Allogeneic hematopoietic stem cell transplantation in patients with low or intermediate-1 myelodysplastic syndrome: A prospective multicenter phase II study based on donor availability on behalf of the GFM & SFGM-TC

Sponsor: French MDS group (GFM)

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Principal Sponsor: Groupe Francophone des Myélodysplasies Service d'Hématologie Séniors Hôpital Saint-Louis / Université Paris 7 1, avenue Claude Vellefaux 75475 Paris cedex 10 **Collaboration:** **SFGM-TC** SOCIETE FRANCAISE DE GREFFE DE MOELLE ET DE THERAPIE CELLULAIRE Service d'Hématologie-Pavillon Marcel Bérard-1G Centre Hospitalier Lyon Sud 165, chemin du Grand Revoyet 69495 Pierre-Bénite	**Protocol Review Committee:** Pr Norbert Vey (Institut Paoli Calmettes, Marseille) Pr Mohamad Mohty (Hôpital St-Antoine, Paris) Dr Myriam Labopin (Hôpital St-Antoine, Paris) **Supported by**: Novartis Neovii

Study contact information

Role in study	Name	Address
Investigators Coordinators	**Marie Robin**	**Service d'hématologie-greffe Hôpital St Louis / Université Paris 7 1 avenue Claude Vellefaux 75475 Paris cedex 10, France Phone : 33(0)1 42 49 96 60 Fax : 33(0)1 42 49 96 36 Email : marie.robin@aphp.fr**
	Marie Sébert	**Service d'hématologie séniors Hôpital St Louis / Université Paris 7 1 avenue Claude Vellefaux 75475 Paris cedex 10, France Phone : 33(0)1 71 20 70 23 Fax : 33(0)1 71 20 70 20 Email : marie.sebert@aphp.fr**
Biological studies	**Claude Preudhomme**	**Laboratoire d'hématologie cellulaire CHRU Lille Bd du Professeur J Leclercq 59037 Lille cedex, France Phone : 33(0)3 20 44 47 83 Fax : 33(0)3 20 44 69 89 Email : claude.preudhomme@chru-lille.fr**
Biostatistics and Data management	**Sylvie Chevret**	**SBIM, Hôpital Saint Louis, 1 avenue Claude Vellefaux 75010 Paris, France Phone : 33(0)1 42 49 97 42 Fax : 33(0)1 42 49 97 45 Email : chevret@dbim.jussieu.fr**
Study Manager	**Fatiha Chermat Rosa Sapena**	**Service d'hématologie séniors Hôpital Saint-Louis / Université Paris 7 1 avenue Claude Vellefaux 75475 Paris cedex 10, France Phone : 33(0)1 71 20 70 59/81 Fax : 33(0)1 71 20 70 38 Email : fatiha.chermat-ext@aphp.fr / rosa.sapena-ext@aphp.fr**

Synopsis

Title	Allogeneic hematopoietic stem cell transplantation in patients with low or intermediate-1 risk myelodysplastic syndrome: A prospective multicenter phase II study based on donor availability on behalf of the GFM & SFGM-TC
Short title	**MDS-ALLO-RISK**
Promotor	Groupe Francophone des Myélodysplasies (GFM)
Coordinators	Marie Robin, PhD Marie Sébert, PhD
Investigators	Centers from the French MDS Group (see list of centers) and centers from the European community
Primary objective	Comparison of survival in patients with or without a matched donor (8/8 at molecular level unrelated donor or matched sibling) at 3 years
Secondary objective	To assess the prognostic factors, and quality of life
Study design	Comparative non-randomized phase II clinical trial
Inclusion criteria	✓ Signed inform consent ✓ Classical IPSS intermediate 1 or low myelodysplastic syndrome associated with at least one poor prognosis feature: 1) Intermediate or higher risk revised IPSS 2) RBC transfusion dependent anemia and failure to 2 or more lines or therapy (including EPO, Lenalidomide or demethylating agent…) with the exception of pure RARS with isolated SF3B1 mutation 3) thrombocytopenia < 20 G/L requiring transfusion 4) neutropenia < 0.5 G/L associated with severe infection (defined as requiring hospitalization) 5) 5q- and lenalidomid failure especially but not only with TP53 mutated ✓ Patient aged ≥ 18 and < 70 years For young patients, 18-45 years, Fanconi disease and dyskeratosis should be ruled out ✓ For whom a transplantation from a matched donor, (8/8 unrelated donor or matched sibling), is considered irrespective of donor availability ✓ Performance status lower than 3 (ECOG 0, 1, or 2) at time of screening ✓ Negative pregnancy test and adequate contraception (including male wishing to father) if relevant ✓ Wash-out of at least 30 days since a previous treatment with Vidaza®, Lenalidomide, EPO or any other treatment inducing cytopenias
Exclusion criteria	✓ Classical IPSS int-2 or high ✓ ARSI with SF3B1 mutation ✓ Transformation into acute myeloid leukemia ✓ Severe active infection or any other uncontrolled severe condition

	✓ Symptomatic cardiac failure ✓ Renal clearance < 60 ml/min ✓ Symptomatic respiratory chronic failure ✓ Aspartate transaminase (AST) or Alanine transaminase (ALT) > 3 x ULN or total bilirubin > 2 x ULN (except moderate unconjugated hyperbilirubinemia due to intramedullary hemolysis or Gilbert syndrome) ✓ Prior malignancy (except in situ cervix carcinoma, limited basal cell carcinoma, or other tumors if not active during the last 3 years) ✓ MDS with the following causal germline disease : Fanconi anemia, GATA2 related syndromes and telomere disorders.
Number of patients	105 (62 in group A, 43 in group B)
Statistical analysis and calculation of number of patients	The objective of this study is to demonstrate an improvement of the overall survival in patients with a donor that is reaching **70% at 36 months compared to 40% in those without a donor.** To test this hypothesis (HR=0.39) based on a two-sided log-rank test, 50 events are required, and 105 patients are needed with a 80% power and type I error rate at 5%, with a probability to identify a donor at 70%
Study duration	Patient recruitment: **36 months** Minimal Patient follow-up: **24 months and until the end of the trial scheduled 60 months after first inclusion (approximately June 2021)** Total duration of the study: **60 months**
Number of centers	20-25
Administration and authorities	**CNIL** **ANSM** **CPP** **Clinicaltrials.gov**

List of abbreviations

AE	**Adverse Event**
ANC	**Absolute Neutrophil Count**
ANSM	**Agence nationale de sécurité du médicament et des produits de santé**
AML	**Acute Myeloid Leukemia**
ALT	**Alanine Aminotransferase (or Serum Glutamate-Pyruvate Transaminase)**
AP	**Alkaline Phosphatase**
AST	**Aspartate Aminotransferase (or Serum Glutamate-Oxylate Transaminase)**
ATG	**Antithymoglobulin**
BM	**Bone Marrow**
CBC	**Complete Blood Count**
CI	**Coordinating Investigator**
CMML	**Chronic Myelomonocytic Leukemia**
CMV	**Cytomegalovirus**
CPK	**Serum creatine phosphokinase**
CPP	**Comité de Protection des Personnes**
CR	**Complete Remission**
CRA	**Clinical Research Assistant**
CRF	**Case Report Form**
CTCAE	**Common Terminology Criteria for Adverse Events**
d	**Day**
DSMB	**Data and Safety Monitoring Board**
EBV	**Epstein Barr Virus**
ECG	**Electrocardiogram**
ECOG	**Eastern Cooperative Oncology Group**
ESA	**Erythropoiesis stimulating agents**
FAB	**French-American-British**
GCP	**Good Clinical Practice**
G-CSF	**Granulocyte-colony stimulating factor**
GFM	**Groupe Francophone des Myélodysplasies**
GVHD	**Graft-versus-host disease**
HLA	**Human leucocytes antigens**
h	**Hour**
HCV	**Hepatitis C Virus**
HSCT	**Hematopoietic Stem Cell transplant**
IPSS	**International Prognostic Scoring System**
LDH	**Lactate Dehydrogenase**
LIC	**Liver iron content**
MAC	**Myelo-ablative conditioning regimen**
MDS	**Myelodysplastic Syndrome**
NRM	**Non relapse mortality**
NTBI	**Non-transferin-bound iron**
OS	**Overall Survival**
PS	**Performance Status**
RBC	**Red Blood Cell**
RCT	**Randomized Controlled Trial**
REB	**Research Ethics Board**
RIC	**Reduced intensity conditioning regimen**
SAE	**Serious Adverse Event**
SGOT	**Serum Glutamate-Oxalate Transferase (or Aspartate Aminotransferase)**
SGPT	**Serum Glutamate-Pyruvate Transferase (or Alanine Aminotransferase)**
ULN	**Upper Limit of Normal**
WBC	**White Blood Cell**
WHO	**World Health Organisation**
SF	**Serum Ferritin**

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1. Background

1.1. Usual management of the lower risk MDS patients

Myelodysplastic syndromes (MDS) are bone marrow stem cell disorders predominating in the elderly, characterized by ineffective hematopoiesis with marrow dysplasia, blood cytopenias and a high risk of transformation to acute myeloblastic leukemia (AML). MDS patients are a heterogeneous group of patients with survival expectancy ranging from a few months to more than 10 years.

Prognosis of MDS is largely determined by an International Prognostic Scoring System (IPSS)[1]. Lower risk patients are defined according to the classical IPSS as the low and intermediate-1 MDS patients meaning that their expected median survivals from the date of diagnosis are 5.7 and 3.4 years according to the original publication in 1997. These patients are characterized by chronic cytopenia and particularly chronic anemia and represent 70% of MDS patients at diagnosis. These patients have poor quality of life due to chronic anemia as well as an increased risk of infections and haemorrhage related to cytopenia[2]. These lower risk patients can also evolve to a higher risk and eventually transform into AML. Concerning treatment management, a watchful-waiting strategy in asymptomatic patients can be an option. Usual treatments consist in an improvement of red blood cell (RBC) count using RBC transfusions or erythropoiesis stimulating agents (ESA) sometimes combined with granulocyte-colony stimulating factor (G-CSF) if neutropenia. Low dose chemotherapy can also be used in these patients (aracytine, melphalan). All these treatments remain palliative. Recombinant erythropoietin or darbopoietin are usually used in lower risk patients without 5q deletion (del 5q) while Lenalidomide is used in patients with del 5q[3,4-6]. Anti-T cell globulin (ATG) are sometimes proposed in cytopenic young patients with marrow hypocellularity, with better response in patients who are HLA DR15[7-10]. In other situation or in cases of refractoriness to usual treatment, there is no consensus[11,12] and prospective studies are encouraged to test drugs that have not been approved yet.

1.2. Poor prognostic features in the lower risk MDS patients

Several evidences have shown that the lower risk patient group is a heterogeneous group and some of these patients will have a poorer outcome than expected according to their IPSS. The MD Anderson Cancer Centre (MDACC) team has developed a clinical and biological score taking into account age in the lower risk MDS patients age (> 60 years), haemoglobin (10 g/dL), platelet count (50 G/L) and marrow blast count (> 3%). This “MDACC” score is able to discriminate 3 groups inside the lower risk with median survival as follow: 5.9, 2.65 and 1.11 years[13]. Furthermore, the IPSS has been recently revised giving 5 categories (instead of 4) which differ slightly with the previous score giving a higher score to 25% of the lower risk according to classical IPSS. This quarter of patients are generally categorized in the “intermediate” risk with a median life expectancy at 3

years[14]. Another poor prognostic factor in the low risk patients is the failure ESA. Kelaidi et al have reported a worsened overall survival in low risk patients refractory or with an early relapse after erythropoiesis-stimulating agents[15] and it has been also confirmed by others[5]. Molecular analyses have recently emerged as potential prognostic markers. Indeed, some somatic mutations can have an adverse impact on outcome, particularly for the lower risk patients. Acquired mutations have been detected in several genes and most frequent mutations (> 5%) have been reported in SF3B1, TET2, RUNX1, ASXL1, SRSF2, TP53, U2AF1, NRAS/KRAS, DNMT3A, ZRSR2, EZH2, less frequently in CBL, IDH2, KRAS, NPM1, NRAS, RUNX1, NPM1, ETV6, SF1, SETBP1, SF3A1, U2AF65, PRPF40B[13,16-19]. Most of these mutated genes are not specific but have been reported present in 50 to 70% of patients with normal cytogenetic. More specifically, lower risk del 5q MDS patients with TP53 mutations appear resistant to lenalidomide and have a higher risk of AML occurrence. TP53 mutations in low-risk myelodysplastic syndromes with del 5q predict disease progression[20-22] while Bejar et al did not retain TP53 mutation as a poor prognostic factors in his low risk MDS cohort[13]. The screening of such somatic mutations is not routinely done yet but still remains under prospective and retrospective investigations before engaging molecular based treatment strategies.

1.3. Iron overload in the lower risk MDS

1.3.1. Physiopathology and potential clinical consequence of iron overload

In physiological conditions, iron released into circulation binds transferrin with high affinity. Transferrin saturation is calculated as a ratio of serum iron to total iron-binding capacity, approximately 30% under normal conditions. When transferrin's iron-binding capacity is exceeded, non–transferrin-bound iron (NTBI) is produced. NTBI is found after transferrin saturation exceeds 80%[23]. Labile plasma iron is redox-active NTBI, permeates cell membranes, and causes cellular damage via production of reactive oxygen species resulting in premature apoptosis, cell death, tissue and organ damage eg, iron-overload–associated liver cirrhosis, diabetes and other endocrinopathies, and cardiomyopathy, and, if untreated, death[24,25]. Clinical data from iron overload comes from transfusion-dependent beta-thalassemia patients that have a natural history of transfusion iron overload resulting in significant morbidity and mortality[26]. Evidence of iron overload in MDS patients is also mounting[27-31] and it is suggested that chronic anemia can lead to increased morbidity, especially as a result of cardiac failure, falls, fatigue, and lower quality of life[2,32]. Transfusion-dependency has been reported as an adverse event in lower risk patients whatever the cytogenetic group according to IPSS decreasing the survival from at least 12 months as compared to non-transfused patients[33]. The number of RBC transfusions required per month has been also reported to progressively decrease survival in low risk MDS patients[34]. However, because MDS occurs in adulthood (when propensity for comorbid diseases is high) when patients have a significantly shorter life expectancy, the impact of iron overload and benefit from iron chelation may be

somewhat different relative to beta-thalassemia patients[2,32,35]. Nevertheless, it has been documented that MDS patients who received regular RBC transfusions will develop an iron overload in the liver and less frequently in the heart[29,36]. Furthermore, in a murine model, it has been reported that iron overload is able to induce hematopoiesis dysfunction, impairing hematopoietic microenvironment[37]. But it is still debated if iron overload is a substantial primary cause of death in MDS patients. Some studies reported a poorer survival in patients with high ferritin level[33].

In the setting of allogeneic transplant, retrospective studies have reported that transfusion burden and/or ferritin levels > 1000 ng/mL were associated with higher risk for non-relapse mortality (NRM), acute GVHD and severe infections[38,39]. Increased risk of infection after HSCT has been also reported in MDS patients with iron overload in a retrospective setting[40-43]. Recently, it has been reported that high NTBI levels in the early phase after HSCT can predict grade III or IV toxicity[44]. Actually, because ferritin is an acute phase reactant, its elevation in serum may betray inflammatory states, including active infection or more advanced disease status, which are expected to confer an adverse prognosis in HSCT independent of iron overload.

The prospective studies lead to conflicting results concerning the impact of iron overload on the post-transplant non-relapse mortality. Armand et al recently published a meta-analysis on the 4 prospective studies focusing on the role of iron overload for post-transplant outcome[45]. Finally, this meta-analyze shows that ferritin level > 1000 ng/L is a risk factor for poorer overall survival in the whole cohort but does not specifically impact the non-relapse mortality specifically and the liver iron content (LIC) (> 5 or > 7 mg/gr) has no clear significant impact on outcome. Nevertheless, when regarding only RIC, LIC (> 7 mg/gr, hazard ratio: 2.2) has an impact on NRM but not on overall survival. Unfortunately, for myelo-ablative conditioning regimen (MAC), LIC and ferritin levels could be tested only for overall survival and showed no impact[46-49]. A subgroup analysis taking only patients with MDS-AML (25%) did not give significant results. Finally, the meta-analyze remains inconclusive because does not bring consistent evidences of mortality related to iron overload. Actually, we don't know if ferritin level, which is reported to have some negative impact on outcome, reflects the iron overload or is a surrogate of severity in these patients.

1.3.2. Treatment of iron overload

Chelating agents have demonstrated that they were efficient for iron depletion. A large prospective study testing deferasirox in 1744 MDS patients receiving chronic RBC transfusions for various reasons reported that serum ferritin and NTBI was decreased under treatment but 66% suffered from reversible adverse events (gastro-intestinal symptoms, skin rash, increase in serum creatinine) requiring dose reduction[50,51].

A more specific study on lower risk MDS patients reported also a normalization of NTBI in all patients but treatment discontinuation mainly due to gastro-intestinal disturbances. Deferasirox reduces serum ferritin and labile plasma iron in RBC transfusion-dependent patients with myelodysplastic syndrome[52].

Except from biological changes with iron chelation agents, it has been also reported that iron chelation itself can induce haematological improvement in MDS patients[53,54].

Concerning improvement of quality of life, one prospective study in MDS transfused patients failed to demonstrate the role of chelation agents[55] but no controlled randomized studies comparing chelation versus placebo are available.

In the setting of transplant, conflicting studies exist reporting an excess of non-relapse mortality in patients with a high ferritin level or who received massive transfusion while prospective studies give conflicting results (see above) failing to demonstrate an association between liver iron overload and post-transplant mortality[56,57].

1.4. Allogeneic hematopoietic stem cell transplantation

Transplantation in MDS patients

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for MDS. Unfortunately, results of this treatment are compromised by NRM occurring in 10 to 40%, while relapses also occur in 10 to 40% of the cases. Long-term survival after HSCT does not exceed 40-50% for these reasons and HSCT is mainly performed in IPSS higher risk MDS patients (ie int 2 and high risk)[58,59,60]. Retrospective studies have demonstrated that higher risk MDS patients benefit from HSCT at time of diagnosis while HSCT should be post-pone in lower risk patients[61,62,63]. Decision to transplant lower risk patients is usually based on disease evolution to a higher risk. The success of HSCT depends from patients, disease and transplant characteristics. Best outcome has been reported in young patients, at low risk who received a graft from an HLA-identical donor[59,64]. In these favourable conditions (low risk in patients younger than 50 years), in a recent Italian registry study, low risk patients transplanted without comorbidities have 94% probability of survival at 5 years[65]. Concerning comorbidities and age, it has been reported that high comorbidities score increased NRM > 50% while elderly are at higher risk of disease progression and NRM, elderly with comorbidities have particular high NRM[66,67]. Concerning the risk related to the disease (high risk according to IPSS), particularly cytogenetics, it is often the most powerful prognostic factor after transplant. Very poor cytogenetic is associated with post-transplant overall survival (OS) < 10%[68]. Some pre-graft treatment (vidaza or chemotherapy) can reduce marrow blast count but rarely induce cytogenetic remission and such a treatment is still debated, due to the potential comorbidities and failure associated with them[69].

Transplant procedure influences also outcome. The advent of reduced intensity conditioning (RIC) regimen has decrease NRM allowing transplantation in older patients (representing the majority of patients with MDS) or with some comorbidities[70,71,72]. In fact, all retrospective studies comparing RIC versus MAC regimen reported similar overall survival because a lower NRM is counterbalanced by a higher relapse rate[73,58,74] and a prospective randomized study in MDS patients has same conclusions (not published yet, presented at ASH 2014, San Francisco, Abstract 320, Kroger et al). In disease in remission, there are few evidences to do MAC in patients older than 50 years while active disease with high blast count are less controlled with HSCT preceded by a RIC[75]. Concerning the source of stem cells, HLA-identical sibling and HLA-identical unrelated donor are the preferred source, given similar results while alternative source of stem cells (HLA mismatched unrelated or unrelated cord blood) seem given worse results in registries studies[76,77].

Finally, Della Porta study perfectly resumes potential risk factors for outcome in their multivariate analysis: age (> 50 years); IPSS-R; comorbidity index; monosomal karyotype; type of donor (sibling versus matched unrelated)[65].

Anti-T Lymphocyte (ATG) in the transplant setting

One of the major drawbacks for HSCT is graft-versus-host disease (GVHD). The use of ATG has been reported to reduce acute and chronic GVHD incidence. Many retrospective studies have reported that acute and chronic GVHD was reduced with ATG use before transplant[78,79] while some other studies reported discordant results. Among them Soiffier et al analyzed 1676 adult patients received a transplant after RIC and the potential impact of ATG[80]. In this study, alemtuzumab, rabbit ATG, horse ATG and no ATG were compared. “ATG” (rabbit and horse ATG were mixed) did not decrease acute GVHD incidence and had a negative impact on survival. This very large study suffers from heterogeneity between groups and ATG dosage which could not be analyzed. A randomized study comparing MAC +/- ATG-Fresenius (ATG-F / GRAFALON) concluded that (1) grade II-IV acute GVHD was reduced in ATG-F (GRAFALON) arm; (2) chronic GVHD was also reduced; (3) probability of survival without immunosuppressive treatment was higher with ATG-F (GRAFALON) (53% vs 17%); without impacting overall survival[81,82]. Another randomized study using Thymoglobulin at 7.5 mg/kg vs 15 mg/kg vs no ATG concluded that there was a decrease of severe acute GVHD and long-term chronic GVHD but an excess of severe infection with higher dose Thymoglobulin[83,84]. Many teams have worked on ATG dosage, particularly in the RIC setting in order to find the right dosage which can prevent GVHD without increasing infection and relapse rates. Dose effect in Thymoglobulin has been reported in RIC with a better dose around 5 mg/kg (only HLA-matched donor)[85,86]. Concerning ATG-F (GRAFALON), usual dose in the setting ranges from 30 to 60 mg/kg[81,87,88] while

lower dosage (5-10 mg/kg) have been experimented without particular lower efficiency[89]. In France, both ATG-F (GRAFALON) and Thymoglobulin have been approved for GVHD prophylaxis in conditioning regimen.

Transplantation in the lower risk patients

In all retrospective studies, the proportion of low risk patients is around 20% indicating that transplant decision has been taken in a substantial proportion of patients. As reported above, survival after transplantation is better in these low risk patients ranging from 50 to 94% according to other potential prognostic factors[60,68,65]. On the other hand, as detailed in the previous section, these lower risk patients have not always prolonged survival as estimated by classical IPSS because other parameters potentially have an impact on their survival. Among these prognostics factors: there are no response to treatment, poor cytogenetic, profound cytopenia requiring regular transfusion, iron overload and some somatic mutations. This is not really known today if all these prognostic factors overlap but some correlation between biology[16] and somatic mutations or between response to treatment and somatic mutations[20-22] have been reported. With one or several of these risk factors, low risk patients have an expected median survival lower than 3 years while post-transplant outcome is expected > 3 years in this category of patients. The aim of the present phase II study is to analyse outcome of the low risk patients with poor prognostic feature with or without a transplant according to donor availability.

2. Type of Study

This is a controlled, non-randomized multicenter, phase II study.

3. Study Objectives

3.1 Primary Objective

The primary objective of the study is to **compare overall survival in patients with or without a matched donor (8/8 unrelated donor or matched sibling) at 36 months.**

3.2 Secondary Objectives

- ✓ Comparison between patients with or without a donor for:
    a. Cumulative incidence of complete response at 36 months
    b. Cumulative incidence of Transformation in AML at 36 months (according to WHO criteria, > 20% blasts in peripheral blood or bone marrow)

Evaluate:

- ✓ Risk factors for outcome (remission, mortality)
- ✓ Risk factors for overall survival (age, disease characteristics…) including iron overload (ferritin, plasmatic iron markers, MRI)
- ✓ The efficiency of chelation will be assessed at 3 months after inclusion for all patients
- ✓ Feasibility of the transplant define by the proportion of patients with a donor who undergo the transplantation (expected between 80 and 95%)
- ✓ Proportion of patients with iron overload (Serum Ferritin (SF) > 1000 ng/mL or RBC transfusion > 20) at time of inclusion and at 16 months after inclusion for non-transplanted patients and 12 months post-transplant for transplanted patients
- ✓ The effect of chelation post-transplant will be measured by SF level: SF < 1000 ng/mL, SF > 1000 ng/mL, SF > 1500 ng/mL and also by percentage of patients with SF decrease of at least 30%
- ✓ Eligibility of post-transplant Exjade® treatment defined by the proportion of patients with iron overload who can benefit from Exjade® after the transplantation (% of patients without contraindication to Exjade® 3 months after transplant) and who can maintained it at least 6 months or until SF < 700 ng/mL
- ✓ Assessment of change of Ferritin level (% of decrease) and MRI at 12 months post-transplant
- ✓ Evolution of innovative iron markers including Non-transferrin binding iron (NTBI), labile plasmatic Iron (LPI) and Hepcidine at time of inclusion (before Exjade®), at 3 months (patients potentially treated by Exjade®) and 16 months post-inclusion for all patients; in transplanted patients these markers will be measured just before conditioning regimen (J-5), just before the transplantation (J0), at D7, 30, 100 and 12 months after transplant. All these markers will be tested **as risk factors for outcome** (survival, non-relapse mortality and progressive disease)
- ✓ Quality of Life study (EORTC questionnaire at registration, 12, 24 and 36 months)
- ✓ Grade III or IV toxicity (hematological and non-hematological) recorded according to NCI CTC criteria version 4.0
- ✓ Cumulative incidence of severe infection at 12 months
- ✓ Risk factors for overall survival and non-relapse mortality after the transplant including iron overload (SF, Non Transferrin Binding Iron)
- ✓ For transplanted patients only: cumulative incidence of neutrophil engraftment on day 60 post-transplant, acute and chronic GVHD at 2 years post-transplant

4. Patient Selection

4.1. Inclusion criteria

1/ Signed Informed consent

2/ Patients should be intermediate 1 or low according to IPSS with at least one poor prognosis feature

- ☐ MDS classified according to revised IPSS at least intermediate
- ☐ RBC transfusion dependent anemia and failure to 2 lines of therapy if eligible (including EPO, Lenalidomide or demethylating agents …) with the exception of pure RARS with isolated SF3B1 mutation
- ☐ thrombocytopenia < 20 G/L requiring transfusion
- ☐ neutropenia < 0.5 G/L associated with severe infection (defined as requiring hospitalization)
- ☐ 5q- and lenalidomid failure especially but not only with TP53 mutated

3/ Age ≥ 18 and < 70 years

For young patients, 18-45 years, Fanconi disease and dyskeratosis should be ruled out by standard test in order to test frequent constitutional genetic alteration.

4/ Patient for whom a transplantation from a matched donor, (8/8 (HLA A, B, C, DRB1) identical at molecular level) unrelated donor or matched sibling), is considered irrespective of donor availability

5/ Performance status 0-2 on the Eastern Cooperative Oncology Group (ECOG) Scale (at time of screening)

6/ Negative pregnancy and adequate contraception (including in male patients wishing to father), if relevant

7/ Wash-out of at least 30 days since a previous treatment with Vidaza®, Lenalidomide, EPO or any other treatment inducing cytopenias

4.2. Exclusion criteria

1/ MDS classified according to classical IPSS as intermediate 2 or High risk

2/ Transformation in Acute Myeloid Leukemia (AML)

3/ ARSI SF3B1 mutated

4/ Severe active infection or any other uncontrolled severe condition

5/ Organ dysfunctions including the following:

- Hepatic: total bilirubin > 2 times upper limit of normal (ULN) (except moderate unconjugated hyperbilirubinemia due to intra medullary hemolysis or Gilbert syndrome), alanine transaminase (ALT) and aspartate transaminase (AST) > 3xULN
- Symptomatic respiratory chronic failure
- Symptomatic cardiac failure
- Renal clearance < 60 ml/min

6/ Prior malignancy (except in situ cervix carcinoma, limited basal cell carcinoma, or other tumors if not active during the last 3 years)

7/ MDS with the following causal germline disease : Fanconi anemia, GATA2 related syndromes and telomere disorders.
Any patient with family history of hematologic disorder and/or dysmorphic syndrome (skeletal malformation ; nail, skin or hair abnormalities ; cardiac abnormalities, growth restriction, pulmonary or hepatitis fibrosis) which

suggest a germline causal mutation of the hematological disease, should send a skin biopsy and blood samples (5 tubes 5 ml with EDTA) to Pr Jean Soulier at Saint-Louis Hospital.

4.3. Discontinuation criteria

All patients will be followed at least 2 years after the date of the last inclusion according to each center policy and disease status (transformation in a higher risk MDS, especially AML) and death (date and cause) will be collected until the end of the protocol and for a maximal duration of 5 years.

5. Number of patients and study duration

- The trial will enroll **105** patients, 62 in group A (donor), 43 in group B (no donor), over a 36-month period
- The whole study will be performed over a 5-year period

6. Therapeutic procedures

Patients will be assigned to group A or B (donor or no donor) after at least 2 months after inclusion to allow the time for donor search.

6.1. Group A: Patients with a donor

- **Iron chelation**

  Patients with iron overload defined as more than 20 blood cell transfusions (since initial diagnosis) or ferritin level > 1000 ng/mL will start Exjade® at 20 mg/kg per day of deferasirox dispersible tablets or 14 mg/kg per day of deferasirox film-coated tablets (when converting a patient from deferasirox dispersible tablets to deferasirox film-coated tablets, the dosage should be decreased by 30%) or will continue Exjade® if they were receiving it before inclusion at a minimal dosage of 20 mg/kg of deferasirox dispersible tablets or 14 mg/kg of deferasirox film-coated tablets or same dose if they were receiving a higher dose. Exjade® will be temporarily discontinued one day before conditioning regimen start or at any moment in case of renal failure (clearance < 60 ml/min). In those patients, Exjade® will be restarted on day 100 in patients with LIC > 5 mg Fe/gr dry weight (defined before the transplantation), except in cases of contraindications including gastro-intestinal disorder (nausea, vomiting, abdominal pain or diarrhea), renal failure (clearance < 60 ml/min), active GVHD, neutropenia < 0.5 G/L, thrombocytopenia < 30 G/L, total bilirubin, AST, ALT > 2xULN, GGT and PAL > 3xULN.

Before restarting Exjade®, complete iron measurements will be repeated (serum iron, serum ferritin, transferrin saturation, NTBI, LPI, hepcidine) and MRI.

The initial dose of Exjade® after transplant will be 10 mg/kg/day of deferasirox dispersible tablets or 7 mg/kg per day of deferasirox film-coated tablets progressively increased to 20 mg/kg/day or 14 mg/kg/day respectively if good tolerance.

Patients with iron overload will be evaluated at each visit by serum ferritin, transferrin and iron in serum; will be evaluated 12 months after transplant by complete iron measurements including serum iron, serum ferritin, transferrin saturation, NTBI, LPI and hepcidine.

Hepcidine and non-transferrin binding iron will be measured in the ancillary biological study (Hôpital Louis Mourier, see appendix 10).

As soon as ferritin reached a level < 700 ng/mL, Exjade® will be discontinued.

- **Transplantation (D0)**

The HSCT should be **scheduled 4 months after the inclusion** either with an HLA-matched sibling or a 8/8 (A,B,C,DRB1) HLA matched unrelated donor.

Preferable source of stem cells will be granulocyte colony-stimulating factor-mobilized peripheral blood stem cells (PB), in case of donor refusal, marrow could be accepted. Minimal CD34+ cells requested will be 3 x 10^6/kg body weight recipient or 2 x 10^8/kg body weight recipient (before in vitro manipulation).

- **GVHD prophylaxis**

GVHD prophylaxis will consist in Cyclosporine on day -1 (per os: 2.5 mg/kg twice a day, with a target residual between 100 and 300) plus Mycophenolate Mofetil (Cellcept®). Mycophenolate Mofetil on day +1 (15 mg/kg twice a day) will be tapered from day 30 to day 45 or stopped when corticosteroids are started to treat an acute GVHD. In the absence of GVHD, Cyclosporine will be progressively tapered from day 120, 25% per 2 weeks until total discontinuation.

D-5	D-4	D-3	D-2	D-1	D0 HSCT	D+1	D+…
-	-	-	-	Start Cyclosporine 2.5 mg/kg twice a day	-	Start MMF (Cellcept®) 15 mg/kg twice a day	**D+30 to D+45:** Stop progressively MMF **Since D+120:** Stop progressively Cyclosporine (-25%

							per 2 weeks until total discontinuation

- **Conditioning regimen**

- Conditioning regimen will be as followed:

-	D-5	D-4	D-3	D-2	D-1	D0
FLUDARABINE mg/m^2	30	30	30	30	30	-
IV BUSULFAN mg/kg	-	-	3.2	3.2	-	-
ATG-FRESENIUS (GRAFALON) mg/kg	-	-	10	10	10	-
CORTICOSTEROIDES mg/kg	-	1	2	2	2	-

Administration of conditioning regimen will follow the following procedure:

- Usual nausea-vomiting prevention
- Hyperhydratation is not required
- Fludarabine will be injected by central venous catheter in 30 minutes
- Busulfan will be injected by central venous catheter in 3 hours once daily or in 2 hours fourth a day
- ATG-Fresenius (GRAFALON) will be preceded by corticosteroides and 5 mg polaramine® or equivalent anti-histaminic. **Heparin should not be administrated during ATG-Fresenius (GRAFALON) administration.** ATG-Fresenius (GRAFALON) will be injected by central venous catheter in 12 hours the first day and 8 hours the second and third day with surveillance of temperature, tension, and pulse every 30 minutes; in cases of hypotension, the perfusion should be discontinued until recovery and restarted at lower velocity.

Anti-infectious prophylaxis

According to national and European guidelines, all patients will received a prophylaxis for herpes simplex (acyclovir or valacyclovir), pneumocystis and toxoplamosis (sulfametoxazole/trimethoprime or sulfadoxine/pyrimethamine or atovaquone) except during aplasia, anti-fungal therapy (fluconazole).

All patients will be monitored for CMV and EBV reactivation by weekly PCR in blood until the third month.

No engraftment

If the patient has acute or late rejection with persistent of severe pancytopenia, a second transplant has to be discussed. The quicker this second transplant is performed, the best chance the patient has to avoid severe infection. According to performance status and comorbidities, a second transplant with same or different donor can be re-scheduled as soon as possible, if possible no later than 3 weeks after rejection diagnosis.

If the patient has an autologous reconstitution with good or moderate hematological reconstitution, or returns to his baseline count, a second allogeneic transplant is not recommended until the disease progresses.

Management of immunosuppressive treatment and donor lymphocyte infusion

As reported in the regimen schema, Mycophenolate Mofetil will be decreased from day 30 and stop on day 45 while Cyclosporine will be decreased on day 120, 25% per 2 weeks in absence of acute GVHD. The optimal dosage of Cyclosporine between D0 and D120 will be between 150 and 350 ng/ml when intravenous and a residual > 150 ng/ml (< 350) when oral route.

There is no systematical preventive donor lymphocyte infusion in this trial (because patients are at low risk).

In some cases, immunosuppressive therapy can continued later if the patient presents acute GVHD until acute GVHD has resolved.

In other cases, if there are some evidences of disease activity and / or mixed chimerism after day 30, immunosuppressive treatment will be discontinued earlier (except if active GVHD). Patients should be evaluated 6 weeks after immunosuppressive discontinuation and in cases of persistence mixed chimerism and/or disease activity proven by marrow analysis, DLI should be planned (at least 6 weeks after immunosuppressive discontinuation) except if the patient had a previous grade II to IV acute GVHD or in patients with active chronic GVHD. The donor can be collected to have a set of 4 donor lymphocytes packages which can be infused in the recipient as follow:

-first: $1x10^6$ CD3+ cells /kg

-second: $0.5x10^7$ CD3+ cells /kg

-third: $1x10^7$ CD3+ cells /kg

-fourth: $0.5x10^8$ CD3+ cells /kg.

Each DLI will be injected after a minimum interval of 6 weeks and immediately stopped in cases of GVHD occurrence. A combined treatment with Vidaza® is also accepted, particularly in the patients with blast excess. In these cases, hypomethylating agents will be used according to their approval (Autorisation de Mise sur le Marché).

If the patient is 100% recipient chimerism, there is no indication to perform DLI. DLI can be done later if the patient has some donor cells (mixed chimerism or donor chimerism) after treatment.

Other treatment: If the transplantation is not performed for any reasons

Patients can benefit from any treatment including phase II prospective studies, or a treatment considered as the best available treatment. There are no unapproved medications which are tested in this trial.

If the disease evolves to a higher risk by acquiring new cytogenetic abnormalities, new cytopenia or an increase in marrow blast including a transformation into AML, a transplant using an alternative donor should be considered (mismatch unrelated donor, unrelated cord blood or mismatch related donor including haplo-identical transplant), if possible in the setting of a trial assessing alternative transplant.

6.2. Group B: Patients without a donor

- **Iron chelation**

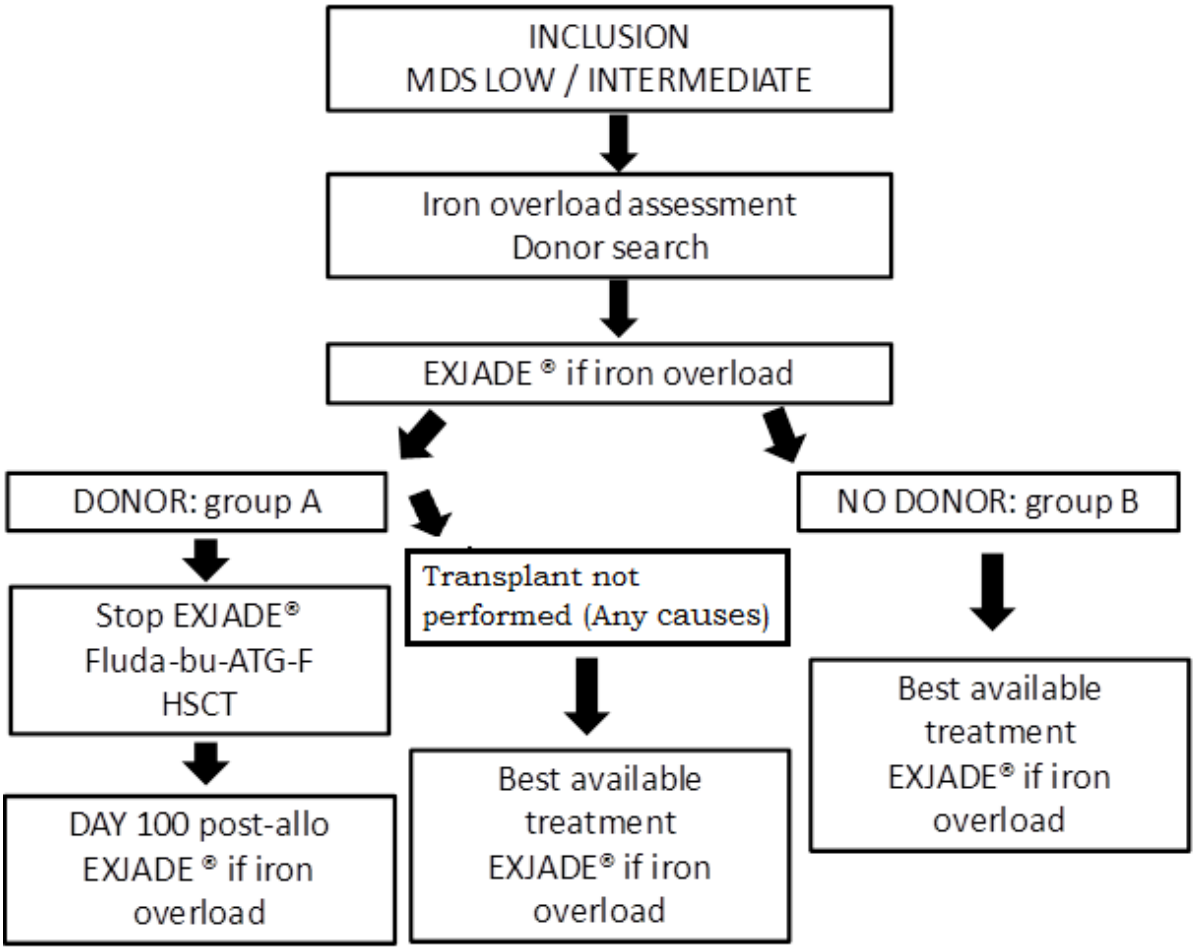
  Iron chelation will be started if baseline ferritin level > 1000 ng/L or in those receiving more than 20 RBC units (or continued if they already received it). Exjade® will be started at 20 mg/kg per day of deferasirox dispersible tablets or 14 mg/kg per day of deferasirox film-coated tablets (when converting a patient from deferasirox dispersible tablets to deferasirox film-coated tablets, the dosage should be decreased by 30%) or will continue if they were receiving it before inclusion at a minimal dosage of 20 mg/kg of deferasirox dispersible tablets or 14 mg/kg of deferasirox film-coated tablets.

- **Other treatment**

  Patients can benefit from any treatment including phase II prospective studies, or a treatment considered as the best available treatment. There are no unapproved medications which are tested in this trial.

If the disease evolves to a higher risk by acquiring new cytogenetic abnormalities, new cytopenia or an increase in marrow blast including a transformation into AML, a transplant using an alternative donor should be considered (mismatch unrelated donor, unrelated cord blood or mismatch related donor including haplo-identical transplant), if possible in the setting of a trial assessing alternative transplant.

6.3. Trial design



7. Centralized biological study (hepcidine, LPI, NTBI)

Serum hepcidin assay:

Serum hepcidin will be measured with a previously validated LC-MSMS method (Thibaud Lefebvre et al, Clin Chem Lab Med 2015). 200 µl of serum samples will be pre-treated by solid phase extraction. Then, hepcidin will be separated on C18 column by ultra high-pressure chromatography (Acquity UPLC - Waters) and detected by MS/MS on a Xevo TQMS (Waters). (13C1815N3)-Hepcidin-25 (PeptaNova GmbH, Sandhausen, Germany) will be used as an internal standard. The method was found to be linear over the analytical range of 1,5 to 200 µg/L (R2 > 0.99). Undetected values for hepcidin (< 0.75 ng/mL) are imputed using a beta law.

Serum NTBI assay:

Total content of Non-Transferrin Bound Iron (NTBI) will be measured by the FeROSTM eLPI kit. This assay measures the iron-specific redox activity in serum. The Reactive Oxygen Species (ROS) will be detected by an oxidation-sensitive probe (DHR), which becomes fluorescent when oxidized by ROS. The assay employs a control reaction where a selective iron chelator blocks redox cycling of iron to specifically identify iron-mediated ROS generation. Comparison of the kinetics of fluorescence generated in the reaction in the presence and absence of the iron chelator provides measure of the total NTBI content.

Iron status measurement:

Serum samples will be used to measure iron, transferrin, ferritin, soluble transferrin receptor and CRP concentrations by routine hospital technology.

8. Study procedures

8.1. At inclusion

After checking the inclusion and exclusion criteria, as well as parameters necessary for stratification, the investigator will request inclusion online.

The following information will be collected:

- Medical history: date of diagnosis and initial disease characteristics (blood counts, bone marrow results, cytogenetic analysis)
- Previous treatments and history of RBC and platelet transfusions

Then, the investigator will:

- Check inclusion criteria
- For young patients, 18-45 years, contact Marie Sébert by e-mail (marie.sebert@aphp.fr)

- Collect the informed consent (clinical +/- biological study)
- Perform clinical examination: weight, height, PS (ECOG, Appendix 1), co-morbidity (Appendix 2-3)
- Perform baseline blood tests including: full blood count and differential, including peripheral blasts count and reticulocytes, AST, ALT, serum bilirubin, LDH, PAL, GGT, creatinine, EPO level, CRP, B12 and folates test
- Urine test: proteinuria
- Perform bone marrow aspiration for morphology, cytogenetics, and correlative biological studies (2 mL EDTA). If this analysis has been done within the 2 months prior to inclusion and if peripheral blood cell counts are stable: a new bone marrow aspiration could be avoided if BM sample have been sent to the CHRU of Lille, Pr Preudhomme laboratory, and SMD molecular analysis have already been performed (ASXL1, TP53, EZH2, ETV6, RUNX1…)
- Send bone marrow (2 ml EDTA) and blood (10 ml EDTA) samples for molecular analysis
- Send serum, 10 ml (2 tubes 5 ml without EDTA or citrate), before Exjade® initiation, to Louis Mourier Hospital for iron biological study
- HLA will be performed in these patients and in their siblings (if available and age < 70 years without cancer, thrombosis or cardiac failure history). If no donor is available among siblings, a matched unrelated donor 8/8 unrelated donor is searched. Antibodies against HLA should also be checked.
- Iron overload will be estimated at registration by the number of transfusions received since initial diagnosis, iron parameters (serum iron, transferrin saturation, serum ferritin)
- **Heart and liver MRI** should be performed within 6 weeks in patients with iron chelation criteria: ferritin level > 1000 ng/mL (can be done also before inclusion, maximally 6 weeks before)
- Pregnancy test, if applicable
- Quality of life questionnaire (EORTC)

8.2. Visits

For all enrolled patients, visits will be performed:

- at inclusion
- every two weeks during the first 4 weeks (first month)
- every 4 weeks from the 5th week to the 26th week (until 6th month)
- every 8 weeks from the 27th week to the 52th week (until 12th month)
- every 12 weeks from the 53th week to the end of study.

At each patient visit, the following information will be registered:

- Grade III to IV adverse events (classified according to NCI- CTCAE 4.0)
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

- Treatment by Exjade® and its dose

- Clinical assessment (every 2 weeks on first month)

- Weight, PS, serious infectious disease (defined in Appendix 4)

- Transfusion history (platelet and RBC) including the research of allo-immunisation (RAI)

- AST, ALT, serum bilirubin, serum creatinine, PAL, LDH, GGT, CRP

- Proteinuria (rapid urine test)

- Pregnancy test, if applicable

- Blood cell count including reticulocytes (every week during the 1th month then every 4 weeks)

- Ferritin level, transferrin level and saturation, plasmatic iron level

- In case of disease progression/relapse suspicion, marrow + cytogenetic analysis

Specific information for specific point (after inclusion):

- Quality of life questionnaire (EORTC) at 12, 24 and 36 months

- Centralized biological study (hepcidine, LPI, NTBI): At 3 and 16 months after inclusion (specific form in Appendix 10)

- Bone marrow aspiration with cytogenetic (local laboratories) and molecular biology (CHRU Lille) will be performed according to local policy but at least once a year

For patients who received a transplant:

- Pre-graft tests including cardiac assessment and respiratory assessment with pulmonary tests
- Centralized biological study (hepcidine, LPI, NTBI): just before conditioning regimen (D-5), just before transplant on day 0, and on day 7, 30, 100, 365 post-transplant
- Between 2 to 3 months after the transplant, a **bone marrow aspiration** (with shipment for biological study: BM 2 ml EDTA)
- At least one post-transplant **chimerism** in blood after engraftment on day 30. The chimerism will be repeated only if the first one is not donor > 95% or in cases a rejection is suspected
- Acute GVHD grade per organ at each visit (Appendix 6)
- Neutrophil engraftment and platelet engraftment (within 60 days post-transplant)
- Chronic GVHD should be noticed according to the NIH classification (Appendix 7), only the first episode but the dosage of corticosteroids and the type of immunosuppressive therapy should be collected in the electronic CRF
- **Heart and liver MRI** 12 months after transplant in patients with iron overload at inclusion

All patients will be followed at least 24 months according to each center policy. After 24 months, disease status (transformation into AML, progression after transplant, persistent complete remission after transplant) and death (date and cause) will be collected until the end of the protocol.

8.3. Diary

8.3.1. Diary for transplanted patient (Group A)

Time from HSCT	Screening D-120 M-4	D-90 M-3	D-60 M-2	D-30 M-1	D0 HSCT	D30 M1	D60 M2	D120 D240 M4-M8	D240 D960 M8-32	D480 M16
Inform consent	X									
Clinical visit	X	X2/ M	X	X	X	X	X	X Every 2 months	X Every 3 months	X
Comorbidity assessment	X			X						
ECOG	X	X	X	X	X	X	X	X	X	X
Blood cell counts (9)	X	X1/ W	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M
Standard biology (1)	X	X	X	X	X	X	X	X	X	X
EPO, folates, B12	X									
Iron plasmatic dosage (2)	X	X	X	X	X	X	X	X	X	X
HLA typing (3)	X	X	X							
Centralized biological study (4)	X*			D-5 Pre-Allo	D0 before HSCT	D7 and D30 post allo		D 100 post Allo		M12 post-Allo
Proteinuria	X	X	X	X	X	X	X	X	X	X
Bone marrow aspiration + blood + cytogenetics (5)	X						D60 to D100 post Allo		M12, 24 &36 post inclusion	
Heart and liver MRI (6)	X			X				X*		M12 post allo
QOL	X								M12, 24 &36 post inclusion	
AE Grade III-IV	X	X	X	X	X	X	X	X	X	X
Pregraft tests (7)			X							
GVHD assessment						X	X	X	X	X
Postgraft blood chimerism (8)						X	X*	X*	X*	
Confirming date of HSCT		X	X	X						

8.3.2. Diary for NON-Tranplanted patient (Group B)

Time from registration	D0 Screening	D30 M1	D60 M2	D90 M3	D120 M4	D150 M5	D180 M6	D240 D365 M8-M12	D365 D990 M12-M33	D1080 M36
Inform consent	X									
Clinical visit	X	X2/M	X	X	X	X	X	X Every 2 months	X Every 3 months	X
Comorbidity assessment	X			X						
ECOG	X	X	X	X	X	X	X	X	X	X
Blood cell counts (9)	X	X1/W	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M
Standard biology (1)	X	X	X	X	X	X	X	X	X	X
EPO, folates, B12	X									
Iron plasmatic dosage (2)	X	X	X	X	X	X	X	X	X	X
HLA typing (3)	X	X	X							
Centralized biological study (4)	X*			X					M16	
Proteinuria	X	X	X	X	X	X	X	X	X	X
Bone marrow aspiration + blood + cytogenetics (5)	X								M12 &24	X
Heart and liver MRI (6)	X			X						
QOL	X								M12 & 24	X
AE Grade III-IV	X	X	X	X	X	X	X	X	X	X

(1) Every visit: Proteinuria, AST, ALT, serum bilirubin, serum creatinine, PAL, LDH, GGT, CRP
(2) Serum ferritin, transferrin, iron plasmatic level
(3) HLA A, B, C, DRB1 molecular level
(4) Centralized study – 2 tubes without EDTA 5 mL to Louis Mourier Hospital, see specificity in appendix 10. *Before Exjade initiation
(5) Inclusion, D60 to D100 post-transplant or once a year for non-transplant patient. Including cytogenetic and centralized shipment for molecular analysis to CHRU Lille (BM 2 ml EDTA + Blood 2 tubes 5 mL with EDTA)
(6) Only for patients with SF > 1000 ng/mL or more than 20 RBC transfusions at time of inclusion. At time of screening, could be done within 6 weeks before or after inclusion. *After transplant, should be done at D100 and before Exjade restarting
(7) Include cardiac and pulmonary assessment
(8) Assessment at D30. *Should be repeated at the next visits only if the first one is not donor > 95% or in cases a rejection is suspected
(9) Including reticulocytes and blast count

9. Statistical analysis

9.1. Sample size

The objective of this study is to demonstrate an improvement of the overall survival in patients with a donor that is reaching 70% at 36 months compared to 40% in those without a donor. To test this hypothesis (HR=0.39) based on a two-sided log-rank test, 50 events are required, and 105 patients are needed with a 80% power and type I error rate at 5%, with a probability to identify a donor at 70%.

9.2. Duration of the study

The study is based on a cohort of patients prospectively enrolled. The main criterion is a right-censored end point that will be compared across groups of patients either transplanted or not, on the basis of a log-rank test. It has been computed that 50 events are required to control for the type I and II error rates, with an estimated sample size of 105 patients. Thus, according to the expected recruitment rate and survival rate, it is supposed that the study will end up 5 years after the trial onset, that is, between 2 and 5 years after patient accrual.

9.3. Statistical analysis

All analyses will be performed in an intent-to-treat approach, whatever the treatment actually performed (either transplantation or not); the basis for comparison will indeed be the selection of a donor or not. Such a design is often considered as a “Mendelian randomization”. Mendelian randomization is a method that allows one to test for, or in certain cases to estimate, a causal effect from observational data in the presence of confounding factors[90]. From a statistical perspective, it is an application of the technique of instrumental variables[91], with genotype acting as an instrument for the exposure of interest. However, Mendelian randomization estimates are especially relevant when the effect of interest is that of a long term population based intervention. Otherwise, a Mendelian randomization approach will generally be qualitatively informative for the direction of effect of a clinical intervention, the genetically derived estimate might not correspond to the magnitude of the effect in practice. Thus, two analyses will be conducted, with two main estimates of intervention effect:

- directly comparing those two groups (donor vs no donor)
- using inverse probability treatment weights, based on the propensity scores to have actually a donor.

Baseline characteristics, overall and complete response rates of the two groups will be compared by non-parametric tests, either the exact Fisher’s exact test for qualitative variables or the Mann-Whitney test for quantitative variables.

Cumulative incidences of AML will be tested with the Gray test considered death as a competitive event. Prognostic factors of event-free and overall survival will be investigated using Cox proportional hazard models. Model assumptions will be checked using a test for proportional hazards and spline smoothing of residuals for the log-linearity assumption.

All statistical analyses will be performed with the R and SAS software packages.

10. Safety assessment

10.1. Assessment of adverse events (AE)

Safety will be assessed continuously during the study. Patients should be questioned at each scheduled visit concerning AEs experienced since last visit. The severity, as graded by the NCI CTC AE v. 4.0 (Cf. Appendix 9), date of onset, and outcome should be recorded for all AEs as well action taken (such as prescription of rescue medication). All concomitant medication taken by the patient will be collected. An adverse event (AE) is defined as any expected or unexpected harmful and unintended occurrence or exacerbation of an event in a clinical trial subject, whether or not related to the trial or the investigational product. It can be a new intercurrent disease, exacerbation of a concomitant disease, an accident, or any other deterioration in the patient's health, including abnormal laboratory findings.

Any medical condition that existed before the start of the study treatment and that remains unchanged or improves must not be recorded as an AE. If a medical condition worsens, it must be recorded as an AE. The diagnosis or syndrome rather than the individual signs or symptoms must be recorded on the AE pages of the case report form. An adverse reaction is any untoward and unintended responses to an investigational medicinal product related to any dose administered.

10.2. Assessment of serious adverse events

An event is considered a serious adverse event (SAE) if it:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization **(except for the transplantation)** or prolongation of existing hospitalization **(expect hospitalization for transplant)**
- Causes permanent disability or serious temporary incapacity
- Causes a congenital anomaly, birth defect or abortion
- Is medically significant

The terms disability and incapacity refer to any clinically significant physical or mental handicap, whether temporary or permanent, that affects the patient's physical activity and/or quality of life.

A medically significant event is any clinical event or laboratory finding considered by the investigator to be serious that does not meet the seriousness criteria defined above. It may pose a risk to the patient and require medical intervention to prevent one of the serious outcomes mentioned previously (e.g. an overdose, second cancer, pregnancy, and new information can be considered medically significant).

The following <u>are not considered</u> as serious adverse events:

- Hospitalization scheduled before the start of the trial and/or stipulated in the protocol (e.g. for biopsy or chemotherapy) or for social reasons
- Aplasia related to conditioning regimen
- Transfusions

The AE pages of the case report form and an SAE form should be completed for each AE considered serious.

For each AE, the investigator shall provide information about the intensity, start and end dates, causality, the action taken, and the outcome.

Definition of an expected serious adverse event (SAE-E)

An expected SAE is an event already mentioned in the most recent version of the Investigator Brochure, or in the Summary of Product Characteristics (SmPC) for medicinal products that have already been granted marketing authorization (MA). This definition also applies to the trial drug when it is administered for the same population but for an unlicensed indication.

Definition of an unexpected serious adverse event (SAE-U)

An unexpected SAE is an event not mentioned in or whose nature, intensity, or clinical course is not consistent with the information in the Investigator Brochure, or in the Summary of Product Characteristics (SmPC) for medicinal products that have already been granted marketing authorization (MA).

Intensity

The term intensity (severity) should not be confused with the term seriousness that serves as a guide for defining reporting obligations.

The intensity of events shall be evaluated according to the excerpt from the CTC-AE classification, version 4.0 (cf. Appendix 9). The intensity of adverse events not listed in this classification will be rated using the following terms:

- Mild (grade 1): does not affect the patient's usual daily activities
- Moderate (grade 2): interferes with the patient's usual daily activities
- Severe (grade 3): prevents the patient's usual daily activities
- Very severe (grade 4): requires intensive care / is life-threatening
- Death (grade 5)

Causal relationship between an adverse event (SAE/AE) and the study treatment

The investigator must determine the cause-effect relationship between administration of the treatment and the incidence of an adverse event (SAE/AE), and rate it as Suspected or Not Suspected, as follows:

Not Suspected:

The timing makes a causal link between the adverse event and administration of the study drug unlikely, or alternatively the concomitant administration of other medicinal products or therapeutic interventions or pre-existing disorders provide sufficient explanation for the observed event.

Suspected:

The timing makes a causal link between the adverse event and administration of the study drug possible, and concomitant administration of other medicinal products, therapeutic interventions and pre-existing disorders do not sufficiently explain the observed event.

10.3. Reporting of SAEs

The investigator must report any SAE that occurs during the study regardless the causality of the event is related or not to the study drug, to the principal investigator and the sponsor, by fax or e-mail. The investigator must complete and send the serious adverse event report form within 24 hours of learning of the serious event to:

GFM
Fatiha Chermat
Service d'Hématologie Séniors
Hôpital Saint-louis
1 avenue Claude Vellefaux
75475 Paris cedex 10
Phone: 33 (0)1 71 20 70 59
FAX: 33 (0)1 71 20 70 38
fatiha.chermat-ext@aphp.fr

The sponsor shall provide NEOVII a copy of all expected or unexpected serious adverse events within 24 hours of learning of the event.

The sponsor shall also provide NEOVII a copy of the annual safety report at the time of its submission to the REB and the regulatory authorities.

QPPV
Name: Dr. Ulrike Mägdefrau

Address: YES Pharma Services GmbH
Bahnstraße 42 - 46
61381 Friedrichsdorf
Germany
Email: vigicare-neovii@yes-services.eu

Deputy QPPV
Name: Dr. Michael Johannes Drexler
Address: Neovii Biotech GmbH
Am Haag 6 + 7
82166 Gräfelfing
Germany
Phone (24 h): +49 (0) 162 4027059
Fax: +49 (0) 89 89 8888 719
Email: drugsafety@neoviibiotech.com

The sponsor shall provide NOVARTIS a copy of all expected or unexpected serious adverse events within 15 days of learning of the event by email to: phv.phfrrv@novartis.com

The sponsor shall also provide NOVARTIS a copy of the annual safety report at the time of its submission to the REB and the regulatory authorities.

Reporting period

All SAEs must be reported:

- Since the signing of consent,

- Up to 30 days following the transplant or following the last study drug therapy

Any delayed serious adverse event that may reasonably be considered related to the treatment(s) described in the protocol or to the study must be reported, and no time limit applies to such adverse events.

For each event, the investigator shall record:

- A description of the event that is as clear as possible, using medical terminology,
- Its intensity,
- Its start and end dates,
- The measures taken and whether or not corrective treatment was required,
- Whether the trial treatment was suspended,
- Its clinical course. If the event was not fatal, it should be monitored until recovery, until the patient has returned to his/her previous condition, or until any sequelae have stabilized,
- The causal relationship between the event and the trial treatment or any trial-related obligations (e.g. a treatment-free period, investigations requested in connection with the trial),

- Any causal link with the trial medication(s), the disorder being treated, another disorder or another treatment. Whenever possible, the investigator must also send the following with the serious adverse event report: a copy of the discharge summary for hospitalization or prolonged hospitalization, a copy of the post-mortem report, a copy of all the results of the diagnostic tests performed, including relevant negative results, together with the normal laboratory values, and any other documents the investigator considers useful and relevant.

All of these documents must be anonymized.

Additional information may be requested by the monitor (by fax, post, telephone, or during a visit).

Nevertheless, any event that qualifies as expected but differs in its intensity, clinical course, or frequency will be considered as an unexpected event by the Pharmacovigilance unit.

Reporting events to the Authorities and the REB

The sponsor shall report the following to the French health products safety agency (ANSM) and the REB concerned:

- Unexpected serious adverse effects that might be related to the investigational product and resulted in death or were life-threatening, at once and within a maximum of seven days from the day the sponsor learned of the event. Additional relevant information shall be reported within a new time limit of eight days following on from the seven-day deadline.

- Other expected serious adverse effects that could be related to the investigational product, at once and within a maximum of fifteen days from the day the sponsor learned of the event. Additional relevant information shall be sent within a new time limit of eight days following on from this fifteen-day deadline.

Follow-up of SAEs

The investigator is responsible for providing appropriate medical follow-up for patients until the event has resolved or stabilized or until the patient's death. Sometimes this may mean that follow-up will extend beyond the patient’s withdrawal from the trial.

The investigator shall send additional information to the sponsor using an SAE report form (ticking the box “Follow-up No. X” to specify that this is a follow-up report and not an initial report) within 48 hours of receiving the information. The investigator also sends the final follow-up once the SAE has resolved or stabilized.

The investigator shall keep the documents about the presumed adverse effect, in case anything needs to be added to the information previously sent.

The investigator shall respond to the sponsor's requests for additional information in order to document the initial report.

11. ETHICAL AND REGULATORY ASPECTS

The clinical trial must be conducted in accordance with:

- the ethical principles of the current version of the Declaration of Helsinki,

- the guideline for Good Clinical Practice of the International Conference on Harmonization (ICH–E6, 07/17/96),

- European Directive 2001/20/EC on the conduct of clinical trials,

- the French Huriet Act (No. 88-1138) dated December 20, 1988 concerning the protection of clinical trial subjects, amended by the French public health law No. 2004-806 dated August 09, 2004,

- the French data protection and civil liberties law No. 78-17 dated January 06, 1978, amended by law 2004-801 of August 06, 2004 regarding the processing of personal data,

- French law No. 2004-800 dated August 06, 2004 concerning bioethics.

Research Ethics Board

Before conducting biomedical research on human subjects, the sponsor is obliged to submit the project to one of the REBs with jurisdiction where the coordinating investigator practices, for its opinion.

The sponsor also submits applications for substantial amendments to the initial project to the REB for its opinion.

Competent Authority

Before conducting a clinical trial or having one conducted, the sponsor of the trial shall apply to the ANSM for authorization.

Subject information and consent

Before biomedical research is conducted on a person, their voluntary, written, informed consent must be obtained, after they have been fully informed by the investigator during a consultation and given enough time to consider their decision.

Information intended for trial subjects must include all of the particulars set out in the French public health law of August 09, 2004 and must be written in straightforward language that the patient can understand.

The consent form must be signed and dated personally by the trial subject and the investigator (the original copy shall be archived by the investigator, and one copy will be given to the trial subject).

The patient information leaflet and informed consent form must be combined into a single document to ensure that all of the information is given to the trial subject.

Responsibilities of the sponsor

The sponsor of the clinical trial is the individual or legal entity that takes the initiative for the biomedical research on human subjects, manages the trial, and ensures that provision has been made for its funding.

The sponsor must be established in the European Community or, failing that, have a legal representative in a member state.

The sponsor's main responsibilities are to:

- take out civil liability insurance,

- request the opinion of the REB on the initial project and substantial amendments,

- request authorization for the initial project and substantial amendments from the competent authority,

- provide information about the trial to the site directors, investigators, and pharmacists,

- report any suspected unexpected serious adverse events related to any of the trial treatments to the competent authority, ANSM and EMEA (European pharmacovigilance database, Eudravigilance), and send this information to the REB and the trial investigators,

- submit the annual safety report to the competent authority and the REB,

- notify the competent authority of the start and end of the trial,

- write the final clinical study report,

- send the trial results to the competent authority, REB, and trial subjects,

- archive essential trial documents in the sponsor's folder for a minimum of 15 years after the trial has ended.

Responsibilities of investigators

The principal investigator of each establishment concerned undertakes to conduct the clinical trial in accordance with the protocol approved by the REB and the competent authority.

The investigator must not make any changes to the protocol without the written authorization of the sponsor and unless the REB and the competent authority have approved the proposed changes.

It is the responsibility of the principal investigator to:

- provide the sponsor with his/her curriculum vitae as well as those of his/her co-investigators,

- identify the members of his/her team who are participating in the trial and define their responsibilities,

- recruit patients once authorized to do so by the sponsor.

It is the responsibility of each investigator to:

- obtain informed consent, personally signed and dated by the subject, before carrying out any trial-specific screening procedures,

- regularly complete the case report form (CRF) of each patient enrolled in the trial and allow the clinical research assistant (CRA) appointed by the sponsor to have direct access to source documents, so that the latter can validate the data on the CRF,

- date, correct, and sign corrections on the CRF of each patient enrolled in the trial,

- accept regular visits by the monitor, and any auditors appointed by the sponsor or inspectors from the regulatory authorities.

All of the trial-related documentation (the protocol, consent forms, case report forms, investigator brochure, etc.) and source documents (laboratory results, X-rays, consultation reports, physical exam reports, etc.) are considered confidential and must be kept in a safe place. The principal investigator must store the data and a patient identification list for a minimum of 15 years after the end of the study.

12. Data Safety Monitoring Board (DSMB)

The DSMB will be set up to safeguard patients, to make sure that the trial is conducted ethically, to evaluate the trial's risk-benefit balance, and to independently review the scientific results during or at the end of the trial. The committee's role is to advise the sponsor, but the final decision on implementing the DSMB proposed recommendations rests with the sponsor.

13. Quality assurance

In order to guarantee the authenticity and credibility of the data in accordance with Good Clinical Practice (GCP), the sponsor shall put in place a quality assurance system which includes:
- Management of the trial in accordance with GFM's procedures,
- Quality control of the investigator site data by the monitor, whose role is to check the data in the case report form for concordance and consistency with the source documents,
- The possible audit of investigator sites.

14. Ownership of the data and confidentiality Central

The investigator undertakes that he/she and anyone who monitors the conduct of the trial will ensure the confidentiality of all of the information provided by GFM until the trial results are published. This confidentiality requirement shall not apply to information that the investigator gives to patients in connection with their participation in the trial or to previously published information.
The investigator undertakes not to publish, disclose, or use any trial-related scientific or technical information, in any way, either directly or indirectly.

Nevertheless, in accordance with Article R 5121-13 of the French public health code, the site and the investigator can give information about the trial:
- to the minister for health,
- to public health inspectors (medical doctors or pharmacists),
- to the Competent Authorities.

No written or verbal comments can be made about the trial without the sponsor's consent, since all of the information provided or obtained while the trial is being conducted legally belongs to the sponsor, which can use the information at its own discretion.

15. Publication policy

All of the information arising from this trial shall be considered confidential, at least until completion of the appropriate analysis and subsequent checks by the trial sponsor, coordinating investigator, and statistician.

Any publications, abstracts or presentations that include results from the trial must be submitted to the sponsor for approval.

Any communications, articles, or presentations must also include a section mentioning the GFM and the organizations that supported the research financially.

The trial’s coordinating investigator will be the main (first or last) author.
The first or last author (depending on the position given to the coordinating investigator) will be jointly selected by the sponsor and the CI.

The following investigators will be cited in order of the number of patients recruited for multicenter trials, or based on their involvement in the protocol and/or the disease. The trial statistician will also be cited.

Similarly, publications of ancillary results (biological studies) shall include the name of the person who carried out the ancillary study, as well as the names of anyone else involved in the ancillary study.

16. Insurance

In accordance with current legislation, insurance has been taken to cover any physical harm or other incapacity that may result from administration of the investigational treatment, in accordance with the study protocol.

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18. APPENDIX

Appendix 1. Performance status assessed by ECOG

GRADE	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:

Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix 2. New York Heart Association (NYHA) Functional Classification

NYHA Class	Symptoms
I	No symptoms and no limitation in ordinary physical activity. E.g., shortness of breath when walking, stair climbing, etc.
II	Mild symptoms (mild shortness of breath and/or angina pain) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity (e.g. walking short distances, ~ >20 - 100m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while at rest, mostly bed bound patients

Appendix 3. SORROR SCORE

Comorbidity	Definition	Score
Arrhytmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	1
Cardiac	Coronary artery disease*, congestive heart failure, myocardial infarction, or EF $\leq$ 50%	1
Inflammatory bowel disease	Crohn disease or ulcerative colitis	1
Diabetes	Requiring treatment with insulin or oral hypoglycemics but not diet alone	1
Cerebrovascular disease	Transient ischemic attack or cerebrovascular accident	1
Psychiatric disturbance	Depression or anxiety requiring psychiatric consult or treatment	1
Hepatic, mild	Chronic hepatitis, bilirubin > ULN to 1.5 x ULN, or AST/ALT > ULN to 2.5 x ULN	1
Obesity	Patients with a body mass index > 35 kg/m^2	1
Infection	Requiring continuation of antimicrobial treatment after day 0	1
Rhumatologic	SLE, RA, polymyositis, mixed CTD, or polymyalgia rheumatica	2
Peptic ulcer	Requiring treatment	2
Moderate or severe renal	Serum creatinine > 205 µmol/L, on dialysis, or prior renal transplantation	2
Moderate pulmonary	DLco and/or FEV1 66%-80% or dyspnea on slight activity	2
Prior solid tumour	Treated at any time point in the patient's past history, excluding nonmelanoma skin cancer	3
Heart valve disease	Except mitral valve prolapsed	3
Severe pulmonary	DLco and/or FEV1 < 65% or dyspnea at rest or requiring oxygen	3
Moderate/severe hepatic	Liver cirrhosis, bilirubin > 1.5 x ULN, or AST/ALT > 2.5 x ULN	3

*One or more vessel-coronary artery stenosis requiring medical treatment, stent, or bypass graft.

EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide.

Appendix 4. Serious infectious disease

Infections which should be considered:

-pneumonia

-severe sepsis defined in **Appendix 5**

-symptomatic bacteriemia

-arthritis / osteomyelitis

-pyelonephritis or prostatis

-profound abscess

-meningitis or encephalitis

-invasive aspergillosis

-candidemia

-CMV disease

-adenoviral disease

-disseminated viral infections (involving at least 2 organs)

-skin or subcutaneous infections

Data concerning benign or asymptomatic infections will not be collected for the protocol:

-superficial fungal infection (skin, mucous)

-upper respiratory tract infection

-sinusitis

-cystitis

-viral infection requiring pre-emptive treatment

-herpes simplex infection

-VZV infections (except severe form leading to pneumonia)

-CMV reactivation

-EBV reactivation

-adenoviral reactivation

Appendix 5. Definition of severe sepsis: at least 2 criteria A and one criteria B

Criteria A	-Cardiac pulse > 90/mn -Respiratory frequency > 20/mn -Mechanical ventilation -Temperature > 38°C or < 36°C
Criteria B	-Systolic blood pressure < 90 mmHg -Diuresis < 30 ml/h ou < 700 ml/24h -PaO2 < 75 mmHg or PaO2/FiO2 < 250 -Encephalopathy (Glasgow score < 14) -Metabolic acidosis -Coagulopathy

Appendix 6. Acute GVHD

GVHD Grading and Staging

Extent of Organ Involvement

State	Skin	Liver	Gut
1	rash on <25% of skin[a]	Bilirubin 2-3 Mg/dl[b]	Diarrhea >500 ml/day[c] or persistent nausea[d]
2	Rash on 25-50% of skin	Bilirubin 3-6 mg/dl	Diarrhea <1000 ml/day
3	Rash on >50% of skin	Bilirubin 6-15 mg/dl	Diarrhea >1500 ml/day
4	Generalized erythroderma with bullous formation	Bilirubin >15 mg/dl	Severe Abdominal pain with or without ileus
Grade[e]			
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	—	Stage 2-3 or	Stages 2-4
IV[f]	Stage 4	Stage 4	—

Source: Przepiorka et al., Bone Marrow Transplant 1995; 15(6): 825-8

[a] = Use "rule of Nines" or burn chart to determine extent of rash.

[b] = Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.

[c] = Volume of diarrhea applies to adults. for pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if an additional cause of diarrhea has been documented.

[d] = Persistent nausea with histologic evidence of GVHD inthe stomach of duodenum.

[e] = Criteria for grading given as minimum degree of organ involvvement required to confer that grade.

[f] = Grade IV may also include lesser organ involvement with an extreme decrease in performance status.

Ap09_1.ppt

Appendix 7. Chronic GVHD

	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 icthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Polikiloderma ☐ 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 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 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

***** AP may be elevated in growing children, and not reflective of liver dysfunction

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LUNGS	☐ No symptoms	☐ Mild symptoms (dyspnea with stair climbing)	☐ Moderate symptoms (dyspnea with level walking)	☐ Severe symptoms (dyspnea at rest; requiring 0_2)
FEV1 ☐	☐ FEV1 > 80% **OR** LFS=2	☐ FEV1 60-79% **OR** LFS 3-5	☐ FEV1 40-59% **OR** LFS 6-9	☐ FEV1 ≤39% **OR** LFS 9-12
DLCO ☐				
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labialagglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable (none – 0,mild -1, moderate -2, severe – 3)

☐ Esophageal stricture or web___ ☐ Pericardial Effusion___ ☐ Pleural Effusion(s)___
☐ Ascites (serositis)___ ☐ Nephrotic syndrome___ ☐ Peripheral Neuropathy___
☐ Myasthenia Gravis___ ☐ Cardiomyopathy___ ☐ Eosinophilia > 500µl___
☐ Polymyositis___ ☐ Cardiac conduction defects___ ☐ Coronary artery involvement___

Í Platelets <100,000/µl ___ Í Progressive onset__

Í **OTHERS:** Specify:_____

Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO is not available, grading using FEV1 should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: > 80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; < 40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12.

Abbreviations: GVHD (graft versus host disease); ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); AST (aspartate aminotransferase); ULN (upper limit of normal)

Mild chronic GVHD involves only 1 or 2 organs or sites (except lung: see below) with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites).

Moderate chronic GVHD involves i) at least one organ or site with clinically significant but no major disability (maximum of score 2 in any affected organ or site), or ii) three or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 will also be considered moderate chronic GVHD.

Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic GVHD

Appendix 8. IWG 2006 response criteria

Modified International Working Group response criteria for altering natural history of MDS

EVALUATION OF RESPONSE FOR MDS

In order to apply, responses must last at least 4 weeks.

1. Complete remission
Bone marrow: less than 5% myeloblasts with normal maturation of all cell lines.
Persistent dysplasia will be noted*†
Peripheral blood:
Hgb $\geq$11 g/dL
Platelets $\geq$ 100 x 10^9/L
Neutrophils $\geq$ 1.0 x 10^9/L
Blasts 0%

2. Partial remission
All CR criteria if abnormal before treatment except:
Bone marrow blasts decreased by at least 50% over pretreatment but still more than 5%
Cellularity and morphology not relevant

3. Marrow CR
Bone marrow: maximum of 5% myeloblasts and decrease by at least 50% over pretreatment
Peripheral blood: if HI responses, they will be noted in addition to marrow CR

4. Stable disease
Failure to achieve at least PR, but no evidence of progression for at least 8 wks

5. Failure
Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pre-treatment.

6. Relapse after CR or PR
At least 1 of the following:
Return to pretreatment bone marrow blast percentage
Decrement of at least 50% from maximum remission/response levels in granulocytes or platelets
Reduction in Hgb concentration by at least 1.5 g/dL or transfusion dependence

7. Cytogenetic response
Complete
Disappearance of the chromosomal abnormality without appearance of new ones

Partial
At least 50% reduction of the chromosomal abnormality

8. Disease progression
For patients with:
Less than 5% blasts: at least 50% increase in blasts to more than 5% blasts
5%-10% blasts: at least 50% increase to more than 10% blasts
10%-20% blasts: at least 50% increase to more than 20% blasts
20%-30% blasts: at least 50% increase to more than 30% blasts
Any of the following:
At least 50% decrement from maximum remission/response in granulocytes or platelets
Reduction in Hgb by at least 2 g/dL
Transfusion dependence

9. Survival
Endpoints:
Overall: death from any cause
Event free: failure or death from any cause
PFS: disease progression or death from MDS
DFS: time to relapse
Cause-specific death: death related to MDS

Modified criteria of the IWG 2006 for hematologic improvement
In order to apply, responses must last at least 8 weeks.

Erythroid response (pretreatment < 11 g/dL)
Hgb increase at least by 1.5 g/dL
Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions / 8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of below or equal to 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response (pretreatment 100 x 10^9/L)
Absolute increase of at least 30x10^9/L for patients starting with more than 20x10^9/L platelets
Increase from less than 20x10^9/L to more than 20x10^9/L and by at least 100%

Neutrophil response (pretreatment < 1.0 x 10^9/L)
At least 100% increase and an absolute increase of at least 0.5x10^9/L

Progression or relapse after HI
At least 1 of the following:
At least 50% decrement from maximum response levels in granulocytes or platelets
Reduction in Hgb by at least 1.5 g/dL
Transfusion dependence

Appendix 9. CTCAE V4

TOXICITY CRITERIA (CTCAE)

Cancer Therapy Evaluation Program

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

Appendix 10. Centralized biological study (Hepcidine, NTBI, LPI)

2 blood Serum tube (tube sec, 5ml)

To be sent from Monday to wednesday to:

Dr LEFEBVRE Thibaud

Service de Biochimie / Centre Français des Porphyries

Hôpital Louis Mourier

178 rue des Renouillers 92701 COLOMBES CEDEX, France
Tel: (33)1 47 60 63 34
Fax: (33)1 47 60 67 03

Frozen samples:

(-20°C for sample sent on the same day or -80°C for sample sent afterward)

FOR NON TRANSPLANT PATIENT	FOR TRANSPLANT PATIENT
At Screening At M3 Post-Inclusion At M16 Post-Inclusion	At Screening At D-5 Pre-Transplant At D0 Pre-Transplant At D7 Post-Transplant At D30 Post-Transplant At D100 Post-Transplant At M12 Post-Transplant

Appendix 11. Study procedures

GROUPE A: Transplanted patients

Time from HSCT	Screening D-120 M-4	D-90 M-3	D-60 M-2	D-30 M-1	D0 HSCT	D30 M1	D60 M2	D120 D240 M4-M8	D240 D930 M8-31	D480 M16
Inform consent	X									
Clinical visit	X	X2/ M	X	X	X	X	X	X Every 2 months	X Every 3 months	X
Comorbidity assessment	X			X						
ECOG	X	X	X	X	X	X	X	X	X	X
Blood cell counts (9)	X	X1/ W	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M
Standard biology (1)	X	X	X	X	X	X	X	X	X	X
EPO, folates, B12	X									
Iron plasmatic dosage (2)	X	X	X	X	X	X	X	X	X	X
HLA typing (3)	X	X	X							
Centralized biological study (4)	X*			D-5 Pre-Allo	D0 before HSCT	D7 and D30 post allo		D 100 post Allo		M12 post-Allo
Proteinuria	X	X	X	X	X	X	X	X	X	X
Bone marrow aspiration + blood + cytogenetics (5)	X						D60 to D100 post Allo		M12, 24 &36 post inclusion	
Heart and liver MRI (6)	X			X				X*		M12 post allo
QOL	X								M12, 24 &36 post inclusion	
AE Grade III-IV	X	X	X	X	X	X	X	X	X	X
Pregraft tests (7)			X							
GVHD assessment						X	X	X	X	X
Postgraft blood chimerism (8)						X	X*	X*	X*	
Confirming date of HSCT		X	X	X						

GROUPE B: NON-Transplanted patients

Time from registration	D0 Screening	D30 M1	D60 M2	D90 M3	D120 M4	D150 M5	D180 M6	D240 D365 M8-M12	D365 D990 M12-M33	D1080 M36
Inform consent	X									
Clinical visit	X	X2/M	X	X	X	X	X	X Every 2 months	X Every 3 months	X
Comorbidity assessment	X			X						
ECOG	X	X	X	X	X	X	X	X	X	X
Blood cell counts (9)	X	X1/W	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M	X1/M
Standard biology (1)	X	X	X	X	X	X	X	X	X	X
EPO, folates, B12	X									
Iron plasmatic dosage (2)	X	X	X	X	X	X	X	X	X	X
HLA typing (3)	X	X	X							
Centralized biological study (4)	X*			X					M16	
Proteinuria	X	X	X	X	X	X	X	X	X	X
Bone marrow aspiration + blood + cytogenetics (5)	X								M12 &24	X
Heart and liver MRI (6)	X			X						
QOL	X								M12 & 24	X
AE Grade III-IV	X	X	X	X	X	X	X	X	X	X

(1) Every visit: Proteinuria, AST, ALT, serum bilirubin, serum creatinine, PAL, LDH, GGT, CRP
(2) Serum ferritin, transferrin, iron plasmatic level
(3) HLA A, B, C, DRB1 molecular level
(4) Centralized study – 2 tubes without EDTA 5 mL to Louis Mourier Hospital, see specificity in appendix 10. *Before Exjade initiation
(5) Inclusion, D60 to D100 post-transplant or once a year for non-transplant patient. Including cytogenetic and centralized shipment for molecular analysis to CHRU Lille (BM 2 ml EDTA + Blood 2 tubes 5 mL with EDTA)
(6) Only for patients with SF > 1000 ng/mL or more than 20 RBC transfusions at time of inclusion. At time of screening, could be done within 6 weeks before or after inclusion. *After transplant, should be done at D100 and before Exjade restarting
(7) Include cardiac and pulmonary assessment
(8) Assessment at D30. *Should be repeated at the next visits only if the first one is not donor > 95% or in cases a rejection is suspected
(9) Including reticulocytes and blast count