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Natural History of Inhibitor Recurrence Following Successful Immune Tolerance Induction

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Abstract Cover Page

Natural History of Inhibitor Recurrence Following Successful Immune Tolerance Induction

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An abstract of
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ABSTRACT

Natural History of Inhibitor Recurrence Following Successful Immune Tolerance Induction

By Ana G. Antun

The formation of factor VIII (fVIII) inhibitory antibodies is a major complication of hemophilia A. Immune tolerance induction (ITI) is successful in up to 70% of patients. The probability and predictors of inhibitor recurrence following successful ITI are unknown. The study's objectives are to determine the influence of discontinuation and adherence of post-ITI prophylaxis on inhibitor recurrence, and the probability of recurrence following successful ITI.

In this multicenter retrospective cohort study, 84 male patients with Hemophilia A who successfully completed ITI based on local institutional criteria were enrolled. Sixty four subjects with fVIII <2% who were considered tolerant following ITI based on a negative inhibitor titer and normalized recovery level and/or half-life were analyzed. Kaplan-Meier method and logistic regression models were used to estimate the probability of inhibitor recurrence at 1, 3 and 5 years and to determine the association between clinical characteristics, including adherence to post-ITI prophylaxis and inhibitor recurrence.

Sixty four (76%) patients with fVIII level < 2% met criteria for tolerance and were included in the analysis: 45 (70.3%) subjects did not have a recurrent inhibitor titer and 19 (29.7%) had at least one inhibitor titer $\geq$ 0.6 BU/ml. The probability of recurrent inhibitor at 1 year was 0.17 (95% CI: [0.05, 0.20]); at 3 years was 0.27 (95% CI: [0.2, 0.4]) and 5 years was 0.35 (95% CI: [0.2, 0.5]). Adherence to prophylactic fVIII infusion was found not to be statistically significant associated with inhibitor-free status (p=0.88). However, the odds of inhibitor recurrence were 11.1 and 9.0 higher in patients who received immunosuppression and in those whose recovery level was < 85%, respectively.

ITI is currently the most effective treatment to eradicate fVIII inhibitors, however 5 years after ITI completion, 30-35% of patients will have at least one inhibitor titer $\geq$ 0.6 BU/ml. A recurrent inhibitor is unlikely after 5 years of ITI. Adherence to post-ITI prophylaxis does not appear to be a major driver of inhibitor recurrence. FVIII recovery level with a cutoff of 85%, and immunosuppression concomitant with ITI were found to be associated with inhibitor recurrence.

Cover Page

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INTRODUCTION

Formation of anti-fVIII inhibitory antibodies (inhibitor) is the major complication of treatment of hemophilia A. Currently immune tolerance induction (ITI) via regular infusion of factor VIII (fVIII) is the most effective method to eradicate the inhibitor and it is successful in up to 70% of the patients [1]. After successful ITI, little is known about the probability of inhibitor recurrence or other parameters that are influential in maintaining tolerance. In clinical practice, continued regular exposure to fVIII (prophylactic treatment) is thought to be imperative for maintaining tolerance, but has not been formally evaluated.

The overall purpose of this study is to estimate predictors of inhibitor recurrence after successful ITI and to determine the impact of poor adherence and discontinuation of prophylactic fVIII treatment on inhibitor recurrence. The patients were identified by retrospective data collection from 12 US Comprehensive Hemophilia Centers.

Some clinical characteristics have been associated with successful ITI but no predictors of inhibitor recurrence following successful ITI have been identified yet. In this study we will analyze factors related to patients, fVIII inhibitor, ITI, and prophylactic treatment to determine their association with inhibitor recurrence. Our primary hypothesis is to test whether fVIII inhibitor recurrence after successful ITI is associated with discontinuation or poor adherence to prophylactic fVIII treatment. As a secondary objective, it is also hypothesized that patients with high inhibitor titer prior to ITI (pre-ITI inhibitor titer), historical peak inhibitor titer,

peak inhibitor titer on ITI and/or patients who received a daily dose of fVIII infusions during ITI are at greater risk of inhibitor titer recurrence.

BACKGROUND

Hemophilia A is a deficiency of fVIII, a vital protein for the formation of a hemostatic clot. It is an inherited disorder, with a defective gene located on the X chromosome. Accordingly, an X-linked pattern of inheritance leads to hemophilia A occurring predominantly in males.

The incidence of Hemophilia A in United States is 1 in 5000 males born and the prevalence is 8 in 100,000 males. [2]

Normal plasma levels of fVIII range from 50 IU/dL to 150 IU/dL. Hemophilia A is classified according to the amount of fVIII present in plasma: 1) Mild, when fVIII levels range between 6% and 49%, [most patients in this category have bleeding problems only after serious injury, trauma or surgery]. 2) Moderate Hemophilia A occurs when fVIII levels range between 1% and 5% of the normal fVIII in blood, [these patients, who are about 15% of hemophilia population, tend to have bleeding following trauma and occasionally spontaneous bleeding]; and 3) Severe Hemophilia A which occurs in about 60% of the hemophilia A population and tend to have a fVIII level <1%, [these patients have bleeding following injury and may have frequent spontaneous bleeding episodes in the muscles and the joints].

The mainstay of hemophilia treatment is replacement of fVIII, which aims to prevent complications that result from prolonged bleeding. FVIII concentrates are prepared from plasma or by using recombinant DNA technology.

The development of antibodies against fVIII is considered by far to be the main complication of the fVIII replacement therapy and represents the most significant risk factor for morbidity and mortality associated with Hemophilia A. The

prevalence of formation of inhibitors is about 5-7% and the cumulative incidence of fVIII inhibitors is estimated to be as high as 33% in patients with severe hemophilia A. [2]

Most patients with hemophilia A are immunologically unresponsive to fVIII replacement therapy but there are a significant proportion of patients, in whom an immune response against fVIII builds up leading to the production of inhibitors of fVIII and subsequent neutralization of the fVIII therapeutic activity. [3] These neutralizing antibodies are called inhibitors as they inhibit the activity of infused factor.

The main goal of the treatment of the inhibitors remains suppression of the production of inhibitor and restoration of a state of responsiveness to fVIII which is achieved by the regular administration of fVIII concentrates until the inhibitor disappears and is currently referred as ITI. ITI is the most effective method to eradicate the inhibitor and is successful in up to 70% of patients. [1]

According with the international consensus (Consensus Proceedings from the Second International Conference on Immune Tolerance Therapy, Bonn 1997, unpublished) successful immune tolerance induction is currently defined as an inhibitor titer < 0.6 BU/ml and normalized fVIII pharmacokinetics (in *vivo* recovery level of > 66% and/or half-life > 6 hours). [4]

Immune tolerance outcomes are influenced by both host and treatment regimen-related factors. The International Immune Tolerance Registry (IITR), The German Registry and North American Immune Tolerance Registry (NAITR) have identified predictors that influence the success of the treatment. Lower pre-ITI

inhibitor titer (< 10 BU/ml), historical peak (< 200 BU/ml); and peak inhibitor titer on ITI (<100 BU/ml) were strongly correlated with time and success of ITI. However, ethnicity has not been shown to influence the outcome. There is no published data to support the role of immunosuppression in primary treatment of ITI or its influence on ITI success. [5][6]

Despite of conflicting data between fVIII dose and success of ITI in NAITR and IITR, a meta-analysis has determined that fVIII dose has not influence in outcomes in those patients with historical inhibitor titers < 200 BU/ml and immediate pre-ITI inhibitor titers < 10 BU/ml. These parameters have been used to define good and poor risk subgroups of ITI. In addition, ITI initiation at younger age and an interval of 5 years or less between the inhibitor onset and the starting of ITI are included in good risk factors group. [7][8] Similar rates of tolerance have been seen using either recombinant or plasma-derived fVIII products. [9]

The proportion of inhibitor recurrence after successful ITI was estimated to be 10%, 4.5% and 2.3% by the NAITR, IITR and PROFIT studies with a median follow up time of 19 months, 9.5 months and 52 months respectively, however the probability of inhibitor recurrence or the parameters that are influential in maintaining tolerance it is not well known or studied. In clinical practice, continued regular exposure to fVIII is thought to be imperative for maintaining tolerance, but has not been formally evaluated. [6][10]

METHODS

Research Goal

The main purpose of this study is to estimate the association between fVIII inhibitor recurrence and discontinuation of prophylactic treatment, factors related to patients, and fVIII inhibitors characteristics while controlling for other clinical and demographics characteristics that are associated with either poor adherence to the treatment or to fVIII inhibitor recurrence. An additional goal is to estimate the probability of fVIII inhibitor recurrence at 1, 3 and 5 years following successful ITI.

Null Hypothesis

There is no association between fVIII inhibitor recurrence and subjects who continue and discontinue prophylactic fVIII infusions following successful ITI or subjects who adhere and do not adhere to prophylactic fVIII infusion following successful ITI. The null hypothesis for the secondary objectives states that there is no difference between subjects with high and low inhibitor titer prior to ITI (pre-ITI inhibitor titer), historical peak inhibitor titer and peak inhibitor titer on ITI, between patients who receive and did not receive daily high dose of replacement fVIII as part of ITI, and between patients who receive and did not receive immunosuppressive therapy in conjunction with ITI.

Alternative Hypothesis

There is an association between fVIII inhibitor recurrence and subjects who discontinued prophylactic fVIII infusions following successful ITI, who are poor

adherent to prophylactic fVIII infusion following successful ITI, who have high pre-ITI inhibitor titer, high historical peak inhibitor titer and high peak inhibitor titer on ITI, who have daily high dose of fVIII as part of ITI, and who receive immunosuppressive therapy in conjunction with ITI.

Study Design

A retrospective cohort study design was used. The main exposure factor was discontinuation of prophylactic fVIII infusion following successful ITI. The main outcome was fVIII inhibitor recurrence after successful ITI.

Patient Selection

All subjects that completed ITI between 1/1/1998 and 8/15/2010 were reviewed to determine if ITI was successful or not. Participating institutions included; University of North Carolina and Emory University/Children's Healthcare of Atlanta; Arizona Hemophilia and Thrombosis Centers at Phoenix Children's Hospital; Mountain States Regional Hemophilia and Thrombosis Center, Aurora Colorado; Children's Hospital of Los Angeles; Blood Center of Wisconsin; Children's Hospital Michigan; Children's National Medical Center, Washington D.C.; Children's Mercy Hospital, Kansas City; Hemophilia Center of Western PA; Miami Comprehensive Hemophilia Center; and Tulane University. All persons who had successfully completed ITI based on the local institutional criteria between 1/1/1998 and 8/15/2010 (11.5 year time span) were enrolled in the study. This 11.5 year time frame was chosen because in 1998 the Centers for Disease Control

and Prevention began the Universal Data Collection project. As a result of the increased attention to data collection, centers may have begun more rigorous record keeping at that time. Although one could include a longer window of time to capture more patients, this would lead to an even more heterogeneous population and reduced validity of the retrospective data. A smaller window, such as 5 years, would provide a more homogenous group, but would limit the ability to estimate long term follow-up and further limit the sample size.

Data Collection

Data was captured from review of medical record prior to 8/15/2011 and recorded on standardized case report form.

Study variables include: subjects demographics (year of birth, race, ethnicity, family history of hemophilia and inhibitor) hemophilia history (fVIII level and *f8* mutation if known), inhibitor characteristics (initial inhibitor titer, peak inhibitor titer prior to start ITI [historical peak], inhibitor titer at the start of ITI), characteristics of ITI regimen (product used, reported first successful course of ITI, dose, frequency of infusion, duration, use of concomitant immune modulating agents and fVIII recovery and half-life at the end of ITI), presence of HIV or HCV at the time tolerance was achieved, and post-ITI prophylaxis (regimen, duration of tapering [from time of tolerance until final prophylaxis regimen], and adherence to prophylactic regimen). To determine adherence, participating medical centers were asked to estimate adherence (> 75%, > 50-75%, 25-50%, and < 25%) during 6 months prior to inhibitor recurrence or the last negative inhibitor titer and also was

categorized into > 80% and < 80%. These percentages were determined by comparison of the actual treatment regimen (as determined by infusion logs/calendars and/or pharmacy information) with the prescribed treatment regimen [(# actual infusions/#prescribed infusions) x 100]. Subjects were considered adherent to post-ITI prophylaxis if they had received > 80% of their prescribed infusions. [3]

Measurements

The primary outcome was fVIII inhibitor recurrence defined as an inhibitor titer (>0.6 BU/ml). Inhibitor titer was measured by using Bethesda assay at each center and expressed in Bethesda Units per milliliter (BU/ml). Historical peak inhibitor titer was defined as the highest inhibitor titer recorded before the start of ITI. The inhibitor titer prior to ITI, (pre-ITI inhibitor titer) was considered the inhibitor titer assayed most immediately prior to the beginning of ITI. The adherence to prophylactic treatment was assessed for the period of 6 months prior to the development of inhibitor recurrence or the negative inhibitor titer. To calculate recovery levels the following formula was used: dose fVIII in IU/kg x 2 and the fVIII half-life at the end of ITI was recorded as reported by the center.

Complete tolerance was defined as a negative inhibitor titer < 0.6 BU/ml and one normalized fVIII half-life (>6 hours) and/or fVIII recovery > 66%. If only inhibitor titers were performed and no measurement of recovery or half-life, then the subject was not included in the data analysis. Follow up time began when tolerance was complete. For subjects who had only inhibitor titers and recovery

measurements, but no formal half-life measurement, the time that the recovery was >66% was considered the point when the patient was first tolerized. If half-life measurement was performed, then the time that the half-life was > 6 hours will be the beginning of the follow-up time.

Follow-up time will begin at the date that the subject was considered to be tolerant and continue until the last recorded negative inhibitor titer or inhibitor recurrence (inhibitor titer > 0.6 BU/ml).

Sample Size and Power Calculations

The sample size was calculated based on an institutional experience where approximately 18 persons were successfully tolerized. Anticipating that an average of 15 persons successfully completed ITI in each center, and then 10 centers would be required to gather 150 persons. If persons are followed for an average 3 years, then 150 subjects would provide 450 person year of follow up.

Power calculations done before the analysis were based on the limited published literature of the influence of discontinuing prophylactic fVIII treatment [1]. Assuming that a 25 % of patients discontinued fVIII prophylactic infusions, 150 patients are needed (38 patient that discontinued fVIII infusions and 112 that continued on fVIII infusions) to achieve a power of 80% with a significance level of 0.05 to detect a difference ranging from 0.17 to 0.24 in the proportion of patients without inhibitor recurrence that discontinued and continued prophylactic fVIII infusions respectively. Power was calculated assuming that under the null

hypothesis, the proportion of patients who do not have inhibitor recurrence ranges from 8% to 21%.

Database Management

Data was entered into REDCap; an electronic data capturing tool. This system is supported by the Emory Research and Health Sciences IT division and hosted through Emory University Technology Services (UTS) on a virtual machine (VM) environment with nightly backup and full redundancy for high application availability and reliability.

Analytic Plan

First the patient related factors, inhibitor characteristics, characteristics related with immune tolerance, post ITI prophylaxis and events related with inhibitor recurrence were reported for all adult patients that successfully complete immune tolerance. Bivariate analysis was used to examine associations between clinical variables and inhibitor recurrence. Two sample equal and unequal variance t-tests were used to compare continuous variables between inhibitor recurrence group when the data were normally distributed. Likewise, Mann-Whitney-U-test and Kolmogorov-Smirnov were used for non-normally distributed variables. Differences in proportions of categorical variables were tested using exact chi square tests. Confidence intervals (CI) were calculated using the Wald method. Significance of the odds ratio was determined using the Chi-square test. All reported p-values were two sided, with $p < 0.05$ considered statistically significant.

Two primary predictors of fVIII inhibitor recurrence were analyzed; characteristic of subjects, inhibitors characteristics and characteristics of ITI were speculated to be different between the group of patients that continue vs. discontinue and those that were adherent vs. not adherent to prophylactic treatment post ITI. To compensate for baseline differences in a relatively small cohort of patients, propensity analysis was utilized. Propensity score was constructed to model the probability of adherence. Variables selected to predict adherence were based on clinical characteristics (i.e., frequency of fVIII infusion during ITI, duration of ITI and pre-ITI inhibitor titer) and patient characteristics (i.e., age, sex, race) that were clinically known or demonstrated to be related to adherence to fVIII prophylactic regimen and/or relapse. Logistic regression was used to model the probability of adherence as predicted by these covariates. Model fit was assessed using model fit statistics and area under the receiver operating curve. Propensity scores were obtained from the predicted probability of adherence and used as a covariate adjustment in all subsequent models.

The outcome of interest was recurrent fVIII inhibitor which was treated in 2 different ways: 1) as a dichotomous variable included in the logistic model and 2) time to event or recurrence of inhibitor with Kaplan Meier curves and Cox proportional models. The inclusion of interaction terms and other terms in the multivariate model was assessed using the Wald test and likelihood ratio test.

Correlation was used to examine collinearity between predictors prior to obtaining the final model. Other predictors of fVIII inhibitor recurrence were analyzed using logistic regression including 3-4 variables. Covariates that were

included in the model were those with a *p*-value< 0.1. Effect modification and confounding was assess fitting interaction terms in each model.

A cut point analysis was performed to determine the fVIII recovery level that best predicts inhibitor recurrence. This involved dichotomizing the recovery level into binary groups and examining sensitivity and specificity of the dichotomous variable to predict relapse.

Time to fVIII inhibitor recurrence was examined using survival analysis. Proportional hazards (PH) assumptions were evaluated for each variable using graphic plot and by including the interaction between the logarithm of time and the predictor variable in each model. Significant interactions with time or non-parallel plots were indications that the PH assumption was not valid. If any of the variables did not meet the proportional hazard assumption, stratification was performed. SAS version 9.1.3 (Cary. NC) was used to perform all analyses.

RESULTS

Descriptive Statistics

After Institutional Review Board approval at 12 US Comprehensive Hemophilia Treatment centers, potential subjects were identified by review of patient databases. One hundred eighteen patients that completed ITI between 1/1/1998 and 8/15/2010 were reviewed to determine if ITI was considered clinically successful or not. Ninety one patients (77%) were considered by their treating center to have successfully completed ITI based on the local institutional criteria during the 11.5 year time span **Table A.** Of those 91 who were considered to have successfully completed ITI, 7 were excluded due to lack of documentation of a negative inhibitor titer at the end of ITI. A total of 84 patients were enrolled in the study.

The criterion for complete tolerance (negative fVIII inhibitor titer and normalized fVIII recovery [>66%] and/or half-life [6 hours]) was met in 79.8% of the patients (n=67) whereas 20% (n=17) did not meet the criteria for tolerance. Of those patients that tolerance was confirmed, 4.5% (n=3) had a fVIII level >2% and were excluded from the analysis, 95.5% (n=64) had a fVIII level < 2%.

Of those 64 patients with a fVIII level < 2%, 64% (n=41) continued on prophylactic fVIII infusions and were adherent to treatment infusing 80% or more of prescribed treatment during the 6 months prior to recurrence of inhibitor or last follow up; 26.5% (n=17) were not adherent to the prophylactic treatment post ITI including one patient that discontinued prophylactic treatment, and 9.3 % (n=6) were unable to assess adherence therefore were excluded from analysis of

adherence. Recurrent fVIII inhibitors were detected in 29% (n=12) of the 41 subjects in the adherent group and 29% (n=5) in the not adherent group **Figure 1.**

Bivariate Analysis

Several parameters were examined as possible predictors of inhibitor recurrence following ITI.

Patient Characteristics

When comparing subjects with and without inhibitor recurrence, our findings showed no statistically significant difference in the median age at the time of inhibitor was diagnosed between the two groups (1.2 and 1.8 years) and no difference in the median age when ITI was started (2.6 and 3.3 years). Amongst those with inhibitor recurrence 79% (n=15) subjects were white vs. 69% (n=31) of those without inhibitor recurrence. These two groups were similarly distributed and no statistically difference between them was found.

Most of the patients in both groups had severe disease with fVIII level <1% except one patient in each group which have a fVIII activity of 1-2% (moderate).

Only 31 subjects in both groups had a genotype testing available. High risk mutations (intron22, intron 1 inversion, large deletions [> 50 base pairs] and nonsense mutations) were seen in 31.6% (n=6) of subjects with and 28.9% (n=13) of subjects without inhibitor recurrence. Seven percent (n=3) in the group without recurrence were HIV positive; 10.5% (n=2) with recurrent inhibitor and 8.9% (n=4) without a recurrent inhibitor had a positive hepatitis C viral load **Table 1.**

Inhibitor Characteristics

The influence of first inhibitor titer (BU/ml), historical peak inhibitor titer prior to ITI (BU/ml), pre-ITI titer (BU/ml) and peak titer during ITI (BU/ml) on outcome were assessed. All four parameters had no statistically significant association with the outcome (*p*-value >0.5) **Table 2.**

The median follow up time was 3.4 years (IQR: 5.7) and the probability of inhibitor recurrence at one, three and five years was 17 % (95% CI, 5-22), 27% (95% CI, 16-42) and 35% (95% CI, 22-46) respectively as shown by the Kaplan-Meier inhibitor free survival curve **Figure 5.**

Immune Tolerance Characteristics

The impact of the time from inhibitor onset to the start of ITI was assessed using both the median time and categorized as a more than or less than 2 years. No association between time from inhibitor onset to the start of ITI with recurrent inhibitor was seen.

No differences were found when comparing ITI regimen-related characteristics among subjects with and without inhibitor: first course of ITI, product used for ITI (recombinant vs. non-recombinant), frequency of the infusions during ITI (once or twice daily vs. three-four times a week), dose of fVIII use for ITI (>< 100 IU/kg/day) and duration of ITI in years.

A total of 5 patients (8%) received immunosuppressive therapy as part of their ITI regimen: (rituximab [n=3], mycophenolate [n=2], cyclophosphamide [n=1], methotrexate [n=1], and plasmapheresis [n=1]). Four of them (80%) had a

recurrent fVIII inhibitor and 1 (20%) who received plasmapheresis did not; the difference between these two groups was statistically significant (*p*-value of 0.007) indicating a possible association with the outcome. The median follow up time was 0.42 years (range 0.08-0.68) Table **3.**

Post Immune Tolerance Characteristics

Of 64 patients included in the analysis, 19 patients (29.7%) with a recurrent inhibitor, and 45 patients (70.3%) without a recurrent inhibitor had achieved tolerance (defined as inhibitor titer of < 0.6 BU/ml as a result of treatment and normal kinetics as measured by factor recovery level >66%). Seven (36.8%) patients in the inhibitor recurrence group and 20 (44.4%) in the no recurrence group met three of the tolerance criteria: inhibitor titer < 0.6 BU/ml, fVIII half-life > 6 hours and fVIII recovery level > 66%. Twelve patients (63%) in the inhibitor recurrence group and 24 (53.3%) in the group without inhibitor recurrence met the tolerance criteria with a negative inhibitor titer < 0.6 BU/ml and fVIII recovery level > 66%. The median recovery level was 79% (range 84) and 90.8 % (range 165) in subjects with and without inhibitor recurrence respectively. This difference was statistically significant with a *p*-value of 0.03 indicating an association with the outcome.

FVIII median half-life and after categorizing into three groups (6-8 hours, 8-10 and >10 hours), and duration of treatment taper following ITI (<1, 1-3, 3-6 and > 6 months) were assessed and found not to be statistically different between groups.

Only one patient (2.2%) in the group without inhibitor recurrence discontinued prophylactic fVIII infusions after tolerance was achieved which was included in the not adherent group Table **3.**

As shown in Table 3, the effect of discontinuation of prophylactic fVIII infusion on fVIII inhibitor recurrence was unable to be determined due to the small sample size and lack of statistical power. Therefore, we shifted our focus to adherence to prophylactic fVIII infusion as a primary predictor of fVIII inhibitor recurrence.

Propensity Score

Characteristics of the patients, the inhibitor, and the immune tolerance were compared between patients that were adherent (n=41) and those that were not adherent (n=17) to prophylactic fVIII infusions following completion of ITI. Three patients (17.6%) in the not adherent group had a historical peak inhibitor titer prior to ITI greater than 200 BU/ml while in the adherent group all the patients had a historical peak inhibitor titer less than 200 BU/ml. Thirty six (87.8%) in the adherent group, and 8 patients (50%) in not adherent group took less than 2 years to complete ITI (*p*-value of 0.005). The time from inhibitor onset to the beginning of immune tolerance was shorter in the adherent group with a median of 0.41 years (5.5 months) compared to 2.4 years in the not adherent group (*p*-value of 0.02). Duration of immune tolerance, time from inhibitor to start ITI, and age at start of ITI were different between groups but were highly correlated with each other, therefore only duration of immune tolerance was included in the model **Table 4.**

In order to have a balanced distribution of covariates between the adherent and not adherent groups, and to adjust for possible confounders a propensity score was estimated using a logistic regression model. Frequency of fVIII infusion during ITI, duration of ITI and pre-ITI inhibitor titer were included in the final propensity score model, ROC was used to determine the quality of the model which was 0.80.

After fVIII recovery level was dichotomized, sensitivity analysis was performed to facilitate the clinical decision making. A recovery level of 85% with a sensitivity of 79% and specificity of 59% (*p*-value of 0.006), was chosen to evaluate the impact of recovery at the end of ITI on inhibitor recurrence **Table 5.**

The propensity score and the variables that were associated with inhibitor recurrence, on bivariate analysis (shown in Table 3-4) were included in the logistic regression analysis. The final model included: fVIII inhibitor recurrence (outcome) = adherence to prophylactic fVIII/propensity score/immunosuppression and fVIII recovery level < or > 85%. Being not adherent to prophylactic fVIII infusions after ITI (adjusted OR= 0.5 (95% CI: 0.06-4.3) was not statistically associated with inhibitor recurrence following successful ITI, (*p*-value: 0.53). The OR associated with receiving immunosuppression at the time of ITI was 11.1 (95% CI: 0.99-125), *p*-value=0.05. The odds of relapsing with a recovery level <85% was 9 times higher than relapsing with >85%, (CI: 1.4-55.6) *p*-value=0.02 **Table 6.** The probability of relapsing up to 5 years in subjects adherent and not adherent to prophylactic fVIII infusions, receiving or not concomitant immunosuppression and starting prophylactic treatment with recovery levels < or > 85% are shown by the Kaplan-Meier inhibitor free survival curves **Figure 2, 3 and 4.**

Proportional hazard model was used to analyze the effect of time to relapse and patient characteristic variables. Immunosuppression was the only variable that did not meet the proportional hazard assumption therefore stratification was performed showing that the odd of relapsing with a recovery level <85% controlling for immunosuppression was 4.2 (95% CI: 0.9-18.7) times higher than with a recovery level > 85%, p-value of 0.06 **Table 7.** A small number of subjects received immunosuppression (5 patients) but it was noted that 4 subjects (80%) had a recurrent inhibitor titer of > 0.6 BU/ml in the first 6 months after completion of ITI.

Variables Influencing fVIII Inhibitor Recurrence

Adherence to prophylactic fVIII infusion appeared not to be a predictor of recurrent fVIII inhibitor when evaluated in the logistic model adjusting for immunosuppression and recovery levels. Therefore adherence was not included in the subsequent model where other clinical parameters were evaluated as possible predictors of inhibitor recurrence **Table 8.**

Logistic regression was used to evaluate demographic and clinical characteristics that could be associated with having a recurrent inhibitor titer following ITI. Each variable assessed in the bivariate analysis was tested in the model, interactions and effect modifications were not found except for a trend toward longer time from inhibitor onset to the start ITI (>2 years). Accordingly, time from inhibitor onset to start of ITI in addition to immunosuppression and recovery levels were all represented in the multivariate logistic regression model. In the final model, time from inhibitor onset longer than 2 years showed an OR of 3.5 (95% CI:

0.69-18.0), *p*-value=0.13, which was not statistically significant. However, receiving concomitant immunosuppression and starting prophylactic treatment with recovery levels < 85% showed again to be independently associated with inhibitor recurrence. The power of the model was assessed with ROC=0.78 **Table 9.** Stratified cox model by immunosuppression was again performed with similar results as shown in **Table 7**.

DISCUSSION

We report the results of a retrospective study that assess fVIII inhibitor recurrence in patient with severe hemophilia A after successfully completing ITI. We hypothesized that discontinuing and poor adherence to prophylactic fVIII therapy would increase the risk of inhibitor recurrence. Unexpectedly, most of the patients continued prophylactic treatment which prevented us from assessing our first question. The analysis did demonstrate a significant risk of recurrent inhibitor, a lack of association of adherence, and a statistically significant association between recovery levels and the use of immunosuppression. Poor adherence to prophylactic treatment was not associated with inhibitor recurrence.

The percentage of fVIII inhibitor recurrence reported in this study was 29.7% which is higher than that reported in the NAITR, IITR and PROFIT studies which were 10%, 4.5% and 2.3% respectively. We reported a median follow up of 39.6 months (3.3 years) longer than 11 and 9.5 months published by the NAITR and IITR but shorter than 53 months compared with the PROFIT study. The probability of recurrence in our study was 17% and 35% at 1 and 5 years respectively. We have found no recurrent inhibitor after 5 years of completion of successful ITI. The use of more strict definition of success ITI by these three studies could explain in part the highest relapse rate reported in our study.

On both bivariate and multivariate level, there is a lack of strong influence of adherence on prophylactic fVIII infusions after successfully completed ITI; in contrast to a belief in clinical practice that adherence to prophylactic fVIII infusions strongly influences inhibitor recurrence.

Although weak associations are difficult to exclude with a small sample size, the point estimate in the analysis was 0.5 consistent with minimal, if any effect. Additionally, in this small cohort, we were unable to assess whether the influence of adherence to fVIII prophylaxis changes over time.

In this study a sensitivity analysis has showed that recovery level of 85% has a sensitivity of 79% and specificity of 59% (*p*-value of 0.006). FVIII recovery level less than 85% was associated with 4 times greater risk of inhibitor recurrence than in those greater than 85%. Currently, a level greater than 66% is used by physician as cut off of normalized recovery level and an indicator of emerging tolerance. Our study differs from published data [1] [9] as we found that the median fVIII recovery level in patient with recurrent inhibitor was lower (79%) and statistically significant (*p*-value 0.03) than those without inhibitor recurrence (90.8%). The only other published assessment of the impact of pharmacokinetics on inhibitor recurrence was from the IITI study which reported a reduced fVIII recovery level ranging between 27% and 61% in 6 patients with recurrent inhibitor during a 12 months follow up time after ITI. This study was limited in addressing the implications of reduced fVIII recovery levels in patient with inhibitor recurrence due to a small sample size and to shorter follow up period. [7] Ideally, this finding could be confirmed in other cohorts or on prospective investigation. If confirmed, not only could help physicians in making more informed decisions about inhibitor recurrence and length of ITI but also could have economic implications.

Second, there is no consensus about the use of immunosuppression as a first line therapy in conjunction with ITI. [10] In our study, a small number of patients

received immunosuppression therapy concomitantly with ITI. Three of the 4 patients who relapsed received immunosuppression with Rituximab. It is not clear if the indication of immunosuppression in conjunction with ITI performed by the treating physician was related with the severity of the bleeding or as a common practice but a confounding by indication is difficult to exclude and could explain some of these findings. In addition the transient effect of Rituximab decreasing the inhibitor level could lead to an earlier withdrawal of ITI as a consequence of a false perception of tolerance. Conclusion regarding this statistically significant difference between patient who received and did not received immunosuppression concomitant with ITI cannot be drawn due to the number of subjects receiving immunosuppression was too small.

The limitations of this analysis were driven by the low power of the study. With a small sample size there is a reduce chance of proving our hypotheses, reduced likelihood of statistically significant results, and increased risk of overestimating the size's effect. Barriers in recruitment of patients in rare diseases populations are major challenges for this type of studies.

Additionally, as is the case with any observational study, there is a concern for unmeasured confounding. Lastly, despite a thorough review of clinical data bases, there is a possibility that some patients were omitted.

The strength of the study is that it was a multi-institutional study representing a broad distribution of patients thereby limiting the variability of institutional practices.

In summary, the data obtained in this study, the first one to address predictors of fVIII inhibitor recurrence following successful ITI, showed that the probability of inhibitor recurrence increased in the first five years after ITI and became less likely after that period of time and is not significantly influenced by adherence to post-ITI prophylaxis. Consideration should be given to increasing the recovery level to 85% or greater as a marker of emerging tolerance. Ideally the influence of recovery level and the use of immunosuppression would be investigated in a prospective study to better elucidate the most effective practice.

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Figure 1: Patients flow chart

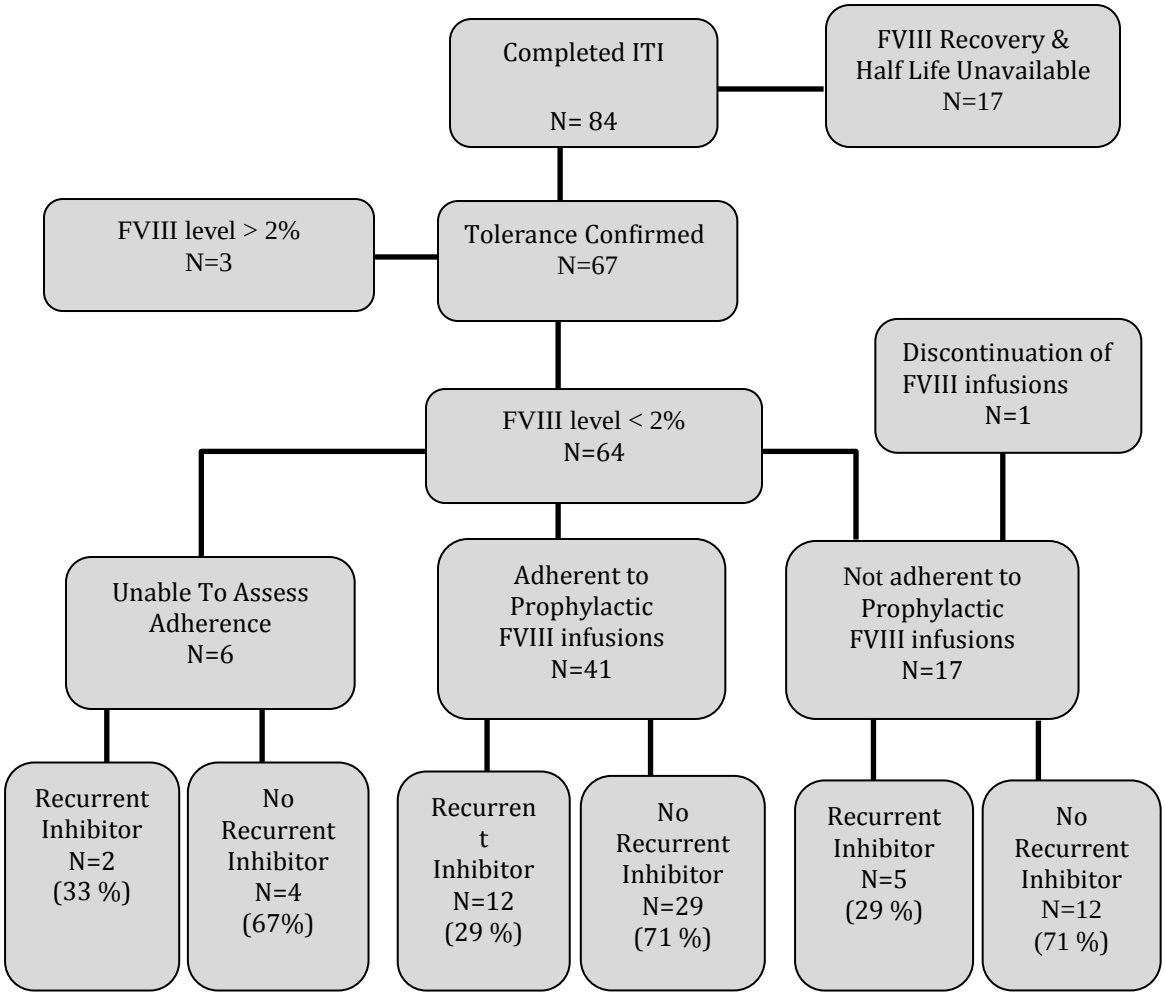

Table 1: Characteristics of subjects with and without inhibitor recurrence

Characteristic	**Recurrent Inhibitor N=19**	**No Recurrent Inhibitor N=45**	***p*-value**
Age at inhibitor onset years, median (range)	1.2 (19.7)	1.83 (30.9)	0.20
Age at start ITI years, median (range)	2.6 (20.0)	3.3 (32.6)	0.55
Race			0.74
White n, (%)	15 (79.0)	31 (69.0)	
Black n, (%)	3 (16.0)	9 (20.0)	
Other n, (%)	1 (5.0)	5 (11.0)	
Hispanic ethnicity n, (%)	6 (32.0)	10 (22.0)	0.53
Hemophilia disease severity			
Severe disease n, (%)	18 (94.8)	44 (98.0)	1.0
Moderate n, (%)	1 (5.2)	1 (2.0)	
Family History of inhibitor n, (%)	11 (57.9)	28 (62.2)	0.75
Genotype (n=31)			0.92
High risk n, (%)*	6 (31.6)	13 (28.9)	
Low risk n, (%) **	4 (21.0)	8 (17.8)	
HIV n, (%)	0	3 (6.7)	
Hepatitis C n, (%)	2 (10.5)	4 (8.9)	0.85

*high risk : intron 22, intron 1 inversion, large deletions and nonsense mutations
** low risk: small deletion or insertion, missense mutation and splice site

Table 2: Characteristics of fVIII inhibitor in patients with and without inhibitor recurrence

Characteristic	**Recurrent Inhibitor N=19**	**No Recurrent Inhibitor N=45**	***p*-value**
First Inhibitor titer (BU/ml), median (range)	6 (127.3)	4.2 (85.3)	0.57
Historical peak inhibitor titer prior to ITI (BU/ml), median (range)	11.2 (543.1)	8.5 (207.3)	0.77
Peak inhibitor titer n, (%)			0.21
>200 BU/ml	2 (10.5)	1 (2.1)	
<200 BU/ml	17 (89.5)	44 (97.8)	
Pre-ITI titer(BU/ml), median (range)	2.0 (11.7)	2.5 (30.0)	0.71
			0.63
>10 BU/ml n, (%)	2 (10.5)	3 (6.7)	
<10 BU/ml n, (%)	17 (89.5)	42 (93.3)	
Peak titer after ITI(BU/ml), median (range)	10 (278.0)	2.7 (275)	0.36

Table 3: Characteristics of immune tolerance induction in patients with and without inhibitor recurrence

Characteristic	Recurrent Inhibitor N=19	No Recurrent Inhibitor N=45	*p*-value
Time from inhibitor onset to start ITI years, median (range)	0.71 (15.9)	0.55(30.9)	0.67
< 2 years	12 (63.2)	32 (71.1)	0.53
>2 years	7 (36.4)	13 (28.9)	
First course ITI n, (%)			
Yes	18 (94.7)	43 (95.6)	1.0
Product used for ITI			0.95
Recombinant n, (%)	17 (89.5)	40 (89.0)	
Non- recombinant n, (%)	2 (10.5)	5 (11.0)	
Frequency of infusion during ITI			0.73
Once or twice daily n, (%)	16 (84.2)	34 (77.3)	
Three-four times week n, (%)	3 (15.8)	10 (22.7)	
Dose of fVIII use for ITI n, (%)			0.52
<100 IU/kg/day	3 (15.8)	12 (26.7)	
>100 IU/kg/day	16 (84.2)	33 (73.3)	
Immunosuppression used during ITI (n, %)	4 (21.1)	1 (2.2)	0.007
Duration of ITI, years median (range)	0.75 (8.0)	1.21 (5.7)	0.42
Tolerance			0.71
Negative titer/recovery > 66%	12 (63)	24 (53.3)	
Negative titer/ half-life > 6h	0	1 (2.3)	
Negative titer/half-life/recovery level	7 (36.8)	20 (44.4)	
Recovery %, median (range)	79 (83.9)	90.8 (165)	0.03*
Half-life hours, median (range)	7(2.8)	8 (8.0)	0.18
			0.41
6-8 hours n, (%)	3 (15.8)	8 (17.8)	
8-10 hours n, (%)	1 (5.3)	3 (6.7)	
>10 hours n, (%)	0	5 (11.1)	
Post ITI prophylaxis discontinuation			
Yes n, (%)	0	1 (2.2)	
No n, (%)	19 (100)	44 (97.8)	
Adherence			0.75
≥80% n, (%)	12 (63.2)	29 (64.4)	
≤80% n, (%)	5 (26.3)	12 (26.7)	
***Kolmogorov-Smirnov two sample test**			

Table 4: Characteristics associated with adherence (>< 80%) to fVIII prophylaxis

Characteristics of adherence			
Characteristic	**Adherent >80% N=41**	**Not Adherent <80% N=17**	***p*-value**
Age at start ITI, median (range)	2.3 (30.0)	10.7 (31.7)	0.003
Historical peak inhibitor titer prior to ITI (BU/ml) (>200 BU/ml)	0	3 (17.6)	
Frequency of infusion ITI n, (%)			0.26
Once or twice a day	34 (85.0)	11 (68.8)	
Three or four times week	6 (15.0)	5 (31.2)	
Duration of ITI, years, median (range)	1.1 (5.3)	1.2 (8.0)	0.56
			0.005
>2 years n, (%)	5 (12.2)	8 (50.0)	
<2 years n, (%)	36 (87.8)	5 (50.0)	
Time from inhibitor onset to start ITI, median (range)	0.41(15.5)	2.4 (18.1)	0.02

Table 5: Sensitivity Analysis of fVIII recovery level

Recovery Level (%)	SENS (%)	SPEC (%)	*p*-value
75	**37**	**68**	**0.7**
80	**63**	**63**	**0.05**
85	**79**	**59**	**0.006**
90	**89**	**50**	**0.003**
95	**89**	**39**	**0.026**

Table 6: Propensity score, logistic regression model

Variables	Adjusted OR	95% C.I.	*p*-value
Adherence (non vs. adherent)	0.5	0.06-4.3	0.53
Immunosuppression (yes vs. no)	11.1	0.99-125.0	0.05
FVIII Recovery (≤85% vs. >85%)	9.0	1.4- 55.6	0.02

Figure 2: Kaplan-Meier curves of fVIII inhibitor recurrent and adherence to prophylactic treatment (n=58)

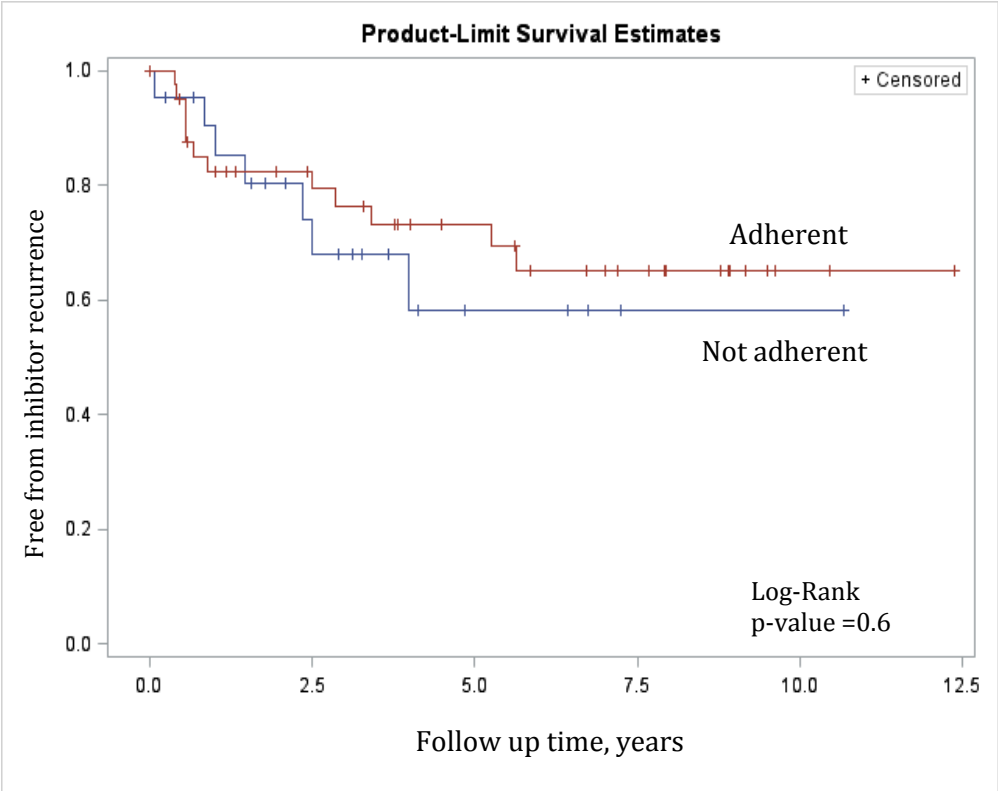

Figure 3: Kaplan-Meier curves of fVIII inhibitor recurrent and use of immunosuppression (n=64)

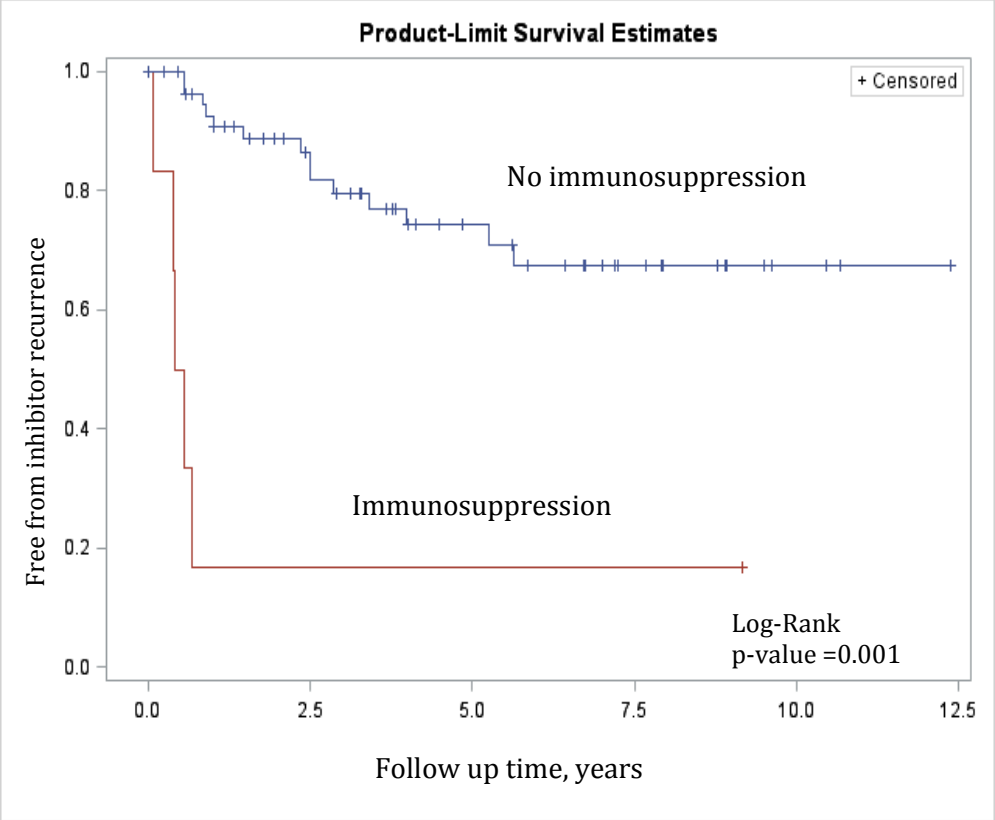

Figure 4: Kaplan-Meier curves of fVIII inhibitor recurrent and recovery level (n=63)

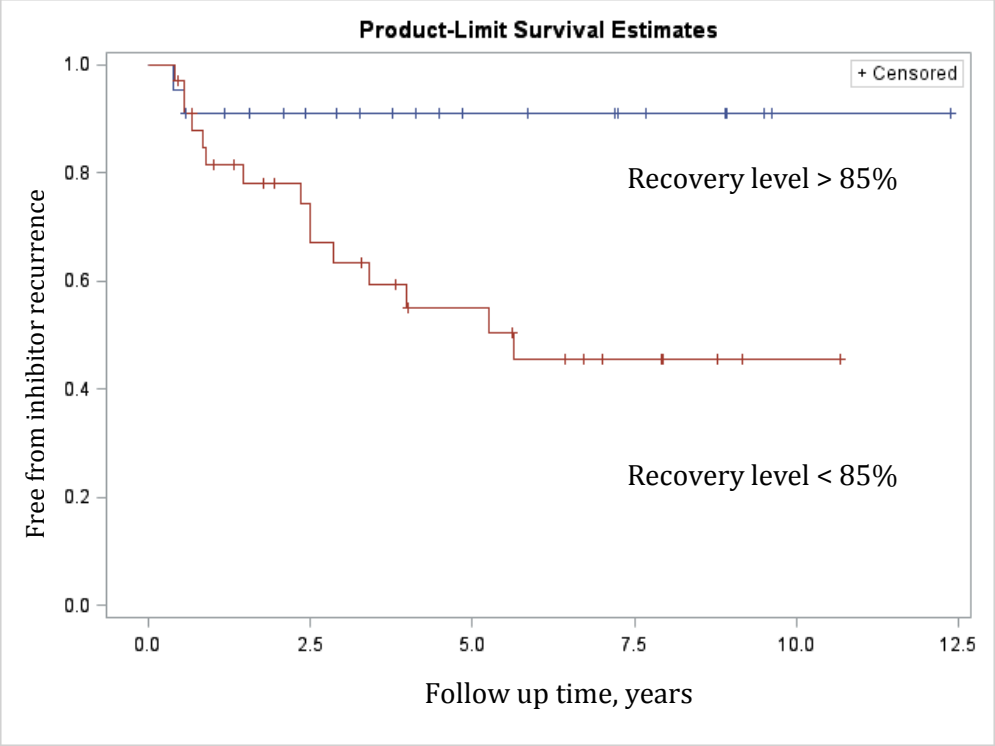

Table 7: Cox Model: follow-up * fVIII inhibitor recurrence (0) = adherence + propensity score + fVIII recovery level 85%, stratified by immunosuppression

Variables	**Adjusted HR**	**95% C.I.**	***p*-value**
Adherence	0.9	0.2-5.2	0.9
FVIII Recovery (≤85% vs. >85%)	4.2	0.9-18.7	0.06

Table 8: Logistic regression additional variables evaluation

Additional Variables	**-2 Log L**	**OR**	***p*-value**	**EM**
Age at start ITI	55.3	1.01	0.97	No
Age at inhibitor onset	55.2	1.01	0.80	No
Race- Black vs white	54.4	1.22	0.30	No
Race- Other vs white	54.2	0.4	0.39	No
Ethnicity: Hispanic	55.2	1.64	0.76	No
Historical peak inhibitor Titer (>200 BU/mL)	54.2	3.57	0.31	No
Titer at start ITI	53.6	0.17	0.25	No
Frequency of ITI fVIII infusions- Weekly	55.1	1.13	0.89	No
Dose of fVIII during ITI< 100 IU/kg/day	55.3	0.91	0.91	No
Time from inhibitor onset to start ITI	53.4	3.03	0.18	No
Peak titer after start ITI	55.3	1.00	0.86	No
EM: effect modification				

Table 9: Final multivariable logistic regression model

Variables	OR	95% CI	*p*-value
fVIII Recovery ≤85% vs. >85%	8.9	1.8-43.4	0.007
Immunosuppression used in ITI	19.6	1.7-227.3	0.017
Time from inhibitor onset to start ITI ≥2 y vs. <2 y	3.5	0.69-18.0	0.13

Figure 5: Kaplan-Meier survival curves to estimate probability of inhibitor recurrence (n=64)

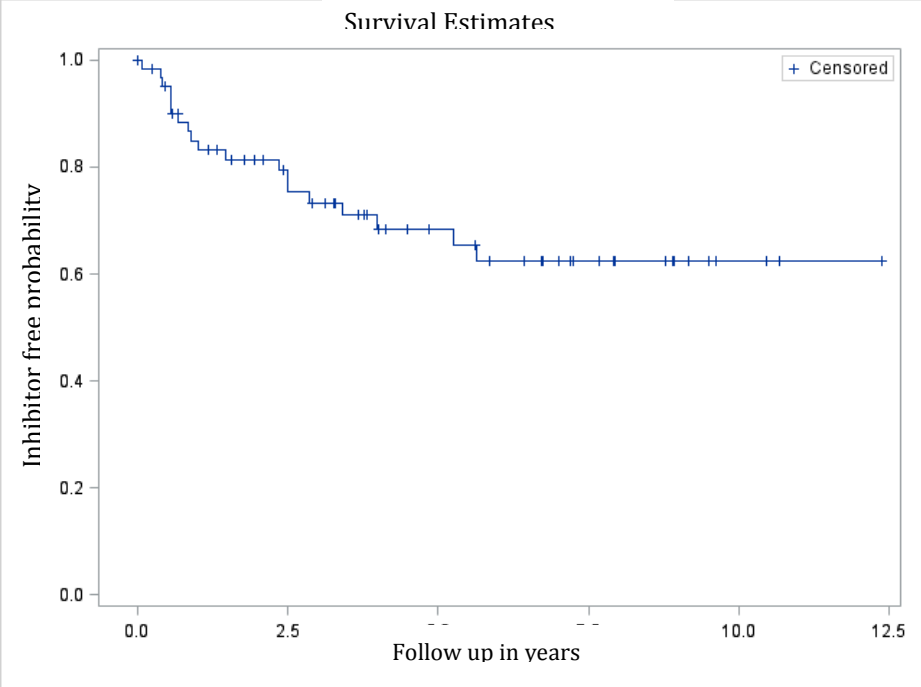

Table A: Patient participation across 12 US Hemophilia Comprehensive Centers

US Comprehensive Hemophilia Treatment Center	**# patients completed ITI by local criteria**
Emory University Hospital	12
Children's Hospital of Michigan	9
University of Colorado Hemophilia and Thrombosis Center	23
Children's National Medical Center	5
Phoenix Children's Hospital	4
Hemophilia Center of Western PA	4
UNC-Chapel Hill	10
Children's Mercy Hospital, Kansas City, MO	7
Children's Hospital Los Angeles	6
Children's Hospital of Philadelphia	2
University of Miami	5
Tulane University	4