Inhibitor incidence in an unselected cohort of previously untreated patients with severe haemophilia B: a PedNet study

by Christoph Male, Nadine G. Andersson, Anne Rafowicz, Ri Liesner, Karin Kurnik, Kathelijn Fischer, Helen Platokouki, Elena Santagostino, Hervé Chambost, Beatrice Nolan, Christoph Königs, Gili Kenet, Rolf Ljung, and Marijke van den Berg

Collaborative Groups: PedNet study group

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Inhibitor incidence in an unselected cohort of previously untreated patients with severe hemophilia B: a PedNet study

Running title: Inhibitor incidence in severe hemophilia B

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**Article summary**

The cumulative inhibitor incidence in an unselected cohort of previously untreated patients with severe haemophilia B was 10.2%, higher than previously reported. Nonsense mutations and deletions with large structural changes comprised all mutations among inhibitor patients and were associated with an inhibitor risk of 26.9% and 33.3%, respectively.
Abstract

The incidence of FIX inhibitors in severe hemophilia B (SHB) is not well defined. Frequencies of 3-5% have been reported but most studies to date were small, including patients with different severities, and without prospective follow-up for inhibitor incidence. Study objective was to investigate inhibitor incidence in patients with SHB followed up to 500 exposure days (ED), the frequency of allergic reactions, and the relationship with genotypes. Consecutive previously untreated patients (PUPs) with SHB enrolled into the PedNet cohort were included. Detailed data was collected for the first 50 ED, followed by annual collection of inhibitor status and allergic reactions. Presence of inhibitors was defined by at least two consecutive positive samples. Additionally, data on factor IX gene mutation was collected. 154 PUPs with SHB were included; 75% were followed until 75 ED, and 43% until 500 ED. Inhibitors developed in 14 patients (7 high-titre). Median number of ED at inhibitor manifestation was 11 (IQR 6.5-36.5). Cumulative inhibitor incidence was 9.3% (95% CI 4.4-14.1) at 75 ED, and 10.2% (5.1-15.3) at 500 ED. Allergic reactions occurred in 4 (28.6%) inhibitor patients. Missense mutations were most frequent (46.8%) overall but not associated with inhibitors. Nonsense mutations and deletions with large structural changes comprised all mutations among inhibitor patients and were associated with an inhibitor risk of 26.9% and 33.3%, respectively. In an unselected, well-defined cohort of PUPs with SHB, cumulative inhibitor incidence was 10.2% at 500 ED. Nonsense mutations and large deletions were strongly associated with the risk of inhibitor development.

The ‘PedNet Registry’ is registered at clinicaltrials.gov; identifier: NCT02979119
Introduction

Hemophilia A (HA) and B (HB) are X-linked inherited bleeding disorders, characterised by deficiency of FVIII and FIX, respectively. HB occurs at a frequency of about 1 in every 25-30,000 newborn males, comprising about 15% of all patients with haemophilia.\(^1\) Patients with severe HB (SHB) have a FIX level <0.01 IU/mL and account for approximately 30-40% of persons with HB.\(^2,3\) Both haemophilia subtypes suffer from recurrent joint bleeds, soft-tissue bleeds, and muscle bleeds. Several studies in adult patients reported a more severe phenotype for HA.\(^4,5\)

Since treatment history has a large effect on the joint outcome of haemophilia, the reason for these different outcomes in adults is still unclear. A study in a large cohort of children did not detect differences in the age of the first joint bleed \(^6\). Longer follow up of patients with SHA and SHB on primary prophylaxis will provide the answers whether clear phenotypic differences exist.

A clear difference between haemophilia subtypes has been observed in inhibitor frequency, with studies reporting incidences of 1-5% in HB\(^2,7-11\) as compared to 25-35% in HA.\(^12,13\) Moreover, in HB, FIX inhibitors occur almost exclusively in the severe form,\(^11\) while FVIII inhibitors occur in both SHA and non-severe HA.\(^14\) The inhibitor incidence in HB is not well defined. Due to small numbers of patients with HB, sufficiently large cohorts of previously untreated patients (PUPs) are difficult to obtain. Thus, no representative prospective studies reporting inhibitor incidence are available to date.\(^15\) Many studies reporting on inhibitor frequency in HB included all severities and even the definition of SHB varied between <0.01 and <0.02 IU/mL. The period of highest risk for inhibitor development is believed to be during the first 50-75 EDs which can take many years during on demand therapy. Studies on HB published before widespread use of prophylaxis might have missed late inhibitors.
Gene defects such as large deletions and nonsense mutations have been reported to increase the risk of inhibitors in HB. The high proportion of missense mutations in HB resulting in circulating amounts of FIX antigen is considered as one of the factors responsible for the overall lower risk of inhibitors. The lower proportion of the severe phenotype of HB compared to HA may be another reason. Moreover, the similarity between FIX and the other vitamin K-dependent factors (FII, FVII, FX) has been hypothesized to render therapeutic FIX less immunogenic.

Although the development of an inhibitor in HB is a rare event, it is associated with significant morbidity, related not only to higher risk of haemorrhagic complications but also to the frequent occurrence of allergic reactions. How often allergic reactions occur in association with inhibitor manifestation is unclear. Nephrotic syndrome often complicates immune tolerance induction treatment in HB and might be one reason for the low success rates of 30-35%. International registries on HB reported on the risk factors for inhibitors such as genotypes but selection of patients in such registries makes it difficult to draw definitive conclusions on their impact.

The objective of our study was to investigate inhibitor development in SHB patients followed for up to 500 ED, the frequency of allergic reactions at inhibitor manifestation, and the effect of FIX genotype on inhibitor risk.
Methods

Patients
The PedNet study is a birth cohort enrolling all PUPs with haemophilia A and B (FVIII or FIX less than 25%) born after January 2000 treated at participating centers. For the current study, we included consecutive PUPs with SHB (factor IX activity <0.01 IU/mL) followed until January 1st, 2018, if informed consent was available, data quality was sufficient, and they had been exposed to FIX concentrate. A list of the participating centres that contributed to this study can be found in the Appendix. Patients who were referred to the participating centres because of the presence of an inhibitor were excluded. Patients were enrolled and followed according to the PedNet study protocol, NCT02979119. Study approval was obtained from every centre’s institutional review boards. Written informed consent was obtained from the parents or guardians of all participants.

Measurements
Baseline information on diagnosis of HB, including age at diagnosis, basal FIX plasma level, FIX gene mutation, ethnicity, family history of haemophilia, family history of inhibitors were collected in uniform web-based case report forms. During the first 50 ED, detailed information on each ED, dose, product type and reason for treatment was collected. After 50 ED, treatment data was collected in annual follow-up forms per individual patient. This included all changes in treatment regimens, bleeds and surgeries, and inhibitor development, and other adverse events. An ED was defined as a calendar day on which one or more infusions of FIX were given.

Genetic analysis
Characterization of mutation in the FIX gene was performed locally at the participating centres and the reports on genotype were centrally evaluated
and adapted to the Human Genome Variation Society nomenclature. To assess the association of FIX genotype with inhibitor development, mutations were categorized according to mutation type (point mutation, deletion, duplication, insertion, polymorphism, complex) and mutation effect (missense, nonsense, frameshift, large structural changes (>50 bp), small structural changes (<50 bp) in frame, silent, splice site mutation, promotor abnormalities).

**Outcomes**
The primary outcome of the study was development of a clinically relevant inhibitor, defined as at least two positive inhibitor titres combined with a decreased in vivo FIX recovery. Inhibitor positivity was defined according to the cut-off level in each individual center’s laboratory, the highest cut-off level used being 0.6 Bethesda Units per mL (BU/mL). Secondary outcome was development of a high-titre inhibitor, defined as the occurrence of an inhibitor with a peak titre of more than 5 BU/mL. The number of ED at inhibitor development was defined as the last ED before the date of the first positive inhibitor test. After a single positive inhibitor test, all subsequent tests and recovery measurements were collected. For this study, additional information was collected regarding the occurrence of allergic reactions at the time of inhibitor development.

Follow-up data on the clinical course of patients who developed an inhibitor is collected and will be reported in a separate manuscript.

**Data analyses**
We used Kaplan-Meier curve survival analysis methods with the number of ED as the time variable. All patients were included in the analyses and censored at the ED of their last follow up. In the analyses using “all clinically relevant inhibitors” as the outcome, censoring occurred at the last exposure day. In the analyses with “high titre inhibitor development” as the outcome, censoring occurred at the last exposure day in non-
inhibitor patients and at the last ED before inhibitor development in patients with low-titre inhibitors. Continuous variables are summarized by median and interquartile range (IQR). Inhibitor incidences are reported as percentage with 95% confidence interval (95%CI). Comparisons of categorial variables between groups were done by Fisher’s exact test.

**Results**

Of a total of 168 consecutive PUPs with SHB identified in PedNet centres during the study period, 14 were excluded for lack of consent (n=2), insufficient data quality (n=5), loss-to-follow up (n=1) or because they were not yet treated with a FIX product (n=7). Thus, 154 (91.6%) patients were included into this analysis. Demographic information of study patients is reported in Table 1. The age at first treatment was median 0.8 years, age at time of study evaluation 9.6 years. About half of patients had a positive family history for haemophilia at diagnosis. Caucasian ethnicity was present in more than 80% of patients. Seventy-seven % of patients were followed until 50 ED, 75% until 75 ED, 68% until 150 ED, and 43% until 500 ED.

Inhibitors were diagnosed in 14 patients, 7 were classified as high-titre and 7 as low-titre. The median number of ED at inhibitor manifestation was 11 (IQR 6.5-36.5), median age was 23.2 months (IQR 12.1-37.1). The cumulative inhibitor incidence at 75 EDs was 9.3% (95%CI 4.4-14.1) for all inhibitors and 5% for high-titre inhibitors. Between 76 and 500 ED, only 1 low-titre inhibitor was diagnosed at 121 ED. The cumulative inhibitor incidence at 500 EDs was 10.2% (95%CI 5.1-15.3) (Table 2; Figure 1).
A positive family history of FIX inhibitors was significantly more frequent (21%) among inhibitor patients compared to non-inhibitor patients (2%) (Table 1). A recombinant FIX product was used initially in 75% and 71% of non-inhibitor and inhibitor patients, respectively. Peak treatment episodes of at least 5 days at initial factor IX exposure occurred in 15% and 14%, respectively. Age at start of prophylaxis was median 1.5 years for non-inhibitor patients and 1.3 years for inhibitor patients, but only 4 inhibitor patients had started prophylaxis before developing the inhibitor. Surgery was performed before ED20 (and before inhibitor development) in 34% and 29% of non-inhibitor and inhibitor patients, respectively.

Factor IX gene mutation genotypes were known from 124/154 (80.5%) patients (Table 3). Overall, the most frequent mutation type were point mutations in 95/124 (76.6%) patients, leading to missense mutations in 58/124 (46.8%) and nonsense mutations in 26/124 (21.0%) patients. Deletions were found in 24/124 (19.4%) patients, causing large structural changes in 15/124 (12.1%) and frameshift in 6/124 (4.8%) patients. Duplications were found in 2.4% and insertions in 1.6% of patients. For more details on mutation type and effect see Table 3.

Among inhibitor patients, the most frequent mutations were nonsense mutations in 7/14 (50.0%), 4 patients with low-titre and 3 with high-titre inhibitors, and deletions with large structural changes in 5/14 (35.7%), 3 with low-risk and 2 with high-risk inhibitors. No other mutations were present among inhibitor patients. In two inhibitor patients, the genotype was not known (no genetic report available in one; no mutation identified in spite of repeated analysis in the other). Figure 2 displays the risk of inhibitor development by mutation. The inhibitor risk for deletions with large structural change was 33.3% (11.8-61.6) and for nonsense mutations 26.9% (95%CI 11.6-47.8). For all other mutations, the inhibitor risk was zero.
Allergic reactions at the time of inhibitor development were observed in 4/14 (28.6%, 95% CI 8.4-58.1) patients, one patient with a high-titre inhibitor and 3 with low titre inhibitors (Table 3). Of these 4 patients, 2 had nonsense mutations, one a large deletion and one the mutation unknown.

Discussion

In this unselected, prospectively followed birth cohort of 154 PUPs with SHB, 14 patients developed an inhibitor. The cumulative inhibitor incidence at 75 EDs was 9.3% for all inhibitors and 5% for high-titre inhibitors. At 500 ED, the cumulative incidence for all inhibitors had increased to 10.2%. Previous large databases of HB patients in general reported inhibitor frequencies of 1.3-2.8% while studies focusing on SHB patients reported inhibitor frequencies of 3.8-4.9%. However, these registries reported inhibitor prevalences rather than prospectively following patients for inhibitor incidence. In the European Haemophilia Safety Surveillance (EUHASS), 5 inhibitors were reported among 72 PUPs with SHB (6.9%). A recent systematic review of PUP studies in SHB reported a summary inhibitor incidence of 10.2%. However, most studies included into this analysis were small (patient numbers between 7 and 72), with inhibitor frequencies from individual studies varying between 5-14%.

How can we interpret the higher incidence of inhibitors in SHB found in our study compared to most previous reports? The PedNet registry represents an unselected birth cohort. In contrast, patients with bleedings in the neonatal period will not be eligible for licensing PUP studies. In the current study on SHB patients, we found that 52% of patients had a negative family history, similar to prospective studies in SHA patients. This frequency is much higher than the 30% patients reported in the
literature. Patients with a negative family history are usually diagnosed after the onset of bleeding, potentially increasing their risk for inhibitors.\textsuperscript{12}

In our cohort of patients born after 2000, all received primary prophylaxis, which made it possible that we could follow them until 500 ED. This long-term follow-up might also have resulted in detecting a higher total number of inhibitors as compared to other studies.\textsuperscript{27} Another reason for the higher incidence in our study may be more frequent testing for inhibitors. Inhibitors received much more attention in the last decades and frequent testing for inhibitors became standard of care. In SHA, increased frequencies of inhibitors have been observed since the nineties.\textsuperscript{12,13} Our group reported that significantly more low-titre inhibitors were diagnosed in SHA after the year 2000.\textsuperscript{29} In the current study of SHB, low-titre inhibitors comprised about half of all inhibitors, and the incidence of high-titre inhibitors was 5%, which is more in line with previous reports.\textsuperscript{2,7,10,11}

A positive family history for inhibitors has been recognized to be an important risk factor, in our patients this was confirmed in 21% of the inhibitor patients. Higher incidences of inhibitors for SHB where reported in Sweden and Ireland that might have been influenced by inclusion of families with more high-risk mutations or a founder effect.\textsuperscript{19,30} No inhibitor patients in our study were related which excludes this so-called “founder” effect.

The relative frequency of factor IX gene mutation types/effects in our cohort was comparable to those reported from other registries.\textsuperscript{9,17} We found 46.8% missense mutations in SHB patients, in contrast to only 11.4% missense mutations among SHA patients in the RODIN study.\textsuperscript{12} A higher prevalence of missense mutations in SHB compared to SHA has previously been reported.\textsuperscript{7,9,12,17,18,31} Missense mutations were mostly found in non-inhibitor patients, consistent with previous reports.\textsuperscript{7,9,16-19} We hypothesize that the high proportion of missense mutations in HB is a
key reason for the overall lower inhibitor incidence compared to HA. Two mutation types/effects were strongly associated with inhibitor development, comprising most mutations among the inhibitor patients: nonsense mutations and deletions with large structural changes both representing null-mutations. Patients with these high-risk mutations had a 27% and 33% risk of developing an inhibitor, respectively. The findings from our representative PUP cohort confirm previous reports, suggesting a strong association between absent endogenous FIX protein due to gross and complete gene deletions and inhibitor development.32 For gross FIX gene defects resulting in absent endogenous FIX protein, the observed risk of inhibitor development (27-33%) is not much different from that in haemophilia A due to gross FVIII gene mutations resulting in absent endogenous FVIII protein (inhibitor risk around 35%).33

Treatment-related factors, such as type of FIX product, peak treatment episodes at initial exposure to FIX concentrate, time of start of prophylaxis, or surgery, were not associated with inhibitor development. However, we must be cautious in interpreting these findings, as the power to identify or rule-out determinants of inhibitor development is limited with the small number of inhibitor patients.

Allergic reactions are more frequently observed in HB compared to HA. Frequencies of allergic reactions reported in the literature for HB vary considerably between 4-60% of inhibitor patients.10,16,21-23 In our cohort, allergic reactions at the time of inhibitor manifestation were observed in 4 (29%) patients, 1 with a high-titre and 3 with low-titre inhibitors. Of these patients, 2 had nonsense mutations, one had a large deletion, and one had the mutation unknown. Large deletions have previously been reported to be associated with allergic reactions.16,22 Data on the course and effect of immune tolerance induction in our cohort are currently collected and will be reported in a future manuscript.
Our study has some limitations, such as the lack of racial diversity which limits generalisability to some extent. Determination of haemophilia severity as well as inhibitor testing was done in local laboratories at the individual centres. A potential disadvantage is insufficient standardization, however, the advantage of this pragmatic approach is better feasibility resulting in a more representative cohort. Although we report the largest consecutive cohort of PUPs with SHB to date, its size still does not allow to comprehensively evaluate whether there is an influence of treatment-related risk factors on inhibitor development.

In conclusion, our study in an unselected cohort of PUPs with SHB found a cumulative inhibitor incidence of 10.2% at 500 ED. Missense mutations were the most frequent mutation type but not associated with inhibitors. Nonsense mutations and large deletions were significantly associated with an increased risk of inhibitor development.
Data sharing statement
For original data, please contact h.marijke.vandenberg@pednet.eu

Acknowledgement
We thank all members of the PedNet study group who are listed in the appendix for providing the data on their HB patients. We thank Ella van Hardeveld and Marloes de Kovel for maintaining the data base and supporting the analysis.

Authorship

Author contribution: C.M., A.R., R.L, K.K., K.F., H.P., E.S., H.C., B.N., C.K., G.K. were responsible for the care for HB patients with inhibitors. C.M., N.A, R.L., M.vdB analyzed the data and drafted the manuscript, all authors contributed to writing the manuscript and have approved its final version.

Conflict-of-interest disclosure: The authors declare no competing financial interests.
Appendix

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* No longer participating as PedNet center
REFERENCES


Table 1. Demographic information of study participants comparing patients without and with inhibitors

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<tr>
<th></th>
<th>Non-inhibitors patients</th>
<th>Inhibitor patients</th>
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<tr>
<td></td>
<td>number (%), or median [IQR]</td>
<td>number (%), or median [IQR]</td>
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<tr>
<td>Number of patients</td>
<td>140</td>
<td>14</td>
</tr>
<tr>
<td>Ethnicity: Caucasian</td>
<td>117 (84%)</td>
<td>13 (93%)</td>
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<tr>
<td>Positive family history of haemophilia</td>
<td>66 (47%)</td>
<td>8 (57%)</td>
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<tr>
<td>Positive family history of FIX inhibitor</td>
<td>3 (2%)†</td>
<td>3 (21%)†</td>
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<tr>
<td>Age at diagnosis (months)</td>
<td>3.3 [0–10.5]</td>
<td>3.1 [0–8.9]</td>
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<tr>
<td>Age at first ED (years)</td>
<td>0.8 [0.4–1.2]</td>
<td>0.9 [0.8–1.5]</td>
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<tr>
<td>Recombinant FIX product at initial exposure</td>
<td>105 (75%)++</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>Peak treatment at initial exposure*</td>
<td>21 (15%)</td>
<td>2 (14%)</td>
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<tr>
<td>Age at start of prophylaxis** (years)</td>
<td>1.5 [1.0–2.1]</td>
<td>1.3 [0.9–3.1]¶</td>
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<tr>
<td>ED until start of prophylaxis</td>
<td>8.0 [5–16]</td>
<td>7.5 [2–18]¶</td>
</tr>
<tr>
<td>Surgery before ED20</td>
<td>48 (34%)</td>
<td>4 (29%)¶¶</td>
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<td>Age at time of study (years)</td>
<td>9.7 [5.6–13.6]</td>
<td>8.8 [6.5–12.3]</td>
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† p=0.008 (Fisher’s exact test);
++ FIX product type unknown in 6 (4.3%) patients;
* peak treatment of at least 5 consecutive exposure days;
** definition of prophylaxis: at least once a week, for 2 consecutive months;
¶ only 5 inhibitor patients had started prophylaxis before inhibitor development;
¶¶ surgery before ED20 and before inhibitor development.
Table 2. Inhibitor development in relation to number of exposure days

<table>
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<th>Exposure days</th>
<th>Patients at risk</th>
<th>All inhibitors</th>
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<th>Cumulative incidence (all inhibitors)</th>
<th>estimate</th>
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<td>103</td>
<td>13</td>
<td>7</td>
<td>9.3</td>
<td>4.4 - 14.1</td>
<td></td>
</tr>
<tr>
<td>76-150</td>
<td>91</td>
<td>14</td>
<td>7</td>
<td>10.2</td>
<td>5.1 - 15.3</td>
<td></td>
</tr>
<tr>
<td>151-250</td>
<td>84</td>
<td>14</td>
<td>7</td>
<td>10.2</td>
<td>5.1 - 15.3</td>
<td></td>
</tr>
<tr>
<td>251-500</td>
<td>52</td>
<td>14</td>
<td>7</td>
<td>10.2</td>
<td>5.1 - 15.3</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Factor IX mutations in study participants comparing patients without and with inhibitors

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Mutation effect</th>
<th>Non-inhibitor patients n=140</th>
<th>Inhibitor patients n=14</th>
<th>Risk of inhibitor by mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n/N, % (95%CI)</td>
</tr>
<tr>
<td>Mutation known</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>124/154 (80.5%)</td>
<td></td>
<td>112 (80.0)</td>
<td>12 (85.7)</td>
<td></td>
</tr>
<tr>
<td>Point mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95/124 (76.6%)</td>
<td>Missense</td>
<td>58 (51.8)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Nonsense</td>
<td>19 (17.0)</td>
<td>7 (50.0)</td>
<td>7/26 (26.9%; 11.6-47.8)</td>
</tr>
<tr>
<td></td>
<td>Splice site</td>
<td>4 (3.6)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Promoter</td>
<td>5 (4.5)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Missing data on effect</td>
<td>1 (0.9)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>Deletion</td>
<td>Large structural change*</td>
<td>10 (8.9)</td>
<td>5 (35.7)</td>
<td>5/15 (33.3%; 11.8-61.6)</td>
</tr>
<tr>
<td>24/124 (19.3%)</td>
<td>Small structural change**</td>
<td>2 (1.8)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Frameshift</td>
<td>6 (5.4)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Splice site</td>
<td>1 (0.9)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>Duplication</td>
<td>Frameshift</td>
<td>2 (1.8)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>3/124 (2.4%)</td>
<td>Small structural change**</td>
<td>1 (0.9)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>Insertion</td>
<td>Frameshift</td>
<td>3 (2.7)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>2/124 (1.6%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation unknown</td>
<td></td>
<td>28 (25.0)</td>
<td>2 (14.3)</td>
<td></td>
</tr>
</tbody>
</table>

* large structural change: >50bp;
** small structural change: <50bp, in frame.
**Figure 1.** Kaplan-Meier survival curve of inhibitor development until 500 exposure days

**Figure 2.** Risk of inhibitor development by mutation
The graph illustrates the risk of inhibitor development across different types of mutations and their effects.

- **Large structural deletion**: Indicates a significant decrease in the risk of inhibitor development, possibly due to the removal of a large structural component.
- **Nonsense**: Shows a moderate risk of inhibitor development, affecting the reading frame and potentially leading to a non-functional protein.
- **Missense, Splice site, Promotor, small deletion**: Indicates a minor to negligible risk of inhibitor development, as the mutations do not significantly alter the protein or its expression.

The horizontal axis represents the mutation type and effect, while the vertical axis shows the risk of inhibitor development in percentage terms.