

Inhibitor development in haemophilia B: an orphan disease in need of attention

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Summary

Factor IX (FIX) inhibitors develop in 1.5–3% of haemophilia B patients. Due to its low incidence compared with that in haemophilia A, few comparable data exist on host and treatment-related risk factors, and immunological processes associated with FIX inhibitor development. Moreover, the safety and efficacy of bypass therapy as well as the outcome predictors of successful inhibitor eradication have been poorly characterised. The lack of a useful evidence-based approach to the diagnosis and management of FIX inhibitors complicates their significant morbidity due to the frequency of allergic reactions that often herald antibody development. This review discusses what is currently known about the epidemiology, natural history and immunology of anti-FIX antibody development. It addresses several special considerations in the approach to the treatment of bleeding and inhibitor eradication. A case is made for moving forward with an integrated international collaboration for the further study of the nature and treatment of this problem.

Keywords: haemophilia B, factor IX deficiency, inhibitors, inhibitor treatment, immune tolerance.

Factor IX (FIX) inhibitors develop in 1.5–3% of haemophilia B patients, with geographically isolated pockets of higher incidence (Briet, 1991; Ljung, 1995; Katz, 1996; Warrier *et al*, 1997; Ljung *et al*, 2001). Given that haemophilia B is also one fifth as common as haemophilia A, FIX inhibitor development is uncommonly encountered in clinical practice. It follows that due to the relatively low incidence of neutralising antibody development in haemophilia B compared to that in haemophilia A, few comparable data exist on host and treatment-related risk factors, as well as the immunological processes associated with FIX inhibitor development. Moreover, the safety and efficacy of bypass therapy in haemophilia B inhibitor patients, as well as the outcome predictors of

successful inhibitor eradication in this population, have been significantly less well characterised than they have for patients with factor VIII (FVIII) inhibitors. The lack of a useful evidence-based approach to the prevention, diagnosis and management of FIX inhibitors exists against a background of greater morbidity associated with this complication. This is due to the frequent occurrence of allergic, anaphylactoid or frankly anaphylactic reactions that accompany and often herald FIX antibody development, a phenomenon that rarely complicates inhibitor development in haemophilia A (Harper *et al*, 1995; Warrier *et al*, 1997; Thorland *et al*, 1999; Warrier, 2005; Kadar *et al*, 2007). This phenomenon further complicates attempts to eradicate FIX inhibitors (Ewenstein *et al*, 1997).

This review discusses what is currently known about the epidemiology, the natural history, the host and treatment-related risk factors, and the immunology of anti-FIX antibody development. It also addresses several special considerations in the clinical approach to both the safe and efficacious treatment of bleeding in the presence of antibody as well as inhibitor eradication, i.e. immune tolerance. A case is made for moving forward with an integrated international collaboration for the scientific and clinical study of the nature and treatment of this orphan disease in need of our attention.

Epidemiology of FIX inhibitors: potential reasons for a low incidence disorder

The published incidence of FIX inhibitors is between 1.5 and 3% of all patients with haemophilia B and between 9 and 23% of severely affected FIX deficient patients (Briet, 1991; Ljung, 1995; Katz, 1996; Warrier *et al*, 1997; Ljung *et al*, 2001). The term *FIX inhibitor* refers to the presence of a high affinity neutralising IgG antibody directed against FIX that develops in response to exogenous FIX exposure, usually following FIX replacement therapy. This rate is much lower than the incidence of up to 33% reported for FVIII inhibitors among haemophilia A patients (DiMichele, 2005). Approximately 80% of the inhibitors that develop to FIX are of the high responding type (Key, 2004). *High responding* defines an immune response characterised by a high titre antibody [≥ 5 Bethesda Units (BU)] and a strong anamnestic response to

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antigen re-exposure that precludes ongoing therapy with specific factor replacement.

Several untested hypotheses for the discrepant incidence of antibody formation between the haemophilias have been proposed and summarised by Warriar (2005). A major potential explanation refers to the overall lower proportion of the severe (plasma factor level <0.01 IU/dl) phenotype in haemophilia B (30–40%) compared with that in haemophilia A (60%) (High, 1995). Overall, more patients with haemophilia B than with haemophilia A have been found to be cross-reacting material positive (CRM+) on the basis of detectable FIX antigen (Ljung *et al*, 2001). Since fewer CRM+ than CRM- individuals with haemophilia B develop inhibitors, it is further postulated that patients with detectable FIX polypeptide (CRM+) develop tolerance to the 'self' protein that extends to the exogenous FIX found in replacement therapy (Ljung *et al*, 2001; Lollar, 2005).

The molecular basis for haemophilia B underlies the observed differences in biochemical phenotype between the haemophilias. The FIX gene (*F9*), cloned in 1982 and sequenced in 1985, maps to position xq27. Although it comprises 34 kilobases (kb) of genomic DNA, only 1.4 kb of a 2.8 kb mRNA is translated (Belvini *et al*, 2005). As of its last update in 2004, the international haemophilia B mutation database, the largest of its kind, has documented the mutational heterogeneity of this disorder: 962 unique molecular events; 87 gross gene deletions; and 4 gene insertions among 2891 patient entries (Green, 2004). Among the 962 distinct gene mutations, most (751) are missense mutations, while 211 represent short (<30 nucleotides) additions, deletions or both. Overall, 75% of the known *F9* mutations in this database are missense mutations. Only 25% represent major gene alterations, among which few are gross (4%) or complete (4%) deletions. (Green, 2004). Interestingly, 60% of the biochemically severe haemophilia B phenotype is a result of missense mutations (Belvini *et al*, 2005) in contrast to only 15% for the severe haemophilia A phenotype (Oldenburg & Pavlova, 2006). The higher prevalence of missense mutations may at least partially explain the higher prevalence of CRM positivity among severe haemophilia B patients.

The international haemophilia B database has also noted the mutations associated with inhibitor development (Green, 2004). Overall, 54 (2%) patients in the database have developed inhibitors, a prevalence rate that validates the historical cohort data. Among these, 23% of patients with gross deletions and 30% of the complete deletion entries have developed FIX inhibitors. An additional 14 inhibitors have been identified in association with 7/211 (3%) distinct small gene alterations. Only 10 inhibitors are represented among 10/751 (1%) missense mutations (Green, 2004).

These data suggest a strong association between absent endogenous FIX protein due to gross and, in particular, complete gene mutations, and inhibitor development. In so far as the haemophilia genotype is a risk factor for inhibitor development, the uncommon prevalence of these high-risk genotypes among

haemophilia B patients may partially explain the lower risk of inhibitor development in FIX-deficient patients.

However, the larger fraction of haemophilia B patients with detectable FIX antigen on the basis of molecular genetics may not provide the sole explanation for the lower incidence of FIX inhibitors. Given that acquired deficiency of FIX is much less common than FVIII, it is thought that FIX may be a less immunogenic protein than FVIII (Largo *et al*, 1974; Carmassi *et al*, 2007). If this is indeed so, the considerable conservation of amino acid sequence among vitamin K-dependent clotting factors (factors II, VII, IX, X, protein C and S) could be a reason for decreased FIX immunogenicity (Warriar, 2005).

Natural history of FIX inhibitors: allergic/anaphylactic(oid) complications

An international registry of FIX inhibitors collected data from 1998 through 2005 under the auspices of the FVIII/IX Subcommittee of the International Society for Thrombosis and Haemostasis (ISTH) that has best characterised the natural history of FIX inhibitor development (Warriar *et al*, 1997; Warriar, 2005). Eighty-eight international registry patients with FIX inhibitors developed their antibodies at a median age of 19.5 months (range 7–156 months) after a median of 11 (range 2–180) exposure days to exogenous FIX replacement therapy. Antibody development was noted to occur in all ethnic groups and in response to therapy with both plasma-derived and recombinant FIX concentrates. The median peak historical inhibitor titre was 30 BU (range 1–1156 BU) (Warriar, 2005).

The manifestation of an allergic, anaphylactic and frank anaphylactic reaction prior to or concomitant with antibody development is a well recognised phenomenon that occurs almost exclusively in conjunction with FIX inhibitor development (Warriar *et al*, 1997; Thorland *et al*, 1999; Warriar, 2005). Fifty-one (58%) of the 88 FIX inhibitors reported to the registry in 2005 were associated with an allergic manifestation (Warriar, 2005).

The aetiology of this allergic co-manifestation remains unclear. The small molecular mass of the FIX protein (55 000 kd) accounts for its extracellular distribution and the potential for mast cell activation and an IgE-mediated hypersensitivity response. Skin and radioallergosorbent testing (RAST) in a few such patients supports this hypothesis, but has yet to be further studied (Ketterling *et al*, 1994). Complement activation by the transient IgG1 antibody formation has been suggested as an alternative immune trigger (Sawamoto *et al*, 1996) and is discussed later in more detail. A third theory proposes that this immune response is triggered by excessive immune complex formation resulting from the high concentration of exogenous FIX protein infused with each treatment. This theory is based on the fact that normal plasma concentrations of FIX (5 µg/ml) exceed that of FVIII (0.1 µg/ml) and the standard dosing for FIX replacement therapy is double that for FVIII deficiency to allow for its increased volume of distribution (Warriar, 2005). However, immune complex

formation has not yet been documented in allergic phenotype patients. Finally, molecular genetic characterisation of the FIX inhibitors, as performed in collaboration with the International Registry, demonstrated that 26% of FIX inhibitor patients whose haemophilia B resulted from a complete gene deletion demonstrated such allergic reactions (Thorland *et al*, 1999). It has been postulated that as *F9* deletions are often extremely large (up to a megabase), co-deletion of immune response modifier genes could trigger this phenomenon in otherwise susceptible patients (Ketterling *et al*, 1994). This hypothesis also remains unstudied and unproven. Such a complex immunological phenomenon is likely to result from a multifactorial trigger. However, the infrequent, unpredictable and often seriously emergent nature of this reaction has so far thwarted any attempts at the coordinated prospective study of the immunology of this life-threatening complication.

Risk factors for FIX inhibitor development

The recent published literature has implicated several host-related and treatment-associated risk factors for the development of FVIII inhibitors (DiMichele, 2005). Potentially important host-related risk factors include positive family history for inhibitors (Astermark *et al*, 2001), African ethnicity (Gill, 1984; Addiego *et al*, 1994) haemophilia genotype (Oldenburg & Pavlova, 2006), and at least two immunogenotypes (Astermark *et al*, 2006a,b). The implicated treatment-associated risk factors include FVIII product type (Goudemand *et al*, 2006; Gouw *et al*, 2007b), age at first exposure to FVIII (Lorenzo *et al*, 2001; Van der Bom *et al*, 2003), as well as intensity/route of and reason for FVIII administration (Von Auer *et al*, 2003; Gouw *et al*, 2007a). Due to the relative infrequency of FIX inhibitor development, few of these risk factors, except for haemophilia B genotype, have so far been similarly explored.

Immunology of factor IX inhibitors

Although our knowledge about the immunology of factor VIII inhibitor development is still rudimentary, even less is currently known about the immunology of FIX inhibitors. In early haemophilia B mouse experiments, single dominant CD4⁺ T-cell epitopes in mice with both C57BL/6 (H2^b) and BALB/C (H2^d) backgrounds proliferated in response to subcutaneous injections with human FIX (Lin *et al*, 1997; Greenwood *et al*, 2003). However, autoreactive CD4⁺ T cells were also noted in normal C57BL/6 mice in the absence of an endogenous immune response to murine FIX, calling into question the specificity of that immune response (Greenwood *et al*, 2003; Lollar, 2005).

Two human cytokine genes, interleukin-10 (*IL-10*) and tumour necrosis factor- α (*TNF*), have recently been linked to factor VIII inhibitor development (Astermark *et al*, 2006a,b). No such data currently exist for FIX inhibitor patients. However, genetic linkage studies in multiple recombinant inbred strains of haemophilia B mice suggested that multiple

gene loci could be linked to the inhibitor response in these animals (Lozier *et al*, 2005). These experiments demonstrated that the major histocompatibility complex (MHC) class II (H-2) and/or K class I-a (Iak) loci were critical to this response (logarithmic odds (LOD) score *c.* 4.8). However, other genes also contributed to FIX antibody development. Noted linkages included polymorphic markers from chromosomes 1 and 10 that approximated the immunoregulatory genes *IL10* and interferon- γ (*IFNG*) (LOD scores *c.* 2.3–2.6).

As is the case with the anti-FVIII antibody response, FIX neutralising antibodies are thought to be polyclonal in nature. Studies by Sawamoto *et al* (1996) first determined that the human anti-FIX antibodies was predominantly IgG4 in nature, based on 10 plasma samples from six haemophilia B inhibitor patients, including five with a history of an allergic phenotype. Interestingly, transient IgG1 subclass antibodies were also detected in all three allergic phenotype patients whose plasma was procured at the exact time of allergic episode. However, IgG1 subclass antibodies could not be detected in plasma samples obtained more remotely (4 d to >4 weeks) from their allergic event from 2/3 of these patients as well as an additional two allergic phenotype inhibitor patients (Sawamoto *et al*, 1996). These data suggest that the allergic response that occurs in some FIX inhibitor patients may be associated with transient IgG1-subclass antibody production. The polyclonal nature of the FIX antibody response was subsequently confirmed by additional studies of haemophilia B inhibitor patient plasma (Christophe *et al*, 2001). Furthermore, the FIX epitopes recognised by the predominantly IgG1 and IgG4 subclass antibodies were noted to include the γ -carboxyglutamic acid (GLA) and serine protease (SP) domains, but not the epidermal growth factor (EGF) and activation peptide (AP) domains (Christophe *et al*, 2001). Functionally, these antibodies inhibited the activated FIX (FIXa)/activated FVIII (FVIIIa) intrinsic FX activation complex through at least two mechanisms, interference with FIX binding to phospholipids as well as phospholipid-independent FIX binding to FVIII light chain (Christophe *et al*, 2001). More *in vitro* and *in vivo* studies are required to confirm these data and to further define the immunological and biochemical nature of the FIX inhibitory antibody response.

Clinical aspects of FIX inhibitor detection, treatment and eradication

Clinical Surveillance and Laboratory Detection of FIX Inhibitors

Although published clinical guidelines for optimal FVIII inhibitor surveillance exist, these do not necessarily apply to FIX inhibitor surveillance, given the differences between the haemophilias in inhibitor epidemiology and natural history. Furthermore, there are no established specific guidelines for clinical FIX antibody surveillance. Based on international data collected through the Haemophilia B Inhibitor Registry, the

risk period for antibody development in susceptible patients with large or complete *F9* deletions occurs early in the patient's exposure history [median of 11 FIX exposure days (EDs)] but may continue for up to 180 EDs (Warrier *et al*, 1997; Warrier, 2005). However, as previously discussed, allergic reaction frequently precedes antibody detection, calling into question the utility of a frequent surveillance protocol. In fact, the severe haemophilia B patient may be better served by a protocol that includes routine early molecular diagnosis to identify the genetically predisposed patient, accompanied by a prolonged period of hospital-based FIX infusion prior to transition to home care in order to observe such a patient closely during the high risk period for inhibitor development. Late onset development of FIX inhibitors in the heavily previously treated patient does not appear to be a major clinical problem; however, the true prevalence of this phenomenon is unknown.

The classical laboratory test used to screen and quantitate anti-FIX inhibitory antibodies is a modified activated partial thromboplastin time (aPTT)-based Bethesda assay using FIX-deficient plasma (Kasper, 1975; Ingerslev, 2005). In the classical Bethesda assay, several dilutions of patient platelet-poor plasma in pooled normal plasma are incubated at 37°C for 2 h. Residual FIX activity is then measured by the one-stage FIX clotting assay. One Bethesda Unit (BU) of inhibitor is defined as the amount of antibody that neutralises 0.5 IU of FIX in 1 ml of plasma (Kasper, 1975). The Nijmegen modification of the Bethesda assay was published in 1995 and was developed in an effort to correct the original assay's lack of specificity in the lower range of FVIII antibody detection (Verbruggen *et al*, 1995). The Nijmegen assay was adopted as the official method for inhibitor quantification by the Factor VIII/IX Subcommittee of the ISTH in 1996 (Giles *et al*, 1998). Although the Nijmegen modification of the Bethesda assay is now sometimes used to also quantitate FIX inhibitors, it is important to note that it has never similarly been validated in this capacity.

The Bethesda assay has historically been plagued by poor intra-laboratory assay correlation due to the multiplicity of aPTT reagents and FIX-deficient plasmas, the inherent imprecision of the one-stage clotting assay, as well as the lack of a consensus international standard for assay equilibration (Barrowcliffe, 2005). Furthermore, there is still no international consensus on the definition of a negative antibody titre, by either Bethesda or Nijmegen assay (Ingerslev, 2005). In an effort undertaken through the FVIII/IX Subcommittee of the ISTH, an international standard for the FVIII inhibitor assay is currently under development in an effort to solve some of its specific problems (S. Raut, personal communication). However, no such standard for the FIX Bethesda test is under study at this time.

In a published study of FIX inhibitors, the Bethesda assay was predictive of a poor response to FIX replacement therapy, suggesting the largely neutralising nature of anti-FIX antibodies (Christophe *et al*, 2001; Lollar, 2005). Recently, newer assay methodologies, such as clot waveform analysis and, to a lesser

extent, the thrombin generation assay, have been found to be useful in the most precise measurement of very low levels of FIX (0–0.1 IU/dl) in *in vitro* experiments (Matsumoto *et al*, 2006). The applicability of these methods, particularly the clot waveform analysis, to the early detection of anti-FIX antibodies has not yet been studied.

Treatment and prevention of major and minor haemorrhage: overview

As a consequence of FIX inhibitor development, minor and major bleeding cannot always be prevented or treated effectively, potentially resulting in increased morbidity and diminished quality of life. Given the paucity of these patients relative to those with FVIII deficiency, few retrospective analyses or prospective studies of the treatment or prevention of bleeding in inhibitor patients have historically involved individuals with anti-FIX antibodies. Therefore, most current standards of care for the treatment for haemorrhage in the presence of inhibitors derive from the study of FVIII inhibitor patients. Accordingly, the ensuing discussion of treatment of the FIX inhibitor patient will largely extrapolate from these published data with exceptions duly noted.

In clinical practice, the therapeutic approach to the treatment and/or prevention of minor and major haemorrhage in the presence of an inhibitor is based on the severity of bleeding, the patient's inhibitor titre, the immunological pattern of anaemnesis, and, specifically for FIX inhibitors, a history of the allergic phenotype. Specific guidelines for the management of bleeding in FIX inhibitor patients were published by the United Kingdom Haemophilia Centre Doctors' Organization (UKHCDO) (Hay *et al*, 2000).

Treatment/prevention of minor/major haemorrhage: low titre/responder inhibitors. In the presence of a low titre and low responding FIX inhibitor, the optimal treatment strategy involves the infusion of FIX concentrate at higher than standard doses (Fig 1). FIX recovery and half-life studies performed in a non-bleeding state are helpful in guiding the choice of dose and dosing frequency in the treatment of

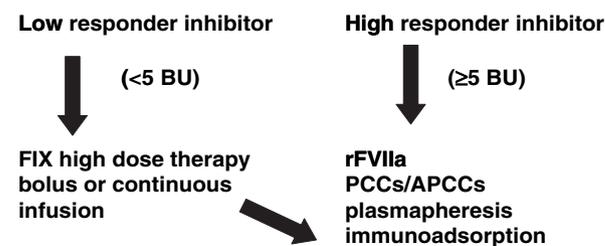


Fig 1. Treatment guidelines for treatment/prevention of major/minor haemorrhage in patients with low and high titre factor IX inhibitors. PCCs/APCCs, prothrombin complex concentrates/activated prothrombin complex concentrates; rFVIIa, recombinant activated FVII; BU, Bethesda unit.

musculoskeletal, soft tissue or mucocutaneous haemorrhage in the home or outpatient setting (Morfini *et al*, 1991).

Treatment for life- or limb-threatening haemorrhage as well as haemostatic prophylaxis during surgery can also be provided with FIX concentrate (Fig 1). Dose and dosing regimen are designed, preferably on the basis of FIX pharmacokinetic studies, to maintain consistently high therapeutic FIX plasma activity levels. These can be achieved through an intensive, closely monitored therapeutic regimen involving either frequent bolus infusion or FIX continuous infusion (CI) delivered through dedicated venous access at an hourly rate calculated on the basis of anticipated daily requirement. Multiple studies of FIX concentrate stability, methodological sterility and optimum FIX delivery by CI support the use of this method (Batorova & Martinowitz, 2006). CI also allows for the flexibility required to provide good haemostasis in the unpredictable or changeable surgical schedule. Frequent monitoring of FIX levels is recommended to accurately estimate the patient's daily FIX requirement in these treatment situations. In the event of a poor therapeutic response, the FIX dose and/or dosing regimen can be augmented based on measured plasma FIX activity. Alternatively, bypass therapy can be instituted under the treatment guidelines provided for the high titre, high responder FIX inhibitor patient (Fig 1).

Treatment/prevention of major/minor haemorrhage: high titre/responder inhibitors. In the case of high titre/high responder inhibitors, when frequent or continuous high dose FIX therapy is ineffective, treatment must either (i) bypass the FIX requirement for clot formation or (ii) remove sufficient antibody through a mechanism of either plasmapheresis or immunoadsorption to permit the temporary administration of high dose FIX (Fig 1). For the treatment of minor (soft tissue, joint or muscle) and most major or life-threatening haemorrhage, as well as for surgical haemostasis, the bypass strategy remains the mainstay of therapeutic practice. Prothrombin complex concentrates (PCCs), activated PCCs (aPCCs) or recombinant activated factor VII (rFVIIa) are used for this purpose. A recent prospective randomised crossover treatment study in FVIII inhibitor patients [FEIBA VH® Novoseven® Comparative study (FENOC study)], demonstrated a similar efficacy of both product types in treating joint haemorrhage, even though the statistical criteria for determining efficacy equivalency were not met (Astermark *et al*, 2007a). Therefore, the current choice of product is often based on patient age, individual historical response to therapy, type and interval between bleed and therapy, potential for anaemnesis, available venous access, parent or patient choice and cost. Indeed, there are several considerations when considering the use and choice of bypass therapy for the FIX inhibitor patient, except in the case of the treatment of the FIX inhibitor patient complicated by the allergic phenotype when first line therapy is usually restricted to the use of rFVIIa. (Brown, 2005; Mehta, 2006).

Firstly, although both classes of available product bypass the requirement for FVIII or IX in the generation of thrombin, their biochemical modes of action differ, suggesting that no single product may be optimal for the treatment of all types of haemorrhage under all circumstances (Negrier *et al*, 2006). However, in the case of the treatment of the FIX inhibitor patient complicated by the allergic phenotype, first line therapy is usually restricted to the use of rFVIIa (Brown, 2005; Mehta *et al*, 2006). Secondly, anaemnesis can be seen with the use of an aPCC due to its FIX content, but is not expected to occur with rFVIIa. Thirdly, optimal dosing regimens for the treatment of bleeding are not well established for either bypassing agent, and the potential efficacy of either product in musculoskeletal and/or surgical prophylaxis still require further post-licensure investigation. Fourthly, the potential for thrombogenicity and or disseminated intravascular coagulation (DIC) exists for both products (Ehrlich *et al*, 2002; Ludlam, 2002). Furthermore, the concurrent use of antifibrinolytic agents, although physiologically justifiable, may increase potential for thrombogenicity and should be used judiciously (Antovic *et al*, 2001). Anecdotally, this is particularly worrisome with the use of aPCCs, making rFVIIa the usual treatment of choice when the concurrent use of antifibrinolytic therapy is required. Finally, in order to definitively address all these issues, standardised laboratory monitoring for both efficacy and toxicity is urgently needed and currently undergoing study (Negrier *et al*, 2006; Young *et al*, 2006).

Activated prothrombin complex concentrates (aPCCs) (FEIBA®). Although early controlled studies demonstrated no difference in efficacy between activated and non-activated PCCs (Lusher *et al*, 1983), aPCCs are now used more frequently in therapeutic practice. FEIBA® (Baxter Healthcare, Glendale, CA, USA) is the only currently licensed product within this class of bypass agents. The active components of FEIBA® [activated factor X (FXa), in combination with prothrombin and trace amounts of activated factors IX and VII] generate thrombin in a complex with activated Factor V (FVa), divalent calcium, and procoagulant membrane (prothrombinase) (Turecek *et al*, 2004).

In one retrospective study, haemostasis was achieved in 50–66% of bleeding episodes with an overall efficacy of 96% noted after 1–3 infusions (Negrier *et al*, 1997). Haemostasis during minor and major surgery with FEIBA® as both second-line and first-line treatment was also reported in the same study and included FIX inhibitor patients. This regimen included an initial dose of 50–100 units/kg and subsequent infusions as needed up to a maximum of 200 units/kg/d. The average number of doses required to control haemorrhage in this retrospective review were 1–3 for musculoskeletal and/or minor bleeding; 3–4 for minor surgery; and 30–90 for major surgery/life-threatening bleeding (Negrier *et al*, 1997). Monitoring for activation of coagulation is recommended during periods of prolonged therapy or frequent dosing. Similar treatment guidelines can be potentially applied to the use of non-activated PCCs.

Recombinant activated factor VII (NovoSeven®). Recombinant factor VII (rFVIIa) (NovoSeven®; Novo Nordisk, Bagsvaerd, Denmark), is also an effective bypassing agent used in the management of inhibitor patients. Although the mechanism of rFVIIa action is incompletely understood, it is thought that this product (i) leads to increased formation of rFVIIa/tissue factor complexes for efficient thrombin generation; (ii) binds to the surface of activated platelets and directly activates FX (independently of the presence of FVIII/FIX) on platelet membrane surfaces (Hoffman & Monroe, 2001); and (iii) enhances thrombin-activatable fibrinolysis inhibitor activation, thus minimising early fibrinolysis (Lisman *et al*, 2002).

In pre-licensure clinical trials of non-surgical bleeding in FVIII inhibitor patients, NovoSeven® was effective in the treatment of 70–100% of joint, muscle, dental, and central nervous system bleeds (Bech, 1996; Lusher *et al*, 1998). The home treatment study, designed to assess efficacy and safety of NovoSeven® for mild-moderate bleeds in inhibitor patients, determined that a mean of 2.2 doses of 90 µg/kg infused every 3 h controlled bleeding with 93% efficacy regardless of bleed site, and with increased efficacy when treated within 8 h of bleed detection (Key *et al*, 1998). For patients undergoing major surgery, NovoSeven® has been shown to be 80–100% effective in restoring haemostasis (Ingerslev *et al*, 1996; Shapiro *et al*, 1998). Furthermore, the NovoSeven® Surgery Study established 90 µg/kg as an effective peri-operative haemostatic dose for more than 90% patients undergoing both minor and major surgery (Shapiro *et al*, 1998). The use of NovoSeven® by continuous infusion remains controversial and is not the standard of care in most institutions (Schulman *et al*, 1998; Brown, 2005).

The licensed dose/dosing regimen for NovoSeven®, as established for FVIII inhibitor patients, is 90 µg/kg administered with a frequency of up to every second hour (Mehta *et al*, 2006). In the experience of some institutions, treatment with a single large bolus dose of NovoSeven® has been reported to rapidly relieve pain in the absence of adverse events (Kenet *et al*, 2003; Mehta *et al*, 2006). *In vitro* studies of rFVIIa – induced thrombin generation also support the rationale for well-conducted *in vivo* dosing trials (Hoffman & Monroe, 2001). NovoSeven® has been evaluated in crossover dose comparison (270 and 90 µg/kg) studies conducted by several investigators who have concluded the two regimens to be comparable in safety and efficacy (Santagostino *et al*, 2006; Kavalki *et al*, 2006). Monitoring for activation of coagulation with aggressive or prolonged therapy is generally recommended.

Although a rationale exists for the use of both aPCCs and rFVIIa in the treatment of FIX inhibitors, a recently published survey of European haemophilia treatment centers documented that while 100% of centres used rFVIIa for the treatment of both haemophilia A and B inhibitor patients, fewer routinely used aPCCs (Astermark *et al*, 2007b). Moreover, fewer centers considered aPCCs as a therapeutic option for adults and children with FIX antibodies (40% and 15%, respectively) than

for adults and children with inhibitors to FVII (85% and 25%, respectively).

Bypass therapy bleeding prophylaxis. The significant morbidity, disability and increased cost of care associated with the musculoskeletal complications of FVIII and IX inhibitor development have been well documented (UKHCDO, 2004), as has the benefit of primary joint prophylaxis in children without inhibitors (Manco-Johnson *et al*, 2005). Safe and effective prophylaxis with bypass therapy is therefore a desirable therapeutic option for all inhibitor patients.

Prophylaxis with twice daily FEIBA® has been used as part of the Bonn immune tolerance protocol for FVIII inhibitors for more than 25 years (Brackmann *et al*, 1996). However, there have been no controlled trials to assess optimal dosing or treatment efficacy/safety. A small cohort study of FVIII inhibitor patients suggested that FEIBA® prophylaxis, with or without concomitant immune tolerance therapy, reduced total bleeds as well as joint bleeds, but did not prevent joint disease progression when used at doses of 50–100 units/kg three to four times weekly (Hilgartner & Makipernaa, 2003). Another observational study prospectively evaluated aPCC prophylaxis (50 U/kg daily to 100 U/kg twice daily) in 22 children ≤6 years with FVIII inhibitors undergoing immune tolerance induction (ITI) (Kreuz *et al*, 2000). The median annual incidence of joint bleeds was 1 (range, 0–6); no life-threatening haemorrhage was observed. Thrombosis was not observed, but has since been reported by others (Carcao *et al*, 2003). A prospective non-randomised study of FEIBA® prophylaxis in inhibitor patients is underway (Leissingner, 2006). However, although few FIX inhibitor patients are likely to be enrolled, their inclusion in this trial is strongly encouraged.

In a recent prospective pilot study of NovoSeven® prophylaxis in FVIII inhibitor patients, bleeding frequency was reduced on both 90 µg/kg and 270 µg/kg daily regimens (Konkle *et al*, 2006). Further studies of NovoSeven® prophylaxis are planned and, again, enrolment of FIX inhibitor patients in this study should be strongly encouraged.

Indications for the use of plasmapheresis/immunoabsorption and high-dose FIX. Rapid and efficient inhibitor titre reduction with plasmapheresis offers patients the temporary option of FIX therapy when the use of bypass agents fails to provide haemostasis or is complicated by DIC. Protein A sepharose column immunoabsorption, a more efficient and reusable system for selective IgG removal, is an option available outside the US (Mariani & Kroner, 2001). Either process significantly reduces anti-FIX antibody levels, allowing for high-dose FIX administration, usually by CI, for surgical prophylaxis, for the treatment of life- or limb-threatening bleeding, or for the initiation of immune tolerance for a high inhibitor titre. A relative indication for this procedure can be pharmaco-economic (Jansen *et al*, 2001). A brisk anaemnetic response can be expected within 5–8 d of FIX re-exposure after

which bypass therapy again becomes the only therapeutic option.

Inhibitor eradication

Immune tolerance induction (ITI) for FIX inhibitors

The historical experience. Given the low incidence of factor IX inhibitors, the historical experience with immune tolerance in haemophilia B is limited to two small series and a total of seven patients for whom the overall success rate with high dose FIX +/- immune modulation was 71% (Brackmann, 1984; Nilsson *et al*, 1986). Additional experience with the Malmö protocol suggested that ITI was successful in six of seven haemophilia B patients treated with the Malmö regimen; however tolerance was lost in one patient within 6 months, and a further attempt to re-induce tolerance was unsuccessful (Freiburghaus *et al*, 1999).

The ITI registries: outcome and outcome predictors. The largest collection of data on ITI in haemophilia B derives from the North American Immune Tolerance Registry (NAITR) and the International Registry for Factor IX Inhibitors.

In the NAITR, only 5/16 (31%) completed courses of ITI in haemophilia B were successful on a median dosing regimen of 100 units/kg/d (range 25–200) (DiMichele *et al*, 2002). Daily dosing regimens and immune modulation were used in 88% and 47% of courses respectively. Plasmapheresis was used in two courses. High purity or monoclonal FIX concentrates were used in 82% of the reported courses (DiMichele *et al*, 2002). Given the paucity of these data, no association between ITI outcome and FIX dose or purity can be established at this time.

The demographics of the NAITR FIX inhibitor cohort are detailed in Table I. Patients with an allergic phenotype in association with these FIX antibodies (10/16) were over-represented in this cohort. ITI complications specific to this group of patients may have been responsible for the high failure rate in the cohort as a whole. Within the allergic subset of subjects, 8/10 failed ITI (DiMichele *et al*, 2002). The rate of adverse events (65%) was 10-fold higher than that in haemophilia A inhibitor subjects, and was not dose-related. Allergic reactions accounted for 79% of the adverse events. All reactions occurred in subjects with a previously identified allergic phenotype (DiMichele *et al*, 2002). Similarly poor outcomes from the International Registry for Factor IX Inhibitors were reported by Warriar (2005) (14% of 34 attempts). Importantly, there were no successes among allergic phenotype patients (Warriar, 2005).

Nephrotic syndrome as a complication of ITI. Nephrotic syndrome as a complication of ITI performed in haemophilia B patients who develop inhibitors in association with an allergic phenotype, was first described by Ewenstein *et al* (1997). In the NAITR experience, the allergic complications of ITI occurred in subjects with a previously

Table I. Factor IX inhibitor subjects in the NAITR: subject demographics and inhibitor characteristics relative to immune tolerance (ITI) outcome.

	Success (n = 5)	Failure (n = 11)
Haemophilia B inhibitors		
Subject demographics		
Factor IX activity <0.01 IU/dl	5/5	11/11
Family history inhibitor	0/5	5/11
Age at ITI (years)*	3.7 (2.4–4.8)	4.6 (0.8–19.6)
Interval (months): inhibitor diagnosis/ITI*	12 (0–31)	47 (0–227)
Inhibitor characteristics		
High responder (≥5 BU)	4/5	9/11
Allergic phenotype	2/5	8/11
Historical peak titre (BU)*	13 (2.4–112)	50 (10–650)
Pre-induction titre (BU)*	5 (3–24)	10.5 (1–19)
Peak titre on ITI (BU)*	20 (3–38)	39.5 (6–59)

NAITR, North American Immune Tolerance Registry; BU, Bethesda Units; ITI, immune tolerance induction.

*Results reported as median (range).

identified allergic phenotype and were accompanied by the development of nephrotic syndrome in 3/10 subjects (DiMichele *et al*, 2002). Aggregate data on this phenomenon, collected through the International Registry for Factor IX Inhibitors, was subsequently published (Warriar, 2005). Based on 13 cases compiled from the international experience, 11 of which were associated with anaphylaxis, this complication presented 8–9 months into the course of high-dose ITI (100–325 units/kg/d) with a sepsis-like presentation in conjunction with periorbital oedema, hypoalbuminaemia and proteinuria. Factor IX deficiency in these patients were the result of either a deletion or stop codon mutation. Factor IX products of all types were implicated. Clinical improvement usually followed cessation of the FIX infusions, but the response to standard therapy with steroids was historically poor. So far, the aetiology of this complication remains unclear. One attempt at immunohistochemical staining of renal tissue obtained by biopsy did not demonstrate FIX immune complexes (Ewenstein *et al*, 1997).

At the current time, given the overall poor ITI success rate as well as the potential for the development of nephrotic syndrome during ITI, most clinicians either do not proceed with ITI or proceed with extreme caution in the allergic phenotype FIX inhibitor patients. This recommendation has also been made by the UKHCDO (Hay *et al*, 2006). If the decision is made to proceed, strong consideration is given to pre-ITI immune desensitisation to FIX as well as to the use of low-dose regimens.

Finally, whether the traditional Malmö regimen, incorporating immunoadsorption for potentially more rapid inhibitor eradication, might obviate the development of nephrotic syndrome in these patients is yet unclear and the proposed subject of further study by a recent international consensus panel (Berntorp *et al*, 2006).

Alternative strategies to immune tolerance

Given the poor success rate with ITI in haemophilia B, there has been anecdotal experience with the use of immunomodulatory therapy with mixed success. Successful tolerance has been induced in a single FIX inhibitor patient using either ciclosporin A (Cross & van den Berg, 2007), or a regimen that included mycophenolate-mofetil, dexamethasone, and intravenous immunoglobulin together with high-dose FIX (Wermes *et al*, 2000). On the other hand, long-term inhibitor eradication was not achieved with the use of rituximab in two haemophilia B inhibitor patients (Mathias *et al*, 2004; Fox *et al*, 2006).

Ultimately, FIX inhibitor prevention may prove to be the best strategy for this group of patients. The immunogenicity of future recombinant FIX proteins produced either by porcine mammary glands or modified FIX constructs developed for gene transfer technology will necessarily have to be carefully assessed in preclinical and prelicensure human clinical trials (Pipe, 2005). The prospect of a role for gene transfer technology in achieving permanent tolerance to FIX is an interesting one. Immunological tolerance with hepatic gene transfer has been achieved in a haemophilia B mouse model (Mingozzi *et al*, 2003; Dobryzski & Herzog, 2005). However, there were many qualifying aspects to the success of this preliminary work. These included the need for high expression (>30 ng/ml) of the FIX transgene product; genotype specificity with tolerance more difficult to achieve in complete gene deletion mice; and the requirement for T-cell naiveté to FIX in the transgenic animals, an immunological state that could be difficult to replicate in humans. Nonetheless, this important proof of principle holds therapeutic promise.

Future considerations

Factor IX inhibitor development is an uncommon complication of a rare and orphan disease, haemophilia B. This reality has made the problem difficult to study from both the clinical and the scientific perspectives. The consequent lack of a disease-specific evidence base for the treatment of patients with haemophilia B who develop inhibitors has been highlighted by this review. Yet, for those patients who are affected, the morbidity associated with alloantibody formation can be severe and potentially life-threatening. Moreover, the safety, efficacy, and optimal dosing of bypass therapy in this group of patients remains largely unknown, and treatment options accessible to haemophilia A inhibitor patients may be limited in some individuals with anti-FIX antibodies. Finally, FIX inhibitors may be very difficult to eradicate using immune tolerance strategies that are effective in 70–80% of haemophilia A inhibitor patients.

In order to move forward, the further understanding of the immunology of FIX inhibitors must become a research priority within the international scientific and funding organisations. Furthermore, both industry and regulatory bodies worldwide

should work together to ensure that the clinical trial design of any future safety, efficacy and/or pharmacovigilance trials of bypass therapeutics include FIX inhibitor patients. Lastly, many questions remain concerning optimal therapy, successful outcome predictors and the complications of ITI in haemophilia B. Prospective randomised studies may not be possible given the low frequency of disease and even lower incidence of inhibitors. However, international registry-based retrospective and prospective data collection could play the key role in the future study of all aspects of FIX antibody eradication, as well as stimulate the critical scientific collaboration that will, in time, bring the much needed solutions.

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