Anti-αIIbβ3 immunization in Glanzmann thrombasthenia: Prevalence and associated risk factors

TAAS

Sponsor code: CHUBX 2019/41

INTERVENTIONAL RESEARCH PROTOCOL INVOLVING THE HUMAN PERSON *(category 2 at risk and minimal constraints)*

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HISTORY OF PROTOCOL UPDATES

VERSION	DATE	REASON FOR UPDATE
1.0	04/05/2020	Soumission initiale au CPP
2.0	09/11/2020	**Modification n°1:** ajout d'un critère de non inclusion, modification de l'âge minimum des mineurs pouvant être inclus, signature du consentement par un tiers, modification du nombre de centres participants, ajout des dosages anticorps anti-HLA

PROTOCOL SIGNATURE PAGE

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CONTENTS

LIST OF ABBREVIATIONS

ANSM	French National Agency for Medicines and Health Products Safety
CPP	Committee for the Protection of Persons
AE	Adverse Event
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
USAR	Unexpected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
GT	Glanzmann Thrombastenia
CRPP	Centre de Référence des Pathologies Plaquettaires

1. SUMMARY OF THE RESEARCH

SPONSOR	CHU de Bordeaux
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TITLE	Anti-$\alpha_{IIb}\beta_3$ immunization in Glanzmann thrombasthenia: Prevalence and associated risk factors. **Thrombasthenia Anti-$\alpha_{IIb}\beta_3$ Antibodies Study (TAAS)**
JUSTIFICATION/ CONTEXT	Glanzmann thrombasthenia (GT) is a rare autosomal recessive disorder caused by the absence or the dysfunction of the $\alpha_{IIb}\beta_3$ integrin (formerly termed the GPIIb–IIIa complex), the most abundant receptor on platelets that mediates platelet aggregation through its binding of adhesive proteins. GT is readily identifiable by platelet function testing, and a lack of platelet aggregation in response to all physiological agonists is unique for this disease. The *ITGA2B* gene encodes for the α_{IIb} subunit, whereas the *ITGB3* gene encodes for β_3. Mutations causing GT can affect either *ITGA2B* or *ITGB3*. In France, GT is particularly frequent in the Gypsy population, mainly represented by the Manouche tribe. The disease is characterized by spontaneous and trauma-related mucocutaneous bleeding, with variable expression ranging from easy bruising to fatal hemorrhages. Generally, local measures will control nasal and superficial bleeding, whereas platelet transfusions are used to control or prevent life-threatening blood loss. Platelet transfusion therapy can become ineffective due to naturally occurring antibodies directed against $\alpha_{IIb}\beta_3$. Such antibodies are produced when patient's immune system comes into contact with normal $\alpha_{IIb}\beta_3$ expressing platelets. Activated recombinant factor VII (rFVIIa) provides an alternative treatment for GT patients who develop such antibodies. However, this therapy is a cost-effective hemostatic agent. Despite reports on characteristics of antibodies from GT patients, there is no currently consensus concerning their frequency, their long-term evolution, or their formation in relation to the nature of the defective gene (*ITGA2B* or *ITGB3*), gene variations or other factors. Although recent results suggest that premature termination mutations may increase the rate of antibody production, further research is needed to confirm that nature of the gene defect may have a causative role in antibody development. Other risk factors have been evaluated, but association was not statistically significant due to small sample size. Moreover, strength and persistence of antibodies may vary among patients with the same mutation, suggesting that other factors, such as immune modifiers genes, play a role in shaping antibody repertoire.

OBJECTIVES	Primary objective: In this project, we aim to correlate risk factors (genetic, therapeutic and socio-demographic factors) to anti-$\alpha_{IIb}\beta_3$ antibodies formation following blood products transfusion (platelets or packed red cells) or pregnancy in a national cohort of GT patients. Secondary goals of this study will be: - To determine the prevalence of anti-$\alpha_{IIb}\beta_3$ antibodies; - To describe the kinetic of immunization following blood transfusion or during pregnancy. - To determine the mechanism of anti-αIIbβ3 antibodies blocking integrin function.
EVALUATION CRITERIA	The main judgment criteria will be the characterization of an anti-$\alpha_{IIb}\beta_3$ immunization and the risk factors associated to it. The secondary judgment criterias are: - Proportion of patients with at least one positive result with the MAIPA assay; - Description of the kinetic of immunization following blood transfusion; - Determination of the mechanism of anti-αIIbβ3 antibodies blocking integrin function by determining the capacity of anti-αIIbβ3 antibodies to impair fibrinogen binding.
INCLUSION CRITERIA	• All patients with a clear diagnosis of Glanzmann Thrombastenia (GT), whatever the subtype of disease. • Affiliated person or beneficiary of a social security scheme. • Free, informed and written consent signed by the participant, or parents or legal representant for the child population, and the investigator (at the latest on the day of inclusion and before any examination required by the research).
EXCLUSION CRITERIA	• Current treatment that may interfere with anti-$\alpha_{IIb}\beta_3$ antibodies detection, such as intravenous immunoglobulins within the previous month. • Patient with a family member already included in the TAAS study • Psychiatric, social or behavioral condition judged to be non-compatible with the respect of the protocol, including good observance of treatment and compliance to follow-up. • Adult protected by the law.

RESEARCH STRATEGIES/ PROCEDURES	**Patients and blood samples** All included GT patients will be enrolled from several national centres during a 6 months period. Antibodies screening will be systematically realized every six months (+/- 2 weeks) and after each last blood transfusion at 7-10 days and one month (+/- 2 weeks), during a period of 18 months. **Clinical Data collection** Clinical records of patients and biological results will be obtained by the national Reference Centre for Platelet Disorders. Patients data will include the following information: (i) age of the patient at first antibody screening; (ii) time after transfusion to antibody detection; (iii) subtype of GT and residual $\alpha_{IIb}\beta_3$ expression; (iv) mutation screening of the *ITGA2B* and *ITGB3* genes; (v) family history of GT and/or antibody formation; (vi) strong or weak antibody development; and (vii) evolution of the antibodies (persistent or transient). Anti-$\alpha_{IIb}\beta_3$ antibodies screening: Indirect MoAb-specific immobilization of platelet antigens (MAIPA) Monoclonal antibody-specific immobilization of platelet antigens (MAIPA) is still considered as the reference method for evaluating the presence of anti-$\alpha_{IIb}\beta_3$ antibodies in GT patients. All the tests will be performed by the principal investigator site (Bordeaux). Antibodies screening will be systematically performed at 7-10 days and one month (+/- 2 weeks) after each last blood transfusion, and every six months (+/- 2 weeks).
STUDY SIZE	We aim to examine a total of 40 GT patients
DURATION OF THE RESEARCH	*Duration of the inclusion period: 6 months *Duration of patient's participation: 18 months *Total duration of the study: 24 months
STATISTICAL ANALYSIS OF THE DATA	The cohort will be analyzed with logistic regression and the outcome variable will be defined as the absence or presence of a current or past history of antibody development. Covariates will include risk factors such as subtype of GT, year of birth, *ITGA2B* or *ITGB3* gene mutations. Selection of risk factors of interest will be performed to identify markers that exhibited effects and analyses will be performed using specific statistical software.
EXPECTED BENEFITS	The advantages of obtaining data from a regional prospective cohort will be to observe the effects of a set of risk factors on antibodies development. Because one of the focus of GT care is identification of subjects at risk of antibodies development, a more complete characterization of risk factors associated with them is warranted. Based on our results, we could propose a risk assessment algorithm and proposals for management of platelet antibody formation against $\alpha_{IIb}\beta_3$ in GT. A better knowledge of the risk factors associated with the immunization of GT patients after platelet transfusion should allow us to rationalize the prescription of costly haemostatic treatments, such as recombiant activated factor VII. Due to its originality and its potential clinical impact, this study could be published in a journal of international importance.

ABSTRACT

This research has been registered at http://www.clinicaltrials.gov/ on *date* under no. *number*.

Anti-$\alpha_{IIb}\beta_3$ immunization in Glanzmann thrombasthenia: Prevalence and associated risk factors. Thrombasthenia Anti-$\alpha_{IIb}\beta_3$ Antibodies Study (TAAS)

CHU Bordeaux is the sponsor of this research.

This research will be conducted with the support Centre de Référence des Maladies Rares- Centre de reference des Pathologies Plaquettaires

- ***Brief summary:*** In this project, we aim to correlate risk factors (genetic, therapeutic and socio-demographic factors) to anti-$\alpha_{IIb}\beta_3$ antibodies formation following blood products transfusion (platelets or packed red cells) or pregnancy in a national cohort of GT patients.
- **Detailed description:**

Glanzmann thrombasthenia (GT) is a rare autosomal recessive disorder caused by the absence or the dysfunction of the $\alpha_{IIb}\beta_3$ integrin, the most abundant receptor on platelets that mediates platelet aggregation through its binding of adhesive proteins. GT is readily identifiable by platelet function testing, and a lack of platelet aggregation in response to all physiological agonists is unique for this disease. The *ITGA2B* gene encodes for the α_{IIb} subunit, whereas the *ITGB3* gene encodes for β_3. Mutations causing GT can affect either *ITGA2B* or *ITGB3*. The disease is characterized by spontaneous and trauma-related mucocutaneous bleeding, with variable expression ranging from easy bruising to fatal hemorrhages. Platelet transfusions are used to control or prevent life-threatening blood loss, but can become ineffective due to naturally occurring antibodies directed against $\alpha_{IIb}\beta_3$. Such antibodies are produced when patient's immune system comes into contact with normal $\alpha_{IIb}\beta_3$ expressing platelets.

There is no currently consensus concerning the frequency, the long-term evolution, or the formation of characteristics of antibodies from GT patients in relation to the nature of the defective gene (*ITGA2B* or *ITGB3*), gene variations or other factors. Research are needed to confirm that nature of the gene defect may have a causative role in antibody development. Moreover, strength and persistence of antibodies may vary among patients with the same mutation, suggesting that other factors, such as immune modifiers genes, play a role in shaping antibody repertoire.

Monoclonal antibody-specific immobilization of platelet antigens (MAIPA) is still considered as the reference method for evaluating the presence of anti-$\alpha_{IIb}\beta_3$ antibodies in GT patients. All the tests will be performed by the principal investigator site (Bordeaux).

- **Primary outcome:** The characterization of an anti-$\alpha_{IIb}\beta_3$ immunization and the risk factors associated to it
- **Secondary outcomes:**
  - Proportion of patients with at least one positive result with the MAIPA assay;
  - Description of the kinetic of immunization following blood transfusion;
  - Determination of the mechanism of anti-αIIbβ3 antibodies blocking integrin function by determining the capacity of anti-αIIbβ3 antibodies to impair fibrinogen binding.
- **Study design:** This is a national, multicenter, prospective cohort study.
- **Eligibility criteria:**
  - inclusion criteria: All patients with a clear diagnosis of Glanzmann Thrombastenia (GT), whatever the subtype of disease
  - exclusion criteria: Current treatment that may interfere with anti-$\alpha_{IIb}\beta_3$ antibodies detection, such as intravenous immunoglobulins within the previous month.
- **Interventions:** All included GT patients will be enrolled from different national centres during a 6 months period. Antibodies screening will be systematically realized every six months (+/- 2 weeks) and after each last blood transfusion at 7-10 days and one month (+/- 2 weeks), during a period of 18 months.

- **Number of subjects:** 40
- **Statistical analysis:** The cohort will be analysed with logistic regression and the outcome variable will be defined as the absence or presence of a current or past history of antibody development. Covariates will include risk factors such as subtype of GT, year of birth, *ITGA2B* or *ITGB3* gene mutations. Selection of risk factors of interest will be performed to identify markers that exhibited effects and analyses will be performed using specific statistical software.
- **Key-words:** glanzmann thrombasthenia; anti-GPIIb-IIIa immunization; MAIPA; platelet transfusions; recombinant activated factor VII

2. SCIENTIFIC JUSTIFICATION AND GENERAL DESCRIPTION

2.1. CURRENT STATE OF KNOWLEDGE

Glanzmann thrombasthenia (GT) is a rare autosomal recessive bleeding disorder caused by inherited defects of the genes encoding the platelet integrin, $\alpha_{IIb}\beta_3$ [1]. The *ITGA2B* gene encodes for the α_{IIb} subunit while the *ITGB3* gene encodes for β_3 [2, 3]. Mutations causing GT can affect either *ITGA2B* or *ITGB3*[3, 4]. The disease is characterized by a lack of platelet aggregation in response to all physiologic stimuli. In France, GT is common within the Manouche tribe, one of the main branches of the French Gypsies [5]. The hypothesis of the existence of a founder mutation in this population was confirmed by us showing how a haplotype core across a 4 cM region was strongly associated with the originally described c.1544+1G>A French Gypsy mutation [5, 6].

Generally, in GT, bleeding is largely mucocutaneous in nature and if minor, local measures, sometimes in conjunction with anti-fibrinolytics, are sufficient; in contrast, platelet transfusions are used to control or to prevent life-threatening blood loss [7]. Unfortunately, platelet transfusion therapy can be followed by an immune response that is usually directed against the deficient $\alpha_{IIb}\beta_3$ [8, 9]. The presence of anti-$\alpha_{IIb}\beta_3$ antibodies is a crucial problem for the management of GT patients. Indeed, naturally occurring antibodies directed against the $\alpha_{IIb}\beta_3$ integrin may cause the removal of or render ineffective transfused donor platelets. Management of pregnancy in women with GT is also a real challenge. One of the major complications relates to foetal immune thrombocytopenia induced by the transplacental passage of the maternal IgG anti-$\alpha_{IIb}\beta_3$ antibodies, which can lead to severe haemorrhage and foetal loss [10, 11].

Previous detailed characterizations of antibodies from GT patients show that they recognize epitopes located on $\alpha_{IIb}\beta_3$ or β_3 and that some can block platelet function as well as cause platelet elimination [12]. So, initial studies of platelet antibody formation against $\alpha_{IIb}\beta_3$ in GT were largely performed on isolated cases and mainly concerned antibody characterization. While the epidemiology of neutralizing antibodies (inhibitors) is well known in patients with hemophilia, it has been poorly studied in GT and the actual frequency of immunization against $\alpha_{IIb}\beta_3$ antibodies remains unknown [13, 14].

The first large case series reporting this complication dates back to 1990 where a very low frequency of 3.5% (6/177) was observed [15]. Fourteen years later, an international survey was conducted in 59 GT patients including 21/54 patients (39%) with current or previous antiplatelet antibodies [16]. More recently, Santoro et al. investigated 16 GT patients for the presence of anti-platelet antibodies and they showed that anti-$\alpha_{IIb}\beta_3$ antibodies were present in 2 of them (12.5%) [17]. In 2012, we reported the results of antibodies screening on a series of 24 GT patients from South-West France dividing them into two cohorts: (i) 16 patients with the French gypsy mutation (c.1544 + 1G>A) within *ITGA2B* that gives platelets totally lacking $\alpha_{IIb}\beta_3$ and (ii) 8 patients carrying other defects of *ITGA2B* or *ITGB3* with different expression levels of $\alpha_{IIb}\beta_3$ [9]. We showed that 81% (13/16) of the French Gypsy patients had developed platelet antibodies against $\alpha_{IIb}\beta_3$. In contrast, only 25% (2/8) of the patients with other GT mutations were concerned. In 2015, a large international study on GT patients showed that 20/83 of them (24%) developed antibodies to $\alpha_{IIb}\beta_3$ [18] and, in the largest international prospective registry of 218 GT patients, 47 (22%) had an history of anti-$\alpha_{IIb}\beta_3$ platelet antibodies [19].

Finally, the incidence of immunization in different studies varies considerably. In addition to patient or treatment related risk factors, diagnostic procedures (possibly due to the disappearance of antibodies at the time of the test, or to insufficient sensitivity of some assays) may influence the frequency of the observed events [9]. Based on the two largest studies published so far, the estimated prevalence of anti-$\alpha_{IIb}\beta_3$ immunization is probably comprised between 20-30%. However, among some ethnic minority groups, frequency may be much greater [9].

Genetics, as well as acquired patient-related factors are likely to influence the process of anti-$\alpha_{IIb}\beta_3$ immunization [8]. Nevertheless, it remains poorly known whether patients can make antibodies or not when exposed to these risk factors. While in hemophilia, some parameters are considered as risk factors for inhibitor development, they have never been really evaluated in GT [20].

Gender

There is currently no scientific data establishing a causal link between gender and the risk of anti-$\alpha_{IIb}\beta_3$ formation. However, of the 20 patients with anti-$\alpha_{IIb}\beta_3$ antibodies reported in the recent international study published by Nurden et al., 14 were female and only 6 were male [18]. This data suggests that female patients could develop anti-$\alpha_{IIb}\beta_3$ antibodies easier than male patients. However, it is also possible that female patients are more exposed to platelet transfusions due to their bleeding challenge (menarche, pregnancy).

Ethnicity

In relation to their factor VIII background haplotypes, the prevalence of factor VIII inhibitors in black patients is about twice that in white patients [20]. Many genetic polymorphisms within the sequenced regions of *ITGA2B* and *ITGB3* generate different haplotypes [21]. The frequencies with which these polymorphisms exist in a population have been shown to be ethnically related. As an example, we previously found that a haplotype of five polymorphic variants covering a 4-cM region was strongly associated with the French Gypsy mutation, suggesting a founder effect [5]. Some of these ethnicity-related polymorphisms could be associated with a higher risk of anti-$\alpha_{IIb}\beta_3$ development, but this has not yet been proved. Haplotypes characterization might be used as a basis for studies on the relationship between integrin genotypes and anti-$\alpha_{IIb}\beta_3$ antibodies formation. These results could determine whether mismatched replacement therapy is a risk factor for the development of anti–$\alpha_{IIb}\beta_3$ antibodies.

Blood product transfusion

Due to the increased risk of bleeding associated with GT, platelet transfusion are given before invasive procedures [19]. Despite the lack of supporting scientific study, concentrates of apheresis platelets, HLA-compatible if needed, are still preferred. Regarding the correlation between transfusion intensity and immunization, it has been previously observed that some immunized patients had been heavily treated with platelet transfusions, while others had not received a very high number of platelet units [9, 17]. Moreover, some of them can be positive after having received just a single platelet concentrate few days earlier [9]. This suggests that it is not necessary to be highly exposed to normal platelets to develop antibodies against $\alpha_{IIb}\beta_3$. Moreover, the time between platelet transfusion and antibody screening shows that platelet antibodies against $\alpha_{IIb}\beta_3$ may still be present after several years [9].

Red blood cells (RBC) administrations may also expose GT patients to the risk of anti-$\alpha_{IIb}\beta_3$ antibody development [22]. This may presumably be due to stimulation of the immune system by the presence of residual platelets in RBC concentrates. This hypothesis was confirmed by Laurian et al. who described the case of a 16-year-old girl with type I GT and a past history of anti-$\alpha_{IIb}\beta_3$ antibodies [22]. While antibodies were no longer detectable, she received RBC concentrates for severe anemia, and antibodies against $\alpha_{IIb}\beta_3$ were found a few weeks later. So, RBC transfusions may expose patients to the risk of anti-$\alpha_{IIb}\beta_3$ development or anamnestic rise and this could be avoided by the use of washed or frozen RBC, which virtually removes all platelets.

Genetic risk factors

Most of GT patients with immunization against $\alpha_{IIb}\beta_3$ are affected by type I disease. Anti-$\alpha_{IIb}\beta_3$ antibody formation has been rarely notified in type II or variant forms. Thus, the risk of immunization is largely restricted to patients whose platelets lack $\alpha_{IIb}\beta_3$ on their platelet surface.

The type of mutations affecting *ITGA2B* or *ITGB3* genes could also influence the risk of developing antibodies [2, 4, 9, 18]. Molecular anomalies associated with GT fall into two groups: (i) those which are highly deleterious leading to inhibition of protein synthesis (null mutations: non-sense or frameshift mutations, splicing defects introducing premature stop codons) where the risk of anti-$\alpha_{IIb}\beta_3$ immunization is probably very high; (ii) and those where a residual synthesis of the integrin may persist (missense mutations) with probably a lower risk of anti-$\alpha_{IIb}\beta_3$ antibodies development. Based on the literature data, most of the mutations known to be associated with anti-$\alpha_{IIb}\beta_3$ antibodies are homozygote or compound heterozygote for premature termination mutations [23, 24]. Exceptions mostly concern homozygote patients for missense substitutions who may produce antibodies after massive platelet transfusions.

Moreover, another point that remains elusive is the concordance rate for antibodies development between GT patients carrying the same mutation. Indeed, while some patients have the same mutation, the strength and persistence of antibodies over time varied between individuals, suggesting that other genetic (e.g. major histocompatibility complex genes, other gene polymorphisms) or environmental factors play key roles in the selection of antibody repertoires [8, 9].

Environmental risk factors

It is still unknown whether GT patients who have not received platelet or RBC transfusions may develop anti-$\alpha_{IIb}\beta_3$ antibodies. Nurden et al. reported an immunized woman belonging to the French gypsy tribe who had not received platelet or RBC transfusions and the cause of her immunization remains unknown. Ghosh et al. studied the prevalence of antiplatelet antibodies in 15 pediatric patients with GT who had not received transfusions [25]. Surprisingly, all of them showed antiplatelet antibodies. They might be produced as a result of molecular homology to bacterial or viral components. Indeed, Chouhan et al. identified bacterial sequences that are similar to the human integrin β-propeller domain [26].

Nevertheless, whether antigenic mimicry to bacterial or viral peptides, is the cause for this finding needs to be determined.

Pregnancy
In GT patients, anamnestic response with rise in antibodies against $\alpha_{IIb}\beta_3$ titre has been previously observed during pregnancy [27, 28], suggesting that immunization may also be a consequence of exposure to fetal platelet antigens. In these cases, absence of platelet-specific antibodies at the start of pregnancy does not preclude their occurrence at a later date.

Therapeutic approach in GT patients with anti-$\alpha_{IIb}\beta_3$ antibodies: recombinant activated factor VII (rFVIIa)
The first successful use of rFVIIa was reported by Tengborn and Petruson in the management of intractable epistaxis in a GT 2-year old child [29]. However, it is only few years later that its use in a patient with anti-$\alpha_{IIb}\beta_3$ antibodies and refractoriness to platelet transfusions was described: a one-year old female successfully treated for severe gastrointestinal bleedings with a standard dose of approximately 90 µg/kg every 2 hours until bleeding stopped. While the mechanism of action of rFVIIa in GT is not fully understood, it is thought that rFVIIa increases thrombin generation by FX activation on the platelet surface. rFVIIa-mediated thrombin formation can restore defective adhesion of $\alpha_{IIb}\beta_3$-deficient platelets to extra-cellular matrix and may induce $\alpha_{IIb}\beta_3$-independent platelet aggregation of washed platelets from GT patients. rFVIIa also interacts with the GPIb-IX-V complex, and this interaction enhances tissue factor-independent thrombin generation mediated by rFVIIa on the activated platelet surface.

Prior to approval of rFVIIa for GT patients with anti-platelet antibodies, a larger international survey of its efficacy and safety was conducted in 59 patients [16]. The results of the survey showed that efficacy was achieved in 29/31 (94%) evaluable procedures, and 77/103 (75%) evaluable bleeding episodes, whereas no significant difference in efficacy was observed between patients with and without platelet antibodies. Data from this survey allowed the suggestion of an optimal rFVIIa regimen (rFVIIa ≥80 µg/kg given at ≤2.5-h intervals for three or more doses) for the treatment of moderate/severe bleeding episodes, but on the other hand, results were not statistically significant concerning minor bleedings.

In the large international GT registry, investigators reported that for the non-surgical bleeds that occurred in patients with a history of antiplatelet antibodies (without distinction between anti-$\alpha_{IIb}\beta_3$ and anti-HLA antibodies) +/- platelet refractoriness, rFVIIa (either alone or with antifibrinolytics) was rated effective in 76% of cases, whereas in the group without antibodies, the rate was 91%. Moreover, the number of rFVIIa doses and overall duration of treatment were greater in patients with a history of antiplatelet antibodies +/- refractoriness to platelets than in subjects without such a history [19].

For surgical procedures, the use of rFVIIa ± antifibrinolytics was rated effective in 88% and 100%, respectively for the antibodies ± refractoriness group and subjects without such a history. Concerning minor procedures, median dose and dosage interval of rFVIIa was very similar for the different patient groups. However, the median cumulative rFVIIa dose range and duration of treatment used were found to be higher in the group with a history of antiplatelet antibodies ± refractoriness.

Globally, these results confirm that rFVIIa is safe in patients with anti-platelet antibodies ± refractoriness, though surprisingly, the effective rate was slightly lower compared to patients without antibodies and the cumulative doses needed were higher, probably due to more serious bleeding episodes. Data from the GTR also pointed that rFVIIa is frequently used off-label for bleeding and surgical procedures, irrespective of platelet antibodies and/or platelet refractoriness. However, rFVIIa has been associated with development of venous or arterial thrombosis during its use and it should be prescribed with caution in patients with thrombosis risk factors. Among patients included in the GTR study, a nonfatal thromboembolic event has been reported in an adult woman treated with rFVIIa + platelets + antifibrinolytics.

2.2. RESEARCH HYPOTHESES AND EXPECTED RESULTS

Our previous published study is the only one that described the natural history of these antibodies in a population of GT patients [9]. However, it has some limitations due to the fact that it is only a retrospective study with bias, such as missing data. It would be interesting to better estimate the prevalence of immunization and its dynamic in a longitudinal and prospective way. Althought it is theoretically maximal after blood transfusion, the kinetics of immunization is not well known. Our hypothesis is that, using a prospective study, it will be easier to better characterize and identify risk factors associated to the development of anti-$\alpha_{IIb}\beta_3$ antibodies in GT patients.

2.3. JUSTIFICATION OF THE METHODOLOGICAL CHOICES

2.3.1. STUDY DESIGN AND STUDY POPULATION SELECTION

The best study design to explore the incidence and risk factors for anti-$\alpha_{IIb}\beta_3$ immunization is a longitudinal prospective cohort study and this is what we intend to conduct.

2.3.2. DEFINITION AND MEASUREMENT OF ANTI- $A_{IIb}B_3$ IMMUNIZATION

Monoclonal antibody-specific immobilization of platelet antigens (MAIPA) is still considered as the reference method for evaluating the presence of anti-$\alpha_{IIb}\beta_3$ antibodies in GT patients. Briefly, platelet-rich plasma (PRP) is obtained by centrifugation and platelets are washed before being resuspended. A volume of the platelet suspension is then sensitized with serum from the patient or control donors. After resuspension, selected platelet glycoprotein-specific monoclonal antibodies directed against $\alpha_{IIb}\beta_3$, β_3 and/or α_{IIb} subunits are added separately and the mixture is incubated at room temperature. Platelets are then washed and solubilized in a lysis solution by incubating overnight at 4°C. In parallel, microtitre plates are coated with antibodies directed against the specific monoclonal antibodies and incubated overnight at 4°C. After several washes, a blocking buffer and a volume of alkaline phosphatase-labelled goat antihuman IgG/M F(ab'2) is added to each well. After incubation, the tray is washed and substrate (paranitrophenylphosphate in diethanolamine buffer) is added and absorbance measured in a microplate reader. Patients' results are calculated as a ratio of their optical density (OD) compared to that of the mean value of a series of control sera.

2.4. RISK/ BENEFIT RATIO

No adverse effects are expected apart from hematoma at the puncture site which should be minimized by adequate local compression after blood collection.

Finally, this study will not be burdensome for the included patients, as only few tubes of peripheral blood will be needed (around 14 mL per blood collection) for this project.

The coordinating investigator must constantly monitor, assess and document risks and ensure that they can be managed satisfactorily.

2.5. EXPECTED BENEFITS

The advantages of obtaining data from a regional prospective cohort will be that we will be able to observe the effects of a set of risk factors on antibodies development.

Because one of the focus of GT care is identification of subjects at risk of antibodies development, a more complete characterization of risk factors associated with them is warranted. This project will be an extensive effort to address this task, and several new candidates that could be potentially predictive in the immune response to the deficient integrin could be identified.

Based on our results, we could propose a risk assessment algorithm and proposals for management of platelet antibody formation against $\alpha_{IIb}\beta_3$ in GT. Our findings might be used to define a risk score based on a combination of additive risk factors. One approach would be to avoid, when possible, platelet transfusions in higher risk patients, since without this exposure antibodies will not be formed. Although platelet transfusions remain the choice for stopping haemorrhage, where bleeding is moderate, rFVIIa appears to represent a good alternative therapeutic option. This could be particularly the case for patients with high risk factors. However, a better knowledge of the risk factors associated with the immunization of GT patients after platelet transfusions should allow us to rationalize the prescription of costly haemostatic treatments, such as rFVIIa.

Studies to evaluate this strategy should be then performed in larger panel of patients, such as a national or international cohort.

On the other hand, creation of a large-scale serum bank of immunized GT patients could be used as a second step to develop fundamental research projects; for example, characterization of pathogenic epitopes essential for antibodies development by an epitope mapping approach. Moreover, there is currently uncertainty about the mechanism of action of anti-$\alpha_{IIb}\beta_3$ antibodies. Indeed, it is not known whether they act by receptor inhibition or by increasing platelet clearance. Then, *in vitro* inhibition of the receptor by platelet antibodies could be studied.

Due to its originality and its potential clinical impact, this study could be published in a journal of international importance.

2.6. JUSTIFICATION OF THE LOW LEVEL OF INTERVENTION

Only few tubes of peripheral blood are needed for this project. In most of the cases, blood samples will be taken every 6 months +/- 2 weeks (maximum of 6 blood samples for all the study). The procedure under consideration involves only minimal risks and constraints for the patient. See also comments on chapter 2.4. In case of any constraints, patients have the opportunity to leave the study at any moment.

3. RESEARCH OBJECTIVES

3.1. MAIN OBJECTIVE

In this project, we aim to identify risk factors (genetic, therapeutic and socio-demographic factors) for anti-$\alpha_{IIb}\beta_3$ antibodies formation following blood products transfusion (platelets or packed red cells) or pregnancy in a national cohort of GT patients.

3.2. SECONDARY OBJECTIVES

- To determine the prevalence of anti-$\alpha_{IIb}\beta_3$ antibodies in a regional cohort of GT patients;
- To describe the kinetic of immunization following blood transfusion.
- To determine the mechanism of anti-$\alpha_{IIb}\beta_3$ antibodies blocking function. For that, *in vitro* studies will be performed by mixing serum of patients with donors' platelets.

4. EVALUATION CRITERIA

4.1. MAIN EVALUATION CRITERION

To characterize of an anti-$\alpha_{IIb}\beta_3$ immunization and the risk factors associated to it.

4.1.1. CHARACTERIZATION OF AN ANTI-GPIIB-IIIA IMMUNIZATION

Monoclonal antibody-specific immobilization of platelet antigens (MAIPA) is still considered as the reference method for evaluating the presence of anti-$\alpha_{IIb}\beta_3$ antibodies in GT patients. Briefly, platelet-rich plasma (PRP) is obtained by centrifugation and platelets are washed before being resuspended. A volume of the platelet suspension is then sensitized with serum from the patient or control donors. After resuspension, selected platelet glycoprotein-specific monoclonal antibodies directed against $\alpha_{IIb}\beta_3$, β_3 and/or α_{IIb} subunits are added separately and the mixture is incubated at room temperature. Platelets are then washed and solubilized in a lysis solution by incubating overnight at 4°C. In parallel, microtitre plates are coated with antibodies directed against the specific monoclonal antibodies and incubated overnight at 4°C. After several washes, a blocking buffer and a volume of alkaline phosphatase-labelled goat antihuman IgG/M F(ab'2) is added to each well. After incubation, the tray is washed and substrate (paranitrophenylphosphate in diethanolamine buffer) is added and absorbance measured in a microplate reader. Patients' results are calculated as a ratio of their optical density (OD) compared to that of the mean value of a series of control sera.

All the tests will be performed by the coordinating investigator site (Bordeaux). Antibodies screening will be systematically realized every six months (+/- 2 weeks) and after each blood transfusion at 7-10 days and one month (+/- 2 weeks).

4.1.2. RISK FACTORS POTENTIALLY INVOLVED

Related to the patient:
- Type of genetic mutation associated with the disease (truncative or missense mutations)
- Age at first blood transfusion
- Sex
- Ethnic origin (such as patients from Gypsy tribe who bear a specific truncative mutation)

Related to the blood products:
- Red blood cells or platelet concentrates
- Number of blood transfusions
- Type of platelet concentrates: apheresis or pooled from several donors

Related to the clinical situation:
- Inflammation (eg: surgery, trauma…)
- Pregnancy
- Blood transfusion in an emergency situation or in a scheduled invasive procedure

4.2. SECONDARY EVALUATION CRITERIA

- To determine the proportion of patients with at least one positive result with the MAIPA assay;

- To describe the kinetic of immunization following blood transfusion.

For that, it will be necessary to repeat antibodies measurements at 7-10 days, one month +/- 2 weeks after blood transfusion, and every 6 months +/- 2 weeks;

- To determine the mechanism of anti-αIIbβ3 antibodies blocking integrin function by determining the capacity of anti-αIIbβ3 antibodies to impair fibrinogen binding.

For that, *in vitro* studies will be performed by mixing serum of patients with washed donors' platelets and inhibition of the integrin will be studied by flow cytometry using:

- PAC-1 (an IgM recognizing activated but unoccupied $\alpha_{IIb}\beta_3$ complexes) purchased from Becton Dickinson (Franklin Lakes, New Jersey, USA).
- Fibrinogen binding study measured with Alexa Fluor 488–labeled human fibrinogen (Molecular probes, Eugene, Oregon, USA).

All the tests will be performed by the principal investigator site (Bordeaux).

5. RESEARCH DESIGN

This is a national, multicenter, prospective cohort study.
The biological test is the MAIPA assay.
The study is multicenter, to be able to enrol the expected number of cases. All centres are specialized in the management of patients with GT through the MHEMO network (Rare Inherited Bleeding Disorders medical network).
All follow-up visits will be performed by one of the investigators identified in each center.

All included GT patients will satisfy diagnostic criteria for the disease by associating mucocutaneous bleeding, inherited defects of $\alpha_{IIb}\beta_3$ expression or function and an absence of platelet aggregation to all agonists. Participants will be enrolled in several sites in France during a 6 months period, through the French Reference Centre for Inherited Platelet Disorders (CRPP). We aim to examine a total of 40 GT patients. Sera from patients will be prepared and decomplemented as previously described.

Clinical records of patients data and biological results will be obtained by the CRPP. Patients data will include the following information: (i) age of the patient at first antibody screening; (ii) time after transfusion to antibody detection; (iii) subtype of GT and residual $\alpha_{IIb}\beta_3$ expression; (iv) mutation screening of the *ITGA2B* and *ITGB3* genes; (v) family history of GT and/or antibody formation; (vi) strong or weak anti-$\alpha_{IIb}\beta_3$ antibody development; (vii) evolution of the anti-$\alpha_{IIb}\beta_3$ antibodies (persistent or transient); and viii) presence of anti-HLA antibodies.

6. ELIGIBILITY CRITERIA

6.1. INCLUSION CRITERIA

- All patients (adult and child) with a clear diagnosis of Glanzmann Thrombastenia (GT), whatever the subtype of disease.
- Affiliated person or beneficiary of a social security scheme.
- Free, informed and written consent signed by the participant or parents or legal representing for the child population, and the investigator (at the latest on the day of inclusion and before any examination required by the research).

The investigator will inform these patients in the presence of the parent authority representative(s). An information note will be provided to the parent authority representatives prior to obtaining their signature on informed consent.
In addition, a specific information note will be given to the minor patient 6 years of age or older who will have to give his (her) agreement to participate by signing an assent form.
For minor patients who become major during the study, a consent form should be signed by these patients to confirm their desire to continue the study.

Where it is impossible for the patient to express his or her consent in writing, the consent may be certified by a family member or, by one of the relatives of the person concerned, provided that this trusted person is independent of the investigator and the sponsor.

6.2. EXCLUSION CRITERIA

- Current treatment that may interfere with anti-$\alpha_{IIb}\beta_3$ antibodies detection, such as intravenous immunoglobulins within the previous month.
- Patient with a family member already included in the TAAS study
- Psychiatric, social or behavioral condition judged to be non-compatible with the respect of the protocol, including good observance of treatment and compliance to follow-up.
- Adult protected by the law.

6.3. FEASIBILITY AND RECRUITMENT PROCEDURES

This study will address a relatively rare event in rare patients. Investigation centers will be members of the MHEMO network. All centers have an excellent experience in the management of GT in adults and children.

A feasibility questionnaire has been already sent to the different investigators. On this basis, we forecast 4-5 patients eligible per center. Duration of the inclusion period will be 6 months.

When possible, patients will be contacted by phone (pre-screening) to suggest that they participate in the study. Thus, those who respond favorably will be received during a pre-inclusion/inclusion visit to explain more precisely the objectives of the study and to sign the informed consent form.

Other mode of recruitment will be previously diagnosed GT patients with regular follow-up, as well as newly diagnosed GT patients. Hospitalized patients, when it is the case, will be also solicited.

7. TREATMENT/ STRATEGY(IES)/ PROCEDURE(S) OF THE RESEARCH

Patients and blood samples

All included GT patients will be enrolled from 8 different centres during a 6 months period. Antibodies screening will be systematically realized every six months (+/- 2 weeks) and after each last blood transfusion at 7-10 days and one month (+/- 2 weeks), during a period of 18 months.

Clinical Data collection

Clinical records of patients and biological results will be obtained by the national Reference Centre for Platelet Disorders. Patients data will include the following information: (i) age of the patient at first antibody screening; (ii) time after transfusion to antibody detection; (iii) subtype of GT and residual $\alpha_{IIb}\beta_3$ expression; (iv) mutation screening of the *ITGA2B* and *ITGB3* genes; (v) family history of GT and/or antibody formation; (vi) strong or weak antibody development; and (vii) evolution of the antibodies (persistent or transient).

Anti-$\alpha_{IIb}\beta_3$ antibodies screening: Indirect MoAb-specific immobilization of platelet antigens (MAIPA)
Monoclonal antibody- specific immobilization of platelet antigens (MAIPA) is still considered as the reference method for evaluating the presence of anti- $\alpha_{IIb}\beta_3$ antibodies in GT patients. All the tests will be performed by the principal investigator site (Bordeaux). Antibodies screening will be systematically performed at 7-10 days and one month (+/- 2 weeks) after each last blood transfusion, and every six months (+/- 2 weeks).

8. CONDUCTING THE RESEARCH

8.1. THE RESEARCH SCHEDULE

- Duration of the inclusion period: 6 months
- Participation duration of each participant: 18 months
- Total duration of the research 24 months

8.2. SUMMARY TABLE OF THE PARTICIPANT FOLLOW-UP

	Pre-inclusion T-*1Week to T0*	Inclusion T 0	Follow-up Visit M6, M12, (+/- 2 weeks)	In Case of blood transfusions during the follow-up period		End of study visit M18 (+/- 2 weeks)
				7-10 days after transfusion	1 months (+/- 2weeks) after transfusion	
Informed consent	✓(R)					
Consentment obtain	✓(R)	✓(R)				
Physical examination [1]		✓	✓			✓
Biological examination [2]		✓(R)	✓(R)	✓(R)	✓(R)	✓(R)
Research on adverse events		✓	✓			✓

[1]Physical examination: *demographic information, weight, size,* medical history

[2]Biological examination: *MAIPA Assay (R) and dosage anti-HLA antibodies (standard of care)*

8.3. PRE-INCLUSION VISIT

8.3.1. COLLECTION OF CONSENT

8.3.1.1. FOR ADULT POPULATION

During the pre-inclusion visit, the investigator informs the participant and answers all questions regarding the purpose, nature of the constraints, foreseeable risks and the expected benefits of the research. The investigator also specifies the participant's rights in the context of a study and checks the eligibility criteria.

A copy of the information note and the consent form is then given to the participant by the investigating doctor. After this information session, the participant has a reflection period. If the participant agrees to participate, the participant and the investigator clearly write their names and surnames, and date and sign the consent form. This must be signed BEFORE ANY CLINICAL OR PARA-CLINICAL EXAMINATION IS CONDUCTED AS REQUIRED BY THE RESEARCH.The copies of the information note and the consent form are then distributed as follows:

- One original copy of the information note and the consent form is given to the participant.
- The other original copy is kept by the investigator (even if the participant moves house during the study period) in a safe place not accessible by third parties.

8.3.1.2. FOR CHILD POPULATION

During the pre-inclusion visit, the investigator informs the parents or legal representing and answers all questions regarding the purpose, nature of the constraints, foreseeable risks and the expected benefits of the research. The investigator also specifies the participant's rights in the context of a study and checks the eligibility criteria.

A copy of the information note and the consent form is then given to either parents or legal representing by the investigating doctor. After this information session, the parents or legal representing have a reflection period. If they agree that their child participates, the parents or legal representing and the investigator clearly write their names and surnames, and date and sign the consent form. This must be signed BEFORE

ANY CLINICAL OR PARA-CLINICAL EXAMINATION IS CONDUCTED AS REQUIRED BY THE RESEARCH.

If the child is 6 years of age or older, a specially adapted information note is provided to explain the research. After this information session, the child has a reflection period with their parents. If the child is agrees to participate, he (she) must clearly write his (her) name and surname, and date and sign an assent.

The copies of the information note and the consent form are then distributed as follows:

- One original copy of the information note and the consent form is given to the parents or legal representing or 6 years old child.
- The other original copy is kept by the investigator (even if the participant moves house during the study period) in a safe place not accessible by third parties.

8.3.2. CONDUCTING THE VISIT

The pre-inclusion visit is provided by the investigator. The pre-inclusion visit takes place between 1 weeks and the day of the inclusion visit. Prior to any research-related examination, the investigator will collect the participant's free, informed and written consent (or that of his/her legal representative for the chid population).

This visit corresponds to a scheduled consultation in the specialized centre for haemorrhagic disorders where the patient is followed. The investigator informs the participant of the constitution of a biological collection and its conservation at the end of the research for scientific purposes of its samples.

8.4. INCLUSION VISIT

The inclusion visit is conducted by the investigating doctor. Once the consent form has been signed, the following clinical and biological examination are performed:

- Information retrieval from medical records such as medical history
- Physical examination such as demographic information, weight, size
- 1 blood sample :
  - For adult population : a volume of 18 ml is collected (3 dry tubes of 6 ml) to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and a volume of 7 ml is collected (1 dry tube of 7 ml) to realize Anti-HLA screening (standard care)
  - For child population: the blood volume collected depends of the weight of the child (cf table in Annexe 1). The maximal volume authorized will be collected in dry tubes, to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and anti-HLA screening (standard of care).

8.5. FOLLOW-UP VISITS

Follow-up visit will be realized with the same frequency in the standard of care by the investigator, as every 6 months.

During this visit the following clinical and biological examination are performed:

- Physical examination such as demographic information, weight, size
- 1 blood sample :
  - For adult population : a volume of 18 ml is collected (3 dry tubes of 6 ml) to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and a volume of 7 ml is collected (1 dry tube of 7 ml) to realize Anti-HLA screening (standard of care)
  - For child population: the blood volume collected depends of the weight of the child (cf table in Annexe 1). The maximal volume authorized will be collected in dry tubes, to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and anti-HLA screening (standard of care).
- Collection of events including transfusion

In case of blood transfusions during the study

If blood products are administered for any reasons, anti-$\alpha_{IIb}\beta_3$ antibodies and Anti-HLA antibodies will be screened one week and 1 month after the last transfusion and medical information regarding this event will be recorded. This screening will allow to observe the kinetic of immunization after each blood transfusion.
In standard of care, when a patient with Glanzmann thrombastenia, followed in a platelet pathology reference center, is transfused, the center in which he is followed is informed by the French institution of the blood (Etablissement Français du Sang – EFS) of this transfusion.
As part of the TAAS study, any patient included will be from the cohort of patients followed at one of the platelet pathology reference centres. Therefore, the principal investigator should be informed of the transfusion of one of these patients and schedule the samples provided for as part of the protocol.

8.6. END OF RESEARCH VISIT

This visit correspond of the 18 months visits. This visit is conducted by the investigator doctor and occurs at the end of the study.

During this visit the following clinical and biological examination are performed:

- Physical examination such as demographic information, weight, size
- 1 blood sample :
  - For adult population : a volume of 18 ml is collected (3 dry tubes of 6 ml) to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and a volume of 7 ml is collected (1 dry tube of 7 ml) to realize Anti-HLA screening (standard of care)
  - For child population: the blood volume collected depends of the weight of the child (cf table in Annexe 1). The maximal volume authorized will be collected in dry tubes, to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and anti-HLA screening (standard of care).
- Collection of events including transfusion

After the study, the patient will then benefit from the usual care.

8.7. RULES FOR STOPPING A PERSON FROM PARTICIPATING IN THE RESEARCH

The study can be interrupted for one of the following reasons:
- withdrawal of consent to participate to the trial,
- major deviation to the protocol.

An abandonment is a decision of an included participant to assert his right to interrupt his participation in the study, at any time during the follow-up, without incurring any prejudice thereby and without having to justify himself.

A withdrawal of consent is a decision of a participant to reconsider his decision to participate in the study and to assert his right to cancel his informed consent, at any time during the follow-up and without incurring any prejudice and without having to justify itself.

The investigator must identify the cause of the abandonment / withdrawal and assess whether it is possible to collect the variable on which the primary endpoint applies at the time of abandonment / withdrawal. Abandonment / withdrawal must be promptly reported to the coordinating investigative center, the sponsor and the methodology and data management center. The reasons and the date of abandonment / withdrawal must be documented in the observation book and in the participant's medical file.

End of study or planned stop of the study: Last visit of the last participant included in the study.

Early termination of study: The clinical study is early (definitively) stopped

8.8. CONSTRAINTS RELATED TO THE RESEARCH AND POSSIBLE COMPENSATION OF PARTICIPANTS

The constraints of participating to the study is additional venous blood sampling realized every six months (about 18 ml of blood for adult population, and volume defined according to patients' weigt for child population) for determination of the presence of anti-αIIbβ3 antibodies.
The patient cannot participate to another study between the inclusion and the end of the study visits.
The patient will not be remunerated for his(her) participation.

8.9. COLLECTION OF BIOLOGICAL SAMPLES

Biological samples will be venous blood samples taken by peripheral route every six months, one week and 1 month after each blood product transfusion.

- For adult population : a volume of 18 ml is collected (3 dry tubes of 6 ml) to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and a volume of 7 ml is collected (1 dry tube of 7 ml) to realize Anti-HLA screening (standard of care)
- For child population: the blood volume collected depends of the weight of the child (cf table in Annexe 1). The maximal volume authorized will be collected in dry tubes, to realize Anti-$\alpha_{IIb}\beta_3$ screening by the MAIPA assay and anti-HLA screening (standard of care)

A biobank at CRB-P (Centre de Ressources Biologiques-plurithématique) will be planned to centralize samples (Anti-αIIbβ3) on the PARS research plateform. The PARS Hématologie offers a specialized service providing support for the development of translational research. This facility, incorporated within the hospital structure, aims to provide a dynamic exploration of the human system in patients in the context of clinical research trials.
Practically, serum will be obtained after centrifugation to 3500 rpm 10' to 18°C at least 1 hour after the sampling and stored frozen in each center (-20°C). These aliquots are monthly sent to the hematology laboratory of the University Hospital of Bordeaux.
The Hematology laboratory analyze aliquots except one, which be sent to a central laboratory to be conserved at -80°C (CRB-P). One aliquot of each sample will be kept 8 years after the end of the study, for further research only on CHU of Bordeaux.

9. MANAGEMENT OF ADVERSE EVENTS AND NEW DEVELOPMENTS

Adverse events / adverse reactions / incidents must be declared to the various health vigilance circuits applicable to each product or practice concerned (vigilance of care, pharmacovigilance, materiovigilance, haemovigilance, cosmetovigilance, etc.) in accordance with the regulations in force.

Registrants must specify that the participant is included in a clinical trial and identify precisely the clinical trial concerned.

If the investigator becomes aware of a breach in patient safety during the research, he/she shall inform the sponsor without delay.

10. STATISTICAL ASPECTS

10.1. CALCULATION OF STUDY SIZE

A sample size of 40 patients was determined by the feasibility of recruitment (rare disease).

The widespread MHEMO network recently approved by the DGOS provides a unique opportunity to set up such a study with 10 participating centres, all having an excellent experience in the management of

haemorrhagic diathesis. We estimate 4-5 patients eligible per centre. A number of 40 patients is therefore realistic for an inclusion period of 6 months.

Finally, significant lost of follow-up is unlikely. The study is relatively short for each patient and is performed in a context where the supervision of a physician experienced in the treatment of haematological diseases is usually asked by the patient itself.

10.2. STATISTICAL METHODS EMPLOYED

The cohort will be analyzed with logistic regression and the outcome variable will be defined as the absence or presence of a current or past history of antibody formation. Covariates will include subtype of GT, year of birth, *ITGA2B* or *ITGB3* gene mutations and other predifined risk factors. Markers that exhibited effects will be identified and analyzed using specific statistical software.

MONITORING OFTHE RESEARCH

10.3. STEERING COMMITTEE

A steering committee of 8 co-investigators involved in the conception of the study is constituted as follows:

- Dr M Fiore (coordinating investigator; University Hospital, Bordeaux)
- Pr MC Alessi, Coordinator of the National Reference Centre (University Hospital, Marseille)
- Dr S Voisin (co-investigator, University Hospital, Toulouse)
- Pr P Sié (co-investigator, University Hospital, Toulouse)
- and the Coordinator of Clinical study mentioned above.

Besides regular web exchange and information on the progress of the study, the committee will meet at the annual meetings of the CRPP, or of the MHEMO network. Interim analysis will be performed every one year, or on demand if a SAE occurred.

The role of steering committee is to validate the cases, to communicate on the advancement of the study to the co-investigators, to discuss all new data relative to the project, including potential amendments, to prepare the final report and publications.

11. RIGHTS OF ACCESS TO DATA AND SOURCE DOCUMENTS

11.1. ACCESS TO DATA

Agreeing to participate in the protocol implies that the investigators will make the documents and personal data that are strictly necessary for the monitoring, quality control and auditing of the research, available in accordance with the laws and regulations in force.

11.2. SOURCE DATA

All information contained in original documents, or in authenticated copies of these documents, relating to clinical examinations, observations or other activities conducted as part of a research study and necessary for the reconstitution and evaluation of the research. The documents in which the source data are saved are called the source documents.
Within the framework of the research, the expected type of source document are medical files and results from an original biological examination.

11.3. DATA CONFIDENTIALITY

In accordance with the legislative provisions in force, persons having direct access to source data will take all the necessary precautions to ensure the confidentiality of information relating to investigational medicinal products, research, participants, especially as regards their identity and the results obtained. These people, like the investigators, are subject to professional secrecy.

During or at the end of the research, the data collected on the participants and sent to the sponsor by the investigators (or any other specialised contributor) will made anonymous. The data must never explicitly mention the names of the persons concerned or their addresses.

All data of participants will be codified with a coded number specific to the research indicating order of inclusion of the subjects. This code compose of the center number (1 number) and patient number (2 number), the first letter of the subject's last name and first name.

The sponsor will ensure that each participant, or parents/legal representing has (have) given his/her/their written agreement for access to the individual data concerning them and strictly necessary for the quality control of the research.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1. GUIDELINES FOR COLLECTING DATA

All the information required by the protocol must be recorded in case report forms and an explanation must be provided for any missing data. The data must be collected as and when they are obtained, and transcribed in these notebooks in a clear and legible way.
All data will be recorded in paper case report form. Incorrect data noted in the case report forms will be clearly crossed out and the new data copied in beside the crossed-out information, with the initials, date and possibly a reason, by the investigator or authorised person who has made the correction.

12.2. QUALITY CONTROL

A clinical researcher appointed by the sponsor will regularly visit each centre investigator, during the implementation of the research, one or more times during research according to the frequency of the inclusions and at the end of the research. During these visits, and in accordance with the risk-based monitoring plan (participant, logistics, impact, resources), the following elements will be reviewed:

- informed consent,
- compliance with the research protocol and the procedures defined therein,
- quality of the data collected in the case report form: accuracy, missing data, consistency of the data with the source documents (medical records, appointment books, originals of laboratory results, etc.).
- management of potential products.

All visits will be the subject of a monitoring report by written report.

12.3. DATA MANAGEMENT

12.3.1. DATA MANAGEMENT SOFTWARE

12.3.1.1. SOFTWARE USED

The data management will be performed using the ACCESS software from the Microsoft Office package (version 10 or later). All analyses will be performed using SPSS® software (SPSS Inc., Chicago, Version 17.0 and later).

12.3.1.2. DATA HOSTING

The database will be located on a shared disk of the Bordeaux CHU network. The server hosting the data is located in a computer bay, in a dedicated lockable room. Only the staff of the Information System Department of the CHU of Bordeaux can access it. This server belongs to an internal network of the company and is not accessible directly from the internet. Access to the internal network of the Bordeaux CHU can only be done from a workstation belonging to the CHU, identified and listed as a client on the internal network of the CHU.

12.3.2. ACCESS TO DATA

Access to a local station of the CHU as well as its internal network requires identification and the allocation of access rights managed by the Information System Department of the CHU of Bordeaux.
Similarly, access to the database requires a second identification, independent of the first one, and protected by a password. The assignment of rights in Reading only or in Reading and Writing is managed by the data management manager of the service.
Each access to the database implements an access register including the identifier, dates and times of connection and disconnection.

12.3.3. DATA ENTRY METHOD

The data collected in paper format by the clinical researcher during the monitoring visit, are entered via in simple entry by clinical research technician, especially dedicated to entry data in the ACCESS®Database.

12.3.4. QUALITY CONTROL OF DATA

Checks are programmed to verify consistency and completeness of the data entered. The data are validated in accordance with the data management plan defined jointly between the coordinating investigator and the Centre for Methodology and Data Management (methodologist, data manager and statistician) in the study data validation plan. The software used is: ACCESS®

12.3.5. MODIFICATIONS/DELETIONS

Any modification/deletion of data in the database will be traced with date, time, and login with the possibility to enter a field explaining the reason for the modification. Only persons with write access rights can modify or delete data from the database.

12.3.6. BACKUP

A final level of protection against data breaches (total or partial loss of data, alteration of data integrity) is set up with the realization of complete daily backups on a second server. Only one backup is present on this server. Finally, a complete weekly backup is performed and saved on an external hard drive, encrypted, stored in a locked drawer. The history and all the weekly backups are kept and accessible on this disk.

12.3.7. FREEZE DATABASE

An initial procedure for Read-only access to the database is decided in order to check that all quality control requests have obtained a validated response. At the end, a copy of the raw data of the database in XML format and in the form of SPSS tables will be made for their analysis according to the defined statistical analysis plan.

12.3.8. DATA TRANSFER

No data transfer to be imported into the database is planned in this study.

12.4. AUDIT AND INSPECTION

An audit may be conducted at any time by persons appointed by the sponsor and independent of the persons conducting the research. Its purpose is to verify the participants' safety and respect for their rights, compliance with applicable regulations and the reliability of data.

An inspection can also be carried out by a competent authority (ANSM for France or EMA in the context of a European study, for example).

The audit, as well as the inspection, can be applied at all stages of the research, from the development of the protocol to the publication of the results and the classification of the data used or produced as part of the research.

Investigators agree to comply with the sponsor's requirements as regards an audit and the competent authority for a research inspection.

13. ETHICAL AND REGULATORY CONSIDERATIONS

The sponsor and the investigator(s) undertake to ensure that this research is carried out in accordance with law no. 2012-300 of 5 March 2012 on research involving the human person, as well as in agreement with Good Clinical Practices (ICH version 4 of 9 November 2016 and the decision of 24 November 2006) and the Declaration of Helsinki (which can be found in full at http://www.wma.net).

The research is conducted in accordance with this protocol. Except in emergency situations that require the implementation of specific therapeutic acts, the investigator(s) undertake(s) to respect the protocol in all points especially with regard to the collection of consent and notification and follow-up of serious adverse events.

This research received a favourable opinion from the Committee for the Protection of Persons (CPP) by Ile de France III CPP and and has been the subject of information to the ANSM.

The CHU de Bordeaux, sponsor of this research, has taken out a civil liability insurance contract with HDI Global SE in accordance with the provisions of the Public Health Code.

The data recorded during this research are the subject of a computerised processing on behalf of CHU de Bordeaux in accordance with the law no. 78-17 of 6 January 1978 on data processing, files and liberties as amended by Law 2004-801 of 6 August 2004.

This research falls within the framework of the "Reference Methodology" (MR-001) in application of the provisions of Article 54 paragraph 5 of the amended law of 6 January 1978 relating to information, files and liberties. This change was approved by the decision of 5 January 2006, updated on 21 July 2016. The CHU de Bordeaux has signed a compliance commitment to this "Reference Methodology".

This research is registered in the European database IDRCB under number 2020-A01285-34

This research has been registered on the site http://clinicaltrials.gov/

After the research, the conservation of the collection of biological samples will be declared to the Minister in charge of research and to the director of the French Regional Health Agency (and submitted to the CPP for an opinion if there was a change of research purpose).

CHANGES TO THE PROTOCOL

Any substantial change, i.e. any change that is likely to have a significant impact on the protection of persons, on the conditions of validity and on the results of the research, on the quality and safety of the products tested, on the interpretation of scientific documents that support the conduct of the research or the way in which the research is conducted, is subject to a written amendment submitted to the sponsor. The latter must obtain, prior to its implementation, a favourable opinion from the CPP, and, where applicable, authorisation from the French National Agency for Medicines and Health Products Safety (ANSM).

Non-substantial changes, i.e, those that do not have a significant impact on any aspect of the research, are communicated to the CPP for information.

All changes are validated by the sponsor, and by all research stakeholders involved in the change, before submission to the CPP, and, where applicable, to the ANSM. This validation may require the meeting of all committees formed for the research. .

All changes to the protocol must be made known to investigators, who are participating in the research. The investigators undertake to respect the content.

Any modification that modifies participant care or the benefits, risks and constraints of the research is the subject of a new information note and a new consent form whose collection follows the same procedure above.

14. PRESERVATION OF RESEARCH DOCUMENTS AND DATA

The following documents related to this research are archived by the investigator in accordance with Good Clinical Practices:

- for a period of 15 years following the end of the research

- The protocol and any modifications to the protocol
- Case report forms (copies)
- Source records of participants who have signed a consent form
- All other documents and correspondence related to research

- for a period of 30 years following the end of the research

- The original copy of the informed consent forms signed by the participants

All of these documents are the responsibility of the investigator during the regulatory archiving period.
No displacement or destruction can be made without the agreement of the sponsor. At the end of the regulatory archiving period, the sponsor will be consulted for destruction. All data, documents and reports may be subject to audit or inspection.

15. FINAL REPORT

Within one year after the end of the research or its interruption, a final report will be prepared and signed by the sponsor and the investigator. This report will be kept at the disposal of the competent authority. The sponsor will transmit to the CPP and, where applicable, to the ANSM, the results of the research in the form of a summary of the final report within one year after the end of the research.

16. RULES FOR PUBLICATION

16.1. SCIENTIFIC COMMUNICATIONS

The data analysis provided by the investigating centres is carried out by Centre de Référence labelled« Maladies Rares » Pathologies Plaquettaires Constitutionnelles.
This analysis gives rise to a written report which is submitted to the sponsor, who will forward it to the Committee for the Protection of Persons and to the competent authority.

Any written or oral communication of the results of the research must have the prior consent of the coordinating investigator and, where appropriate, of all committees that were established for the research.

The coordinating investigator undertakes to make available to the public both negative and inconclusive and positive research results.

The publication of the main results must mention the name of the sponsor, all the persons who helped in the inclusion or follow-up of participants in the research, methodologists, biostatisticians and data managers who participated in the research, health vigilance personnel who participated in the safety analysis of the participants, members of the committee(s) constituted for the research and the possible involvement of the source of funding. International rules of writing and publication will be taken into account (*The Uniform Requirements for Manuscripts*of the ICMJE, April 2010).

16.2. COMMUNICATION OF RESULTS TO PARTICIPANTS

In accordance with law no. 2002-303 of 4 March 2002, the participants are informed, at their request, of the overall results of the research.

16.3. TRANSFER OF DATA

Data management is provided by Centre de Référence labellisé « Maladies Rares » : Pathologies Plaquettaires Constitutionnelles. The conditions for the transfer of all or part of the research database are decided by the research sponsor are the subject of a written contract.

BIBLIOGRAPHIC REFERENCES

[1] Nurden AT. Glanzmann thrombasthenia. Orphanet J Rare Dis. 2006;1:10.
[2] Nurden AT, Fiore M, Pillois X, Nurden P. Genetic testing in the diagnostic evaluation of inherited platelet disorders. Seminars in thrombosis and hemostasis. 2009 Mar;35:204-12.
[3] Fiore M, Nurden AT, Nurden P, Seligsohn U. Clinical utility gene card for: Glanzmann thrombasthenia. Eur J Hum Genet. 2012 Oct;20.
[4] Nurden AT, Fiore M, Nurden P, Pillois X. Glanzmann thrombasthenia: a review of ITGA2B and ITGB3 defects with emphasis on variants, phenotypic variability, and mouse models. Blood. 2012 Dec 1;118:5996-6005.
[5] Fiore M, Pillois X, Nurden P, Nurden AT, Austerlitz F. Founder effect and estimation of the age of the French Gypsy mutation associated with Glanzmann thrombasthenia in Manouche families. Eur J Hum Genet. 2011 Sep;19:981-7.
[6] Fiore M, Nurden AT, Vinciguerra C, Nurden P, Pillois X. Rapid diagnosis of the French gypsy mutation in Glanzmann thrombasthenia using high-resolution melting analysis. Thrombosis and haemostasis. 2010 Nov 3;104:1076-7.
[7] Di Minno G, Coppola A, Di Minno MN, Poon MC. Glanzmann's thrombasthenia (defective platelet integrin alphaIIb-beta3): proposals for management between evidence and open issues. Thrombosis and haemostasis. 2009 Dec;102:1157-64.
[8] Fiore M, d'Oiron R, Pillois X, Alessi MC. Anti-alphaIIb beta3 immunization in Glanzmann thrombasthenia: review of literature and treatment recommendations. British journal of haematology. 2018 Apr;181:173-82.
[9] Fiore M, Firah N, Pillois X, Nurden P, Heilig R, Nurden AT. Natural history of platelet antibody formation against alphaIIbbeta3 in a French cohort of Glanzmann thrombasthenia patients. Haemophilia. 2012 May;18:e201-9.
[10] Siddiq S, Clark A, Mumford A. A systematic review of the management and outcomes of pregnancy in Glanzmann thrombasthenia. Haemophilia. 2011 Sep;17:e858-69.
[11] Leticee N, Kaplan C, Lemery D. Pregnancy in mother with Glanzmann's thrombasthenia and isoantibody against GPIIb-IIIa: Is there a foetal risk? Eur J Obstet Gynecol Reprod Biol. 2005 Aug 1;121:139-42.
[12] Levy-Toledano S, Tobelem G, Legrand C, Bredoux R, Degos L, Nurden A, Caen JP. Acquired IgG antibody occurring in a thrombasthenic patient: its effect on human platelet function. Blood. 1978 Jun;51:1065-71.
[13] Nurden AT. Glanzmann thrombasthenia: the need for epidemiological studies. J Thromb Haemost. 2009 Nov;7:1875-7.
[14] Hay CR, Palmer B, Chalmers E, Liesner R, Maclean R, Rangarajan S, Williams M, Collins PW. Incidence of factor VIII inhibitors throughout life in severe hemophilia A in the United Kingdom. Blood. 2011 Jun 9;117:6367-70.
[15] George JN, Caen JP, Nurden AT. Glanzmann's thrombasthenia: the spectrum of clinical disease. Blood. 1990 Apr 1;75:1383-95.
[16] Poon MC, D'Oiron R, Von Depka M, Khair K, Negrier C, Karafoulidou A, Huth-Kuehne A, Morfini M. Prophylactic and therapeutic recombinant factor VIIa administration to patients with Glanzmann's thrombasthenia: results of an international survey. J Thromb Haemost. 2004 Jul;2:1096-103.
[17] Santoro C, Rago A, Biondo F, Conti L, Pulcinelli F, Laurenti L, Perrone MP, Baldacci E, Leporace A, Mazzucconi MG. Prevalence of allo-immunization anti-HLA and anti-integrin alphaIIbbeta3 in Glanzmann Thromboasthenia patients. Haemophilia. 2010 Sep 1;16:805-12.
[18] Nurden AT, Pillois X, Fiore M, Alessi MC, Bonduel M, Dreyfus M, Goudemand J, Gruel Y, Benabdallah-Guerida S, Latger-Cannard V, Negrier C, Nugent D, Oiron RD, Rand ML, Sie P, Trossaert M, Alberio L, Martins N, Sirvain-Trukniewicz P, Couloux A. Expanding the Mutation Spectrum Affecting alphaIIbbeta3 Integrin in Glanzmann Thrombasthenia: Screening of the ITGA2B and ITGB3 Genes in a Large International Cohort. Human mutation. 2015 May;36:548-61.

[19] Poon MC, Di Minno G, d'Oiron R, Zotz R. New Insights Into the Treatment of Glanzmann Thrombasthenia. Transfusion medicine reviews. 2016 Apr;30:92-9.
[20] Oldenburg J, Pavlova A. Genetic risk factors for inhibitors to factors VIII and IX. Haemophilia. 2006 Dec;12 Suppl 6:15-22.
[21] Pillois X, Nurden AT. Linkage disequilibrium amongst ITGA2B and ITGB3 gene variants in patients with Glanzmann thrombasthenia confirms that most disease-causing mutations are recent. British journal of haematology. 2016 Nov;175:686-95.
[22] Laurian Y, Tisseron-Maury B, Bibi Triki T, Kaplan C, Gaudelus J. Red blood cell transfusion in patients with type 1 Glanzmann's thrombasthenia. J Thromb Haemost. 2005 Oct;3:2346-7.
[23] Nurden AT, Kunicki T, Nurden P, Fiore M, Martins N, Heilig R, Pillois X. Mutation analysis for a patient with Glanzmann thrombasthenia who produced a landmark isoantibody to the alphaIIbbeta3 integrin. J Thromb Haemost. 2010 Aug;8:1866-8.
[24] Kashiwagi H, Kiyomizu K, Kamae T, Nakazawa T, Tadokoro S, Takiguchi S, Doki Y, Kanakura Y, Tomiyama Y. Molecular analysis of a patient with type I Glanzmann thrombasthenia and clinical impact of the presence of anti-alphaIIbbeta3 alloantibodies. Int J Hematol. 2011 Jan;93:106-11.
[25] Ghosh K, Kulkarni B, Shetty S, Nair S. Antiplatelet antibodies in cases of Glanzmann's thrombasthenia with and without a history of multiple platelet transfusion. Indian journal of human genetics. 2009 Jan;15:23-7.
[26] Chouhan B, Denesyuk A, Heino J, Johnson MS, Denessiouk K. Conservation of the human integrin-type beta-propeller domain in bacteria. PloS one. 2011;6:e25069.
[27] Vivier M, Treisser A, Naett M, Diemunsch P, Schmitt JP, Waller C, Tongio MM, Hemmendinger S, Lutz P. [Glanzmann's thrombasthenia and pregnancy. Contribution of plasma exchange before scheduled cesarean section]. Journal de gynecologie, obstetrique et biologie de la reproduction. 1989;18:507-13.
[28] Monrigal C, Beurrier P, Mercier FJ, Boyer-Neumann C, Gillard P. [Glanzmann's thrombasthenia and pregnancy: a case and review of the literature]. Annales francaises d'anesthesie et de reanimation. 2003 Nov;22:826-30.
[29] Tengborn L, Petruson B. A patient with Glanzmann thrombasthenia and epistaxis successfully treated with recombinant factor VIIa. Thrombosis and haemostasis. 1996 Jun;75:981-2.

APPENDICES

- ANNEXE 1 : VOLUME DE PRÉLÈVEMENT SANGUIN EN FONCTION DU POIDS DE LA PERSONNE

Volume maximal de sang pouvant être prélevé en fonction du poids corporel

Poids en kilos	Volume sanguin total en mL	Volume maximal par prélèvement en mL (=2.5% du volume sanguin total)	Volume total maximal [recherche + soin (le cas échéant)] par période de 30 jours en mL	Taux minimum d'hémoglobine requis au moment du prélèvement en mL	Taux minimum d'hémoglobine requis au moment du prélèvement si le patient a une pathologie respiratoire ou cardiovasculaire en mL
1	100	2.5	5	7.0	9.0-10.0
2	200	5	10	7.0	9.0-10.0
3	240	6	12	7.0	9.0-10.0
4	320	8	16	7.0	9.0-10.0
5	400	10	20	7.0	9.0-10.0
6	480	12	24	7.0	9.0-10.0
7	560	14	28	7.0	9.0-10.0
8	640	16	32	7.0	9.0-10.0
9	720	18	36	7.0	9.0-10.0
10	800	20	40	7.0	9.0-10.0
11-15	880-1200	22-30	44-60	7.0	9.0-10.0
16-20	1280-1600	32-40	64-80	7.0	9.0-10.0
21-25	1680-2000	42-50	64-100	7.0	9.0-10.0
26-30	2080-2400	52-60	104-120	7.0	9.0-10.0
31-35	2480-2800	62-70	124-140	7.0	9.0-10.0
36-40	2880-3200	72-80	144-160	7.0	9.0-10.0
41-45	3280-3600	82-90	164-180	7.0	9.0-10.0
46-50	3680-4000	92-100	184-200	7.0	9.0-10.0
51-55	4080-4400	102-110	204-220	7.0	9.0-10.0
56-60	4480-4800	112-120	224-240	7.0	9.0-10.0
61-65	4880-5200	122-130	244-260	7.0	9.0-10.0
68-70	5280-5600	132-140	264-280	7.0	9.0-10.0
71-75	5680-6000	142-150	284-300	7.0	9.0-10.0
76-80	6080-6400	152-160	304-360	7.0	9.0-10.0
81-85	6480-6800	162-170	324-340	7.0	9.0-10.0
86-90	6880-7200	172-180	344-360	7.0	9.0-10.0
91-95	7280-7600	182-190	364-380	7.0	9.0-10.0
>96	7680-8000	192-200	384-400	7.0	9.0-10.0

D'après Blood Volume Guidelines V1.1, 30 November 2015 Stellenbosch University, Health Research Ethics Committee (HREC)