# Developments in modern hemophilia care

Hassan, S.

# Chapter 4

**Factor VIII products and inhibitor development in previously treated patients with severe or moderately severe haemophilia A: a systematic review**

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## Summary

### Background

Patients with severe haemophilia A who have been treated extensively with factor VIII (FVIII) products face a low but potentially serious risk of inhibitor development. It is unknown why these patients break immunological tolerance and data on product-related immunogenicity is scarce.

### Aims

To summarize the currently available evidence on the relationship between inhibitor development and recombinant FVIII product type in previously treated patients with severe haemophilia A.

### Methods

Longitudinal studies were included that reported on de novo inhibitor formation in patients with baseline FVIII activity levels less than 0.02 IU/ml who had been treated with FVIII for at least 50 days. Pooled incidence rates of inhibitor development according to product types were calculated using a random intercept Poisson regression model.

### Results

Forty-one independent cohorts were included, 39 patients developed de novo inhibitors during 19,157 person-years of observation. The overall incidence rate was 2.06 per 1000 person-years (p-y) with a 95% confidence interval (CI95) of 1.06-4.01. According to product type, the pooled incidence rate was 0.99 (CI95: 0.37-2.70) per 1000 p-y for patients treated with Advate, 5.86 (CI95: 0.25-134.92) per 1000 p-y for those treated with Kogenate/Helixate, 1.35 (CI95: 0.66-2.77) per 1000 p-y for Kogenate FS/Helixate NexGen, 12.05 (CI95: 1.53-94.78) per 1000 p-y for Refacto and 4.64 (CI95: 0.82-26.43) per 1000 p-y for Refacto AF.

### Conclusion

These results suggest that some products may be associated with increased immunogenicity. However, the low incidence of inhibitors in PTPs and the differences in study design may cause significant variation in estimates of risk.

## Introduction

The development of factor VIII (FVIII)-specific neutralizing antibodies (inhibitors) remains the most important treatment complication in patients with congenital haemophilia A. Inhibitor development is associated with increased morbidity and mortality[1-3] and occurs primarily during the first 50 days of treatment with FVIII[4,5] after a median of 14.5 days of exposure to FVIII (IQR: 9.75-20.0)[6]. Patients who have been treated with FVIII for more than 50 days, also termed previously treated patients (PTPs), are relatively tolerant to FVIII and inhibitor development is rare[7], with a reported rate of 2.14 per 1000 person-years[8]. It has been suggested that inhibitor incidence follows a bimodal distribution and that at older age the risk of developing inhibitors increases again[9].

Knowledge about immunogenicity of recombinant FVIII (rFVIII) products in PTPs is scarce, which is largely due to the rarity of inhibitor development during this phase of replacement therapy. In addition, findings on a differential inhibitor rate among rFVIII products in PTPs might seem conflicting[7, 10]. The observed differences in immunogenicity between rFVIII products may be explained by product characteristics such as the specific amino acid sequence, culture conditions, stabilizing agents and/or post-translational modifications.[11]

Two previous meta-analyses have assessed product-related immunogenicity in previously treated haemophilia A patients.[7, 10] Several new studies have been published since the latest review (published in 2013), which is one of the reasons to perform a new meta-analysis. Moreover, a new meta-analysis is needed with methods that can appropriately handle rare event situations and differences in follow-up time among included studies.

The objective of this systematic review and meta-analysis was to quantify and compare the current knowledge on incidences of inhibitor formation according to rFVIII product type among PTPs affected with severe or moderately severe haemophilia A.

## Methods

A systematic literature review was performed to identify studies that assessed de novo inhibitor development in PTPs with severe or moderately severe haemophilia A who were treated exclusively with one brand of rFVIII. The Meta-analysis of Observational Studies in Epidemiology (MOOSE)[12] and Strengthening of Reporting of Observational Studies in Epidemiology (STROBE)[13] guidelines were followed.

**Inclusion/exclusion criteria**

*Types of studies*
All longitudinal studies that assessed de novo inhibitor development and that reported total, mean or median follow-up time in person-years were eligible. Original articles, letters published in peer-reviewed journals and meeting abstracts were eligible for inclusion. There was no restriction on date of publication or language. We excluded case-control studies, case-series, cross-sectional studies, studies with a follow-up time of less than 3 months, studies with fewer than 10 patients, studies in which treatment for surgery was the main goal, pharmacokinetic studies and studies with duplicate data. Authors of studies in which inhibitor incidences were not reported separately for PTPs were asked to provide these data. In case these data were not provided, these studies were excluded.

*Type of patients*
All patients with severe moderately severe haemophilia A (baseline FVIII activity ‹ 0.02 IU/ml) with at least 50 days of prior exposure to FVIII, were eligible. Furthermore, only patients that were exclusively treated with one brand of rFVIII during the observation period were eligible. Studies that also included patients with fewer than 50 days of exposure to FVIII were only included when separate results were available for the subset of patients with more than 50 days of exposure to FVIII.

*Types of rFVIII products*
rFVIII product type (analysed according to brand) was the determinant in the primary analysis. The following brands were included; Advate (Shire), Kogenate (Bayer), Kogenate FS/Bayer (Bayer), Helixate (Bayer), Helixate FS/NexGen (CSL Behring), Refacto (Wyeth), Refacto AF (Pfizer). Also included were GreenGene F (Green Cross), Kovaltry/Iblias (Bayer), NovoEight (Novo Nordisk), Nuwiq (Octapharma) and Recombinate (Baxter). Kogenate and Helixate users were grouped into one category. Similarly, Kogenate FS/Bayer and Helixate FS/NexGen users were grouped together.

For the secondary analyses, rFVIII products were also categorised according to length (full-length vs B-domain deleted) and the cell line used for production (Chinese hamster ovary cells, baby hamster kidney cells or human embryonic kidney cells). Lastly, rFVIII products were also categorised according to generation; first-generation products (human/animal proteins in production and final formulation), second-generation products (human/animal proteins in production but not in final formulation), third-generation products (no human/animal proteins used in production or final

formulation) and fourth-generation products (no human/animal proteins used in production or final formulation and human embryonic kidney cells used as cell line). Studies performed with extended half-life rFVIII products were excluded, mainly since there were not enough studies done with these products.

*Type of endpoints*

The primary endpoint was de novo inhibitor development defined as the first occurrence of an inhibitor according to the cut-off used by the investigators of the original studies. The secondary outcome was high titre de novo inhibitor formation, defined as a peak inhibitor titre of at least 5 Bethesda Units (BU)/mL.

**Search strategy**

We searched the following databases; PubMed, Embase, Web of Science, Cochrane database and CINAHL. The search strategy was designed and supervised by an experienced librarian (J.W. Schoones, MA, Walaeus Library, Leiden University Medical Center). The initial search was performed in February 2016. Additional studies were included by monthly searches in PubMed up to November 2017. (search terms are reported in supplemental figure S1)

**Study selection and data extraction**

Two reviewers (S. Hassan and A. Cannavò) independently scanned all titles and abstracts to select articles for further scrutiny. Full text versions of each selected article were reviewed to assess eligibility. Inclusion of an article was determined by consensus between the two reviewers. Consultation of a third reviewer (J.G. van der Bom) was carried out in case of disagreement. To avoid multiple counting of patients included in more than one study, recruitment periods and catchment areas were recorded and, if needed, authors were contacted for clarification. Data were extracted independently by two investigators (S. Hassan and A. Cannavò). A structured electronic data extraction form was used. When the required data were missing, the original investigator(s) were contacted for further information.

**Quality assessment**

The methodological quality of each article was assessed using the Downs and Black checklist[14]. For the non-comparative studies in our systematic review, only items relevant to this study design were scored (18 of the 27 items from the original checklist[14]). The modified Downs and Black checklist contained 8 items about reporting accuracy, 3 items about external validity, 6 items concerning internal validity and 1 item about study power. Eight items that were only applicable to comparative studies

(i.e. all items about randomisation, blinding, concealment of treatment allocation and confounding) and one item about the use of p-values were removed. The wording of some questions was modified to provide clearer scoring criteria to improve consistency among raters. (supplemental table S2) Each item could be scored as "no" or "unknown" which yielded 0 points or "yes" which yielded 1 point. The overall score was derived by adding up each item score, each study could score between 0-18 points. Two reviewers (A. Cannavò and S. Hassan) evaluated each article independently and a third reviewer (J.G. van der Bom) was consulted in case of any discrepancy.

**Data analysis**

*Statistical analysis*

The total inhibitor incidence rate and high titre inhibitor incidence rate in PTPs was estimated for each study as the number of de novo inhibitors divided by the number of person-years on a given rFVIII product. Conventional random effects meta-analysis methods (such as the DerSimonian-Laird random-effects method) are biased when the outcome of interest is rare, also when continuity corrections are applied[15]. Therefore, we pooled the incidence rates of the individual studies and calculated the pooled incidence rate ratio (IRR) of inhibitor development according to product type using a random intercept Poisson regression model[16]. Heterogeneity was explored by estimating the between-study variance ($^2$) as well as visually assessing the extent to which the confidence intervals of the individual studies overlapped. As the most frequently used product, we used Advate as the reference category in the analysis according to product type.

*Sensitivity analysis*

To verify whether the results were robust to changes in methodology two sensitivity analyses were conducted. In the first sensitivity analysis, we restricted the main analysis to studies that only reported information for severe patients (baseline FVIII activity ‹ 0.01 IU/ml ). In the second sensitivity analysis, we restricted the main analysis to large studies (i.e. studies with › 150 person-years of follow-up time).

*Summary of findings*

The main results of the product comparisons (including an overall quality assessment) are also summarized in a "summary of findings" table (table 3), according to the GRADE approach.[17]

## Results

### Included studies

A flowchart of the literature search is reported in figure 1 and the search terms are reported in supplemental figure S1 (see appendix). In total, 1605 articles were screened on their title and abstract. Eighty-two unique articles were reviewed in full, of these, 52 articles were excluded. Thirty articles[18-47] were selected for the analysis, four additional articles[48-51] were included after monthly searches on PubMed. Most articles reported on a single cohort of patients using one brand of rFVIII product, whereas three articles[23, 25, 26] provided information on multiple cohorts. Fischer et al[23] reported on five cohorts using different rFVIII products, Recht et al[26] reported on 2 cohorts with slightly different inclusion criteria and Hay et al[25] reported on three cohorts using different rFVIII products. In total, 34 articles reporting on 41 cohorts were included[18-51]. Characteristics of the 52 excluded papers are reported in supplemental table S1, references to the 52 excluded papers (labelled S1-S52) are also reported in supplemental table S1. Eighteen articles did not separately report inhibitor incidence and follow-up time for severe or moderately severe PTPs (but were otherwise eligible for inclusion). The corresponding authors were contacted but did not provide additional data. Consequently, these 18 articles were excluded from the meta-analysis. (supplemental table S1)

### Study characteristics

Overall, 39 patients developed inhibitors during 19,157 person-years of observation. (table 1) One study did not provide information on the total number of patients[23], therefore, the overall number of patients included in this meta-analysis is unknown. Seven studies evaluated Advate (6043 person-years, 6 inhibitors), four studies evaluated Kogenate or Helixate (537 person-years, 5 inhibitors), ten studies evaluated Kogenate FS/Bayer or Helixate FS/NexGen (7386 person-years, 10 inhibitors), three studies evaluated Refacto (609 person-years, 7 inhibitors) and four studies (containing 5 cohorts) evaluated Refacto AF (3226 person-years, 10 inhibitors).

Furthermore, one study used GreenGene F (56 person-years, 1 inhibitor), three studies used Kovaltry/Iblias (165 person-years, 0 inhibitors), three studies used NovoEight (551 person-years, 0 inhibitors), three studies used Nuwiq (85 person-years, 0 inhibitors) and two studies evaluated Recombinate (499 person-years, 0 inhibitors). Because of the small sample sizes, studies evaluating GreenGene F, Kovaltry/Iblias, NovoEight, Nuwiq and Recombinate were only included when calculating the overall incidence rate but were excluded from product-specific analyses. In total, 12 studies were excluded (1356 person-years, 1 inhibitor).

**Table 1.** Study characteristics.

| **Advate** | | | | | | |
|---|---|---|---|---|---|---|
| **Author** | **Year** | **Study design** | **Country** | **Inclusion criteria** | **INH testing** | |
| Blanchette [33] | 2008 | Clinical trial | US, Europe | ≤ 2%, EDs ≥ 50 | 3 months | |
| Den Uijl [36] | 2009 | Registry | The Netherlands | Any severity, EDs ≥ 50 | 12 months | |
| Valentino [38] | 2012 | Clinical trial | US, Europe | ≤ 2%, EDs ≥ 150 | 3 months | |
| Fukutake [19] | 2014 | Surveillance | Japan | Any severity, EDs ≥ 4 | Unknown | |
| Hay (cohort 2) [25*] | 2015 | Surveillance | UK | ≤ 1%, 12 months of prior treatment | 6 months | |
| Oldenburg [21**] | 2010 | Surveillance | US, Europe | Any severity, All previous EDs | routine detection | |
| Fischer (cohort 1) [23] | 2015 | Registry | Europe | ‹1%, EDs› 50 | routine detection | |
| **Kogenate, Helixate** | | | | | | |
| Aygören-Pürsün [8] | 1997 | Clinical trial | Germany | ‹ 15%, EDs› 100 | 3 months | |
| Seremetis[20] | 1999 | Clinical trial | US, Europe | ‹5%, EDs › 50 | Monthly (at beginning), every 6 months (at end) | |
| Yoshioka[30] | 2006 | Clinical trial | Japan | Any, EDs › 50 | At months 0-3-6-9-12-18-24 | |
| Singleton[32] | 2007 | Retrospective survey | Ireland | Any severity, All previous EDs | routine detection | |
| **Kogenate FS/Bayer, Helixate FS/Nexgen** | | | | | | |
| Abshire[22] | 2000 | Clinical trial | North America, Europe | ‹2%, EDs≥ 100 | week 0-4-12-24, months 12-18-24 | |
| Musso[34] | 2008 | Surveillance | Europe | ‹2%, EDs› 0 | routine detection | |
| Delumeau[35] | 2008 | Surveillance | Japan | Any severity, All previous EDs | routine detection | |
| Youn [18] | 2009 | Surveillance | Taiwan | Any severity, All previous EDs | routine detection | |
| Collins[37] | 2010 | Clinical trial | US, Europe | ‹1%, EDs› 100 | baseline and 13 months | |
| Manco-Johnson[41*] | 2013 | Clinical trial | Worldwide | ‹2%, EDs≥ 150 | 0 and 3 months, 1, 2 and 3 years | |
| Lalezari [27*] | 2014 | Clinical trial | Worldwide | ‹1%, EDs≥ 150 | Week 1-2-3-7-12-26-38-52 | |
| Gouider [45] | 2015 | Surveillance | Worldwide | ‹4%, all previous EDs | routine detection | |
| Hay (cohort 3) [25*] | 2015 | Surveillance | UK | ≤ 1%, 12 months of prior treatment | 6 months | |
| Fischer (cohort 2) [23] | 2015 | Registry | Europe | ‹1%, EDs› 50 | routine detection | |

| | Sample size | Follow-up (person-years) | Follow-up (exposure days) | Inhibitors | Age |
|---|---|---|---|---|---|
| | 53 | 56 | 8268 | 0/0 | Mean 3.1 years (SD, 1.5) |
| | 71 | 213 | - | 0/0 | Median 25 years (range, 0.5-67) |
| | 73 | 97 | - | 0/0 | Median 26 years (range, 7-59) |
| | 271 | 542 | . | 0/0 | Median 24 years (range, 0-81) |
| | 118 | 118 | - | 0/0 | Switchers: mean 25 years (IQR 13-44)<br>Non-switchers: mean 22 years (IQR 14-33) |
| | 348 | 361 | 30972 | 1/0 | 29.9% ‹ 12 years<br>10.4% 12-16 years<br>59.3% ≥ 16 years |
| | - | 4656 | - | 5/- | - |
| | | | | | |
| | 22 | 22 | 1507 | 0/0 | Median 27 years (range:2-62) |
| | 54 | 254 | 12204 | 1/1 | Median 25 years (range:1-72) |
| | 74 | 121 | 7134 | 4/0 | Mean 24 years (range:1-73) |
| | 84 | 140 | - | 0/0 | 51.1%: › 18 years<br>11.7%: 13-18 years<br>37.2%: ≤ 12 years |
| | | | | | |
| | 71 | 119 | 11867 | 0/0 | NA: mean 22.6 years (SD: 10.2)<br>EU: mean 32.6 years (SD: 13.3) |
| | 181 | 352 | 33847 | 0/0 | Mean 23.6 years (range:0.1-71) |
| | 323 | 409 | - | 1/0 | Mean 23.7 years (SD, 16.6) |
| | 38 | 34 | - | 0/0 | Mean 20.3 years (SD, 15.6) |
| | 20 | 22 | 2231 | 0/0 | Mean 36.4 years (SD, 3.5) |
| | 84 | 143 | 11676 | 0/0 | Median 30.6 years (range, 15-50) |
| | 72 | 56 | 8834 | 0/0 | Mean 34.4 years (range, 13-64) |
| | 118 | 236 | - | 1/0 | Mean 13.8 years (SD, 13.6) |
| | 509 | 509 | - | 1/1 | Switchers: mean 25 years (IQR 13-44)<br>Non-switchers: mean 22 years (IQR 14-33) |
| | | 5506 | - | 7/- | - |

| **Refacto** | | | | | | |
|---|---|---|---|---|---|---|
| **Author** | **Year** | **Study design** | **Country** | **Inclusion criteria** | **INH testing** | |
| Gringeri [24] | 2004 | Cohort study | Italy | ‹1%, EDs≥ 50 | 3 months | |
| Pollmann [31] | 2007 | Surveillance | Germany, Austria | Any severity, All previous EDs | routine detection | |
| Fischer (cohort 3) [23] | 2015 | Registry | Europe | ‹1%, EDs› 50 | routine detection | |
| **Refacto AF** | | | | | | |
| Recht (cohort 1) [26] | 2009 | Clinical trial | Worldwide | ≤ 2%, EDs≥ 150 | Months 0-1-3-6 | |
| Recht (cohort 2) [26] | 2009 | Clinical trial | Worldwide | ≤ 2%, EDs≥ 250 | Months 0-1-3-6 | |
| Lopez [43*] | 2015 | Clinical trial | Europe | ‹1%, EDs› 150 | At 1, 10-15, 50 EDs and then every 6 months | |
| Hay (cohort 1) [25*] | 2015 | Registry | UK | ≤ 1%, EDs› 50 or 12 months of prior treatment | 6 months | |
| Fischer (cohort 4) [23] | 2015 | Registry | Europe | ‹1%, EDs› 50 | routine detection | |
| **GreenGene F** | | | | | | |
| Hyun [44] | 2015 | Clinical trial | Korea | ≤ 2%, EDs› 150 | 3 months | |
| **Kovaltry, Iblias** | | | | | | |
| Kavakli [46] | 2015 | Clinical trial | Worldwide | ‹1%, EDs≥ 150 | - | |
| Ljung [47] | 2016 | Clinical trial | Worldwide | ‹1%, EDs≥ 50 | Months 0-1-2-6 | |
| Saxena [50] | 2016 | Clinical trial | Worlwide | ‹1%, EDs≥ 150 | - | |
| **NovoEight** | | | | | | |
| Kulkarni [39] | 2013 | Clinical trial | Worldwide | ≤ 1%, EDs› 50 | At 6/8 study visits | |
| Lentz [40] | 2013 | Clinical trial | Worldwide | ≤ 1%, EDs› 150 | At 8/9 study visits | |
| Lentz [49] | 2016 | Clinical trial | Worldwide | ≤ 1%, EDs› 50 | Every 6 months | |
| **Nuwiq** | | | | | | |
| Lissitchkov [42] | 2015 | Clinical trial | Europe | ≤ 1%, EDs› 150 | EDs 1, 2, 10–15, months 3 and 6. | |
| Tiede [48] | 2016 | Clinical trial | Europe | ≤ 1%, EDs› 150 | - | |
| Lissitchkov [51] | 2017 | Clinical trial | Europe | ≤ 1%, EDs› 150 | At baseline and study completion | |

| | Sample size | Follow-up (person-years) | Follow-up (exposure days) | Inhibitors | Age |
|---|---|---|---|---|---|
| | 25 | 12.5 | 610 | 1/1 | Median 31 years (range:6-60) |
| | 188 | 387 | 55259 | 2/1 | Mean 26.3 years (range:0-67) |
| | - | 209 | - | 4/- | - |
| | | | | | |
| | 94 | 62 | 6741 | 2/0 | Median 24 years (range: 12-60) |
| | 110 | 48 | 6860 | 1/0 | Median 19 years (range: 7-70) |
| | 208 | 207 | 19552 | 0/0 | Mean 30.5 years (SD:13) |
| | 571 | 571 | - | 4/1 | Switchers: mean 25 years (IQR 13-44)<br>Non-switchers: mean 22 years (IQR 14-33) |
| | - | 2338 | - | 3/- | - |
| | | | | | |
| | 70 | 56 | 6397 | 1/- | Mean 31.9 years (SD, 9.6) |
| | | | | | |
| | 79 | 79 | - | 0/0 | Median 28.5 years (range: 14-59) |
| | 50 | 25 | 3650 | 0/0 | Mean 6.4 years (SD, 3.0) |
| | 61 | 61 | - | 0/0 | Mean 31.5 years (SD, 12.7) |
| | | | | | |
| | 63 | 24 | 3780 | 0/0 | Mean 6.1 years (SD, 2.9) |
| | 150 | 75 | 12750 | 0/0 | Mean 28 years (SD, 11.8) |
| | 199 | 452 | 72320 | 0/0 | |
| | | | | | |
| | 32 | 16 | 2723 | 0/0 | Mean 37.3 years (SD, 13.6) |
| | 22 | 20 | 1030 | 0/0 | Mean 39.6 years (SD, 14.1) |
| | 66 | 49 | 6612 | 0/0 | Mean 33.6 years (SD, 9.89) |

| Recombinate | | | | | | |
|---|---|---|---|---|---|---|
| **Author** | | **Study design** | **Country** | **Inclusion criteria** | **INH testing** | |
| White [29] | | Clinical trial | Worldwide | ≤ 5%, EDs›200 | - | |
| Fischer (cohort 5) [23] | | Registry | Europe | ‹1%, EDs› 50 | routine detection | |

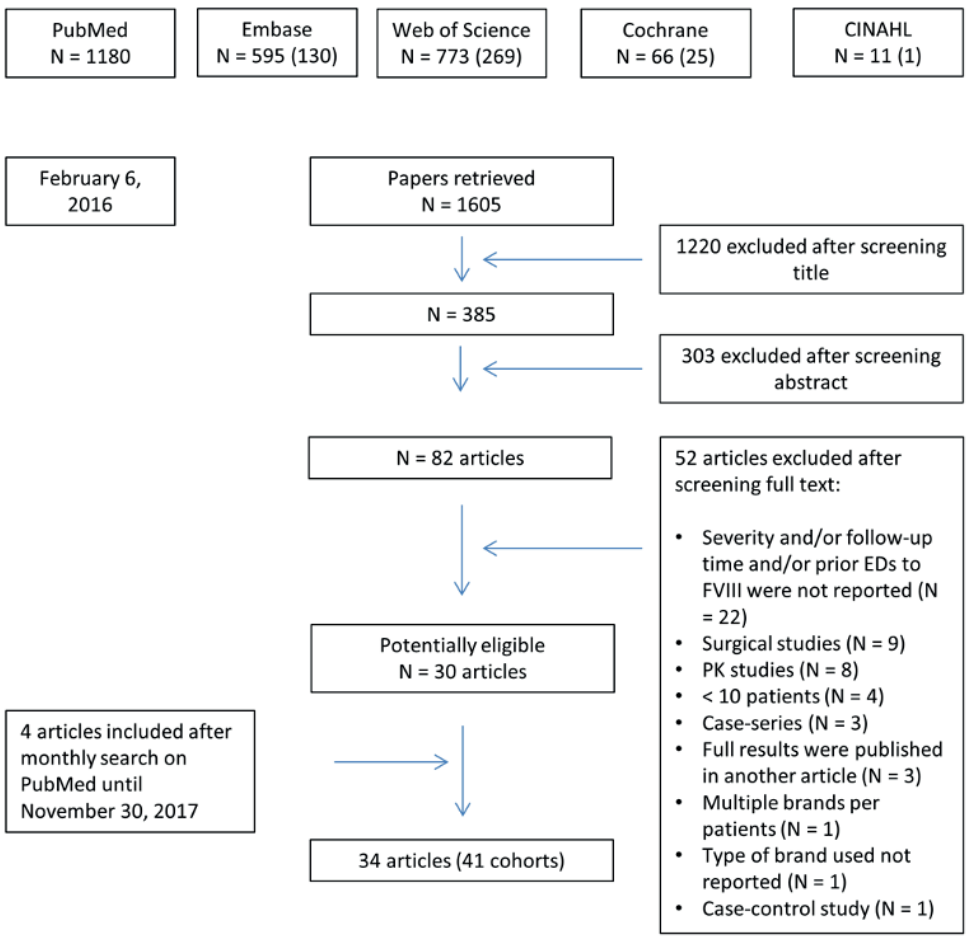
* Possible overlap with EUHASS registry.[21]

** Patient recruitment period not reported, unclear if there is any overlap with EUHASS registry.[21]

| | Sample size | Follow-up (person-years) | Follow-up (exposure days) | All Inhibitors / High-titre inhibitors | Age |
|---|---|---|---|---|---|
| | 67 | 248 | - | 0/0 | 33% › 18 years<br>67% ≥ 18 years |
| | | 251 | - | 0/- | - |

**Figure 1**. Flowchart of the search strategy (number of unique reports are indicated in parentheses).

The search was run on February 6, 2016. Two additional studies were included by performing monthly searches on Pubmed until November 30, 2017.




We found similar methodological quality across studies with the modified Downs and Black checklist (median score: 11, range: 6-16), except for two studies with a high risk of bias which were published as a letter to the editor[18] (score: 6) and a conference poster[19] (score: 8). (supplemental table S2) The majority of studies were similar in quality, therefore, we did not perform a sensitivity analysis based on methodological quality.

### Risk of inhibitor formation according to recombinant rFVIII product

*Overall incidence rate and incidence rate per rFVIII product*

The overall inhibitor incidence rate among previously treated patients was 2.06 per 1000 person-years with a 95% confidence interval (CI95) of 1.06-4.01). The incidence rate of inhibitor formation was 0.99 (CI95: 0.37-2.70) per 1000 person-years for Advate, 5.86 (CI95: 0.25-134.92) per 1000 person-years for Kogenate/Helixate, 1.35 (CI95: 0.66-2.77) per 1000 person-years for Kogenate FS/Helixate NexGen, 12.05 (CI95: 1.53-94.78) per 1000 person-years for Refacto and 4.64 (CI95: 0.82-26.43) per 1000 person-years for Refacto AF (figure 2).

*Inhibitor formation by product*

Compared with Advate, the pooled incidence rate ratio (IRR) was 9.77 (95%CI: 1.97-48.41) for Kogenate/Helixate, 1.51 (95%CI: 0.34-6.69) for Kogenate FS/Helixate NexGen, 14.40 (95%CI: 2.84-72.94) for Refacto and 4.81 (95%CI: 0.99-23.34) for Refacto AF. (table 2). Compared with full-length rFVIII, the pooled IRR for B-domain-deleted rFVIII was 4.80 (CI95: 1.32-17.40). Compared to rFVIII products derived from Chinese hamster ovary (CHO) cells, the pooled IRR was 0.62 (CI95: 0.17-2.34) for rFVIII products derived from baby hamster kidney (BHK) cells. Compared to second generation rFVIII products, the pooled IRR was 2.54 (CI95: 0.45-14.27) for first generation rFVIII products and 0.75 (CI95: 0.21-2.66) for third-generation rFVIII products. (table 2)

*Sensitivity analysis*

The sensitivity analyses showed that the results for each rFVIII brand varied significantly with changes to methodology. (supplemental table S4 and S5) However, this can be partly explained by the low number of studies per brand. Furthermore, the results of the sensitivity analyses were roughly in line with the results of the main analysis with regards to the overall incidence rate and when rFVIII products were analysed according to length, cell line and generation. Nevertheless, this shows that that the most important results of the main analysis are not very robust to changes in methodology. (supplemental table S4 and S5)

## Discussion

This meta-analysis comprehensively reviews published reports of rFVIII products in relation to immunogenicity among previously treated patients with haemophilia. In total, 34 studies reporting on 41 cohorts were included with 39 inhibitor events and

19,157 person-years of observation. The incidence rate among PTPs was 2.06 per 1000 person-years (CI95: 1.06-4.01).

Formal comparisons of products yielded a statistically significant higher incidence of inhibitors among patients using Kogenate/Helixate and Refacto when compared with Advate, but not Kogenate FS/Helixate NexGen or Refacto AF. Taken as a whole, B-domain deleted rFVIII products were associated with an increased risk of inhibitor formation when compared to full-length rFVIII products. However, the overall quality of evidence was low, mainly due to the high risk of bias and confounding, lack of power to detect an effect in most studies (given the rare outcome) and the lack of consistency among studies evaluating the same rFVIII product. Therefore, the aforementioned results have to be interpreted with caution (Table 3).

**Comparison with previous reviews**

The overall incidence of inhibitors in PTPs in our study corroborates earlier findings[8, 52-55]. Recently, two previous systematic reviews have evaluated the association between rFVIII product type and inhibitor formation in PTPs[7, 10].

In 2011, the first of the two meta-analyses was published, its focus was mainly on the risk of inhibitor formation with B-domain deleted rFVIII products compared to full-length rFVIII products[10]. This meta-analysis included prospective studies of patients who were treated for more than 50 exposure days at baseline. A mixed effects Cox proportional hazards model with study as a random effect was used to pool and compare studies. Due to incomplete reporting, individual follow-up time was estimated for most non-inhibitor patients. Fourteen out of 29 studies in the previous meta-analysis were also included in our current meta-analysis. The following 9 studies were included in the previous meta-analysis but excluded from the current meta-analysis; 3 surgical studies [S7, S27, S28], 1 case-series [S2], 2 studies that did not adequately report prior exposure to FVIII [S49, S51] and 3 studies that did not adequately report follow-up time [S39, S41, S46] (see supplemental table S1 for references of excluded studies). Similar to our study, this meta-analysis found a statistically significantly higher risk of inhibitor formation in previously treated patients using B-domain deleted rFVIII, compared to previously treated patients using full-length rFVIII (HR: 7.26, CI95: 2.12–24.9).

A more recent meta-analysis from 2013 did not report any differences in immunogenicity[7]. Thirteen out of 33 studies in this previous meta-analysis were also included in the current meta-analysis. The following 11 studies were included in the previous

**Figure 2.** Incidence rates of inhibitor development per study.

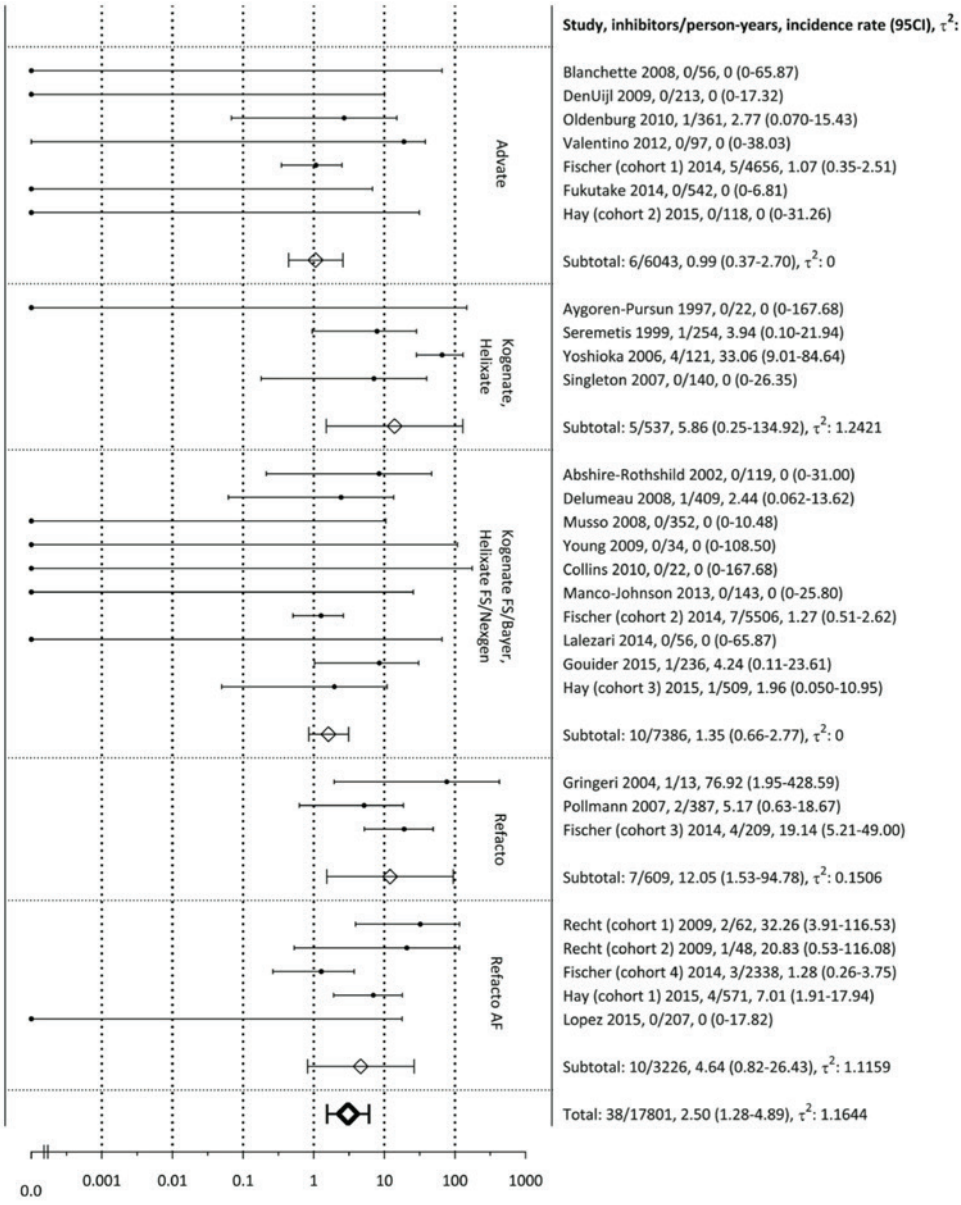


**Table 2.** Pooled incidence rates and incidence rate ratios of inhibitor development by product type.

| Variable | N | Inhibitors/ p-y | Pooled inhibitor incidence rate per 1000 p-y (CI95) | between-study variance ($^2$) | Incidence rate ratio (CI95) |
|---|---|---|---|---|---|
| Overall (main products only): | 29 | 38/17801 | 2.50 (CI95: 1.28-4.89) | 1.1644 | |
| **Product** | | | | | |
| Advate | 7 | 6/6043 | 0.99 (CI95: 0.37-2.70) | 0 | Ref |
| Kogenate/Helixate | 4 | 5/537 | 5.86 (CI95: 0.25-134.92) | 1.2421 | 9.77 (CI95: 1.97-48.41) |
| Kogenate FS/Helixate NexGen | 10 | 10/7386 | 1.35 (CI95: 0.66-2.77) | 0 | 1.51 (CI95: 0.34-6.69) |
| Refacto | 3 | 7/609 | 12.05 (CI95: 1.53-94.78) | 0.1506 | 14.40 (CI95: 2.84-72.94) |
| Refacto AF | 5 | 10/3226 | 4.64 (CI95: 0.82-26.43) | 1.1159 | 4.81 (CI95: 0.99-23.34) |
| **rFVIII length**[1] | | | | | |
| Full-length rFVIII | 21 | 21/13966 | 1.46 (CI95: 0.59-3.59) | 0.8967 | Ref |
| B-domain deleted rFVIII | 8 | 17/3835 | 6.93 (CI95: 2.28-21.08) | 0.9980 | 4.80 (CI95: 1.32-17.40) |
| **Cell line**[2] | | | | | |
| CHO-cells | 15 | 23/9878 | 3.01 (CI95: 1.20-7.54) | 1.3115 | Ref |
| BHK-cells | 14 | 15/7923 | 1.96 (CI95: 0.63-6.15) | 1.0564 | 0.62 (CI95: 0.17-2.34) |
| **rFVIII generation**[3] | | | | | |
| Second-generation rFVIII | 13 | 17/7995 | 2.66 (CI95: 1.06-6.66) | 0.7128 | Ref |
| First-generation rFVIII | 4 | 5/537 | 5.86 (CI95: 0.25-134.92) | 1.2421 | 2.54 (CI95: 0.45-14.27) |
| Third-generation rFVIII | 12 | 16/9269 | 1.95 (CI95: 0.70-5.40) | 0.9157 | 0.75 (CI95: 0.21-2.66) |

[1] Full-length rFVIII (Kogenate/Helixate, Kogenate FS/Helixate NexGen and Advate) is compared with B-domain deleted rFVIII (Refacto and Refacto AF).

[2] rFVIII derived from CHO-cells (Refacto, Refacto AF and Advate) is compared with rFVIII derived from BHK-cells (Kogenate/Helixate and Kogenate FS/Helixate NexGen).

[3] First Generation rFVIII (Kogenate/Helixate) is compared with second generation rFVIII (Refacto and Kogenate FS/Helixate NexGen) and third generation rFVIII (Advate and Refacto AF).

meta-analysis but excluded from the current meta-analysis; 3 surgical studies [S7, S27, S28], 3 studies that did not report haemophilia severity and/or prior EDs to FVIII [S43, S49, S9], 4 studies that did not report follow-up time [S40, S41, S46, S47] and 1 study in which the type of FVIII brand used was not specified [S10] (see supplemental table S1 for references of excluded studies). The method of Laird and Mosteller was used to pool study results. Crude proportions of inhibitor development for each FVIII product were indirectly compared by evaluating whether statistically significant between-groups heterogeneity existed according to the Cochran's Q statistic. The crude proportion of inhibitor development was 1.0% (CI95: 0.5%-1.8%) for Advate, 2.6% (CI95: 1.6%-4.4%) for Kogenate (first generation) and 1.9% (CI95: 1.1%-3.4%) for Refacto (first generation).

No statistically significant Q-statistic was found based on the type of FVIII concentrate (Q statistic = 6.854, P = 0.077), this was confirmed by a univariate meta-regression analysis (these results were not shown). Cochran's Q, however, is not a sensitive tool for assessing heterogeneity as it has low power to detect heterogeneity if the event rate is very low[56], and hence this meta-analysis at most indicated the absence of gross differences by product.

In this meta-analysis Kogenate/Helixate and Kogenate FS/Helixate NexGen were categorized and analysed as one product group, complicating comparisons between individual rFVIII products. Further, only information on the cumulative incidence of inhibitor formation (i.e., the numbers of events per persons) per product was provided without correcting for study follow-up time. It is mentioned in the article that "similar results were obtained when the incidence rate was calculated as events per person-years" (however, these data were not shown). As development of inhibitors to FVIII is dependent on exposure to FVIII and therefore follow-up time, the reporting of incidence rates is preferred over proportions of inhibitor patients. In addition, conventional data pooling methods (such as the one used in the aforementioned meta-analysis) are based on large sample approximations which produce biased estimates when applied to studies with very low event rates[56], which is the case in inhibitor development in PTPs.

**Study strengths and limitations**

*Study strengths*

The last review included studies up to January 2013. Of the 41 cohorts included in this analysis, 14 cohorts were published after this date.

In contrast to previous reviews, the inhibitor incidence rate was the main study outcome. This was preferred over the cumulative inhibitor incidence as the main outcome because the study duration was not identical across studies and over the hazard rate as the main outcome because most studies did not report the follow-up time of non-inhibitor patients. Unlike earlier reviews, we also directly compared the pooled inhibitor incidence rates of all major rFVIII products with each other.

Standard meta-analysis methods (e.g. the DerSimonian-Laird random effects method) can give biased results when applied inappropriately. Firstly, the effect estimate and standard error of each study are usually correlated. Secondly, pooling studies with zero events leads to computational errors, this is often avoided by applying a continuity correction. Lastly, the within-study distribution of the effect estimate is assumed to be normal, this assumption is often violated when the event rate is very rare. The meta-analysis model used in this review, a random intercept Poisson regression model, avoids the aforementioned problems[16].

**Table 3.** Summary of findings.

| **Main recombinant FVIII products compared to Advate in previously treated patients with severe haemophilia A** | | | | | |
|---|---|---|---|---|---|
| **Intervention**: Kogenate/Helixate | | | | | |
| Outcomes | **Absolute effects*** (95% CI) | | Relative effect (95% CI) | Nº of person-years (studies) | Certainty of the evidence (GRADE) |
| | **Risk with Advate** | **Risk with Kogenate/ Helixate** | | | |
| Inhibitor incidence assessed with: Bethesda assay | 0.99 per 1,000 | **5.86 per 1,000** (0.25 to 134.92) | **RR 9.77** (1.97 to 48.41) | 6580 (11 non-comparative observational studies) | ⨁◯◯◯ VERY LOW |
| **Intervention**: Kogenate FS/Helixate NexGen | | | | | |
| Outcomes | **Absolute effects*** (95% CI) | | Relative effect (95% CI) | Nº of person-years (studies) | Certainty of the evidence (GRADE) |
| | **Risk with Advate** | **Risk with Kogenate FS/Helixate NexGen** | | | |
| Inhibitor incidence assessed with: Bethesda assay | 0.99 per 1,000 | **1.35 per 1,000** (0.66 to 2.77) | **RR 1.51** (0.34 to 6.69) | 13429 (17 non-comparative observational studies) | ⨁◯◯◯ VERY LOW |

| **Intervention**: Refacto | | | | | |
|---|---|---|---|---|---|
| Outcomes | **Absolute effects*** (95% CI) | | Relative effect (95% CI) | Nº of person-years (studies) | Certainty of the evidence (GRADE) |
| | **Risk with Advate** | **Risk with Refacto** | | | |
| Inhibitor incidence assessed with: Bethesda assay | 0.99 per 1,000 | **12.05 per 1,000** (1.53 to 94.78) | **RR 14.40** (2.84 to 72.94) | 6652 (10 non-comparative observational studies) | ⨁◯◯◯ VERY LOW |
| **Intervention**: Refacto AF | | | | | |
| Outcomes | **Absolute effects*** (95% CI) | | Relative effect (95% CI) | Nº of person-years (studies) | Certainty of the evidence (GRADE) |
| | **Risk with Advate** | **Risk with Refacto AF** | | | |
| Inhibitor incidence assessed with: Bethesda assay | 0.99 per 1,000 | **4.64 per 1,000** (0.82 to 26.43) | **RR 4.81** (0.99 to 23.34) | 9269 (12 non-comparative observational studies) | ⨁◯◯◯ VERY LOW |

* **The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

**CI:** Confidence interval; **RR:** Risk ratio

**GRADE Working Group grades of evidence**
**High certainty:** We are very confident that the true effect lies close to that of the estimate of the effect
**Moderate certainty:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
**Low certainty:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
**Very low certainty:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

*Limitations - Random variation*

The pooled results have to be interpreted with caution due to the low number of inhibitors within each product type, which give rise to significant random variation as indicated by the broad confidence intervals. Furthermore, haemophilia severity, follow-up time and the prior number of exposure days to FVIII were not accurately reported in several studies (supplemental table S1), these studies were excluded (after attempts to retrieve this information by contacting the corresponding authors). Due to the low event rate overall, the absence of these studies in the meta-analysis may have significantly impacted our results.

*Limitations - Confounding*

As no comparative studies were found, we could only compare single-arm trials in our analysis of inhibitor formation by product type. Due to differences in the distri-

bution of genetic/treatment-related risk factors, comparing single-arm trials may be misleading.

Many studies also included moderately severe patients (the exact proportion varied per study). If moderately severe patients are at a significantly lower risk of inhibitor formation, then this could have confounded our results.

Compared to on-demand treatment, patients on prophylactic treatment are exposed to more units of FVIII over a given time period and are therefore at a higher risk of inhibitor formation. Correcting for this problem by using exposure days to FVIII instead of person-years as the unit of time in the main analysis was not feasible due to the low number of studies that accurately reported the total number of exposure days to FVIII.

Adjustment for other potential confounders such as F8 genotype, ethnicity, family history and surgery was not possible due to incomplete reporting (supplemental table S3). Overall, there is a moderate chance of confounding, mainly due to variables that may have influenced the physician's choice of rFVIII product (F8 genotype, family history of inhibitors).

*Limitations - Bias*
The cut-off level and screening frequency of the inhibitor assays, which could have influenced the reported number of low-titre inhibitors, varied across studies. This could have introduced misclassification bias and consequently over- or underestimation of inhibitor incidences. Patients in market approval studies undergo more intensive screening for inhibitors. (Transient) low-titre inhibitors that were not detected before the study or at study baseline may be detected after inclusion. Due to this, newer products for which data is mainly available from market approval studies may seem more immunogenic than older products which have also been evaluated in post-approval studies.

Over time, the screening intensity has increased, possibly leading to an increased detection of low-titre inhibitors in newer studies. However, screening intensity was slightly higher among older products (Kogenate/Helixate and Refacto) when compared to newer products (Kogenate FS/Helixate NexGen and Refacto AF). (table 1) This observation is in line with our results, as Kogenate/Helixate and Refacto were also the most immunogenic products in our analysis. Correcting for this problem by only analysing high-titre inhibitors was not feasible due to the very low number of high-titre inhibitors overall.

In addition, there could have been some overlap between 5 studies (that evaluated Advate, Kogenate FS/Helixate NexGen or Refacto AF) and the EUHASS registry[23] (table 1) Double counting could have led to over- or underestimating inhibitor incidences and producing overly narrow confidence intervals. Because Advate was used as the reference product, reported incidence rate ratios for all product types would also be biased. Overall, double counting could have influenced the main results.

Many patients were treated with a different FVIII product before study inclusion (especially in market approval trials). Consequently, increased immunogenicity due to product switching could have biased the results. However, there have been several national product switches and there was no evidence of increased immunogenicity.[57]

**Biological explanation of a causal effect**

Several differences between rFVIII products could explain the reported results. Second- and third generation full length rFVIII products vary slightly in their FVIII amino acid sequence. Furthermore, differences in product formulation such as culture conditions and stabilizing agents could also be relevant. Lastly, the type of cell culture used for production such as CHO cells, BHK cells or, more recently HEK 293 cells, leads to rFVIII products with different post-translational modifications that may influence immunogenic potential[11].

**Implications of these results for future research**

Comparing single-arm trials may be misleading due to bias and confounding. Single-arm trials are useful for identifying extremely immunogenic products but less suitable for detecting smaller effects (e.g. the difference in inhibitor risk found in the studies by Peyvandi et al[2] or Gouw et al[58]). Nevertheless, these studies could be used more effectively if a standardized data reporting system was used. This system should include all relevant variables such as known genetic/treatment-related confounders.[59] Lastly, future research should focus on using study designs that are appropriate for evaluating rare outcomes (i.e. case control studies).

## Conclusion

These results suggest that some products may be associated with increased immunogenicity. However, these findings should be interpreted with caution, both the low incidence of inhibitors in PTPs and the differences in study design may cause significant variation in estimates of risk.

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**Supplemental Table S1.** List of excluded papers (including references).

| First author | Year | Reason for exclusion | Product | Patients | Inhibitors |
|---|---|---|---|---|---|
| Sennet [S1] | 2004 | Case series | Refacto | 2 | 0 |
| Keeling [S2] | 2006 | Case series | Refacto | 3 | 3 |
| Ishaku [S3] | 2015 | Case-control study | - | 48 | 3 |
| Kocher [S4] | 2012 | Multiple brands per patient | rFVIII and pdFVIII | 119 | 0 |
| Von Auer [S5] | 2005 | Case series | - | 10 | 10 |
| Giles [S6] | 1998 | Severity and prior exposure to FVIII were not reported | rFVIII | - | - |
| Auerswald [S7] | 2013 | Surgery | rFVIII and pdFVIII | 29 | 0 |
| Batorova [S8] | 2012 | Surgery, cross-sectional study | - | 742 | 9 |
| Siegmund [S9] | 2010 | Prior exposure to FVIII was not reported | rFVIII and pdFVIII | 118 | 0 |
| Aznar [S10] | 2014 | Type of brand used not accurately reported | rFVIII and pdFVIII | 97 | 9 |
| Xuan [S11] | 2014 | Prior exposure to FVIII and follow-up were not reported. | - | 926 | 40 |
| Martinowitz [S12] | 2011 | Study on pharmacokinetics | Novoeight, Advate | 23 | - |
| Lambert [S13] | 2007 | Study on pharmacokinetics | Refacto | 14 | - |
| Di Paola [S14] | 2007 | Study on pharmacokinetics | Refacto, Advate | 18 | 0 |
| Kelly [S15] | 1997 | Study on pharmacokinetics | rFVIII | 10 | - |
| Barnes [S16] | 2006 | Study on pharmacokinetics | Kogenate-FS | 20 | - |
| Kessler [S17] | 2005 | Study on pharmacokinetics | BDD rFVIII, pdFVIII | 18 | - |
| Mulcahy [S18] | 2005 | Only treatment during surgery and/or severe bleeding | rFVIII | 12 | 2 |
| Mannucci [S19] | 1994 | Prior exposure to FVIII was not reported | Kogenate | 51 | 0 |
| Lalezari [S20] | 2013 | Follow-up and inhibitor information not reported | Kogenate FS | 68 | - |
| Shah [S21] | 2015 | Study on pharmacokinetics | Kovaltry | 45 | - |
| Tuddenham [S22] | 2010 | Early findings of a study, results of full study were published later | - | - | - |
| Oldenburg [S23] | 1995 | Prior exposure to FVIII, severity and follow-up were not reported | Kogenate, Recombinate | 112 | - |
| Ewenstein [S24] | 2004 | Prior exposure to FVIII and follow-up were not accurately reported | Recombinate/ Bioclate | - | - |
| Lusher [S25] | 2005 | Follow-up was not reported | Refacto | 218 | 33 |
| Jiménez-Yuste [S26] | 2015 | Study on pharmacokinetics | NovoEight | 76 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| Windyga [S27] | 2010 | Surgery | Refacto AF | 30 | 1 |
| Négrier [S28] | 2008 | Surgery | Advate | 58 | 0 |
| Meijer [S29] | 2015 | Surgery | Kogenate FS | 25 | 0 |
| Santagostino [S30] | 2015 | Surgery | Novoeight | 33 | 0 |
| Scharrer [S31] | 2000 | Surgery | Kogenate FS | 15 | 1 |
| Martinowitz [S32] | 2009 | Surgery | Kogenate FS | 14 | 0 |
| Shirahata [S33] | 2000 | < 10 patients | Kogenate FS | 5 | 0 |
| Zanon [S34] | 1999 | < 10 patients treated with rFVIII products | rFVIII and pdFVIII | 62 | 7 |
| Fukui [S35] | 1991 | < 10 patients | Kogenate | 5 | 0 |
| Prezotti [S36] | 2015 | Follow-up and prior exposure to FVIII were not reported | Advate | 346 | 5 |
| Chen [S37] | 2012 | Prior exposure to FVIII was not reported | Advate | 40 | 0 |
| Rubinger [S38] | 2008 | Prior exposure to FVIII was not reported | Kogenate-FS | 274 | 0 |
| Roussel-Robert [S39] | 2003 | Follow-up was not reported | Refacto | 70 | 4 |
| Tarantino [S40] | 2004 | Follow-up was not reported | Advate | 108 | 1 |
| Shi [S41] | 2007 | Follow-up was not reported | Kogenate-FS | 49 | 0 |
| Rea [S42] | 2009 | Prior exposure to FVIII was not reported | Refacto | 33 | 1 |
| Bacon [S43] | 2011 | Prior exposure to FVIII was not reported | Advate | 96 | 1 |
| Zhang [S44] | 2011 | Prior exposure to FVIII was not reported | Advate | 58 | 1 |
| Chang [S45] | 2015 | < 10 patients | Refacto AF | 8 | 4 |
| Smith [S46] | 2005 | Follow-up was not reported | Refacto | 60 | 3 |
| Vidovic [S47] | 2010 | Follow-up was not reported | Kogenate-FS | 306 | 0 |
| Pollmann [S48] | 2013 | Subanalysis of earlier report (duplicate data) | - | - | - |
| Schwartz [S49] | 1990 | Prior exposure to FVIII was not reported accurately, long-term results are published in later report | Kogenate | 107 | 8 |
| Rothschild [S50] | 2002 | Subanalysis of earlier report (duplicate data) | - | - | - |
| Petrini [S51] | 2009 | Prior exposure to FVIII was not reported | Refacto | 57 | 0 |
| Klukowska [S52] | 2015 | Follow-up not reported | Nuwiq | 59 | 0 |

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**Supplemental Table S2.** Quality assessment score. The methodological quality of each article was assessed using a modified Downs and Black checklist.

| Study | REPORTING | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 |
|---|---|---|---|---|---|---|---|---|---|
| | | Is the aim clearly described? | Are the main outcomes clearly described in the methods? | Are the characteristic of the patients clearly described? | Are the interventions of interest clearly described? | Are the main study findings clearly described? | Does the study provide estimates of the random variability in the data for the main outcomes? | Have all important adverse events that may be a consequence of the intervention been reported? | Have the characteristics of patients lost to follow-up been described? |
| Abshire [22] | | * | * | * | * | * | | * | |
| Aygören-Pürsün [28] | | * | * | * | * | * | | * | * |
| Blanchette [33] | | * | * | * | * | * | | * | * |
| Collins [37] | | * | * | * | * | * | | * | * |
| Delumeau [35] | | * | * | * | * | * | | * | |
| Den Uijl [36] | | * | * | * | * | * | | * | |
| Fischer [23] | | * | * | * | * | * | * | | * |
| Fukutake [19] | | * | * | * | | * | * | | |
| Gouider [45] | | * | * | * | * | * | | * | * |
| Gringeri [24] | | * | * | * | * | * | | | |
| Hay [25] | | * | * | * | * | * | * | * | |
| Hyun [44] | | * | * | * | * | * | | * | * |
| Kavakli [46] | | * | * | * | * | * | | * | * |
| Kulkarni [39] | | * | * | * | * | * | | * | * |
| Lalezari [27] | | * | * | * | * | * | | * | |
| Lentz [40] | | * | * | * | * | * | * | * | * |
| Lentz [49] | | * | * | | * | * | | * | |

| | EXTERNAL VALIDITY | | | | INTERNAL VALIDITY | | | | | | POWER | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Q9 | Q10 | Q11 | | Q12 | Q13 | Q14 | Q15 | Q16 | Q17 | | Q18 | |
| | Were the subjects asked to participate representative of the entire population? | Were those subjects who were prepared to participate representative of the entire population from which they were recruited ? | Were the staff, places and facilities representative of the treatment the majority of patients receive? | | If any of the results of the study were based on *data dredging*, was this made clear? | Do the analyses adjust for different lengths of folluw-up of patients? | Were the statistical tests used to assess the main outcomes appropriate? | Was compliance with the interventions reliable? | Were the main outcome measures used accurate (valid and reliable)? | Were losses of patients to follow-up taken into account? | | Did the study have sufficient power? (>100 person-years) | SCORE |
| | * | | * | | * | | | | * | | | * | 11 |
| | * | | * | | * | | | | * | | | | 11 |
| | * | | * | | * | | | | * | * | | | 12 |
| | * | | * | | * | | | | * | | | | 11 |
| | | | * | | * | | | | * | | | * | 10 |
| | * | | * | | * | | | * | * | * | | * | 13 |
| | * | * | * | | * | * | * | | * | * | | * | 16 |
| | * | | * | | | | | | | | | * | 8 |
| | * | | * | | * | | | * | * | | | * | 13 |
| | * | | * | | * | | | | * | | | | 9 |
| | * | | * | | * | * | * | * | * | | | * | 15 |
| | | | * | | * | | | * | * | | | | 10 |
| | * | | * | | * | | | * | * | | | | 12 |
| | * | | * | | * | * | * | * | * | * | | | 15 |
| | * | | * | | * | | | * | * | | | | 11 |
| | * | | * | | * | | | * | * | * | | * | 15 |
| | * | | * | | * | | | * | * | | | * | 11 |

| Study | REPORTING | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 |
|---|---|---|---|---|---|---|---|---|---|
| | | Is the aim clearly described? | Are the main outcomes clearly described in the methods? | Are the characteristic of the patients clearly described? | Are the interventions of interest clearly described? | Are the main study findings clearly described? | Does the study provide estimates of the random variability in the data for the main outcomes? | Have all important adverse events that may be a consequence of the intervention been reported? | Have the characteristics of patients lost to follow-up been described? |
| Lissitchkov (2015) [42] | | * | * | * | * | * | | * | * |
| Lissitchkov (2017) [51] | | * | * | * | * | * | | * | * |
| Ljung [47] | | * | * | * | * | * | | * | |
| Lopez [43] | | * | * | * | * | * | | * | |
| Manco-Johnson [41] | | * | * | * | * | * | | * | * |
| Musso [34] | | * | * | * | * | * | | * | |
| Oldenburg [21] | | * | * | * | * | * | * | * | * |
| Pollmann [31] | | * | * | * | * | * | | * | |
| Recht [26] | | * | * | * | * | * | | * | |
| Saxena [50] | | * | * | * | * | * | | * | * |
| Seremetis [20] | | * | * | * | * | * | | * | |
| Singleton [32] | | * | * | * | * | * | | | |
| Tiede [48] | | * | * | * | * | * | | * | * |
| Valentino [38] | | * | * | * | * | * | | * | * |
| White [29] | | * | * | * | * | * | | * | |
| Yoshioka [30] | | * | * | * | * | * | | * | |
| Young [18] | | * | * | * | | | | | |

| | EXTERNAL VALIDITY | | | | INTERNAL VALIDITY | | | | | | | POWER | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Q9 | Q10 | Q11 | | Q12 | Q13 | Q14 | Q15 | Q16 | Q17 | | Q18 | |
| | Were the subjects asked to participate representative of the entire population? | Were those subjects who were prepared to participate representative of the entire population from which they were recruited ? | Were the staff, places and facilities representative of the treatment the majority of patients receive? | | If any of the results of the study were based on *data dredging*, was this made clear? | Do the analyses adjust for different lengths of folluw-up of patients? | Were the statistical tests used to assess the main outcomes appropriate? | Was compliance with the interventions reliable? | Were the main outcome measures used accurate (valid and reliable)? | Were losses of patients to follow-up taken into account? | | Did the study have sufficient power? (>100 person-years) | SCORE |
| | * | | * | | * | | | | * | | | | 11 |
| | * | | * | | * | | | * | * | | | | 12 |
| | * | | * | | * | | | * | * | | | | 10 |
| | * | | * | | * | | | | * | | | * | 11 |
| | * | | * | | * | | | * | * | | | * | 13 |
| | * | | * | | * | | | | * | | | * | 11 |
| | * | | * | | * | | | * | * | | | * | 14 |
| | * | | * | | * | | | * | * | | | * | 12 |
| | * | | * | | * | | | | * | | | * | 11 |
| | * | | * | | * | | | * | * | | | | 12 |
| | * | | * | | * | | | | * | | | * | 11 |
| | * | | * | | * | | | * | * | | | * | 11 |
| | * | | * | | * | | | | * | | | | 11 |
| | * | | * | | * | | | * | * | * | | | 13 |
| | * | | * | | * | | | | * | | | * | 11 |
| | | | * | | * | | | | * | | | * | 10 |
| | | | * | | * | | | | * | | | | 6 |

**Supplemental table S3.** information on the distribution of potential confounders in the studies that were included in the main analysis.

| Author | Family history of inhibitors | Treatment type (prophylaxis/on-demand) |
|---|---|---|
| **Advate** | | |
| Blanchette [33] | - | 90.6% prophylaxis<br>3.8% on-demand<br>5.7% on-demand/prophylaxis |
| Den Uijl [36] | - | 65.9% prophylaxis, 34.1% on-demand |
| Valentino [38] | - | Patients were treated with on-demand regimen during the first 6 months, and then with a prophylactic regimen for the following 6 months |
| Fukutake [19] | - | 53.4% Prophylaxis<br>30.7% On-demand<br>15.9% Mixed |
| Hay (cohort 2) [25] | - | - |
| Oldenburg [21] | - | 57.0% Prophylaxis<br>43.0% On-demand |
| Fischer (cohort 1) [23] | - | - |
| **Kogenate, Helixate** | | |
| Aygören-Pürsün [28] | - | - |
| Seremetis [20] | - | - |
| Yoshioka [30] | - | - |
| Singleton [32] | - | 52.1% Prophylaxis<br>38.3% On-demand<br>9.6 % Unknown |
| **Kogenate FS/Bayer, Helixate FS/NexGen** | | |
| Abshire [22] | - | Prophylaxis: 43.8% N. America, 60.7% EU<br>On-demand: 12.1% N. America, 12.9% EU |
| Musso [34] | - | 31.8% Prophylaxis |

| | F8 genotype | Ethnicity | Surgery during follow-up |
|---|---|---|---|
| | | | |
| | 40% intron 22 inversion<br>27% missense mutation<br>13% nonsense mutation<br>11% frameshift mutation<br>5% deletion<br>2% intron 1 inversion<br>2% splice defect | 90.6% Caucasian<br>5.6% African-American<br>3.8% Unspecified | 5 patients underwent a surgical procedure |
| | - | - | 27 surgical procedures |
| | - | 87.7% White<br>5.5% Hispanic<br>4.1% Black/African-American<br>1.4% Asian<br>1.4% Other | - |
| | - | 100% Asian | - |
| | - | - | - |
| | - | 90.8% Caucasian<br>3.3% Black<br>1.5% Asian<br>3.6% Other | 16 surgical procedures<br>(15 patients) |
| | - | - | - |
| | | | |
| | - | - | - |
| | - | - | 25 surgical procedures (22 patients) |
| | - | 100% Asian | 10 patients underwent at least one surgical procedure (not included in analysis) |
| | - | - | - |
| | | | |
| | - | - | 22 surgical procedures<br>(15 patients) |
| | - | 81.8% White<br>1.4% Black<br>0.9% Asian<br>3.6% other | 46 surgical procedures<br>(37 patients) |

| Author | Family history of inhibitors | Treatment type (prophylaxis/on-demand) |
|---|---|---|
| Delumeau [35] | - | 17.6% prophylaxis |
| Young [18] | - | 12.9% regular prophylaxis<br>87.1% Other |
| Collins [37] | - | Patients were treated on-demand for 6 months, followed by 7 months prophylaxis |
| Manco-Johnson [41] | - | 50% prophylaxis<br>50% on-demand |
| Lalezari [27] | - | 100% prophylaxis |
| Gouider [45] | - | 60.2% prophylaxis |
| Hay (cohort 3) [25] | - | - |
| Fischer (cohort 2) [23] | - | - |
| **Refacto** | | |
| Gringeri [24] | - | 100% on demand |
| Pollmann [31] | - | 81 patients treated on prophylaxis for at least one treatment-year<br>39 patients treated on-demand for at least one treatment-year |
| Fischer (cohort 3) [23] | - | - |
| **Refacto AF** | | |
| Recht (cohort 1) [26] | - | 100% prophylaxis |
| Recht (cohort 2) [26] | - | 100% prophylaxis |
| Lopez [43] | - | 74% prophylaxis, 25% on-demand, 1% Other |
| Hay (cohort 1) [25] | - | - |
| Fischer (cohort 4) [23] | - | - |

| | F8 genotype | Ethnicity | Surgery during follow-up |
|---|---|---|---|
| | - | 100% Asian | - |
| | - | 98.6% Asian<br>1.4% Caucasian | - |
| | - | 95% White<br>5% Hispanic | - |
| | - | 90.5% White<br>2.4% Asian<br>7.1% Hispanic | - |
| | - | - | - |
| | - | 81.2% Caucasian<br>2.2% Black<br>5.4% Asian<br>5.9% Other | 18 surgical procedures<br>(15 patients) |
| | - | - | - |
| | - | - | - |
| | | | |
| | - | - | - |
| | 47.0% Intron 22 inversion<br>16.7% Missense mutation<br>10.8% Small deletion or insertion | 100% Caucasian | - |
| | - | - | - |
| | | | |
| | - | 94.7% White<br>5.3% Other | Surgery during study was not permitted |
| | - | 86.4% White<br>13.6% Other | 9 patients underwent at least one surgical procedure |
| | - | 96.6% White<br>1.0% Asian<br>0.5% Black<br>1.9% Other | - |
| | - | - | - |
| | - | - | - |

**Supplemental Table S4.** Sensitivity analysis. Main analysis restricted to studies that only reported information for severe patients (baseline FVIII activity <0.01 IU/ml).

| Variable | N | Inhibitors/ p-y | Pooled inhibitor incidence rate per 1000 p-y (CI95) | Between-study variance ($\tau^2$) | Incidence rate ratio (CI95) |
|---|---|---|---|---|---|
| Overall (main products only) | 20 | 30/16181 | 1.88 (CI95: 0.72-4.92) | 1.6120 | |
| Product | | | | | |
| Advate | 4 | 5/5529 | 0.62 (CI95: 0.11-3.46) | 0 | Ref |
| Kogenate/Helixate | 3 | 4/283 | 6.34 (CI95: 0.01-7819.34) | 1.8502 | 15.63 (CI95: 3.84-63.63) |
| Kogenate FS/Helixate NexGen | 8 | 9/7031 | 1.28 (CI95: 0.58-2.82) | 0 | 1.42 (CI95: 0.44-4.55) |
| Refacto* | 2 | 5/222 | - | - | - |
| Refacto AF | 3 | 7/3116 | 2.30 (CI95: 0.19-28.48) | 0.4149 | 2.48 (CI95: 0.73-8.45) |
| rFVIII length[1] | | | | | |
| Full-length rFVIII | 15 | 18/12843 | 1.14 (CI95: 0.30-4.33) | 1.4962 | Ref |
| B-domain deleted rFVIII | 5 | 12/3338 | 5.19 (CI95: 0.85-31.77) | 1.3310 | 4.49 (CI95: 0.70-28.58) |
| Cell line[2] | | | | | |
| CHO-cells | 9 | 17/8867 | 2.13 (CI95: 0.52-8.67) | 1.7264 | Ref |
| BHK-cells | 11 | 13/7314 | 1.62 (CI95: 0.32-8.09) | 1.5944 | 0.74 (CI95: 0.12-4.53) |
| rFVIII generation[3] | | | | | |
| Second-generation rFVIII | 10 | 14/7253 | 2.40 (CI95: 0.65-8.83) | 1.1894 | Ref |
| First-generation rFVIII | 3 | 4/283 | 6.34 (CI95: 0.01-7819.34) | 1.8502 | 3.56 (CI95: 0.44-28.77) |
| Third-generation rFVIII | 7 | 12/8645 | 1.35 (CI95: 0.44-4.12) | 0.3568 | 0.47 (CI95: 0.10-2.23) |

[1] Full-length rFVIII (Kogenate/Helixate, Kogenate FS/Helixate NexGen and Advate) is compared with B-domain deleted rFVIII (Refacto and Refacto AF).

[2] rFVIII derived from CHO-cells (Refacto, Refacto AF and Advate) is compared with rFVIII derived from BHK-cells (Kogenate/Helixate and Kogenate FS/Helixate NexGen).

[3] First Generation rFVIII (Kogenate/Helixate) is compared with second generation rFVIII (Refacto and Kogenate FS/Helixate NexGen) and third generation rFVIII (Advate and Refacto AF).

* Not enough studies for analysis.

**Supplemental Table S5.** Sensitivity analysis. Main analysis restricted to large studies (i.e. studies with › 150 person-years of follow-up time).

| Variable | N | Inhibitors/ p-y | Pooled inhibitor incidence rate per 1000 p-y (CI95) | Between-study variance ($\tau^2$) | Incidence rate ratio (CI95) |
|---|---|---|---|---|---|
| Overall (main products only) | 15 | 30/16750 | 2.06 (CI95: 1.09-3.91) | 0.5248 | |
| Product | | | | | |
| Advate | 4 | 6/5772 | 1.04 (CI95: 0.28-3.81) | 0 | Ref |
| Kogenate/Helixate* | 1 | 1/254 | - | - | - |
| Kogenate FS/Helixate NexGen | 5 | 10/7012 | 0.88 (CI95: 0.29-2.69) | 0 | 1.37 (CI95: 0.33-5.75) |
| Refacto* | 2 | 6/596 | - | - | - |
| Refacto AF | 3 | 7/3116 | 2.30 (CI95: 0.19-28.48) | 0.4149 | 2.16 (CI95: 0.47-9.86) |
| rFVIII length[1] | | | | | |
| Full-length rFVIII | 10 | 17/13038 | 1.83 (CI95: 1.15-2.91) | 0 | Ref |
| B-domain deleted rFVIII | 5 | 13/3712 | 4.07 (CI95: 1.01-16.39) | 0.7253 | 3.20 (CI95: 1.09-9.40) |
| Cell line[2] | | | | | |
| CHO-cells | 9 | 19/9484 | 2.23 (CI95: 0.79-6.28) | 0.9128 | Ref |
| BHK-cells | 6 | 11/7266 | 1.51 (CI95: 0.70-3.29) | 0 | 0.68 (CI95: 0.18-2.51) |
| rFVIII generation[3] | | | | | |
| Second-generation rFVIII | 7 | 16/7608 | 2.77 (CI95: 0.94-8.18) | 0.6663 | Ref |
| First-generation rFVIII | 1 | 1/254 | - | - | - |
| Third-generation rFVIII | 7 | 13/8888 | 1.49 (CI95: 0.58-3.86) | 0.2716 | 0.51 (CI95: 0.14-1.83) |

[1] Full-length rFVIII (Kogenate/Helixate, Kogenate FS/Helixate NexGen and Advate) is compared with B-domain deleted rFVIII (Refacto and Refacto AF).

[2] rFVIII derived from CHO-cells (Refacto, Refacto AF and Advate) is compared with rFVIII derived from BHK-cells (Kogenate/Helixate and Kogenate FS/Helixate NexGen).

[3] First Generation rFVIII (Kogenate/Helixate) is compared with second generation rFVIII (Refacto and Kogenate FS/Helixate NexGen) and third generation rFVIII (Advate and Refacto AF).

* Not enough studies for analysis.

**Supplemental Figure S1.** Search strategy

**Pubmed**

(((“Factor VIII”[Mesh] OR “Factor VIII”[tw] OR “Factor 8”[tw] OR “Thromboplastinogen”[tw] OR “Hyate-C”[tw] OR “Hyate C”[tw] OR “Factor VIIIC”[tw] OR “F VIII-C”[tw] OR “F VIII C”[tw] OR “FVIII”[tw] OR antihemophilic factor*[tw] OR anti-hemophilic factor*[tw] OR antihaemophilic factor*[tw] OR anti-haemophilic factor*[tw] OR “Factor VIIIa”[tw] OR “Coagulation Factor VIIIa”[tw]) AND (“recombinant”[tw] OR “Recombinant Proteins”[Mesh]) AND (“INH”[tw] OR “inhibitor development”[tw] OR “inhibitors development”[tw] OR (inhibitor*[tw] AND (develop*[tw] OR occurence*[tw])) OR inhibitor*[tw] OR “inhibitory”[tw])) **OR** (“Factor VIII/antagonists and inhibitors”[Mesh] AND (“recombinant”[tw] OR “Recombinant Proteins”[Mesh])) **OR** ((“Advate”[tw] OR “rAHF-PFM”[tw] OR “Refacto”[tw] OR “Refacto AF”[tw] OR “Kogenate-FS”[tw] OR “Kogenate”[tw] OR “Helixate”[tw] OR “Helixate-FS”[tw] OR “Recombinate”[tw] OR “Xyntha”[tw]) AND (“INH”[tw] OR “inhibitor development”[tw] OR “inhibitors development”[tw] OR (inhibitor*[tw] AND develop*[tw]) OR inhibitor*[tw] OR “inhibitory”[tw] OR “antagonists and inhibitors”[Subheading])) **OR** ((“Advate”[ti] OR “rAHF-PFM”[ti] OR “Refacto”[ti] OR “Refacto AF”[ti] OR “Kogenate-FS”[ti] OR “Kogenate”[ti] OR Helixat*[ti] OR “Recombinate”[ti] OR “Xyntha”[ti] OR recombinant factor VIII*[ti])) **OR** ((“Factor VIII”[majr] OR “Factor VIII”[ti] OR “Factor 8”[ti] OR “Thromboplastinogen”[ti] OR “Hyate-C”[ti] OR “Hyate C”[ti] OR “Factor VIIIC”[ti] OR “F VIII-C”[ti] OR “F VIII C”[ti] OR “FVIII”[ti] OR antihemophilic factor*[ti] OR anti-hemophilic factor*[ti] OR antihaemophilic factor*[ti] OR anti-haemophilic factor*[ti] OR “Factor VIIIa”[ti] OR “Coagulation Factor VIIIa”[ti]) AND (“concentrates”[tw] OR “concentrate”[tw]) AND (“INH”[ti] OR “inhibitor development”[ti] OR “inhibitors development”[ti] OR (inhibitor*[ti] AND (develop*[ti] OR occurence*[ti])) OR inhibitor*[ti] OR “inhibitory”[ti]) AND (“Clinical Study”[Publication Type] OR “Epidemiologic Studies”[Mesh] OR “Support of Research”[Publication Type]))) AND (“Hemophilia A”[Mesh] OR “hemophilia”[tw] OR “haemophilia”[tw] OR hemophil*[tw] OR haemophil*[tw]) NOT (“Animals”[mesh] NOT “Humans”[mesh]) NOT ((acquired haemophil*[ti] OR acquired haemophil*[ti]) NOT Congenital*[ti])

**Embase**

(((*”blood clotting factor 8”/ OR “Factor VIII”.ti,ab OR “Factor 8”.ti,ab OR “Thromboplastinogen”.ti,ab OR “Hyate-C”.ti,ab OR “Hyate C”.ti,ab OR “Factor VIIIC”.ti,ab OR “F VIII-C”.ti,ab OR “F VIII C”.ti,ab OR “FVIII”.ti,ab OR antihemophilic factor*.ti,ab OR anti-hemophilic factor*.ti,ab OR antihaemophilic factor*.ti,ab OR anti-haemophilic factor*.ti,ab OR “Factor VIIIa”.ti,ab OR “Coagulation Factor VIIIa”.ti,ab) AND (“recom-

binant".ti,ab OR exp *"Recombinant Protein"/) AND ("INH".ti,ab OR "inhibitor development".ti,ab OR "inhibitors development".ti,ab OR (inhibitor*.ti,ab ADJ5 (develop*.ti,ab OR occurence*.ti,ab)) OR inhibitor*.ti OR "inhibitory".ti OR *"blood clotting factor 8 inhibitor"/)) OR ((*"recombinant blood clotting factor 8"/ OR "Advate".ti,ab OR "rAHF-PFM".ti,ab OR "Refacto".ti,ab OR "Refacto AF".ti,ab OR "Kogenate-FS".ti,ab OR "Kogenate".ti,ab OR "Helixate".ti,ab OR "Helixate-FS".ti,ab OR "Recombinate".ti,ab OR "Xyntha".ti,ab) AND ("INH".ti,ab OR "inhibitor development".ti,ab OR "inhibitors development".ti,ab OR (inhibitor*.ti,ab ADJ5 develop*.ti,ab) OR inhibitor*.ti OR "inhibitory".ti OR *"blood clotting factor 8 inhibitor"/))) AND ("Hemophilia A"/ OR "Hemophilia"/ OR "hemophilia".ti,ab OR "haemophilia".ti,ab OR hemophil*.ti,ab OR haemophil*.ti,ab) AND exp "Humans"/ NOT ((acquired haemophil*.ti OR acquired haemophil*.ti) NOT Congenital*.ti) NOT "conference review".pt NOT "conference abstract".pt

**Web of Science**

(((TS=("blood clotting factor 8" OR "Factor VIII" OR "Factor 8" OR "Thromboplastinogen" OR "Hyate-C" OR "Hyate C" OR "Factor VIIIC" OR "F VIII-C" OR "F VIII C" OR "FVIII" OR antihemophilic factor* OR anti-hemophilic factor* OR antihaemophilic factor* OR anti-haemophilic factor* OR "Factor VIIIa" OR "Coagulation Factor VIIIa") AND TS=("recombinant" OR "Recombinant Protein") AND TI=("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 (develop* OR occurence*)) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor")) **OR** (TS=("recombinant blood clotting factor 8" OR "Advate" OR "rAHF-PFM" OR "Refacto" OR "Refacto AF" OR "Kogenate-FS" OR "Kogenate" OR "Helixate" OR "Helixate-FS" OR "Recombinate" OR "Xyntha") AND TI=("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 develop*) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor"))) AND TS=("Hemophilia A" OR "Hemophilia" OR "hemophilia" OR "haemophilia" OR hemophil* OR haemophil*) NOT TI=(animal* OR "rat" OR "rats" OR "mice" OR "mouse") NOT TI=((acquired haemophil* OR acquired haemophil*) NOT Congenital*)) OR (((TI=("blood clotting factor 8" OR "Factor VIII" OR "Factor 8" OR "Thromboplastinogen" OR "Hyate-C" OR "Hyate C" OR "Factor VIIIC" OR "F VIII-C" OR "F VIII C" OR "FVIII" OR antihemophilic factor* OR anti-hemophilic factor* OR antihaemophilic factor* OR anti-haemophilic factor* OR "Factor VIIIa" OR "Coagulation Factor VIIIa") AND TS=("recombinant" OR "Recombinant Protein") AND TS=("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 (develop* OR occurence*)) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor")) **OR** (TI=("recombinant blood clotting factor 8" OR "Advate" OR "rAHF-PFM" OR "Refacto" OR "Refacto AF" OR "Kogenate-FS" OR "Kogenate" OR "Helixate" OR "Helixate-FS" OR

"Recombinate" OR "Xyntha") AND TS=("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 develop*) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor"))) AND TS=("Hemophilia A" OR "Hemophilia" OR "hemophilia" OR "haemophilia" OR hemophil* OR haemophil*) NOT TI=(animal* OR "rat" OR "rats" OR "mice" OR "mouse") NOT TI=((acquired haemophil* OR acquired haemophil*) NOT Congenital*))

**Cochrane**
(((("blood clotting factor 8" OR "Factor VIII" OR "Factor 8" OR "Thromboplastinogen" OR "Hyate-C" OR "Hyate C" OR "Factor VIIIC" OR "F VIII-C" OR "F VIII C" OR "FVIII" OR antihemophilic factor* OR anti-hemophilic factor* OR antihaemophilic factor* OR anti-haemophilic factor* OR "Factor VIIIa" OR "Coagulation Factor VIIIa") AND ("recombinant" OR "Recombinant Protein") AND ("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 (develop* OR occurence*)) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor")) **OR** (("recombinant blood clotting factor 8" OR "Advate" OR "rAHF-PFM" OR "Refacto" OR "Refacto AF" OR "Kogenate-FS" OR "Kogenate" OR "Helixate" OR "Helixate-FS" OR "Recombinate" OR "Xyntha") AND ("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 develop*) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor"))) AND ("Hemophilia A" OR "Hemophilia" OR "hemophilia" OR "haemophilia" OR hemophil* OR haemophil*)

**CINAHL**
(((("blood clotting factor 8" OR "Factor VIII" OR "Factor 8" OR "Thromboplastinogen" OR "Hyate-C" OR "Hyate C" OR "Factor VIIIC" OR "F VIII-C" OR "F VIII C" OR "FVIII" OR antihemophilic factor* OR anti-hemophilic factor* OR antihaemophilic factor* OR anti-haemophilic factor* OR "Factor VIIIa" OR "Coagulation Factor VIIIa") AND ("recombinant" OR "Recombinant Protein") AND ("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 (develop* OR occurence*)) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor")) **OR** (("recombinant blood clotting factor 8" OR "Advate" OR "rAHF-PFM" OR "Refacto" OR "Refacto AF" OR "Kogenate-FS" OR "Kogenate" OR "Helixate" OR "Helixate-FS" OR "Recombinate" OR "Xyntha") AND ("INH" OR "inhibitor development" OR "inhibitors development" OR (inhibitor* ADJ5 develop*) OR inhibitor* OR "inhibitory" OR "blood clotting factor 8 inhibitor"))) AND ("Hemophilia A" OR "Hemophilia" OR "hemophilia" OR "haemophilia" OR hemophil* OR haemophil*)