FONDAZIONE BIANCHI BONOMI
Piazza Castello, 2 – 20121 Milan, Italy

TYPE 3 VON WILLEBRAND INTERNATIONAL REGISTRIES INHIBITOR PROSPECTIVE STUDY

(3WINTERS-IPS)

EXTENDED

A 8-year International Registries and
Prospective Study on VWD Type 3 Patients

STUDY CODE: ABB-11-01

PROTOCOL VERSION: Amendment 2, 28th January 2020

Scientific Coordination and Supervision:

PROF. A. B. FEDERICI

With the Working Group on VWD3 recognized by the
European Association of Haemophilia and Allied Disorders (EAHAD)
and Sub-Committee on VWF Scientific Standardization Committees of the
International Society on Thrombosis and Haemostasis (SC-VWF, SSC-ISTH)

TABLE OF CONTENTS

STUDY APPROVAL SIGNATURES

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Signature

30/01/2020
Date

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Date

ABBREVIATIONS

AC	Assistant Coordinator
AE	Adverse Event
ANGDYS	Angiodysplasia
CP	Cell Pellet
CRF	Case Report Form
CRO	Contract Research Organization
DDAVP	Desmopressin
DMC	Data Monitoring Committee
EAHAD	European Association of Haemophilia and Allied Disorders
EC	Ethics Committee
FC	Financial Coordinator
FVIII	Factor VIII
FVIII:C	Factor VIII clotting activity
GCP	Good Clinical Practice
GIB	Gastro-Intestinal Bleeding
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
ISTH	International Society on Thrombosis and Haemostasis
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
PC	Publication Coordinator
PPP	Platelet Poor Plasma
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Steering Committee
SS	Study Supervisor
US	United States (of America)
VWD	Von Willebrand Disease
VWD3	Type 3 Von Willebrand Disease
VWF	Von Willebrand factor
VWF:Ag	Von WIllebrand factor Antigen
VWF:RCo	Von Willebrand Ristocetin Cofactor
WHO	World Health Organization
WP	Working Package

INVESTIGATOR'S AGREEMENT

Study title: **Type 3 Von Willebrand International Registries Inhibitor Prospective Study - (3WINTERS-IPS) - EXTENDED**

Investigator name: Prof./Dr. _____

Center address: _____

_____

Center Number: |____|____|____|____|

I declare that I have exhaustively discussed the objectives of this study and the contents of this protocol with the representatives of the Sponsor/Steering Committee/CRO.

I agree to conduct the study described in this protocol according to the procedures and ethical and patient safety aspects reported herein, and in accordance with Good Clinical Practice and the Helsinki Declaration.

If the Sponsor decides for whatever reason and at any time to suspend the study or end it prematurely, this will be communicated to me in writing. If I decide to withdraw from performing the study, I will immediately inform the Sponsor/Steering Committee/CRO of this decision in writing.

Principal Investigator

_____ _____

Signature *Date*

STUDY SYNOPSIS

Study title	**Type 3 Von Willebrand International Registries Inhibitor Prospective Study - (3WINTERS-IPS) - EXTENDED**
Study background	Von Willebrand Disease (VWD) is the most common inherited bleeding disorder, characterized by a quantitative (VWD types 1 and 3) and/or qualitative (VWD types 2A, 2B, 2M and 2N) deficiency of von Willebrand factor (VWF), the large multi-functional plasma glycoprotein that plays a major role in early phases of haemostasis. VWD type 3 (VWD3) is due to virtually complete deficiency of VWF and, for this reason, has been also described as "severe VWD". Recurrent Gastro-Intestinal Bleeds (GIB) is one of the most challenging complications encountered in the management of patients with VWD. The commonest cause is angiodysplasia (ANGDYS), but often no cause is identified due to the difficulty in making the diagnosis. In recent years, research from several laboratories has identified multiple roles for VWF in the control of vascular function. Globally, these findings provide the first possible explanation for the presence of ANGDYS in patients with VWD. These vascular malformations in the gastrointestinal (GI) tract are characterized by fragile, leaky mucosal vessels. Combined with the hemostatic dysfunction, these can lead to severe intractable bleeding including GIB. VWD3 patients by definition are characterized by undetectable levels of VWF antigen (VWF:Ag) in plasma and by reduced concentrations (< 10 IU/dL) of factor VIII (FVIII). These baseline levels usually do not increase in plasma following desmopressin (DDAVP), the drug which can release VWF from endothelium. VWD3 is inherited as a recessive trait and heterozygous relatives have mild or no bleeding symptoms. The prevalence of VWD3 is very low, ranging from 0.1 to 5.3 per million and differing considerably between countries. The highest rate is found in Iran and the lowest in southern Europe. However, the actual prevalence of VWD3 is still unknown in most countries, due to the lack of retrospective or prospective studies. Although rare, VWD3 is of major interest because of its severe clinical presentation, the need for replacement therapy with plasma-derived and/or recombinant VWF concentrates and the risk of occurrence of anti-VWF inhibitors after the infusion of VWF concentrates, for which risk factors have not been systematically determined.
Study objectives	• International network among European (approximately 125 cases) and Iranian (approximately 125 cases) centers; • Prospective enrollment of at least 250 VWD3 patients using a common database online; • Detailed information about previous bleedings and exposure to plasma-derived and/or recombinant VWF concentrates of identified VWD3 patients;

	• Bleeding severity score of identified VWD3 patients calculated with a common questionnaire; • Collection of plasma and DNA samples from all the identified VWD3 patients enrolled for centralized analyses; • Confirmation of the local VWD3 diagnosis using centralized tests; • Evaluation of VWF gene defects, VWF phenotype and risk of anti-VWF inhibitors through common methods; • Evaluation of potential correlations between phenotypic results (including markers of angiogenesis) and GIB occurrence; • Objective evaluation of severity of GIB in VWD3 patients; • Assessment of frequency and sites of bleeding in VWD3 patients followed-up for 2 prospective observation periods (2 years each: 2017-2018 and 2020-2021); • Efficacy assessment of the plasma-derived and/or recombinant VWF concentrates used to treat VWD3 (on demand versus prophylaxis) using the most objective criteria for efficacy during 2 prospective observation periods (2 years each: 2017-2018 and 2020-2021); • Evaluation of the efficacy and safety of plasma-derived and/or recombinant VWF concentrates in the treatment of GIB during 2 prospective observation periods (2 years each: 2017-2018 and 2020-2021), in comparison to the use of anti-angiogenetic agents within the standard clinical setting.
Study design	No-profit, investigators initiated, multi-center, European-Iranian observational, retrospective and prospective study on patients with diagnosis of Type 3 von Willebrand Disease.
Study Centers	A total of 20 Investigational sites will be involved in this project in 9 European countries: Finland, France, Germany, Hungary, Italy, The Netherlands, Spain, Sweden, UK and other 7 sites in Iran.
Study population	A cohort of **at least 250 patients** with diagnosis of Type 3 von Willebrand Disease will be enrolled using homogenous and standardized criteria.

Eligibility criteria	**Inclusion Criteria** • All ages, both genders; • Informed Consent obtained (parents will sign for children); • Previous documented diagnosis of VWD3 (VWF antigen **undetectable or <5 U/dL**); • Detailed information on inherited pattern, history of bleeding, previous exposure to blood products; • Availability of plasma and DNA samples at enrolment. **Exclusion Criteria** • Patient who, at the enrolment, are not available for follow-up.
Study procedures	The work planned to achieve the objectives of the project will be divided in two parts: • **the first part** deals with standardized criteria for enrolment, collection of retrospective clinical and laboratory data, further characterization of clinical and laboratory parameters to be confirmed by centralized laboratories, prevalence of anti-VWF inhibitors and standardized assays to measure these anti-VWF inhibitors, advanced laboratory tests to further identify VWD3 patients, mutations analyses of the VWF gene; • **the second part** of the study for the first time deals with the prospective clinical observation in a large cohort of VWD3 patients all previously well characterized by an international panel of experts to assess number, types and risk factors for bleeding in the first part of the study and to assess the efficacy and safety of plasma-derived and/or recombinant VWF concentrates used to treat them.
Study timelines	• Study period: First part of the study (retrospective survey and centralized confirmation): approximately 36 months (years 2014 to 2016). Second part of the study (prospective observation): approximately 24 months (years 2017 – 2018). Confirmation of clinical phase data: approximately 12 months (year 2019).

	Second prospective observation: approximately 24 months (years 2020 – 2021). Total study duration: approximately 96 months. • Individual subject participation: First part of the study: approximately 1 month. Second part of the study: 4 years (2 prospective observation periods of 2 years each: 2017-2018 and 2020-2021).
Data points	**First part of the study** • Informed Consent collection • Eligibility Criteria verification • Patient ID Assignment • Demographics • Bleeding History and Previous Use of Blood Components • General Laboratory Test with Local Assays for VWD3 Diagnosis • Family History in parents and relatives • Blood Withdrawal for Central Laboratory Assessment • Mutation analysis in VWD3 • Inhibitors assessment **Optional (in patient relatives)** • Informed Consent collection • Historical information • Blood Sampling for Local Laboratory Assessment **Second part of the study** • Bleeding episodes • Use of plasma-derived and/or recombinant VWF Concentrates and comparison between patients treated with VWF concentrates under on demand versus secondary long-term prophylaxis regimens • Blood Withdrawal for Central Laboratory Assessment only in case of anti-VWF inhibitors • Concomitant medication and Adverse Events • Patients with recurrent GIB who might benefit of treatment with VWF concentrates and correlation of angiogenesis markers with previous GIB episodes recorded

Statistical Analysis	Analytic techniques will be pertinent to the observational design of the registry. Basic analyses will be descriptive and associative.
Study management	Sintesi Research S.r.l, Via Matteo Bandello, 6 – 20123 Milan (Italy) has been assigned to coordinate and manage the study conduction in every phase.

1. STUDY BACKGROUND

Von Willebrand Disease (VWD) is the most common inherited bleeding disorder, characterized by a quantitative (VWD types 1 and 3) and/or qualitative (VWD types 2A, 2B, 2M and 2N) deficiency of von Willebrand Factor (VWF), the large multifunctional plasma glycoprotein that plays a major role in early phases of Haemostasis. Recurrent Gastro-Intestinal Bleeds (GIB) is one of the most challenging complications encountered in the management of patients with VWD. The commonest cause is angiodysplasia (ANGDYS), but often no cause is identified due to the difficulty in making the diagnosis. In recent years, research from several laboratories has identified multiple roles for VWF in the control of vascular function. In vivo studies in the VWF-deficient mouse have demonstrated a role for VWF in vascular development and angiogenesis. In vitro inhibition of VWF expression using siRNA in HUVEC results in increased proliferation, migration and angiogenesis. Other studies have suggested that VWF is also involved in controlling blood vessel permeability, although its effect on this crucial vascular property may be organ-specific and depend on tissue microenvironment. Globally, these findings provide the first possible explanation for the presence of ANGDYS in patients with VWD. These vascular malformations in the gastrointestinal (GI) tract are characterized by fragile, leaky mucosal vessels. Combined with the hemostatic dysfunction, these can lead to severe intractable bleeding including GIB.

Interestingly, VWD patients can show GIB and ANGDYS; whether the same pathways are implicated remains to be established. The direct role of VWF in the development of ANGDYS is best exemplified in the condition known as “Heyde’s Syndrome”. The GIB in Aortic Stenosis (AS) was indeed shown to be proportional to the severity of AS and to be reversible following replacement of the aortic valve. The pathophysiology of this association is thought to be the unfolding of the VWF due to the high shear as the blood flows through the stenotic valve making it more readily accessible to proteolysis by the VWF-cleaving protease ADAMTS13. Similar VWF defects have been recently observed in patients exposed to Cardiovascular Assisted Devices (CAD).

GIB with or without ANGDYS can be difficult to diagnose and treat. The diagnosis is challenging due to accessibility, especially when the lesions are in the small bowel, and the requirement that active bleeding be present at the time of the examination for optimal identification.

Whilst treatment of acute bleeding is fairly standard, a major issue is the recurrent nature of the GIB and its prevention. Multiple physical and pharmacological therapies have been reported but none proven to be fully effective in every case. A further difficulty is the low frequency of the complication in a rare disease, making collaboration desirable among Centers to study the disorder. The optimal treatment to prevent recurrences of GIB episodes remains still unknown.

Since a specific abnormality of VWF is always present in VWD patients with recurrent GIB with or without ANGDYS, the main objective of the 3WINTERS-EXTENDED project is to demonstrate if the regular (on demand and/or secondary long-term prophylaxis) administration of plasma-derived and/or recombinant VWF concentrates can reduce the number of recurrent GIB episodes in VWD patients.

VWD3 patients by definition are characterized by undetectable levels of VWF antigen (VWF:Ag) in both plasma and by reduced concentrations (< 10 IU/dL) of factor VIII (FVIII). These baseline levels usually do not increase in plasma following desmopressin (DDAVP), the drug which can release VWF from endothelium. Therefore VWD3 patients must be treated with exogenous VWF contained in VWF concentrates.

VWD3 is inherited as a recessive trait and heterozygous relatives have mild or no bleeding symptoms. The prevalence of VWD3 is very low, ranging from 0.1 to 5.3 per million and differing considerably between countries. The highest rate is found in Iran and the lowest in southern Europe. However, the actual prevalence of VWD3 is still unknown in most countries, due to the lack of retrospective or prospective studies. In the Italian registry on Hemophilia and allied disorders organized on behalf of the Italian Association of Hemophilia Center, 96 VWD3 patients (5.8%) have been recently identified among the 1650 cases (prevalence of 1.6 per million)**:** however, many clinical and laboratory parameters for diagnosis and treatment of VWD3 are still not available. Other national registries on VWD have been organized in several counties (France, Germany, Iran, The Netherlands, Spain, UK, USA) but data on prevalence of VWD3 are not available so far. Although rare, VWD3 is of major interest because of its severe clinical presentation, the need for replacement therapy with plasma-derived and/or recombinant VWF concentrates and the risk of occurrence of anti-VWF inhibitors after the infusion of VWF concentrates, for which risk factors have not been systematically determined.

2. OBJECTIVES AND EXPECTED ACHIEVEMENTS

Aims of this project are to evaluate: 1) the prevalence, clinical and laboratory parameters of a large cohort (at least 250 cases) of patients with local diagnosis of VWD3 enrolled by European (approximately 125 cases) and by Iranian (approximately 125 cases) centers using homogeneous and standardized criteria; 2) role of VWF phenotypic data measured with standardized clinical and laboratory markers on the bleeding tendency; 3) frequency of bleeding and the requirement for VWF concentrates in VWD3; 4) correlation between clinical and molecular markers and bleeding tendency, response to therapy with plasma-derived and/or recombinant VWF concentrates and risk of anti-VWF inhibitors.

2.1 Measurable Objectives

1) International network among European (approximately 125 cases) and Iranian (approximately 125 cases) centers;
2) Prospective enrollment of at least 250 VWD3 patients using a common database online;
3) Detailed information about previous bleedings and exposure to plasma-derived and/or recombinant VWF concentrates of identified VWD3 patients;
4) Bleeding severity score of identified VWD3 patients calculated with a common questionnaire;
5) Collection of plasma and DNA samples from all the identified VWD3 patients enrolled for centralized analyses;
6) Confirmation of the local VWD3 diagnosis using centralized tests;
7) Evaluation of VWF gene defects, VWF phenotype and risk of anti-VWF inhibitors through common methods;
8) Evaluation of potential correlations between phenotypic results (including markers of angiogenesis) and GIB occurrence;
9) Objective evaluation of severity of GIB in VWD3 patients;
10) Assessment of frequency and sites of bleeding in VWD3 patients followed-up for 4 years (2 prospective observation periods of 2 years each: 2017-2018 and 2020-2021);
11) Efficacy assessment of the plasma-derived and/or recombinant VWF concentrates used to treat VWD3 (on demand versus prophylaxis) using the most objective criteria for efficacy during the 4-year observation period (2 prospective observation periods of 2 years each: 2017-2018 and 2020-2021);
12) Evaluation of the efficacy and safety of plasma-derived and/or recombinant VWF concentrates in the treatment of GIB during the 4-year observation period (2 prospective observation periods of 2 years each: 2017-2018 and 2020-2021), in comparison to the use of anti-angiogenetic agents within the standard clinical setting.

2.2 Expected Achievements

1) Natural history, predictors, clinical and molecular markers for bleeding in a large cohort of patients with VWD3 identified in developed (Europe) and developing (Iran) countries;
2) Common clinical and lab methods to identify VWD3 patients;
3) Guidelines for management of VWD3 without and with anti-VWF inhibitors.

3. STUDY DESIGN AND PROJECT WORKING PACKAGES

This is a no-profit, investigators initiated, multi-center, European-Iranian observational, retrospective and prospective study on patients with diagnosis of Type 3 von Willebrand disease (VWD3). Patients meeting the enrolment criteria will be consecutively enrolled at each participating Center and data entered in the register. Upon confirmation of VWD3 diagnosis from the Central Laboratories, the patients will enter the second part of the study and will be prospectively observed for 24 months (2 years). After confirmation of clinical phase data (taking approximately 12 months), the patients will be observed within a second prospective period of 24 months (2 years) to collect additional information related to study endpoints.

A total of 20 Investigational sites will be involved in this project in 9 European countries: Finland, France, Germany, Hungary, Italy, The Netherlands, Spain, Sweden, UK and other 7 sites in Iran. The investigational sites' names and address are detailed separately.

3.1 General description of the project

The work planned to achieve the objectives of the project will take place over a 8-year period and will be divided in three parts: a) the first part deals with standardized criteria for enrollment, collection of retrospective clinical and laboratory data, further characterization of clinical and laboratory parameters to be confirmed by centralized laboratories, prevalence of anti-VWF inhibitors and standardized assays to measure these anti-VWF inhibitors, advance laboratory tests to further identify VWD3 types, mutations analyses of the VWF gene; b) the second part of the study for the first time deals with the prospective clinical observation in a large cohort of VWD3 patients all previously well characterized by an international panel of experts to assess number, types and risk factors for bleeding in at least 250 patients and to assess the efficacy and safety of plasma-derived and/or recombinant VWF concentrates used to treat them; c) the third part of the study (after 12 months of evaluation of the collected data by the Study Coordinators) will replicate the second part to collect additional information related to study endpoints aiming to a better characterization of the observed patients.
This will be achieved through the implementation of a Working Packages Plan as indicated in Appendix 3 to this study protocol.

3.1.1 First part of the Study

The first critical phase of the project, to be completed over the initial 36-month period, will be the recruitment of at least 250 patients with VWD3, approximately 125 in Europe and 125 in Iran. After the approval of the study protocol of the Study by the reference Ethics Committee for each Center and the organization of the network within a common database, the enrolled patients will be characterized by clinical and family history at the Centers (WP1).

A detailed clinical history to aid diagnosis will be obtained by interview and use of a questionnaire previously tested in a large cohort of VWD1 patients. In case of VWD3 patients, detailed history about previous exposure to blood products will be collected and blood samples obtained will be separately coded and stored (WP2).

Basic laboratory testing with use of WHO International plasma standards for VWF/FVIII activities will be an essential part of this initial recruitment and analyses as detailed in WP2. All basic laboratory tests data (WP2) and patient and family details (WP1) will be sent in coded form to the Coordinators for data storage as outlined in WP8. Each Center will also obtain the best possible patient and optionally family resource, basic laboratory tests as performed locally: these tests will be confirmed centrally by laboratories known for their expertise (WP3). Initial and confirmatory data will be included into the database.

Advanced tests for the evaluation of antibodies against VWF in each enrolled family will be centralized in a few expert laboratories (WP4). Intra-platelet VWF quality and quantity will be assessed in all VWD3 patients and will be measured centrally following receipt of a lysed platelet preparation (WP4). An important part of the project will be the analysis of results obtained with all the advanced tests leading to detailed analysis of their value as markers for the diagnosis and management of VWD3, particularly when compared to the basic tests as in WP2.

Mutation analysis will start only when most of the patients will have been enrolled and evaluated at the end of the retrospective survey (month 24th). Since this gene analyses might require more time, the central laboratories involved in this first part of the study will continue their tests also during the second part of the study and should provide all data completed within month 48th. The presence of VWF gene defects will be correlated with the presence of inhibitors (WP5).

3.1.2 Second & third parts of the Study

The novelty of this project consists in the correlation between clinical and molecular markers and bleeding tendency as well as with response to therapy with VWF concentrates and risk of anti-VWF inhibitors. Therefore the second part should start only when all clinical, laboratory and molecular markers will be available at the end of the first part of the study.

Clinical and laboratory predictors of bleeding (WP6) will be evaluated in all patients with confirmed diagnosis of VWD3 who can be followed prospectively for 24 months and, after 12 months of evaluation of the collected data by the Study Coordinators, the patients will be observed for additional 24 months to collect additional information related to study endpoints aiming to a better characterization of the observed patients.

Bleeding severity score, baseline levels of VWF and FVIII, the presence of anti-VWF inhibitors and several modifiers will be tested as clinical and laboratory predictors of bleeding in VWD3.

The current treatments of VWD3 patients will be also evaluated during this study (WP7). It is important to say that during the 48-month follow-up (2 observation phases), all the VWD3 patients will continue the current type of treatment using the same type of VWF concentrate with or without FVIII available in their own countries at the time of enrollment in this study. The information on the amounts of concentrates (U/month or U/year), the number of exposure days/year, the efficacy and safety of each VWF concentrates used in these patients will be collected and will be available at the end of the study. Moreover, the previous exposure to VWF concentrates will be analyzed as well as the frequency of the major side effects, including anaphylaxis in VWD3 with anti-VWF inhibitors.

Efficacy assessment of the VWF concentrates used to treat VWD3 using the most recent objective criteria for efficacy. Site and type of bleedings and specific approaches to VWD3 patients with anti-VWF inhibitors will be recorded.

The use of plasma-derived and/or recombinant VWF concentrates will be evaluated comparing the regimens adopted for patients treated with VWF concentrates under on demand versus secondary long-term prophylaxis.

In addition, a detailed evaluation of type, severity and characterization of GIB episodes and of the type, dosage and frequency of administration of VWF concentrates related to GIB will be performed in a small group of patients only (WP7b). The type of diagnosis and site of bleeding should be evaluated at each bleeding episode by performing a complete endoscopic examination of the GI tract, including esophagogastroduodenoscopy, colonoscopy and videocapsule enteroscopy, unless the diagnosis has been confidently reached by one the exams and/or the clinical presentation is indicative of the recurrence of a previously diagnosed event. The severity of each event, either retrospectively or prospectively collected, will be evaluated according to the Blatchford Score (see below) when data can be accurately obtained:

Calculation of prognostic severity of GIB episodes		
Blood Nitrogen	<18.2 mg/dL (<6.5 mmol/L)	0 points*
	≥18.2 and <22.4 mg/dL (≥6.5 and <8 mmol/L)	2 points
	≥22.4 and <28 mg/dL (≥8 and <10 mmol/L)	3 points
	≥28 and <70 mg/dL (≥10 and <25 mmol/L)	4 points
	≥70 mg/dL (≥25 mmol/L)	6 points
Hemoglobin	MALE: ≥13 g/dL (≥130 g/L)	0 points*
	MALE: ≥12 and <13 g/dL (≥120 and <130 g/L)	1 point
	MALE: ≥10 and <12 g/dL (≥100 and <120 g/L)	3 points
	FEMALE: ≥12 g/dL (≥120 g/L)	0 points*
	FEMALE: ≥10 and <12 g/dL (≥100 and <120 g/L)	1 point
	MALE / FEMALE: <10 g/dL (<100 g/L)	6 points
Blood Pressure	≥110 mmHg	0 points*
	100 to 109 mmHg	1 point
	90 to 99 mmHg	2 points
	<90 mmHg	3 points
Other markers	Heart Rate: ≥100 per minute	1 point
	Melena at presentation	1 point
	Syncope at presentation	2 points
	Presence of hepatic disease	2 points
	Presence of cardiac failure	2 points

** A score of zero is associated with a low risk of the need for endoscopic intervention.*

Should all the above parameters be not available, a "simplified score" should be proposed, which includes:

- Signs of hypovolemia (tachycardia > 100 bpm and arterial pressure < 100 mmHg; alternatively a shock index (RPM/AP > 1));
- Signs of ongoing bleeding (hematemesis or proctorragia or melena);
- Hgb < 10 g/dL;
- Transfusional need of > 2 units during the event.

On the long term, particularly in case of occult or non-acute bleeding, the entity of transfusional need for time unit is considered an accurate measure of GI bleeding and will be recorded for each patient.

In case of the few patients with GIB recurrence an additional blood sample will be collected upon request and approval by the Steering Committee and shipped to the Central Laboratory deputed to the analysis of angiogenesis markers (Angiopoietin-1, Angiopoietin-2, Osteoprotegerin, Galectin-3, CXCL8/IL-8, Tie-2, VEGF) in order to evaluate the presence of ANGDYS.

All the clinical and laboratory data collected during this 8-year project will provide the actual information on the management of this rare but complex inherited bleeding disorder and will allow preparation of novel guidelines on VWD3 diagnosis and treatment (WP8) based on the most recent results of a prospective study.

3.2 General Management of the Study

The overall objective of this proposal is to utilize and combine clinical knowledge and technical expertise of clinicians well known for their ability to diagnose and manage patients with VWD3 and scientists well known for their innovative research in the field of thrombosis and Haemostasis. This international combination of clinical and technical expertise will allow us to substantially improve the available information on the clinical aspects of rare VWD3 as well as clinical and laboratory predictors of the bleeding diathesis of these patients. This effort is highly relevant, as it will inform us on issues like duration of hospitalization, frequency of doctor visits and severity and frequency of bleeding. Little if any information on these VWD3 patients to be investigated by this study is available to Haematologists, Patient Organizations and Health Authorities. Moreover, our studies will allow the translation from clinical observations to basic research and back, which will contribute significantly to our understanding of the marked variability observed between patients with this rare inherited bleeding disorder.

We expect that via the execution of this study, we will be able to establish:

a) An international database with clinical and laboratory information of patients with VWD3;

b) A better understanding of the basic molecular mechanisms of VWD3 that will allow a more specific therapeutic approach to these VWD3 patients.

Taken together, this international collaborative effort will translate in better diagnostic and therapeutic tools to manage this rare but severe bleeding disorder.

The proposed project involves 8 different Working packages in order to reach the objectives necessary to improve the knowledge on the basic molecular mechanisms of VWD3. The information available in this International Registry (European and Iranian) will be exchanged and compared among the different partners involved in this 8-year study by using a common database. Start and completion dates, responsibilities, involvement and work duties of the different partners will be reported in each Working package. A detailed description for each and every Working Package will be available separately in Appendix 3.

4. STUDY DURATION AND TIMELINES

Study period:

- First part of the study (retrospective survey and centralized confirmation): approximately 36 months (years 2014 to 2016)
- Second part of the study (prospective observation): approximately 24 months (years 2017 – 2018)
- Confirmation of clinical phase data: approximately 12 months (year 2019)
- Second prospective observation: approximately 24 months (years 2020 – 2021)
- Total study duration: approximately 96 months

Individual subject participation:

- First part of the study: approximately 1 month
- Second part of the study: 4 years (2 prospective observation periods of 2 years each: 2017-2018 and 2020-2021).

5. STUDY POPULATION

A large cohort of at least 250 patients with diagnosis of Type 3 von Willebrand Disease will be enrolled in Europe (approximately 125 cases) and in Iran (approximately 125 cases) at approximately 27 investigational sites using homogenous and standardized criteria (20 in Europe + 7 in Iran).

5.1 ELIGIBILITY CRITERIA

All patients with diagnosis of Type 3 von Willebrand Disease will be enrolled.

Inclusion criteria:

- Male and female of any age, including infants, children, adolescent and adults
- Informed Consent obtained (parents should sign for patients < 18 y.o.)
- Previous Diagnosis of VWD3 (VWF antigen: undetectable or <5 U/dL)
- Detailed information on inherited pattern, history of bleeding, previous exposure to blood products
- Availability of plasma and DNA samples

Exclusion criteria:

- VWD3 patients who may not be available for follow-up

5.2 ENROLMENT STRATEGIES

The recruitment of at least 250 patients with VWD3 as determined by a family history and basic laboratory tests will be completed within 36 months.

All the Centers involved will gather retrospective data on patients already diagnosed at the Center as VWD3 patients and who are eligible for this study.

Each and every subject will be asked to read, understand and sign the Informed Consent Form to authorize his participation in the study before entering into the study.

Optional: for relatives who accept the following will be collected:

- Informed Consent collection
- Historical Information
- Blood Sampling for Local Laboratory Assessment

6. STUDY PROCEDURES

6.1 ENROLMENT – FIRST PART OF THE STUDY

6.1.1 Enrolment visit

Before any screening activity may be performed, a signed and dated Informed Consent form must have been obtained from patient. The medical history (demography, bleeding history, previous treatments, previous diagnosis and familiarity of VWD3) will be collected to assess a subject's eligibility for this study. If the patient satisfy the enrolment criteria a blood sample of 20 ml will be withdrawn for adults and 10 ml for children. For children below 12 y.o. blood sampling should be part of the regular management adapted to the age.

The central laboratory evaluations are detailed in the following paragraph 7.

Each blood sample will be stored locally at -70°C and subsequently sent to the designed Central Laboratory for the assessment. If a -70° C freezer is not available at sites, a -20° C freezer can be used but the shipment to central laboratory should be done within 3 months from the sampling.

All samples will be collected and processed according to the central laboratory manual. Handling and shipment of the samples and the materials will be described in the manual as well.

Medical history will include concomitant diseases, concomitant medications and previous treatments for VWD3, if any. A familiar pattern form will be evaluated. Past bleeding episode(s) history and severity score will be recorded on CRF.

For the optional family members the same procedures will be followed.

6.2 FOLLOW-UP – SECOND PART OF THE STUDY

6.2.1 Control visit

Upon confirmation of the diagnosis of VWD3 from the assigned Central Laboratory, the patient will enter into the second part (prospective observation) of the study. Due to the observational nature of the study, all patients will be treated at each Investigational site according to the Center standard clinical practice with none interference in the treatment and care in relation to this study. The patients will be asked to visit the site regularly according to the standard clinical practice at each investigational site (at least once in a year). No additional visits nor instrumental assessments will be required.

At each visit all information about bleeding events will be collected and recorded on subject's case history and on CRF. All medications taken, prescription or over-the counter continued at the start of the study or started during the study must be documented in source documents and entered in the CRF.

AEs and SAEs will be recorded/communicated according to the applicable procedure.

Bleeding episode(s) occurred between visits will be recorded on CRF. In details, the points below itemized will be checked:

- date and site
- bleeding score
- treatment

Additional blood withdrawal of 5 ml will be performed only in case of anti-VWF inhibitors development for confirmation at Central Laboratory.

6.2.2 Final visit

Bleeding episode(s) occurred since previous visit will be recorded on CRF.

VWF containing concentrates and Concomitant Medications, on-going at the end, discontinued or stopped during the study will be reported.

AEs and SAEs will be recorded and reconciled with source documents.

7. DATA POINTS

The site will determine whether the patient qualifies for the study before entering the patient into the registry. Sites will assign a patient identification (ID) number to each patient that enters the registry. Therefore, the patient ID will consist of 11 digits as follows:

- **Site code**: composed by two capital letters for the Country and two number for the site (centrally assigned)
- **Family code**: composed by the capital letter "F" and followed by two numbers starting from 01 and assigned in sequential order to identify the family in case two or more patients are relatives
- **Generation**: can be I, II, III or IV
- **Individual code**: composed by the capital letter "P" (patient) or "R" (relative) to specify if the subject is a VWD3 patient or a relative (in case any relative of VWD3 patient is enrolled – optional) and followed by two numbers starting from 01 and assigned in sequential order.

Example: **|N|L|0|1|-|F|0|1|-|II|-|P|0|1|**

The patient ID number will be used to identify the patient in the study and must be used on all study documentation related to that patient and must not be changed at any time. Entry is defined as the day the patient consents to participate.

The following data will be collected for each and every patient included in the study and who signed the Informed Consent Form before enrolment.

First part of the study

1. Informed Consent collection – If Informed Consent is required, the patient will sign Informed Consent before the patient ID is assigned and data collection. The Informed Consent process will be clearly documented into the patient's chart.
2. Eligibility Verification – The eligibility criteria will be confirmed for the patient. If the patient meets the eligibility criteria, the patient will be entered into the registry.
3. Patient ID Assignment – Once all the eligibility criteria have been confirmed, the patient will be assigned a patient 11 digits ID number.
4. Demographics – Age, race and gender of the patient will be documented.
5. Bleeding History and Previous Use of Blood Components – The year, month and day the patient was diagnosed for VWD3; the Site and Score of bleeding for the calculation of the Bleeding Severity Score and the type of Blood Products used with reference to the year of first exposure and the units used will be documented. The negativity/positivity to Blood-borne infections (HCV and HIV) will be assessed too.
6. General Laboratory Test with Local Assays for VWD3 Diagnosis - In case a Local assays has not been done within 1 year ,a blood sample will be collected at enrolment to determine locally the following parameters: Hemoglobin, MCV, Leucocytes, Platelet count, MPV, Ferritine, Prothrombin Time, PTT, PTT mix, either Bleeding Time or Closure Time, FVIII:C, VWF:RCo and VWF:Ag.
7. Family History in the Parents and relatives Tree and Pedigree.
8. Blood Withdrawal for Central Laboratory Assessment for VWD3 patients – A venous blood sample will be withdrawn from the patients for a total amount of 20 ml for adults and 10 for children. These amount of samples will be used to optain both citrated Platelet Poor Plasma (PPP) for VWF assays and Cell Pellet (CP) for DNA extraction. For PPP analysis each tube will be filled in with 0.3 ml (the total amount of 0.3 ml tubes should be at least 24 for adults and 12 for children) while in case of CP analysis the sample should be divided equally into at least 2-4 tubes respectively for children and adults. All these tubes must be stored locally at – 80 C° .and sent to the European Central Laboratories. For the analysis of PPP, CP and DNA, samples will be evaluated in 5-6 assigned European Laboratories. The details of the Central Laboratories will be distributed separately (Working Packages).

9. Optional: Bleeding history and blood withdrawal for Local Laboratory Assessment will be collected for subject's parents and relatives who agree to participate.

Second part of the study

1. Bleedings – All bleeding experienced during the study will be documented and detailed for number, types and risk factors for all patients with confirmed diagnosis of VWD3.
2. VWF Concentrates – All different VWF-containing concentrates used during the study will be documented and described in quantities, efficacy and safety for all patients enrolled in the study. The number of patients treated with plasma-derived and/or recombinant VWF concentrates under on demand versus secondary long-term prophylaxis regimens will be evaluated.
3. Concomitant medication and Adverse Events – all other medication in use and all adverse events occurred during the study course will be collected and entered in CRF.
4. Patients with recurrent GIB who might benefit of treatment with VWF concentrates and correlation of angiogenesis markers with previous GIB episodes recorded – within the identification of a pool of VWD3 patients with GIB according to standardized and objective criteria, the data collected retrospectively in these patients will be evaluated and correlated with phenotype (including markers of angiogenesis) and genotype centralized results. This will help identifying potential correlations between phenotype/genotype and GIB occurrence. The VWD3 patients with GIB occurrence will be tested centrally for angiogenesis markers (Angiopoietin-1, Angiopoietin-2, Osteoprotegerin, Galectin-3, CXCL8/IL-8, Tie-2, VEGF) in order to evaluate the presence of ANGDYS. In addition, the efficacy and safety of plasma-derived and/or recombinant VWF concentrates used to treat patients' GIB during the prospective observation period, in relation to the use of anti-angiogenetic agents within the standard clinical setting, will be evaluated.

Additional blood withdrawal of 5 ml will be performed only in case of anti-VWF inhibitors development for confirmation at Central Laboratory.

8. STATISTICAL ANALYSIS

8.1 PRIMARY ANALYSIS

Consistently with the objectives of the retrospective and prospective observational study and appropriate to the data being collected, a Statistical Analysis Plan (SAP) will be developed. Given

the observational design of the registry, the primary analysis will be descriptive and associative. The SAP will describe, in the form of annotated tables, figures and listings, the analyses to be conducted.

Analyses will first present the patient clinical and demographic characteristics of the patients being treated for VWD3 at the investigational sites in Europe and in Iran. Next, analyses will be conducted to identify clinical and laboratory predictors of bleeding.

The SAP will also provide details on how the effectiveness variables will be derived, how the missing values will be handled, and how these variables will be analyzed.

8.2 STATISTICAL ANALYSIS

Analytic techniques will be pertinent to the observational design of the registry. Basic analysis will be descriptive and associative. Tables of baseline clinical and demographic characteristics, treatment patterns, clinical outcomes, and health care resource utilization will be compiled for all patients, and other techniques may be employed to assess association of covariates, including treatments and outcomes (including clinical and laboratory outcomes).

9. PROJECT MANAGEMENT AND COORDINATION

Fondazione Bianchi Bonomi shall assign Sintesi Research S.r.l, Via Matteo Bandello, 6 – 20123 Milan (Italy) to coordinate and manage the study conduction in every phase. Sintesi Research will assure to act in compliance with the local guidelines and legislations, in the respect of the protocol and timelines agreed in the same.

Sintesi Research, thorough its personnel, will be responsible for the following:

1) Request of authorization for study conduction – Sintesi Research will perform all the necessary steps to obtain the authorization for the study conduction in all the investigational sites involved in Europe (documents preparation, request of authorization dossier preparation and submission)
2) Sites activation – upon authorization from the Central and Local Competent Authorities and Ethics Committees accordingly to the country specific requirements and procedures, the sites will receive the personal access details for the project data base; the Investigator Site File containing the documentation, template, forms and training material for the study conduction.
3) Study management – the assigned Project Manager will coordinate and manage the survey conduction particularly (but not limited to) in the following activities:

a. He/she will supervise the sites and patients enrolment thorough the web site and the direct communication with site personnel;
b. He/she will be responsible to maintain contact with and supervise the blood samples shipments from the investigational sites to the reference central laboratories;
c. He/she will guarantee the standardization of the study procedures and the distribution of the right and proper documentation to the sites;
d. He/she assists and supports the sites for any issue occurring during the study course
e. He/she takes all the measure to guarantee that the patient privacy is protected and the data quality respect the standards required.
f. He/she verifies that the study conduction always occurs in compliance with all the applicable regulations.

A web based project portal and database (www.vwd-3winters-ips.com) will be developed by Sintesi Research for the overall study management and data collection. The portal will be used as virtual archive for the study documentation, communication, updates, and to share information, experiences, opinion among participants. The database will serve to collect the data points as specified in Paragraph 6 of this study protocol. Each single site member involved in the project will confidentially receive personal access to the web site and at the level of functionality assigned to his/her role in the study.

The on-site monitoring of the Centers is not planned. The sites will be monitored remotely through the web site. Nevertheless, for those Centers that require patient Informed Consent the Sponsor's monitors may, on occasion, visit the site during the study to ensure GCP Guidelines have been followed to protect patient confidentiality.

10. SAFETY

10.1Safety Information

An adverse event (AE) is defined as any new medical problem or exacerbation of an existing problem, associated with the use of a drug in humans, whether or not the event is considered drug related. A serious adverse event (SAE) includes any event that results in any of the following outcomes:

a) death

b) life-threatening, i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred. It does not include an event that, had it occurred in a more severe form, might have caused death.

c) persistent or significant disability/incapacity

d) requires in-patient hospitalization or prolongs hospitalization

e) congenital anomaly/birth defect

f) other medically significant events that, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above, e.g., allergic bronchospasm requiring intensive treatment in an emergency room or home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

Non-serious adverse events are all adverse events that do not meet the criteria for a "serious" adverse event. For adverse events associated with the use of any other treatment, the investigator will be encouraged to contact the manufacturer or the regulatory authorities to report the adverse event. A copy of any form submitted to the manufacturer or regulatory authorities must be submitted to Sponsor.

An Independent Data Monitoring Committee (DMC) will be appointed for data monitoring and will be extended for the entire duration of the prospective observation.

The designed DMC members are:

Craig KESSLER, MD
Lombardi Cancer Ctr.
Georgetown University
Washington, USA

David LILLICRAP
Queens University
Kingston, Canada

Robert R MONTGOMERY
Department of Pediatrics Blood Center
Milwaukee, USA

11.ETHICAL ASPECTS

The 3WINTERS-IPS study is Sponsored by a Non-Profit Organization, Angelo Bianchi Bonomi Foundation, Milan, Italy, and it will be conducted in collaboration with Centers belonging to the Group on VWD3 recognized by the European Association of Haemophilia and Allied Disorders (EAHAD) and Sub-Committee on VWF Scientific Standardization Committees of the International Society on Thrombosis and Haemostasis (SC-VWF, SSC-ISTH). This study must be conducted in compliance with the protocol and all other applicable local laws and regulatory requirements. Each study site will seek approval by an IRB or EC according to regional requirements. The IRB/IEC

will evaluate the ethical, scientific and medical appropriateness of the study. Further, in preparing and handling CRF and EDC, the investigator, sub-investigator and their staff will take measures to ensure adequate care in protecting patient privacy. To this end, a patient identification code will be used to identify each patient.

11.1Study supervision

The 3WINTERS-IPS Steering Committee (SC) whose members are all the Partners Responsible persons, will supervise the conduction of the study. The SC will be regularly updated on the study progress by the Study Project Manager and it is the organ which finally will take any decision on any issues that should derive from the study conduction.

11.2Privacy of personal data

In order to maintain patients' privacy, all CRFs, study reports and communications will identify the patient by the assigned patient number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the patient's original medical records for verification of data entered into the CRFs and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations. The data collected properly coded will remain within the investigational Center where the patient is recruited, the Central Laboratories involved for clinical and molecular analysis, the study Sponsor and the Members appointed for data review (DMC and SC) and verification (CRO).

11.3Confidentiality

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to the study purposes only. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations, in particular the General Data Protection Regulation (GDPR) n. 2016/679 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data.

The Sponsor ensures that the personal data will be:

- processed fairly and lawfully
- collected for specified, explicit, and legitimate purposes and not further processed in a way incompatible with these purposes
- adequate, relevant, and not excessive in relation to said purposes
- accurate and, where necessary, kept current

Explicit consent for the processing of personal data will be obtained from the patients before collection of data. Such consent should also address the transfer of the data to other entities and to other countries. The patients have the right to request through the investigator access to personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of study subjects confidential.

11.4Protocol compliance

The Investigator will conduct the study in compliance with the protocol provided by the Sponsor, and given approval/favourable opinion by the IRB/EC and the appropriate regulatory authority(ies). Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB/EC approval/favourable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB/EC may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favourable opinion for minor change(s) in ongoing studies that have the approval/favourable opinion of the IRB/EC. The Sponsor or its designees will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

11.5Protocol modifications

Neither the investigator nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the Sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified. In situations requiring a departure from the protocol, the investigator or other physician in attendance will contact the appropriate Sponsor representative by fax or telephone (see Contact Information pages provided separately in the Investigator Site File). If possible, this contact will be made before implementing any departure from the protocol. In all cases, contact with the Sponsor must be made as soon as possible in order to discuss the situation and agree on an appropriate

course of action. The data recorded in the CRF and source document will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it. When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to patients, the Investigator will contact the Sponsor or its designees, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the CRF and source documentation.

11.6Subject identification register and subject screening log

The investigator agrees to complete a subject identification register to permit easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness. The subject identification register will be treated as confidential and will be filed by the investigator in the Investigator Site File. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by initials and assigned number only. The investigator must also complete a subject-screening log, which reports on all subjects who were seen to determine eligibility for inclusion in the study.

12. PUBLICATION POLICY

The results of this study will be presented at scientific meetings and/or published in a peer reviewed scientific or medical journal. A Publications Committee, comprised of investigators participating in the study, as appropriate, will be formed to oversee the publication of the study results, which will reflect the experience of all participating study Centers.

All reports (including abstracts) and publications shall be approved by the Steering Committee prior to submission. The authorship of publications shall reflect the input of individual collaborators into the work and recognize the role of the project. The aim is to include at least one author from each participating Center, however this is subject to specific journal requirements.

Studies performed with a recruited family and relating to the project should be reported as part of the project. Studies performed with a recruited family that are not related to the project may be published by the individual investigator after consultation with the Publications Committee and should make reference to the project.

13.GENERAL REFERENCES

1. Marchese M, De Cristofaro R, Federici AB, Biondi A, Petruzziello L, Tringali A, Spada C, Mutignani M, Ronconi P, Costamagna G. "Duodenal and gastric Dieulafoy's lesions in a patient with type 2A von Willebrand's disease." Gastrointest Endosc. 2005 Feb;61(2):322-5.

2. Tosetto A, Rodeghiero F, Castaman G, Bernardi M, Bertoncello K, Goodeve A, Federici AB, Batlle J, Meyer D, Mazurier C, Goudemand J, Eikenboom J, Schneppenheim R, Budde U, Ingerslev J, Vorlova Z, Habart D, Holmberg L, Lethagen S, Pasi J, Hill F, Peake I. "Impact of plasma von Willebrand Factor levels in the diagnosis of type 1 von Willebrand Disease: results from a multicenter European study (MCMDM-1VWD)." J Thromb Haemost. 2007 Apr;5(4):715-21.

3. Federici AB. "Highly purified VWF/FVIII concentrates in the treatment and prophylaxis of von Willebrand Disease: the PRO. WILL Study." Haemophilia. 2007 Dec;13 Suppl 5:15-24.

4. Federici AB, Mannucci PM, Castaman G, Baronciani L, Bucciarelli P, Canciani MT, Pecci A, Lenting PJ, De Groot PG. "Clinical and molecular predictors of thrombocytopenia and risk of bleeding in patients with von Willebrand Disease type 2B: a cohort study of 67 patients." Blood. 2009 Jan 15;113(3):526-34.

5. Gritti G, Cortelezzi A, Bucciarelli P, Rezzonico F, Lonati S, La Marca S, Silvestris I, Federici AB. "Circulating and progenitor endothelial cells are abnormal in patients with different types of von Willebrand Disease and correlate with markers of angiogenesis." Am J Haematol. 2011 Aug;86(8):650-6.

6. Abshire TC, Federici AB, Alvárez MT, Bowen J, Carcao MD, Cox Gill J, Key NS, Kouides PA, Kurnik K, Lail AE, Leebeek FW, Makris M, Mannucci PM, Winikoff R, Berntorp E; VWD PN. "Prophylaxis in severe forms of von Willebrand's disease: results from the von Willebrand Disease Prophylaxis Network (VWD PN)." Haemophilia. 2013 Jan;19(1):76-81.

7. Castaman G, Goodeve A, Eikenboom J; European Group on von Willebrand Disease. "Principles of care for the diagnosis and treatment of von Willebrand Disease." Haematologica. 2013 May;98(5):667-74.

8. Federici AB, Bucciarelli P, Castaman G, Mazzucconi MG, Morfini M, Rocino A, Schiavoni M, Peyvandi F, Rodeghiero F, Mannucci PM. "The bleeding score predicts clinical outcomes and replacement therapy in adults with von Willebrand Disease." Blood. 2014 Jun 26;123(26):4037-44.

9. Abdul-Kadir R, McLintock C, Ducloy AS, El-Refaey H, England A, Federici AB, Grotegut CA, Halimeh S, Herman JH, Hofer S, James AH, Kouides PA, Paidas MJ, Peyvandi F, Winikoff R. "Evaluation and management of postpartum hemorrhage: consensus from an international expert panel." Transfusion. 2014 Jul;54(7):1756-68.

10. Makris M, Federici AB, Mannucci PM, Bolton-Maggs PH, Yee TT, Abshire T, Berntorp E. "The natural history of occult or angiodysplastic gastrointestinal bleeding in von Willebrand Disease." Haemophilia. 2015 May;21(3):338-42.

11. Federici AB. "Clinical and laboratory diagnosis of VWD." Haematology Am Soc Haematol Educ Program. 2014 Dec 5;2014(1):524-30.

12. Holm E, Abshire TC, Bowen J, Álvarez MT, Bolton-Maggs P, Carcao M, Federici AB, Gill JC, Halimeh S, Kempton C, Key NS, Kouides P, Lail A, Landorph A, Leebeek F, Makris M, Mannucci P, Mauser-Bunschoten EP, Nugent D, Valentino LA, Winikoff R, Berntorp E. "Changes in bleeding patterns in von Willebrand Disease after institution of long-term replacement therapy: results from the von Willebrand Disease Prophylaxis Network." Blood Coagul Fibrinolysis. 2015 Jun;26(4):383-8.

13. Randi AM, Laffan MA. "Von Willebrand Factor and angiogenesis: basic and applied issues." J Thromb Haemost. 2016 Oct 25.

14. Federici AB. "Current and emerging approaches for assessing von Willebrand Disease in 2016." Int J Lab Haematol. 2016 May;38 Suppl 1:41-9.

15. De Jong A, Eikenboom J. "Developments in the diagnostic procedures for von Willebrand Disease." J Thromb Haemost. 2016 Mar;14(3):449-60.

16. Abshire T, Cox-Gill J, Kempton CL,4, Leebeek FW, Carcao M, Kouides P, Donfield S, Berntorp E. "Prophylaxis escalation in severe von Willebrand Disease: a prospective study from the von Willebrand Disease Prophylaxis Network." J Thromb Haemost. 2015 Sep;13(9):1585-9.

17. Engelen ET, van Galen KP, Schutgens RE. "Thalidomide for treatment of gastrointestinal bleedings due to angiodysplasia: a case report in acquired von Willebrand syndrome and review of the literature." Haemophilia. 2015 Jul;21(4):419-29.

18. Holm E, Abshire TC, Bowen J, Álvarez MT, Bolton-Maggs P, Carcao M, Federici AB, Gill JC, Halimeh S, Kempton C, Key NS, Kouides P, Lail A, Landorph A, Leebeek F, Makris M, Mannucci P, Mauser-Bunschoten EP, Nugent D, Valentino LA, Winikoff R, Berntorp E. "Changes in bleeding patterns in von Willebrand Disease after institution of long-term replacement therapy: results from the von Willebrand Disease Prophylaxis Network." Blood Coagul Fibrinolysis. 2015 Jun;26(4):383-8.

19. Franchini M, Mannucci PM. "Gastrointestinal angiodysplasia and bleeding in von Willebrand Disease." Thromb Haemost. 2014 Sep 2;112(3):427-31.

20. Randi AM, Laffan MA, Starke RD. "Von Willebrand Factor, angiodysplasia and angiogenesis." Mediterr J Haematol Infect Dis. 2013 Sep 2;5(1):e2013060.

21. Thachil J, Hay CR, Campbell S. "Tamoxifen for recurrent bleeds due to angiodysplasia in von Willebrand's disease." Haemophilia. 2013 Sep;19(5):e313-5.

22. Castaman G, Federici AB, Tosetto A, La Marca S, Stufano F, Mannucci PM, Rodeghiero F. "Different bleeding risk in type 2A and 2M von Willebrand Disease: a 2-year prospective study in 107 patients." J Thromb Haemost. 2012 Apr;10(4):632-8.

23. Starke RD, Ferraro F, Paschalaki KE, Dryden NH, McKinnon TA, Sutton RE, Payne EM, Haskard DO, Hughes AD, Cutler DF, Laffan MA, Randi AM. "Endothelial von Willebrand Factor regulates angiogenesis." Blood. 2011 Jan 20;117(3):1071-80.

24. Veyradier A, Balian A, Wolf M, Giraud V, Montembault S, Obert B, Dagher I, Chaput JC, Meyer D, Naveau S. "Abnormal von Willebrand Factor in bleeding angiodysplasias of the digestive tract." Gastroenterology. 2001 Feb;120(2):346-53.

25. Bowers M, McNulty O, Mayne E. "Octreotide in the treatment of gastrointestinal bleeding caused by angiodysplasia in two patients with von Willebrand's disease." Br J Haematol. 2000 Mar;108(3):524-7.

26. Van Belle E, Rauch A, Vincent F, Robin E, Kibler M, Labreuche J, Jeanpierre E, Levade M, Hurt C, Rousse N, Dally JB, Debry N, Dallongeville J, Vincentelli A, Delhaye C, Auffray JL, Juthier F, Schurtz G, Lemesle G, Caspar T, Morel O, Dumonteil N, Duhamel A, Paris C, Dupont-Prado A, Legendre P, Mouquet F, Marchant B, Hermoire S, Corseaux D, Moussa K, Manchuelle A, Bauchart JJ, Loobuyck V, Caron C, Zawadzki C, Leroy F, Bodart JC, Staels B, Goudemand J, Lenting PJ, Susen S. "Von Willebrand Factor Multimers during Transcatheter Aortic-Valve Replacement." N Engl J Med. 2016 Jul 28;375(4):335-44.

27. Susen S, Rauch A, Van Belle E, Vincentelli A, Lenting PJ. "Circulatory support devices: fundamental aspects and clinical management of bleeding and thrombosis." J Thromb Haemost. 2015 Oct;13(10):1757-67.

28. Blatchford O, Murray WR, Blatchford M. "A risk score to predict need for treatment for upper-gastrointestinal haemorrhage." Lancet. 2000 Oct 14;356(9238):1318-21.

29. Gerson LB, Fidler JL, Cave DR, Leighton JA. "ACG Clinical Guideline: Diagnosis and Management of Small Bowel Bleeding." Am J Gastroenterol. 2015 Sep;110(9):1265-87.

30. Strate LL, Gralnek IM. "ACG Clinical Guideline: Management of Patients With Acute Lower Gastrointestinal Bleeding." Am J Gastroenterol. 2016 Apr;111(4):459-74.

31. Jackson CS, Gerson LB. "Management of gastrointestinal angiodysplastic lesions (GIADs): a systematic review and meta-analysis." Am J Gastroenterol. 2014 Apr;109(4):474-83.

32. Martin-Grace J, Tamagno G. "Somatostatin analogs in the medical management of occult bleeding of the lower digestive tract." Gastroenterol Res Pract. 2015;2015:702921.

33. Holleran G, Hall B, Breslin N, McNamara D. "Long-acting somatostatin analogues provide significant beneficial effect in patients with refractory small bowel angiodysplasia: Results from a proof of concept open label mono-centre trial." United European Gastroenterol J. 2016 Feb;4(1):70-6.

34. Eikenboom JC. (2001) Congenital von Willebrand disease type 3: clinical manifestations, pathophysiology and molecular biology. Best Pract Res Clin Haematol 14, 365-379.

35. Sadler JE, Budde U, Eikenboom JC et al. (2006) Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost 4, 2103-2114.

36. von Willebrand EA. (1926) Hereditär Pseudohemofili. Finska Läkaresällskapets Handlingar 68, 87-112.

37. Nilsson IM. (1999) Commentary to Erik von Willebrand's original paper from 1926 'Hereditar pseudohemofili'. Haemophilia 5, 220-221.

38. Nilsson IM, Blomback M, Blomback B. (1959) von Willebrand's disease in Sweden. Its pathogenesis and treatment. Acta Med Scand 164, 263-278.

39. Mannucci PM, Bloom AL, Larrieu MJ et al. (1984) Atherosclerosis and von Willebrand factor. I. Prevalence of severe von Willebrand's disease in western Europe and Israel. Br J Haematol 57, 163-169.

40. Weiss HJ, Ball AP, Mannucci PM. (1982) Incidence of severe von Willebrand's disease (letter). N Engl J Med 307, 127

41. Berliner SA, Seligsohn U, Zivelin A et al. (1986) A relatively high frequency of severe (type III) von Willebrand's disease in Israel. Br J Haematol 62, 535-543.

42. Iorio A, Oliovecchio E, Morfini M et al. (2008) Italian Registry of Haemophilia and Allied Disorders. Objectives, methodology and data analysis. Haemophilia 14, 444-453.

43. Federici AB, Mannucci PM. (2007) Management of inherited von Willebrand disease in 2007. Ann Med 39, 346-358.

44. Silwer J. (1973) von Willebrand's disease in Sweden. Acta Paediat Scand 238, 1-159.

45. Lak M, Peyvandi F, Mannucci PM. (2000) Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. Br J Haematol 111, 1236-1239.

46. Federici AB, Castaman G, Mannucci PM. (2002) Guidelines for the diagnosis and management of von Willebrand disease in Italy. Haemophilia 8, 607-621.

47. Rodeghiero F, Castaman G, Tosetto A et al. (2005) The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multiCenter study. J Thromb Haemost 3, 2619-2626.

48. Tosetto A, Rodeghiero F, Castaman G et al. (2006) A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multiCenter European study (MCMDM-1 VWD). J Thromb Haemost 4, 766-773.

49. Federici AB, Bucciarelli P, Castaman G et al. (2007) Incidence and determinants of bleeding in different types of von Willebrand disease:results of the first prospective multiCenter study on 814 Italian patients Blood 110, 713

50. Cattaneo M, Federici AB, Lecchi A et al. (1999) Evaluation of the PFA-100 system in the diagnosis and therapeutic monitoring of patients with von Willebrand disease. Thromb Haemost 82, 35-39.

51. Mannucci PM, Lattuada A, Castaman G et al. (1989) Heterogeneous phenotypes of platelet and plasma von Willebrand factor in obligatory heterozygotes for severe von Willebrand disease. Blood 74, 2433-2436.

52. Mannucci PM, Federici AB. (1995) Antibodies to von Willebrand factor in von Willebrand disease. Adv Exp Med Biol 386, 87-92.

53. Federici AB. (2008) Clinical and molecular markers of inherited von Willebrand disease type 3: are deletions of the VWF gene associated with alloantibodies to VWF? J Thromb Haemost 6, 1726-1728.

54. Veltkamp JJ, van Tilburg NH. (1973) Detection of heterozygotes for recessive von Willebrand's disease by the assay of antihemophilic-factor-like antigen. N Engl J Med 289, 882-885.

55. Shelton-Inloes BB, Chehab FF, Mannucci PM et al. (1987) Gene deletions correlate with the development of alloantibodies in von Willebrand disease. J Clin Invest 79, 1459-1465.

56. Ngo KY, Glotz VT, Koziol JA et al. (1988) Homozygous and heterozygous deletions of the von Willebrand factor gene in patients and carriers of severe von Willebrand diease. Proc Natl Acad Sci USA 85, 2753-2757.

57. Schneppenheim R, Krey S, Bergmann F et al. (1994) Genetic heterogeneity of severe von Willebrand disease type III in the German population. Hum Genet 94, 640-652.

58. Eikenboom JCJ, Castaman G, Vos HL et al. (1998) Characterization of the genetic defects in recessive type 1 and type 3 von Willebrand disease patients of Italian origin. Thromb Haemost 79, 709-717.

59. Schneppenheim R, Castaman G, Federici AB et al. (2007) A common 253-kb deletion involving VWF and TMEM16B in German and Italian patients with severe von Willebrand disease type 3. J Thromb Haemost 5, 722-728.

60. Peake IR, Liddell MB, Moodie P et al. (1990) Severe type III von Willebrand's disease caused by deletion of exon 42 of the von Willebrand factor gene: Family studies that identify carriers of the condition and a compound heterozygous individual. Blood 75, 654-661.

61. Mancuso DJ, Tuley EA, Castillo R et al. (1994) Characterization of partial gene deletions in type III von Willebrand disease with alloantibody inhibitors. Thromb Haemost 72, 180-185.

62. Baronciani L, Cozzi G, Canciani MT et al. (2000) Molecular characterization of a multiethnic group of 21 patients with type-3 von Willebrand disease. Thromb Haemost 84, 536-540.

63. Abuzenadah AM, Gursel T, Ingerslev J et al. (1999) Mutational analysis of the von Willebrand factor gene in 27 families from Turkey with von Willebrand disease Thromb Haemost 82 (Suppl), 283

64. Mohl A, Marschalek R, Masszi T et al. (2008) An Alu-mediated novel large deletion is the most frequent cause of type 3 von Willebrand disease in Hungary. J Thromb Haemost 6, 1729-1735.

65. Xie F, Wang X, Cooper DN et al. (2006) A novel Alu-mediated 61-kb deletion of the von Willebrand factor (VWF) gene whose breakpoints co-locate with putative matrix attachment regions. Blood Cells Mol Dis 36, 385-391.

66. Cooper DN, Krawczak M. (1990) The mutational spectrum of single base-pair substitutions causing human genetic disease: patterns and predictions. Hum Genet 85, 55-74.

67. Zhang ZP, Lindstedt M, Falk G et al. (1992) Nonsense mutations of the von Willebrand factor gene in patients with von Willebrand disease type III and type I. Am J Hum Genet 51, 850-858.

68. Eikenboom JCJ, Ploos van Amstel HK, Reitsma PH et al. (1992) Mutations in severe type III von Willebrand's disease in the Dutch population: candidate missense and nonsense mutations associated with reduced levels of von Willebrand factor messenger RNA. Thromb Haemost 68, 448-454.

69. Bahnak BR, Lavergne JM, Rothschild C et al. (1991) A stop codon in a patient with severe type III von Willebrand disease. Blood 78, 1148-1149.

70. Zhang ZP, Falk G, Blombäck M et al. (1992) Identification of a new nonsense mutation in the von Willebrand factor gene in patients with von Willebrand disease type III. Hum Molec Gen 1, 61-62.

71. Zhang ZP, Blombäck M, Egberg N et al. (1994) Characterization of the von Willebrand factor gene (vWF) in von Willebrand disease type III patients from 24 families of Swedish and Finnish origin. Genomics 21, 188-193.

72. Hagiwara T, Inaba H, Yoshida S et al. (1996) A novel mutation Gly 1672->Arg in type 2A and a homozygous mutation in type 2B von Willebrand disease. Thromb Haemost 76, 253-257.

73. Gupta PK, Saxena R, Adamtziki E et al. (2008) Genetic defects in von Willebrand disease type 3 in Indian and Greek patients. Blood Cells Mol Dis 41, 219-222.

74. Casana P, Martinez F, Haya S et al. (2000) Q1311X: a novel nonsense mutation of putative ancient origin in the von Willebrand factor gene. Br J Haematol 111, 552-555.

75. Surdhar GK, Enayat MS, Lawson S et al. (2001) Homozygous gene conversion in von Willebrand factor gene as a cause of type 3 von Willebrand disease and predisposition to inhibitor development. Blood 98, 248-250.

76. Baronciani L, Cozzi G, Canciani MT et al. (2003) Molecular defects in type 3 von Willebrand disease: updated results from 40 multiethnic patients. Blood Cells,Mol & Dis 30, 264-270.

77. Gupta PK, Adamtziki E, Budde U et al. (2005) Gene conversions are a common cause of von Willebrand disease. Brit J Haematol 130, 752-758.

78. Eikenboom JCJ, Vink T, Briët E et al. (1994) Multiple substitutions in the von Willebrand factor gene that mimic the pseudogene sequence. Proc Natl Acad Sci USA 91, 2221-2224.

79. Zhang ZP, Falk G, Blombäck M et al. (1992) A single cytosine deletion in exon 18 of the von Willebrand factor gene is the most common mutation in Swedish von Willebrand disease type III patients. Hum Molec Gen 1, 767-768.

80. Xie F, Wang X, Cooper DN et al. (2007) Compound heterozygosity for two novel mutations (1203insG/Y1456X) in the von Willebrand factor gene causing type 3 von Willebrand disease. Haemophilia 13, 645-648.

81. Castaman G, Giacomelli SH, Coppola A et al. (2008) Molecular bases of type 3 von Willebrand disease in Italy: report on 12 families Blood Transfus Suppl 3, 44

82. Gazda H, Budde U, Krey S et al. (1997) Delta C in exon 18 of the von Willebrand factor gene is the most common mutation in patients with severe von Willebrand disease type 3 in Poland Blood 90 (Suppl 1), 94b

83. Wetzstein V, Budde U, Oyen F et al. (2006) Intracranial hemorrhage in a term newborn with severe von Willebrand disease type 3 associated with sinus venous thrombosis. Haematologica 91, 163-165.

84. Krawczak M, Cooper DN. (1991) Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment. Hum Genet 86, 425-441.

85. Efstratiadis A, Posakony JW, Maniatis T et al. (1980) The structure and evolution of the human beta-globin gene family. Cell 21, 653-668.

86. Zhang ZP, Blombäck M, Nyman D et al. (1993) Mutations of von Willebrand factor gene in families with von Willebrand disease in the Aland Islands. Proc Natl Acad Sci USA 90, 7937-7940.

87. Mohlke KL, Nichols WC, Rehemtulla A et al. (1996) A common frameshift mutation in von Willebrand factor does not alter mRNA stability but interferes with normal propeptide processing. Br J Haematol 95, 184-191.

88. Eikenboom JCJ, Reitsma PH, van der Velden PA et al.(1993) Instability of repeats of the von Willebrand factor gene variable number tandem repeat sequence in intron 40. Br J Haematol 84, 533-535.

89. Antonarakis SE. (1998) Recommendations for a nomenclature system for human gene mutations. Nomenclature Working Group. Hum Mutat 11, 1-3.

90. Mertes G, Ludwig M, Finkelnburg B et al. (1994) A G+3-to-T donor splice site mutation leads to skipping of exon 50 in von Willebrand factor mRNA. Genomics 24, 190-191.

91. Zhang ZP, Lindstedt M, Blombäck M et al. (1995) Effects of the mutant von Willebrand factor gene in von Willebrand disease. Hum Genet 96, 388-394.

92. Allen S, Abuzenadah AM, Hinks J et al. (2000) A novel von Willebrand disease-causing mutation (Arg273Trp) in the von Willebrand factor propeptide that results in defective multimerization and secretion. Blood 96, 560-568.

93. Titapiwatanakun R, Guenther JC, Asmann YW et al. (2007) Novel Mutations in Types 2 & 3 von Willebrand Disease and Correlation with von Willebrand Factor Multimer Patterns. Blood 110, 2136

94. Montgomery RR, Jozwiak MA, Hutter JJ et al. (1999) A homozygous variant of the von Willebrand factor (VWF) that fails to c-terminal dimerize resulting in loss of VWF multimers larger than dimer Blood 94 (Suppl 1), 443a

95. Schneppenheim R, Budde U, Drewke E et al. (1999) Cysteine mutations of von Willebrand factor correlate with different types of von Willebrand disease Thromb Haemost 82 (Suppl), 283

96. Enayat MS, Guilliatt AM, Surdhar GK et al. (2001) Identification of five novel mutations in families with type 3 von Willebrand's disease Thromb Haemost 86 (Suppl), P1810

97. Castaman G, Lattuada A, Mannucci PM et al. (1995) Factor VIII: C increases after desmopressin in a subgroup of patients with autosomal recessive severe von Willebrand disease. Br J Haematol 89, 147-151.

98. Tjernberg P, Castaman G, Vos HL et al. (2006) Homozygous C2362F von Willebrand factor induces intracellular retention of mutant von Willebrand factor resulting in autosomal recessive severe von Willebrand disease. Br J Haematol 133, 409-418.

99. Castaman G, Eikenboom JC, Lattuada A et al. (2000) Heightened proteolysis of the von Willebrand factor subunit in patients with von Willebrand disease hemizygous or homozygous for the C2362F mutation. Br J Haematol 108, 188-190.

100. Baronciani L, Federici AB, Cozzi G et al. (2008) Expression studies of missense mutations p.D141Y, p.C275S located in the propeptide of von Willebrand factor in patients with type 3 von Willebrand disease. Haemophilia 14, 549-555.

101. Voorberg J, Fontijn R, Calafat J et al. (1991) Assembly and routing of von Willebrand factor variants: the requirements for disulfide-linked dimerization reside within the carboxy-terminal 151 amino acids. J Cell Biol 113, 195-205.

102. Peake IR, Bowen D, Bignell P et al. (1990) Family studies and prenatal diagnosis in severe von Willebrand disease by polymerase chain reaction amplification of a variable number tandem repeat region of the von Willebrand factor gene. Blood 76, 555-561.

103. Mannucci PM. (2004) Treatment of von Willebrand's Disease. N Engl J Med 351, 683-694.

104. Federici AB, Mazurier C, Berntorp E et al. (2004) Biologic response to desmopressin in patients with severe type 1 and type 2 von Willebrand disease: results of a multiCenter European study. Blood 103, 2032-2038.

105. Castaman G, Lethagen S, Federici AB et al. (2008) Response to desmopressin is influenced by the genotype and phenotype in type 1 von Willebrand disease (VWD): results from the European Study MCMDM-1VWD. Blood 111, 3531-3539.

106. Cattaneo M, Moia M, Della Valle P et al. (1989) DDAVP shortens the prolonged bleeding times of patients with severe von Willebrand disease treated with cryoprecipitate. Evidence for a mechanism of action independent of released von Willebrand factor. Blood 74, 1972-1975.

107. Mannucci PM, Chediak J, Hanna W et al. (2002) Treatment of von Willebrand disease with a high-purity factor VIII/von Willebrand factor concentrate: a prospective, multiCenter study. Blood 99, 450-456.

108. Federici AB, Baudo F, Caracciolo C et al. (2002) Clinical efficacy of highly purified, doubly virus-inactivated factor VIII/von Willebrand factor concentrate (Fanhdi) in the treatment of von Willebrand disease: a retrospective clinical study. Haemophilia 8, 761-767.

109. Bello IF, Yuste VJ, Molina MQ et al. (2007) Fanhdi, efficacy and safety in von Willebrand's disease: prospective international study results. Haemophilia 13 Suppl 5:25-32., 25-32.

110. Federici AB, Barillari G, Zanon E et al. (2008) Efficacy and safety of highly-purified, doubly virus-inactivated VWF/FVIII concentrates in patients with von Willebrand disease: an Italian retrospective study on 120 cases. Haemophilia Submitted.

111. Dobrkovska A, Krzensk U, Chediak JR. (1998) Pharmacokinetics, efficacy and safety of Humate-P in von Willebrand disease. Haemophilia 4 Suppl 3, 33-39.

112. Lillicrap D, Poon MC, Walker I et al. (2002) Efficacy and safety of the factor VIII/von Willebrand factor concentrate, haemate-P/humate-P: ristocetin cofactor unit dosing in patients with von Willebrand disease. Thromb Haemost 87, 224-230.

113. Franchini M, Rossetti G, Tagliaferri A et al. (2003) Efficacy and safety of factor VIII/von Willebrand's factor concentrate (Haemate-P) in preventing bleeding during surgery or invasive procedures in patients with von Willebrand disease. Haematologica 88, 1279-1283.

114. Federici AB, Castaman G, Franchini M et al. (2007) Clinical use of Haemate P in inherited von Willebrand's disease: a cohort study on 100 Italian patients. Haematologica 92, 944-951.

115. Gill JC, Ewenstein BM, Thompson AR et al. (2003) Successful treatment of urgent bleeding in von Willebrand disease with factor VIII/VWF concentrate (Humate-P): use of the ristocetin cofactor assay (VWF:RCo) to measure potency and to guide therapy. Haemophilia 9, 688-695.

116. Thompson AR, Gill JC, Ewenstein BM et al. (2004) Successful treatment for patients with von Willebrand disease undergoing urgent surgery using factor VIII/VWF concentrate (Humate-P). Haemophilia 10, 42-51.

117. Lethagen S, Kyrle PA, Castaman G et al. (2007) von Willebrand factor/factor VIII concentrate (Haemate P) dosing based on pharmacokinetics: a prospective multiCenter trial in elective surgery. J Thromb Haemost 5, 1420-1430.

118. Stadler M, Gruber G, Kannicht C et al. (2006) Characterisation of a novel high-purity, double virus inactivated von Willebrand Factor and Factor VIII concentrate (Wilate). Biologicals 34, 281-288.

119. Favaloro EJ, Lloyd J, Rowell J et al. (2007) Comparison of the pharmacokinetics of two von Willebrand factor concentrates [Biostate and AHF (High Purity)] in people with von Willebrand disorder. A randomised cross-over, multi-Center study. Thromb Haemost 97, 922-930.

120. Shortt J, Dunkley S, Rickard K et al. (2007) Efficacy and safety of a high purity, double virus inactivated factor VIII/von Willebrand factor concentrate (Biostate) in patients with von Willebrand disorder requiring invasive or surgical procedures. Haemophilia 13, 144-148.

121. Menache D, Aronson DL, Darr F et al. (1996) Pharmacokinetics of von Willebrand factor and factor VIIIC in patients with severe von Willebrand disease (type 3 VWD): estimation of the rate of factor VIIIC synthesis. Cooperative Study Groups. Br J Haematol 94, 740-745.

122. Goudemand J, Scharrer I, Berntorp E et al. (2005) Pharmacokinetic studies on Wilfactin, a von Willebrand factor concentrate with a low factor VIII content treated with three virus-inactivation/removal methods. J Thromb Haemost 3, 2219-2227.

123. Borel-Derlon A, Federici AB, Roussel-Robert V et al. (2007) Treatment of severe von Willebrand disease with a high-purity von Willebrand factor concentrate (Wilfactin): a prospective study of 50 patients. J Thromb Haemost 5, 1115-1124.

124. Bergamaschini L, Mannucci PM, Federici AB et al. (1995) Postranfusion anaphylactic reaction in a patient with severe von Willebrand disease: role of complement and alloantibodies to von Willebrand factor. J Lab Clin Med 125, 348-355.

125. Ciavarella N, Schiavoni M, Valenzano E et al. (1996) Use of recombinant factor VIIa (NovoSeven) in the treatment of two patients with type III von Willebrand's disease and an inhibitor against von Willebrand factor. Haemostasis 26 (Suppl 1), 150-154.

126. Boyer-Neumann C, Dreyfus M, Wolf M et al. (2003) Multi-therapeutic approach to manage delivery in an alloimmunized patient with type 3 von Willebrand disease. J Thromb Haemost 1, 190-192.

127. Berntorp E, Petrini P. (2005) Long-term prophylaxis in von Willebrand disease. Blood Coagul Fibrinolysis 16 Suppl 1, S23-S26

128. Federici AB, Gianniello F, Canciani MT et al. (2005) Secondary long-term prophylaxis in severe patients with von Willebrand disease: an Italian cohort study Blood 106, 507a

129. De Meyer SF, Vanhoorelbeke K, Chuah MK et al. (2006) Phenotypic correction of von Willebrand disease type 3 blood-derived endothelial cells with lentiviral vectors expressing von Willebrand factor. Blood 107, 4728-4736.

APPENDIX 1

STUDY FLOWCHART

Activity	FIRST PART retrospective phase (approx. 1 year) + diagnosis confirmatory phase (approx. 1 year)	SECOND PART first observation period (approx. 2 years) + confirmation of clinical phase data (approx. 1 year) + second observation period (approx. 2 years)		
	Enrolment Visit	**Enrolment Visit**	**Control visit***	**Final Visit**
	1 month	4 years (including two observation periods of 2 years each)		
Informed Consent	x			
Eligibility verification	x			
Patient ID assignment	x			
Demographics	x			
Medical History	x			
Previous diagnosis of VWD3	x			
Bleeding History	x			
Previous Treatments for VWD3	x			
Family history in parents and relatives	x			
Blood sample for local assessment	x	X (if not performed within 1 year before the study start)		x
Blood sample for Central Lab. assessment	x			
Bleedings		x	x	x
VWF concentrates	x	x	x	x
Concomitant medications	x	x	x	x
Adverse Events		x	x	x

**** a Control Visit should be performed at least once in a year according to the standard clinical practice at each investigational site***

APPENDIX 2

MEMBERS OF THE WORKING GROUP ON THE 3WINTERS-IPS STEERING COMMITTEE

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APPENDIX 3

WORKING PACKAGES

- WP1a Study documents set-up and finalization
- WP1b Network and Data Base of the study
- WP1c Regulatory process for the study
- WP1d Study initiation and patients' recruitment
- WP2 Collection of retrospective data & blood sample withdrawal for VWD3 diagnosis confirmation
- WP3a Centralized evaluation of VWF antigen (VWF:Ag) levels for the VWD3 diagnosis confirmation
- WP3b Centralized evaluation of FVIII levels (FVIII:C, FVIII:Amidolytic, FVIII:Ag) levels for VWD3 diagnosis confirmation
- WP3c Centralized evaluation of VWF multimer profile for VWD3 diagnosis confirmation
- WP3d Centralized evaluation of anti-VWF inhibitors presence in the VWD3 patients enrolled
- WP3e Centralized evaluation of VWF propeptide levels for VWD3 diagnosis confirmation
- WP3f Centralized confirmation of VWD diagnosis (genotype)
- WP4 Number, types and risk factors of bleeding in patients with confirmed VWD3 diagnosis
- WP5 Quantities, Efficacy and Safety of different plasma-derived or recombinant VWF concentrates in patients with confirmed VWD3 diagnosis
- WP6 Regulatory process for the study amendment related to 2-year extension of prospective observation
- WP7a Evaluation of Gastro-Intestinal Bleeding recurrence in patients with confirmed VWD3 diagnosis
- WP7b Centralized evaluation of angiogenesis markers in patients with confirmed VWD3 diagnosis experiencing recurrent GIB
- WP8 Recommendations on the use of plasma-derived or recombinant VWF concentrates in patients with confirmed VWD3 diagnosis

APPENDIX 4

PRELIMINARY INFORMATION COLLECTED DURING THE STUDY