



**MEMORANDUM**

**Department of Health and Human Services  
Public Health Service  
Food and Drug Administration  
Center for Biologics Evaluation and Research**

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**To:** File of STN 125398/0, Roman Drews and Debbie Cordaro

**From:** Zuben E. Sauna (OBRR/DH/LH)

**Through:** Tim Lee, Acting Chief, LH/DH/OBRR

**Subject:** Review of assays used to assess immunogenicity in patients treated with NovoThirteen (recombinant human FXIII A subunit) in studies conducted by Novo Nordisk as part of the above referenced original BLA.

**CC:** Charles Maplethorpe

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This memorandum summarizes the review of the assays used to assess the incidence of inhibitory anti-drug antibodies to NovoThirteen (a recombinant form of the A Subunit of human coagulation factor XIII [FXIII]). Based on my review, I conclude that the assay development and validation are performed adequately, and the assays are suitable to be used in clinical studies to assess the levels of antibodies against the product. However, some minor clarifications are required from Novo Nordisk, which can either be included in the CR letter or addressed by the clinical reviewer.

**Background**

NovoThirteen is a recombinant form of the A Subunit of human coagulation factor XIII, intended to be administered to patients with Coagulation Factor XIII A-subunit deficiency on a monthly basis for routine prophylaxis of bleeding. The product will be supplied in a glass vial with a content of 2500 IU (15 mg), with another glass vial containing 3.2 mL water for injection for reconstitution of the product. NovoThirteen is produced by fermentation as an intracellular protein in baker's yeast (*Saccharomyces cerevisiae*) followed by downstream purification.

**Summary**

The applicant has submitted the results of the immunogenicity studies in section 5.3.1.4 of the original BLA. This section includes the validation studies as well as identification and characterization of anti-FXIII antibodies in plasma samples obtained from the clinical studies F13CD-1725 and NN1841-3788. A list of all studies related to assay development and the results from clinical samples is given below:

A] ASSAY DEVELOPMENT	STUDY #
Validation of an (b) (4) for the detection of anti-rFXIII antibody in human plasma	VAL-10025 A 00

Validation of an (b) (4) for the detection of anti-rFXIII antibodies in human plasma (EDTA) and primate serum	0665/674
Validation of neutralizing antibody assay for human FXIII deficient plasma	208239
Validation of the Dade Behring (Marburg, Germany) Berichrom <sup>®</sup> FXIII assay	VAL-10005 A 00
Validation of the FXIII assay previously validated by (b) (4) using a Dade Behring analyzer, re-validated using a (b) (4)	
Validation of the Dade Behring (Marburg, Germany) Berichrom <sup>®</sup> FXIII assay in human EDTA plasma	208311
Partial validation of a FXIII activity assay (modified Berichrom) for use in normal human EDTA plasma	210267
Validation of an (b) (4) to quantify FXIII in human plasma	VAL-10030 A 00
Validation of an (b) (4) to identify FXIII Total A2 sub-unit in human plasma (EDTA)	0665/676
Validation of an ELISA to quantify FXIII[A2B2] tetramer in human plasma	VAL-10010
Validation of an ELISA to quantify FXIII[A2B2] tetramer in human plasma (EDTA)	205514
Validation of an (b) (4) to quantify FXIII Free B in human plasma	VAL-10011
Validation of an (b) (4) to quantify FXIII Free B in human plasma (EDTA)	0665/680
Validation of three analytical methods for the determination of FXIII in human plasma (EDTA)	2209/003
Validation of a FXIII activity assay (b) (4) for use in human EDTA plasma	209177
.Non-regulatory assessment of recovery and dilutional linearity of FXIII in monkey and human plasma (EDTA) using (b) (4)	0665/758
Incurred sample reproducibility of FXIII activity in samples from trial F13CD-1725	209015
Incurred sample reproducibility of FXIII activity in samples from trial NN1841-3788	210347
Long term stability of rFXIII spiked in human EDTA plasma	207135
Long term stability of three rFXIII sub-units in monkey and human EDTA plasma	0665/742
<b>B] CLINICAL RESULTS</b>	
Determination of anti-FXIII antibodies in human plasma (EDTA) using (b) (4)	0665-979-A
Determination of the isotype and cross reactivity of anti-FXIII Abs in human plasma (EDTA) using (b) (4)	0665-979-B
Analysis of human plasma (EDTA) samples for the detection of FXIII subunit A2B2 using (b) (4)	0665-976
Analysis of human plasma (EDTA) samples for the detection of Factor XIII subunit A2 using (b) (4)	0665-977
Analysis of human plasma (EDTA) samples for the detection of Factor XIII subunit FreeB using (b) (4)	0665-978
Analysis of anti-FXIII inhibitors in samples that screened positive for anti-FXIII antibodies	209127
Determination of anti-FXIII Antibodies in Human plasma (EDTA) Using (b) (4)	8223273
Determination of isotype and cross reactivity of anti-FXIII antibodies in human plasma (EDTA) using (b) (4)	8223273

## Assay development

Validation of (b) (4) to detect anti-FXIII antibody in human plasma (citrate):

(b) (4)



(b) (4)

[Redacted text block]

Validation of (b) (4) to detect anti-FXIII antibody in human plasma (EDTA) and primate serum ((b) (4); NN reference number 206140):

Method:

(b) (4)

[Redacted text block]

[Redacted text block]

(b) (4)



Validation of NN1841 neutralizing antibody assay for human FXIII deficient plasma:

Method:

(b) (4)



Validation of the Dade Behring (Marburg, Germany) Berichrom<sup>®</sup> FXIII assay:

Background:

For quantification of FXIII activity in patient samples, the Berichrom<sup>®</sup> FXIII assay was selected by the applicant. This method was developed by Dade Behring (Marburg, Germany) for the measurement of FXIII activity in plasma. The Berichrom<sup>®</sup> assay is

commercially available for clinical use in Europe and for research purposes in the United States.

Method:

(b) (4)



Validation of the Dade Behring Berichrom<sup>®</sup> FXIII assay in human EDTA plasma:



(b) (4)

FXIII assay previously validated using a Dade Behring analyzer, re-validated using a (b) (4) analyzer:

Method:

The Berichrom<sup>®</sup> assay (described above) has been validated using a Dade Behring analyzer. In the current validation, the method in the Dade Behring FXIII assay kit was performed using a (b) (4) analyzer instead of the Dade Behring analyzer. Based on the data presented, the use of the alternate analyzer appears to be acceptable.

Validation of an (b) (4) to quantify FXIII in human plasma:

Method:

(b) (4)

Validation of an (b) (4) to identify FXIII Total A2 subunit in human plasma (EDTA):

Background:

This study was initiated to confirm a method for the determination of FXIII Total A2 subunit in human plasma with standard concentration ranges of (b) (4) was acceptable for use in clinical studies and to assign acceptable ranges of new QC material. After the study had started, it was learnt that the initial validation work had been for the determination of FXIII in human plasma (Citrate) while future clinical studies were to involve human plasma (EDTA) samples. This cross-validation was included in this study to show the acceptability of analysis in human plasma (EDTA).

Internal reference:

Purified rFXIII prepared by the applicant (Lot: (b) (4) ) was used as the primary reference material (PRM) and stored at (b) (4)

Results:

The experimental parameters determined from this study are as follows:

(b) (4)

Validation of an ELISA to quantify FXIII [A<sub>2</sub>B<sub>2</sub>] tetramer in human plasma:

Method:

An enzyme immunoassay (ELISA) for the quantification of FXIII [A<sub>2</sub>B<sub>2</sub>] tetramer in human plasma, MTD-FXIII-M0020, was validated. (b) (4)

(b) (4)

Validation of an (b) (4) to quantify FXIII Free B subunit in human plasma:

Method:

(b) (4)

Non-regulatory assessment of recovery and dilutional linearity of FXIII in monkey and human plasma (EDTA) using (b) (4)

A study was conducted by the sponsor to assess the feasibility of determining recovery of the 3 FXIII subunit combinations (FXIII Free B, Total A<sub>2</sub> and A<sub>2</sub>B<sub>2</sub>) in monkey and human plasma (EDTA).

The in-house reference materials for the three subunit combinations were prepared by the applicant and carried the following lot numbers: FXIII rA<sub>2</sub>B<sub>2</sub> Lot (b) (4) rFXIII A<sub>2</sub> Lot (b) (4) and FXIII human Free B sub-unit purified Lot (b) (4)

Recovery of these FXIII subunits in human and monkey plasma was within 25% of the nominal concentration (i.e., (b) (4) recovery) and recovery improves as concentration increases. The recovery of FXIII Free B in human plasma and FXIII total A<sub>2</sub> in Monkey plasma, however, was less than 25%. For the former recovery ranged from (b) (4) % and for the latter from (b) (4) %.

Stability studies of rFXIII spiked in human and monkey plasma:

This study determined the stability of the three FXIII subunit combinations (FXIII Free B, Total A<sub>2</sub> and A<sub>2</sub>B<sub>2</sub>) in monkey and human plasma when stored at (b) (4) period:

(b) (4)

(b) (4)

**Clinical results**

Determination of anti-FXIII antibodies in human plasma (EDTA) using (b) (4) (Clinical study F13CD-1725):

A total of 629 human plasma samples representing 31 patients from the clinical study F13CD-1725 were analyzed for the presence, specificity and quasi-quantification of anti-Factor XIII antibodies, using a tiered approach (see above). The assay used in this clinical investigation utilizes rFXIII to capture any anti-FXIII immunoglobulins present in the plasma test samples. The detection antibody is a (b) (4)

(b) (4) The validation of this assay is described above (see (b) (4)

The analysis was performed in three tiers:

(b) (4)

[Redacted content]

**Samples from 4 patients were found to be positive for anti-FXIII antibodies.**

Determination of the isotype and cross reactivity of anti-FXIII antibodies in human plasma (EDTA) using (b) (4)

A total of 36 human plasma samples (4 patients) from clinical study F13CD-1725 were analyzed for the presence, specificity and isotype of anti-Factor XIII antibodies, using an (b) (4) Four subjects were tested and 3 were

found to have produced IgM isotype antibodies early in the dosing schedule. This immune response to dosing was transient and did not lead to a secondary IgG response. The fourth subject exhibited only slightly raised IgG levels after dosing and no significant IgM response.

Samples used in this study were the ones that tested positive in the 3-tiered analyses of samples from patients enrolled in study F13CD-1725 described above (Determination of anti-FXIII antibodies in human plasma (EDTA) using (b) (4)

Analysis of anti-FXIII inhibitors in antibody screening positive samples:

Samples from patients that tested positive for anti-FXIII antibodies (see above) were analyzed for anti-FXIII neutralising activity (inhibitors). Samples were obtained from 4 patients (21 plasma samples) that tested positive for anti-rFXIII antibodies by (b) (4) in clinical trial F13CD-1725. The inhibitory action of the anti-FXIII antibodies was based on a (b) (4) assay for (b) (4) assay). No samples were found to be positive for neutralizing antibodies against rFXIII through (b) (4) analytical runs based on a cut-off of >12% inhibition in (b) (4). These results are presented in Table 1 of section 5.3.1.4.3 reproduced below:

**Neutralising activity of (b) (4) positive samples, sorted by subject:**

Run	Sample	Sample Id	Neutralization (%)	Neutralizing anti-FXIII antibodies (positive/negative)
2	7	(b) (6)	-15	negative
2	8		-2	negative
3	7		-18	negative
3	8		-9	negative
4	10		-6	negative
6	5		-4	negative
6	6		-2	negative
8	9		1	negative
2	11		-17	negative
2	12		-4	negative
3	11		-11	negative
3	12		-9	negative
4	11		-6	negative
4	7		-19	negative
4	8		-2	negative
4	9		-8	negative
5	7		-10	negative
5	8		-2	negative
5	9		1	negative
5	10		-3	negative
7	6		-3	negative

This data needs some clarification. The “V” probably refers to the visit number of the patient. However it is not clear how long the patient has been on the regimen and how many doses of FXIII the patient has received. Furthermore the sponsor states, “All test

results come from (b) (4) individual runs performed.” Were additional multiple replicates performed for the data presented here? Were any of those samples positive for FXIII-neutralizing activity?

Determination of anti-FXIII antibodies in human plasma (EDTA) using (b) (4) (Clinical study NN1841-3788):

A three-tiered analyses of 189 human plasma samples (50 subjects) from clinical study NN1841-3788 was performed as described above for samples from the study F13CD-1725. Antibodies were detected in only one individual, but no measurable antigen specific antibodies were determined to be present in these samples.

**Immune response to yeast host cell proteins (HCP) in patients treated with rFXIII in clinical trials**

In the clinical phase 1 trials, yeast IgE antibodies were investigated using two different assays (For details see Module 2.7.1). In the majority of the trials, a modified (b) (4) (b) (4) ) was used according to the manufacturer’s instructions. The assay uses an (b) (4)

in patient samples. The assay sensitivity is (b) (4) . In one trial (NN1810-3733), the (b) (4) method was applied (b) (4) . The assay uses a (b) (4) .

A listing of antibodies to baker’s yeast in individual patients (Table 16.2.8.3) shows that the level of antibodies in individuals treated with 50 IU/kg of FXIII and a placebo. Antibody levels were measured at visits 1 and 10. In all instances, levels of antibodies observed in individuals treated with the FXIII were comparable to those treated with the placebo. In addition, no adverse events of injection site reactions have been reported in the clinical trial program (as of 30 Nov 2010). Eleven non-serious events have been reported and the sponsor claims that causality is unlikely (we defer the evaluation to the Clinical Review Branch for these claims).

**Recommendations**

The sponsor in this submission has followed state-of-the-art norms (as determined by publications in the literature, guidance documents from the EMA and draft guidance from the FDA) for development of assays, reagents for negative and positive controls, storage and quality control of these reagents, assay execution and analyses.

As is desirable, a multi (3)-tiered approach was adopted for screening for anti-FXIII antibodies and the criteria for the initial screening had cut-off criteria to minimize false negatives. In addition assays were developed to detect and identify the antibody isotypes. The selection of the assay format and the bioassay for detecting inhibitory antibodies also appears suitable for the purpose. Reagents used in these assays as well as their storage conditions were adequately characterized.

However, the following clarifications are required, which if not resolved internally should be included in the CR letter:

1. With reference to the assessment of freeze-thaw stability of quality control (QC) reagents described in Module 5.3.1.4.3, (“Final report: validation of an (b) (4) for the detection of anti-FXIII antibodies in human plasma (EDTA) and primate serum using (b) (4) Section 3.6, Pg. 17), please describe the handling of patient samples, specifically the number of freeze/thaw cycles they have experienced, and limit the number of freeze/thaw cycles for the QC reagents and samples to (b) (4) in subsequent studies

2. With reference to Module 5.3.1.4.3, (“Analysis of anti-FXIII inhibitors in antibody screening positive samples from Trial ID: F13CD-1725”, Section 3, Table 1, Pg. 9) which reports on the analysis of anti-FXIII inhibitors in antibody screening positive samples,

- a. Please specify the duration at which the samples were taken, and the number of doses the patient has received.
- b. Novo Nordisk states “All test results come from (b) (4) individual runs performed.” Please clarify if additional multiple replicates were performed for the data presented in this section, and if any of those samples were tested positive for FXIII-neutralizing activity.

(However, as these results are part of the clinical study we would defer to the Clinical Review Branch in terms of sampling, sample size and analysis of these results.)

3. With reference to the determination of anti-FXIII antibodies in human plasma (EDTA) using (b) (4) Clinical study F13CD-1725 provided in Module 5.3.1.4.3 (Determination of anti-FXIII Antibodies in Human plasma (EDTA) Using (b) (4) section 2.13.1, Pg. 21), please describe the derivation of the normalization factor (b) (4) using negative control CR09/0665/305.