
49) Valentino LA. Assessing the benefits of FEIBA prophylaxis in haemophilia patients with inhibitors. Haemophilia 2010; **16**: 263-71.

50) Brackmann HH, Schwaab R, Effenberger W, et al. Hemophilia treatment. Side effects during immune tolerance induction. Haematologica 2000; **85**: 75-7.
51) Young G, McDaniel M, Nugent DJ. Prophylactic recombinant factor VIIa in haemophilia patients with inhibitors. Haemophilia 2005; **11**: 203-7.
52) Morfini M, Auerswald G, Kobelt RA, et al. Prophylactic treatment of haemophilia patients with inhibitors: clinical experience with recombinant factor VIIa in European Haemophilia Centres. Haemophilia 2007; **13**: 502-7.
53) Jimenez-Yuste V, Alvarez MT, Martin-Salces M, et al. Prophylaxis in 10 patients with severe haemophilia A and inhibitor: different approaches for different clinical situations. Haemophilia 2009; **15**: 203-9.
54) Young G, Auerswald G, Jimenez-Yuste V, et al. PRO-PACT: retrospective observational study on the prophylactic use of recombinant factor VIIa in hemophilia patients with inhibitors. Thromb Res 2012.
55) Gupta S, Siddiqi AE, Soucie JM, et al. The effect of secondary prophylaxis versus episodic treatment on the range of motion of target joints in patients with haemophilia. Br J Haematol 2013; **161**: 424-33.
56) Carcao M, Lambert T. Prophylaxis in haemophilia with inhibitors: update from international experience. Haemophilia 2010; **16** (Suppl 2): 16-23.
57) DiMichele DM, Hoots WK, Pipe SW, et al. International workshop on immune tolerance induction: consensus recommendations. Haemophilia 2007; **13** (Suppl 1): 1-22.
58) Lenk H. The German Registry of immune tolerance treatment in hemophilia--1999 update. Haematologica 2000; **85**: 45-7.
59) Brackmann HH, Oldenburg J, Schwaab R. Immune tolerance for the treatment of factor VIII inhibitors--twenty years' 'bonn protocol'. Vox Sang 1996; **70** (Suppl 1): 30-5.
60) Oldenburg J, Schwaab R, Brackmann HH. Induction of immune tolerance in haemophilia A inhibitor patients by the 'Bonn Protocol': predictive parameter for therapy duration and outcome. Vox Sang 1999; **77** (Suppl 1): 49-54.
61) Mauser-Bunschoten EP, Nieuwenhuis HK, Roosendaal G, van den Berg HM. Low-dose immune tolerance induction in hemophilia A patients with inhibitors. Blood 1995; **86**: 983-8.
62) German Medical Association. Indications and recommended doses for treating patients with factor VIII inhibitors in hemophilia A. In: *Cross-Sectional Guidelines for Therapy with Blood Components and Plasma Derivatives*. Executive Committee of the German Medical Association on the recommendation of the Scientific Advisory Board; 2008. p. 91.
63) Coppola A, Margaglione M, Santagostino E, et al. Factor VIII gene (F8) mutations as predictors of outcome in immune tolerance induction of hemophilia A patients with high-responding inhibitors. J Thromb Haemost 2009; **7**: 1809-15.
64) Freiburghaus C, Berntorp E, Ekman M, et al. Immunoadsorption for removal of inhibitors: update on treatments in Malmo-Lund between 1980 and 1995. Haemophilia 1998; **4**: 16-20.
65) Auerswald G, Spranger T, Brackmann HH. The role of plasma-derived factor VIII/von Willebrand factor concentrates in the treatment of hemophilia A patients. Haematologica 2003; **88**: EREP05.
66) Dimichele D. The North American Immune Tolerance Registry: contributions to the thirty-year experience with immune tolerance therapy. Haemophilia 2009; **15**: 320-8.
67) Mariani G, Ghirardini A, Bellocco R. Immune tolerance in hemophilia-principal results from the International Registry. Report of the factor VIII and IX Subcommittee. Thromb Haemost 1994; **72**: 155-8.
68) Mariani G, Kroner B, Immune Tolerance Study G. Immune tolerance in hemophilia with factor VIII inhibitors: predictors of success. Haematologica 2001; **86**: 1186-93.
69) Franchini M, Mannucci PM. Inhibitors of propagation of coagulation (factors VIII, IX and XI): a review of current therapeutic practice. Br J Clin Pharmacol 2011; **72**: 553-62.
70) Greninger DA, Saint-Remy JM, Jacquemin M, et al. The use of factor VIII/von Willebrand factor concentrate for immune tolerance induction in haemophilia A patients with high-titre inhibitors: association of clinical outcome with inhibitor epitope profile. Haemophilia 2008; **14**: 295-302.
71) Gringeri A, Musso R, Mazzucconi MG, et al. Immune tolerance induction with a high purity von Willebrand factor/VIII complex concentrate in haemophilia A patients with inhibitors at high risk of a poor response. Haemophilia 2007; **13**: 373-9.
72) Kurth MA, Dimichele D, Sexauer C, et al. Immune tolerance therapy utilizing factor VIII/von Willebrand factor concentrate in haemophilia A patients with high titre factor VIII inhibitors. Haemophilia 2008; **14**: 50-5.
73) Astermark J, Morado M, Rocino A, et al. Current European practice in immune tolerance induction therapy in patients with haemophilia and inhibitors. Haemophilia 2006; **12**: 363-71.
74) Garvey B. Rituximab in the treatment of autoimmune haematological disorders. Br J Haematol 2008; **141**: 149-69.
75) Franchini M, Mengoli C, Lippi G, et al. Immune tolerance with rituximab in congenital haemophilia with inhibitors: a systematic literature review based on individual patients' analysis. Haemophilia 2008; **14**: 903-12.
76) Sorensen B, Johansen P, Christiansen K, et al. Whole blood coagulation thrombelastographic profiles employing minimal tissue factor activation. J Thromb Haemost 2003; **1**: 551-8.
77) de Paula EV, Kavakli K, Mahlangu J, et al. Recombinant factor VIIa analog (vatreptacog alfa [activated]) for treatment of joint bleeds in hemophilia patients with inhibitors: a randomized controlled trial. J Thromb Haemost 2012; **10**: 81-9.
78) Holmberg HL, Lauritzen B, Tranholm M, Ezban M. Faster onset of effect and greater efficacy of NN1731 compared with rFVIIa, aPCC and FVIII in tail bleeding in hemophilic mice. J Thromb Haemost 2009; **7**: 1517-22.
79) Moss J, Scharling B, Ezban M, Moller Sorensen T. Evaluation of the safety and pharmacokinetics of a fast-acting recombinant FVIIa analogue, NN1731, in healthy male subjects. J Thromb Haemost 2009; **7**: 299-305.
80) Sorensen B, Persson E, Ingerslev J. Factor VIIa analogue (V158D/E296V/M298Q-FVIIa) normalises clot formation in whole blood from patients with severe haemophilia A. Br J Haematol 2007; **137**: 158-65.
81) Allen GA, Persson E, Campbell RA, et al. A variant of recombinant factor VIIa with enhanced procoagulant and antifibrinolytic activities in an in vitro model of hemophilia. Arterioscler Thromb Vasc Biol 2007; **27**: 683-9.
82) Mahlangu JN, Coetzee MJ, Laffan M, et al. Phase I, randomized, double-blind, placebo-controlled, single-dose escalation study of the rFVIIa variant BAY 86-6150 in hemophilia. J Thromb Haemost 2012; **10**: 773-80.
83) Karpf DM, Sorensen BB, Hermit MB, et al. Prolonged half-life of glycoPEGylated rFVIIa variants compared to native rFVIIa. Thromb Res 2011; **128**: 191-5.
84) Sen P, Ghosh S, Ezban M, et al. Effect of glycoPEGylation on factor VIIa binding and internalization. Haemophilia 2010; **16**: 339-48.
85) Toschi V. OBI-1, porcine recombinant factor VIII for the potential treatment of patients with congenital hemophilia A and alloantibodies against human Factor VIII. Curr Opin Mol Ther 2010; **12**: 617-25.
86) Parker ET, Craddock HN, Barrow RT, Lollar P. Comparative immunogenicity of recombinant B domain-deleted porcine factor VIII and Hyate: C in hemophilia A mice presensitized to human factor VIII. J Thromb Haemost 2004; **2**: 605-11.
87) Kempton CL, Abshire TC, Deveras RA, et al. Pharmacokinetics and safety of OBI-1, a recombinant B domain-deleted porcine factor VIII, in subjects with haemophilia A. Haemophilia 2012; **18**: 798-804.
88) Abshire TC, Brackmann HH, Scharrer I, et al. Sucrose formulated recombinant human antihemophilic factor VIII is safe and efficacious for treatment of hemophilia A in home therapy--International Kogenate-FS Study Group. Thromb Haemost 2000; **83**: 811-6.
89) Tarantino MD, Collins PW, Hay CR, et al. Clinical evaluation of an advanced category antihaemophilic factor prepared using a plasma/albumin-free method: pharmacokinetics, efficacy, and safety in previously treated patients with haemophilia A. Haemophilia 2004; **10**: 428-37.
90) Young G, Cooper DL, Gut RZ, Investigators H. Dosing and effectiveness of recombinant activated factor VII (rFVIIA) in congenital haemophilia with inhibitors by bleed type and location: the experience of the Haemophilia and Thrombosis Research Society (HTRS) Registry (2004-2008). Haemophilia 2012; **18**: 990-6.

Arrived: 29 October 2012 - Revision accepted: 16 May 2013
Correspondence: Keith Gomez
"Katherine Dormandy" Haemophilia Centre and Thrombosis Unit
Royal Free London NHS Foundation Trust
Pond Street, Hampstead, London NW3 2QG, UK
e-mail: k.gomez@ucl.ac.uk