International workshop on immune tolerance induction: consensus recommendations[1]

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Summary. Although immune tolerance induction (ITI) has been used for 30 years to eliminate inhibitors and restore normal factor pharmacokinetics in patients with hemophilia, there is a paucity of scientific evidence to guide therapeutic decision-making. In an effort to provide direction for physicians and hemophilia treatment center staff members, an international panel of hemophilia opinion leaders met to develop consensus recommendations for ITI in patients with severe and mild hemophilia A and hemophilia B. These recommendations draw on the available published literature and the collective clinical experience of the group and are rated based on the level of supporting evidence .

Keywords: factor VIII, factor IX, hemophilia A, hemophilia B, immune tolerance induction, inhibitors

Introduction

The development of inhibitors that neutralize the function of factor VIII (FVIII) or factor IX (FIX) is the most serious complication associated with the treatment of haemophilia. Inhibitors occur in up to 30% of patients with severe haemophilia A (FVIII <1% of normal) [1–3], 0.9–7% of patients with mild to moderate haemophilia A [4] and 3% of patients with haemophilia B [3]. The presence of an inhibitor does not increase mortality, but it complicates treatment and increases disease-related morbidity because bleeding episodes do not respond to standard therapy [5]. While large doses of factor can usually overcome low-titre inhibitors ($\leq$5 Bethesda units (BU) [1]), high-titre inhibitors generally render factor replacement ineffective, and bypassing therapy is required [6]. Two bypassing agents are currently available: the activated prothrombin complex concentrate (aPCC), FEIBA® (Baxter AG, Vienna, Austria), and recombinant-activated FVII (rFVIIa; NovoSeven®, Novo Nordisk, Bagsvaerd, Denmark). Both products have been shown to control at least 80% of bleeding episodes associated with high-titre inhibitors, including perioperative bleeding [7–13]. However, their haemostatic efficacy is difficult to predict [6,14] and does not reach the overall success rates obtained with specific factor replacement in patients without inhibitors [6,15]. Furthermore, no laboratory assays are available that correlate with dosing or efficacy or predict complications [6]. Consequently, inhibitor patients, particularly those with high-titre, high-responding inhibitors, are at increased risk for uncontrollable haemorrhage, devastating joint disease and subsequent disability [16].

To reduce these risks and improve quality of life, immune tolerance induction (ITI) is usually attempted to eliminate high-responding (anamnestic) FVIII inhibitors of recent onset and restore normal factor pharmacokinetics [14]. ITI may also be performed, albeit far less frequently, in patients with high-titre FIX inhibitors. The Bonn protocol, first described in 1977 [17], is the prototype from which subsequent

ITI regimens evolved. All regimens utilize ongoing, frequent, uninterrupted exposure to FVIII or FIX over a period of a few months to two or more years [18] with the goal of inducing antigen-specific tolerance [14,19]. Despite three decades of clinical experience with this modality, there is a lack of scientific evidence to direct ITI decision-making. Supporting data primarily come from small institutional studies and the three major ITI registries (International [20], German [21] and North American [22]). With regard to registry data, it was retrospectively collected several years ago, and the registries differed in terms of demographics; collection methods; and, in some instances, endpoints. The International Immune Tolerance Induction (I-ITI) Study [23], the first prospective, randomized, controlled trial of immune tolerance, was launched in July 2002 and currently involves 96 centres in 25 countries. This study is expected to resolve some of these unanswered questions, but the final results may not be known for several years, as subject accrual is ongoing.

To provide haemophilia caregivers with interim guidance, an international panel of haemophilia opinion leaders gathered in New York, NY, on 9–11 June 2006, and again in London, England, on 14–16 September 2006, to accomplish two goals: First, to critically evaluate the quality of the published literature on the clinical use of ITI, associated outcomes, and outcome-predictive variables in patients with inhibitors to FVIII or FIX. Secondly, to formulate consensus on a set of recommendations for ITI that draw on both the available published literature and the collective clinical experience of the group. These recommendations have been developed into decision-tree algorithms that physicians and haemophilia treatment centre (HTC) staff members may utilize and individualize according to the unique needs of each patient. The background,

rationale and support for the algorithms are described in detail. Each practice recommendation has been assigned a rating based on the level of available supporting evidence (Table 1) [24]. In addition to these algorithms, the panel developed a list of research priorities. This investigational call-to-action is presented at the end of the supplement.

We are grateful for the opportunity to be guest editors of this supplement to *Haemophilia* and to work with our colleagues from Europe and North America to develop the recommendations for ITI. The names and affiliations of the co-authors in alphabetical order are:

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Table 1. Levels of evidence [24].

Level	Evidence
Ia	Meta-analysis of randomized controlled trials (RCT)
Ib	At least one RCT
IIa	At least one well-designed, controlled study without randomization
IIb	At least one other type of well-designed, quasi-experimental study
III	Well-designed, non-experimental, descriptive studies (e.g. comparative studies, correlational studies, and case–control studies)
IV	Expert committee reports or opinions and/or clinical evidence offered by respected authorities

Immune tolerance induction in patients with severe haemophilia A

Overview of inhibitor formation

Studies in previously untreated patients (PUPs) with severe haemophilia A suggest that most FVIII inhibitors develop early in life (median age, 1.7–3.3 years), with the highest risk during the first 50 exposure days to factor concentrate [25,26]. Although genetic markers indicating a predisposition to inhibitor development are not fully identified, several other host factors are known to influence the risk of inhibitor development. In addition to the type and severity of haemophilia, these include FVIII gene mutations, primarily large defects in multiple domains [27]; polymorphisms in genes involved in the regulation of the immune system [28–30], which suggest that inhibitor formation is a polygenic process; family history, linked to approximately a threefold increased risk of inhibitor development [31]; and African heritage, which doubles the risk [32]. In addition, differences in the major histocompatibility complex, a critical component of the immune system, may contribute to inhibitor development [33]. The likelihood of inhibitor formation may also be increased by environmental factors, such as age at first factor exposure [34], intensive FVIII exposure during infancy [35], immunologic challenge (e.g. infection, surgery, immunization) [36], or choice of FVIII replacement concentrate (i.e. recombinant or plasma-derived) [37], but their roles in the aetiologic process remain less certain. The entire ITI algorithm for patients with severe haemophilia A is presented in Fig. 1. The following sections discuss the various components of this algorithm.

When to Start ITI

In 1999, Kroner [38] published a meta-analysis of the data from the International Immune Tolerance Registry (IITR) and the North American Immune Tolerance Registry (NAITR). Collectively, these data included information on 437 patients with haemophilia A and B evaluated for ITI. Among the putative predictors of ITI success, only two were found to be statistically significant in this meta-analysis: historical peak inhibitor titre and titre at the initiation of ITI. ITI was successful in 82% of patients whose peak titres were <50 BU, and as the historical titre increased, success rates diminished (Table 2). This inverse correlation between historical peak titre and successful tolerance was also observed in the German ITI registry [21].

Table 2. IITR and NAITR data meta-analysis: outcome by maximum historical titre [38].

	Maximum historical titre (BU)				
	<50	50–200	201–500	>500	Total
IITR *n* (%)					
Success	64 (82)	24 (65)	9 (56)	17 (50)	114
Failure	14 (18)	13 (35)	7 (44)	17 (50)	51
Total (*P* = 0.004)	78	37	16	34	165
NAITR *n* (%)					
Success	71 (82)	18 (69)	4 (40)	1 (7)	94
Failure	16 (18)	8 (31)	6 (60)	13 (93)	43
Total (*P* = 0.001)	87	26	10	14	137

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IITR, International Immune Tolerance Registry; NAITR, North American Immune Tolerance Registry.

While the maximum historical inhibitor titre may influence decisions about whether to attempt ITI, the pre-ITI titre is relevant to decisions about when to start immune tolerance. An inhibitor titre of <10 BU immediately before ITI initiation positively affected both the likelihood of success and the time required to achieve tolerance in the IITR and NAITR (Table 3) [22], the German registry [21], the Spanish registry [39] and in several institutional studies [40–44]. There is some suggestion that waiting until the inhibitor titre falls below 5 BU may further improve response [22], but this has yet to be firmly established. Thus, reasonable evidence supports the recommendation that ITI should generally be deferred until the inhibitor titre has dropped below 10 BU, although this may not be the absolute cutoff. This postponement typically delays the start of ITI by 3–6 months. According to an early progress report from the I-ITI study, the median delay between inhibitor development and a drop in the inhibitor titre below 10 BU has been 6 months [23]. Despite waiting for the inhibitor titre to fall, the median age of the children at the start of ITI was only 25 months. Close surveillance of inhibitory antibody levels during the waiting period is essential to ensure the prompt initiation of immune tolerance once the titre falls sufficiently or alternative action if it does not. During this interval, exposure to FVIII should be avoided or minimized by discontinuing FVIII prophylaxis (if applicable) and treating intercurrent bleeding on-demand with bypassing therapy [18]. rFVIIa is the preferred agent for acute haemorrhage management in this setting [18] since FEIBA, which contains residual FVIII antigen, may cause anamnesis [26].

When bleeding is life- or limb-threatening or has the potential to cause significant morbidity
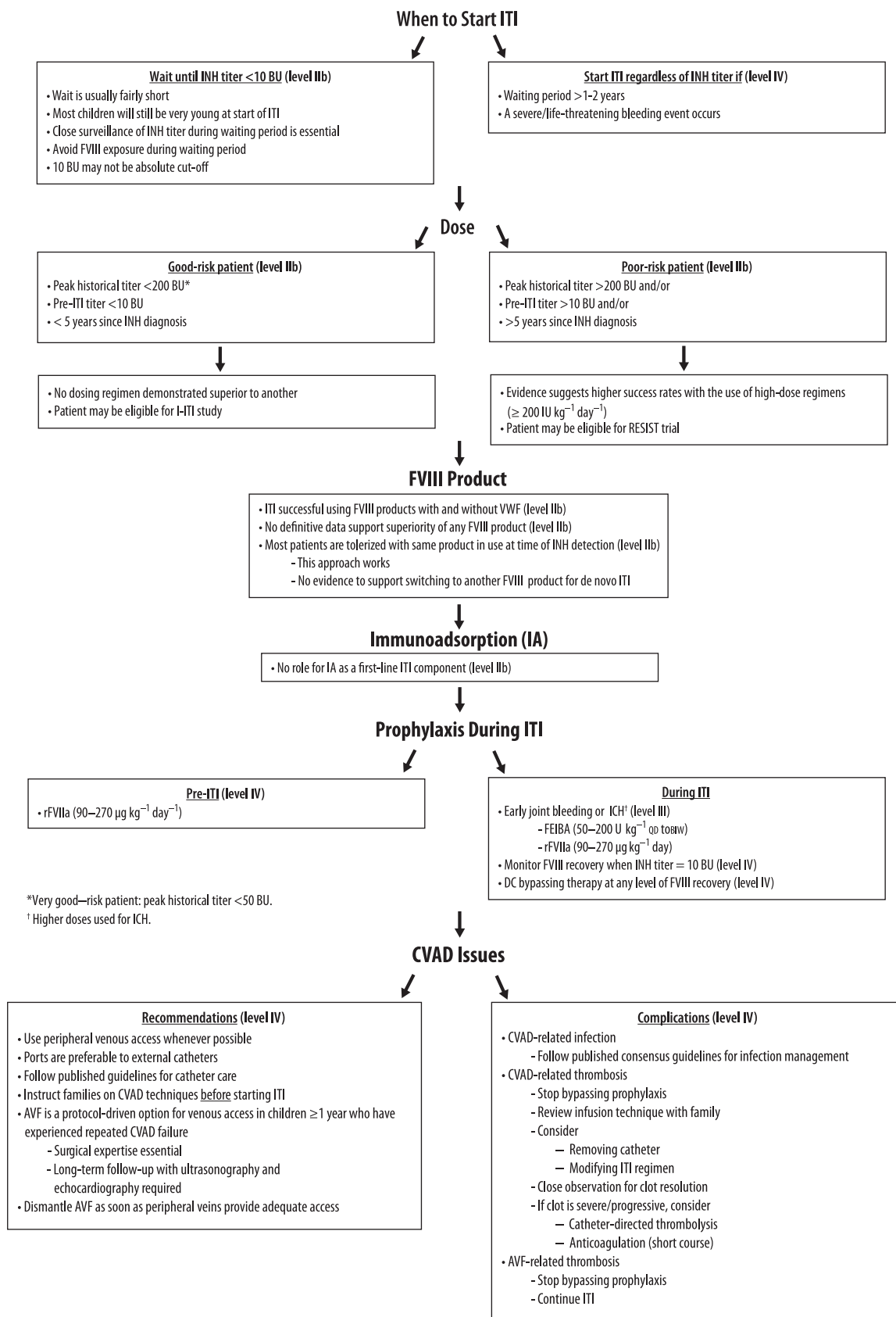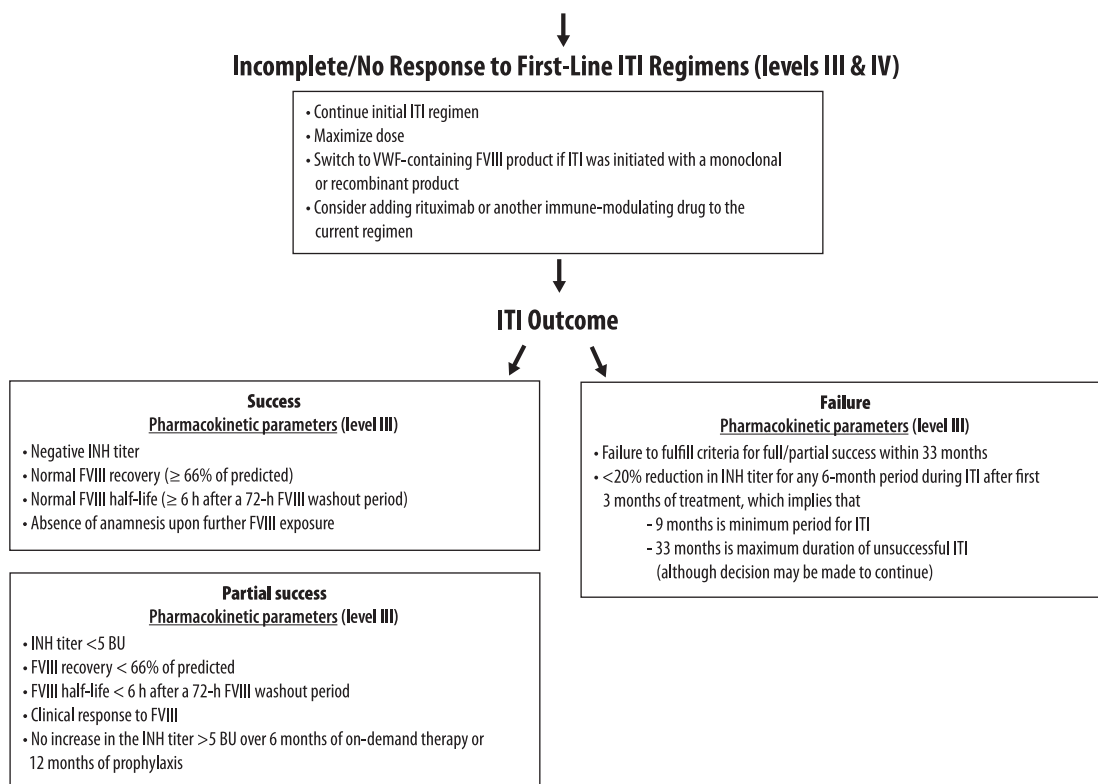
When to Start ITI

Wait until INH titer <10 BU (level IIb)
- Wait is usually fairly short
- Most children will still be very young at start of ITI
- Close surveillance of INH titer during waiting period is essential
- Avoid FVIII exposure during waiting period
- 10 BU may not be absolute cut-off

Start ITI regardless of INH titer if (level IV)
- Waiting period >1–2 years
- A severe/life-threatening bleeding event occurs

Dose

Good-risk patient (level IIb)
- Peak historical titer <200 BU*
- Pre-ITI titer <10 BU
- < 5 years since INH diagnosis

Poor-risk patient (level IIb)
- Peak historical titer >200 BU and/or
- Pre-ITI titer >10 BU and/or
- >5 years since INH diagnosis

- No dosing regimen demonstrated superior to another
- Patient may be eligible for I-ITI study

- Evidence suggests higher success rates with the use of high-dose regimens ($\geq$ 200 IU kg^{-1} day^{-1})
- Patient may be eligible for RESIST trial

FVIII Product
- ITI successful using FVIII products with and without VWF (level IIb)
- No definitive data support superiority of any FVIII product (level IIb)
- Most patients are tolerized with same product in use at time of INH detection (level IIb)
    - This approach works
    - No evidence to support switching to another FVIII product for de novo ITI

Immunoadsorption (IA)
- No role for IA as a first-line ITI component (level IIb)

Prophylaxis During ITI

Pre-ITI (level IV)
- rFVIIa (90–270 µg kg^{-1} day^{-1})

During ITI
- Early joint bleeding or ICH† (level III)
    - FEIBA (50–200 U kg^{-1} QD toBIW)
    - rFVIIa (90–270 µg kg^{-1} day)
- Monitor FVIII recovery when INH titer = 10 BU (level IV)
- DC bypassing therapy at any level of FVIII recovery (level IV)

*Very good—risk patient: peak historical titer <50 BU.
† Higher doses used for ICH.

CVAD Issues

Recommendations (level IV)
- Use peripheral venous access whenever possible
- Ports are preferable to external catheters
- Follow published guidelines for catheter care
- Instruct families on CVAD techniques <u>before</u> starting ITI
- AVF is a protocol-driven option for venous access in children ≥1 year who have experienced repeated CVAD failure
    - Surgical expertise essential
    - Long-term follow-up with ultrasonography and echocardiography required
- Dismantle AVF as soon as peripheral veins provide adequate access

Complications (level IV)
- CVAD-related infection
    - Follow published consensus guidelines for infection management
- CVAD-related thrombosis
    - Stop bypassing prophylaxis
    - Review infusion technique with family
    - Consider
        — Removing catheter
        — Modifying ITI regimen
    - Close observation for clot resolution
    - If clot is severe/progressive, consider
        — Catheter-directed thrombolysis
        — Anticoagulation (short course)
- AVF-related thrombosis
    - Stop bypassing prophylaxis
    - Continue ITI

Fig. 1. Severe haemophilia A: recommended ITI algorithm.

Incomplete/No Response to First-Line ITI Regimens (levels III & IV)

- Continue initial ITI regimen
- Maximize dose
- Switch to VWF-containing FVIII product if ITI was initiated with a monoclonal or recombinant product
- Consider adding rituximab or another immune-modulating drug to the current regimen

ITI Outcome

Success
Pharmacokinetic parameters (level III)
- Negative INH titer
- Normal FVIII recovery (≥ 66% of predicted)
- Normal FVIII half-life (≥ 6 h after a 72-h FVIII washout period)
- Absence of anamnesis upon further FVIII exposure

Failure
Pharmacokinetic parameters (level III)
- Failure to fulfill criteria for full/partial success within 33 months
- <20% reduction in INH titer for any 6-month period during ITI after first 3 months of treatment, which implies that
    - 9 months is minimum period for ITI
    - 33 months is maximum duration of unsuccessful ITI (although decision may be made to continue)

Partial success
Pharmacokinetic parameters (level III)
- INH titer <5 BU
- FVIII recovery < 66% of predicted
- FVIII half-life < 6 h after a 72-h FVIII washout period
- Clinical response to FVIII
- No increase in the INH titer >5 BU over 6 months of on-demand therapy or 12 months of prophylaxis

AVF = arteriovenous fistula; BU = Bethesda units; CVAD = central venous access device; DC = discontinue; IA = immunoadsorption; ICH = intracranial hemorrhage; I-ITI study = International Immune Tolerance Study; INH = inhibitor; ITI = immune tolerance induction; RESIST study = Rescue Immunotolerance Study; rFVIIa = recombinant activated factor VII; VWF = von Willebrand factor.

Fig. 1. Continued.

Table 3. IITR and NAITR data meta-analysis: outcome by Pre-ITI titre [38].

	Pre-ITI titre (BU)				
	<10	10–20	21–50	>50	Total
IITR n (%)					
Success	65 (79)	16 (76)	5 (36)	15 (53)	101
Failure	17 (21)	5 (24)	9 (64)	13 (46)	44
Total (P = 0.002)	82	21	14	28	145
NAITR n (%)					
Success	73 (87)	9 (50)	5 (38)	6 (33)	93
Failure	11 (13)	9 (50)	8 (62)	12 (67)	40
Total (P = 0.001)	84	18	13	18	133

IITR, International Immune Tolerance Registry; NAITR, North American Immune Tolerance Registry.

(e.g. haemarthrosis with risk of target joint development), it may be prudent to start ITI prior to the desired decline in titre to <10 BU. A significant association between outcome and time from inhibitor diagnosis to ITI was observed in the IITR, although this association was only of borderline significance in the NAITR [38]. Nonetheless, success rates for both registries were at their lowest after 5 years (51% for the IITR, 57% for the NAITR). Consequently, if the waiting period for the inhibitor to decline extends beyond 1–2 years, the advantages and disadvantages of beginning ITI at a titre higher than 10 BU should be considered.

Consensus recommendations for when to start ITI (Fig. 1)

1 Postpone the initiation of ITI until the inhibitor titre has dropped to <10 BU, although an even lower inhibitor titre may be more beneficial (level IIb).
   a. The waiting time is usually short, and most children will still be very young at the start of ITI.
   b. Closely monitor inhibitory antibody levels during the waiting period to ensure that ITI is started promptly once the titre falls sufficiently.
   c. Avoid FVIII exposure during the waiting period.
2 Consider starting ITI regardless of the inhibitor titre if (level IV)
   a. The inhibitor titre does not fall below 10 BU within a 1–2 year period of close observation.
   b. A severe life- or limb-threatening bleeding event occurs.

FVIII dose

Dosing regimens

Three primary therapeutic regimens have been developed for inhibitor eradication (Table 4). The high-dose Bonn protocol currently calls for the administration of FVIII at a dosage of 150 IU kg^{-1} twice daily [41]. FEIBA (50 U kg^{-1} b.i.d.) is added in patients at high risk of bleeding and is continued until the inhibitor titre falls below 1 BU. The Bonn protocol is intensive for patients and families and very costly because of high-FVIII consumption.

Table 4. Primary therapeutic regimens used for ITI.

Bonn protocol (high-dose regimen) [41]
  FVIII 150 IU kg^{-1} b.i.d.
  FEIBA 50 U kg^{-1} b.i.d. for patients at high risk of bleeding
  Comments
    Intensive for patients and families
    Very costly due to high FVIII consumption
van Creveld (Dutch) Protocol (low-dose regimen) [45]
  FVIII 25 IU kg^{-1} q.o.d.
  Decrease dose each time absolute FVIII recovery is >30%
  Continue until a prophylactic dose (10–15 IU kg^{-1} q.o.d.) is
    reached
  Comments
    Less demanding for patients and families than the Bonn
      regimen
    More economical
Malmö Protocol (immunomodulation plus high-dose FVIII) [40]
  Immunoadsorption followed by
  Immunosuppression with cyclophosphamide followed by IVIG
    replacement
  FVIII
  Comments
    Requires hospitalization
    May allow quick completion of ITI, resulting in cost savings
    No longer used
Other Protocols
  Gruppo: 50–100 IU kg^{-1} week^{-1} plus IM [49]
  Rocino: 100 IU kg^{-1} day^{-1} [44]
  Smith: 100 IU kg^{-1} b.i.d., then 100 IU kg^{-1} day^{-1} [42]

b.i.d., twice daily; IVIG, intravenous immune globulin; IM, immunomodulation; q.o.d., every other day.

In the low-dose van Creveld (Dutch) regimen, FVIII is administered every other day at a dose of 25 IU kg^{-1} [45]. The dose is decreased each time the absolute FVIII recovery exceeds 30%. These reductions are continued until a prophylactic FVIII dose of 10–15 IU kg^{-1} three times weekly is reached.

The Malmö protocol utilizes extracorporeal immunoadsorption with protein A columns as needed to remove high-titre inhibitory antibodies (>10 BU) [40,46]. This process is followed by immunomodulation using intravenous and oral cyclophosphamide and intravenous immune globulin (IVIG) combined with high-dose FVIII (Bonn regimen). Hospitalization is required, but tolerization has been reported in as little as 3–4 weeks. The Malmö protocol is no longer used in Sweden for ITI because the long-term follow-up showed that responses were not always durable [40,46,47], and that success rates were no higher than for regimens that do not use immunomodulation [40]. Furthermore, immunoadsorption may be difficult in small children [18], and the use of cyclophosphamide is potentially associated with an increased risk of myelosuppression, leukemia and sterility [48]. These side effects of cyclophosphamide are thought to be dose-related, however, and may not occur at the doses used in the Malmö protocol. In addition to these ITI regimens, various intermediate-dose protocols have been developed at and are used by individual HTCs worldwide [42,44,49].

Dosing considerations

Controversy persists regarding the roles of FVIII dose and dosing regimen as predictors of ITI success [19]. While many investigators have reported similar rates of successful tolerization (63–91%) despite the use of widely disparate therapeutic dosing regimens [40,42,44,49–51], the registries generated varied data in this regard [20,38,52–54]. In the IITR, daily FVIII doses of at least 200 IU kg^{-1} resulted in a statistically significant improvement in outcome (86%; $P = 0.001$) [55]. In contrast, the NAITR observed a significant inverse relationship between FVIII dose intensity and overall ITI success. Success rates were 80% for daily doses of <50 IU kg^{-1}, compared with 41% for doses of 200 IU kg^{-1} day^{-1} or higher ($P = 0.01$) [54]. Tolerization was achieved more rapidly, however, when higher doses were used (≥50 IU kg^{-1} day^{-1}) [53,54].

A meta-analysis of the two registries found that FVIII dosing had no impact on ITI success rates for 'good-risk' patients with historical inhibitor titres below 200 BU and pre-ITI titres of <10 BU [38]. There are strong proponents of the exclusive use of high-dose ITI because of longstanding institutional experience [51]. However, consideration of the comparative cost-effectiveness and reduced morbidity associated with lower-dose, non-daily regimens [56] may be crucial to the broader availability of ITI in both the developed and developing world. To this end, the ongoing I-ITI study is evaluating the effect of FVIII dose on the overall ITI success rate and time to success by randomizing 150 good-risk, severe haemophilia A subjects to receive FVIII doses of either 200 IU kg^{-1} daily or 50 IU kg^{-1} three times weekly

for up to 33 months [23]. The need for reliable, unbiased data is crucial, and it is recommended that all ITI patients be enrolled either in a prospective, randomized clinical trial or an international registry [57,58].

In contrast to good-risk patients, dose may be important for 'poor-risk' patients, defined as those with a peak historical inhibitor titre >200 BU and/or a pre-ITI titre higher than 10 BU and/or time since inhibitor diagnosis exceeding 5 years. According to the meta-analysis of the IITR and NAITR data, only high-dose regimens ($\geq$200 IU kg^{-1} day^{-1}) offer the most reasonable chance of ITI success for individuals with poor prognostic parameters [38]. Only one of 23 (4%) poor-risk patients treated with daily doses of <200 IU kg^{-1} were successfully tolerized, whereas higher doses effectively eradicated inhibitors in 12 of 18 patients (67%). ITI-naive patients with poor prognoses will be eligible to participate in the Rescue Immunotolerance (RESIST) Study, a companion trial to the I-ITI study, that is scheduled to start in the near future and will randomize patients to receive high-dose ITI (200 IU kg^{-1} day^{-1}) with either recombinant FVIII (rFVIII) or a plasma-derived von Willebrand factor (VWF)-containing FVIII concentrate [19]. Enrollment of poor-risk patients in prospective, comparative clinical trials and a trial of dose maximization among those who fail low-dose ITI is strongly recommended.

Consensus recommendations for ITI dosing (Fig. 1)

1 Among good-risk patients (i.e. peak historical titre <200 BU, pre-ITI titre <10 BU, <5 years since diagnosis), no dosing regimen has been shown to be superior to another. These patients may be eligible to participate in the I-ITI study (level IIb).
2 Among poor-risk patients (i.e. peak historical titre >200 BU and a pre-ITI titre >10 BU and/or >5 years since inhibitor diagnosis), the evidence suggests a higher success rate with the use of high-dose regimens ($\geq$200 IU day^{-1}). These patients may be eligible to participate in the RESIST Study (level IIb).

FVIII product

The choice of FVIII product to achieve inhibitor eradication is another matter of debate. High ITI success rates (73–91%) have been reported for patients treated with monoclonal (plasma-derived human FVIII produced using immunoaffinity chromatography and a monoclonal antibody to FVIII) and recombinant FVIII concentrates [42,44,59–62],

yet some data suggest that plasma-derived FVIII (pdFVIII) concentrates rich in VWF increase the likelihood of successful tolerization.

A longitudinal study conducted at the HTC in Frankfurt, Germany, showed that success rates using a high-dose ITI protocol had declined from 91% (19/21 patients) to 37.5% (six of 16 patients) with the introduction of high-purity concentrates [63,64]. However, when patients who had an unsatisfactory response to ITI using monoclonal or recombinant FVIII were switched to concentrates containing high amounts of VWF, eight of 10 (80%) experienced complete eradication of their inhibitors. Similarly, a compilation of data on ITI therapy from HTCs in Bonn and Bremen, Germany, showed that before 1990, ITI was successful in 44 of 51 patients (86%) treated exclusively with intermediate-purity FVIII/VWF concentrates [65]. Success rates dropped to 55% between 1990 and mid-1999, when high-purity FVIII products without VWF were primarily used. With the resumed use of FVIII/VWF concentrates in July 1999, success rates increased to 71%. Specifically, 23 of 28 patients (82%) undergoing ITI with FVIII/VWF were successfully tolerized, compared with six of 14 patients (43%) treated with rFVIII.

Other researchers have also observed the enhanced efficacy of VWF-containing concentrates in achieving tolerization. Orsini *et al.* [66] reported that ITI was successful in eight of eight patients undergoing ITI with a high-purity FVIII/VWF product. In addition, prospective surveillance conducted by Gringeri showed that six of 13 patients at high risk for a poor response to ITI, including three patients who had previously failed ITI, were successfully tolerized using a high-purity FVIII/VWF concentrate [67]. The remaining seven patients, who were still undergoing ITI, converted from high- to low-responder status.

One explanation for these favourable findings is that VWF, in addition to being a critical component of haemostasis [68] and playing key roles in FVIII function, production and stabilization [68,69], may also modulate FVIII immunogenicity [70], thereby influencing ITI outcome. Inhibitors react with and bind to active sites on the FVIII molecule, primarily with epitopes in the A2, A3, C1 and C2 domains [71] VWF binds to similar epitopes on the FVIII molecule as inhibitors directed against the C2 domain, which may result in epitope masking [72,73]. Consequently, a small subset of FVIII antibodies may be less inhibitory to FVIII complexed with VWF compared with monoclonal and recombinant FVIII products [74–77].

The potentially beneficial effects of VWF notwithstanding, immune tolerance is a multifactorial process, and variables other than VWF contribute to differences in success rates [73]. Moreover, it is unclear if a switch from monoclonal or recombinant FVIII to VWF-containing concentrates was responsible for successful tolerization or if inhibitor eradication simply resulted from an extended duration of ITI. In other words, the patients may have become immune tolerant had their original treatment regimens been continued longer. Meta-analysis of data from the IITR and NAITR found no association between outcome and treatment product [38]. In the IITR, 96% of patients were treated with intermediate-purity products; in the NAITR, monoclonal and recombinant products were predominantly used. Yet the distribution of success and failure was equivalent: 70% success, 30% failure. Furthermore, no prospective, randomized trial has compared VWF/FVIII with monoclonal or recombinant concentrates. Thus, at the present time, there is no definitive evidence demonstrating the superiority of any FVIII product, although the RESIST trial may provide this data in the future. Most patients are tolerized using the product they were receiving when they developed an inhibitor [15]. This approach is effective, and switching to another FVIII product for *de novo* ITI is not recommended.

Consensus recommendations for FVIII product (Fig. 1)

1 ITI is successful using FVIII products with and without VWF (level IIb).
2 No data support the superiority of any FVIII product (level IIb).
3 Most patients are tolerized with the same FVIII product in use at the time of inhibitor detection. This approach works, and there is no evidence to support switching to another FVIII product for *de novo* ITI (level IIb).

Immunoadsorption

Extracorporeal immunoadsorption, a component of the Malmö ITI protocol when necessary [40] involves selectively removing immunoglobulins and immune complexes using *Staphylococcal* protein A or anti-immunoglobulin columns [78]. The process, which eliminates approximately 70–90% of circulating inhibitory antibodies [79], appears to be more useful for patients with acquired haemophilia than for those with severe congenital haemophilia with inhibitors [80]. Accordingly, there is no role for immunoadsorption as a first-line component of ITI.

Consensus recommendations for immunoadsorption (Fig. 1)

1 There is no role for immunoadsorption as a first-line component of ITI (level IIb).

Prophylaxis during ITI

The results of the randomized controlled US Joint Outcome Study, reported by Manco-Johnson in 2005, established the efficacy of FVIII prophylaxis in preventing joint damage in patients with severe haemophilia A without inhibitors [81]. Other investigators have shown that FVIII prophylaxis indirectly improves academic performance [82] and quality of life [83]. Increasingly, prophylaxis with bypassing therapy is being used for patients with FVIII inhibitors, as they are at increased risk for joint disease and severe haemorrhage [16] owing to the difficulty in controlling bleeding episodes [84].

FEIBA has been a component of the Bonn ITI regimen for 30 years [17], and it is currently used in patients at high risk for bleeding [51] Kreuz *et al.* [85] prospectively evaluated FEIBA prophylaxis in 22 children, aged 0.1–6 years, with high-titre inhibitors (>5 BU) undergoing ITI. FEIBA was administered at a dose of 50 U kg^{-1} daily, with the dosage increased up to 100 U kg^{-1} twice daily for breakthrough bleeding. The median annual incidence of joint bleeding during FEIBA prophylaxis was one (range, 0–6), and no patient suffered a life-threatening haemorrhage. No evidence of arthropathy was seen in six of eight patients evaluated radiographically; and in the remaining two patients, joint pathology was minimal. Valentino prospectively studied three inhibitor patients, aged 3.7–24.1 years, who received concomitant FEIBA prophylaxis at a daily dose of 100 U kg^{-1} during ITI [86]. The incidence of joint bleeding prior to the initiation of prophylactic therapy ranged from 2.5 to 4.08 per 100 days and declined to 0.0–1.42 per 100 days during treatment, a reduction of 65–100%. FEIBA was well tolerated and did not cause thrombosis.

Historically, rFVIIa is less likely to be used for prophylaxis outside of the perioperative setting because of its shorter half-life (approximately 3 h in adults [87] and even more rapid clearance rates in children [88]), compared with FEIBA (4–7 h) [89]. Although future clinical practice may change subsequent to the pilot data presented by Konkle et al. [90] on the efficacy of rFVIIa in inhibitor patients not concomitantly receiving ITI, a current review of the literature identified only one case study describing the successful use of rFVIIa prophylaxis during immune

tolerance. Saxon et al. [91] reported that rFVIIa at a dose of 90 μg kg^{-1} once daily significantly reduced the number of target joint bleeding episodes in a pre-school-aged boy undergoing ITI. The efficacy of rFVIIa in this patient may indicate that rFVIIa may have a longer biologic effect than is indicated by its half-life, according to the authors [91].

Potential drawbacks to the concurrent use of bypassing agent prophylaxis during ITI include the high cost and risk of thrombosis [92]. When bypassing therapy is used outside the ITI setting, thrombotic risk is very low. Whether the same holds true when these products are used concurrently with ITI is unknown.

By avoiding an inflammatory environment in which the tolerogenic delivery of FVIII is not possible, prophylaxis during immune tolerance may prevent major haemorrhage, thereby shortening the duration of ITI and improving the likelihood of success. However, data supporting such a hypothesis are limited. In patients undergoing immune tolerance who experience early joint bleeding or intracranial haemorrhage (ICH), prophylaxis with a bypassing agent may be considered. The dose of FEIBA recommended based on reports of bypassing-agent prophylaxis during ITI [85,86,91] and outside the ITI setting [93,94] is 50–200 U kg^{-1} every day to twice weekly, and the recommended dose of rFVIIa is 90–270 μg kg^{-1} daily [90]. The higher ends of the dosing ranges should be used for prophylaxis following ICH because of the significant risk of recurrent bleeding [95] FVIII recovery should be monitored frequently once the inhibitor titre drops to 10 BU or below (following anamnesis triggered by ITI initiation). In an attempt to prevent the sporadic development of catheter-associated vessel thrombosis that has been reported with the concurrent use of ITI and bypassing agents [96], it is the consensus opinion that prophylaxis with bypassing agents should be discontinued when any level of FVIII recovery is detected.

When ITI is postponed to allow the inhibitor titre to decline to <10 BU, FEIBA is not recommended for the first-line prophylaxis because of its potential to protract anamnesis [18]. The use of rFVIIa is preferred in this setting at a recommended dose of 90–270 μg kg^{-1} daily.

Consensus recommendations for prophylaxis during ITI (Fig. 1)

1 Prophylaxis should be considered if patients continue to bleed frequently while awaiting or on ITI (level III).

2 rFVIIa at a dose of 90–270 μg kg^{-1} daily is preferred for prophylaxis when ITI is delayed to allow the inhibitor titre to decline to <10 BU (level IV).
3 Prophylaxis with FEIBA (50–200 U kg^{-1} daily to twice weekly) or rFVIIa (90–270 μg kg^{-1} day^{-1}) may be considered for patients undergoing ITI who experience early joint bleeding or ICH (level III).
4 Monitor FVIII recovery when the inhibitor titre drops to 10 BU (level IV).
5 Discontinue bypassing therapy at any level of FVIII recovery (level IV).

Central venous access device issues

General recommendations
Uninterrupted and uncomplicated venous access is essential in children undergoing ITI. Peripheral venipuncture is the first choice [97], and some HTCs initially attempt immune tolerance using regimens that involve less frequent FVIII administration, as this may facilitate peripheral venipuncture [18]. Nonetheless, central venous access devices (CVADs) are often necessary for ITI in young children, particularly for high-dose regimens [97].

Two major types of long-term CVADs are available: tunnelled and fully implantable (referred to as *ports*) and tunnelled external catheters [98]. Among haemophilia patients receiving long-term therapeutic interventions, subcutaneously implanted single-reservoir ports are the most commonly used CVADs [99]. In patients without inhibitors, ports are preferable to external devices [97] because they are less likely to become infected by pathogens than are external catheters [100]. The panel supports this recommendation for inhibitor patients as well. Published guidelines [97] for catheter care should be meticulously followed by physicians and nurses. Furthermore, because long-term CVAD use requires considerable commitment from caregivers and patients, comprehensive education should be provided before ITI is initiated and reinforced periodically thereafter.

Complications

Infection
Infection is the major complication associated with CVADs used in haemophilia [99,101,102], and it is the most common cause for their removal [98] CVAD infection occurs more frequently in haemophilia patients with inhibitors [98,101,103], possibly due to subcutaneous bleeding around the port that facilitates entrance of Gram-positive bacteria

through the skin, a situation unique to the pediatric inhibitor population [104]. Infection rates are particularly high among inhibitor patients undergoing ITI, ranging from 50% to 83%, according to a review by van den Berg *et al.* [104]. Increased CVAD access for daily FVIII administration may explain this higher incidence [101]. The development of catheter infections may cause interruptions in ITI that prolong tolerization or lead to treatment failure by provoking an anamnestic increase in the inhibitor titre [15,18].

Preliminary data from the I-ITI study confirm that CVAD infection occurs frequently during immune tolerance [23,105]. Thirteen of 36 subjects (36%) enrolled in the trial as of January 2006 had developed at least one catheter infection. Furthermore, ITI success rates were observed to be lower, failure rates higher, and response to treatment slower in these individuals than in subjects without infection. Specifically, five subjects (38%) with infection achieved a negative inhibitor titre after a median of 14 months on ITI, compared with 17/22 uninfected patients (77%) who achieved the same status after a median of 7.5 months. Another five (38%) of the 13 subjects with a history of CVAD infection have thus far met the criteria for ITI failure. Published guidelines on CVAD care [98] provide clinical strategies for the prevention of catheter-related infection as well as catheter management in the presence of a single or recurrent infection.

Thrombosis
Thrombotic events associated with CVADs may result in catheter occlusion or catheter-related thrombosis [98], and the risk of such events increases with the duration of catheter placement [106]. A meta-analysis by Valentino *et al.* [98] of 48 studies involving a total of 2704 patients and 2973 CVADs found a pooled incidence of thrombosis of 0.056 per 1000 catheter days. The presence of an inhibitor did not significantly affect thrombosis incidence, in contrast to infection rate.

When a CVAD-related deep vein thrombosis (DVT) is detected, the panel recommends discontinuing bypassing prophylaxis (if used) and reviewing infusion techniques with the family. Consideration should be given to removing the catheter and transitioning to peripheral venipuncture and also to modifying the ITI regimen. Clot resolution may occur spontaneously after catheter removal or even when the catheter is left in place [98]. Patients should be monitored closely, and if a severe clot fails to resolve or if it progresses, catheter-directed thrombolysis or a short course of anticoagulation should be considered.

Mechanical dysfunction
Mechanical problems may also occur with ports [98], although this complication is less common than infection or thrombosis [101]. Mechanical dysfunction may lead to catheter removal [101], and all such events should be reported to the appropriate governmental regulating agency.

Arteriovenous fistulae
An infectious or thrombotic complication may limit the long-term use of a CVAD [107]. Arteriovenous fistulae (AVF), considered the vascular access of choice for haemodialysis patients because of their durability and low complication rate [108], are a protocol-driven option for venous access in children aged 1 year and older who have experienced repeated CVAD failure. Santagostino *et al.* [107] prospectively studied the long-term safety and feasibility of AVF in 27 children with severe haemophilia A, including 14 with inhibitors. The AVF were regularly and successfully used at home by 26 patients (96%) for a median of 29 months. Venous thrombosis occurred in one patient after 9 months, but symptoms spontaneously disappeared, and the AVF was used for an additional 9 months.

Adapting AVF to the requirements of children with haemophilia requires surgical expertise, and long-term follow-up with ultrasonography and echocardiography is necessary. All AVF should be dismantled as soon as peripheral veins provide adequate vascular access. Although ITI can continue if an AVF-related thrombosis occurs, prophylactic bypassing therapy should be stopped.

Consensus recommendations for CVAD issues (Fig. 1)

1 Use peripheral venous access whenever possible (level IV).
2 Ports are preferable to external catheters because of the significantly lower risk of infection (level IV).
3 Follow published guidelines for catheter care and infection prevention (level IV).
4 Instruct families on CVAD techniques before starting ITI (level IV).
5 If an infection develops, follow published guidelines on CVAD management (level IV).
6 If a CVAD-related DVT occurs, discontinue bypassing prophylaxis (if used) and review infusion techniques with family (level IV) in addition to the following:
   a. Consider removing the catheter and transitioning to peripheral venipuncture (level IV).
   b. Consider modifying the ITI regimen and future bypass therapy regimens (level IV).

7 Monitor closely for clot resolution (level IV).

8 If a severe clot fails to resolve or progresses, consider (level IV)
    a. Catheter-directed thrombolysis
    b. A short course of anticoagulation

9 An AVF is a protocol-driven option for venous access in children at least 1 year of age who have experienced repeated CVAD failure (level IV).
    a. Surgical expertise is essential in adapting AVF to the requirements of children with haemophilia (level IV)
    b. Long-term follow-up of AVF with ultrasonography and echocardiography is necessary (level IV)
    c. Dismantle an AVF as soon as peripheral veins provide adequate vascular access (level IV).
    d. ITI can continue if an AVF-related thrombosis develops, but stop prophylactic bypassing therapy (level IV).

Incomplete or lack of response to ITI

The time to ITI success is extremely variable, ranging from a few months to two or more years [18]. In the NAITR, while successful outcomes were achieved more quickly in patients who received FVIII doses of at least 50 IU kg^{-1} daily, the majority of patients treated with lower doses were successfully tolerized, albeit more slowly [26]. Thus, one strategy for patients who demonstrate no response or an incomplete response to ITI is to give the current treatment regimen additional time to produce results, particularly if a low-dose regimen is being used for reasons of preferential or default use of peripheral access. In the IITR, daily doses of at least 200 IU kg^{-1} were associated with statistically significantly higher success rates [55]. Although these data cannot be extrapolated to second-line or salvage ITI strategies, an alternative approach used in clinical practice is to maximize the ITI dose.

A switch to an intermediate-purity VWF-containing FVIII product may be an option for patients whose response to immune tolerance using monoclonal or recombinant FVIII is suboptimal. Given the small repository of published evidence suggesting that VWF-containing concentrates improve the likelihood of successful tolerization [64–67], coupled with the predominant use of intermediate-purity products in the IITR to achieve the reported outcome with the first-line ITI [22], consideration may be given to changing to a FVIII/VWF product.

The addition of rituximab to the ITI regimen may improve response rates in patients who remain difficult to tolerize [109,110]. Rituximab is a monoclonal antibody against CD20-positive B cells that has shown promise in the treatment of acquired haemophilia (autoantibodies) [111,112]. However, published experience with this agent in the treatment of alloantibodies associated with congenital haemophilia is limited to approximately two dozen patients [109,113–123]. Among these patients treated with rituximab at a dose of 375 mg m^{-2} given once weekly for 4 weeks, 16 (73%) achieved an undetectable inhibitor based on the Bethesda assay. After 90 days–2 years of follow-up, 55% remained in remission. Interpretation of these data is hampered by the variable regimens used, some of which included steroids, vincristine, or cyclosporine [116,117], and the likelihood of preferential reporting of positive outcomes. Nonetheless, the 22 patients compose a group with poor prognostic indicators, and a 55% response rate is encouraging. Treatment of patients using a standardized protocol and the reporting of consecutive patients is a necessary first step to collecting interpretable data.

Consensus recommendations for managing incomplete or lack of response to ITI (Fig. 1)

1 Continue the initial ITI regimen, particularly if a low-dose regimen is being used for reasons of preferential or default use of peripheral access (level IV).

2 Maximize the ITI dose if a lower dose regimen is being used and adequate venous access exists (level IV).

3 Consider switching to a FVIII/VWF product if ITI was initiated with a monoclonal or recombinant product (level III).

4 Consider adding rituximab or another immune-modulating drug to the current regimen (level III).

ITI outcome definitions

Success

Pharmacokinetic parameters
The pharmacokinetic parameters currently used to define ITI success in patients with severe and mild haemophilia A were established by consensus at the Second International Conference on Immune Tolerance held in Bonn, Germany, in 1997. These parameters include an undetectable inhibitor level (<0.6 BU), FVIII plasma recovery ≥66% of predicted, FVIII half-life ≥6 h after a 72-h FVIII washout period, and the absence of anamnesis upon further FVIII exposure [57]. This definition has been adopted by the I-ITI study [23], which defined partial

success as a reduction of the inhibitor titre to <5 BU; FVIII recovery of less than 66% of predicted; and FVIII half-life of <6 h after a 72-h FVIII washout period associated with clinical response to FVIII therapy and no increase in the inhibitor titre exceeding 5 BU over a 6-month period of on-demand treatment or 12 months of prophylaxis [124].

Potential alternative definitions of ITI success based on measurable parameters of immunologic tolerance and/or the emerging biochemistry of FVIII clearance were explored by the panel. However, none were thought to be sufficiently definitive at this time to supplant or complement the current definition.

Cost-effectiveness as a defining parameter of ITI success
ITI success may also be defined on the basis of its cost-effectiveness. The majority of direct costs associated with the care of haemophilia patients are attributable to clotting factor replacement therapy [125]. These costs are greater in patients with high-titre inhibitors because they require bypassing therapy, which is more expensive. Costs may further increase as a result of the outlier effect caused by a small percentage of inhibitor patients who require a disproportionate amount of treatment.

An analysis of 314 patients enrolled in the IITR found that 70% of patients with good prognostic indicators (i.e. pre-ITI titre <10 BU, <5 years since inhibitor diagnosis, high-dose ITI regimen) achieved tolerance within 1 year, whereas those with poor prognostic indicators required 2.5 years of treatment to achieve comparable results [126]. The total cost of ITI in a patient with good prognostic indicators was estimated to range from US $900 000 to $1.8 million, depending on whether plasma-derived or recombinant product was used. This cost increased to an estimated US $6.3 million–12.6 million in a patient with poor prognostic indicators.

Colowick *et al.* [127] modelled the cost-effectiveness of ITI as a long-term strategy for inhibitor management. Their Markov model showed that while the cost of immune tolerance in a 5-year-old child approached $1 million, successful ITI would result in a lifetime reduction in the need for replacement clotting factor (including bypassing therapy) of $1.7 million. Moreover, successful tolerization increased life expectancy by 4.6 years.

An alternative strategy to the Markov decision model developed by Colowick is to compare ITI intervention costs and outcomes, whether or not successful, with those of patients with lifelong high-titre inhibitors. Such a comparison would require meticulously gathered cost and quality of life (QOL)

data from representative cohorts of inhibitor patients. Recently completed studies that have captured some of this information [128–131] may make this feasible in the future. Cost-effectiveness as a measure of ITI success is also being examined by the I-ITI study. At the current time, however, cost-effectiveness parameters could not be incorporated into a definition of ITI success.

Failure

Pharmacokinetic parameters
The pharmacokinetic definition of ITI failure currently adopted by the I-ITI study was also established by consensus at the Second International Conference on Immune Tolerance. It is defined as failure to achieve a full or partial success within 33 months or the failure to achieve an ongoing reduction in the inhibitor titre of at least 20% during each interim, non-overlapping, 6-month period after the first 3 months of ITI (in the absence of documented infection) [124]. This definition implies that 9 months is the minimum treatment period and 33 months is the maximum reasonable duration of unsuccessful ITI.

In clinical practice, ITI is sometimes continued beyond 33 months, and a resistant minority of patients may take 3 years or more to achieve tolerance [132]. Clinical improvement, including fewer bleeding episodes and improved QOL, may favour continuing ITI [128]. However, economic considerations often determine how long ITI is pursued [20].

Potential alternative definitions of ITI failure based on measurable parameters of immunologic tolerance and/or the emerging biochemistry of FVIII clearance were discussed. However, none were thought to be sufficiently definitive at this time to supplant or complement the current definition.

Consensus recommendations for defining ITI outcome (Fig. 1)

1 Pharmacokinetic parameters of success: (level III)
    a. A normal inhibitor titre
    b. Normal FVIII recovery (≥66% of predicted)
    c. Normal FVIII half-life (≥6 h) after a 72-h FVIII washout period
    d. Absence of anamnesis upon further FVIII exposure
2 Pharmacokinetic parameters of partial success: (level III)
    a. An inhibitor titre <5 BU
    b. FVIII recovery <66% of predicted

    c. FVIII half-life <6 h after a 72-h FVIII wash-out period

    d. Clinical response to FVIII

    e. No increase in the inhibitor titre >5 BU over a 6-month period of on-demand therapy or a 12-month period of prophylaxis

3 Pharmacokinetic parameters of failure: (level III)

    a. Failure to fulfil criteria for full or partial success within 33 months

    b. Less than a 20% reduction in the inhibitor titre for any 6-month period during ITI after the first 3 months of treatment, which implies that

        i. 9 months is the minimum period for ITI;

        ii. 33 months is the maximum duration of ITI, although the decision may be made to continue immune tolerance

Immune tolerance induction in patients with mild haemophilia A

Compared with alloantibodies that develop in severe FVIII deficiency, inhibitors associated with mild FVIII deficiency tend to arise later in life, reflecting the infrequency of bleeding episodes in this patient population and the resultant decreased need for treatment [133].

Inhibitors in mild haemophilia A are often associated with a familial predisposition and the presence of high-risk mutations that induce a conformational change in the FVIII molecule resulting in a functional FVIII defect [133]. In a cohort of mild haemophilia A patients with inhibitors described by Hay et al. [134], 41% of treated family members also had a history of FVIII inhibitors. High-risk mutations in the FVIII gene and intensive replacement therapy, such as for surgery, trauma, or muscle bleeding, are other risk factors for inhibitor development in mild haemophilia A. An inhibitor titre and baseline FVIII level should be obtained for all patients with mild haemophilia A and a family history of inhibitors or when genotyping reveals the presence of high-risk mutations. Screening should be repeated at each clinic visit if the patient has received FVIII in the interim and should be performed both preoperatively and postoperatively. When patients with mild haemophilia A undergo surgery, FVIII replacement therapy should be closely monitored, as it is common for inhibitors to present with postoperative haemostatic failure [133].

Bleeding manifestations associated with mild haemophilia A and inhibitors are similar to those observed with acquired antibodies (alloantibodies) [135,136]. Patients may develop severe and persistent bleeding, often in soft tissue and the gastrointestinal and genitourinary tracts, that is relatively or completely refractory to FVIII replacement therapy and may be life-threatening [133]. Desmopressin acetate (DDAVP) and other therapies that avoid FVIII exposure (e.g. bypassing agents) should be used for the management of bleeding episodes in patients with mild haemophilia A and inhibitors in circumstances where the response to testing is adequate. Both low-titre and high-titre alloantibodies may disappear spontaneously but may recur when the patient is re-exposed to FVIII [133].

Inhibitors in patients with mild haemophilia A also resemble autoantibodies in their poor response to traditional ITI [136]. The Malmö regimen may be more effective than other protocols in completely eradicating inhibitors associated with mild haemophilia A, according to published reports, but the number of patients reported on is too small to reach any firm conclusions [133]. If immune tolerance is being considered, it is advisable to first rechallenge the patient with FVIII, keeping in mind that a lack of anamnesis does not mean the inhibitor will not return upon subsequent rechallenge [133,135]. No recommendations can be made for ITI, but if treatment is initiated, the data should be reported to national and international registries. A retrospective collection of data is currently being performed in France and Belgium, in an effort to increase knowledge and improve the understanding of mild haemophilia A complicated by an inhibitor [135]. Preliminary results from this group of patients, who have a significantly lower median age than that reported by Hay [133], suggest a higher response rate to ITI [135]. Nonetheless, these success rates are still lower than those reported for severe haemophilia A.

Consensus recommendations for ITI in patients with mild haemophilia A (Fig. 2):

1 There can be no recommendations for ITI in mild haemophilia A.

2 If ITI is initiated, report data to national and international registries.

Immune tolerance induction in patients with haemophilia B

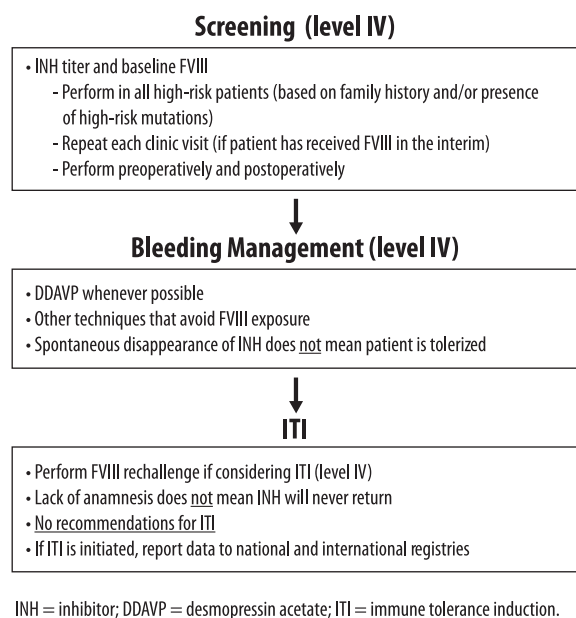Approximately 3% of patients with haemophilia B develop inhibitors [3], and more than 80% of these

Screening (level IV)

- INH titer and baseline FVIII
  - Perform in all high-risk patients (based on family history and/or presence of high-risk mutations)
  - Repeat each clinic visit (if patient has received FVIII in the interim)
  - Perform preoperatively and postoperatively

↓

Bleeding Management (level IV)

- DDAVP whenever possible
- Other techniques that avoid FVIII exposure
- Spontaneous disappearance of INH does <u>not</u> mean patient is tolerized

↓

ITI

- Perform FVIII rechallenge if considering ITI (level IV)
- Lack of anamnesis does <u>not</u> mean INH will never return
- <u>No recommendations for ITI</u>
- If ITI is initiated, report data to national and international registries

INH = inhibitor; DDAVP = desmopressin acetate; ITI = immune tolerance induction.

Fig. 2. Mild haemophilia A: recommended management algorithm.

alloantibodies are high-responding [137]. The development of inhibitors in patients with haemophilia B is generally associated with the absence of FIX antigen caused by large or complete deletions or major derangements of the FIX gene [138]. These mutations are also linked to severe allergic anaphylactoid or life-threatening anaphylactic reactions to FIX concurrent with the appearance of an inhibitor [139]. This is a well-recognized phenomenon that occurs almost exclusively in conjunction with FIX inhibitor development.

The aetiology of allergic reactions remains unclear. The small molecular mass of the FIX protein (55 000 kDa) accounts for its extracellular distribution, and this feature of FIX may contribute in some way to hypersensitivity reactions in haemophilia B patients [139]. A second theory ties anaphylactic reactions to excessive immune complex formation resulting from a large infused load of exogenous FIX protein with each treatment. The standard dose of FIX is 40–80 IU kg^{-1}, which exposes patients to 200–400 μg of exogenous protein. In contrast, standard dosing for FVIII deficient patients is 25–50 IU kg^{-1}, which exposes them to just 2.5–5.0 μg of factor [138,139]. Finally, molecular genetic characterization of FIX inhibitors reported to the registry showed that FIX inhibitor patients whose haemophilia B resulted from complete gene deletions had a 26% risk for anaphylactic reactions [140]. It has been postulated that since FIX gene deletions are sometimes extremely large [141], codeletion of

immune response modifier genes could trigger allergic responses in otherwise susceptible patients [139].

To date, the immunology of both FIX inhibitor development and its associated allergic phenomena have been difficult to study in a definitive way. However, FIX genotyping may identify children at greatest risk for inhibitor development and anaphylaxis [138] and should be performed for every patient with severe haemophilia B at the time of initial presentation [58].

Allergic-type reactions in patients with inhibitors preclude the use of FIX-containing products unless patients are thoroughly desensitized. Consideration may be given to desensitization to improve the management of bleeding episodes, but the response is inconsistent. Moreover, even after patients become desensitized, most continue to have recurrent allergic reactions to FIX that require premedication with antihistamines and steroids [138].

The success of ITI in eradicating FIX inhibitors is low, particularly in patients with the allergic phenotype [22]. Among 16 haemophilia B subjects in the NAITR who completed ITI, five had a successful outcome using FIX therapeutic dosing regimens that ranged from 43 to 200 IU kg^{-1} daily [22]. This group included one patient with an inhibitor-associated allergic phenotype and three whose inhibitors were not allergic in nature. Adverse events were reported during 11 of 17 (65%) ITI courses administered to individuals with FIX inhibitors, a frequency 10 times higher than that for persons with FVIII inhibitors. The 11 courses were complicated by 14 reactions to treatment. Allergic reactions accounted for 11 of 14 adverse events and occurred in the 10 registry subjects already known to exhibit allergic reactions to FIX replacement therapy subsequent to the development of inhibitors. Of those 11, three were severe reactions and the cause of premature ITI cessation. Overall, allergic reactions represented the major reason for failure in at least four of 11 unsuccessful courses of ITI in haemophilia B.

The International Society on Thrombosis and Haemostasis (ISTH) Registry on FIX Inhibitors data reveal that ITI was successful in only five of the 34 patients (15%) in whom it was attempted, and two of those patients had low-responding inhibitors [137]. Moreover, 13 of the 34 patients (38%) developed nephrotic syndrome during ITI [137]. An association between allergic reactions to FIX in inhibitor patients undergoing ITI and the development of nephrotic syndrome was also noted in the NAITR cohort [22]. In all, three of 10 such subjects developed nephrotic syndrome while undergoing immune tolerance, accounting for three of 14 reported

adverse events. This complication is primarily associated with a history of allergic phenotype inhibitors [142,143] and has been observed with all FIX products [143]. Although there have been reports of return to normal renal function with cessation of ITI alone, corticosteroid therapy has been required by some patients with variable success [142]. Nephrotic syndrome has not been reported in patients undergoing ITI for FVIII inhibitors, which may reflect the lower amounts of FVIII in plasma, compared with FIX (approximately 1:50 on a mg mL^{-1} basis) [137,142].

An alternative immunosuppressive strategy for the treatment of FIX inhibitors described by German researchers has been successful in a single patient [144]. A 5-year-old boy with severe haemophilia B underwent ITI using a regimen that included mycophenolate-mofetil, dexamethasone and intravenous immunoglobulin together with high-dose FIX. After 8 weeks, his inhibitor had disappeared, FIX recovery and half-life had normalized, and he did not develop nephrotic syndrome.

Desensitization may be needed prior to ITI initiation, but this process does not transform risk from poor to good, nor does it preclude the development of nephrotic syndrome. The decision to attempt ITI for FIX inhibitors must be very carefully weighed against the relatively high-risk of adverse reactions and the relatively low likelihood of success, particularly in those patients whose inhibitor is associated with an allergic phenotype [58]. Consequently, if immune tolerance is initiated, particularly in that subset of patients, physicians are urged to proceed with great caution and to perform routine urinalyses during ITI to detect proteinuria and early nephrotic syndrome [138].

Consensus recommendations for ITI in patients with haemophilia B (Fig. 3):

1 There can be no recommendations for ITI in haemophilia B.
2 If ITI is initiated, proceed with caution and perform routine urinalyses during treatment to evaluate for nephrotic syndrome (level IV).
3 Parameters for ITI success and failure established by consensus for FVIII inhibitors cannot be applied to define success in haemophilia B.

Research priorities

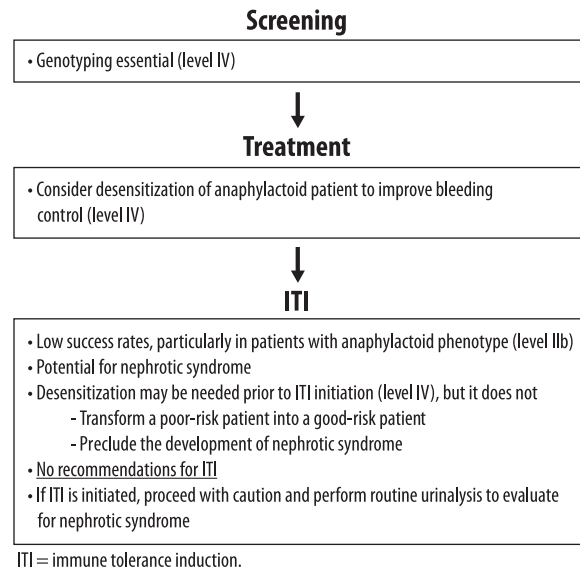Immune tolerance is a costly process that is unsuccessful in approximately 20–40% of patients with

Screening

- Genotyping essential (level IV)

↓

Treatment

- Consider desensitization of anaphylactoid patient to improve bleeding control (level IV)

↓

ITI

- Low success rates, particularly in patients with anaphylactoid phenotype (level IIb)
- Potential for nephrotic syndrome
- Desensitization may be needed prior to ITI initiation (level IV), but it does not
  - Transform a poor-risk patient into a good-risk patient
  - Preclude the development of nephrotic syndrome
- No recommendations for ITI
- If ITI is initiated, proceed with caution and perform routine urinalysis to evaluate for nephrotic syndrome

ITI = immune tolerance induction.

Fig. 3. Haemophilia B: recommended management algorithm.

severe haemophilia A [136] and a far greater percentage of those with mild haemophilia A and haemophilia B [137]. Moreover, many patients are not candidates for ITI because of the presence of long-standing inhibitors, the expense of treatment, unfavourable risk-benefit ratios, or patient/family issues. Thus, inhibitory antibodies are persistent in a substantial number of individuals, severely jeopardizing their health and the quality of their lives. To design optimal and cost-effective therapies that prevent and treat inhibitors requires deepened understanding of the immunology of inhibitor formation and of the mechanisms that promote tolerance. The following high-priority research areas were identified by the International Consensus Panel:

Immunologic aspects of inhibitor development
Antibody formation against FVIII is a CD4-positive (CD4$^+$) T cell-dependent process that requires T-cell interaction with antigen-presenting cells together with T-cell and B-cell interaction [145]. The failure to activate regulatory CD4+ cells may play a crucial role in the development of FVIII inhibitors and is an area that requires ongoing investigation [146].

Immunology of FVIII tolerance
Tolerance is an active process that can be both induced and antigen-specific [147]. Although the basic mechanisms of the immune response to FVIII in the setting of factor replacement therapy are being elucidated, further study is needed to clarify the relevance of these mechanisms in the context of successful ITI and, ultimately, gene therapy [146].

Haemophilia and inflammatory cytokine mediator genotypes

Haemophilia genotype and the genotype for host immune responses influence the risk of inhibitor development [29,36]. Whether these genotypes also influence the response to ITI is unclear and is a focus of ongoing investigations.

Role of FVIII dose in ITI success

This parameter is under intensive study and, it is hoped, will be clarified through the I-ITI study.

Role of FVIII purity in ITI success

In vitro experiments showed that VWF prevents the interaction of FVIII with endocytic receptors that degrade and eliminate FVIII from the circulation [148]. In these studies, increasing the molar ratio of VWF to FVIII above the physiologic ratio of 50:1 decreased the endocytosis of FVIII by dendritic cells. Increasing plasma concentrations of VWF may decrease the amount of FVIII presented to T lymphocytes, thereby reducing T-cell activation. Findings from the RESIST trial and an ongoing German observational study by Kreuz and colleagues may determine if VWF/FVIII products improve ITI success rates or if VWF is simply a surrogate marker for other beneficial components present in pdFVIII. Other questions related to the role of VWF/FVIII interaction that may be explored:

1 The role of endogenous VWF levels on ITI success
2 Correlation of ABO blood grouping to ITI success
3 Differences in immunologic processing of VWF-bound FVIII and unbound ('free') FVIII.
4 Differences in efficacy between high-purity pdFVIII and VWF-containing pdFVIII.

Role of infection, catheter infection and catheter thrombosis on ITI success

Both infection and thrombosis are common complications in patients with CVADs [97,99,101,102]. Their true impact on ITI success may be clarified in the I-ITI study.

Alternative strategies for treating bleeding episodes and inducing tolerance

Preclinical studies suggest that innovative strategies, such as bypassing inhibitors with human/porcine FVIII hybrid concentrates, blocking or neutralizing inhibitors using peptide decoys or anti-idiotypic antibodies, administering FVIII to the nasal or gut mucosa, and gene transfer technology, may enhance the management of bleeding events in inhibitor patients or improve the likelihood of successful tolerization. Clinical trials are needed to determine if the results obtained in the laboratory and in animals translate to success in patients.

Immunologic definition of ITI success/failure

Despite the ubiquitous use of pharmacokinetic parameters to define ITI success or failure, these parameters vary considerably among patients, and no firm evidence supports the defining values [57]. Furthermore, these parameters may be insufficient, given that approximately 4.3% of patients relapse after 'successful' ITI, according to registry data [55], an observation also noted in the ongoing IITI study. An immunologic definition of success may provide more useful information on when to start or stop ITI; how to tailor immune tolerance to the individual patient; how to maintain tolerance after ITI; and, in general, how to better manage haemophilia A and B.

Immunology of inhibitor development and tolerance in mild haemophilia A

It is unclear whether patients with mild haemophilia A who undergo 'successful' ITI achieve true tolerance, or if they remain at risk for anamnesis upon reexposure to FVIII. An immunologic definition may be essential to determine the success or failure of immune tolerance in this subset of inhibitor patients. The findings from the French-Belgium registry will be of critical importance in identifying useful inhibitor prevention and management strategies.

Alternative strategies for ITI in haemophilia B patients with inhibitors associated with anaphylaxis

ITI is unlikely to be successful in haemophilia B patients with the anaphylactoid phenotype [138,149]. Improving upon these success rates requires an enhanced understanding of the immunology of haemophilia B-related anaphylaxis.

Conflict of interest statements

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