CLINICAL PROTOCOL

General Information

Title:
EFFICACY OF PLATELETS HAVING UNDERGONE PATHOGEN REDUCTION

Short title:
EFFIPAP

ClinicalTrials.gov Identifier:
NCT01789762
http://clinicaltrials.gov/ct2/show/NCT01789762?term=EFFIPAP&rank=1

Phase IV randomised non-inferiority therapeutic clinical trial

Study products: platelet concentrates (both pooled random donor and apheresis platelets) prepared in plasma versus platelet concentrates prepared in additive solution versus platelet concentrates treated with the Intercept® system

Methodology:
Multicenter randomized therapeutic trial
Blinding: product labelling, patient, bleeding assessor, treating MD, local investigator and data-management are blinded; personnel receiving/dispensing platelet product is not blinded

Principal Investigator:
Prof. Frédéric Garban: Établissement Français du Sang

Haematology centres involved and clinical investigators
- Angers (CHU) Prof. Norbert Ifrah
- Besançon (CHU) Prof. Eric Deconinck
- Brest (CHU), Dr Catherine Le Niger
- Clermont Ferrand (CHU) Prof. Jacques Olivier Bay
- Dijon (CHU) Dr. Denis Caillot
- Grenoble (CHU haematology clinic of Prof. Jean Yves Cahn) Prof. Frédéric Garban
- Lille (CHU) Prof Ibrahim Yakoug Agua
- Lyon (Hospices Civils de Lyon-Lyon Sud, department of Prof. Mauricette Michallet) Dr. Hélène Labussière
- Marseille (Institut Paoli Calmette Hématologie, Prof. Norbert Vey) Dr. Patrick Ladaigue
- Paris (Assistance Publique Hôpitaux de Paris – Créteil) Prof. Catherine Cordonnier
- Paris (Assistance Publique Hôpitaux de Paris – St Antoine) Prof. Mohamad Mohty
- Rennes (CHU, department of Prof. Thierry Lamy de la Chapelle) Dr. Stanislas Nimubona
- St Priest en Jarez (Institut de cancérologie de la Loire) Dr. Christiane Mounier

**Etablissement Français du Sang steering committee:**
- Prof. Pierre Tiberghien (national coordinator representing the sponsor)
- Prof. Frédéric Garban (Principal Investigator)
- Dr. Suzanne Assari
- Dr. Anne François
- Dr. Dominique Legrand
- Dr. René Tardivel,
- Prof. Philippe Bierling
- Dr. Françoise Hau
- Dr. Chantal Jacquot

Prof. Jean Luc Bosson (CIC of Grenoble representative)
Ms. Carole Rolland (Project Manager – Contact person)

**Sponsor:** Etablissement Français du Sang  
**Representative of the sponsor:** Prof. Pierre Tiberghien  
EFS siège, avenue du Stade France 93218 La plaine Saint Denis, France

**Coordinating centre of the study:**
Centre d’Investigation Clinique – Inserm 003 – Equipe THEMAS (Prof. Jean Luc Bosson)  
CHU de Grenoble, Pavillon Taillefer - 38043 Grenoble Cedex 09, France  
Tel.: +33 (0)4 76 76 50 40  Fax: +33 (0)4 76 76 52 42
## PROTOCOL – SUMMARY

**Therapeutic randomized phase 4 Study**

**Study centers:** Haematology departments of the following institutions: CHU d’Angers, CHU de Besançon, CHU de Clermont Ferrand, CHU de Dijon, CHU de Grenoble, CHU de Lille, Hospices civils de Lyon (Lyon Sud), Institut Paoli Calmette (Marseille), Assistance Publique Hôpitaux de Paris (Créteil - Saint Louis ; St Antoine), Institut de Cancérologie de la Loire (St Priest en Jarez), CHU de Rennes. Établissement Français du Sang (French Blood Service)

**Principal Investigator:** Prof. Frédéric Garban (Établissement Français du Sang)

**Investigators:**

*Haematology centres involved and clinical investigators*

- Angers (CHU) Prof. Norbert Ifrah
- Besançon (CHU) Prof. Eric Deconinck
- Clermont Ferrand (CHU) Prof. Jacques Olivier Bay
- Dijon (CHU) Dr. Denis Caillot
- Grenoble (CHU haematology clinic, Prof. Jean Yves Cahn) Prof. Frédéric Garban
- Lille (CHU) Prof Ibrahim Yakougu Agua
- Lyon (Hospices Civils de Lyon-Lyon Sud, department, Prof. Mauricette Michallet) Dr. Hélène Labussière
- Marseille (Institut Paoli Calmette Hématologie, Prof. Norbert Vey) Dr. Patrick Ladaïque
- Paris (Assistance Publique hôpitaux de Paris – Créteil) Prof. Catherine Cordonnier
- Paris (Assistance Publique Hôpitaux de Paris – St Antoine) Prof. Mohamad Mohty
- Rennes (CHU, department of Prof. Thierry Lamy de la Chapelle) Dr. Stanislas Nimubona
- St Priest en Jarez (Institut de cancérologie de la Loire) Dr. Christiane Mounier

**Établissement Français du Sang steering committee:**

- Prof. Pierre Tiberghien (national coordinator as sponsor representative)
- Prof. Frédéric Garban (Principal Investigator)
- Dr. Suzanne Assari
- Dr. Anne François
- Dr. Dominique Legrand
- Dr. René Tardivel,
- Prof. Philippe Bierling
- Dr. Françoise Hau
- Dr. Chantal Jacquot

**Clinical Investigation Centre (CIC) of Grenoble University Hospital**

Prof. Jean Luc Bosson, CIC Head
Ms. Carole Rolland, Project Manager

**Sponsor:** Établissement Français du Sang

**Methodology:**

Multicentre, randomized therapeutic trial
Blinding: product labelling, patient, bleeding assessor, treating MD, local investigator and data-management are blinded; personnel receiving/dispensing platelet product is not blinded.

**Primary objective:**
Non-inferiority trial involving 3 transfusion arms of platelet concentrates (buffy-coat-derived pooled platelet concentrates (PPC) or apheresis platelets concentrates (APC) equally)
- Platelet concentrates having undergone pathogen reduction (Intercept® amotosalen and UVA process)
- Platelet concentrates in plasma
- Platelet concentrates in additive solution

- **Primary endpoint:**
  Incidence of grade 2 or higher (WHO) haemorrhagic episodes

<table>
<thead>
<tr>
<th>Secondary objectives:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Incidence of haemorrhagic episodes, grade 1 and higher</td>
</tr>
<tr>
<td>2) Number of serious grade 3-4 haemorrhagic episodes</td>
</tr>
<tr>
<td>3) Number of minor grade 1 haemorrhagic episodes</td>
</tr>
<tr>
<td>4) 24 hours corrected platelet count increment (CCI)</td>
</tr>
<tr>
<td>5) Number of transfusions of platelet concentrates and red blood cells</td>
</tr>
<tr>
<td>6) Transfusion intervals</td>
</tr>
<tr>
<td>7) Transfusion side effects, grade 2 or higher</td>
</tr>
<tr>
<td>8) Occurrence of anti-platelet antibodies (Anti-HLA, anti-HPA)</td>
</tr>
<tr>
<td>9) Occurrence of refractory reactions to platelet transfusions</td>
</tr>
<tr>
<td>10) Validation of a new haemorrhagic evaluation: EFS scale</td>
</tr>
<tr>
<td>11) Variation in hematocrit and hemoglobin levels (Amendment added before start of inclusions)</td>
</tr>
<tr>
<td>12) Number of bleeding days of WHO grade equal to or greater than 2 (Amendment Sept 2014)</td>
</tr>
</tbody>
</table>

**Main inclusion criteria (target population of the study):**
- Patients aged 18 years or older with haematological malignancy
- Hospitalisation for aplasia with expected thrombopaenia less than 10 g/l and requiring 2 or more platelet transfusions (long-term aplasia)

**Main exclusion criteria:**
- Patients with known platelet transfusion refractoriness, related or not to an anti-HLA alloimmunization (thus, patient already known as requiring HLA-compatible platelets)
  Note: The presence of anti-HLA antibody is not « per se » a criterion of non-inclusion. Detection of anti-HLA Ab is not required for the inclusion.
- Patient requiring CMV Ab negative  blood products  Patient known to require specific platelet transfusion protocol (HLA compatible platelets, washed platelets)
- Patient requiring curative dose anticoagulant treatment at the time of inclusion (vitamin K antagonists, heparin (both low molecular weight and non-fractionated), anti-IIa and Xa at curative doses for treatment or prophylaxis of arterial or venous thromboembolic disease as part of the treatment for cardiac valvulopathy and complications of atrial fibrillation).
  Note: Anticoagulant treatment with prophylactic doses is allowed (prevention of thrombosis or management of DICD for example). The introduction of anticoagulant after inclusion in the trial treatment at curative doses after inclusion does not imply exclusion from the study
(Amendment Feb 2014)
- Pregnant women, adults under protective services and persons deprived of libert

<table>
<thead>
<tr>
<th>Product tested:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet concentrates (PPC or APC) in plasma, in additive solution, or in additive solution and treated with the Intercept® process</td>
</tr>
<tr>
<td>The study concerns a total of 6 labile blood products authorised by the National Agency for the Safety of Pharmaceuticals and Health Products (ANSM) (formerly AFSSAPS) and currently available in France.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total number of patients:</th>
<th>840 (amended Nov. 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total duration of the study:</td>
<td>3 years (amended April 2015)</td>
</tr>
<tr>
<td>Duration of the study per patient:</td>
<td>30 days</td>
</tr>
</tbody>
</table>
CONTENTS

GENERAL INFORMATION .......................................................................................................................................................... 1

PROTOCOL – SUMMARY .......................................................................................................................................................... 3

CONTENTS ......................................................................................................................................................................................... 6

STUDY BACKGROUND .............................................................................................................................................................. 8

PLATELET TRANSFUSION ........................................................................................................................................................... 8

ADVERSE EFFECTS OF PLATELETS TRANSFUSION ................................................................................................................ 10

PATHOGEN REDUCTION FOR PLATELET CONCENTRATES ............................................................................................ 11

PLATELET CONCENTRATES AVAILABLE IN FRANCE ........................................................................................................ 13

PATHOGEN REDUCED PLATELETS CONCENTRATES IN FRANCE .......................................................................................... 13

THE EFFIPAP TRIAL ............................................................................................................................................................... 14

PATIENT SELECTION ............................................................................................................................................................... 15

STUDY OBJECTIVES .............................................................................................................................................................. 17

PRIMARY OBJECTIVE AND PRIMARY ENDPOINT ........................................................................................................ 17

SECONDARY OBJECTIVES AND SECONDARY ENDPOINTS ................................................................................................ 17

RESEARCH DESIGN ............................................................................................................................................................... 18

TYPE OF STUDY ........................................................................................................................................................................ 18

GENERAL ORGANISATION OF THE STUDY AND DIAGRAM .................................................................................................. 18

PATIENT CHARACTERISTICS .................................................................................................................................................. 22

METHODS OF PATIENT RECRUITMENT ................................................................................................................................... 22

INCLUSION CRITERIA ................................................................................................................................................................. 22

EXCLUSION CRITERIA ................................................................................................................................................................. 23

VARIABLES MEASURED AND METHODS OF MEASUREMENT ............................................................................................ 24

CLINICAL PARAMETERS ............................................................................................................................................................ 24

LABORATORY PARAMETERS ........................................................................................................................................................ 24

DATA COLLECTED DIRECTLY IN THE CASE REPORT FORM .................................................................................................. 25

TREATMENTS COMPARED ....................................................................................................................................................... 26

TREATMENT ................................................................................................................................................................................ 26

TECHNICAL DATA ON THE TESTED BLOOD PRODUCTS ...................................................................................................... 28

PLATELET CONCENTRATES IN ADDITIVE SOLUTION: ........................................................................................................... 28

PLATELET CONCENTRATES TREATED BY PATHOGEN INACTIVATION TECHNOLOGY COMBINING AMOTOSALEN AND UVA (INTERCEPT®). ...................................................................................................................... 28

PLATELET CONCENTRATES IN PLASMA .................................................................................................................................... 28

TRANSFUSION ............................................................................................................................................................................... 28

TRACEABILITY ................................................................................................................................................................................ 29

RANDOMIZATION METHOD ..................................................................................................................................................... 29

PRODUCT LABELING ................................................................................................................................................................. 29

PRODUCT TRACEABILITY .......................................................................................................................................................... 30

PROCEDURE FOR UNBLINDING ................................................................................................................................................ 32

DATA COLLECTION AND MANAGEMENT ................................................................................................................................. 33

DATA COLLECTION ................................................................................................................................................................. 33

DATA MONITORING ................................................................................................................................................................. 33

STATISTICAL ANALYSIS OF THE MEASURED PARAMETERS .................................................................................................. 34

CALCULATION OF THE NUMBER OF PATIENTS ........................................................................................................................................... 34
STUDY BACKGROUND

Platelet transfusion

Platelet transfusion support has long been a mainstay in the haematological management of patients treated with high-dose chemotherapy or hematopoietic transplantation, as well as in cases of aplastic anaemia. Used initially to cope with the prevention and treatment of haemorrhaging from thrombopaenia, platelets are now used by most teams first for prophylactic purposes in order to obtain or maintain a sufficient number of platelets relative to the patient’s clinical condition or in accordance with the planned technical procedures. Recommendations exist both for prophylactic and therapeutic use. Important questions on platelet dose were raised in the 1990’s with a focus on large platelet doses. The use of platelet transfusions is essential in cases of long-term (over 10 days) thrombopaenia such as in patients with aplastic anaemia, acute leukaemia, or in the setting of intensive chemotherapy or hematopoietic stem cell transplantation. Despite this being an essential and commonly prescribed treatment in cases of long-term aplasia, there are still many questions concerning transfusion practices and the basic principles required to optimise platelet transfusion support. The main questions focus on (i) transfusion strategies that are primarily based on older studies (therefore without taking into consideration changes in the preparation methods of currently available products), and even without definitive decision based on current products with regard to prophylactic or therapeutic transfusions, (ii) the choice of evaluation methods: platelets recovery/survival, clinical evaluation of bleeding, (iii) the statistical and methodological quality of the studies, and (iii) the choice of study populations. Nearly all studies focus on situations of stable aplasia, most often excluding patients who are the greatest consumers of platelets (with coagulation...

3 Slichter SJ. Evidence-based platelet transfusion guidelines. Hematology ASH Educ Program. 2007:172-8
disorders, anti-HLA antibodies), not to mention the near total absence of clinical studies concerning qualified patients who are refractory to platelet transfusions.

The optimal dose of platelet transfusion may be defined as the dose to ensure patient safety (prevention of hemorrhagic syndrome and decreased induced side-effects) at the cost of a total amount of transfused platelets as low as possible. Current guidelines recommend the use in France of a standard dose of $0.5 \times 10^{11}$ for 7 to 10 kg of weight. The recommended doses are often higher in children ($0.7 \times 10^{11}$)\(^7\).

Larges platelets doses (>1x10\(^{11}\)/10 kg) significantly increased post-transfusion platelet increments and the interval between transfusions in some studies\(^4\). In contrast, others studies have found the effectiveness of a strategy of using lower doses of platelets justified by a reduction in adverse effects, overall financial cost of transfusions and impact on the availability of CP. One study\(^8\) comparing low doses of platelets to higher doses not adjusted for the weight of the patient (1.5 to 3 x 10\(^{11}\) versus 3-6 x 10\(^{11}\) ) had to be stopped because of the occurrence of a greater number of severe bleeding (WHO grade > 4) in the group of patients receiving low doses of platelets. In a second one\(^9\), comparing doses of 1.10 x 10\(^{11}\)/m2 body surface area (approximately 0.25 x 10\(^{11}\)/10 Kg weight) to two or four fold doses did not find any difference in the occurrence of bleeding (WHO grade > 4) between the three groups. In contrast, a significant decrease in platelet consumption (20 % to 50 % of the low dose group compared to the other two), and an increase in the number of transfusion episodes for the low dose group was found.

The most relevant mean to assess the effectiveness of transfusions should be the appearance or not of hemorrhagic syndrome and particularly the occurrence of severe hemorrhage involving life-threatening.

Transfusion threshold can be regarded as a platelet count below which there is a significant risk of a hemorrhagic syndrome. The occurrence of serious bleeding is rare below a platelet count of 10 G / L. That is why such a threshold has been endorsed for patients without others risk (such bleeding, fever, coagulation defect). Furthermore, it has been demonstrated that the platelet count does not in itself constitute an accurate reflection of the risk of bleeding, except in extreme cases of thrombocytopenia less than 5 G / l.

Despite the scarcity of published comparative large-scale studies, professional agreements have resulted in various transfusion thresholds depending on the different clinical situations. These agreements have been published in several countries, including France, as recommendations for good clinical practice.

Several recent studies have brought the evaluation of platelet products to the forefront with a primary endpoint being the impact on the occurrence of bleeding episodes, variable dose policies and transfusion strategies. With this platelet support, haemorrhagic mortality in long-term aplasia has decreased but not disappeared entirely, and around 60% to 70% of patients will present with haemorrhagic episodes of varying severity during a stable phase of long-term aplasia.

**Adverse effects of platelets transfusion**

Platelet concentrates are blood products, and as with all blood products, they expose recipients to adverse effects that can be categorized as following:

1) Immunological risks: fever – chills, secondary allergic effects, more seldom and more serious: post-transfusion purpura, graft-versus-host disease, and TRALI (transfusion related acute lung injury: a respiratory distress syndrome of complex origin, involving immunologic processes). Most of these effects are associated with the presence of antibodies (against various allogeneic targets) and/or mediators implicated in inflammation.

2) Microbiologic risks: The relative risk of viral transmission (hepatitis, HIV) is now extremely low owing to highly sensitive serological and genomic testing as well as efficient donor selection. However, a significant risk of bacterial transmission remains, especially for PC which are stored at room temperature. Presently, bacterial infections transmitted by PC transfusion are responsible for 0 to 2 deaths per year in France\(^\text{12}\). Furthermore, “emerging” pathogens such as West Nile virus or Dengue virus and Hepatitis E virus remain a transfusion risk since serologic or genomic screening for these infectious agents are not always widely available and/or or totally reliable.

**Pathogen reduction for platelet concentrates.**

Several pathogen reduction methods have been developed among which the Intercept technology that combines the use of a psoralene (Amotosalen), an intercalation agent, with UVA irradiation\(^\text{13}\). A major concern is the impact of these technologies on the integrity of blood components, and for platelets concentrates the efficacy to stop or prevent bleeding. Out of four published randomized studies,\(^\text{14,15,16,17}\) the Hovon study\(^\text{16}\) was associated with a significant excess of haemorrhage in the arm using the Intercept\(^\text{®}\) procedure. Such a trend for increased (low-grade) bleeding associated with Intercept-treated platelets was also reported in the expanded safety analysis\(^\text{18}\) of the Sprint study\(^\text{15}\). Of note, the two other studies were not designed with bleeding as the primary outcome.

As mentioned previously a limited number of clinical studies have been examined the clinical impact of pathogen reduction procedures on transfused products. The Hovon study\(^\text{16}\), which randomised 3

---

\(^{12}\) http://www.afssaps.fr/var/afssaps_site/storage/original/application/365a97e590280fb1192c05a838cb97bb.pdf
arms (one arm with plasma, one with additive solution and one with inactivation procedure) showed a higher frequency (just beyond threshold level of significance) of haemorrhagic events in the platelet arm with the pathogen inactivation process, but it was a secondary objective of the study (the primary objective being the transfusion outcome). As the trial had been stopped prematurely, the strength did not seem to be sufficient for statistically confirming this excess of haemorrhagic syndromes on a larger scale. However, more recently, a meta-analysis\textsuperscript{19} concluded that Intercept-treated platelets might increase the risk of all and clinically significant (albeit not severe) bleeding complications. To our knowledge there are presently 2 on-going trials exploring in a prospective randomized fashion the impact of pathogen reduction technology on platelet function as assessed in vivo by bleeding occurrence and severity: the dutch PREPAReS study\textsuperscript{20} comparing Riboflavine + UV-B treated platelets (Mirasol) vs control untreated platelets in plasma, and the Italian IPTAS study\textsuperscript{21} comparing Amotosalen + UV-A platelets (Intercept) as well as Mirasol platelets versus control untreated platelets in additive solution.

With regard to the adverse effects linked to the transfusion of platelets treated by Intercept\textsuperscript{®}, there are still questions on the possible increased incidence of respiratory distress, as was reported in the SPRINT study but which was not confirmed by re-analyzing the cases on the basis of detailed clinical investigations\textsuperscript{22}. More recently, an experimental study nevertheless reported evidence in favour of pulmonary trapping of platelets that are irradiated by UV\textsuperscript{23}. Finally, a possible increase incidence of refractoriness to platelet transfusion has been suggested in the SPRINT study\textsuperscript{15,18}. Observations of this type, which deserve to be better documented, were also reported in France by ETS la Reunion.

\textsuperscript{19} Vamvakas EC. Meta-analysis of the studies of bleeding complications of platelets pathogen-reduced with the Intercept system. Vox Sang. 2012; 102(4):302-16
\textsuperscript{20} Pathogen Reduction Evaluation & Predictive Analytical Rating Score PREPAReS Study: Pathogen Reduction Evaluation & Predictive Analytical Rating Score. NTR Number NTR2106
\textsuperscript{21} Italian Platelet Technology Assessment Study (IPTAS) ClinicalTrials.gov Identifier: NCT01642563
Platelet concentrates available in France

Platelet concentrates are obtained either from several whole blood donations pooled random donor platelets concentrates (PPC, buffy-coat method), or from a single donor having undergone an aphaeresis procedure, apheresis platelets concentrates (APC). PPC and APC have been shown to have equal efficacy. The platelet products used for blood transfusion in France are authorised by ANSM (formerly AFSSAPS) and published in the Journal Officiel (JO, French government bulletin).

As a broad outline, the following platelet products are presently authorized in France:

- Platelet concentrates in plasma
- Platelet concentrates in additive solution: the platelet product undergoes partial extraction of the plasma (about 62%) and an additive solution is added to the suspension (two commercial solutions are used by the EFS: SSP+® MacoPharma and Intersol® Fenwall)
- Platelet concentrates treated with a pathogen inactivation procedure (Intercept® marketed by Cerus) with Intersol or SSP+ as additive solution

Pathogen reduced platelets concentrates in France

In France, Intercept is presently in use for platelets concentrates in 2 specific settings: in the DOM (French overseas departments: Martinique, Guadeloupe, Guyane and Ile de la Réunion) due to the particular epidemiological context (increased exposure to mosquito-transmitted virus such as Dengue or Chikungunya), and in the Alsace region in a pilot setting. Results from the Alsace experience, published in part in 2011, include a reduced incidence of allergic reactions in relation...
with the use of additive solution (vs plasma), no cases of transfusion-mediated bacterial contamination and an increased number of platelet transfusion per patient.

A workshop on pathogen attenuation in platelets was organised by the EFS on 23rd of May 2011 on the use of pathogen-reduced platelets to prevent transfusion-transmitted infections and to provide an informed assessment of the risk/benefits and costs/benefits ratio of such an approach. Different European approaches were analysed through the experiences of three countries. The medical director of Sanquin (Netherlands) stressed that the haemostatic quality of platelets administered to the patient is a health safety parameter, and he intends to pursue clinical investigations before decisions are made as to the possible implementation pathogen-reduced platelets. With this in mind, a study to evaluate the haemostatic efficacy of an alternative inactivation procedure (Mirasol technology, riboflavin + UV) has been initiated in the Netherlands (PREPAReS study mentioned previously). A similar point of view was expressed by the medical director of the Frankfurt Red Cross, who initiated a study in cardiac surgery comparing the haemostatic efficacy, as evaluated by biological parameters, of Intercept® platelets versus non-treated platelets. Finally, the director of the Lausanne transfusion Centre described the conditions of the recent introduction (2010) of Intercept® platelets in Switzerland following the death of a young patient from a bacterial infection transmitted by a platelet transfusion. In conclusion, it was recalled that pathogen inactivation technology per se is associated with a high potential for health safety improvement and that its eventual use appears probable. However as of now, the benefit/risk ratio associated with the implementation of the presently available inactivation technologies requires further studies with clinically-relevant endpoints.

The Effipap trial

In this context, EFS has decided to promote a randomized clinical trial whose objective is to verify the non-inferiority of Intercept-treated platelets in terms of bleeding frequency compared with untreated platelets in plasma or untreated platelets in additive solution.

The trial comprises 3 treatments arms:
1) An arm qualified as historical control drawing on the platelets produced in plasma. These platelets are currently still produced in France, albeit in small amounts.

2) An arm qualified as the current control arm, referring to platelets prepared in additive solution, currently the majority of platelets produced in France.

3) An arm qualified as the experimental arm: platelets treated by pathogen inactivation process (Amotosalen + UVA: Intercept®). The inactivation process requires the use of platelets in additive solution and not platelets in plasma.

The use of 2 control arms is justified by the persisting limited availability of clinically-relevant data pertaining to the use platelets in additive solution in comparison to platelets in plasma. A reduced CCI has been reported with the use of platelets in additive solution\(^2^9\). Therefore a comparison restricted to Intercept-treated platelets (inevitably in additive solution) with non-treated platelets in additive solution, as in the Eurosprite\(^1^4\) and Tessy\(^1^7\) studies, will not reveal any additional effects of the additive solution might have per se on clinically relevant endpoints.

The non-inferiority reference criteria will be of 12.5%, whereas the clinical endpoint is set with a high frequency of expected events (haemorrhages grade ≥2: about 60% and grade 3 and 4 of approximately 5% to 7%) in accordance with the findings of the PLADO study\(^3^0\).

**PATIENT SELECTION**

Patient selection is a determining factor for the relevance of this study. We will therefore have broad inclusion criteria consistent with everyday practice, particularly including patients who have coagulation disorders (disseminated intravascular coagulation) or anti-HLA antibodies. As these patients are routinely excluded from clinical trials with platelets, the data in the literature for such patients is therefore very limited. They are also large consumers of labile blood products, with a mean of 30 blood products during the period of chemotherapy induction (acute myelogenous


acute leukaemia). The methods of inclusion will be simplified as much as possible, as will the clinical and biological follow-up, so as to be applied easily without major modifications in the management of patients usually followed for acute leukaemia, autologous or allogeneic transplantation (early phase only).

In France, the large majority of patients meeting the selection criteria of the study will moreover be included in national clinical trials on first-line management of leukaemia. All these patients included in first-line clinical trials will necessarily be transfused with platelets, and in addition will benefit from the clinical and biological evaluation of the other trials conducted independently. In addition, evaluated blood products in the EFFIPAP trial are authorized products available in France. Conversely, excluding all patients included in another clinical trial would remove any interest in a trial that would then be limited to a marginal patient population.

As the primary objective is clinical, particular attention will be given to the quality of its assessment through a daily clinical evaluation independent from the clinician in charge of the patient. There is an unresolved difficulty in the evaluation of haemorrhagic symptoms in thrombopaenia due to the particularities of the scale, which is the international standard at this time (WHO scale attached Schedule B). Several studies question the systems of evaluation including adjudication done by investigators outside the clinical site, a method used by Heddle. Each method has its advantages and disadvantages; We therefore established simplified collection scales based on the physical examination, the patient interview and the patient file (scales in Schedule B and C).

A reduction in the frequency of adverse events related to allergic transfusion in the experimental arm in comparison to platelets in plasma is expected. On the other hand, we will be unable to demonstrate the benefits of the experimental arm with regard to reduction in the number of infectious bacterial episodes due to the very low frequency of these incidents. This factor therefore cannot be a study endpoint.

The demonstration of non-inferiority of the Intercept® platelets compared with platelets stored in plasma or in additive solution with regard to haemostatic efficacy of transfused platelets will be a strong argument with regard to a more generalized use of Intercept® platelets. Moreover, the safety data will be important for defining possible restrictions on this type of platelet product. Finally, the data regarding transfusion needs will also inform as to potential adaptation of blood collection and blood product preparation policies.

Blood donation is valuable, and this study must not lead to excessive loss of platelet products. Being authorised and available products, the protocol PC not used in protocol patients will be transfused to other patients through the normal distribution system.

**STUDY OBJECTIVES**

*Primary objective and primary endpoint*

- **Primary objective:**

  The objective of this study is to evaluate non-inferiority with regard to prevention and control of haemorrhage:
  
  - of platelet concentrates in additive solution (Intersol®) and treated by pathogen inactivation (Intercept®: amotosalen + UVA)

  Compared with:
  
  - untreated platelet concentrates in plasma
  - untreated platelet concentrates in additive solution (Intersol®)

  These three type of blood products are available and authorised by ANSM (formerly AFSSAPS).

- **Primary endpoint:**

  The primary endpoint is the proportion of patients with one or more grade 2 or higher (WHO) haemorrhagic event.

  This endpoint will be associated with the prospective validation of a new bleeding scale called “EFS scale”, which is a scale based on the level of therapeutic intervention.

*Secondary objectives and secondary endpoints*

- To evaluate the transfusion needs, transfusion outcomes and safety.
The secondary objectives/endpoints are:

1. Incidence of haemorrhagic episodes (grade 1 and higher)
2. Number of grade 3-4 haemorrhagic episodes
3. Number of minor grade 1 haemorrhagic episodes
4. Transfusion outcome in platelets (CCI) at 24 hours
5. Number of transfusions of platelet concentrates and red blood cells
6. Transfusion intervals
7. Safety (transfusion side effects) grade 2 or higher
8. Presence of anti-platelet antibodies (Anti-HLA)
9. Occurrence of refractory reactions to platelet transfusions
10. Validation of a new haemorrhagic evaluation: EFS scale
11. Evaluation of hematocrit and haemoglobin levels between arms (Amendment added before start of inclusions)
12. Number of bleeding days of WHO grade equal to or greater than 2 (Amendment Sept. 2014)

Patients that will also be transfused with packed red blood cells, and will be tested for red blood cell alloimmunization.

The evaluation of platelet function at 1 hour (by thromboelastogram) and recovery (CCI at 1 hour) after transfusion will only be performed in a limited number of clinical centers according to the availability of the techniques and at the discretion each clinical investigator. Thromboelastogram analysis requires fresh platelets. An ancillary study involving a maximum of a 100 patients, will provide a functional database, which is currently unavailable, in a homogenous patient population.

**RESEARCH DESIGN**

*Type of study*

- Clinical trial (Therapeutic)
- Multicentre
- Randomised
- Phase IV
- With individual direct benefit

*Blinding: product labelling, patient, bleeding assessor, treating MD, local investigator and data-management are blinded; personnel receiving/dispensing platelet product is not blinded*

*General organisation of the study and diagram*

Experimental diagram
**Practical conduct of the study**

This study will begin in May 2013 and last for 2 years.

- **Screening visit:**
  - Inclusion / exclusion criteria verified.
  - Information to patient and acknowledgement of consent.
  - Recording of patient history and prior treatments
  - Recording of concurrent treatments at the time of inclusion.
  - General physical examination
  - “Standard” laboratory tests: complete blood count, platelets, electrolytes, CRP, serum protein electrophoresis, ALT/AST, alkaline phosphatase, bilirubin, PT, PTT, fibrinogen, research for DIC, research for anti-platelet antibodies (anti HLA-HPA)
  - Blood group (or available group card)
  - Antibody screening (Anti-red blood cell antibodies)

- **Daily monitoring**
  - Recording of concurrent treatments.
• Physical examination with research for haemorrhagic syndrome, questionnaire (Annex C)
• CBC
• Recording of clinical factors of transfusion safety
• Traceability of transfusions (transfusion file)
• Standard hemovigilance follow-up; platelet function before transfusion and 1 hour after (thromboelastogram), according to availability of each centre (optional in this study).

• Exit visit
  • Recording of concurrent treatments.
  • General physical examination
  • Standard lab tests (see above) + anti-platelets (anti-HLA, anti-HPA)
  • RBC antibody screening
  • Recording of transfusion safety clinical factors
  • Traceability of transfusions (transfusion file)
  • Standard haemovigilance follow-up
  • Additional but optional recommended research for anti-HLA antibodies approximately 21 days after last transfusion (or during the next hospitalisation)

Laboratory tests will be carried out in the laboratories of each clinical centre; they will not differ from the standard tests. Research for anti-HLA antibodies will be done according to the applicable national EFS protocol involving Luminex screening, followed by identification in case of positive result. Screening for other anti-leukocyte / platelet alloimmunization will be performed either immediately, if the laboratory technical platform of EFS centre is adapted, or secondarily after transport to an EFS laboratory with technical resources.

Investigation procedures conducted and differences compared with standard management

No additional clinical visit or examination is planned with regard to the standard follow-up of patients treated in haematology for long duration aplasia as part of the care for haemopathies or haematopoietic stem cell transplantations.
The study of platelet functions, which is based on routine exams but requires specific equipment, is optional and depends on the ability of each centre, as its financing is independent of the main protocol.

However, for questions of reliability and reproducibility, evaluation of the haemorrhagic syndrome will be done in each clinical centre by the local medical personnel, specifically trained and independently of the team in charge of the patient and financed by the study organisation. Bleeding evaluation will be carried out blinded to the treatment arms by using both a physical examination and a patient history, in addition to the collection of information from the nurse’s notes and medical file.

Haemorrhagic syndrome will be evaluated according to the scale attached Schedule C, and then a WHO score will be attributed, as previously published. Bleeding will also be evaluated according to a new clinical scale called “EFS scale”, which is based on the level of transfusion therapeutic intervention. There will therefore be a prospective and comparative evaluation of these scores. The different scores are listed in Schedule B.

**Chronological synoptic table of visits and tests**

<table>
<thead>
<tr>
<th>STUDY PERIOD</th>
<th>INCLUSION</th>
<th>TRIAL PERIOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinic visits</td>
<td>Inclusion over 15 days</td>
<td>Daily</td>
</tr>
<tr>
<td>Inclusion /exclusion criteria</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Signed consent</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Patient history</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Concurrent treatments</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Physical examination</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CBC</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Haemorrhagic score</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Safety</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>RBC antibodies screen</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>“Standard” laboratory tests other than CBC</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
Note: Anti-HLA research will be systematically proposed at the patient’s next visit (around 21 days after the last platelet transfusion of the study), but it is optional.

**PATIENT CHARACTERISTICS**

*Methods of patient recruitment*

*Inclusion criteria*

- Patients aged 18 years or older
- Patient hospitalised for bone marrow aplasia, with expected stay of over 10 days and in principle requiring platelet transfusion support (at least twice). In practice this includes:
  - acute leukaemia, at diagnosis or at induction or at consolidation (if the consolidation meets the criteria of aplasia of long duration with hospitalisation)
  - other haematological malignancy with central thrombopaenia, whether or not related to treatment, expected to last over 10 days and expected thrombopaenia less than 10 g/l (at least two times).
  - autologous haemopoietic stem cell transplantation, not related to myeloma
  - allogeneic haemopoietic stem cell transplantation in the first 30 days after the start of conditioning

Note: a patient who is hospitalised and might meet the inclusion criteria (and is in need of at least 2 additional transfusions) can be included in the protocol, even if he/she was already received a platelet transfusion.

- Signed informed consent

- Patients with DIC can be included; they will undergo a separate analysis.

- A negative pregnancy test is necessary before inclusion in all women of childbearing age
• Possible participation in biomedical research involving a medicinal product or a diagnostic strategy or treatment.

Note: Patients already (or likely to be) included in a first-line therapeutic protocol may be included, with knowledge that the objective of the study does not concern a medicinal product as such, but health products (labile blood products), which will necessarily be administered to patients. These first-line protocols are usually academic and/or relevant to national groups and involve the target population of the study.

Exclusion criteria

• Signed consent absent
• Patient included in this trial previously during a prior aplasia episode.
• Patient requiring curative anticoagulant treatment at the time of inclusion (vitamin K antagonists, heparin (LMWH and NFH), anti-IIa and Xa at curative doses for treatment or prophylaxis of arterial or venous thromboembolic disease (TED) or as part of the treatment for cardiac valvulopathy and complications of atrial fibrillation).

Note: An anticoagulant treatment with prophylactic doses is allowed (prevention of thrombosis or management of DICD for example). The introduction of anticoagulant treatment at curative doses after inclusion does not imply exclusion from the study (Amendment Feb. 2014)

• Thrombocytopenia due to increased platelet destruction
• Pregnancy
• Patient requiring washed platelet concentrates (i.e., with residual plasma less than that remaining during the addition of an additive solution) due to previous intolerance to platelets (cf IgA deficiency, history of major allergic reaction)
• Patient requiring CMV negative blood products
• Patients with platelet transfusion refractoriness, related or not to an anti-HLA alloimmunization (thus, patient already known as requiring HLA-compatible platelets)

Note: this exclusion criterion is the known platelet transfusion refractoriness. The presence of anti-HLA antibody is not « per se » a criterion of not inclusion. A negative anti-HLA screening test is not required for the inclusion in the study.

• “Protected” adults and persons deprived of liberty
VARIABLES MEASURED AND METHODS OF MEASUREMENT

Clinical parameters

Evaluation of bleeding will be mostly clinical. Paraclinical explorations will be performed with regards to the patient’s condition and according to the usual management of the patients at each centre.

Recording the evaluation will be done by the medical personnel according to the scale attached in Schedule C and published by Slichter et al. These personnel will be trained according to the specification of the study.

This evaluation is retrospectively done daily over the last 24 hours. It includes a physical examination and a systems history, recording of concurrent treatments and paraclinical parameters from the patient’s medical file and the history as well as interview of the medical doctor managing the patient.

On each day of the study, the summary of the haemorrhagic syndrome will be classified according to the WHO scale between grade 0 and grade 4. A rating using the EFS scale will be done as well.

An internal group of experts in the study will meet to review the scaling of haemorrhagic syndromes and to implement a simplified adjudication system inspired from the model that had been recently published and discussed by Heddle.

Laboratory parameters

Laboratory parameters measured and methods used

- “Standard” laboratory tests: complete blood count, platelets, electrolytes, CRP, serum protein electrophoresis, ALT/AST, alkaline phosphatase, bilirubin
EFFIPAP Protocol, translated from french

- **Immunologic testing**: Anti-HLA antibodies
- **Serologic testing**: HIV 1+2, HCV, hepatitis B, HTLV I+II, CMV
- The study of platelet function is considered optional since it is only available in certain centres. It requires a laboratory study consent, which is available in Annex A.

Labelling

- The tubes sent directly to the laboratories of the participating centres will be labelled with the usual labels.

*Data collected directly in the case report form*

All of the above-described data will be directly recorded in the study case report form. Data will also be collected for products platelet transfusions (amount of platelets, age of the concentrated preparations, RBC groups)

The data collection will be done through the use of an electronic case report form, which will enable quality control of the data in real time (control at the data entry plus immediate control by a clinical research associate with requests of rapid correction). This electronic case report form is managed by the Société Clininfo in Lyon.

The electronic case report form also enables precise monitoring of the inclusions as well as end of treatment. The feasibility of this type of approach has been demonstrated by the success of the Optimev study in which close to 400 vascular physicians participated and included (simple epidemiological study) over 8000 patients in 12 months, with an erroneous data rate of less than 3%.

The data concerning the administration of labile blood products as required legally (MEMORANDUM DGS/DHOS/AFSSAPS No. 03/ 582 dated 15 December 2003) will be taken from the transfusion file, including haemovigilance data.
TREATMENTS COMPARED

Treatment

The is a non-inferiority study comparing 3 types of homologous platelet concentrate preparations authorised in France (equally originating from APC or PPC, according to availability of the stocks):

- Arm A platelet concentrates in additive solution (Intersol®):
- Arm B platelet concentrates in additive solution and treated with the Intercept® system (amotosalen and UVA treatment)
- Arm C platelet concentrates in plasma

In terms of organisation and homogeneity of the study within each arm, all the platelet products of Arms A and C will be "T-cells - inactivated" (irradiated products except for those treated by Intercept®). As a reminder, the amotosalen + UV technology inactivates residual leucocytes and thus prevents the risk of post-transfusion GVHD. For the packed red blood cells, they will be irradiated according to the requirements defined by each centre and by the national recommendations.

The platelets will be prepared according to standard practices and procedures of the Etablissement Français du Sang, with all the study products authorized for routine use (Journal Officiel dated 28 November 2010). Each EFS blood product production platform will provide the platelets of the 3 study arms.

There is no distinction made between the type of product (APC or PPC) considered equivalent with regard to efficacy and safety (review by Heddle et al25).

The delivery of the platelets by the EFS will be name specific as per standard procedures. Evaluators will be blinded as to the treatment arm.

Transfusion rules:
Platelet quantity

The quantity of platelets per transfusion for both APC and PPC will be according to ANSM recommendations: 0.5 to 0.7 $10^{11}$ for 7 kg of weight, i.e., 0.5 $10^{11}$ for 7 to 10 kg of weight.

A
variation of $0.5 \times 10^{11}$ will be deemed acceptable relative to the prescription in order to adapt to the product availability.

Different doses can be used in patients who are refractory to platelet transfusions (two ineffective transfusions according to the definition from ANSM (formerly AFSSAPS) or possibly in patients with known anti-HLA antibodies. If a patient becomes refractory to platelet transfusions in the presence of anti-HLA antibodies and subsequently requires HLA-matched transfusions, the patient will be withdrawn from the study.

Platelet transfusion criteria:
The main criteria is the platelet number (prophylactic transfusion)
Platelets ≤10 G/l
The threshold may be increased (20 G/l or more according to the clinical situation) if fever of 39°C, anticoagulant treatment, presence of DIC or additional coagulation disorder or following treatment of a severe haemorrhagic episode secondary to active haemorrhagic lesion or postoperatively (cf below).

Therapeutic (or pre-therapeutic) transfusions will also be possible and will be explicitly documented in the CRF:
Invasive procedure or surgery and surgical consequences
Haemorrhagic syndrome

**Transfusions of packed red blood cells:**
A haemoglobin level >80g/l will be ensured. The transfusion support will follow standard haematology practices.

**Rules of product delivery**
Platelet delivery will be carried out in a standard fashion with the following restriction: due to the blinding, the label on the bag will not mention the characteristics of the product, but only the name of the protocol and the identity of the recipient.
According to the state of the available platelet stock, the distribution priorities are:
Priority 1: treatment arm
Priority 2: ABO compatibility

**Compatibility**

ABO compatibility will be respected. However in emergency situations, it transfusion of ABO incompatible platelet products will be allowed (a serum/plasma sampling will be taken before transfusion for subsequent anti-HLA/anti-HPA research).

**Technical data on the tested blood products**

*Platelet concentrates in additive solution:*

These are platelets usually produced and distributed by the EFS in France. See Schedule E

*Platelet concentrates treated by pathogen inactivation technology combining Amotosalen and UVA (Intercept®):*

These are platelets usually produced and delivered on a routine basis by the EFS in Alsace, Reunion, Martinique and Guadeloupe. See Schedule E

*Platelet concentrates in plasma*

These are platelets that were routinely produced and delivered by the EFS until 2010 (but which are still in use in a limited number of locations).
The platelets are stored in the donor plasma without further transformation. See Schedule E

**Transfusion**

Transfusions will be performed per current rules in France (MEMORANDUM DGS/DHOS/AFSSAPS No. 03/ 582 dated 15 December 2003), applicable in each institution.
Traceability

Traceability will be ensured through retention of the transfusion file (MEMORANDUM DGS/DHOS/AFSSAPS No. 03/ 582 dated 15 December 2003) and per current rules in France. This traceability will not be disrupted by the fact that the study is conducted with blinding conditions.

Randomization method

The randomization list will be drawn up by Clininfo (Lyon) before the study begins, and kept by the same company and the EFS distribution sites participating in the study throughout the length of the study until the database is frozen.

The randomisation will be centralised with stratification per centre with random sized blocks.

It will be done by the physician investigator on the e-CRF. After verification of the inclusion and exclusion criteria and receipt of the written consent, the investigator will randomise the patient directly on the e-CRF. A randomisation number corresponding to the treatment will then be assigned.

Once the randomisation number is allocated, it will be considered as assigned to an included patient, even if the patient leaves the trial prematurely before having received it.

Product labeling

As all products are "T-cells - inactivated" (irradiation products except for those treated by Intercept), a label mentioning Effipap protocol will be printed at this time without reference as to the type of blood product

Traceability will be ensured through retention of the transfusion file (MEMORANDUM DGS/DHOS/AFSSAPS No. 03/ 582 dated 15 December 2003). Only the mention of “EFFIPAP protocol” will replace the types. The label will contain all the usual information:

- Platelet type: APC or PPC
**EFFIPAP Protocol, translated from French**

- Expired (date) at (time)
- Transfuse immediately upon reception in the care unit
- Platelet contents = xxx $10^{11}$
- Blood group
- CMV negative if requested
- Irradiated (all LBP will be "T-cells - inactivated" (irradiated products except for those treated by Intercept) in the EFFIPAP study,)
- Donation barcode
- Do not expose to cold
- Volume = XXX ml
- EFFIPAP PROTOCOL
- EFS XXX site XX

**Product traceability**

The prescription sheet of the labile blood products usually used by the hospital departments will be collected by the referring physician. This sheet contains all the useful information for the preparation of treatments:

- Prescriber identity (name, department, date, unit, telephone number)
- Patient identity
- Urgency: critical, immediately critical, relative
- Transfusion scheduled: date, time
- Number of packed red blood cells: homologous, autologous
- Phenotype, matched, CMV negative, washed, irradiated (even if not checked, all treatments will be "T-cells - inactivated" (irradiation products except for those treated by Intercept)
- Patient weight
- Last blood count
- Number of fresh, frozen plasma: homologous, autologous
- Indication: coagulopathy, acute haemorrhage and overall deficiency of coagulation factors, rare deficiency in coagulation factor
- Date last research for RBC antibody screening. Negative/positive result

Once the prepared products conform to a randomisation result, a courier will come to collect the product. The courier will come to the department of the investigator with the delivery form and the bag.

The delivery form contains all the usual information for a transfusion.
EFFIPAP Protocol, translated from french

Contact information for the department where the patient is
Name of the prescriber
Prescription no.

Information about the patient:
Surname
First name
Date of birth
Sex
EFS patient identification no. with barcodes (matches the bag)

Immunohaematological data – transfusion instructions
Blood group, today’s date
Phenotype
RH
Last anti red blood cell screening: date results given,
Transfusion instructions: remarks
Transfusion protocols: remarks
Distribution remarks
Barcodes: Product, Number / batch, group, phenotype, Qualification

The mention of “EFFIPAP protocol” will replace the type.

The person in the investigator’s department who receives the bags must sign the delivery form and will place it in the patient’s transfusion file.

Received by XXX
Date XXX
Time XXX
Check at reception in conformity YES/NO

The following statements must appear:

Last check in the presence of the patient is obligatory before the transfusion:
1 last check of concordance of the documents for all LBPs
2 last check of compatibility for RBCs, including autologous
Start the transfusion within 6 hours following reception.
The person in the department of the investigator who receives the platelet product in the department should provide information in the following fields of the traceability sheet:

- **Product:** Attach the removable sticker on the bag
- **Transfusion:** done by (name, quality)
- **Document concordance check:** yes/no
- **Last bedside check compatible:** yes/no
- **Transfusion:** date and time
- **Remarks and patient identity if changed**
- **Transfusion incident:** record it and notify the haemovigilance contact person or EFS

The yellow copy must be placed in the patient’s transfusion file for traceability.
The pink copy is intended for haemovigilance.

**Procedure for unblinding**

Unblinding will only be done when knowledge of the treatment allotted to the patient is necessary for his/her medical care.

Operating procedure: the investigator who needs to know the group his/her patient was randomised to may contact the referring physician from EFS who prepared the treatment.

During the call, the reference person must record on a document:

- The contact information of the doctor, whether or not investigator, or pharmacist who called (surname, first name, qualification of the requester, telephone number).
- The name of the study.
- The date of the call, the description of the clinical event that was the reason for the call: type of event, start date, methods of management.
- Whether the event is classified as a serious adverse effect (cf definitions).
- The follow-up to this call: unblinding, course of action to take to investigate the effect and make a diagnosis, treatment of the effect.
- Is a specific treatment or support planned after the unblinding?

1) If not, there is no indication for unblinding. If the person insists, before unblinding, call:

Prof. Frédéric Garban +33 (0)4 76 76 49 90
If no one can be reached, proceed with unblinding.

2) If yes, proceed with unblinding.

The randomisation code is available from the reference lists distributed to the EFS for preparing the bags.

This information will be sent within a maximum of 24 hours during the week or as soon as the resumption of work following a day off or a weekend at the Clinical Investigation Center by fax at +33 (0)4 76 76 52 42

In all cases, the sponsor must be informed of the unblinding.

**Data collection and management**

**Data collection**

All the data from the patient interview and physical examination collected in a file dedicated to the protocol and available for consultation by the sponsor will consist of the source data. This file will be electronic.

Through his/her participation agreement, the investigator promises to respect the experimental protocol, the Good Clinical Practices and the applicable laws. He/she guarantees the authenticity of the collected data within the framework of the study and accepts the legal provisions authorising the sponsor of the study to set up a quality control.

The statistical analysis will only be done after verification of the data entry and the data consistency. The data will be archived by the CIC of Grenoble.

**Data monitoring**

Data monitoring will be carried out on all collected data by the CIC (Centre of Clinical Investigations i.e. academic clinical research organization) at the university hospital of Grenoble. The data monitoring will be based on 100% of the inclusion/exclusion criteria, consents and SAE. A random selection of 20% of the files will be made for monitoring 100% of the clinical data. A monitoring report will be written by the CRA and kept in the study file.
Statistical analysis of the measured parameters

Calculation of the number of patients

Assumption:
We based our calculations of the number of patients necessary on a non-inferiority threshold of 12.5% for an expected grade 2 and over haemorrhage incidence that is estimated at 60%, which conforms to studies published in Blood (ref 13) and New England (ref 9). This hypothesis of 60% is actually slightly lower compared to these studies (around 70%), but it is justified by a noticeably different recruitment in our trial and by the probable progress in the quality of the overall care proposed to these types of patients in clinical haematology units.
This non-inferiority threshold should be read keeping in mind the fact that the primary clinical endpoint is set with a high frequency of expected events (grade ≥2 haemorrhage): about 60% but with a decreased frequency of serious haemorrhagic events (grade 3 and 4 haemorrhage of about 5% to 7%), as confirmed in a very recent study on patients with long-term aplasia. Otherwise this non-inferiority threshold is very close to that of 11% as chosen in the Italian IPTAS study mentioned previously.
We used an alpha risk of 0.05, a power of 80%, and we adapted our threshold according to the Bonferroni correction ($\alpha_{\text{Bonferroni}} = \alpha / k$, with $k=$ number of arms).
The calculation was done with N Query software, version 7.

A total of 270 patients are expected to be included per arm, i.e. 810 patients for the entire population.
There was no consideration given to replacement methods or follow-up of persons in case of premature stopping of treatment, as the transfusion protocol is rarely refused by the patient.
Following a recruitment analysis, it was decided to include an additional 30 patients to replace the incorrectly included patients who never received the study treatment (amended Nov. 2015)
Data analysis strategy

The primary endpoint will be analysed as on the per protocol patient population. As this is a 3-arm efficacy trial, which is a priori equivalent, adaptation of the alpha risk by Bonferroni method will be applied for the 2 by 2 comparisons between the 3 therapeutic modalities of the study.

The risk of type I error (possibly after adaptation for multiple tests) is the usual threshold usual alpha = 0.05. Evaluation of the incidence density of haemorrhagic syndrome will be presented with a 95% confidence interval according to the tables of binomial law. The baseline patient characteristics will be shown in a comparative table according to the treatment group.

The description of data will use the usual parameters (percentage, median and interquartile).

The usual supplementary criteria for therapeutic trials (relative risk and 95% CI, relative risk reduction and 95% CI, number of patients required to avoid an event) will also be presented.

There is no intermediate analysis planned between the groups in this protocol.

Data analysis site and software used

Management of the data, the statistical analysis and the archiving of the database (after database freeze procedure) will be done under the supervision of the statistician Prof. Jean Luc Bosson and Ms. Sophie Thoret and Ms. Audrey Guyard, biostatistical engineers of CIC in Grenoble. The statistical analysis will be done with STATA software, version 12 on OS X (StataCorp LP 4905 Lakeway Drive College Station, Texas 77845 USA). Equiv'Test software (Statcon, Germany) will be used for the non-inferiority or equivalence analysis.

Analysis site:
CHU de Grenoble, CIC – BP 217 - 38043 Grenoble Cedex 09 France
Tel.: +33 (0)4 76 76 50 40    Fax: +33 (0)4 76 76 52 42

A detailed STATISTICAL ANALYSIS PLAN is described in

Safety evaluation

As these are LBPs, double monitoring of side effects will be done: first, with respect for the internal haemovigilance circuit of all care institutions, and second, as imposed by clinical trials regulations. And the vigilance is assured specifically in this study by the national vigilance service of the
Etablissement Français du Sang, which was previously centralized in the name of the sponsor by CIC of Grenoble (contact person Ms. Carole Rolland).

**Definition of an adverse event**

- **Definition of the adverse event:**
  Any harmful medical manifestation occurring in a person who participates in biomedical research, whether or not this manifestation is related to the research or the product on which the research is based.
  
  An adverse effect may be any unfavourable or unexpected sign (including abnormal laboratory results), any symptom or temporary disorder associated with the use of the product, with or without relation to the product.

- **Definition of the serious adverse event:**
  A serious adverse event is an adverse event with one of the following consequences:
  - death
  - life-threatening condition
  - hospitalisation or extension of hospitalisation
  - disability or incapacity that is clinically significant and temporary or permanent
  - congenital anomaly or malformation.

- **Definition of an unexpected adverse event:**
  Any adverse effect of the product in which the nature, severity or course does not correspond to the information mentioned in the summary of product characteristics when it is authorised, and in the investigator's brochure when it is not authorized.

- **Expected adverse events:**
  - Due to long-term aplasia relative to serious malignant haemopathy:
    - death
    - septic shock
    - respiratory failure related to haemorrhage, infection, tumour progression, metabolic cause
- any documented infection (due to immune deficiency secondary to neutropaenia)
- any haemorrhagic syndrome (due to bone marrow failure)
- kidney failure and disturbances in the hepatic work-up related to the concurrent treatments required for the haematologic disease and associated complications
- heart failure from fluid overload syndrome
- anaemia (due to bone marrow failure)

- Related to the blood transfusion:
  - fever - chills
  - blood pressure changes
  - allergic reactions
  - TRALI
  - infectious events (they cannot be easily distinguished from those directly related to aplasia)
  - the general side effects described in the annual haemovigilance report

- Related to the haematological disease:
  - Death
  - Relapse of any kind

**Monitoring committee**

A safety committee comprised of 3 persons independent of the trial will be organised and will meet together during the study to check the safety related to the study treatment.

Review will be performed, every 200 inclusions, by the independent safety committee on the overall mortality of each arm and bleeding grade of class 3 or 4. The imputability will be the responsibility of this committee and also of the haemovigilance/pharmacovigilance of the study; The committee acting as an additional warning against haemovigilance/pharmacovigilance of the study.

We propose the concept of threshold to bring the committee to consider the suspension of the study by defining the threshold as a 10% rate of death from all causes.
All bleeding events severe grade 3 or 4 shall be transmitted immediately to the haemovigilance / pharmacovigilance in accordance with the regulations

This committee may advise a possible stop to the study. The practical modalities of monitoring will be decided by this committee.

Modalities of their detection and their documentation

All serious adverse effects will be reported, respectively, by the investigator of each centre to the sponsor and by the sponsor to the competent authority (ANSM).

The unexpected serious adverse events will also be declared to the Institutional Review Board.

Persons to notify in the event of occurrence

- Sponsor: EFS by the intermediary of CIC from CHU of Grenoble:
  Tel.: +33 (0)4 76 76 50 40 / Fax.: +33 (0)4 76 76 52 42
  Ms. Carole Rolland (CarRolland@chu-grenoble.fr)
- Biovigilance cells as part of UMAGRIS.

Projected calendar of the study

- Duration of the study per patient: the first of the following criteria met:
  - 30 days or hospital discharge
  - death
  - spontaneous platelet count >50 g/l
  - side effects related to the grade 3 or higher platelet transfusion, involving the use of washed /additive solution platelets
- Total duration of the study: 3 years (amended April 2015)
- Projected date for the start of inclusions: 01 May 2013
- Projected date for the end of inclusions: 01 December 2015 (amended April 2015)
- Projected date for the end of the study: 01 May 2016 (amended April 2015)
Premature stopping of the study

Stopping the study for participating patients

For the patient’s benefit, the investigators can have him/her leave the study in progress. A study exit evaluation will be carried out, which notes the reason for the premature study exit:

- withdrawal of consent
- death
- serious adverse event
- protocol violation
- lost to follow-up.

After premature exit from the study, the patient will receive medical follow-up under the responsibility of the investigators.

Stopping of the study by the sponsor

The sponsor can stop the study at any time for the following reasons:

- inability of the investigator to include the patients according to the projected calendar
- absence of signed consent
- major protocol violations
- incomplete or erroneous data concerning the injected product.
- notification by the independent safety committee of the study end

An intermediate evaluation of death (safety evaluation) from haemorrhage out of the first 250 patients will be carried out by the monitoring committee, so as to be aware of excess haemorrhagic mortality specific to one arm; the trial will be stopped if there is more than 10% haemorrhage death as the main cause of death in one arm.
**Stopping of the study by the investigator**

When an adverse event is deemed severe by the investigator and can threaten the health of patients, the investigator may stop the study in accordance with the sponsor.

**Material and legal aspects**

**Feasibility of the study**

The EFFIPAP trial proposed project is part of the national blood transfusion operator strategy to offer more dependable products in terms of safety, and with identical clinical efficacy. The targeted patient population is extensive, so as to reflect real-life conditions and to represent most of the platelet transfusion situations in haematology (other than occasional support).

**Perspectives and expected consequences**

- **Direct individual benefit for patients in the study.**
  Patients included in this study will benefit from standard medical follow-up. They may have reduced risk of blood transmitted pathogens.

- **Improving transfusion practices**

- **Longer term perspectives**
  1- This French multicentric, randomized study with a clinical primary endpoint can be considered as a counterpart of other European studies in Netherlands and Italy
  2- This study could provide strong results in terms of public health to develop or not at a large scale the procedures of platelet pathogen reduction

**Written informed consent**
In accordance with Good Clinical Practices and the legal provisions in effect, all preselected patients will be informed beforehand by the investigator of the study objectives, its methodology, its duration, its foreseeable risks and constraints and projected medical management modalities at the end of research, including if the study is stopped before its term. It will notably be specified to the patient that he/she is completely free to refuse to participate in the study or to withdraw his/her consent at any time, without incurring any liability or suffering any harm from doing so. A document summarising the information provided by the investigator will be given to him/her (Annex A).

After being assured of the proper understanding of the information provided, the investigator will request the patient’s written consent for participating in the study. If he/she accepts, the patient will sign the consent form (Annex A) before the study begins.

**Professional secret, confidentiality**

The investigator is held to respect professional secrecy. The collected data, including the results of the analyses, will be made anonymous by all appropriate means. The sponsor and his/her representatives are subjected to the same obligations of professional secrecy as the investigator.

The present document are returned to the investigator on a confidential basis and should only be returned or communicated to persons specifically involved in the trial, with the agreement of or at the request of the coordinator.

**Insurance contract**

Contract no.: 128559

Société Hospitalière d’Assurances Mutuelles.

18 rue Edouard Rochet, 69372 Lyon cedex France

**Funding of the study**

This study is funded by the Etablissement Français du Sang.
Institutional review board for biomedical research

C.P.P Sud Est V de Grenoble, Rez-de-Chaussée Haut, C.H.U. de Grenoble,
38043 Grenoble Cedex 09 France

Amendments to the protocol

The possible extension of the trial will be subject to an amendment submitted for opinion to the IRB and the competent authority.

Anonymity of patients participating in the study

The forms in the case report forms will only bear the patient’s initials (first letter of the surname and first letter of the first name) and an anonymous number. Only this number will be computerised. The computerised sheet used for data entry and the treatment of data will be subjected to a declaration of the French National Commission for Information Technology and civil Liberties (reference methodology MR001).

In accordance with the “Information and Liberties” law, patients will be informed that the data will not be treated name specific and computerised and that they may have access to the data concerning them either from the investigator or from the intermediary of their doctor of choice. (law dated 6 January 1978 modified relative to the computer, to files and to liberties).

Quality Assurance Department

The clinical part of the study will be done in accordance with Good Clinical Practices.

Authorisation of the research site
According to Article L1121-13 of the French Public Health Code, no authorisation of site is required for this study; it does not require other procedures besides those usually practised in the departments where the study takes place. Furthermore, persons participating in the study, as well as the donors, do not present with distinct clinical conditions that are outside the field of expertise of the department.

**Publications**

All the data collected during this study are the property of the study sponsor and cannot be communicated in any case to a third party without the written agreement of the investigator. All publications will mention the Etablissement Français du Sang. For the main publication of the study, the principle investigator will be the first author.

Any publication or communication (oral or written) will be decided from a common agreement between the investigators and will respect the international recommendations: "Uniforms Requirements for Manuscripts Submitted to Biomedical Journals" ([http://www.cma.ca/publications/mwc/uniform.htm](http://www.cma.ca/publications/mwc/uniform.htm)): notably, an authorship will be proposed to each clinical centre participating in the study and to each member of the steering committee according to his/her actual participation, and to the CIC of Grenoble for its collaboration by bringing its methodological expertise and taking charge of the statistical operations.

**Archiving**

All of the files from the study will be archived for 15 years under the responsibility of the sponsor.

The source documents, case report forms, the originals of the consent forms, the signed protocol must be stored by the investigator for a minimum of 15 years from the end of the study.

In the name of the study sponsor, the coordinator organises the storage in appropriate premises of the following documents:

- Protocol and schedules, amendments.
- Case report forms (originals) with annex documents.
- Follow-up document of the clinical study.¹
- All the administrative documents and letters related to the study.
- Study report.
DATE and SIGNATURES

This protocol was read and approved. Date:

29/06/2012

Coordinating investigator
Professor Frédéric Garban
EFS Rhône-Alpes

For the sponsor
Professor Pierre Tiberghien
EFS headquarters

Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HLA</td>
<td>Anti-Human Leukocyte Antigen</td>
</tr>
<tr>
<td>Anti-HPA</td>
<td>Anti-Human Platelet Alloantigen</td>
</tr>
<tr>
<td>CCI</td>
<td>Correct Count Increment</td>
</tr>
<tr>
<td>CIC</td>
<td>Clinical Investigation Centre</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated Intravascular Coagulation</td>
</tr>
<tr>
<td>APC</td>
<td>Aphaeresis platelet concentrates</td>
</tr>
<tr>
<td>EFS</td>
<td>Établissement Français du Sang (French Blood Service)</td>
</tr>
<tr>
<td>PPC</td>
<td>Pooled random whole blood donor platelets concentrates</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>LBP</td>
<td>Labile Blood Products</td>
</tr>
<tr>
<td>PPR</td>
<td>Percent platelet recovery</td>
</tr>
<tr>
<td>TRALI</td>
<td>Transfusion Related Acute Lung Injury</td>
</tr>
</tbody>
</table>
Appendix A: information and consent forms

PATIENT INFORMATION FORM

Document prepared pursuant to the Public Health Code

Coordinating investigator:
Prof. Frédéric Garban
Investigator: Dr. ………………………….

Address:
Centre d’Investigation Clinique de
Grenoble, CHU de Grenoble BP 217
38043 Grenoble Cedex 09 France
Tel. +33 (0)4 76 76 50 40
Fax +33 (0)4 76 76 52 42
Sponsor: Établissement Français du Sang
(French Blood Service)

EVALUATION OF THE EFFICACY OF PLATELETS TREATED WITH PATHOGEN INACTIVATION PROCEDURE

Dear Sir, Madam,

You are presently hospitalised for the care of a haematological disease. Its treatment requires or will require blood transfusions, particularly platelet (blood component) transfusions to reduce the risk of bleeding. This risk is related to the decrease in your production of platelets.

There are several platelet products in France. They are authorised and useable under normal and usual conditions of blood transfusion. We are inviting you to participate in a study to show the equivalency between different platelet products. It is called a statistical non-inferiority study. It aims to compare two types of authorized platelet concentrates to another authorized product that has undergone a pathogen inactivation treatment (Intercept procedure).
The pathogen inactivation treatment is a procedure that inhibits the proliferation of:
- viruses and bacteria
- residual cells of the donor in general.

There is no need to know the specific potential infectious agent. This treatment consists of:
- the injection of a photosensitive product
- the action of ultraviolet rays (invisible light rays).

This is done during the preparation of the platelet product. This procedure renders all the nucleic acids unusable, including the nucleic acids (DNA) of microorganisms. It is a well-known treatment and authorised in France. It has been systematically used in the Alsace region for several years. All the biological characteristics of the platelets treated as such are known and unmodified. They do not have nuclei. But it is known that during the preparation procedure, a small quantity of platelets is lost. Overall, this last finding is not significant:
- the products are transfused based on the number of platelets present in the bag after treatment
- the dose of transfused platelets is adjusted to the weight of each patient.

The question raised by this study is very important. It aims to show in a large number of patients that the pathogen inactivation treatment in the platelets does not alter their properties of protecting patients from bleeding. This will enable the EFS (Etablissement Français du Sang) to propose the necessary data to the health authorities with regard to the potential widespread use of platelet pathogen inactivation procedures in France. This has already been the case for plasma. There is no such study that is independent of any industrial partners in this domain. Before thinking about the widespread use of the procedure, a large-scale study should confirm the data:
- in the literature
- in the Alsace region.

This study concerns the largest possible number of patients. It is intended to reflect the normal prescription conditions in haematology. It does not expect to have data other than that from the usual care of haematology patients. It does not change anything in the management of:
- your haematological disease
- or your treatment.

The rules for prescribing blood products are not changed compared to the usual reference frameworks used in haematology.
**Conduct of the study**

**Step 1: selection**

The physician confirms the inclusion and exclusion criteria before you can agree to participate.

**Step 2: inclusion**

If you are eligible, your doctor asks you to give your written consent for this study. If you give your informed consent, the procedure is conducted as such:

The Clinical Investigation Centre of CHU in Grenoble is managing the study. It will randomly assign you:

- a number
- one treatment arm out of 3 study arms.

The information is sent to EFS. It will deliver the products. They all identically qualified, being:

1. platelet concentrates in plasma
2. platelet concentrates in additive solution
3. platelet concentrates with pathogen inactivation treatment

The study is done under double-blind conditions: neither your haematologist nor you yourself will know what type of platelets you will have. The EFS will make up a large stock of treated products. There will be no shortage in the availability of the transfusion, even in an emergency, for all patients in the study. In all cases, you will have a blood product authorised by the health authorities. You will receive it or can receive it independent from this study.

**Step 3: transfusion itself and follow-up**

If your health status requires one or several platelet transfusions, you will have the platelets corresponding to your treatment arm. They will be delivered:

- according to the rules of blood group compatibility applicable to platelet products
- and in a quantity calculated according to the usual recommendations based on your weight.

The entire transfusion procedure will be as that done in the haematology department where you are. It will be done in accordance with the rules concerning transfusion.

Bleeding and its management will be monitored daily. A member of the medical team from the hospital where you are will come to see you every day to:

- examine you
- and question you about bleeding and events that will have occurred since your first transfusion.

He/she is independent from the doctors of the care unity where you are. The data collection from your medical file, and thus the laboratory data, will be taken and recorded anonymously in a central file, which is declared to the CNIL (French national commission for data protection). In accordance with the legal provisions relative to computers, to files and to liberties, you have the right to access and to rectification. You also have the right to object to the transmission of data covered by professional secrecy, which is likely to be used as part of this research and to be treated. You can also access, either directly or through the intermediary of a doctor of your choice, all of your medical data through application of the provisions of Article L 1111-7 of the Public Health Code. These rights are exercised through a doctor who follows you as part of the research and who knows your identity.

You are free to accept or to refuse to participate in this research, and you can withdraw your consent at any time, without incurring any liability or suffering any harm from doing so.

The duration of follow-up in this study is 30 days maximum after the first platelet transfusion done as part of this trial. But if the length of your hospital stay is shorter, the duration of follow-up will be that of your hospitalisation.

**Participation in another research study:**
You may participate simultaneously in another biomedical study on your disease or a medicinal product.

**Additional biological study – depending on the availability of the centre:**
An optional study is proposed using the blood samples on the platelet functions before and one hour after transfusion. This would involve one 7 ml tube of blood before and after transfusion. The study of platelet functions will not be available in all the centres and for all patients. It will depend on the technical conditions and particular organisation of each centre. But it is planned systematically. It will be offered to you individually if the conditions of organisation in your centre are compatible.

You will incur no additional costs due to your participation in this study. It is part of the usual management of your disease. You will not receive a compensation.

To participate in this research, you should be affiliated with a social security regime.

The Sud-Est V Institutional Review Board gave a favourable opinion on XX/XX/XXXX.
The French National Agency for Medicines and Health Products Safety gave its authorisation on XX/XX/XXXX, for performing this research. The Établissement Français du Sang took all the provisions laid down by law on the protection of persons (SHAM insurance contract no.128559; 18, rue Edouard Rochet, 69372 Lyon Cedex 08, France).

CONSENT FOR PARTICIPATION

EVALUATION OF THE EFFICACY OF PLATELETS TREATED WITH PATHOGEN INACTIVATION PROCEDURE

Sponsor: Établissement Français du Sang (French Blood Service)
Investigator responsible for the study: Prof. Frédéric Garban (Établissement Français du Sang)

Contact information: Centre d’Investigation Clinique de Grenoble, CHU de Grenoble BP 217
38043 Grenoble Cedex 09 France
Tel. +33 (0)4 76 76 50 40 Fax +33 (0)4 76 76 52 42

I ACCEPT TO PARTICIPATE IN THIS RESEARCH UNDER THE CONDITIONS SPECIFIED ABOVE.

If I desire, I have the right to refuse to participate in this research or to withdraw my consent at any time, without incurring any liability or suffering any harm from doing so. I will then inform Dr……………………………… about it. The data concerning me will remain strictly confidential. I only authorise them to be seen by persons subjected to professional secrecy, who are collaborating in this research. I may ask the physician investigators for any additional information at any time.

I accept that the data recorded at the time of this study may be subjected, after being made anonymous, to computerized treatment by the sponsor or on his/her behalf. I have noted that my right for access as foreseen by the computer and liberty law is exercised at any time.

I have received a detailed information sheet. I have received a copy of the present document; I have been informed that one copy will also be kept by the organisers under conditions that guarantee confidentiality, without incurring any liability or suffering any harm from doing so. The doctor who invited me in the study assured me that I could participate in the proposed research despite my participation in another. I have been informed that, in accordance with the rules on biomedical research, the institutional Review Board of Grenoble provided a favourable opinion on XX/XX/XXXX and the French National Agency for Medicines and Health Products Safety gave its authorisation on XX/XX/XXXX for the performance of this research.

☐ I give my consent for the study of platelet functions*
☐ I do not give my consent for the study of platelet functions*

*Cross out the unnecessary statement

Patient name: ____________________________
Date: ____________________________
Signature of the patient

Name of the investigator: ____________________________
Signature of the investigator
Appendix B: bleeding evaluation scales and scores

Echelle de l’OMS modified according to Slichter et al

<table>
<thead>
<tr>
<th></th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral and nasal</td>
<td>&gt; Oropharyngeal bleeding – total duration of all episodes in previous 24 hours ≤ 30 minutes*&lt;br&gt; &gt; Petechiae of oral mucosa&lt;br&gt; &gt; Epistaxis – total duration of all episodes in previous 24 hours ≤ 30 minutes*</td>
<td>&gt; Oropharyngeal bleeding – total duration of all episodes in previous 24 hours &gt; 30 minutes*&lt;br&gt; &gt; Epistaxis – total duration of all episodes in previous 24 hours &gt; 30 minutes*</td>
<td>♦ Any bleeding requiring RBC transfusion over routine transfusion needs**</td>
</tr>
<tr>
<td>Skin, soft tissue,</td>
<td>&gt; Petechiae of skin&lt;br&gt; &gt; Purpura ≤ 1 inch diameter&lt;br&gt; &gt; One or more spontaneous hematomas in the soft tissue or muscle &gt; 1 inch</td>
<td>&gt; Purpura &gt; 1 inch diameter&lt;br&gt; &gt; Spontaneous hematoma in deeper tissues&lt;br&gt; &gt; Joint bleeding (confirmed by aspiration, imaging study or other accepted technique)</td>
<td>♦ Any bleeding requiring RBC transfusion over routine transfusion needs**</td>
</tr>
<tr>
<td>musculoskeletal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>&gt; Positive stool occult blood test</td>
<td>&gt; Melanotic stool&lt;br&gt; &gt; Hematochezia – visible red blood mixed in stool, not requiring a transfusion&lt;br&gt; &gt; Hematemesis – Grossly visible blood in emesis or in nasogastric drainage tube (not related or secondary to swallowed blood)</td>
<td>♦ Any bleeding requiring RBC transfusion over routine transfusion needs**</td>
</tr>
</tbody>
</table>
| Genitourinary | • Any biochemical or microscopic Hb/RBCs without red urine  
  • Abnormal vaginal bleeding (Unexpected bleeding out of normal cycle OR Bleeding heavier than normal OR Breakthrough bleeding (patient on hormonal therapy to prevent bleeding)) with spotting | • Gross/visible hematuria without need for transfusion  
  • Abnormal vaginal bleeding (Unexpected bleeding out of normal cycle OR Bleeding heavier than normal OR Breakthrough bleeding (patient on hormonal therapy to prevent bleeding)) more than spotting | • Any bleeding requiring RBC transfusion over routine transfusion needs** |
| Pulmonary | • Hemoptysis – Visible blood  
  • Blood in broncho-pulmonary lavage, or blood tinged sputum (excluding those with nose or oropharyngeal bleeding) | • Any bleeding requiring RBC transfusion over routine transfusion needs** |
| Body Cavity | • Visible blood in body cavity fluid (e.g. red cells apparent in fluid aspirate) short of criteria for Grade 3 or 4 | • Grossly bloody body cavity fluids and organ dysfunction with symptoms, and/or need to intervene (e.g. to aspirate), and/or need for transfusion |
| Central Nervous System | • Retinal bleeding without visual impairment  
  • Lumbar puncture with blood (>5 RBC/μL in CSF on microscopic analysis and non-traumatic tap), no symptoms and no visible red color | • Lumbar puncture with visible red color in absence of symptoms, and non-traumatic tap |
<table>
<thead>
<tr>
<th>Invasive Sites</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; Bleeding at invasive sites (venipuncture sites, intravenous lines or catheter exit sites): active oozing at site for a cumulative total of &gt; 1 hour in the previous 24 hours</td>
</tr>
<tr>
<td></td>
<td>◦ Any bleeding requiring RBC transfusion over routine transfusion needs**</td>
</tr>
<tr>
<td>Hemodynamic Instability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; Any bleeding associated with moderate hemodynamic instability (hypotension; &gt;30mmHg fall or &gt;30% decrease in either systolic or diastolic blood pressure) and requiring RBC transfusion over routine transfusion needs**</td>
</tr>
</tbody>
</table>

* Count actual bleeding (i.e. “running out” or need for basin, Kleenex, towel, etc) not minor bleeding
** Red cell transfusion must be specifically related to treatment of bleeding within 24 hours of onset of bleeding

GRADE 4:

◦ Any bleeding associated with severe hemodynamic instability (hypotension; >50mm/Hg fall or >50% decrease in either systolic or diastolic blood pressure, with associated tachycardia (heart rate increase of ≥ 20% for 20 minutes) and requiring RBC transfusion over routine transfusion needs

◦ Fatal bleeding from any source

◦ Retinal bleeding with visual impairment (Visual impairment is defined as a field deficit, and patients with suspected visual impairment require an ophthalmologic consult for documentation)

◦ CNS symptoms with non-traumatic bloody lumbar puncture

◦ CNS bleeding on imaging study with or without dysfunction
**EFS scale (interventional scale)**

Level 0 : No bleeding

Level 1 : Minor skin or mucous membrane bleeding that does not require platelet transfusion

Level 2 : Skin or mucous membrane bleeding that requires a therapeutic platelet transfusion

Level 3 : Persistent or recurrent bleeding that requires one or several therapeutic platelet transfusions per day on at least two consecutive days

Level 4 : Bleeding with blood loss that requires transfusions of packed red blood cells or a surgical intervention or similar (endoscopic procedure) with haemostatic objective.

Level 5 : Bleeding involving death of the patient as the main cause of death
Appendix C: Bleeding evaluation

The evaluation will be done with a questionnaire administered to the patient, a physical examination and the collection of data from the medical file on a daily basis.

<table>
<thead>
<tr>
<th>Evaluation of new bleeding (in last 24 hours) on</th>
<th><strong>/</strong>/<strong>/</strong>/<strong>/</strong>/__</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d d / m m / y y y y</td>
</tr>
</tbody>
</table>

**ENT**

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
</table>

If yes, specify

<table>
<thead>
<tr>
<th>Oropharyngeal bleeding &lt; 30 accumulated minutes</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngeal bleeding &gt; 30 accumulated minutes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Petechiae</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Duration &gt; 30 accumulated minutes/24 hours</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Haemostatic packing (coalgan)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Surgical packing (compression packing)</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Skin tissues**

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
</table>

If yes, specify

| Petechiae on the skin                          | No | Yes |
| Purpura < 2.5 cm?                              | No | Yes |
| Purpura > 2.5 cm?                              | No | Yes |
| Spontaneous haematoma (muscle or superficial tissue) > 2.5? | No | Yes |
| Spontaneous haematoma (deep tissues)           | No | Yes |
| Haemarthrosis                                  | No | Yes |

**Tube**

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
</table>

If yes, specify

| Melena (without transfusion)                    | No | Yes |
| Rectal haemorrhage (without transfusion)        | No | Yes |
| Haematemesis (excluding swallowed epistaxis)    | No | Yes |

**Genitals**

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
</table>

If yes, specify

| Microscopic haematuria or equivalent            | No | Yes |
| Macroscopic haematuria                         | No | Yes |
| Abnormal gynaecological bleeding by spotting   | No | Yes |
| (menses abnormally abundant, other gynaecological bleeding) | No | Yes |
| Abnormal gynaecological bleeding besides spotting | No | Yes |
| (menses abnormally abundant, other gynaecological bleeding) | No | Yes |

**Respiratory system**

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
</table>

If yes, specify

| Haemoptysis                                    | No | Yes |
| Alveolar haemorrhage at BAL                    | No | Yes |
### EFFIPAP Protocol, translated from french

<table>
<thead>
<tr>
<th>Cavities</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, specify</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloody effusion of serous membrane</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Bloody effusion of serous membrane with consequence</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>(requiring drainage or evacuation or even requiring transfusion support)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemorrhage of the mucous membranes documented on endoscopic exam</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nervous system</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, specify</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemorrhage to ocular fundus without visual impact</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Retinal haemorrhage with visual impact (confirmed by ophthalmologist)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-traumatic LP with more than 5 RBC/µl</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-traumatic haemorrhagic LP</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cerebral haemorrhage or meningitis documented by imagery (without LP)</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes, specify</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-traumatic bleeding ( &gt; accumulated 1 hour/24 hrs)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Bleeding associated with moderate haemodynamic instability (hypotension or decrease of 30 mmHg or more of 30% of the diastolic or systolic BP and requiring a transfusion)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Bleeding associated with haemodynamic instability (hypotension or decrease of 50 mmHg or more of 50% of the diastolic or systolic BP with tachycardia and requiring a transfusion)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Other severe haemorrhage documented by imaging exam</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Other haemorrhage listed in patient interview (and for which there is objective proof or clinical observation)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Other severe haemorrhage (documented by puncture of a haemorrhagic fluid without other known cause)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Death by haemorrhage</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

## Appendix D: Definition and management of platelet refractory state

Any suspicion of platelet refractory state should prompt verification of the transfusion outcome between 15 minutes and one hour after the first transfusion.

Then a second transfusion of ABO compatible and fresh (<3 days) platelets with test between 15 minutes and one hour.
Calculation of the CCI (corrected count increment) or the percent platelet recovery (PPR)

- Percent platelet recovery (PPR) or the platelet transfusion outcome

\[
PPR = \left(\text{Platelet count after transfusion} - \text{Platelet count before transfusion}\right) \times \text{weight (kg)} \times 0.075
\]

Number of platelets transfused (\(x \ 10^{11}\))

Corrected Count Increment (CCI)

\[
CCI = \left(\text{Platelet count after transfusion} - \text{Platelet count before transfusion}\right) \times \text{body surface (m²)} \times 100
\]

Number of platelets transfused (\(x \ 10^{11}\))

* This number should appear on the APC and PRDP labels

Platelet count expressed as G.L.\(^{-1}\)

Refractory state = PPR < 0.2 or CCI < 7

In case of platelet over consumption: the possibility of multiple daily therapeutic transfusions may be considered, without exceeding \(1 \ 10^{11} / 7\text{kg platelets in 3 times /day.}\)

In case of a refractory state: anti-HPA/HLA antibody screening will be recommended as well as, if warranted, HLA-compatible platelets

**Appendix E: summary of platelet product characteristics**

Aphaeresis platelet concentrates and mixtures of standard platelet concentrates provided in this protocol meet the specificities described in the “Decision dated 20 October 2010 establishing the list and the characteristics of labile blood products”, which was published in the Journal Officiel of the French Republic dated 28 November 2010.
Definition

- Pooled random donor platelets

The standard homologous platelet concentrates is a platelet suspension obtained aseptically from a unit of homologous adult whole blood. It presents in plasma medium as an iridescent liquid without visible signs of haemolysis.

The mixture of standard homologous platelet concentrates, coming from different donors (6 maximum) and with the same ABO blood group, is a platelet suspension obtained aseptically from several units of homologous adult whole blood. It is prepared in the same authorized, closed, sterile and nonpyrogenic container. With mild and continuous agitation, the mixture presents in plasma medium as an iridescent liquid without visible signs of haemolysis.

- Homologous aphaeresis platelet concentrates

Platelet suspension obtained aseptically in a donor who was deemed medically capable. It is collected in an authorized, closed, sterile and nonpyrogenic container. With mild and continuous agitation, the mixture presents in plasma medium as an iridescent liquid without visible signs of haemolysis.

Characteristics

A- Pooled random donor platelets (PPC)

- Volume between 80 and 600 ml taking into account the anticoagulant and additive solution.
- Contents in platelets ≥ 1.10^{11}
- corrected pH, at end of storage, at + 22 °C ≥ at 6.4
- the platelets may be stored in plasma or with additive solution of a supplementary storage solution. In this case, the proportion of residual plasma will be 20 to 40%. The solution used will be Intersol ®, which has an authorisation from the ANSM (formerly AFSSAPS) for the pathogen inactivation procedure through the Intercept® procedure.

These mixtures may also benefit the following secondary transformations:

- Irradiation (PPC D irradiated) without modification of the characteristics described above
- Treatment for attenuation of pathogenic agents and inactivation of T lymphocytes by Amotosalen (PPC-IA), with the following characteristics:
  - Contents in platelets between 2.2 and 6.10^{11}
  - Concentration in residual Amotosalen ≤ 2 µM
  - Additional storage solution required for treatment
B- Aphaeresis platelet concentrates (APC)

- Maximum volume: 600 ml, without taking into account the volume of the anticoagulant and storage solution.
- Minimal contents in platelets: 2.10^11
- corrected pH to +22°C at end of storage ≥ 6.4

The APC can be stored in the same solutions as the PPC and can benefit from the same transformations.

The storage time of the APC and PPC is 5 days after slow and continuous agitation at a temperature between + 20°C and + 24°C.

The APC and PPC may or may not benefit from CMV negative qualification.

Identification:
PPPC and APC of this protocol will be identified by an identical label without mention of the name and transformation in order to preserve the blinding.
Traceability will be guaranteed by the product code and the sample number or mixture number.

Appendix F: Detailed Final Statistical Analysis Plan

Data analysis strategy
Analysis of the main outcome is performed on the per-protocol patient population as recommended for non-inferiority testing. A second test will be performed on the intention to treat population.
For the secondary endpoints, the analysis is conducted with intention to treat and is complemented by a per-protocol analysis.
Statistical analyzes are carried out using Stata 13.1 software.

Population
Initially, a per protocol statistical analysis is planned for the main outcome, and an intention-to-treat analysis for secondary endpoints. Patients in the per protocol population are those who have received at least one transfusion of the product corresponding to their randomization arm. The intention-to-treat population is patients who would have received at least one transfusion, but not necessarily corresponding to their randomization arm. Patients who received no transfusion during the follow-up period will not be included in the analysis.

Comment: In fact no patients with uniquely transfusions that were off-protocol were recorded.
However, 56 patients received at least one transfusion that did not correspond to their randomization arm. Nevertheless, it was foreseen in the protocol that in the event of a therapeutic emergency or product unavailability, a patient could be transfused with a product that did not correspond to their randomization arm. This was the case for these 56 patients. Thus, they were included in the per protocol population, which is in fact the same as the intention-to-treat population.

Population description: characteristics

A table of patient characteristics by randomization arm will be presented. The characteristics described will be:

- Sex, age, BMI, pregnancy, follow-up duration, reason for hospitalization, thrombocyte count at inclusion;
- Results of clinical examination at inclusion: respiratory and cardiac parameters, any tumoral syndrome, fever, documented infection, splenomegaly, hepatomegaly, previous transfusion of platelets or plasma, red blood cell count.

Quantitative variables will be described according to the data distribution:

- Normally distributed data: mean, standard deviation
- Not-normally distributed data: median, 25th percentile and 75th percentile.

Qualitative variables will be expressed in numbers and percentages.

The amount of missing data will be indicated.

Main Outcome

This is a non-inferiority trial with 3 transfusion arms:

- Platelet concentrates in additive solution: reference treatment (used in routine practice in France)
- Platelet concentrates in plasma: historical treatment (was previously used routinely in France and is still used routinely in many countries including the US)
- Platelet concentrates in additive solution treated by pathogen reduction: Intercept® amotosalen / UVA

These arms are randomly and blindly assigned and recorded as 1, 2 or 3.

The primary endpoint is the incidence of grade 2 or higher hemorrhagic episodes according to WHO criteria. This is the maximum daily hemorrhagic grade occurring in a patient during study follow-up. This grade is allocated daily according to predefined rules and is based on the presence of a hemorrhagic syndrome, the nature of the hemorrhagic problem (as defined by the WHO) and confirmed for the entire period by an expert adjudication committee. It is this adjudicated grade that will be used for the main outcome. If this is missing (in the case of no access to the hospitalization report), the daily maximum grade, among grades allocated daily after imputation, is used. This composite grade serves to fulfill the main outcome. It will be called the PDC grade.
The incidence rate of hemorrhagic syndrome in terms of the PDC grade will be presented with a 95% confidence interval according to the tables of binomial law.

Non-inferiority will be checked in the per protocol population by comparing the arms two by two, keeping the blinding as long as possible. Thus 6 non-inferiority tests will be performed from the PDC grade.

The “Two-one-sided test” will be used. Given the multiplicity of tests (new treatment versus one, and versus the other control) a Bonferroni correction will be applied with threshold ($\alpha$) of 2.5%. Using this 2.5% threshold, the confidence interval for the difference between the two arms will be given at 95%, using the Wald method for “two-one-sided test”.

The margin for non-inferiority is fixed at 12.5% based on the literature and clinical considerations.

Thus, an incidence of hemorrhagic episodes of grade 2 or higher in one arm that is lower than in the other will show non-inferiority if the upper limit of the confidence interval of the difference is less than 12.5%.

On lifting the blinding, the relative risk of hemorrhagic episodes of grade 2 or higher (and confidence interval) in the experimental arm vs reference arm, and vs the historic arm, can be given.

**Additional analyzes**

Non-inferiority analysis will be also carried out using the daily maximum hemorrhagic grade, with automatic scoring as defined by the WHO for a per protocol population.

*Comment: This analysis is not included in the present article.*

**Missing Data Management Strategy**
If the daily hemorrhagic grade is missing for the last day, it will be considered that the patient has been followed until the day before actual discharge or transfer. The date of end of follow-up will be modified.

If a patient is recorded as ending the study due to a spontaneous platelet count > 50 G/L for at least 3 consecutive days (following transfusions), and the patient has missing data for the last 3 days before discharge (last day in the study), the daily hemorrhagic grade during this time will be recorded as 0, meaning absence of hemorrhagic syndrome.

A single missing value will be replaced by the average of the two values which surround it rounded to the lower integer. If an isolated missing value is the first one of the follow-up period, the next value will be used to fill the missing value. If an isolated missing value is the last of the follow-up period, the previous value will be used to fill the missing value.

Several sequences of missing data on the daily hemorrhagic grade may occur for a patient. Each sequence of missing data will be replaced if and only if the total number of days missing does not exceed 15% of the total length of the patient’s follow-up (15% rounded up to nearest integer). The following strategies will be used to replace missing data:

• A sequence is missing at the beginning of the follow-up period: next observation carried backwards assigns the next known score after the missing sequence to the missing one.
• A sequence is missing at the end of the follow-up period: last observation carried forward assigns the last known score before the missing sequence to the missing one.
• Sequence of one or several items of missing data with non-missing data before and after the sequence: “Last” and “Next” assigns the average of the person's last known and next known observation to the missing value. The score is rounded down to the nearest whole number if needed. (e.g. mean (1+2) =1)


If a patient has missing data greater than 15% of the total, for the daily hemorrhagic grade no replacement will be made (e.g. If the total stay is 30 days, the amount of missing data accepted is 4 days). The patient will be excluded from analyses needing the daily hemorrhagic grade; particularly for the secondary endpoint n°. 12 (number of days with grade 2 or higher hemorrhagic episodes).
Secondary objectives

Secondary objective n° 1: Density of incidence of hemorrhagic episodes (grade 1 and higher)
The density of incidence of hemorrhagic episodes grade 1 and higher will be calculated from the number of hemorrhagic episodes in the intention-to-treat population (those who received at least one transfusion during follow-up and who had at least one hemorrhagic syndrome classed as Grade 1 or higher, based on PDC grade) relative to the duration of follow-up. This incidence will be presented for the whole population and by randomization arm. Depending on the data distribution, significant difference between the three arms will be tested by an ANOVA or a Kruskal-Wallis test. If there is a significant difference, a comparison of the arms 2 by 2 is planned in a post-hoc analysis, using either a Scheffe test or a Wilcoxon rank test.
On lifting the blinding, if there is a significant difference between the three arms, the relative risk of hemorrhagic episodes in the experimental arm vs. reference arm, and vs. historical arm will be given.
This analysis could be repeated for the daily hemorrhagic data.
Comment: This analysis is not included in the present article

Secondary objective n° 2: Frequency of severe hemorrhagic episodes (grade 3 or 4)
The frequency of grade 3 or 4 bleeding will be calculated using the PDC grade. The result will be presented for the whole population, then for each arm, with its 95% confidence interval. A Chi-squared test will be performed to compare the occurrence of Grade 3 or 4 episodes per patient in the different arms. If there is a significant difference, a difference will be tested by applying the Chi-squared test 2 by 2 between the arms.
This analysis could be repeated on the daily hemorrhagic data.

Secondary objective n° 3 Frequency of minor hemorrhagic episodes (grade 1)
The frequency of grade 1 bleeding will be calculated using the PDC grade. The result will be presented for the whole population, then for each arm, with its 95% confidence interval. A Chi-squared test will be performed to compare the occurrence of grade 1 episodes in the different arms. As for objective 2, if there is a significant difference, a difference will be tested by applying the Chi-squared test 2 by 2 between the arms.
This analysis could be reproduced on the daily hemorrhagic data.
Comment: This analysis is not included in the present article
**Secondary objective n° 4** Transfusion outcome as the platelet corrected count increment (CCI) at 24 hours

The CCI will be calculated for each transfusion using the difference between the platelet count on the day of transfusion and the platelet count on the day after transfusion, divided by platelet dose transfused \(\times 10^{11}\) and adjusted on body surface area.

Body surface area is calculated according to Dubois’s method: 
\[
BSA = (W^{0.425} \times H^{0.725}) \times 0.007184
\]

If a patient receives several transfusions on the same day, then no CCI will be calculated on this day, because laboratory analyses are made once a day (not necessarily in the short time before the second transfusion). No CCI can be calculated for transfusions given on the last day of follow-up because no laboratory analysis is performed after the follow-up period.

As well as CCI per transfusion, a mean CCI will be calculated for each patient for their whole follow-up period.

*Patients receiving more transfusions are often those with the poorest transfusion outcome.* Thus, a difference in CCI between the 3 arms will be tested, for the mean and for the first transfusion, by an ANOVA or a Kruskal-Wallis test according to the data structure. If there is a significant difference, arms will be compared 2 by 2, either using a Scheffe test or a Wilcoxon rank test, in post-hoc analysis.

**Secondary objective n° 5** Number of transfusions of platelet concentrates and red blood cells

The number of transfusions of platelet concentrates will be calculated on the basis of data from the “Transfusion case report form” and number of transfusions of red blood cells will be calculated from data in the “RBC and Plasma Transfusion form”. The latter is the total of pouches transfused. Results will be presented for the whole population, then for each arm. A difference in number of transfusions (platelets or RBC) between the 3 arms will be tested with an ANOVA or a Kruskal-Wallis test according to the data structure. If there is a significant difference, the arms will be compared 2 to 2, using either a Scheffe test or a Wilcoxon rank test, in post-hoc analysis.

**Secondary objective n° 6** Platelet transfusion intervals

The interval, in days, between two transfusions will be calculated for each transfusion, except the last one. As every patient did not receive 3 or more transfusions, the analysis will be restricted to the two first transfusions for each patient, in order to keep strong statistical power.

Depending on the distribution of the interval between the two first transfusions, an ANOVA or a Kruskal-Wallis test will be performed. If there is a significant difference, the study arms will be
compared 2 to 2, using either a Scheffe test or a Wilcoxon rank test, in a post-hoc analysis. For easier reading, this interval could be presented as “class”.

**Secondary objective n° 7** Safety (transfusion side effects) grade 2 or higher
Side effects are recorded throughout the study. Different report forms are used to record all medical events, severe adverse events and adverse events linked to the transfusion. The number of transfusion side effects will be based on the events linked to the transfusion according to the clinician. These events were recorded as text, so a classification was needed. This was realized by the principal investigator blinded to the study arm. He received only event texts which were selected by the statistician (also blinded) according to the local clinician’s judgement as whether “related to transfusion”, plus the date, bleeding events, and transfusions delivered. 
The proportion of these events will be calculated as a percentage of transfusions.
The proportion of patients having transfusion side effects will be also calculated as a percentage of patients.
As only small number of events is expected a Fischer Exact test will be performed to determine whether there is a significant difference in both the proportion of transfusions and the proportion of patients having transfusion side effects for each type of effects.

**Secondary objective n° 8** Occurrence of anti-platelet antibodies (Anti HLA, anti HPA)
The presence of anti-platelet antibodies is recorded by the clinician either in the “Adverse events form”, or in the “Exit form”. A variable will be created based on the text in these forms; 1 in case of anti-platelet antibodies, and 0 otherwise.
Thus, the proportion of patients developing anti-platelet antibodies will be presented for the whole population and for each arm, with its 95% confidence interval. A Chi-squared test will be used to compare the occurrence of anti-platelet antibodies in the different arms. If there is a significant difference, the difference in study arms will be tested by applying the Chi-squared test 2 to 2 in a post-hoc analysis.
*Comment: This analysis is not included in the present article*

**Secondary objective n° 9** Occurrence of refractoriness to platelet transfusion
Occurrence of refractoriness is be described by the clinician in the “Transfusion case report form”. Unfortunately the corresponding two variables showed a lot of missing data, without the possibility of replacement. Some clinicians recorded this information in the “Adverse events form”, or in the “Exit form” as a text. A variable could be generated based on this textual
declaration, and completed with data from the “Transfusion case report form”, encoding 1 if there was refractoriness, and 0 otherwise.

As this item is sensitive, another variable will be generated using the CCI at 24h. The literature relates to low CCI for refractoriness. Thus we considered the threshold of 4.5 (as in\textsuperscript{16}) to define treatment failure, corresponding to refractoriness.

Thus, both variables describing refractoriness (from textual declaration and from 24h CCI<4.5) will be presented for the whole population and then for each arm, with its 95% confidence interval. The existence of a difference between arms will be tested by a Chi-squared test. This will be tested 2 by 2 in a post-hoc analysis.

Comment: Only an analysis of CCI<4.5 is included in the present article

Secondary objectives \textsuperscript{10} Validation of a new hemorrhagic scale: the EFS scale

The Etablissement Français de Sang proposes a bleeding scale that is different from the WHO bleeding scale. The 5 items of this scale were completed each day by the clinician or nurse. In order to validate this EFS scale, correlation with the daily grade allocated according to WHO criteria, after imputation, will be tested using a Wilcoxon rank-sum test.

Using the maximum daily EFS grade, the ability of the EFS scale to predict the number of platelet transfusions received by the patient will be tested with an ordinal Logit model.

Comment: This analysis is not included in the present article

Secondary objective n° \textsuperscript{11}: Variations in hematocrit and hemoglobin levels

Depending on the proportion of missing data for the hematocrit, a repeated measures ANOVA will be performed on daily recorded hematocrit values. Variability between the study arms will be tested taking into account variations over time. The interaction between treatment and time will also be tested.

An ANOVA or a Kruskal-Wallis test, depending on data distribution, will be performed to test differences between the study arms. If there is a significant difference, a post-hoc analysis will compare the arms 2 to 2, using either a Scheffe test or a Wilcoxon rank test.

Comment: This analysis is not included in the present article

Secondary objective n° \textsuperscript{12}: Number of days with bleeding of grade 2 or higher

The number of days with hemorrhagic episodes of grade 2 or higher will be calculated from the daily grade allocated according to WHO criteria, after imputation. The result will be presented for the whole population, then for each arm. Depending on the data structure, the difference in the number of hemorrhagic episodes of grade 2 or higher between the 3 arms will be tested by an
ANOVA or a Kruskal-Wallis test. If there is a significant difference, a post-hoc analysis will compare the arms 2 to 2, using either a Scheffe test or a Wilcoxon rank test.