Evaluation of an optimized allogeneic hematopoietic stem cell transplantation protocol with post-transplant cyclophosphamide in patients aged 40 to 60 years old with acquired aplastic anemia refractory or in relapse after immunosuppression

APARR

INTERVENTIONAL RESEARCH PROTOCOL

Project code: APHP230832 / IDRCB n°: 2024-A00262-45

Version N°1.4 dated 24/06/2024

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SIGNATURE page for a research PROTOCOL

Research code number: APHP230832

Title: Evaluation of an optimised allogeneic hematopoietic stem cell transplantation (HSCT) protocol including cyclophosphamide post-transplantation in patients aged 40 to 60 years old with acquired aplastic anemia refractory or in relapse after immunosuppression

Version no.1.4 dated 24/06/2024

The study will be carried out in accordance with the protocol, with current good practices and with statutory and regulatory requirements.

Coordinating Investigator Pr Peffault de Latour Bone marrow transplantation Unit Hôpital Saint-Louis Paris	Date: ……/……/……. Signature: 05 juillet 2024 | 17:08 CEST DocuSigned by: Mr PEFFAULT DE LATOUR Régis EF094FED015C4E8...
Sponsor Assistance Publique – Hôpitaux de Paris Délégation à la Recherche Clinique et à l'Innovation - DRCI (Clinical Research and Innovation Department) Hôpital Saint Louis 1 avenue Claude Vellefaux 75010 PARIS	Date: ……/……/……. Signature: 08 juillet 2024 | 09:41 CEST DocuSigned by: Yannick VALLET 86016AAFDBC044F...

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CHANGE HISTORY

N°	Date of authorization	Changes

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1 SUMMARY

Full title	Evaluation of an optimized allogeneic hematopoietic stem cell transplantation protocol with post-transplant cyclophosphamide in patients aged 40 to 60 years old with acquired aplastic anemia refractory or in relapse after immunosuppression
Acronym/reference	APARR
Coordinating investigator	Régis PEFFAULT DE LATOUR
Sponsor	Assistance Publique – Hôpitaux de Paris
Scientific justification	Hematopoietic Stem Cell Transplantation (HSCT) is a major therapeutic option for patients with acquired aplastic anemia (AA). Improved survival has been achieved in patients younger than 40 years old, thanks to better donor selection, conditioning regimens and graft versus host disease prophylaxis, together with improved supportive care. However, this has never been the case for patients over the age of 40 for whom the overall survival after HSCT never exceeded 60% at 5 years using available sibling or unrelated donor **(Ref#3)**. Thus, the **standard immunosuppressive therapy (IST)** associating cisclosporine, horse anti-thymocyte globuline, together with the addition of eltrombopag (EPAG) is now considered as the standard of care in adult patients older than 40 years with AA **(ref#1**). Indeed, outcomes for patients who are refractory to first-line IST remain poor because of the lack of other non-transplant treatment and because of **the poor results of transplantation at this age (Ref#2, 37)**. During the past 2 decades, there has been a significant decrease in infection-related mortality in this population after initial IST but risk of hemorrhage and long-term fatigue are still present. Moreover, clonal evolution including paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML) still occur in the long-term with a grim prognosis for MDS/AML **(Ref#2)**. The overall survival of such adult patients with acquired AA refractory or in relapse after IST outside HSCT is thus about 60% at 2 years **(ref #2).** Recently, new strategies to prevent acute and chronic graft versus host disease, including T-cell replete grafts with administration of post-transplantation cyclophosphamide (PTCy), have revolutionized the field. These changes extended the option of HSCT primary for patients with refractory/relapse AA who lack an HLA matched donor **(Ref#4)**. More recently the optimized Baltimore protocol using PTCy strategy also appears to improve engraftment and reduce graft versus host disease (GvHD) in other more classical setting (sibling and matched unrelated HSCT outside AA) **(Ref#5)**. One of the first cause of death after HSCT in patients older than 40 years of age with refractory/relapse AA is GvHD and the use of the optimized Baltimore protocol for all type of donors (haplo but also sibling and matched unrelated

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	transplantation) within the field of AA rapidly appears promising **(Ref#6&7)**. Using an optimized HSCT procedure (marrow as source of stem cells and a PTCy strategy) not only in haplo-identical donor setting but also in case of an available matched sibling or unrelated donor might prevent drastically GvHD and eventually be practice changing. Evaluating this new transplant protocol as a new strategy is the main objective of “APARR”.
Main objective and primary endpoint	**Main objective:** To demonstrate a benefit in terms of the 2-year GRFS (Graft Versus Host Disease {GvHD} and primary and secondary graft failure-Free Survival) from 50% (historical rates in patients with refractory/relapse AA undergoing HSCT (REF#8) up to 70% using an optimized HSCT procedure (marrow as source of stem cells and a PTCy strategy) on patients over 40 years old and not only in haplo-identical donor setting. **Primary endpoint:** 2-year GRFS following HCST This primary composite endpoint is measured as the time from HSCT to the first of the following events: primary and secondary graft failures, grade 3-4 acute GVHD, severe chronic GVHD and death.
Secondary objectives and endpoints	**Secondary objectives** To demonstrate a benefit in terms of clinical and biological outcomes: - primary and secondary graft failure - mortality, overall survival - quality of life -treatment related morbidity and notably severe infections, cardiac toxicities - chimerism - immune reconstitution **Secondary endpoints** • 100-day engraftment (3 consecutive days with neutrophiles >0.5 G/L and 7 consecutive days with platelets >20 G/L) with donor chimerism > 85 % on the total blood. • Absolute numbers of neutrophils and platelets at M1, M3, M6, M12, M24 and day of last platelet and red blood cell transfusions • Acute GvHD incidence grade 2-4 at M3 • Chronic GvHD incidence at M24 • Severe chronic GvHD at M24 • Secondary graft failure at M12 and M24 • Severe infections (CTCAE grade 3-4) at M1, M3, M6, M12, M24 • Incidence of cardiac toxicities at M12 • Incidence of CMV and EBV infection at M12 • Mortality at M12, M24 • Overall survival at 12 months and 24 months

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	• Quality of life questionnaire at pre-transplant, M6, M12, M24 • Chimerism at M1, M3, M6, M12, M24 • Immune reconstitution at M1, M3, M6, M12, M24 (T lymphocytes, CD4, CD8, B, NK lymphocytes and gammaglobulines)
Design of the study	A phase II multicenter, national, prospective, single-arm trial.
Population of study participants	Patients aged from 40 to 60 years with refractory/relapse AA after IST eligible to HSCT.
Inclusion criteria	Patients • Aged from 40 to 60 years old • Suffering from acquired refractory severe idiopathic aplastic anemia after at least 6 months treatment with anti-thymocyte globulin and cyclosporine with t Eltrombopag or in relapse • Allograft validated in the National Multidisciplinary expertise meetings of the French reference centre for aplastic anemia • With an available geno-identical donor or 10/10 matched donor or haploidentical donor • With the absence of donor specific antibody detected in the patient with a MFI < 1500 (antibodies to the distinct haplotype between donor and recipient) • Usual criteria for HSCT: ▪ ECOG ≤ 2 ▪ No severe and uncontrolled infection ▪ Cardiac function compatible with high dose of cyclophosphamide ▪ With adequate organ function ASAT and ALAT ≤ 3N, conjugated bilirubin ≤ 2N (or total bilirubin ≤ 2N if not available), clearance creatinine ≥50ml / min • With health insurance coverage • Women of childbearing potential and men must use contraceptive methods during their participation to the research and for 12 months and 6 months after the last dose of cyclophosphamide, respectively. • Having signed a written informed consent NB: The authorized contraceptive methods are: For women of childbearing potential and in absence of permanent sterilization: -oral, intravaginal or transdermal combined hormonal contraception, -oral, injectable or transdermal progestogen-only hormonal contraception, -intrauterine hormonal-releasing system (IUS), -sexual abstinence (need to be evaluated in relation to the duration of clinical trial and the preferred and usual lifestyle of the participants).

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	For men in absence of permanent sterilization: sexual abstinence, condoms. Individuals must meet all of the inclusion criteria as verified at the screening / inclusion visit to be eligible to participate at the study.
Exclusion criteria	**Patients** • With morphologic evidence of clonal evolution (patients with isolated bone marrow cytogenetic abnormalities are also eligible excepted chromosome 7 abnormalities and complex karyotype). • With seropositivity for HIV or HTLV-1-2 or active hepatitis B or C and associated hepatic cytolysis • Cancer in the last 5 years (except basal cell carcinoma of the skin or "in situ" carcinoma of the cervix) • Pregnant (βHCG positive) or breast-feeding • Yellow fever vaccine and all others live virus vaccines within 2 months before transplantation and during the research • With uncontrolled coronary insufficiency, recent myocardial infarction <6-month, current manifestations of heart failure according to NYHA (II or more), ventricular ejection fraction <50% • With renal failure with creatinine clearance <50ml /min • Any contraindication mentioned in the SmPC and the Investigator's brochure of all medicinal products planned to be used in the trial including conditioning regimen, GVHD prophylaxis, prevention of EBV reactivation, infection prophylaxis • Known allergy or intolerance to all medicinal products and/or excipients planned to be used in the trial including conditioning regimen, GVHD prophylaxis, prevention of EBV reactivation, infection prophylaxis, according to Investigator's brochure and SmPC. • Who have any debilitating medical or psychiatric illness, which precludes understanding the inform consent as well as optimal treatment and follow-up • Under legal protection (tutorship or curatorship) • Under state medical aid • Participation to another interventional trial on a medicinal product or cell therapy Individuals meeting any of the exclusion criteria verified at the screening / inclusion visit will be ineligible to participate at the study.
Transplants modalities	1/**<u>Conditioning regimen</u>** Thymoglobulin (0.5/mg/kg à D-9, 2 mg /kg at D-8 and 2.5 mg/kg à D-7), Fludarabine (30mg/m2/day i.v: day -6 to day -2), pre-transplant, Cyclophosphamide (14.5 mg/kg/day i.v: day -6 and day -5), and Total Body Irradiation (2 Gray on day -1).

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	2/**<u>Stem cell source</u>** Bone Marrow only. Target of 4×10^8 nucleated cells/kg recipient body weight. Granulocyte colony stimulating factor is given subcutaneously starting on day +5 at 5 mg/ kg/day until the absolute neutrophil count is greater than 1.5×10^9/L for 3 days. 3/ **<u>GVHD prophylaxis</u>** Cyclophosphamide 50 mg/Kg/day at D+3 and D+4. Tacrolimus (0,2 à 0,3 mg/kg/day *per os* divided into 2 doses or 0.05 to 0.1 mg/kg/d IVSE) and mycophenolate (MMF) will begin from D+5. In absence of GvHD, MMF will be stopped between D35 and D45 and Tacrolimus at day 365. 4/ **<u>Prevention of EBV reactivation</u>** Rituximab 150mg/m2 intravenously at Day+5 post HSCT (except patients and their donor with EBV serology and EBV PCR negative). Each infusion of Rituximab will be preceded by administration of anti-pyretic and an antihistaminic, e.g. paracetamol and diphenhydramine NB: However, it is permissible to delay the administration of rituximab by 24 hours if D+5 occurs on a Sunday for greater safety at the discretion of the investigator
Interventions added for the study	No additional test or specific examinations except the quality-of-life questionnaire. Rituximab is considered as added by the research and provided by the sponsor.
Expected benefits for the participants and for society	Outcomes for adult patients with refractory/relapse AA older than 40 years remain poor. HSCT is the sole therapeutically valid option but results have always been very disappointing and transplantation is currently rarely possible. An optimized HSCT protocol not only in haplo-identical donor setting but also in case of an available matched sibling or unrelated donor might prevent GvHD and eventually drastically improve the outcome of HSCT in patients older than 40 years with refractory/relapse AA. There are direct benefits for patients since no curative and safe options are available so far. In the absence of transplantation, patients aged 40 years or more with refractory/relapse AA after IST are thus exposed to infections risk, hemorrhage, prolonged fatigue but also on the long-term clonal evolution including paroxysmal nocturnal hemoglobinuria (PNH) as well as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), both with a grim prognosis. A global health care benefit will also be possible since those patients won't need any more medical support including hospitalization but also daily hospital care (antibiotics or need for transfusions).
Risks and burdens added by the study	Rituximab injection Risk level of the study: D

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Practical implementation	Indication of HSCT in patients with refractory/relapse AA aged more than 40 years who are refractory or in relapse after first-line IST
Number of participants included	52 patients
Number of centres	28 centers affiliated at SFGM-TC in France
Duration of the study	Inclusion period: 36 months Participation period (BMT + follow-up): 24 months Total duration: 60 months
Number of enrolments expected per site and per month	0,05 patient/centre/month
Statistical analysis	**Sample size calculation** A two-sided, one-sample log-rank test calculated from a sample of 52 subjects achieves 80% power at a 0.05 significance level to detect a 2-years GRFS of 70% in the new group when the 2-years GRFS in the historic control group is 50% if all patients are followed 2 years. **Analysis of primary endpoint** Several interim analyses of the primary outcome measure are planned once every 12 months (unless less than 20 enrolled patients), based on a Bayesian approach. At the end of the trial (accrual+follow-up), GRFS will be estimated using Kaplan Meier method with its corresponding 95% confidence interval and compared to historical rate using one sample two-sided log-rank test. An additional terminal analysis will use external patients from an observational cohort, with propensity score weighting, to ensure an unbiased comparison and enhance the level of evidence of the treatment effect.
Funding sources	PHRC-N 2022
Study will have a Data Safety Monitoring Board	Yes

2 SCIENTIFIC JUSTIFICATION FOR THE STUDY

2.1 Hypothesis for the study

Outcomes for adult patients with aplastic anemia (AA) and aged more than 40 years who are refractory or in relapse after first-line IST remain poor. Hematopoietic stem cell transplantation (HSCT) is the sole therapeutically valid option but results have always been very disappointing and transplantation is rarely possible because of unacceptable toxicity. Using an optimized HSCT protocol (marrow as source of stem cells and a PTCy strategy) not only in haplo-identical donor setting but also in case of an available matched sibling or unrelated donor might prevent GvHD and eventually drastically improve the outcome of HSCT in patients with refractory/relapse AA after IST.

Overall survival is not reflecting anymore the main goal in older patients undergoing HSCT. Considering beyond simple OS, the use of GRFS (**G**raft Versus Host Disease (GvHD) and **R**elapse/rejection-**F**ree **S**urvival) may thus be a more meaningful clinical study endpoint by

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allowing for greater accuracy in assessing patient outcomes and thus benefits from the experimental approach.

The main hypothesis of this study is that, in patients aged 40 years or above, with refractory/relapsed AA, applying a new HSCT strategy based on marrow stem cells, whichever the donor type (haplo-identical, related or unrelated donor), together with a haplo-identical conditioning platform with PTCy, may be beneficial, in terms of the 2-year GRFS - that should increase from 50% (historical controls, ref#8) to 70%. This primary composite endpoint is measured as the time from HSCT to first of four events: primary and secondary graft failures, grade 3-4 acute GVHD, severe chronic GVHD and death.

2.2 Description of knowledge relating to the condition involved

Hematopoietic Stem Cell Transplantation (HSCT) is a major therapeutic option for patients with acquired aplastic anemia (AA). Improved survival has been achieved in patients younger than 40 years old, thanks to better donor selection, conditioning regimens and graft versus host disease prophylaxis, together with improved supportive care. However, this has never been the case for older patients over the age of 40 for whom the overall survival after HSCT never exceeded 60% at 5 years using available sibling or unrelated donor **(Ref#2,3, 37)**.

Thus the standard immunosuppressive therapy (IST) with addition of eltrombopag (EPAG) to cisclosporine and horse anti-thymocyte globuline is now considered as the standard of care in adult patients older than 40 years with acquired aplastic anemia (AA) **(ref#1)**

Recently, new strategies to prevent acute and chronic graft versus host disease, including T-cell replete grafts with administration of post-transplantation cyclophosphamide (PTCy), have revolutionized the field. These changes extended the option of HSCT primary for patients with refractory/relapse AA who lack an HLA matched donor **(Ref#4)**. More recently the optimized Baltimore protocol (using PTCy strategy) also appears to improve engraftment and reduce graft versus host disease (GvHD) in other more classical setting (sibling and matched unrelated HSCT outside AA) **(Ref#5)**. One of the first cause of death after HSCT in patients older than 40 years of age with refractory/relapse AA is GvHD and the use of the optimized Baltimore protocol for all type of donors (haplo but also sibling and matched unrelated transplantation) within the field of AA rapidly appears promising **(Ref#6&7)**.

Indeed, outcomes for patients who are refractory to first-line IST remain poor because of the lack of other non-transplant treatment and **the poor results of transplantation at this age (Ref#2, 37)**. During the past 2 decades, there has been a significant decrease in infection-related mortality in this population after initial IST but risk of hemorrhage and long-term fatigue are still present. Moreover, clonal evolution including paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML) still occur in the long-term with a grim prognosis for MDS/AML **(Ref#2)**. The overall survival of such adult patients with acquired AA refractory or in relapse after IST outside HSCT is thus about 60% at 2 years **(ref #2).**

In summary, in adult patients aged over 40 years with acquired aplastic anemia, <u>the standard therapeutic option is a standard immunosuppressive treatment (IST) combining ciclosporin and horse anti-thymocyte globulin, together with the administration of eltrombopag (EPAG).</u> In case of refractory/relapsed disease, the best treatment (but not a standard) is HSCT from related or unrelated bone-marrow in case the patient is eligible for transplantation. However, transplantation is currently rarely possible because of the unacceptable rate of toxicity related to it after the age of 40.

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Using the optimized HSCT protocol (marrow as source of stem cells together with a haplo-identical conditioning platform including PTCy) not only in haplo-identical donor setting but also in case of an available matched sibling or unrelated donor might prevent drastically GvHD and eventually be practice changing in this particular population of patients. Evaluating this new HSCT strategy for refractory/relapsed AA in this patient age category, is the main objective of "APARR".

2.3 Summary of relevant pre-clinical experiments and clinical trials

The unmet need – an unacceptable toxicity of HSCT in patients with refractory/relapse SAA > 40 years of age since decades

Age is known to be a strong negative predictor of survival in patients undergoing an HSCT for acquired AA, with higher mortality in patients >40 years of age **(Ref#9)**, and this has been constantly confirmed in several large studies **(Ref#10,11,12,13,14)**. A multivariate analysis recently confirmed the lack of improvement in term of overall survival in 2010-2015, as compared with 2001 to 2009 (HR 0.95 95%CI 0.73-1.24; p=.7) **(Ref#15)**. Overall, using the classical HSCT procedure, HSCT over the age of 40 years continues to carry a significant risk of mortality, which has not been reduced in the current era.

This is the reason why current guidelines firmly recommend the use of first-line immunosuppression for patients over the age of 40.

On the other hand, tremendous progress has been done very recently regarding IST for patients with acquired AA. The addition of eltrombopag (EPAG) to standard IST associating cisclosporine, horse anti-thymocyte globuline lead to almost 80% response rate and is now considered as the standard of care in older patients **(ref#1)**. However, 20% of patients remain refractory to immunosuppression and 30 to 40% of patients will relapse after this treatment with very few treatment options today outside supportive care with antibiotics and transfusion support.

> There is thus an urgent need to improve HSCT outcomes in those older patients to be able to propose to them an acceptable treatment option in case of refractoriness or relapse after IST.

Successful haplo-identical transplantation using PTCy in patients with aplastic anemia

A more recent strategy for haploidentical (haplo) related donor SCT (haplo-SCT) have improved drastically outcomes using T-cell replete grafts with administration of post-transplantation cyclophosphamide (PTCy, which targets alloreactive T cells generated early after an HLA- mismatched transplant, sparing regulatory T cells and leaving unaffected the non-dividing hematopoietic stem cells) and standard post-transplant immune suppression with a calcineurin inhibitor (CNI) and mycophenolate mofetil. Preliminary results in a little number of patients with refractory SAA at Kings college (London, UK) and John Hopkins (Baltimore, USA) were promising **(Ref#16 and 17)**. We also showed on behalf of the Severe Aplastic Anemia Working Party of the European Blood and Marrow Transplantation group very promising results on 36 patients (median age 42 years) transplanted between 2010 and 2017 The 1-year overall survival was about 78% with low rate GvHD suggesting that this approach might be a valid option in this particular poor clinical situation **(Ref#18)**. Since then, the Baltimore group published last year a confirmatory study with clear messages on the use of PTCy in patients with aplastic anemia in the context of haplo-identical donor **(Ref#19):**

1) despite HLA barriers, cure rates for patients with acquired AA following HLA-haploidentical BMT using a non-myeloablative conditioning regimen and PTCy exceed 80% overall survival with low rates of GVHD and eventually TRM

2) the results were drastically improved by added anti-thymocyte globuline (2.5 mg/Kg total dose) to the "classical" Baltimore protocol **(Ref#20)**

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3) the recommended source of stem cells is bone marrow (over peripheral blood stem cells) due to the low incidence of GvHD with bone marrow grafts. Bone marrow grafts should aim to collect 4×10^8 mononuclear cells per Kg ideal body weight of the recipient since grafts containing $<2.5 \times 10^8$ mononuclear cells per Kg ideal body weight have inferior engraftment **(Ref#21)**.
The results were so good that our colleagues from United States are now proposing this approach upfront (first line) in young patients **(Ref#19)**. In France, a prospective phase 2 clinical trial is open to confirm those good results in young patients with refractory aplastic anemia (Haploidentical allogeneic hematopoietic stem cell transplantation with post-transplant cyclophosphamide in patients with acquired refractory aplastic anemia: a nationwide phase II study “HAPLO-EMPTY” – funded by PHRC-N 2019 NCT05126849)

> The successful performance of haploidentical transplants with PTCy was a transformative advance in the field of alternative (haplo-identical) HSCT in patients with SAA

Expansion of PTCy GvHD prophylaxis to matched unrelated donor HSCT in other diseases than aplastic anemia
The success of PTCy in enabling successful transplantation across HLA-barriers with very low rates of acute and chronic GVHD led to the next inevitable question-would this work just as well or better in the HLA-matched setting? This question has been a burning topic for research in the recent years.
To explore this further, the BMT-CTN 1203 (PROGRESS-I) trial was launched with the goal of identifying the most promising GVHD prophylaxis regimen which could challenge the existing paradigm of calcineurin inhibitor / methotrexate in HLA-matched unrelated transplantation. Patients were randomly assigned to one of three experimental arms namely PTCy/Tac/MMF (PTCy-based), Tac/MTX/bortezomib (Bort-based) or Tac/MTX/maraviroc (MVC-based). The primary end-point in this trial was GVHD free relapse free survival (GFRS), measured as the time from transplant to first of four events: onset of grade III-IV aGVHD, cGVHD requiring systemic immunosuppression, disease relapse or death in an intent to treat analysis. Best results were obtained in the PTCy-based cohort **(Ref#22)**.
These promising results in the PTCy arm led to the development of BMT CTN 1703, a large multi-center randomized study (n = 428) comparing PTCy/Tac/MMF to Tac/MTX in the reduced intensity setting using only peripheral blood stem-cells (PBSC) in myeloid and lymphoid malignancies (NCT03959241). This practice changing trial completed accrual a year ahead of schedule despite the COVID pandemic and the results were presented at the 2022 American Society of Hematology Meeting (**Ref#23)**. The primary end-point in this study was 1-year GRFS. The trial met its primary end-point with GRFS favoring PTCy/ Tac/MMF at 52.7% compared to Tac/MTX at 34.9% (HR 0.641, $p < .001$) thus re-defining the standard for GVHD prophylaxis in RIC HLA- matched unrelated transplantation. This was secondary to lower rates of acute and chronic GVHD.
In the meantime, a similar but smaller randomized study was conducted by the HOVON group in Europe (HOVON-96), the final results of which were recently published again in favor of PTCy **(Ref#24)**.

> PTCy is becoming the standard GvHD prophylaxis in HSCT from unrelated donor outside aplastic anemia

Use of PTCy GvHD prophylaxis to matched sibling and unrelated donor HSCT in patients with aplastic anemia
PTCy was also reported to be used in patients with AA undergoing HSCT. First report was about small case series from the Baltimore group, including old patients at high risk of graft failure and GvHD due to massive transfusions and alloimmunization, demonstrated its feasibility **(Ref#25)**. Research also investigated the possibility to use PTCy as the sole GvHD prophylaxis compared to CsA and MTX in patients receiving PBSC, owing lower rates of grade

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II-IV acute GvHD (22.2% vs 37.1%, p = 0.56) and chronic GvHD (22.7% vs 63.6%, p = 0.013) **(Ref#26)**. The combination of PTCy with standard immunosuppression (CsA + MTX) has proven superiority to CsA + MTX alone in patients receiving PBSC – who are known to have higher rates of graft failure and GvHD – with lower grade II-IV acute GvHD (22-6% vs 52.2%, p = 0.0015) but similar chronic GvHD (16.7% vs 26%, p = 0.306) **(Ref#27)**.
The most promising results using marrow as source of stem cells and PTCy strategy were reported by Baltimore in 2020 in 3 patients receiving matched sibling, and 7 receiving unrelated (6 matched, 1 1-antigen mismatched) donor allografts. All patients were reported alive, fully engrafted, with no GVHD. All patients were off IST at time of publication. Two reactivated CMV and none EBV. Moreover, all 9 patients who had had evidence of PNH clone presence before HSCT were negative when tested posttransplant. One female (BMT performed with 200 cGy TBI) did become pregnant naturally at 4.5 years post-HSCT but had spontaneous loss at 14 weeks because of placental insufficiency. Again, in the same patient, a natural pregnancy occurred at 5 years post-BMT, and she is currently in the third trimester of an uncomplicated pregnancy **(Ref#28).**
Several expert international centers are now experimenting this approach of HSCT using PTCy in patients with AA from sibling or matched unrelated donor **(Ref#6).** In Saint-Louis hospital, we used this approach in 4 patients with refractory AA transplanted with unrelated donors who were at high risk of acute and chronic GvHD using bone marrow as source of stem cells and PTCy according to Baltimore protocol **(Ref#19)**. The results were very promising, reason why we would like now assess this strategy in “APARR”, the actual proposal.

> PTCy has been used in HSCT from sibling and unrelated donor in patients with refractory/relapse AA with promising results

GRFS as a new endpoint in patients HSCT with SAA
In contrast to HSCT in hematological malignancies, the challenges in HSCT for patients with aplastic anemia remains the achievement of sustained engraftment without significant clinical alloreactivity because no graft-versus-tumor effect is needed to achieve long term survival. Long term follow-up studies repeatedly reported that GVHD strongly impairs quality of life and plays a pivotal role in the occurrence of late complications including secondary cancers. Thus avoiding GVHD is a particularly important aim in HSCT for SAA **(Ref#29,30,31)**. Beyond high OS rate, an SAA-adapted composite endpoint GVHD and rejection free survival (GRFS) may thus be more accurate to assess patient outcomes and may be a meaningful clinical study endpoint. Very recently, we assess GRFS in a large cohort of patients with aplastic anemia **(Ref#8).** On behalf of the SAAWP of the EBMT, we analyzed GRFS in 479 patients with idiopathic SAA who underwent HSCT in 2 conventional situations: i) upfront HSCT from a matched related donor (MRD) (upfront cohort, 209 patients), and ii) HSCT for relapsed or refractory SAA (rel/ref cohort, 270 patients). Relevant events for GRFS calculation included graft failure, grade 3-4 acute GVHD, severe chronic GVHD, and death. In the relapse/refractory cohort (n=270), which is the subject of APARR, 5-year GRFS was 61%. Age was one of the main factors significantly increasing the risk of death (HR: 1.04, 95% CI [1.02-1.06], p<0.001) (as well as acute GVHD (HR: 1.03, 95% CI [1.00-1.07], p=0.041) and chronic GVHD (HR: 1.04 95% CI [1.01-1.08], p=0.032). On a sub analysis of this cohort in patients aged between 40 and 60 years, the 2-year GRFS was 50%, which thus represents the “standard” rate. With the results described in the previous section, the use of bone marrow as source of stem cells and the aplastic anemia Baltimore protocol, we believed we can reach a 2-year GRFS was 70% in this experimental approach **(see section 13.2 for hypotheses justification)**.

>SAA-adapted composite endpoint GVHD and primary and secondary graft failure *free survival (GRFS) is more accurate to assess patient outcomes as clinical study endpoint.*

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2.4 Description of the population to be studied and justification for this choice of participants

Patients aged from 40 to 60 years with acquired refractory AA or in relapse after 6 months treatment with anti-thymocyte globulin, cyclosporine and Eltrombopag, with an available donor (geno-identical donor or 10/10 matched donor or haploidentical donor with no or <1500 MFI anti-HLA antibodies) and of course eligible to HSCT will be included.

- Outcomes for adult patients with AA aged more than 40 years who are refractory or in relapse after first-line IST remain poor. Hematopoietic stem cell transplantation (HSCT) is the sole therapeutically valid option but results have always been disappointing in patients aged 40 years or older and HSCT is rarely possible. The first cause of death after HSCT in those patients with refractory/relapse SAA is still graft versus host disease (GvHD). This is the reason why we decided to limit our study to patients aged between 40 and 60 years **with a transplantation strategy (Baltimore approach) know to limit the risk of GvHD.**
- New strategies to prevent GvHD, including T-cell replete grafts with administration of an optimized new protocol using post-transplantation cyclophosphamide (PTCy), have revolutionized the field, notably in haplo-identical donor setting. Using marrow as source of stem cells and a PTCy strategy not only in haplo-identical donor setting but also in more classical case (available matched sibling or unrelated donor) might drastically prevent GvHD.
- Eligibility to HSCT is following usual criteria:
  > ECOG ≤ 2
  > No severe and uncontrolled infection
  > Cardiac function compatible with high dose of cyclophosphamide
  > Adequate organ function: ASAT and ALAT ≤ 3N, conjugated bilirubin ≤ 2N (or total bilirubin ≤ 2N if not available), creatinine clearance ≥50ml / min

2.5 Identification and description of the transplant's modalities

Transplantation modalities are following previous published results **(REF#4)**
The conditioning regimen will consist of Fludarabine (30mg/m2/day i.v.: day -6 to day -2), pre-transplant cyclophosphamide (14.5 mg/kg/day i.v.: day -6 and day -5), and Total Body Irradiation (2 Gray on day -1).

a) The stem cell source will be bone marrow.
b) Granulocyte colony– stimulating factor is given subcutaneously starting on day +5 at 5 mg/ kg/day until the absolute neutrophil count is greater than 1.5 × 109/L for 3 days.
c) GVHD prophylaxis will consist in rabbit ATG dosed at 0.5/mg/kg à D-9, 2 mg /kg at D-8 and 2.5 mg/kg à D-7 and cyclophosphamide 50 mg/Kg/day at D+3 and D+4. Tacrolimus (residual rate 10-15 microg/L) and mycophenolate (MMF) will begin from D+5. In absence of GvHD, MMF will be stopped at D35 and Tacrolimus at day 365.
d) Moreover, all patients **except patients and their donor with EBV serology and EBV PCR negative** will receive 1 injection of an anti-CD20 monoclonal antibody (rituximab) (150 mg/m2) to prevent Epstein-Barr virus (EBV) reactivation (day+5), as recommended in this situation (Ref#32).

2.6 Summary of the known and foreseeable benefits and risks for the Clinical Trial participants

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Outcomes for adult patients with AA aged more than 40 years who are refractory or in relapse after first-line IST remain poor. HSCT is the sole therapeutically valid option but results have always been very disappointing so far and HSCT is rarely possible. Using marrow as source of stem cells and a PTCy strategy not only in haplo-identical donor setting but also in case of an available matched sibling or unrelated donor might prevent GvHD and eventually drastically improve the outcome of HSCT in this setting.
There are direct benefits for patients since no curative and safe options are available so far. In the absence of transplantation, patients aged 40 years or more with SAA refractory or in relapse after IST are thus exposed to infections risk, hemorrhage, prolonged fatigue but also clonal evolution including paroxysmal nocturnal hemoglobinuria (PNH). Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) might also occur in the long-term with a grim prognosis. A global health care benefit will also be possible since those patients won't need medical support anymore including hospitalization but also daily hospital care (antibiotics or need for transfusions).

The risks are associated with hematopoietic stem cell transplantation, which is the purpose of the study, and can be described as follows:
- Primary (non-engraftment) and secondary (rejection) graft failures
- Acute graft-versus-host disease (all grades)
- Chronic graft-versus-host disease
- Severe infectious complications (bacterial, fungal, viral and parasites)
- Toxicity might also associated digestive disorders, mucositis, asthenia, skin rash.
- Additionally, high-dose cyclophosphamide may also exhibit cardiac toxicity
- Patients will also be exposed to long-term complications such as osteonecrosis, cataract, fatigue, kidney dysfunction, liver dysfunction, lung dysfunction (GvHD related or not)

Due to the age range of the population, patients might be exposed to other complications not only related to HSCT but also to their age. Moreover, the types of complications are similar to those ones presented at younger ages but the number of complications is usually higher in patients aged 40 years or more and undergoing HSCT.

3 OBJECTIVES

3.1 Primary objective

The main objective is to demonstrate a benefit in terms of the 2-year GRFS (Graft Versus Host Disease {GvHD} and primary and secondary graft failure-Free Survival) from 50 % (historical rates in patients with refractory/relapse AA undergoing standard HSCT using bone marrow from a related/unrelated donor (REF#8)) up to 70 % using an optimized HSCT protocol (bone marrow as source of stem cells and a haplo-identical conditioning platform including PTCy) on patients over 40 years old and not only in haplo-identical donor setting.

3.2 Secondary objectives

The secondary objectives will evaluate the following clinical and biological outcomes:
- primary and secondary graft failure
- mortality, overall survival
- quality of life
- treatment related morbidity and notably severe infections, cardiac toxicities
- chimerism
- immune reconstitution

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4 STUDY DESIGN

4.1 Study endpoints

4.1.1 Primary endpoint

2-year GRFS.

GRFS is a composite right-censored endpoint, defined as the time from HSCT to the first of the following events:

- primary graft failure, defined as the absence of engraftment from aplasia at day 60 after graft (D0) (i.e., persistence of neutrophils< 500 AND platelets < 20 Giga/L)
- secondary graft failure, defined as the reoccurrence of aplasia after engraftment (defined as both occurrence of neutrophils< 500 for 3 days and platelets < 20 Giga/L for 7 consecutive days)
- grade 3-4 acute GVHD, according to the MAGIC CONSORTIUM 2016
- severe chronic GVHD, according to the NIH classification
- death, whatever the cause

4.1.2 Secondary endpoints

- 100-day engraftment (3 consecutive days with neutrophils >0.5 G/L and 7 consecutive days with platelets >20 G/L) with donor chimerism> 85% on the total blood.
- Absolute numbers of neutrophils and platelets at M1, M3, M6, M12, M24 and day of last platelet and red blood cell transfusions
- Acute GvHD incidence grade 2-4 at M3
- Chronic GvHD incidence at M24
- Severe chronic GvHD at M24
- Secondary graft failure at M12 and M24
- Severe infections (CTCAE grade 3-4) at M1, M3, M6, M12, M24
- Incidence of cardiac toxicities at M12
- Incidence of CMV and EBV infection at M12
- Mortality at M12, M24
- Overall survival at 12 months and 24 months
- Quality of life questionnaire (pre-transplant, M6, M12, M24)
- Chimerism at M1, M3, M6, M12, M24
- Immune reconstitution at M1, M3, M6, M12, M24 (T lymphocytes, CD4, CD8, B, NK lymphocytes and gammaglobulines)

4.2 Description of research methodology

4.2.1 Design of the study

A phase II multicenter, French, single-arm trial.

4.2.2 Number of participating sites

This is a national multi-center study including adult transplant centers of the SFGM-TC (28 centres).

- **Recruitment centres**

Patients will be recruited in the hematology units and referred to the transplant team for the pre-transplant assessment.

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4.2.3 Identification of participants

The participants in this research will be identified as follows:
Site number. (3 digits) - Sequential enrolment number for the site (4 digits) - surname initial - first name initial.
This reference number is unique and will be used for the entire duration of the study.

5 IMPLEMENTATION OF THE STUDY

The start of the clinical trial is the inclusion of the first patient.

No additional test or specific examinations are performed for research. The patient assessment is performed in the usual care of allogeneic transplant according to JACIE procedure.

5.1 Screening visit

The screening visit takes place between D-60 and D-30 before transplant. The investigator checks the eligibility criteria and proposes the study to the patient. Information about the protocol is delivered by the transplant physician in charge of the patient. Concomitantly, the case of the patient will be discussed during the National Multidisciplinary expertise meetings of the French reference centre for aplastic anemia (bi-monthly).

A specialized consultation in reproductive medicine should be proposed.

Whose consent must be obtained	Who informs the individual and collects their consent	At what point the individuals are informed	At what point the consent is obtained
Patient	The transplant physician (investigator of research)	At the screening visit	At the inclusion visit (baseline visit) and after a reflection period of at least 15 days

5.2 Baseline visit (inclusion) (D-10 minimum before transplant)

At this visit, the consent of the patient will be collected at the latest by D-10 before transplant. A Patient Information Sheet and consent form are given to the patient by the investigator; the original is conserved by the investigator and the third copy for the sponsor.
Patients, after signing written informed consent, will be included by the investigators on eCRF CleanWeb™. The physician will receive a confirmation of the inclusion by email.

The patient assessment is performed in the usual care of allogeneic transplant.
Data collected for the research:

- Physical examination

- Reports of patient and disease history including biology, diagnosis, specific AA treatments
- Weight and height
- ECOG performans status assessment
- Sorror score of comorbidities
- Complete physical examination
- HLA compatibility check between recipient and donor

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- Pre-transplant bone marrow aspiration for smear and cytogenetics done before inclusion (within 6 to 8 weeks)
-Electrocardiogram done at screening visit or no less than 2 months before inclusion
- Echocardiogram with evaluation of left ventricular ejection done at screening visit or no less than 2 months before inclusion.
- Evaluation of the cardiovascular risk factors (dyslipidemia, HBP, obesity, diabetes, smoking) done at screening visit.
- Pulmonary function tests including at least Forced Expiratory Volume in 1 second (FEV1) and Forced vital capacity (FVC) done at screening visit or no less than 2 months before inclusion
- Respiratory function tests done at screening visit or no less than 2 months before inclusion
- Liver ultrasound and doppler echography (baseline values)

· Biological test

- Complete Blood count: neutrophil, monocyte, lymphocyte, haemoglobin, platelet (screening and inclusion visit)
- ABO and Rh typing Blood cell
- Chemistry panel (serum electrolytes with creatinine, calcium, glucose, uric acid, magnesium levels, ferritin, CRP)
- Liver function tests (transaminases, alkaline phosphatase, gamma-GT and bilirubine)
- Circulating protein electrophoresis
- Four pregnancy test (for women of childbearing potential) before starting any treatment*
- Search of anti-HLA antibodies with LUMINEX technology (DSA)
- Chimerism markers' identification
- Marrow aspiration with Karyotype analysis within 6 to 8 weeks before HSCT

** Serum or urine pregnancy test with a sensitivity of at least 25 mIU/mL (for women of childbearing potential) can be used indifferently.*

· Infectious assessment

- Viral serologies: Serology for hepatitis B (Antigen HBs, Ig antiHBc) and C, Aspergillus antigen, EBV (IgG and M), CMV (IgG and M), HSV (IgG and M), HIV (2 Elisa tests), HTLV-1 and 2 (IgG), toxoplasmosis (IgG and M), TPHA and VDRL
- PCR: HIV, B and C hepatitis

· Imaging

- Dental radiography
- Total body CT scan

This assessment is performed according to the practice of the investigator.

- Quality of Life (EORTC QLQ-C30- v3)

- Preservation of the fertility:

a. For women: it may be proposed a stimulation for the collection of follicles and secondary ovocytes in order to realize either gamete vitrification or in vitro fertilisation followed by embryo preservation (technique reserved for couples). As with cryopreservation of gonadal tissue, the stimulation and transvaginal punctures required for these techniques may be contraindicated by thrombocytopenia or neutropenia. In a woman with a stable partner, embryo freezing is theoretically possible but rarely compatible with the emergency of management.

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b. For men: it is essential to propose a consultation at the CECOS (Centre for the Study and Conservation of Sperm) for the collection and cryopreservation of sperm.

5.3 Follow-up visits

Patients are monitored daily **during initial hospitalization** to detect possible complications of procedure or GvHD occurrence. Once patients are discharged from the hospital, the follow-up will be done according to each centre policy and protocol requirement but at least once a week until months 3 and then in consultation on a regular basis lifelong.

During the first 100 days, and after the patient has left the hospital, biological testing (hepatic and renal) are performed twice a week, as part of standard of care. The eCRF will include a check of the performance of these weekly biological testing (yes/no question).

Specifically and only for the **specific population of patients aged from 50 to 60,** the file of patient must be presented again on the National Multidisciplinary expertise meeting of the French reference centre for aplastic anemia after the patient has reached 100 days post-graft and at each event listed in section 11.3.2.2.1 “Other events that require the investigator to notify without delay the sponsor”.

The minimum expected length of hospitalization is 21 days.

The daily monitoring includes:
Physical examination of the patient and safety assessment by collection of all adverse events/serious adverse events likely to occur as well as all actions taken because of these AEs. These AEs will be grading according to the CTC-AE scale except acute GvHD is grading according to MAGIC CONSORTIUM 2016 criteria (Réf#29), Chronic GvHD according to the NIH classification 2015 (Ref#30).

Data will be collected in the eCRF as follow:

- ☛At M1, M2, M3/D+100, M6, M12 and M24 after transplantation
- Disease assessment
- Complete Blood count chemistry assessment with kidney and liver test, (also at graft), Aspergillus antigen, toxoplasmosis according to risk of infection, PCR for CMV, EBV, adenovirus, HHV-6
- Acute GvHD: date of apparition, maximal grade, steroid response
- Chronic GVH: date of apparition, maximal grade, steroid response
- Cardiologic monitoring:
  Electrocardiogram will be checked before the infusion of cyclophosphamide and repeated in association to a dosage of troponine and proBNP on a daily basis for 3 consecutive days after the administration of cyclophosphamide and repeated after if any doubt. Weight measure will be done twice a day to identify quickly cardiac problems during 3 weeks then once a day until D+100. A new echocardiography will be immediately done if necessary. The patient will also be monitored continually during the perfusion of cyclophosphamide (JACIE procedure)
  Physical cardiac exam, electrocardiogram and cardiac echography at M3, M12 and M24
- All adverse events (AEs) will be recorded. All AEs (except GvHD) shall be graded according to CTC-AE Toxicity Grading Scale (v5.0).
- CD3(in case of mixed chimerism i.e. <95% total population)/CD4/ CD3/ CD8 /B lymphocytes and NK cells protide electrophoresis at D+45, M3/D100, M6, M12, M24 and ferritin levels at M1, M3, M6, M12, M24
- Chimerism evaluation at M1, M3/D100, M6, M12, M24

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- Pulmonary function tests including at least Forced Expiratory Volume in 1 second (FEV1) and Forced vital capacity (FVC) at M3/D +100
- Chest X-ray and dental radiography at M1, M2, M3, M12,
- Respiratory function tests at M3/D100, M12, M24
- Quality of life at M6, M12, M24
- Status of patient (alive/dead) and cause of death

5.4 Early termination visit

Same assessment as the M24 visit.

5.5 Expected length of participation and description of the chronology and duration of the study.

Duration of enrolment period	36 months
The length of participation for participants, of which:	
- Duration of follow-up period after graft	24 months
- Total study duration	60 months

The end of the research is defined by the last follow-up of the last allograft patient.

5.6 Table or diagram summarising the chronology of the study and recorded data

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Recorded data on eCRF	***Screening visit (D-60 to D-30)***	***Inclusion (baseline visit) (D-10 minimum)***	***D0 = graft***	***Immediate post graft monitoring daily***	***M1+/- 3d***	***M2+/- 3d***	***M3/ D100***	***M6+/- 7d***	***M12+/- 7d***	***M24+/- 7d***
National Multidisciplinary expertise meeting of the French reference centre for aplastic anemia	*x*						*x*			
Patient Information	*X*									
Signature of the consent form		*x*								
Inclusion exclusion criteria check	*X*	*x*								
Disease history	*X*									
STANDARD OF CARE										
Beta HCG (1)		*D-10*								
Physical examination (see paragraph 5.2 for complete baseline)		*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*
Imaging (Chest X-ray, Dental radiography)Total body CT scan (only at inclusion)		*x*			*x*	*x*	*x*		*x*	
Myelogram (Medullar Karyotype) within 6 to 8 weeks before HSCT		*x*								
Respiratory function Evaluation (2)		*x*					*x*		*x*	*x*
Cardiac monitoring (a) - dosage of troponine and proBNP (β) (3)		*x*		*x (β)*			*x*		*x*	*x*
Biological test (3)		*x*	*x*	*x*	*X*	*x*	*x*	*x*	*x*	*x*
Viral Serology (4) and PCR: HIV, B and C hepatitis		*x*			*x*	*x*	*x*	*x*	*x*	*x*
Chimerism					*x*		*x*	*x*	*x*	*x*
Evaluation GvHD				*x*	*x*	*x*	*x*	*x*	*x*	*x*
Lymphocyte phenotyping: CD3/CD4/ CD8/ / B lymphocytes (CD19) and NK cells (CD56),		*x*			*D+45*		*x*	*x*	*x*	*x*
Adverse events/serious adverse event			*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*
ADDED BY THE RESEARCH										
Infusion of Rituximab (only if serology or PCR of donor or patient are positive)				*D+5*						
Beta HCG (1)				*D+5, D+15*	*D+45*					
Quality of life questionnaire (EORTC QLQ-C30-V3)		*X*						*X*	*X*	*X*

(1) βHcG : before start treatment of MMF D+5 and 8-10 days after the first test : D+15 and D+45 (monthly pregnancy tests recommended)

(2) Pulmonary function tests including Forced Expiratory Volume in 1 second (FEV1),Forced vital capacity (FVC), VEMS, CVF, DLCO at inclusion and M3 ; at M12, M24 : VEMS, CVF, DLCO

(3) Cardiac monitoring a) *Electrocardiogram and echocardiography at baseline visit or less than 2 months before inclusion for all patients (β) : Electrocardiogram will be checked before the infusion of cyclophosphamide and repeated in association to a dosage of troponine and proBNP on a daily basis for 3 consecutive days after the administration of cyclophosphamide. Weight measure will be done twice a day to identify quickly cardiac problems during 3 weeks then once a day until D100. A new echocardiography will be immediately done if necessary. The patient will also be monitored continually during the perfusion of cyclophosphamide. For all patients, a systematic screening (physical cardiac exam, electrocardiogram and cardiac echography) will be done at M3, M12 and M24.*

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(3) Biological test: Complete Blood count, ABO and Rh typing Blood cell, Chemistry panel (serum electrolytes with creatinine, calcium, glucose, uric acid, magnesium levels, ferritin, CRP), Liver function tests (transaminases, alkaline phosphatase, gamma-GT and bilirubine), Circulating protein electrophoresis, HLA compatibility check between recipient and donor, Search of anti-HLA antibodies with LUMINEX technology (DSA)

(4) Viral serology: hepatitis B (Antigen HBs, Ig antiHBc) and C, Aspergillus antigen, EBV (IgG and M), CMV (IgG and M), HSV (IgG and M), HIV (2 Elisa tests), HTLV-1 and 2 (IgG), toxoplasmosis (IgG and M), TPHA and VDRL at baseline. Aspergillus antigen, toxoplasmosis according to risk of infection, PCR for CMV, EBV, adenovirus, HHV-6 at M1 until M24.post transplantation

The minimum expected length of hospitalization is 21days

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5.7 Distinction between standard care and study

TABLE: "Standard care" vs. "additional interventions" required specifically for the study

Procedures and treatments to be provided during the study	Procedures and treatments associated with standard of care	Procedures and treatments added for the study
Treatments	– Allogenic transplantation, conditioning regimen, GVHD prophylaxis as well as infection prophylaxis – HSCT overall follow-up	– Rituximab 150mg/m2 at D5 post-transplant and administration of anti-pyretic and an antihistaminic
Hospitalizations-Consultations	– The minimum expected length of hospitalization is 21 days	None
Biology test	– All biology test included marrow aspiration with Karyotype analysis in the 6 weeks before HSCT – β-HCG: 1 before graft	– β-HCG: 3 tests after Graft
Imaging	– Cardiac monitoring, Dental radiography, Total Body CT scan, Chest X ray	None
Questionnaire Quality of life		– At baseline, M6, M12 and M24

6 ELIGIBILITY CRITERIA

6.1 Inclusion criteria

Patients:

- Aged from 40 to 60 years old
- Suffering from acquired refractory severe idiopathic aplastic anemia after at least 6 months treatment with anti-thymocyte globulin, cyclosporine with Eltrombopag or in relapse
- Allograft validated in the National Multidisciplinary expertise meetings of the French reference centre for aplastic anemia
- With an available geno-identical donor or 10/10 matched donor or haploidentical donor
- With the absence of donor specific antibody detected in the patient with a MFI < 1500 (antibodies to the distinct haplotype between donor and recipient)
- Usual criteria for HSCT:
    - ECOG ≤ 2
    - No severe and uncontrolled infection

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    - Cardiac function compatible with high dose of cyclophosphamide
    - With an adequate organ function ASAT and ALAT ≤ 3N, conjugated bilirubin ≤ 2N (or total bilirubin ≤ 2N if not available), clearance creatinine ≥ 50ml / min
- With health insurance coverage
- Women of childbearing potential and men must use contraceptive methods during their participation to the research and for 12 months and 6 months after the last dose of cyclophosphamide, respectively.
- Having signed a written informed consent

NB: The authorized contraceptive methods are: For women of childbearing age and in absence of permanent sterilization:

- oral, intravaginal or transdermal combined hormonal contraception,
- oral, injectable or transdermal progestogen-only hormonal contraception,
- intrauterine hormonal-releasing system (IUS),
- sexual abstinence (need to be evaluated in relation to the duration of clinical trial and the preferred and usual lifestyle of the participants).

For men in absence of permanent sterilization: sexual abstinence, condoms.
https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf)

Individuals must meet all of the inclusion criteria as verified at the screening / inclusion visit to be eligible to participate at the study.

6.2 Exclusion criteria

Patients:

- With morphologic evidence of clonal evolution (patients with isolated bone marrow cytogenetic abnormalities are also eligible excepted chromosome 7 abnormalities and complex karyotype).
- With seropositivity for HIV or HTLV-1-2 or active hepatitis B or C and associated hepatic cytolysis
- Cancer in the last 5 years (except basal cell carcinoma of the skin or “in situ” carcinoma of the cervix)
- Pregnant (βHCG positive) or breast-feeding
- Yellow fever vaccine and all others live virus vaccines within 2 months before transplantation and during the research
- With uncontrolled coronary insufficiency, recent myocardial infarction < 6-month, current manifestations of heart failure according to NYHA (II or more), ventricular ejection fraction <50%
- With renal failure with creatinine clearance <50ml /min
- Any contraindication mentioned in the SmPC and the Investigator’s brochure of all medicinal products planned to be used in the trial including conditioning regimen, GVHD prophylaxis, prevention of EBV reactivation, infection prophylaxis
- Known allergy or intolerance to all medicinal products and/or excipients planned to be used in the trial including conditioning regimen, GVHD prophylaxis, prevention of EBV reactivation, infection prophylaxis, according to Investigator’s brochure and SmPC.
- Who have any debilitating medical or psychiatric illness, which precludes understanding the inform consent as well as optimal treatment and follow-up
- Under legal protection (tutorship or curatorship)
- Under state medical aid
- Participation to another interventional trial on a medicinal product or cell therapy

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Individuals meeting any of the exclusion criteria as verified at the screening / inclusion visit will be ineligible to participate at the study.

6.3 Clinical selection and Inclusion criteria of a donor

The clinical selection will follow the JACIE standard.

1) First choice is a sibling donor if available
2) An unrelated matched donor will be used as second choice
3) In case no HLA matched donor is available, eligible patient with excellent general status might benefit within the protocol of an haplo-identical transplantation

6.3.1 Age range criteria of a donor

According to the French Low, the authorized age is:

- For Sibling or Haplo-identical donor:
    - Aged 18 to 70 years old.
    - If no adult fulfills inclusion criteria, a minor donor may be chosen. In that case, the management of minor donors ≤ 18 years old will be done by a pediatrician, including the bone marrow harvest, and parents of minor donors will give their assessment as the donor's legally authorized representative in accordance with applicable laws and regulations: this will be documented.
- For unrelated matched donor:
    - Aged 18 to 50 years old.
    - However, the age limit can be raised to 60 years because stem cell source used will be only bone marrow.

6.3.2 Exclusion criteria of a donor

-Donor who is unable to tolerate a bone marrow harvest or receive general anesthesia, for psychological or medical reasons.
-Donor refusing bone marrow harvest
-Pregnancy in the donor
If haplo-identical donor: presence of donor specific antibody (DSA) with a MFI ≥ 1500 detected in the patient

6.4 Recruitment procedure

The protocol is carried out by the French aplastic anemia of reference centre and the Société Francophone de Greffe de Moëlle et de Thérapie Cellulaire (SFGM-TC) so most of the members of SFGM-TC will participate to this research. The French biomedicine agency and patients' association also support this proposal.

Refractory aplastic anemia for this study population concerns about 30% of the 150 new patients per year (60% of patients aged 40 years or more) receiving immunosuppression (anti-thymocyte globulin, cyclosporine and Eltrombopag) for this disease in the French Reference / SFGM-TC network, which illustrates the feasibility of the study.

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6.5 Termination rules

6.5.1 Criteria and procedures for prematurely terminating the transplant procedure including auxiliary medical products.

The transplant procedure, once started, cannot be interrupted unless the patient dies.
So, there is no particular disposition /procedure in case of prematurely terminating the transplant procedure.

6.5.1.1 Premature treatment discontinuation criteria related to warnings mentioned in the SmPC of auxiliary medical products

In case of occurrence of an adverse reaction and in the physician's opinion requiring treatment discontinuation as described in the SmPC of Tacrolimus and Mycophenolate Mofetil.

Medical management care:

- For the MMF, it will be stopped or decreased faster in case of unexpected prolonged cytopenias and in case of digestive disorders (diarrhea).
- For the tacrolimus, it will be stopped in case of severe hepatic and renal insufficiency and replaced by corticosteroides at 0.5mg/kg (day 7 to 14), 1mg/kg (day 15 to 29), 0.5mg/kg (day 30 to 45), 0.25mg/kg (day 46 to 60), taper to zero (day 61 to 84) (Ref #39). If hepatic and renal function improve, tacrolimus will be reintroduced at half dose. This will be discussed at Multidisciplinary meetings.
-

6.5.2 Criteria and procedure for premature withdrawal of a participant from the study

- Participants may exit the study at any time and for any reason. They will be followed in any case as part of a classic care follow-up.
- Before procedure of graft, the investigator can temporarily or permanently withdraw a participant from the study for any safety reason or if it is in the participant's best interests. If the transplant procedure has been initiated, then the patient will not be discharged from the study.
- Participant lost to follow-up: the participant cannot be located. The investigator must make every effort to reconnect with the participant (and document his attempts in the source file), at least to determine whether the participant is alive or dead

If a participant exits the study prematurely by withdrawing consent, any data collected prior to the date of premature exit may still be used.

The case report form must list the various reasons why the participant has discontinued the study:

- ☐ Another medical issue
- ☐ Explicit withdrawal of consent
- ☐ Lost to follow-up
- ☐ Death

6.5.3 Follow-up of participants following premature withdrawal from the study

Beside consent withdrawal, there is no reason for premature trial discontinuation, except if a second transplant is envisaged and if the patient can be included in the HaploRescue protocol (NCT05126186, Pr Peffault de Latour, AP-HP sponsor); then this is the only case of end of study.

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In all cases, either full or partial discontinuation of the study, the participants included in the study will be monitored until the end of the study scheduled in the protocol, unless consent withdrawal. In this later case, any data collected prior to the date of premature exit may still be used unless explicit decision from the patient.

6.5.4 Full or partial discontinuation of the study

AP-HP as sponsor or the Competent Authority can prematurely discontinue all or part of the study, temporarily or permanently, further to the recommendations of the Data Safety Monitoring Board in the following situations:

- first of all, if suspected unexpected serious adverse reactions (SUSARs) are observed requiring a reassessment of the benefit-risk ratio for the study.

Similarly, AP-HP, as the sponsor, or the Competent Authority may decide to prematurely discontinue the study due to unforeseen issues or new information about the transplant procedure in light of which the objectives of the study or clinical programme are unlikely to be achieved.

AP-HP as sponsor reserves the right to permanently suspend enrolment at any time if it appears that the inclusion objectives are not met.

If the study is discontinued, the participants included in the study will be monitored until the end of their participation.

If the study is prematurely discontinued for safety reasons, the decision and justification will be provided by the sponsor (AP-HP) to the Competent Authority and to the Ethics Committee without undue delay but not later than in 15 days of the date of the temporary halt or early termination. It shall include the reasons for such action and specify follow-up measures, along with recommendations from the Data Safety Monitoring Board in the case of substantial modification.

- ***Early and temporary discontinuation of the study with inclusions suspended***

The criteria for suspension of inclusion in the population 50-60 years are:

- Excessive premature mortality defined as 50% in the first 3 months among the first 10 patients aged 50-60 years.

If, for safety reason, the inclusion of patients aged 50-60 is stopped, the trial will be temporarily suspended, a substantial modification of the inclusion criteria “age” and other necessary modifications will be submitted for approval to the Competent Authority and Ethics Committee. The decision and follow-up measures will be discussed with the DSMB.

For patients aged 50-60 enrolled in the study, the management care will be:

- the patient will be monitored until the end of their participation, as set forth in the protocol.

7 TRANSPLANT PROCEDURE

7.1 Transplants modalities

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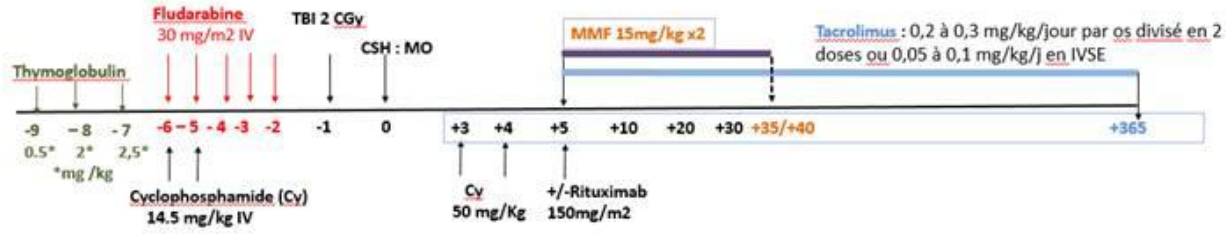
Transplantation modalities are standard routine care as define in JACIE (Joint Accreditation Committee ISTC EBMT) shared by all transplantation centers in France (https://www.ebmt.org/jacie-accreditation)

7.1.1 Conditioning regimen

The conditioning regimen will consist of Fludarabine (30mg/m^2/day i.v.: day -6 to day -2), pre-transplant cyclophosphamide (14.5 mg/kg/day i.v.: day -6 and day -5), and Total Body Irradiation (2 Gray on day -1).
However, it is allowed to realize TBI before Fludarabine for local planning reasons.
Dosage adaptation will be possible depending on renal function according to SmPC recommendations and proper clinical use of the medication.http://sitegpr.com/fr/



7.1.2 Type of stem cell source

The stem cell source will be **only** bone marrow. The bone marrow collection is carried out according to the practice of each centre with a target yield of 4 × 10^8 nucleated cells/kg recipient ideal body weight and infused on day 0, not exceeding the donor volume of 20ml/kg. Granulocyte colony stimulating factor was given subcutaneously starting on day +5 at 5 mg/ kg/day until the absolute neutrophil count was greater than 1.5 × 10^9/L for 3 days.
If the graft is less rich than the minimum target dose, it can be administered at the discretion to the physician.

7.1.3 GVHD prophylaxis

GVHD prophylaxis with consisted in:

a) Thymoglobulin® dosed at 0.5 mg/kg on day −9, 2 mg /kg at D-8 and 2,5 mg/kg on day-7,
b) Cyclophosphamide 50 mg/Kg/day at D+3 and D+4. The injection of cyclophosphamide will be accompanied by systematic injection of Mesna (50 mg / kg) for the prevention of urinary toxicity. The dose of Mesna is twice the one of cyclophosphamide divided in 4 injections per day of 30 minutes each. The first injection of Mesna is performed at the time of cyclophosphamide injection and then 3 hours, 6 hours and 9 hours after it. Patients must not receive any immunosuppressive agents between the graft infusion and until day +5.
c) Tacrolimus 0,2 à 0,3 mg/kg/day per os divided into 2 doses or 0.05 to 0.1 mg/kg/d IVSE. Tacrolimus is given for 9 months maintaining a level of the drug at 10 to 15 µg/L, then tapering between 9 and 12 months. For Tacrolimus, in case of use of posaconazole or voriconazole (anti-fungal), the posology must be reduced by 2 or 3 respectively. Tacrolimus will be stopped at day 365.
d) Mycophenolate mofetil (MMF) 15 mg/kg twice a day will begin from D+5. In absence of GvHD, MMF will be stopped between D35 and D45.

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7.1.4 Prevention of EBV reactivation

All patients will receive 1 injection of an anti-CD20 monoclonal antibody (rituximab) (150 mg/m2) to prevent Epstein-Barr virus (EBV) reactivation (day+5), as recommended in this situation (Ref #32) except patients and their donor with EBV serology and EBV PCR negative.
However, it is possible to delay the administration of rituximab at D+6 if D+5 occurs on a Sunday for greater safety at the discretion of the investigator
Premedication consisting of an anti-pyretic and an antihistaminic, e.g., paracetamol and diphenhydramine, should always be given before administration of Rituximab.

7.1.5 Infection Prophylaxis

Prophylactic and curative anti-infectious treatments (antibiotics, antivirals, antifungals) will be administered according to the ECIL recommendations (www.kobe.fr/ecil workshops, recommendations).

- Prevention of fungal infections will be done by 3rd generation azols according to ECIL5 (adapted to the SCT risk group) *https://www.ebmt.org/Contents/Resources/Library/ECIL/Pages/ECIL.aspx*
- Prevention of HHSV and VZV reactivation: aciclovir 250mg/m² x3/day IV then valciclovir: 500 mg/day po.
- Prevention of CMV infections will be done by Letermovir (adapted to the SCT risk group) (Ref#33)
- Patients received standard Pneumocystis jiroveci, toxoplasmosis and anti-herpes and varicella prophylaxis for 1 year.
- Prevention of encapsulated bacteria: Oracilline® 50 000 UI/kg x 2/day (starting after engraftment)
- Monthly polyvalent immunoglobulins will begin in case of hypogammaglobulinemia (<4 g / L)

❖ Management of toxicities:

- Antibiotics (Aminosides, Vancomycine), antivirals (Foscavir), and antifungals (ambisome) will be adapted to the renal function. Voriconazole and posaconazole will be adapted to the hepatic function, Cymevan to cytopenias. These adaptations will be regularly carried out in the transplantation department.

7.2 Authorized and prohibited treatments (medicinal, additional medicinal, surgical), *including rescue medications*

The investigator should be verified that patients should not have a contraindication tp treatments used in the study.

7.2.1 Authorized treatments

Anti-infectious treatments (antibiotics, antivirals, antifungals), transfusions, growth factors according to usual practice of each centre are authorized.

7.2.2 Treatments forbidden

Yellow fever vaccine and all others live virus vaccine are not authorized for 2 years after HSCT

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7.2.3 Treatments not recommended

- For cyclophosphamide

- Phenytoin
- Pentostatin

- For Fludarabine

- Pentostatin
- Dipyridamole or other inhibitor of adenoside captation

Patients receiving Benzodiazepines, Carbamazepine, Corticosteroids, Chloral hydrate, Phenobarbital Rifampicin, should be closely monitored for signs of toxicity
With the exception of the drugs listed above and the other drugs, in reference with the SPC "associations to be considered", will be administered according to the usual practice of the center and at the discretion of the investigator.

7.2.4 Management of primary or secondary graft failure

Patient case must be discussed during the bi-monthly National Multidisciplinary expertise meeting of the French reference center for aplastic anemia. We may propose according to specific patients features a second transplantation or a best supportive care including growth factors, transfusions support and antibiotics if needed. The same follow up will be planned for these patients until the end of the study.

8 ADDITIONAL MEDICINAL PRODUCTS TO TRANSPLANT PROCEDURE SUPPLIED BY THE SPONSOR

8.1 Rituximab

Posology for clinical trial: Rituximab will be given in one injection: 150 mg/m^2, 330 mg max at Day+5.
It is possible to delay the administration of rituximab at D+6, if D+5 occurs on a Sunday for greater safety at the discretion of the investigator.

Presentation: Rituximab will be provided by the sponsor as 100 mg vials concentrate for solution for infusion. Each box of one vial will be labelled for this study according to the Good Manufacturing Practices under the responsibility of the Département des Essais Cliniques de l'Agence Générale des Equipements et Produits de Santé (AGEPS).

Supplies:
The shipments to the hospital pharmacies will be insured by the DEC of AGEPS.

The hospital pharmacist (with respect to usual procedures) will confirm receipt in writing of all batches of study medication sent and maintain an accurate accounting of them.

Dispensing:
Pharmacies will dispense rituximab infusion bag specifically labelled for each patient on the basis of a specific prescription.

Storage:
Treatments should be stored in the refrigerator (between + 2° C and + 8° C).
Keep the package in the outer carton in order to protect from light.

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From a microbiological point of view, the prepared infusion solution should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2°C - 8°C, unless dilution has taken place in controlled and validated aseptic conditions.

Administration:
The prepared Rituximab solution should be administered as an intravenous infusion through a dedicated line.
Rituximab should be administered under the close supervision of an experienced healthcare professional and in an environment where full resuscitation facilities are immediately available. Premedication consisting of an anti-pyretic and an antihistaminic, should always be given before each administration of Rituximab. Patients should be closely monitored for the onset of cytokine release syndrome. Patients who develop evidence of severe reactions, especially severe dyspnoea, bronchospasm or hypoxia should have the infusion interrupted immediately.
Accountability and destruction: will be made by the CRA at the end of the study in the pharmacies.
A pharmacy manual will describe supplies, storage, dispensing, administration, accountability and destruction.

8.2 Traceability information and monitoring compliance for the Rituximab

Each injection will be recorded on a specific traceability document.

9 EFFICACY ASSESSMENT

9.1 Description of efficacy endpoints assessment parameters

9.1.1 GRFS

GRFS (Graft Versus Host Disease {GvHD} and Relapse/rejection-Free Survival) is defined by the time from HSCT to first of four events: primary and secondary graft failure, grade 3-4 acute GVHD, severe chronic GVHD and death.

9.1.2 Overall Survival

Overall Survival is defined as the time between HSCT and death.

9.1.3 Acute GvHD

Acute GvHD is defined according to MAGIC CONSORTIUM 2016 criteria (19). Each organ is rated with the diagnosis in stage, which allows to define a grade. Similarly, the clinician is asked to rate the maximum grade of acute GvHD over the period and maximum grade date. Histological documentation is recommended for GI GVHD.

9.1.4 Chronic GvHD

Chronic GvHD is defined according to the NIH classification published in 2005 (20). The diagnosis of chronic GVHD is retained if there is a distinctive sign (1 alone is sufficient) or evocative signs associated with a supplementary examination in favor (biopsy, for example). We then define:
A- Classical chronic GvHD in patients with only evidence of chronic GvHD
B- The overlap syndrome when a patient presents both signs of acute GvHD and chronic GvHD
C- Late acute GvHD which corresponds to exclusive signs of acute GvHD without signs of chronic GvHD occurring after J100.
The severity of chronic GvHD is defined by the number of affected organ (Appendix).

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The severity of chronic GvHD is defined by the number of affected organs.

Affected organ	Mild	Moderate					Severe		
Number of organ affected	1 or 2 without significant dysfunction	≥3	**or**	≥ 1	**or**	lung	≥ 1	Or	lung
Score of the achievement of each organ	1 (except lung)	1		2		1	3		≥2

9.2 Anticipated methods and timetable for measuring, collecting and analysing the efficacy data

The parameters for assessing efficacy were collected according to the schedule in table paragraph 5.6.

10 SPECIFIC STUDY COMMITTEE

10.1 Scientific Steering Committee

1. Missions: The scientific steering committee will define the general organization and the conduct of the research. He will determine the initial methodology and oversee the trial.
He will propose procedures to be followed during the study, acknowledging any recommendations from the Data Monitoring Committee. The sponsor retains the decision-making authority.
2. Members of the committee: Pr Régis Peffault de Latour, Pr Sylvie Chevret, Isabelle Brindel, project manager, aplastic anemia French reference center and for the DRCI: Project manager (URC and Promotion) and Clinical Research Assistant.

11 SAFETY ASSESSMENT - RISKS AND BURDEN ADDED BY THE STUDY

Regarding this research, biovigilance applies for the donor. The vigilance of clinical trials applies.

11.1 Description of Safety endpoints assessment parameters

The safety assessment shall be done by collecting all adverse events that occur during the research. All adverse event (except GvHD and MVO) shall be graded according to CT-CAE Toxicity Grading Scale (v5.0). Acute GvHD shall be graded according to MAGIC CONSORTIUM 2016 classification (19), chronic GvHD shall be graded according to NIH classification. Veno-occlusive disease shall be graded according to EMBT 2023.

11.2 Anticipated methods and timetable for measuring, collecting and analysing the safety endpoints

Adverse events shall be collected according to the schedule in table of paragraph 5.6 of the protocol.

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11.3 Recording and reporting adverse events

11.3.1 Definitions

According to Article R.1123-46 of the *Code de la Santé Publique* (French Public Health Code)

- **Adverse event**

Any untoward medical occurrence in a study participant, which does not necessarily have a causal relationship with the study or with the product subject to the study.

- **Adverse reaction**

An adverse event occurring in a study involving human participants when this event is related to the study or to the product being studied.

- **Adverse reaction to an investigational medicinal product**

Any untoward and unwanted reaction to an investigational medication regardless of the dose administered.

- **Serious adverse event or reaction**

Any adverse event or reaction that results in death, threatens the life of the participant enrolled in the study, requires hospitalisation or prolongs existing hospitalisation, causes a severe or long-term disability or handicap, or results in a congenital abnormality or deformity, and in the case of a medication, regardless of the dose administered.

- **Unexpected adverse reaction to an investigational medicinal product**

Any adverse reaction to the product whose nature, severity, frequency or outcome is inconsistent with the standard safety information described in the summary of the product characteristics, or in the investigator's brochure if the product is not authorised.

Pursuant to article R. 1123-46 of the Code de la Santé Publique and the opinion of the clinical trial sponsor not relating to a health product (ANSM):

- **Emerging safety issue**

Any new information that may lead to a reassessment of the risk/benefit ratio of the trial or of the product under investigation, modifications in the investigational medicinal product use, the conduct of the clinical trial or the clinical trial documents, or a suspension, interruption or modification of the clinical trial or of similar trials.
For studies involving the first administration of a health product in healthy volunteers: any serious adverse reaction.
Examples:
a) any clinically significant increase in the frequency of an expected serious adverse reaction;
b) suspected unexpected serious adverse reactions in patients who have terminated their participation in the clinical trial which have been notified to by the investigator to the sponsor , as well as potential follow-up reports;
c) any new fact relating to the conduct of the clinical trial or the development of the investigational medicinal product, if the new fact is likely to affect participant safety
Examples:

- a serious adverse event likely to be related to the trial's interventions and diagnostic procedures and which may impact the conduct of the clinical trial,
- a significant risk for the trial participants such as a lack of efficacy of the investigational medicinal product used in the treatment of a life-threatening disease,
- significant safety results from a recently completed study on animal subjects (such as a carcinogenicity study),

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- the premature termination, or temporary suspension, of a trial conducted with the same investigational medicinal product in another country, for safety reasons
- an unexpected serious adverse reaction related to a non-investigational medicinal product required to conduct the trial, (e.g.: “challenge agents”, rescue treatment)

d) recommendations from the Data Safety Monitoring Board (DSMB), if applicable, if they are relevant to the safety of the participants
e) any unexpected serious adverse reaction reported to the sponsor by another sponsor of a clinical trial carried out in a different country but relating to the same medication

11.3.2 The role of the investigator

The investigator must **assess the seriousness of each adverse event** and record all serious and non-serious adverse events in the case report form (eCRF).
The investigator must **document** serious adverse events **as thoroughly as possible** and provide a definitive medical diagnosis, if possible.

The investigator must **assess the intensity** of the adverse events by using a rating scale for adverse events appended to the protocol (CTC-AE V5.0), MAGIC CONSORTIUM 2016 classification for acute GvHD, NIH classification for chronic GvHD, EBMT 2023 for MVO.

The investigator must **assess the causal relationship** between serious and none serious adverse events and the procedure added by the study.

The method used by the investigator is based on 2 causality terms (EVCTM method):

- Related
- Not related

Note: the investigator will report to the Agence de la Biomédecine (French health competent authority for biovigilance) and to the sponsor all adverse events occuring in the donor and serious incident without delay as soon as the investigator becomes aware

11.3.2.1 Serious adverse events that require the investigator to notify the sponsor without delay

As per Article R.1123-49 of the *Code de la Santé Publique* (French Public Health Code), the investigator notifies informs the sponsor without delay on the day he become aware of any serious adverse event which occurs during a study that meets the description in line 1º of Article L.1121-1 of the *Code de la Santé Publique*, with the exception except any event which is listed in the protocol (see section 10.2.2) and, if applicable, in the investigator’s brochure as not requiring notification.

A serious adverse event is any untoward medical occurrence that:

1- results in death
2- is life-threatening to the participant enrolled in the study
3- requires hospitalisation or prolongation of existing hospitalisation
4- results in persistent or significant disability/incapacity
5- is a congenital anomaly/birth defect

Medication errors, pregnancies and uses outside what is foreseen in the protocol, including misuse and abuse of the product, require the investigator to notify the sponsor without delay.

11.3.2.2 Specific features of the protocol

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11.3.2.2.1 Other events that require the investigator to notify without delay the sponsor

- Adverse events deemed “medically significant” (i.e. considered as “serious”)
  - Primary and secondary graft failure
  - Severe dyspnoea, bronchospasm or hypoxia-related to Rituximab (CTCAE grade ≥3)
  - Secondary neoplasia (excepted basal cell carcinoma of the skin or “in situ” carcinoma of the cervix).
  - Veno-occlusive disease according to EMBT 2023 (appendix 19.4)
  - Thrombotic microangiopathy (CTCAE grade ≥3)
  - Bronchiolitis obliterans (all grade)
  - Neurological disorders (coma, convulsion, encephalitis) occurring until one month after HSC transplant (CTCAE grade ≥3).
  - Cardiac toxicities (CTCAE grade ≥3) occurring until one month after HSC transplant, including cardiogenic shock.
  - Post-transplant lymphoproliferative disorders.

The investigator must notify the sponsor without delay on the day the investigator becomes aware of these adverse events, in the same manner and within the same deadline as for serious adverse events (see above).

- ***In utero* exposure**

The investigator must notify the sponsor without delay on the day the investigator becomes aware of any pregnancy that occurs during the study, even if it is not associated with an adverse event.
If the investigational medicinal product is genotoxic, every case of maternal or paternal exposure must be notified.

- Exposure **while breastfeeding**

Exposure while breastfeeding occurs if an infant or a child may have been exposed to a medicinal product *via* the breast milk of a mother being treated with an investigational medicinal product.
Even if it is not associated with an adverse event, the investigator must notify the sponsor without delay on the day the investigator becomes aware of the exposure while breastfeeding.

11.3.2.2.2 Serious adverse events that do not require the investigator to notify the sponsor without delay

These serious adverse events are only recorded in the case report forms. A data retrieval of the case report forms will be implemented for serious adverse events every 3 months by the CTU (URC Saint Louis) and will be transmitted to the safety department: expertisecsi.drc@aphp.fr

- *Normal and natural course of the condition*:
  - Scheduled inpatient hospitalization for monitoring the condition under investigation [with no deterioration in the subject's medical condition compared to baseline]
  - Inpatient hospitalization for routine treatment or monitoring the condition under investigation, not associated with a deterioration in the subject's medical condition
  - Emergency inpatient hospitalization upon enrollment or prolongation of hospitalization upon enrollment for monitoring the condition under investigation
  - Worsening of the condition under investigation before the start of the study procedure (including conditioning regimen) excluding those leading to death

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- In case of primary or secondary graft failure: SAE occurring after the second transplant (if the second transplant is performed)
- Others conditions:
  - In case of disturbance of biological values corresponding to an adverse event of grade ≤ 3 and no other symptoms (fever, etc.) associated with this adverse event, this event will not require the investigator to notify the sponsor as a serious adverse event but only in the case report form.
  - All post graft complication grade ≤ 3 (including conditioning regimen and graft prophylaxis), except those listed in section 11.3.2.2.1

These serious adverse events are only recorded in the case report forms.

- *Special circumstances*
  - Hospitalisation for a pre-existing illness or condition
  - Hospitalisation for a medical or surgical treatment scheduled prior to the study
  - Admission for social or administrative reasons

11.3.2.3 Period during which SAEs must be notified without delay by the investigator to the sponsor

The investigator must notify the sponsor without delay of any serious adverse events as defined in the corresponding section:
- from the date on which the participant begins the conditioning regimen
- throughout the whole follow-up period required for the trial
- Indefinitely, if the SAE is likely to be due to the procedures of transplant including conditioning regimen and GVHD prophylaxis (e.g. serious reactions that could appear long after exposure to the medication, such as cancers or congenital abnormalities).

In case of primary or secondary graft failure: from the date on which the participant begins the conditioning regimen until the second transplant process (including conditioning regimen)(if the second transplant is performed).

11.3.2.4 Procedures and deadlines for notifying the sponsor

The initial notification of an SAE must be provided in a written report signed by the investigator using a SAE notification form specific to the study and intended for this purpose (in the case report form).

Each item in this form must be completed by the investigator so that the sponsor can carry out the appropriate analysis.

The initial notification to the sponsor of a serious adverse event must be quickly followed by an additional detailed written report (or reports) so that the case outcome may be monitored by the Safety Department or to provide further information.

Whenever possible, the investigator will provide the sponsor with any documents that may be useful (medical reports, laboratory test results, results from additional examinations, etc.). These documents must be non-identifying. In addition, the documents must include the following: study acronym, number and participant's initials.

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Any adverse event will be monitored until fully resolved (stabilisation at a level considered acceptable by the investigator, or return to the previous state) even if the participant has left the study.

The initial notification, the SAE follow-up reports and all other documents must be sent to the sponsor's Safety Department by email (eig-vigilance.drc@aphp.fr). It should be noted that it is possible to send SAE reports to the Safety Department by fax to +33 (0)1 44 84 17 99 only in the event of a failed attempt to send the SAE report by email (in order to avoid duplication).

For studies which use e-CRFs

- the investigator completes the SAE notification form in the e-CRF, then validates, prints, and signs the form before sending it by email;
- if it is not possible to connect to the e-CRF, the investigator should complete, sign, and send the SAE notification form to the Safety Department. As soon as the connection is restored, the SAE notification form in the e-CRF must be duly completed.

The investigator must respond to all requests from the sponsor for additional information.
For all questions relating to the notification of an adverse event, the Safety Department can be contacted via email: vigilance.drc@aphp.fr.

For cases of *in utero* exposure, the investigator will complete the Notification and Follow-up form for a pregnancy occurring during participation in a study".
The investigator must monitor the pregnant woman throughout her pregnancy or until the pregnancy is terminated, and must notify the sponsor of the outcome of the pregnancy using this form.
If the outcome of the pregnancy falls within the definition of a serious adverse event (miscarriage, pregnancy termination, foetal death, congenital abnormality, etc.), the investigator must follow the procedure for reporting SAEs.
The initial pregnancy notification, the SAE follow-up reports, and any other documents will be sent to the sponsor according to the same procedures specified herein.
If it was the father who was exposed, the investigator must obtain the pregnant woman's permission before collecting information about the pregnancy.

11.3.3 Role of the sponsor

The sponsor, represented by its Safety Department, shall continuously, throughout the trial, assess the safety of each investigational medicinal product throughout the study.

11.3.3.1 Analysis and declaration of serious adverse events

The sponsor assesses:

- the **seriousness** of all the adverse events reported
- the **causal relationship** between these events and each investigational medicinal product and/or specific interventions/procedures/examinations added by the study and any other treatments,
  All serious adverse events which the investigator and/or the sponsor reasonably believe may have a causal relationship with the investigational medicinal product are classed as suspected serious adverse reactions.
- the **expected or unexpected nature** of the serious adverse reactions
  Any serious adverse reaction is considered to be unexpected when the nature, severity or progression are not consistent with information pertaining to the interventions/procedures/practiced acts and/or administered products over the course of the study.
  The sponsor, represented by its Safety Department, assesses the expected/unexpected nature of a serious adverse reaction based on the information described below**.**

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- For serious adverse events likely to be related to the study procedures (conditioning regimen, HSCT graft, GVHD prophylaxis): Refer to the Investigator's Brochure and Summary of Products Characteristics of medicinal products

Serious adverse events likely to be related to the study procedures:
The sponsor will report all suspected unexpected serious adverse reactions (SUSARs), within the regulatory time frame, to the ANSM (French Health Products Safety Agency):
• The sponsor must send the initial report immediately upon learning of the unexpected serious adverse reaction if is fatal or life-threatening, or otherwise within 15 days from learning of any other type of unexpected serious adverse reaction;
• All additional, relevant information must be declared by the sponsor in the form of monitoring reports within a period of 8 calendar days starting from when the sponsor had this information.

Note: the investigator will report to the Agence de la Biomédecine (French health competent authority for biovigilance) and to the sponsor all adverse events occuring in the donor and serious incident without delay as soon as the investigator becomes aware.
The sponsor will report to the ANSM the adverse events occurring in the donor and serious incidents without delay as soon as the sponsor becomes aware.
As a reminder, regarding this research, biovigilance applies for the donor.

The sponsor must notify all the investigators involved about any information that could adversely affect the safety of the research participants.

11.3.3.2 Analysis and declaration of other safety data

Pursuant to article 1123-46 of the Code de la Santé Publique, a new development is defined by any new data that may lead to a reassessment of the study's risk-benefits ratio or studied product, to modifications in the use of this product, in the conduct of the study, or documents pertaining to the study, or to suspend or halt or modify the study protocol or similar studies.

The sponsor will inform the competent authority and the Research Ethics Committee without delay upon knowledge of any emerging safety issues and, if applicable, describe what measures have been taken.

Following the initial declaration of any emerging safety issues, the sponsor will address to competent authorities any additional relevant information about the emerging safety issue in the form of a follow-up report, which must be sent no later than 7 days from learning of the information.

11.3.3.3 Annual safety report

Once a year for the duration of the clinical trial, the sponsor must draw up an annual safety report (ASR or annual safety report), which includes, in particular:
- a safety analysis for the research participants,
- a list of all the suspected serious adverse reactions that occurred in France in the concerned study during the period covered by the report,
- summary tables including all of the SAEs that have occurred since the start of the study.

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The annual safety report must be sent no later than 60 days after the anniversary of the date on which the first participant was included in the study.

11.3.4 Data Safety Monitoring Board (DSMB)

A DSMB will be set up for this trial. The DSMB must hold its first meeting before the first subject is enrolled. The DSMB's preliminary meeting should take place before the protocol submission to competent health authority (ANSM) and Ethics committee.

The members of the DSMB are:

- Alicia Rovo, Hematology department, Bern, Suisse
- Xavier Poire, Hematology department, Université de Louvain, Belgique
- Jacques Emmanuel Galimard, statistician, Université de Paris

The DSMB's principal missions and their operating procedures are described below and in the DSMB chart of the clinical trial.
The DSMB has a consultative role. The decision concerning the conduct of the clinical trial relies on the sponsor.

General information about the DSMB
The DSMB makes recommendations to the sponsor about the continuation, modification or termination of the research. The recommendations that the DSMB can make are:
- to continue the research with no modifications
- to continue the research with a modification to the protocol and/or to the monitoring of subjects
- to temporarily halt inclusions
- to permanently terminate the research in light of:
    - o safety data: serious adverse reactions
    - o efficacy data: proven futility or efficacy

The DSMB is appointed by the sponsor and is made up of at least 3 people with no connection to the research, including at least one clinician specialized in the pathology being studied and one specialist in the medication being studied (or a pharmacologist/pharmacovigilance specialist), and possibly a methodologist/biostatistician.
The DSMB has a consultative role in advising the sponsor on safety issues such as tolerance and re-assessment of the benefit-risk ratio during the research. The DSMB must hold its preliminary meeting before the first inclusions of the first subject and ideally before the protocol is submitted to the competent authority and the CPP.

Definition of the DSMB's missions:
- Validation of the research methodology: The proposed methodology for the clinical trial will be validated by the DSMB so that it does not jeopardise the safety of subjects, in particular relating to the inclusion and randomisation methods.
- Validation of tolerance monitoring methods:
    - o nature of the evaluated parameters
    - o frequency of the evaluations, consultation schedule
- Validation of termination criteria:
    - o criteria for terminating a subject's participation for tolerance reasons
    - o criteria for the temporary or permanent termination of the research (leading to the establishment of certain recommendations ("stopping rules"))
- Modification of the protocol and recommendations:
    - o In light of the interim analyses of the primary endpoint if one arm seems to be clearly in defavour of patients

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o In light of the analysis of tolerance data for the research, the DSMB can, when applicable: propose substantial modifications in order to modify certain data, in particular relating to the protocol (inclusion and non-inclusion criteria, monitoring, additional exams, etc.). Likewise, the DSMB can issue any recommendations it deems useful in order to best ensure the safety of the research subjects and to maintain a favorable benefit-risk balance throughout the research.

Definition of the DSMB's operating methods:

The DSMB will meet every 15 patients **to carefully check the data in terms of observed deaths and GVHD events. The DSMB will also review the results of the scheduled interim analyses of the trial.**

Meetings modalities (open session, then closed sessions) and frequency will be detailed in the DSMB charter at the latest before inclusion of the first patient.

Modalities and format expected for the transmission of SAE from the sponsor to the DSMB will be detailed in the DSMB charter at the latest before inclusion of the first patient.
The sponsor retains decision-making authority. The sponsor delivers its decision, with justification, and DSMB reports to the Competent Authority (ANSM) and when applicable to the CPP.

12 DATA MANAGEMENT

12.1 Data collection procedures

Data entry will be performed by each centre by authorized persons.

12.2 Identification of data recorded directly in the CRFs which will be considered as source data

No data.

12.3 Right to access data and source documents

12.3.1 Data access

In accordance with GCPs:
- the sponsor is responsible for ensuring all parties involved in the study agree to guarantee direct access to all locations where the study will be carried out, the source data, the source documents and the reports, for the purposes of the sponsor's quality control and audit procedures or inspections by the competent authority
- the investigators will ensure the persons in charge of monitoring, quality control and auditing or inspecting the interventional study involving human participants have access to the documents and personal data strictly necessary for these tasks, in accordance with the statutory and regulatory provisions in force (Articles L.1121-3 and R.5121-13 of the French Public Health Code)

12.3.2 Source documents

Source documents are defined as any original document or item that can prove the existence or accuracy of a data or a fact recorded during the study. These documents will be kept in

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accordance with the regulations in force by the investigator or by the hospital in the case of a hospital medical file.:

In this study, source documents are: medical files, original biological examination results, summary from imaging examinations.

12.3.3 Data confidentiality

The persons responsible for the quality control of clinical studies (Article L.1121-3 of the *Code de la Santé Publique* [French Public Health Code]) will take all necessary precautions to ensure the confidentiality of information relating to the investigational medicinal products, the study, the study participants and in particular their identity and the results obtained.
These persons, as well as the investigators themselves, are bound by professional secrecy (in accordance with the conditions set out in Articles 226-13 and 226-14 of the *Code Pénal* [French Criminal Code]).
During and after the research involving human participants, all data collected concerning the participants and sent to the sponsor by the investigators (or any other specialised collaborators) will be rendered non-identifying.
Under no circumstances shall the names and addresses of the participants involved be shown. Only the participant's initials will be recorded, accompanied by an encoded number specific to the study indicating the order of enrolment.
The sponsor will ensure that each participant has given written permission for any personal information about him or her which is strictly necessary for the quality control of the study to be accessed.

12.4 Data processing and storage of research documents and data

12.4.1 Data entry

Non-identifying data will be entered electronically via a web browser.

12.5 Data ownership

AP-HP is the owner of the data. The data cannot be used or disclosed to a third party without its prior permission.

13 STATISTICAL ASPECTS

13.1 Description of statistical methods to be used including the timetable for the planned interim analyses

Several interim analyses are planned (see below).
The interim and primary analyses will be performed on the ITT population.
A secondary analysis will be performed on the per-protocol population
Descriptive statistics will present median and interquartile range, or mean and standard deviation for quantitative variables and count and percentages for qualitative variables

Interim analyses
The interim analyses will take place every 12 months, unless less than 20 enrolled patients. They will use a Bayesian inference frawework.
We will compute the posterior mean and a credible band for the GRF survival function, as well as the posterior mean for the hazard of event, from a piecewise exponential prior with Gamma distributed independent heights (Castillo and Van der Pas (2020). Multiscale Bayesian survival analysis. <arXiv:2005.02889).

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Assuming an exponential GRFS distribution, we will compute the posterior probability that the hazard of event is above -0.03 (corresponding to the expected survival of 50% at month 24).
These results will be provide to the DSMB as a toll for decision making.
The R Package 'BayesSurvival' will be used.

Primary endpoint analysis
The primary endpoint (GRFS) will be estimated and plotted using the Kaplan-Meier estimator, along with its 95% Confidence Interval using Greenwood's formula.
The 2-year GRF rate estimation will be compared to the historical rate using a one sample, two-sided log-rank test.

Secondary endpoints
All time to event endpoints with no competing risks (that is, assuming a non-informative censoring) will be analysed similarly to GRFS.
For time to event endpoints with competing risk setting, cumulative incidence of the endpoint will be estimated in a competing-risks setting, plotted with its corresponding 95%CI.
For binary endpoints, proportion will be calculated with its corresponding exact binomial 95%CI
For quantitative endpoints, summary statistics (median and IQR or mean and SD) will be presented.

Subgroup analyses
Two subgroups of interest are defined based on the type of HSCT donor:
1/ haplo-identical HSCT donors
2/ sibling or matched unrelated donors

Analysis of primary and secondary endpoints, and the additional PS-based analysis (see below) will be repeated specifically for these 2 subgroups. Interaction tests of Gail and Simon will be used.

PS-based analysis
To enhance the level of evidence of this single arm trial, we will add a comparison to external controls (#Ref 34-35). With access to individual patient data from an observational cohort (#Ref 8), we will perform a causal inference analysis with Propensity Score (PS) weighting analysis (#Ref 36).
Primary endpoint and secondary endpoints will be compared between patients from the trial and patients from the cohort and using IPW methods (with ATT weights).
The propensity score will include already known prognostic factors, among which time between diagnosis and BMT, age, type of donor and disease type at time of BMT and observed prognostic factors.
Effect of GRFS will be compared using a Cox proportional hazard weighted model.
For secondary time-to-event endpoints, either Cox or Cox-cause specific weighted models will be used, depending on the presence of competing risks.
For binary endpoints, weighted logistic model will be used.

13.2 Calculation hypotheses for the number of participants required and the result

Hypotheses:
Historical rates of GRFS (Graft Versus Host Disease (GvHD) and Relapse/rejection -Free Survival) has been set up to 50% in refractory/relapse AA undergoing HSCT (REF#8). In this later publication, main causes of GRFS failure have been identified in the subgroup of patients >40 years: 1. aGvHD (6%), 2. cGVHD (8%), 3. graft failure (10%), death (30%). We hypothesized the improvement in GRFS according to those 4 endpoints:

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- The combination of ATG and PTCy has been shown efficacy in GvHD prophylaxis (REF#4, 7 et 28) but the rates coming from the historical cohorts are already very low (cf point 1. and 2.).
> We thus do not think to gain any GRFS benefit on this endpoint.

- The risk of rejection has been significantly reduced adding a small dose of Total Body Irradiation (TBI) for all alternative HSCTs in SAA (REF #37, 39). First report of the Baltimore platform was about small case series from the Baltimore group, including old patients at high risk of graft failure and GvHD due to massive transfusions and alloimmunization, demonstrated its feasibility (Ref#25). The conditioning regimen of APARR should thus improve engraftment since 1/3 only of patients will receive alternative donor BMT (2/3 of the patients will receive a matched family or unrelated donor BMT).
> We thus plan a 5% graft failure rate (versus 10% in the historical controls) using a TBI-based conditioning regimen.

- PTCy was firstly reported in the context of haplo-identical transplantation with regular overall survival >90% (REF #4, 7, 17 et 28). The most promising results using marrow as source of stem cells and PTCy strategy were reported by Baltimore in 2020 in 3 patients receiving matched sibling, and 7 patients receiving unrelated (6 matched, 1 1-antigen mismatched) donor allografts. All patients were reported alive, fully engrafted, with no GVHD. All patients were off IST at time of publication (Ref#28). Several expert international centers are now experimenting this approach of HSCT using PTCy in patients with AA from sibling or matched unrelated donor (Ref#6). In Saint-Louis hospital, we used this approach in 4 patients with refractory AA transplanted with unrelated donors who were at high risk of acute and chronic GvHD using bone marrow as source of stem cells and PTCy according to Baltimore protocol (Ref#19). The results were very promising with all the 4 patients alive at last follow-up.
> We thus plannned a 15% reduction in overall mortality (versus 30% in the historical controls) using this approach.

In conclusion, the reduction to 5% graft failure (versus 10%) and 15% overall survival (versus 30%) compared with the historical controls (GRFS = 50%) lead to a target endpoint of GRFS of 70% at 2 years.

A two-sided, one-sample log-rank test calculated from a sample of 52 subjects achieves 80% power at a 0.05 significance level to detect a proportion GRFS of 0.70 in the new group when the proportion GRFS in the historic control group is 0.50. These proportions GRFS are for a period of 24 months. The probability that a subject experience an event during the study is 0,25. The expected number of events during the study is 11. It is assumed that the GRFS time distributions of both groups are approximated reasonably well by the Weibull distribution with a shape parameter of 1,00.

14 QUALITY CONTROL AND ASSURANCE

Every research project involving human participants managed by AP-HP is ranked according to the projected risk incurred by study participants using the classification system specific to AP-HP sponsored interventional research involving human participants.

14.1 General organisation

The sponsor must ensure the safety and respect of individuals who have agreed to participate in the study. The sponsor must implement a quality assurance system to best monitor the implementation of the study in the investigation centres.

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For this purpose, the sponsor shall assign Clinical Research Associates (CRA) whose primary role is to carry out regular follow-up visits at the study locations, after having carried out the initial visits.
The purpose of monitoring the study, as defined in Good Clinical Practices (GCP section 5.18.1), is to verify that:

- the rights, safety and protection of the research participants are met
- the data reported are exact, complete and consistent with the source documents
- the study is carried out in accordance with the protocol in force, the GCPs and the statutory and regulatory provisions in force

14.1.1 Strategy for centre opening

The strategy for opening the centres established for this study is determined using the appropriate monitoring plan.

14.1.2 Scope of centre monitoring

In the case of this **D** risk study the appropriate monitoring level has been determined based on the complexity, the impact and the budget for the study. Therefore, in agreement with the coordinating investigator, the sponsor has determined the logistical score and impact, resulting in a study monitoring level to be implemented: **level high**.

14.2 Quality control

A Clinical Research Associate (CRA) appointed by the sponsor will be responsible for the good completion of the study, for collecting, documenting, recording and reporting all handwritten data, in accordance with the Standard Operating Procedures applied within the DRCI (Clinical Research and Innovation Department) and in accordance with Good Clinical Practices as well as the statutory and regulatory requirements.

The investigator and the members of the investigator's team agree to make themselves available during regular Quality Control visits carried out by the Clinical Research Associate. During these visits, the following elements will be reviewed depending on the monitoring level:

- written consent
- compliance with the study protocol and with the procedures defined therein
- quality of the data collected in the case report form: accuracy, missing data, consistency of the data with the "source" documents (medical files, appointment books, original copies of laboratory results, etc.)
- management of the treatments used

14.3 Case report forms

All information required by the protocol must be entered in the case report forms. The data must be collected as and when they are obtained, and clearly recorded in these case report forms. Any missing data must be coded.
Every site will have access to the electronic case report forms via a web-based data collection system. Investigators will be given a document offering guidance on using this tool.
When the investigators complete the case report form via the Internet, the CRA can view the data quickly and remotely. The investigator is responsible for the accuracy, quality and relevance of all the data entered. In addition, the data are immediately verified as they are entered, thanks to consistency checks. To this end, the investigator must validate any changes to the values in the case report form. An audit trail will be kept of all changes. A justification can be added when applicable, as a comment.
The investigator must archive a copy of the authenticated document that was issued to the sponsor.

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14.4 Management of non-compliances

Any events that occur as a result of non-compliance – by the investigator or any other individual involved in running the study – with the protocol, standard operating procedures, good clinical practices or statutory and regulatory requirements must be recorded in a declaration of non-compliance and sent to the sponsor.

These non-compliances will be managed in accordance with the sponsor's procedures.

14.5 Audits/inspections

The investigators agree to consent to the quality assurance audits carried out by the sponsor as well as the inspections carried out by the competent authorities. All data, documents and reports may be subject to regulatory audits and inspections. These audits and inspections cannot be refused on the grounds of medical secrecy.

An audit can be carried out at any time by individuals appointed by the sponsor and independent of those responsible for the research. The aim of the audits is to ensure the quality of the study, the validity of the results and compliance with the legislation and regulations in force.

The persons who manage and monitor the study agree to comply with the sponsor's requirements and with the competent authority regarding study audits or inspections.

The audit may encompass all stages of the study, from the development of the protocol to the publication of the results, including the storage of the data used or produced as part of the study.

14.6 Principal Investigator's commitment to assume responsibility

Before starting the study, each investigator will give the sponsor's representative a copy of his/her updated personal *curriculum vitæ*, signed and dated less than one year, with his/her RPPS number (*Répertoire Partagé des Professionnels de Santé*, Collective Database of Health Professionals, for France). The CV must include any previous involvement in clinical research and related training.

Any conditions, such as economic interests and institutional affiliations that might influence the impartiality of the investigators shall be presented.

Each investigator will commit to comply with legislation and to conduct the study in line with GCP, in accordance with the Declaration of Helsinki. Each investigator will give the sponsor's representative a GCP certificate dated fewer than three years ago.

The Principal Investigator at each participating site will sign a commitment of responsibility (standard DRCI document) which will be sent to the sponsor's representative.

The investigators and their staff will sign a delegation of duties form specifying each person's role and will provide their CVs.

15 ETHICAL AND LEGAL CONSIDERATIONS

15.1 Methods for informing research participants and obtaining their consent

In accordance with Article L1122-1-1 of the *Code de la Santé Publique* (French Public Health Code), no interventional research involving human participants can be carried out on a person

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without his/her freely given and informed consent, obtained in writing after the person has been given the information specified in Article L.1122-1 of the aforementioned Code.

A reflection period of **15 days** is given to the individual between the time when he or she is informed and when he or she signs the consent form.
The person's freely-given, written, informed consent will be obtained by the principal investigator or a physician representing the investigator before the person is enrolled in the study.

A copy of the information note and consent form, signed and dated by the research participant and by the principal investigator or the physician representing the investigator will be given to the individual prior to their participation in the study. The principal investigator or the physician representing him/her will keep a copy.

In addition, the investigator will specify in the person's medical file the person's participation in the research, the procedures for obtaining his/her consent as well as the methods used for providing information for the purpose of collecting it. The investigator will retain one copy of the signed and dated consent form.

At the end of the study, one copy will be placed in a tamper-proof sealed envelope containing all the consent forms. This envelope will be archived by the sponsor.

15.2 Prohibition from participating in another clinical study or exclusion period set after the study

No exclusion period of participation after the participant has finished this study is defined in the context of this research.

The participant may not enroll in another interventional study protocol on a medicinal product or cell therapy involving human participants for the duration of his or her participation without consulting with the physician monitoring him or her in the context of the study. Indeed, participation in another interventional trial can be considered by investigator as long as it does not influence the main criteria of this present research.

The participants can however participate in other non-interventional studies or in minimal risk and constraint study that does not involve therapeutic strategies, but this should be reported to the physician who follows it in the present research.

15.3 Authorisation for the research location

In France, the study will be carried out in treatment units on individuals presenting a clinical condition for which the units are specialised and who require interventions that are routinely performed at those units. Therefore, the research location does not require specific authorisation.

15.4 Legal obligations

15.4.1 Role of the sponsor

Assistance Publique Hôpitaux de Paris (AP-HP) is the sponsor of this study and by delegation, the DRCI (Clinical Research and Innovation Department) carries out the study's missions in accordance with Article L.1121-1 of the *Code de la Santé Publique* (French Public Health Code). Assistance Publique - Hôpitaux de Paris reserves the right to halt the study at any time for medical or administrative reasons. In this case, notification will be sent to the investigator.

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15.4.1 Request for approval from the CPP (Research Ethics Committee)

Prior to starting the study, AP-HP, as sponsor, must obtain for this interventional study involving human participants concerning a medicinal product for human use, approval from the appropriate CPP (Research Ethics Committee), within the scope of its authority and in accordance with in force legislation and regulatory requirements.

15.4.2 Request for authorisation from ANSM

Prior to starting the study, AP-HP, as sponsor, must obtain authorisation from the ANSM (French Health Products Safety Agency) for the interventional study involving human participants not concerning a health product, within the scope of the ANSM's authority and in accordance with in force legislation and regulatory requirements.

15.4.3 Procedures relating to data protection regulations

The computer file used for this research is implemented in accordance with French (amended "Informatique et Libertés" law governing data protection) and European (General Data Protection Regulation – GDPR) regulations.

Commitment to comply with "Reference Methodology" MR-001:
This research is governed by the CNIL (French Data Protection Agency) "Reference Methodology for processing personal data used within the scope of health research" (amended MR-001). AP-HP, as sponsor of the research, has signed a declaration of compliance with this "Reference Methodology"

15.4.4 Amendments to the research

Any substantial modification to the protocol by the coordinating investigator must be sent to the sponsor for approval. After approval is given, the sponsor must obtain approval from the CPP (Research Ethics Committee) and authorisation from the competent authority (ANSM) within the scope of their respective authorities, before the amendment can be implemented.
The information note and the consent form can be revised if necessary, in particular in case of a substantial amendment to the study or if adverse reactions occur.

15.4.5 Final study report

The final report for the research involving human participants referred to in Article R1123-67 of the *Code de la Santé Publique* (French Public Health Code) is written and signed by the sponsor and the investigator. A report summary drafted according to the reference plan of the competent authority must be sent to the competent authority within a period of one year following the end of the study, i.e., the end of the participation of the last participant in the study.

15.4.6 Archiving

Specific documents for an interventional study involving human participants not concerning a health product will be archived by the investigator and the sponsor for 30 years after the end of the research.

This indexed archiving includes, in particular:

- A sealed envelope for the investigator containing a copy of all the information notes and consent forms signed by all individuals at the centre who participated in the study;
- A sealed envelope for the sponsor, containing a copy of all the information notes and consent forms signed by all individuals at the centre who participated in the study;
- "Study" binders for the Investigator and the sponsor, including (non-exhaustive list) :

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- the successive versions of the protocol (identified by the version number and its date), and any appendices
- the competent authority authorisations and Research Ethics Committee decisions
- any correspondence
- the enrolment list or register
- the appendices specific to the research
- final study report

- The data collection documents

16 FUNDING AND INSURANCE

16.1 Funding sources

This study is performed by PHRC N 2022.

16.2 Insurance

For the duration of the study, the Sponsor will take out an insurance policy covering the sponsor's own public liability, as well as the public liability for all the physicians involved in the study. The sponsor will also provide full compensation for any damages caused by the study to the participant enrolled and their beneficiaries, unless the sponsor can prove that the harm is not the fault of the sponsor or any collaborator. Compensation cannot be refused on the grounds of a third-party act or the voluntary withdrawal of the person who initially consented to participate in the study.

Assistance Publique - Hôpitaux de Paris (AP-HP) has taken out insurance with HDI - GLOBAL SE through BIOMEDIC-INSURE for the full study period, which covers its own public liability and that of any collaborator (physician or research staff), in accordance with Article L.1121-10 of the Code de la Santé Publique (French Public Health Code).

17 PUBLICATION RULES

The author(s) of any publication relating to this study must include the APHP among their affiliations and name the sponsor AH-HP (DRCD) and the source of funding, if funded by a call for tenders (e.g. national PHRC); a copy of the publication must be sent to the sponsor.

17.1 Mention of AP-HP affiliation for projects sponsored by AP-HP

- If an author has several affiliations, the order in which the institutions are mentioned (AP-HP, University, INSERM, etc.) is unimportant
- However, if the study is funded in the context of an internal AP-HP call for tender, the first affiliation must be "AP-HP"
- Each of these affiliations must be identified by an address and separated by a semicolon (;)
- The AP-HP institution must feature under the acronym "AP-HP" first in the address, specifically followed by: AP-HP, hospital, department, city, postcode, France

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17.2 Mention of the sponsor AP-HP (DRCI) in the acknowledgements of the text

The sponsor was Assistance Publique – Hôpitaux de Paris (Direction de la Recherche Clinique et de l'Innovation)"

17.3 Mention of the financial backer in the acknowledgements of the text

The study was funded by a grant from Programme Hospitalier de Recherche Clinique - PHRC 2022 (French Ministry of Health).

This study has been registered on the website http://clinicaltrials.gov/ under number

18 BIBLIOGRAPHY

1- Peffault de Latour R, Kulasekararaj A, Iacobelli S et al. Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia. N Engl J Med. 2022 Jan 6;386(1):11-23. doi: 10.1056/NEJMoa2109965.
2- Young NS. Aplastic Anemia. N Engl J Med. 2018 Oct 25;379(17):1643-1656.
3- Bacigalupo A. How I treat acquired aplastic anemia. Blood. 2017 Mar 16;129(11):1428-1436. doi: 10.1182/blood-2016-08-693481.
4- DeZern AE, Eapen M, Wu J et al. Haploidentical bone marrow transplantation in patients with relapsed or refractory severe aplastic anaemia in the USA (BMT CTN 1502): a multicentre, single-arm, phase 2 trial. Lancet Haematol. 2022 Sep;9(9):e660-e669. doi: 10.1016/S2352-3026(22)00206-X. Epub 2022 Jul 27. Clinical Trial.
5- Gooptu M, Bolan˜os-Meade J, Koreth J. Expanding post-transplant cyclophosphamide to matched unrelated donor transplants and beyond Blood Rev 2023 Feb 3;101053. doi: 10.1016/j.blre.2023.101053. PMID: 36822991 DOI: 10.1016/j.blre.2023.101053
6- Bacigalupo A, Benintende G.Bone marrow transplantation for acquired aplastic anemia: What's new. Best Pract Res Clin Haematol. 2021 Jun;34(2):101284. doi: 10.1016/j.beha.2021.101284. Epub 2021 Jul 7.
7- DeZern A and Brodsky R. Combining PTCy and ATG for GvHD prophylaxis in non-malignant diseases. Combining PTCy and ATG for GvHD prophylaxis in non-malignant diseases. Blood Rev 2022 Sep 26;101016. doi: 10.1016/j.blre.2022.101016.
8- Devillier R, Eikema DJ, Dufour C et al. Graft-versus-host disease and relapse/rejection free survival after allogeneic transplantation for idiopathic severe aplastic anemia: a comprehensive analysis from the SAAWP of EBMT. Haematologica. 2023 Mar 23.doi: 10.3324/haematol.2022.281876. PMID: 3695116
9-Gupta V, Eapen M, Brazauskas R, et al. Impact of age on outcomes after bone marrow transplantation for acquired aplastic anemia using HLA- matched sibling donors. Haematologica. 2010;95(12):2119-2125.
10-Maury S, Bacigalupo A, Anderlini P, et al; Severe Aplastic Anemia Working Party, European Group for Blood and Marrow Transplantation (EBMT- SAAWP). Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a com- parison with conventional conditioning regimen. Haematologica. 2009; 94(9):1312-1315.
11- Maury S, Aljurf M. Management of adult patients older than 40 years refractory to at least one immunosuppressive course: HLA-identical sibling HSCT using fludarabine-based conditioning. Bone Marrow Transplant. 2013;48(2):196-197.
12-Passweg JR, Socie´ G, Hinterberger W, et al. Bone marrow transplantation for severe aplastic anemia: has outcome improved? Blood. 1997;90(2): 858-864.
13-Bacigalupo A, Brand R, Oneto R, et al. Treatment of acquired
severe aplastic anemia: bone marrow transplantation compared with immunosuppressive therapy–The European Group for Blood and Marrow Transplantation experience. Semin Hematol. 2000;37(1):69-80.

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14- Devillier R, Dalle JH, Kulasekararaj A, et al. Unrelated alternative donor transplantation for severe acquired aplastic anemia: a study from the French Society of Bone Marrow Transplantation and Cell Therapies and the Severe Aplastic Anemia Working Party of EBMT. Haematologica. 2016;101(7):884-890.
15-Giammarco S, PeffaultdeLatour R, Sica S et al. Transplant outcome for patients with acquired aplastic anemia over the age of 40: has the outcome improved? blood® 26 APRIL 2018 | VOLUME 131, NUMBER 17
16-Clay J, Kulasekararaj AG, Potter V, et al. Nonmyeloablative peripheral blood haploidentical stem cell transplantation for refractory severe aplastic anemia. Biol Blood Marrow Transplant 2014;20(11):1711–6.
17-DeZern AE, Zahurak M, Symons H et al. Alternative donor transplantation with high-dose post-transplantation cyclophosphamide for refractory severe aplastic Anemia. Biol Blood Marrow Transplant 2017;23(3): 498–504.
18-Prata PH, Ekima DJ, Afansyev B et al. Haploidentical transplantation and posttransplant cyclophosphamide for treating aplastic anemia patients: a report from the EBMT Severe Aplastic Anemia Working Party. Bone Marrow Transplant. 2020 Jun;55(6):1050-1058. doi: 10.1038/s41409-019-0773-0. Epub 2019 Dec 16.
19- DeZern AE, Eapen M, Wu J et al. Haploidentical bone marrow transplantation in patients with relapsed or refractory severe aplastic anaemia in the USA (BMT CTN 1502): a multicentre, single-arm, phase 2 trial. Lancet Haematol. 2022 Sep;9(9):e660-e669. doi: 10.1016/S2352-3026(22)00206-X. Epub 2022 Jul 27.
20-Luznik L, O'Donnell PV, Symons HJ, et al. HLA- Haploidentical bone marrow transplantation for hematologic malignancies using Nonmyeloablative conditioning and high-dose, Posttransplantation cyclophosphamide. Biology of blood and marrow transplantation. J Am Soc Blood Marrow Transplant 2008;14:641–50.
21- Niederwieser D, Pepe M, Storb R et al. Improvement in rejection, engraftment rate and survival without increase in graft-versus-host disease by high marrow cell dose in patients transplanted for aplastic anaemia. Br J Haematol 1988;69(1):23–8.
22-Bolan̋os-Meade J, Reshef R, Fraser R, et al. et al. Three prophylaxis regimens (tacrolimus, mycophenolate mofetil, and cyclophosphamide; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc) versus tacrolimus and methotrexate for prevention of graft-versus-host disease with haemopoietic cell transplantation with reduced- intensity conditioning: a randomised phase 2 trial with a non-randomised contemporaneous control group (BMT CTN 1203). Lancet Haematol 2019;6: e132–43.
23-Holtan S. Post-transplant cyclophosphamide, tacrolimus, and mycophenolate Mofetil as the new standard for graft-versus-host disease (GVHD) prophylaxis in reduced intensity conditioning: results from phase III BMT CTN 1703. In: ASH; 2022. Available at, https://ash.confex.com/ash/2022/webprogram/Paper171463. html [Accessed January 10, 2023].
24- Broers AEC, de Jong CN, Bakunina K, et al. Posttransplant cyclophosphamide for prevention of graft-versus- host disease: results of the prospective randomized HOVON-96 trial. Blood Adv 2022;6:3378–85.
25- Dezern AE, Luznik L, Fuchs EJ, et al. Post-transplantation cyclophosphamide for GVHD prophylaxis in severe aplastic anemia. Bone Marrow Transplant 2011 Jul;46(7):1012–3. https://doi.org/10.1038/bmt.2010.213. Epub 2010 Sep 13. PMID: 20838390; PMCID: PMC4161041.
26-GeorgeB, PnN, DevasiaAJ, et al. Post-transplantcyclophosphamideassolegraft-versus-hostdiseaseprophylaxisisfeasible in patients undergoing peripheral blood stem cell transplantation for severe aplastic anemia using matched sibling donors. Biol Blood Marrow Transplant 2018 Mar;24(3):494–500. https://doi.org/10.1016/j.bbmt.2017.10.034. Epub 2017 Oct 31.
27-Kwon M, Bail´en R, Pascual-Casco´n MJ, et al. Posttransplant cyclophosphamide vs cyclosporin A and methotrexate as GVHD prophylaxis in matched sibling transplantation. Blood Adv 2019 Nov 12;3(21):3351–9. https://doi.org/10.1182/ bloodadvances.2019000236.

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28- Amy E DeZern, Marianna L Zahurak, et al. Haploidentical BMT for severe aplastic anemia with intensive GVHD prophylaxis including posttransplant cyclophosphamide Blood Adv 2020 Apr 28;4(8):1770-1779. doi: 10.1182/bloodadvances.2020001729.
29-Deeg, H. J. et al. Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. Blood 87, 386–392 (1996).
30-Deeg, H. J. et al. Long-term outcome after marrow transplantation for severe aplastic anemia. Blood 91, 3637–3645 (1998).
31-Vo, P., Onstad, L., Flowers, M. E. & Storb, R. Cancers after HLA-matched related bone marrow transplantation for aplastic anemia. Bone Marrow Transplant 57, 83–88 (2022).
32-Dominietto A, Tedone E, Soracco M, et al. In vivo B-cell depletion with rituximab for alternative donor hemopoietic SCT.Bone Marrow Transplant. 2012;47(1):101-106.
33- Chemaly RF, Ullmann AJ, Stoelben S et al. Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. N Engl J Me. 2014 May 8;370(19):1781-9. doi:10.1056/NEJMoa1309533.
34- Thorlund K, Dron L, Park JJH, Mills EJ. Synthetic and External Controls in Clinical Trials - A Primer for Researchers. Clin Epidemiol. 2020 May 8;12:457-467. doi: 10.2147/CLEP.S242097. PMID: 32440224; PMCID: PMC7218288
35- Lambert J, Lengliné E, Porcher R, Thiébaut R, Zohar S, Chevret S. Enriching single-arm clinical trials with external controls: possibilities and pitfalls. Blood Adv. 2022 Dec 19:bloodadvances.2022009167. doi: 10.1182/bloodadvances.2022009167. Epub ahead of print. PMID: 36534147
36- Rosenbaum PR, Rubin DB. The central role of the propensity score in observational studies for causal effects. *Biometrika*, 70(1):41–55, 1983.
37- Peffault de Latour R. Transplantation for bone marrow failure: current issues. *Hematology Am Soc Hematol Educ Program*. 2016 Dec 2;2016(1):90-98.
38- Bacigalupo A, Sica S. Alternative donor transplants for severe aplastic anemia: current experience. *Semin Hematol*. 2016;53(2):115-119
39- Puckrin R, Chi Fung Kwan A. Corticosteroids as graft-versus-host disease prophylaxis for allogeneic hematopoietic cell transplant recipients with calcineurin inhibitor intolerance. Cytotherapy, 25(2023)1101-1106.

19 LIST OF ADDENDA

The addendum not included here are attached independently of the protocol. These addenda can be modified (change of addendum version) without modifying the protocol version.

19.1 List of investigators

19.2 Serious Adverse Events notification form

19.3 Pregnancy notification form

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19.4 Questionnaires or scales

19.4.1 Quality of life questionnaire

EORTC QLQ-C30-V3

19.4.2 Scales

19.4.2.1 CTC-AE -Toxicity Grading scale for determining the severity of adverse event

https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

19.4.2.2 Acute GVH according to MAGIC CONSORTIUM 2016

According to Harris et al. Biology of Blood and Marrow Transplantation 2016; 22 (1): 4-10

1. GVHD target organ staging

GVHD Target Organ Staging

Stage	Skin (active erythema only)	Liver (bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	< 2 mg/dl	No or intermittent nausea, vomiting or anorexia	Adult: < 500 ml/day or <3 episodes/day Child: < 10 ml/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2–3 mg/dl	Persistent nausea, vomiting or anorexia	Adult: 500–999 ml/day or 3–4 episodes/day Child: 10–19.9 ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25 – 50% BSA	3.1–6 mg/dl	-	Adult: 1000–1500 ml/day or 5–7 episodes/day Child: 20 – 30 ml/kg/day or 7–10 episodes/day
3	Maculopapular rash > 50% BSA	6.1–15 mg/dl	-	Adult: >1500 ml/day or >7 episodes/day Child: > 30 ml/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation > 5% BSA	>15 mg/dl	-	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

2. Overall clinical grade (based upon most severe target organ involvment) :

- Grade 0: No stage 1-4 in any of the organs

- Grade I: Stage 1-2 in the skin with no liver or upper or lower digestive tract involvement

- Grade II: Stage 3 in the skin and/or stage 1 in the liver and/or stage 1 in the upper or lower digestive tract

- Grade III: Stage 2-3 liver and/or stage 2-3 low bowel + stage 0-3 skin and/or stage 0-1 high bowel

- Grade IV: Stage 4 skin, liver or low GI with stage 0-1 high GI

19.4.2.3 Chronic GVH

According to according to the NIH classification published in 2005 (selon Filipovitch et al.,BBMT 2005).

The diagnosis of chronic GVHD is made if there is a distinctive sign (1 alone is sufficient) or evocative signs associated with a supplementary examination in favor (biopsy, for example). We then define:
A- Classical chronic GvHD in patients with only evidence of chronic GvHD
B- The overlap syndrome when a patient presents both signs of acute GvHD and chronic GvHD
C- Late acute GvHD, which corresponds to exclusive signs of acute GvHD without signs of chronic GvHD occurring after J100.

The severity of chronic GvHD is defined by the number of affected organs.

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Affected organ	Mild	Moderate					Severe		
Number of organ affected	1 or 2 without significant dysfunction	≥3	**or**	≥ 1	**or**	lung	≥ 1	Or	lung
Score of the achievement of each organ	1 (except lung)	1		2		1	3		≥2

Manifestation de la GVHD chronique

Dans le cas de manifestations cliniques parallèles comme un épisode infectieux ou une réaction médicamenteuse, cette évaluation ne sera pas prise en compte.

Un Karnofsky < 60% avec une perte de poids > 15% et des infections récurrentes sont en général des signes de GVHD chronique sévère.

Manifestation de GVHD chronique

Les anomalies cliniques selon les organes touchés permettant d'évaluer la GVHD chronique sont les suivantes :

Peau Erythème, sécheresse, prurit, changement de pigmentation (vitiligo, hyperpigmentation) plaques papulosquameuses, nodules, exfoliation, rash maculo-papulaire ou urticaire, sclérodermie, morphée (une ou plusieurs lésions lisses indurées et circonscrites)

Ongles Onychodystrophie, onycholyse, striés, fendus.

Cheveux Canitie prématurée (cuir chevelu, cils, sourcils), alopécie, amincissement du cuir chevelu, raréfaction de la pilosité corporelle.

Bouche Sécheresse, brûlures, gingivite, mucite, atrophie gingivale, érythème, lichen, ulcères, atrophie labiale, changement de pigmentation, contracture de la bouche, caries dentaires.

Yeux Sécheresse, brûlures, photophobie, douleur, larmoiement, sensation de grain de sable

Organes génitaux Sécheresse, sténose vaginale, dyspareunie, érythème vulvaire, atrophie génitale, lichen

Foie Élévation du bilan hépatique sanguin sans autre cause connue. En l'absence d'une autre atteinte organique, une biopsie est nécessaire pour confirmer le diagnostic.

Poumons Bronchiolite oblitérante, toux, sifflements, dyspnée d'effort, bronchites chroniques ou sinusites.

Tube digestif Anorexie, nausées, vomissements, perte de poids, diarrhées, dysphagie, malabsorption.

Fasciite Ankylose et réduction des mouvements, avec occasionnellement gonflement, douleurs, crampes, érythème et induration, atteignant le plus fréquemment les avant- bras les poignets et les mains, les chevilles, les jambes et les pieds, incapacité d'étendre les poignets sans fléchir les doigts ou les coudes, contractures.

Muscles Faiblesse proximale, crampes.

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Squelette	Arthralgies proximales des articulations des os du bassin, et parfois d'articulation moins importantes
Séreuses	Douleurs pulmonaires ou cardiaques secondaires à une pleurésie ou une péricardite.

Gradation de GVHD chronique par organe :

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	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN

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19.4.2.4 Gradation of severity of MVO

According to Bone Marrow Transplantation (2023) 58:749–754; https://doi.org/10.1038/s41409-023-01992-8

Table 3. Severity grading of SOS/VOD in adults.

	Mild[a]	Moderate[a]	Severe	Very severe – MOD[b]
Time since clinical symptoms of SOS/VOD	>7 days	5–7 days	≤4 days	Any time
Bilirubin (mg/dl)	≥2 and <3	≥3 and <5	≥5 and <8	≥8
Bilirubin kinetic			Doubling within 48 h	
Transaminases	≤2 × normal	>2 and ≤5 × normal	>5 and ≤8 × normal	>8 × normal
Weight increase			≥5%	≥10%
Renal function (creatininemia)	Baseline at transplant	<1.5 × baseline at transplant	≥1.5 and <2 × baseline at transplant	≥2 × baseline at transplant or diagnosis of MOD[b]

Patients belong to the category that fulfilled 2 or more criteria. If patients fulfilled 2 or more criteria in 2 different categories, they must be classified in the most severe category between both.
[a]In case of presence of 2 or more risk factors for SOS/VOD, patients should be in the upper grade.
[b]Patients with multiple organ dysfunction (MOD) must be classified as very severe, MOD is defined as ≥2 organs from the SOFA score with a score ≥2 or an increase ≥2 or organ dysfunction for patients with underlying organ involvement (Table 4).

Table 4. Sequential Organ Failure Assessment (SOFA) adapted from Vincent et al., [33].

Organ System, Measurement	0	1	2	3	4
Respiration PaO2/FiO2, mmHg	Normal	<400	<300	<200 with respiratory support	<100 with respiratory support
Coagulation Platelets x10^3/mm^3	Normal	<150	<100	<50	<20
Liver bilirubin, mg/dL (µmol/l)	Normal	1.2–1.9 (20–32)	2.0–5.9 (33–101)	6.0–11.9 (102–204)	>12.0 (>204)
Cardiovascular hypotension	Normal	MAP < 70 mmHg	Dopamine <5 or dobutamine (any dose)[a]	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Central Nervous System Glasgow Coma Score	Normal	13–14	10–12	6–9	<6
Renal Creatinine, mg/dL (µmol/l) or Urine output	Normal	1.2–1.9 (110–170)	2.0–3.4 (171–299)	3.5–5.0 (300–440) or <500 mL/day	>5.0 (>440) or <200 mL/day

[a]Adrenergic agents administered for at least 1 h (doses given are in mcg/kg/min).
Abbreviations: *MAP* mean arterial pressure

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