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# The Immunogenicity of ReFacto AF (moroctocog alfa AF-CC) in previously untreated patients with haemophilia A in the UK

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## Abstract

**Introduction**: Factor VIII inhibitor development is currently the most serious complication of the treatment of Haemophilia A. Differences in manufacturing and the molecular structure of brands of recombinant factor VIII have led to speculation that concentrates may differ in immunogenicity[1-4]. This has led to a regulatory focus on the immunogenicity of the factor VIII concentrates both before and after licensure. **Aim**: To collect post-marketing data on >100 UK previously untreated patients (PUPs) treated exclusively with ReFacto AF until at least 50 EDs. **Methods**: The United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) National Haemophilia Database (NHD) identified a consecutive cohort of patients with severe haemophilia A (<0.01 IU/l) whose first treatment was with ReFacto AF and collated a report of inhibitor incidence and inhibitor risk-factors. **Results**: 103 boys reached 50 EDs within the study period; 68 (66.0%) did not develop an inhibitor and 35 (34.0% [95% CI 24.7 – 43.3%]) did, of which 15 (14.6% [95% CI 7.6 – 21.5%]) were high titre. Inhibitors arose after a median (interquartile range) 11 (7-16) EDs. Inhibitors were significantly associated with high risk mutations and non-significantly associated with non-white ethnicity. **Conclusion**: Inhibitor incidence in a single country population of ReFacto AF PUPs was similar to that previously described. Low and high titre inhibitors were detected after a similar number of EDs, contrasting with previous data, probably reflecting standardised inhibitor monitoring within the UK.

## Introduction

Pivotal studies in previously untreated patients (PUP) studies are open, non-comparative and, with a relatively small sample-size, have very limited statistical power. They are therefore unsuitable for the detection of small differences in immunogenicity[5-7]. The clinical trial setting and strict entry criteria also limit the extent to which the results of such studies may be extrapolated to the general patient population. For this reason, post-authorisation surveillance has assumed increasing importance in assessing less frequent side-effects and subtler differences in comparative immunogenicity of factor VIII products and in providing a real-world perspective. Indeed, some product differences have become apparent only after follow-up of 400 or more PUPs over periods of ten years or more[7-9].

The UK National Haemophilia Database (NHD) registers and monitors all patients with severe haemophilia A as part of a prospective program of pharmacovigilance and ongoing investigation into the risk factors and potential prevention of factor VIII inhibitor development[9-12].

Following market authorisation of ReFacto AF (Pfizer, Walton Oaks, Tadworth, UK) in 2009, the European Medicine Agency requested that Pfizer conduct a post-authorisation safety surveillance registry of ReFacto AF in PUPS in usual care settings. The UKHCDO Data Collection was supplemental to the Pfizer registry data and enabled Pfizer to secure the requisite numbers for ReFacto AF. This study was to include at least 100 consecutive UK patients treated exclusively with ReFacto AF for at least 50 exposure days (ED). In 2010, the UK National Procurement Exercise awarded 47% of the contract for supply of factor VIII to Pfizer Ltd (ReFacto AF). Consequently, 34% of UK PUPs were allocated to ReFacto AF on a contractual basis from 2010.

## Methods

The United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) NHD was used to identify consecutive children with severe haemophilia A (<0.01 IU/l) whose first treatment was with ReFacto AF. PUPs were selected to be treated with ReFacto AF or other products non-randomly to achieve contractual volume requirements. Most large Haemophilia Centres allocate product brand to PUPs on a rotational basis but this is not universal. Patients diagnosed outside the UK were excluded because their previous treatment history was unknown.

UKHCDO guidelines recommend inhibitor testing of PUPS every third exposure day until 20 EDs and then every 5$^{th}$ ED until 50 EDs[10]. Inhibitor tests were conducted locally using either a Bethesda or Nijmegen assay or a pharmacokinetic test. All laboratories participate in a national quality control exercise and are inspected and accredited by Clinical Pathology Accreditation (CPA).

Adverse events including new factor VIII inhibitors are reported to NHD electronically when they occur. Inhibitor reports require a second confirmatory test. The continued presence of an inhibitor is indicated in each quarterly report from Haemophilia Centres to the NHD. Each inhibitor report to the NHD includes details of the laboratory method

used and the normal range for the local laboratory. Some low-level inhibitors (below the limit of detection of the Bethesda assay) are diagnosed based on a reduced half-life and/or recovery, in keeping with UKHCDO guidelines[10]. High-titre inhibitors are defined as those with a recorded peak inhibitor titre ≥ 5 Bethesda Units (BU), whereas low-titre inhibitors are those where the recorded peak inhibitor titre is < 5 BU. EMA defined thresholds for inhibitor titre were utilised in this study, with titres ≥5 BU considered high titre and titres ≥0.6 and <5 BU considered lower titre[13]. A sub-analysis including UKHCDO pharmacokinetic definitions is described. Transient inhibitors were identified by examining the number of calendar quarters in which each inhibitor was reported to be present, and defined as those which were reported in just one quarter.

The number of factor VIII EDs prior to first inhibitor detection was provided in the corresponding adverse event report. For patients who did not develop an inhibitor, the number of EDs to date was estimated by dividing their total exposure to ReFacto AF by the mean units used per exposure day in 50 inhibitor-free UK RODIN patients, as previously described[9].

Clinical and demographic data were extracted from the NHD, or requested from UK Haemophilia Centres. Age at first treatment is estimated as the mid-point of the quarter of first treatment, or at the mid-point of date of birth and the end of the quarter of first treatment. Family history was defined as any confirmed history of haemophilia and/or factor VIII inhibitor development in a first or second degree relative. Ethnicity is categorised as white or non-white. FVIII mutations are categorized as high risk (large deletions, nonsense mutations, intron 1 and 22 inversions), low risk (small deletions and insertions, missense mutations, splice site mutations), awaiting categorisation or unknown (no mutation detected or testing status unknown). Intron 22 inversions were included in the high-risk mutation category and also analysed separately, given previous reports suggesting that it was associated with an intermediate risk of inhibitor development.

### *Statistics*

Descriptive statistical analysis includes medians, interquartile ranges (IQR) and Kaplan-Meier (K-M) estimates of probabilities of inhibitor development within fifty exposure days (EDs), P(t≤50), with 95% confidence intervals (CI). Time to inhibitor development, measured in EDs, is illustrated using K-M curves. Differences in K-M curves are tested using the log rank test.

## Results

Of the 113 eligible patients identified between 2010 and 2017, 103 have either progressed to 50 EDs without developing an inhibitor (n=68, 66.0%) or developed a confirmed inhibitor prior to 51 EDs (n=35, P(t≤50)=0.33, [95% CI 0.25 – 0.43]) (Figure 1). These inhibitors were first detected between 2010 and 2017.

There was one confirmed report of a transient inhibitor, and for a further four patients, inhibitors were reported only once, and thus also considered transient. A further seven non-inhibitor patients are yet to reach 50 EDs, and three non-inhibitor patients switched products prior to 51 EDs.

Inhibitors prior to 51 EDs were identified after a median (iqr) 11 (7-16) EDs. High-titre inhibitors arose in 15 (P(t≤50)=0.16, [95% CI 0.10 – 0.25]) patients before 51 EDs, arising after a median of 11 (8-16) EDs. Low-titre inhibitors were reported in 20 (P(t≤50)=0.20, [95% CI 0.14 – 0.30]) patients before 51 EDs, arising at a median of 11 (6.25-15) EDs.

The seven non-switching inhibitor-free patients who have not reached 50 EDs in this study had been followed up for an estimated median (iqr) 7.5 (4.5-10.5) calendar months by 31st December 2017, and 4.1 (1.6-12.2) exposure days. They were first treated in 2016 or 2017. The three patients who switched products were initially treated with up to 1250 units of ReFacto AF as PUPs before they switched to either Advate (n=2), after 1 and 2 EDs, or to Nuwiq, after 2 EDs. These ten patients with incomplete follow-up are included in the Kaplan-Meier analysis (Figure 2) prior to censoring at their most recent exposure to ReFacto AF, or the point at which they changed products.

Additionally, five patients were reported to have been given up to 3000 units of other products (3 Advate, 1 Kogenate, 1 Helixate Nexgen), presumably in error. All were treated previously and subsequently exclusively with ReFacto AF. Rather than excluding them or censoring their data, they have been treated as 'protocol variations' and their full follow-up has been included in the analysis.

Most patients (n=96) (Table 1) were of white ethnicity. Non-white patients were more likely to develop inhibitors (6/12 (P(t≤50)=0.49, [95% CI 0.26 – 0.79])) than white patients (29/91 (P(t≤50)=0.31, [95% CI 0.23 – 0.42])) before 51 EDs (p=0.30) and this difference was more marked for high titre inhibitors (3/12 (P(t≤50)=0.30, [95% CI 0.10 – 0.68])) and 12/91 (P(t≤50)=0.15, [95% CI 0.09 – 0.24])), respectively (p=0.30).

FVIII mutation was reported for 105 patients. Most had a high-risk mutation (n=79), whilst 26 had a low risk mutation. The high-risk mutation patient group developed significantly more inhibitors before 51 EDs (29/71 (P(t≤50)=0.40, [95% CI 0.30 – 0.52])) than the low risk mutation patient group (4/25 (P(t≤50)=0.16, [95% CI 0.06 – 0.37])) (p=0.03). Figure 3 shows time to inhibitor detection by mutation group. The proportion of patients with intron 22 inversions who developed inhibitors prior to 51 EDs (23/54 (P(t≤50)=0.42, [95% CI 0.30 – 0.56])) was greater than that for the other 'high-risk' mutations (6/17 (P(t≤50)=0.35, [95% CI 0.17 – 0.61])). This difference was not statistically significant (p=0.55).

A family history of haemophilia was reported in 62 patients, of whom thirteen had a family history of inhibitors. Patients without a family history of haemophilia were significantly more likely to develop an inhibitor prior to 51 EDs than those with a family history of haemophilia (p=0.04). Patients with a family history of inhibitors were significantly more likely to develop a high titre inhibitor prior to 51 EDs than those without a family history of inhibitors (p=0.01).

Excluding inhibitors, there were four reported adverse events (intracranial haemorrhage, rash, poor efficacy, and post-operative bleed following insertion of a central line device). The patient with the post-operative bleed had an inhibitor detected one month earlier, after 14 EDs. The patient with poor efficacy had been treated for intracranial haemorrhage, and was suspected to have an inhibitor due to the increased dosing required to maintain levels. However, no inhibitor was detectable on testing, and the patient is therefore coded as inhibitor-free after 50 EDs in this report. A sensitivity analysis was performed recoding this patient as a pre-51 EDs inhibitor case (as it meets the UKHCDO criteria), in which the statistical association between family history of haemophilia A and inhibitors lost significance. The outcomes of the remaining statistical tests described above were not affected by this reclassification.

A further sensitivity analysis was performed to investigate the effect of the 'protocol variations' described above in which five patients had been administered small amounts of other factor VIII products. When these patients were excluded, the association between high-risk mutations and inhibitors prior to 51 EDs lost statistical significance, as did the association with family history of haemophilia A. The outcomes of the other statistical tests were unaffected.

Follow-up of patients who were inhibitor-free after fifty EDs (n=68) was available for a median (iqr) 431 (257-816) days. Inhibitors were detected after fifty EDs in two of these patients, after 52 and 160 EDs.

## Discussion

This report describes the occurrence of inhibitors in a consecutive cohort of PUPs whose first treatment was with ReFacto AF. The incidence of inhibitors observed is comparable with previous published reports of PUP studies[7,8,14,15]. The cumulative probability of inhibitor development over time is shown in a Kaplan-Meier plot (Figure 1). The wide confidence intervals shown illustrate the degree of uncertainty around estimates of immunogenicity of factor VIII products, using samples which are typically around this size. Were one to attempt a comparative trial, a much larger sample would be required to demonstrate a difference in immunogenicity.

Approximately one third of of all UK PUPs who started treatment since the end of 2009 are included in the cohort. Patients were non-randomly selected to use ReFacto AF to fulfil national procurement volume committments. The distribution of inhibitor risk factors such as ethnicity, F8 genotype and family history of inhibitors appears representative and is very similar to that previously reported for the whole UK PUP cohort treated during the period 2000-2011 and using all available products[9]. There is therefore no evidence of systematic bias in allocating patients to ReFacto AF.

As expected, subjects with high risk genotypes had significantly more inhibitors. The association between high risk genotypes and high titre inhibitors did not reach statistical significance, possibly because of the small numbers of patients analysed.

High- and low-titre inhibitors were detected after similar median EDs (11 in both groups). In the past, low titre inhibitors were thought to tend to arise later than high-

titre inhibitors. Our results suggest that historically later detection of low-titre inhibitors may be attributable to a sampling artefact, and that low-titre inhibitors may be detected earlier when patients are tested more frequently, as has become current clinical practice.

In conclusion, we report that in a consecutive cohort of PUPs treated with ReFacto AF in the UK the proportion developing inhibitors was similar to previous PUP studies of ReFacto AF and comparable with PUP studies of other recombinant factor VIII concentrates. The confidence intervals around these estimates are wide, however, indicating that althought there was no evidence of increased immunogenicity associated with ReFacto AF, A formal comparison of the immunogenicity of factor VIII concentrates would require a much larger sample.

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## Authorship:

The first draft of this manuscript was written by MM, BPP and CRMH. The remaining authorship contributed data to the study and provided editorial input into subsequent drafts. Statistical analysis was conducted by BPP.

## Conflict of Interest Statement:

EC Has received speaker's honoraria from Roche, CSL Behring and Boehringer Ingelheim.

CRMH has acted as an advisor to Pfizer and has been a member of their speaker's bureau. He has also received speaker's honoraria from Pfizer, Shire, Sobi Biotest and Novo Nordisk.

MM, PWC, MR, AW and BPP have no conflicts to declare

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## Table 1: Time to inhibitor by clinical and demographic characteristics

| | Recruited (N=113)* | Completed (N=103) | No inhibitor prior to 51st exposure day (n=68) | Inhibitors prior to 51st exposure day (n=35) | High titre inhibitors prior to 51st exposure day (n=15) |
|---|---|---|---|---|---|
| **Age at first exposure to factor VIII (months), median (interquartile range)** | 8 (2-13) | 8 (4-13) | 8 (2-13) | 10 (6-13) | 11 (8-16) |
| **Ethnicity** | **n** | **n** | **n (probability)** | **n (probability)** | **n (probability)** |
| White | 96 | 91 | 62 (0.69) | 29 (0.31) | 12 (0.15) |
| Non-white | 17 | 12 | 6 (0.51) | 6 (0.49) | 3 (0.30) |
| **Genotype** | **n** | **n** | **n (probability)** | **n (probability)** | **n (probability)** |
| High risk | 79 | 71 | 42 (0.60) | 29 (0.40) | 13 (0.21) |
| Low risk | 26 | 25 | 21 (0.84) | 4 (0.16) | 1 (0.05) |
| Not known | 8 | 7 | 5 (0.71) | 2 (0.29) | 1 (0.14) |
| **Family history of Haemophilia A** | **n** | **n** | **n (probability)** | **n (probability)** | **n (probability)** |
| Yes | 62 | 57 | 42 (0.74) | 15 (0.26) | 7 (0.13) |
| No | 51 | 46 | 26 (0.57) | 20 (0.43) | 8 (0.21) |
| **Family history of inhibitor** | **n** | **n** | **n (probability)** | **n (probability)** | **n (probability)** |
| Yes | 13 | 12 | 8 (0.67) | 4 (0.33) | 4 (0.33) |
| No | 49 | 45 | 34 (0.76) | 11 (0.24) | 3 (0.07) |
| Not applicable | 51 | 46 | 26 (0.57) | 20 (0.43) | 8 (0.21) |

*Includes patients who switched prior to 50 exposure days (n=3) and non-inhibitor patients yet to reach 50 exposure days (n=7)

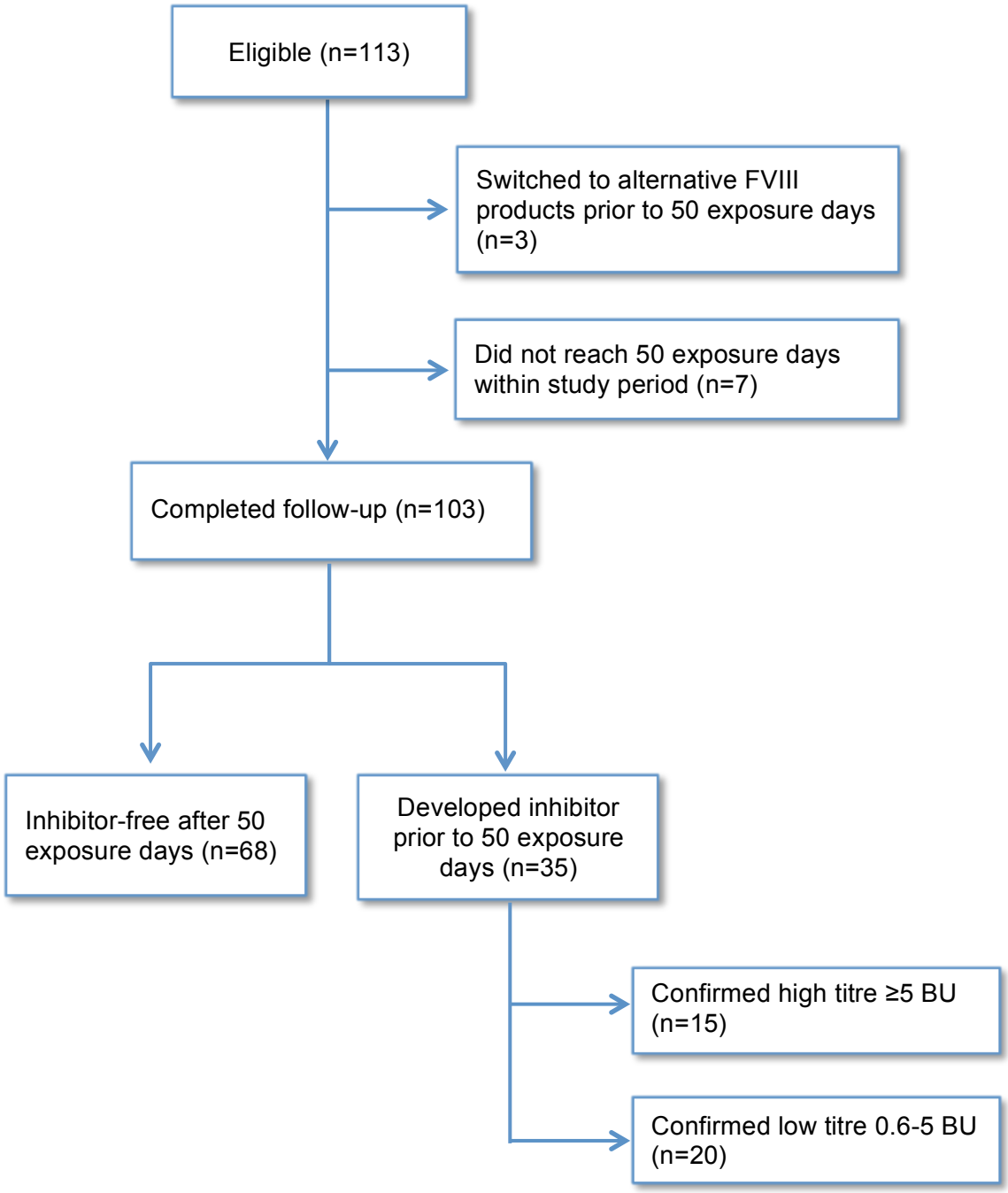
Eligible (n=113)
Switched to alternative FVIII products prior to 50 exposure days (n=3)
Did not reach 50 exposure days within study period (n=7)
Completed follow-up (n=103)
Inhibitor-free after 50 exposure days (n=68)
Developed inhibitor prior to 50 exposure days (n=35)
Confirmed high titre ≥5 BU (n=15)
Confirmed low titre 0.6-5 BU (n=20)

Figure 2 - Time to inhibitor detection

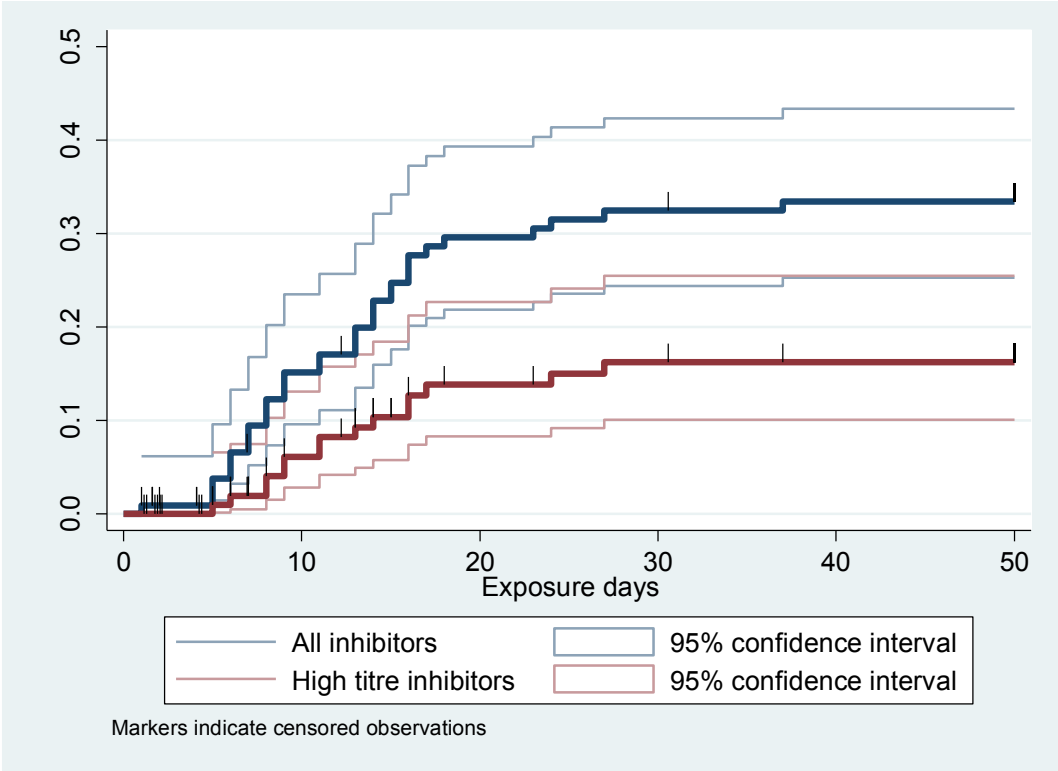
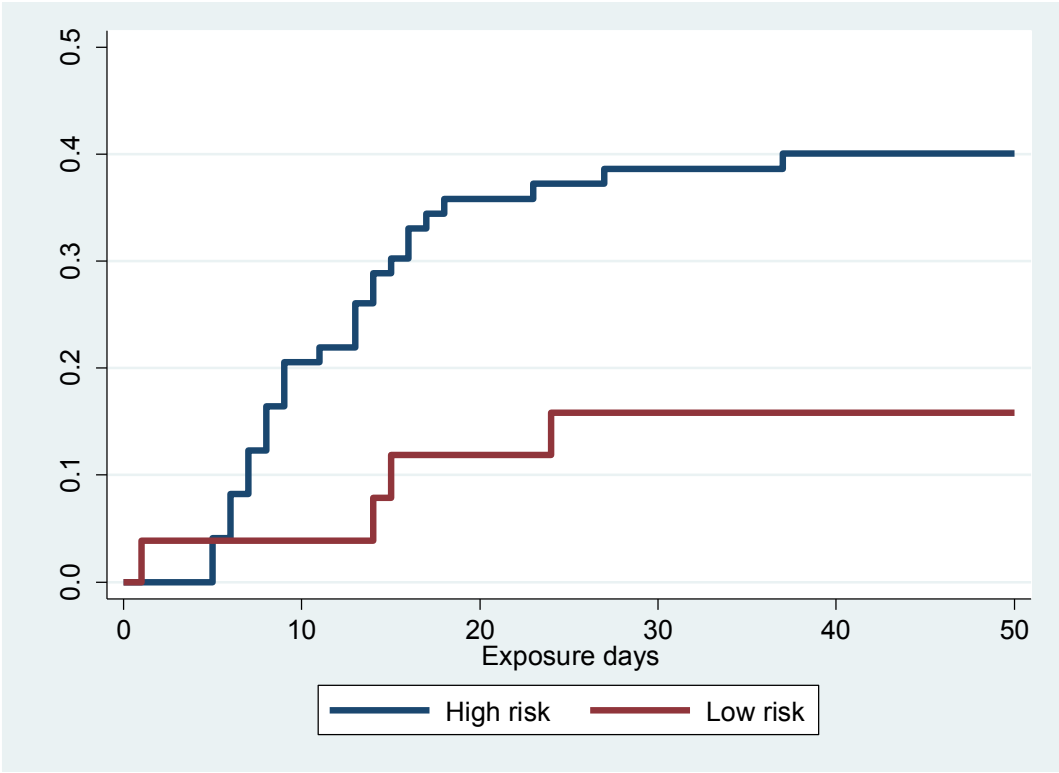
Figure 3 - Time to inhibitor detection by genotype