Name of Sponsor/Company **CFR** Name of Finished Product: **Nanocog alfa** Name of Active Ingredient: **recombinant FIX**	Individual Study Table Referring to Part of the Dossier Volume: Page:	

Title of Study: **Relazioni tra genotipo del FIX e Farmacocinetica del FIX ricombinante, Nanocog alfa, in pazienti affetti da emofilia B**

Investigators: **Prof. Francesco Bernardi, Dr.Massimo Morfini, Prof. Paola Giordano, Dr. Paolo Radossi, Dr. Dorina Cultrera, Dr. Rita Carlotta Santoro, Prof. Donato Gemmati, Dr. Claudio Molinari, Dr. Maria Santa Napolitano, Prof. Paolo Gresele, Dr. Antonio Chistolini, Prof. Giancarlo Castaman**

Study centre(s): **Azienda Ospedaliero Universitaria Consorziale Policlinico di Bari - Ospedale Civile (Azienda Ulss n. 2 Marca Trevigiana – Distretto di Asolo)-Via Ospedale, 18 – 31033 Castelfranco Veneto (TV) - Azienda Ospedaliera-Universitaria Policlinico V. Emanuele-Presidio Ospedaliero Ferrarotto Catania - Azienda Ospedaliera Pugliese Ciaccio - Azienda Ospedaliero-Universitaria di Ferrara - Dipartimento di Emato-Oncologia Pediatrica IRCCS Giannina Gaslini - L.go G. Gaslini, 5 16147 Genova Quarto(GE) - Azienda Ospedaliera Universitaria e Policlinico Paolo Giaccone - Azienda Ospedaliera di Perugia Ospdale S. Maria della Misericordia - Azienda Ospedaliera Universitaria Policlinico Umberto I - Azienda Ospedaliero-Universitaria Careggi**

Publication (reference): **F9 missense mutations impairing factor IX activation are associated with pleiotropic plasma phenotypes. Branchini A, Morfini M, Lunghi B, Belvini D, Radossi P, Bury L, Serino ML, Giordano P, Cultrera D, Molinari AC, Napolitano M, Bigagli E, Castaman G, Pinotti M, Bernardi F; GePKHIS Study Group of AICE.**
J Thromb Haemost. 2022 Jan;20(1):69-81. doi: 10.1111/jth.15552. Epub 2021 Oct 24. PMID: 34626083

Studied period (years): **Two years** (date of first enrolment) **10.9.2018** (date of last completed) **17.7.2020**	Phase of development: N.A. **Nonacog alpha has been approved by the European Commission for treatment of hemophilia B on August 27 1997**

Objectives: **To investigate the relation among F9 genotypes- rFIX expression levels of F9 mutations - PK parameters**

Methodology: **pharmacokinetics, F9 genotyping, site-directed mutagenesis, recombinant FIX expression of variants, transient expression in eukaryotic cell, FIX antigen assay by ELISA, analysis of association between PK parameters/F9 mutation types**

Number of patients (planned and analysed): **60/20**

Diagnosis and main criteria for inclusion:
patients suffering from severe or moderately severe hemophilia B (FIX: C <2 IU / dL)

Test product, dose and mode of administration, batch number:
IV infusion of 45 (range 22-67) IU / kg of Nonacog alfa

Duration of treatment: **Single-dose pharmacokinetics, up to 96 hours**

Reference therapy, dose and mode of administration, batch number:
Substitutive therapy/prophylaxis, IV infusion of 45 IU / kg of Nonacog alfa (several batch numbers according to the availability of the drug in each Hemophilia Center)

Criteria for evaluation:
Efficacy**: N.A.**
Safety**: N.A.**
Association of pharmacokinetics parameters with FIX genotypes**: Detected with specific mutations at the FIX activation sites**

Statistical methods:
Non-Compartmental Analysis (NCA), Compartmental analysis according to the One and Two-compartment models (OCM and TCM), Best fitting. ANOVA, t-test, correlation analysis-Pearson coefficients

SUMMARY - CONCLUSIONS

Barriers encountered in the study are listed as follows:

i) the submission and approval from a number of independent Ethics Committees (ECs), needed to recruit HB patients in more than 10 different centers in Italy, were laborious and time-consuming

ii) The procedure was prolonged by several minor comments from ECs to be formally addressed.

iii) The EC of one Center in Milan, expected to perform centralized FIX:c coagulation assays, did not approve the GePKHIS protocol, and asked for comparison of PK of Nonacog alfa with extended half-life recombinant FIX (EHLrFIX) in the recruited patients. Consequently, we did not enroll patients from this large center (Milan).

iv) Consequently, the protocol was modified and an additional large reference center (Florence) was enrolled to perform centralized coagulation assays. This has required a “de novo” EC procedure.

v) Covid-19 related issues have impeded from February 2019 the recombinant expression analysis of the last mutations discovered in patients

vi) New EHLrFIX products have been available to the Italian Hemophilia Centers during the ECs approval processes. As a consequence, part of the severe HB patients in prophylaxis with Nonacog alfa, expected to be enrolled in GePKHIS, have been switched to the EHLrFIX. This would have prevented further enrollment, which at the end led us to conclude it.

Overall, the enrollment rate in GePKHIS has been significantly decreased. However, the main scientific objectives have been achieved as detailed in the conclusion.

EFFICACY RESULTS**: N.A**
SAFETY RESULTS: **N.A.**
CONCLUSION: **Properties of FIX variants may exert effects on distribution of endogenous (plasma, residual) and infused (on demand/prophylaxis, nonacog alfa) FIX molecules.**
Our results suggest that combination of the amounts and quality of specific plasma FIX variants (FIX activation sites), which predict noticeable amounts of endogenous FIX, may exert positive effects on distribution of infused FIX molecules.

Date of the report: **April 28th 2022**

Professor Francesco Bernardi