CENTRE HOSPITALIER
UNIVERSITAIRE DE NANTES

PHASE 2 STUDY OF A REDUCED-TOXICITY MYELOABLATIVE CONDITIONNING REGIMEN USING FLUDARABINE AND FULL DOSES OF IV BUSULFAN IN PEDIATRIC PATIENTS NOT ELIGIBLE FOR STANDARD MYELOABLATIVE CONDITIONING REGIMENS

N° EudraCT: 2011-004764-30
N° Reference CPP: 49/11
N° Reference CHU: BRD 11/6-N

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PROTOCOL AT A GLANCE

1. PRIMARY ENDPOINT

To assess transplant-related mortality (TRM) at one year after allo-HSCT prepared by a "reduced toxicity myeloablative" conditioning regimen in young patients (children and adolescents) with hematologic malignancies.

2. SECONDARY ENDPOINTS

- Incidence of engraftment,
- Incidence and severity of acute GVHD,
- Incidence and severity of chronic GVHD,
- Rate of relapse,
- Relapse-free survival,
- Overall Survival,
- Immune Recovery

3. TYPE OF THE STUDY

Phase II, open, prospective, multicenter study

4. INCLUSION CRITERIA

- Children and adolescents aged over 12 months and under 18 years
- Availability of an HLA identical family donor or an HLA-matched unrelated donor (10/10 or 9/10 if the mismatch level is at the level of HLACw)
- Informed consent signed by legal representative and confirmed by children (if applicable)
- Diagnosis of a hematologic malignancy which is a candidate for allo-HSCT, but not eligible for standard or conventional myeloablative conditioning regimens because of high risk for toxicity. Are considered as criteria of non-eligibility for standard or conventional myeloablative conditioning: a history of autologous or allogeneic stem cell transplantation, comorbidities or medical history predictive of a prohibitive rate of TRM and toxicity with the use of standard high dose chemotherapy and / or radiotherapy as judged by the referring physician (details provided in the full protocol).

5. TREATMENT PLAN

The conditioning regimen will include:
- IV fludarabine (30 mg/m²/day for 5 days)
- IV Busulfan (Busilvex® 3.2 mg/kg/day for 4 days) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- Anti-thymocyte globulines (Thymoglobuline®, 2.5 mg/kg/day for 2 days).

- J-6 : Fludarabine (30 mg/m²)+ Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-5 : Fludarabine (30 mg/m²) + Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-4 : Fludarabine (30 mg/m²) + Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-3 : Fludarabine (30 mg/m²) + Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-2 : Fludarabine (30 mg/m²) + Thymoglobuline® (2,5 mg/Kg)
- J-1 : Thymoglobuline® (2,5 mg/Kg)
- J0 : Graft infusion

Prophylaxis of GVHD will be provided by Cyclosporine A (CSA) alone in case of a family donor or combined to mycophenolate mofetil (MMF) in case of an unrelated donor.
After selection based on the inclusion criteria, patients with an HLA identical donor (family or not) and who have accepted to participate to this study will be included after signing the informed consent by their legal representative.
The registration of patients will be performed centrally, and will use an electronic CRF. As in transplant protocols, patients are monitored daily from the day of transplantation until the last day of hospitalization. Subsequently, the monitoring frequency will be adapted according to standard criteria. "Supportive care" will be provided according to each centre practice

6. STATISTICS

An exact procedure for Ahern in one step is used to detect a maximum rate of TRM of 10% at one year post allograft, which would correspond to a difference of 15% whereas 25% as overall mortality rate for standard myeloablative conditioning in this population. With a significance level of 0.05 and a power of 0.80, 45 patients will be necessary. Anticipating that 5-10% of patients will not be evaluable for the main question (not meeting the criteria for inclusion or non-performance of allo-HSCT), 50 patients will be included in total. Every patient included in the study and who actually received the allogeneic stem cell graft will be considered in the final analysis. A descriptive analysis will be performed on the qualitative and quantitative characteristics of donors and patients as well as primary and secondary criteria. The survival analysis will be carried out by estimating the Kaplan-Meier, and by calculating the cumulative incidences of all relevant outcomes.

7. STUDY DURATION

- Inclusion: 24 months
- Monitoring of last patient: 12 months
- Total Project Duration: 36 months

Glossary of Abbreviations

AE	Adverse event
ALL	Acute Lymphocytic Leukemia
Allo-SCT	Allogeneic Stem Cell Transplantation
AML	Acute Myeloid Leukaemia
BM	Bone Marrow
BUN	Blood Urea Nitrogen
CR	Complete response
CRF	Case report form
CSA	Cyclosporine A
CTC	Common toxicity criteria
DFS	Disease-free Survival
DSUR	Development Safety Update Report
EC	Ethics Committee
ECOG	Eastern Cooperative Oncology Group
EME	European Medecine Agency
GCP	Good clinical practice
G-CSF	Granulocyte - colony stimulating factor
GVHD	Graft Versus Host Disease
HSCT	Hematopoietic Stem Cell Transplantation
ICH	International Conference on Harmonization
IT	Intrathecal
MedDRA	Medical Dictionary for Regulatory Activity
MUD	Matched Unrelated Donor
NCI	National Cancer Institute
TRM	Transplant-related Mortality
OS	Overall Survival
PB	Peripheral Blood
PBSC	Peripheral Blood Stem Cell
PI	Principal Investigator
RI	Relapse Incidence
RIC	Reduced Intensity Conditioning
SAE	Serious adverse event
SUSAR	Suspected unexpected Serious Adverse Reaction
TRM	Transplant Related Mortality

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1. STUDY RATIONAL AND BACKGROUND

1.1. The Role of IV Busulfan and Fludarabine as a myeloablative conditioning regimen for allogeneic stem cell transplantation

Busulfan [1, 4-bis-(methanesolfonoxyl) butane] is a bifunctional alkylating agent, which was first described by Haddow and Timmis (Haddow 1953). Since the demonstration of its potent antitumor effects, it has been used extensively for the treatment of malignant disease, especially hematologic malignancies and myeloproliferative syndromes (Galton 1953, Ambs 1971, Canellos 1985, Hughes 1991). Its use in high-dose combination chemotherapy was explored with oral busulfan in combination with cyclophosphamide as pretransplant conditioning therapy for patients undergoing autologous or allogeneic marrow transplantation (Santos 1983, Lu 1984, Yeager 1986, Tutschka 1987, Peters 1987, Geller 1989, Grochow 1989, Dix 1996, Sheridan 1989, Clift 1994, Schwertfeger 1992, Vaughan 1991). High-dose busulfan (Bu), most commonly in combination with cyclophosphamide (Cy), has proven to be an effective antileukemic regimen when used in conjunction with hematopoietic stem cell support. A comparison between BuCy and cyclophosphamide (Cy) combined with total body irradiation (TBI) for preparation of patients with a hematologic malignancy undergoing allogeneic marrow transplantation illustrated that the BuCy regimen was better tolerated than and associated with survival and relapse probabilities that compare favorably with a Cy-TBI regimen (Clift 1994).

1.2. Busulfan Oral Formulation

Busulfan was originally available only as an oral formulation. Oral busulfan has several serious shortcomings. When used in high-dose combination regimens, serious side effects in the liver and lungs were often encountered (Collis 1980, Koch 1976, Oakhill 1981, Santos 1983). Several investigators reported veno-occlusive disease (VOD) of the liver leading to fatal liver failure, as the most serious side effect (Yeager 1986, Geller 1989, Grochow 1989, Dix 1996). Neurologic disturbances such as grand mal seizures, and severe nausea and vomiting were also frequently encountered (Grigg 1989, Marcus 1984, Martell 1987, Sureda 1989, Vassal 1990). It was impossible to predict which patients would develop liver failure, and it was further unknown whether the liver failure was due to toxicity from the systemic busulfan or whether it was mainly due to a first-pass phenomenon. Based on the limited information regarding busulfan pharmacokinetics, it appears however patients who absorbed a large fraction of the ingested dose, with a prolonged high busulfan plasma concentration, were at increased risk for developing serious side effects (Grochow 1989, Grochow 1993, Dix 1996). Another disadvantage with oral busulfan is that patients who developed severe nausea and vomiting shortly {within one-half to two (1/2-2) hours} after a dose has been delivered will lose part or all of the dose, and it will be virtually impossible to accurately determine how much of the dose has been lost in a vomiting subject. Furthermore, the intestinal absorption of any delivered drug may be influenced by the patient's nutritional status, and by concurrent administration of other drugs affecting the intestinal microenvironment, as well as by whether the patient has eaten in close proximity to ingestion of the administered drug dose and, finally, by the inherent biological variability in intestinal absorption between different patients (Benet 1985).

1.3. Intravenous busulfan formulation

Due to the above mentioned uncertainties, oral administration of high-dose busulfan carried with it an inherent safety problem both from the potential danger of inadvertent overdosing with a risk for lethal toxicities, as well as from the hazard of suboptimal underdosing the patient with an inadvertently high potential for recurrent or persistent malignancy after the marrow transplant. With this background, a parenteral formulation of busulfan was developed (IV Busulvex™) with complete bioavailability and absolute dose assurance. In addition, cyclophosphamide, which traditionally had been combined with Bu

for its immunosuppressive rather than antileukemic effects, has recently been shown to compete with Bu in the liver for Glutathione in its degradation, and may produce synergistic hepatic toxicity.

1.4. Rationale for using fludarabine in combination with IV Busulfan

Fludarabine is an effective drug against hematologic malignancies and appears less toxic than cyclophosphamide in many studies. Fludarabine inhibits repair of DNA crosslinks induced by alkylating agents and is likely synergistic with busulfan if administered prior to the DNA alkylating agent (Gandhi and Plunkett, 2000). Fludarabine has been used in a number of studies in BMT preparative regimens and appears an effective immunosuppressive regimen to prevent rejection and relatively well tolerated in terms of toxicity (Giralt et al 2002)

1.5. Experience with the busulfan-fludarabine regimen

The transplant group at MD Anderson (Houston, USA) explored a combination of daily Bu and the nucleoside analog fludarabine (Flu) as pretransplant conditioning therapy for patients with AML or MDS (protocol ID01-011) (de Lima et al, 2004). The combination of Flu (40 mg/m2 daily for 4 days) and intravenous busulfan (130 mg/m^2 daily for 4 days) has been actively investigated as a myeloablative, reduced toxicity preparative regimen that allowed engraftment of allogeneic progenitor cells from both related and unrelated donors with adequate disease control and acceptable toxicities also in older patients (up to age 65 years). The MD Anderson experience was published recently (De Lima et al., 2008). 67 patients received BuCy2 and subsequently 148 patients received Bu-Flu. The investigators used a Bayesian method to compare outcomes between these non-randomized patients. The groups had comparable pretreatment characteristics, except that Bu-Flu patients were older (46 vs. 39 years, $p< 0.01$), more often had unrelated donors (47.3% vs. 20.9%, $p< 0.0003$), and had shorter median follow-up (39.7 vs. 74.6 months). To account for improved supportive care and other unidentified factors that may affect outcome ("period" effects), 78 AML patients receiving Melphalan-Flu ("MF"), treated in parallel during this time (1997 to 2004) were used to estimate the period effect; The MF patients' outcomes worsened during this period. Therefore, the period effect is unlikely to explain the greatly improved outcome with Bu-Flu. Patients transplanted with Bu-Flu in CR1 had a 3 year overall survival and event-free-survival (EFS) of 78% and 74%, respectively, while CR1 patients younger than age 41 had a 3-year EFS of 89%. These results supported replacing BuCy±ATG with Bu-Flu±rabbit-ATG.

1.6. Experience with reduced intensity conditioning regimens in children and adolescents

Over the past decade, reduced-intensity conditioning (RIC) regimens have become a well-established approach in adult patients, offering curative allogeneic hematopoietic stem cell therapy to older persons and patients with comorbidities, rendering them otherwise ineligible for myeloablative procedures (Mohty et al. 2010). Because pediatric patients generally tolerate more intensive transplantation approaches, myeloablative regimens have continued to be the preferred approach in all but the highest-risk persons. In addition, although most RIC regimens use peripheral blood stem cells (PBSCs), many pediatric centers have preferred umbilical cord blood (CB) and bone marrow (BM) to PBSCs because of the lack of demonstration of a survival advantage with PBSCs in pediatric recipients and a hesitancy to collect PBSCs from minor donors (Pulsipher et al., 2005 and 2006).
Data regarding the safety and efficacy of RIC approaches to treat hematologic malignancies in pediatric patients are limited to single institution studies, and the role of this approach in pediatric cancer has yet to be defined (Pulsipher et al., 2009; Satwani et al. 2008) Moreover, experience from the adult population suggests that RIC regimens can allow for toxicity and TRM reduction on the short term at the cost of a higher disease relapse rate (Mohty et al. 2007 and 2010)

With these issues in mind, we sought to develop a modified so-called "reduced toxicity conditioning" regimen based on the combination of IV. Busulfan, Fludarabine and antithymocyte globulin (ATG) with the aim to deliver high dose myeloablation that would allow optimal disease control while minimizing toxicity.
As part of this protocol, strict eligibility criteria were established that defined subgroups at very high risk for transplantation-related mortality (TRM) and/or with a history of previous myeloablative transplantation. In addition to previous transplantation, inclusion criteria included patients with significant organ dysfunction, active fungal infection, or those receiving unrelated donor transplantation in advanced disease phases.

2. STUDY ENDPOINTS

2.1 Principal endpoint

To assess transplant-related mortality (TRM) at one year after allogeneic hematopoietic stem cell transplantation prepared by a "reduced toxicity myeloablative" conditioning regimen

2.2 Secondary endpoints

- Incidence of engraftment (neutrophils and platelets recovery after transplantation)
- Incidence and severity of acute GVHD
- Incidence and severity of chronic GVHD
- Rate of disease relapse at one year after transplantation
- Disease-free survival at one year after transplantation
- Overall Survival at one year after transplantation
- Immune Recovery (to be determined in a subgroup of patients)

3. STUDY DESIGN

This is a prospective, multicenter, non-randomized Phase II study that will include a total number of 50 patients included over a period of 2 years.

4. PATIENTS SELECTION

4.1 Inclusion criteria

- Children and adolescents aged over 12 months and under 18 years
- Availability of an HLA identical family donor or an HLA-matched unrelated donor (10/10 or 9/10 if the mismatch level is at HLACw for an unrelated donor)
- Informed consent signed by patient's legal representative, parent(s) or guardian (cf p13)
- Diagnosis of a hematologic malignancy which is a candidate for allo-HSCT, but not eligible for standard or conventional myeloablative conditioning regimens because of high risk for toxicity.

- Are considered as criteria of non-eligibility for standard or conventional myeloablative conditioning:
➔ a history of autologous or allogeneic stem cell transplantation
➔ comorbidities or medical history predictive of a prohibitive rate of TRM and toxicity with the use of standard high dose chemotherapy and / or radiotherapy.

➔ In terms of comorbidities, patients are defined as being at significant risk of TRM by:
(1) the presence of organ system dysfunction or severe systemic infections known to increase the risk of TRM with standard myeloablative transplantation regimens
(2) a history of previous myeloablative allogeneic or autologous transplantation
(3) undergoing unrelated donor transplantation in a third or higher complete remission (CR)
(4) a combination of toxicities that put the patient at high risk (>50%) of TRM with myeloablative transplantation. Patients entering by this criterion, require consultation between the local principal investigator (PI) and the study central coordinator.

➔ Definitions of qualifying organ system dysfunction are as follows:
(1) Pulmonary: carbon monoxide lung diffusion capacity (DLCO), forced expiratory volume in 1 second (FEV1), or forced vital capacity (FVC) less than 60% but not less than 30% predicted. Patients too young for pulmonary function tests with suspected pulmonary toxicity should be assessed by a consulting pulmonologist. If the pulmonologist judges the child to have moderate to severe pulmonary disease, they are qualified for inclusion.
(2) renal: creatinine clearance less than 60 but not less than 30 mL/m per 1.73 m2 or requiring dialysis;
(3) hepatic: transaminases more than 4 times normal but not more than 10 times normal or total bilirubin

more than 2.0 mg/dL but not more than 3.0 mg/dL or evidence of synthetic dysfunction with an international normalized ratio more than 2.0
(4) cardiac: ejection fraction less than 50% but not less than 30%.
(5) Patients with severe systemic fungal, bacterial, or other opportunistic infections (eg, atypical mycobacterium) that responded after a minimum of 2 weeks of therapy, but were persistent at the time of trial entry (eg, multiple pulmonary nodules that were shrinking but still visible), are eligible to enroll in the study. ***Progressive infections despite therapy are not allowed, and viral infections do not qualify patients for the study.***

- Eligible hematologic malignancies treatable with allogeneic hematopoietic cell transplantation include: acute and chronic leukemias, myelodysplasia [MDS], or lymphomas.
➔ Patients with ALL are required to be in morphologic remission (<5%blasts), whereas patients with acute myelogenous leukemia (AML) not in stringent CR are allowed (Patients not in CR should be discussed with the PI on a case per case basis).
➔ Patients with juvenile myelomonocytic leukemia (JMML) and MDS are required to have less than 5% blasts, and those with chronic myelogenous leukemia have to be in first chronic phase, accelerated phase, or subsequent chronic phase with less than 5% blasts.

4.2 Exclusion criteria

The presence of one exclusion criteria makes a patient unable to enter the protocol:
- Patient has been administered any other systemic chemotherapeutic drug (including Gemtuzumab) within 21 days prior to trial enrollment and start of the conditioning regimen. Hydroxyurea is permitted if indicated to control induction refractory disease, and IT chemotherapy is allowed if indicated as maintenance treatment for previously diagnosed leptomeningeal disease, that has been in remission for at least 3 months prior to enrollment on this study.
- Active infection. Protocol PI will be final arbiter if there is uncertainty regarding whether a previous infection is resolved.
- Children and adolescents who are not older than 12 months and under 18 years
- A donor who is HLA mismatched at the level of more than one locus.
- Poor performance status (Lansky ‹ 50%)
- Life expectancy is severely limited by concomitant illness and expected to be <12 weeks.
- Left ventricular ejection fraction $\leq$ 30%. Uncontrolled arrhythmias or symptomatic cardiac disease.
- Symptomatic pulmonary disease. FEV1, FVC and DLCO $\leq$30% of expected corrected for hemoglobin.
- Creatinine clearance less than 30 mL/m per 1.73 m2 or requiring dialysis
- Evidence of chronic active hepatitis or cirrhosis. If positive hepatitis serology, discuss with Study Chairman and consider liver biopsy.
- Effusion or ascites >1L prior to drainage.
- HIV-positive.
- Female pregnancy
- Absence of effective contraception among boys and girls of childbearing potential (that contraception should be continued until 6 months after stopping treatment)
- Breastfeeding
- Patient’s legal representative, parent(s) or guardian not able to sign informed consent.
- children's refusal
- Hypersensitivity to rabbit proteins, to the active substance or to any of the excipients of experimental products

➔ Prior to entry into the trial, the participating investigators (or designated assistant) will explain to each patient, and/or his/her legal representative, parent(s) or guardian, the nature of the trial, its purpose, procedures, expected duration, alternative therapy and the benefits and risks involved in trial participation. Each patient, parent and/or legal guardian will be given the opportunity to ask questions and will be informed of the right of the patient to withdraw from the trial at any time without prejudice. After this explanation and before entering the trial, the patient or his/her legal representative, parent(s) or guardian, will voluntarily sign and date an informed consent statement.

5. INCLUSION PROCEDURES, TREATMENT SCHEMA AND CALENDAR OF EVALUATIONS

5.1 Patients selection and registration

Patients are included if:
- they fulfil the inclusion criteria defining eligibility for "reduced-toxicity conditioning" regimen
AND
- an evaluation for organ functions has not revealed any exclusion criteria as defined,
AND
- a suitable related or unrelated donor has been identified
A specific "Registration form" will be used:
➔ An inclusion number will be attributed to the patient when he/she has met the inclusion/exclusion criteria and consent has been obtained.
This number will be automatically generated by connecting to the electronic CRF using the Capture System software accessible from the https://www.dirc-hugo-online.org/csonline web site.

5.2 Donor selection

Only HLA identical family donors OR unrelated donors with matching in 10/10 alleles (HLA-A, B, C, DRB1, DQB1) OR maximum of one allele or antigen mismatch (the mismatch level is at the level of HLACw for unrelated donors) OR family donor with maximum one allele mismatch will be selected for the purpose of this study. After informed consent, HLA-identical family donors will undergo a clinical and biological evaluation according to the national recommendations of the «Agence de Biomedecine».

➔ Donor selection is performed on the basis of a high resolution (4 digits) typing of HLA-A, B, C, DRB1, DQB1. The final selection of the most suitable donor is at the discretion of the transplanting physician. An HLA identical sibling donor or an unrelated donor matched in 10/10 alleles are equally considered as first choice donor. Health and age of the donor, CMV serostatus, a history of pregnancy and blood transfusion have to be considered in the donor selection process. Both family and unrelated donors up to 1 antigen or allele mismatch are acceptable.

➔ Bone marrow and stem cells harvest: this will be performed according to the national recommendations of the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire (SFGM-TC) and the "Agence de Biomedecine". In case of G-CSF-mobilized peripheral blood stem cells (PBSC), a total number of 6.0x10^6 CD34+ cells per kilogram recipient's body weight should be targeted with an acceptable minimum of 2.0 x 10^6 CD34+ cells per kilogram recipient's body weight
In the case of bone marrow, a total of $\geq 2x10^{8}$ mononuclear cells per kilogram recipient's body weight should be targeted.
In case of poor stem cell mobilisation or poor marrow harvest, this will be reported in the CRF, however, since this is not within the influence or responsibility of the transplant physician or the sponsor or the principle investigator of the study, this fact will not be regarded as a protocol violation.
Grafts are transfused without any further manipulation such as T-cell depletion and CD34+ selection are not permitted. Transplantation should be performed within 72 hours from start of first apheresis.
The production of PBSC or BM graft follows the standard operation procedures of the participating centers or, in case of an unrelated donor, of the donor search centers. It is the responsibility of the producing institution to perform the stem cell or bone marrow harvest according to GMP guidelines and national and international law.

5.3 Treatment plan

The conditioning regimen will include:
- IV fludarabine (30 mg/m²/day for 5 days)
- IV Busulfan (Busilvex® 3.2 mg/kg/day for 4 days) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- Anti-thymocyte globulines (Thymoglobuline®, 2.5 mg/kg/day for 2 days).

- J-6 : Fludarabine (30 mg/m²)+ Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-5 : Fludarabine (30 mg/m²) + Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-4 : Fludarabine (30 mg/m²) + Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-3 : Fludarabine (30 mg/m²) + Busilvex® (3.2 mg/kg) (the Busulfan dose is to be adapted to the weight of the child according to the drug label)
- J-2 : Fludarabine (30 mg/m²) + Thymoglobuline® (2,5 mg/Kg)
- J-1 : Thymoglobuline® (2,5 mg/Kg)
- J0 : Graft infusion

➔Administration regimen:

- Busulfan:
  - 3.2 mg / kg / day, i.e. 12.8 mg / kg total dose By slow intravenous administration in four daily injections of 0.8 mg / kg (hospital pharmacy preparation)
  - With prophylactic anti-comitial treatment (exemple, Valium® morning and evening on the day before administration of Busulfan, then until the day following (inclusive) the final day of administration of Busulfan)

➔ In small children the recommended IV Busulfan dose is:

Body weight (Kg)	Busilvex® dose (mg/kg)
< 9	1.0
9 à < 16	1.2
16 à 23	1.1
> 23 à 34	0.95
> 34	0.8

In small children (weight < 9 kg), in case of obesity, in case of known liver dysfunction, or in case of a recent use of Gemtuzumab, a PK analysis may be recommanded for dose ajustment. This should be done according to each center standard pratice when it comes to the use of IV Busulfan.

- Anti-thymocyte Globulin (Thymoglobuline®) 2.5 mg/Kg/day for 2 days
  - 2.5 mg / kg / day, i.e. 5 mg / kg total dose
  - By intravenous infusion over eight to 12 hours
  - On Day-2 and Day-1
  - Premedication using corticosteroids and antihistamines (depending on the routine practice in each participating centre)

- Fludarabine:
For All brand drugs of fludarabine, the use is not recommended in children due to a lack of data on safety and/or efficacy. For exemple, the RCP of Fludara® states that "the safety and effectiveness of Fludara® in children have not been established. The use of Fludara® is not recommended in children".

The coordinator justifies its use in such indication. An argument has been written to health authorities

➔ GVHD prophylaxis:
- Cyclosporine A alone (CSA; 3 mg/Kg IV from day-3) in case of a family donor
- Cyclosporine A (CSA; 3 mg/Kg IV from day-3) and Mycophenolate Mofetil (MMF; 600 mg/m² twice per day from day-3) in case of MUD.
IV CSA continuous infusion daily, to be changed to oral dosing whenever tolerated.
As a general guideline, MMF and CSA can be tapered after transplant starting from day 60 and 90, respectively, if no GVHD is present.

➔ Graft infusion at day 0 and premedication according to local practice

➔ Supportive care: will be performed according to each participating centre usual practice. As in standard transplant protocols, patients are monitored daily from the day of transplantation until the last day of hospitalization. Subsequently, the monitoring frequency will be adapted according to standard criteria

➔ CNS prophylaxis (for patients with previous CNS involvement)
IT are allowed according to standard protocols beginning day +30.

➔ Other treatments administration
Treatment administration for Fludarabine and IV Busulfan should be done according to the protocol. All other concomitant medications shall follow standard transplant procedures as per local procedures (e.g. Antiemetics should be administered per institutional guidelines prior to the first dose of Bu and continued on a fixed schedule through 12-24 hours after the last dose of Bu.

➔ IF NOT MANDATORY OR NECESSARY, THE USE OF PARACETAMOL SHOULD BE AVOIDED DURING BUSULFAN ADMINISTRATION, SINCE IT INTERFERES WITH THE METABOLISM OF BUSULFAN AND MAY CONTRIBUTE TO SERIOUS LIVER DAMAGE.

➔ Other drugs known to interfere with the metabolism of fludarabine and/or busulfan should not to be concomitantly used during the chemotherapy administration up to and including the day of transplantation. In particular, voriconazole, itraconazole, and metronidazole as well as Tyrosine-kinase inhibitor therapy must be omitted for at least 10 days prior to admission for transplantation on this program since these agents have well described interference with busulfan. They can be resumed on or after the day of the stem cell transplant as indicated for the individual patient.

5.4. Safety Committee

An independent safety committee (composed of experts in the field) will be established by the sponsor to assess at regular intervals the progress of the trial, the safety data, and the critical efficacy endpoints, and to recommend to the sponsor whether to continue, modify, or stop the trial.
During the study, meetings of the Independent Data Monitoring Committee will be organised periodically (i.e. on inclusion of five, 10, 15, and 24 patients), every year for the DSUR.
The members will receive a copy of SUSAR.
The members of this committee are listed in the APPENDIX 15.

The members of the Independent Committee agree to participate in safety committee by signing a participation agreement.

5.5. Calendar of evaluations (APPENDIX 9)

5.5.1 Evaluation before inclusion into the study

Function of organ systems have to be documented before inclusion, as outlined in the inclusion criteria. This includes physical exam, chest x-rax, ECG, echocardiogram, lung function test, liver function tests, pancreas function tests, creatinine, BUN, total protein. In addition, blood cell counts including a differential count of white blood cells (and a bone marrow aspiration whenever appropriate) is required.

5.5.2 Evaluation before start of conditioning

A routine exam of clinical chemistry values is performed according to local standards. For examination of chimerism, samples from patient and donor have to be collected and stored.

5.5.3 Evaluation of early toxicity

- day of neutrophil (>500/µl) and platelets (first of three days with >20G/l without transfusion) engraftment
- performance status
- maximum toxicity with respect to mucositis, liver, pancreas, kidney, lung, heart, neurological system according to CTC criteria (cf. appendices)
- infections (bacteremia, fungemia, invasive fungal infection, CMV reactivation and disease, other viral reactivation or infection),
- acute GVHD (cf. appendices)
- Bone marrow aspiration with evaluation of morphological response (whenever appropriate) as well as chimerism from peripheral blood

5.5.4 Evaluation during follow up

At 3 months from allo-SCT (d +90 to +120)

- performance status
- maximum toxicity with respect to mucositis, liver, pancreas, kidney, lung, heart, neurological system according to CTC criteria (cf. appendices)
- infections (bacteremia, fungemia, invasive fungal infection, CMV reactivation and disease, other viral reactivation or infection)
- acute GVHD (cf. appendices)
- date of discontinuation of immunosuppressive medication (if appropriate)
- blood counts
- bone marrow aspiration with evaluation of morphological response (if appropriate) as well as chimerism from peripheral blood

At 6 and 12 months after allo-SCT

- performance status
- maximum toxicity with respect to mucositis, liver, pancreas, kidney, lung, heart, neurological system according to CTC criteria (cf. appendices)
- infections (bacteremia, fungemia, invasive fungal infection, CMV reactivation and disease, other viral reactivation or infection)
- grade of acute and chronic GVHD (cf. appendices); GVHD is classified according to clinical symptoms.
- date of discontinuation of immunosuppressive medication
- blood counts
- bone marrow aspiration with evaluation of morphological response (if appropriate)

At J7 J14, J21, M5, M6, M9 and M12, **the maximum volume of blood sample at each visit will be between 10 and 15 ml.**
At J28, J56 and J90-J120, due to the chimerism analysis, **the maximum volume of blood sample at each visit will be between 22 and 25 ml.**

6. EVALUATION CRITERIA

6.1 Primary endpoint

Evaluation of the cumulative incidence of TRM at 12 months after transplantation

6.2 Secondary endpoints

- Incidence of engraftment defined as the first day of neutrophil (>500/µl for 3 consecutive days). Engraftment failure is defined as neutrophil <500/µl at day+42 after allo-SCT.
- Evaluation of overall (OS) and disease-free survival (DFS) at 1 year after transplantation
- Cumulative incidence of relapse, death from disease, and non-relapse mortality (NRM)
- Cumulative incidences and severity of acute and chronic Graft-versus-Host disease
- Immune Recovery parameters

7. SAFETY ASPECTS AND ADVERSE EVENTS

7.1 Adverse Event (AE)

An adverse event (AE) is any noxious, unintended, or untoward medical event occurring at any dose that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values. Any medical condition that was present prior to study treatment and that remains unchanged or improved should not be recorded as an AE. A diagnosis or syndrome should be recorded on the AE page of the Case Report Form rather than the individual signs or symptoms of the diagnosis or syndrome.
All AEs will be recorded by the Investigator(s) from the time of signing the informed consent until the end of the designated follow-up period.
An Adverse event is not necessary related to a drug.

7.2 Serious Adverse Event (SAE)

A serious adverse event is any event occurring irrespective of the dose (including overdose) and that:

- ☐ Results in death
- ☐ Is life-threatening1
- ☐ Requires inpatient hospitalization or prolongation of existing hospitalization
- ☐ Results in persistent or significant disability or incapacity2
- ☐ Is a congenital anomaly or birth defect
- ☐ Is an important medical event3 (as reported in IME list)

1 "Life-threatening" means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.
2 "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.
3 Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

7.3 adverse drug reaction (or effect)

All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

7.4 Definition of an expected adverse event (AE-E)

An "expected" adverse event is one, the nature or severity of which is consistent with information already available in the relevant source document(s).
A serious adverse event fulfils with seriousness definition as mentioned in paragraph 7.2
The expected adverse events in this study can be summarized as follow:

- related to the disease itself = disease symptoms in relation with relapse or progression
- related to study products (fludarabine/Busulfan/Thymoglobuline®) according to the drug brochures (please see appendices)
    - o The most frequent events are those:
    - Related to immunosupression such as infectious complications (candidosis, herpes-zoster, pneumonia, bacteremia, septicemia, septic choc,…)
    - Related to hematological toxicity (anemia, leuco-neutropenia with or without fever, thrombocytopenia, aplasia..)
    - Related to toxicity on the mucosal and cutaneous tissues: mucositis, rash, dermatitis ,alopecia…
    - Related to digestive toxicity (nausea, vomiting, anorexia..)
    - Related to renal impairement (increase of serum creatinine, renal failure..)
    - Related to liver and pancreatic toxicity (increase of ASAT, ALAT, serum bilirubin, or increase of pancreatic enzymes)
    - Related to central or peripheral neurological toxicity
        i. anxiety, agitation, somnolence
        ii. peripheral neuropathy
- related to fertility and teratogenicity concerns:

Busulfan : Ovarian suppression and amenorrhoea with menopausal symptoms commonly occur in pre-menopausal patients. Busulfan has caused embryofoetal lethality and malformations in pre-clinical studies There are no adequate data from the use of either busulfan in pregnant woman. A few cases of congenital abnormalities have been reported with low-dose oral busulfan, not necessarily attributable to the active substance, and third trimester exposure may be associated with impaired intrauterine growth.Women of childbearing potential have to use effective contraception during and up to 6 months after treatment.
busulfan can impair fertility in male too . Impotence, sterility, azoospermia, and testicular atrophy have been reported in male patients. Therefore, men treated with Busilvex® are advised not to father a child during and up to 6 months after treatment and to seek advice on cryo-conservation of sperm prior to treatment because of the possibility of irreversible infertility due to therapy with Busilvex®.

All adverse effects not previously described in the drug brochures should be considered as unexpected AEs

- Related to the allogeneic transplantation procedure itself such as acute GVHD, aplasia, etc.
- Related to other concomitant treatments (e.g. growth factors, analgesic drugs, anti-emetic drugs, immunosuppressive therapy):
- Related to immunosuppressive drugs
- Related to other condition (crash, accident...)
- Related to the consequence of a preexisting medical condition

7.5 Definition of an unexpected adverse event (AE-E)

An "unexpected" adverse event is one, the nature or severity of which is not consistent with information already available in the relevant source document(s). Until source documents are amended, expedited reporting is required for additional occurrences of the reaction.

As part of this protocol, serious adverse events that will be considered are those > grade 3. Except for death, AE related to disease progression or to the allogeneic transplant procedure itself will not be communicated to the sponsor irrespective of their severity. They only have to be reported in the eCRF.

The declaration modalities of serious adverse events is detailed in Appendix N°12

7.6 Classification of severity

For both adverse events (AE) and severe AE (SAE), the investigator(s) must assess the severity of the event. The severity of the AEs will be graded on a scale going from 1 to 5 according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events Version 3.0 (NCI CTCAE). The NCI CTCAE V3.0 can be viewed on-line at the following NCI web site:

http://ctep.cancer.gov/reporting/ctc.html. If a specific event is not included in the NCI CTCAE toxicity scale, the following scale should be used to grade the event:

Grade	Definition
1	**Mild** Awareness of sign, symptom, or event, usually transient, requiring no special treatment and generally not interfering with usual daily activities
2	**Moderate** Discomfort that causes interference with usual activities; usually ameliorated by basic therapeutic manoeuvres
3	**Severe** Incapacitating with inability to do usual activities or significantly affects clinical status and warrants intervention. Hospitalization may or may not be required
4	**Life-threatening** Immediate risk of death; requires hospitalization and clinical intervention.
5	**Death**

7.7 Classification of relationship/causality of adverse events (SAE/AE) to study drug

The investigator(s) must determine the relationship between the administration of study drug and the occurrence of an AE/SAE as "not reasonable possibility" or "reasonable possibility" as defined below:

Reasonable possibility: The temporal relationship of the adverse event to study drug administration makes a causal relationship possible, and other medications, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

Not reasonable possibility: The temporal relationship of the adverse event to study drug administration makes a causal relationship unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.

The sponsor of the trial shall determine the causality.

7.8 Procedure in case of a serious adverse event

The declaration should be made by fax to 02 53 48 28 36 using the specific declaration form in appendix 12, and within a delay of 24 hours (opening hours).

Direction de la Recherche CHU de Nantes, 5 allée de l'Ile Gloriette, 4093 NANTES Cedex1 Fax : 02 53 48 28 36 Tel: 02 53 48 28 35

Precise recommendations for the investigator are detailed in a guideline.

7.9 Serious Adverse Event Reporting

Reporting of Adverse Events to Regulatory Authorities and the Ethics Committee

The sponsor will inform all relevant regulatory authorities and the ethics Committee according to mandatory rules:

- of all relevant information about serious unexpected adverse events suspected to be related to the study medication that are fatal or life threatening as soon as possible, and in any case no later than seven days after being aware of such a case. Relevant follow-up information for these cases will subsequently be submitted within an additional eight days.
- of all other serious unexpected events suspected to be related to the study medication as soon as possible , but within a maximum of fifteen days of first knowledge by the investigator.

7.10 Annual safety report (DSUR)

A safety report will be produced annually at time of the anniversary of the clinical trial authorization issued by the competent authority.
This report comprises three parts: report on patient safety, "line-listing" of SAE and comprehensive summary of study status.
The report is produced by the sponsor of research in collaboration with the principal investigator. This report will be submitted to the relevant authorities by the sponsor within 60 days from the above anniversary date.
In addition, an independent data safety committee will be constituted to assist the sponsor and the principal investigator in case of difficulties.

8. CRITERIA OF PREMATURE DISCONTINUATION OR PATIENT EXIT FROM THE STUDY

A premature discontinuation is defined when a patient selected in a trial ceases its participation before the end of study.
The criteria for premature discontinuation of the study are:
- Patient's refusal to continue the study
- Interruption of the study as per the sponsor's decision or per the regulatory agencies
- Cancellation of allo-SCT

In case of premature discontinuation during the selection period, the patient will be replaced and his data will not be taken into account for analysis. However, monitoring of patients who have undergone allo-SCT

will be pursued, even if they have ceased their participation in the trial. In addition, monitoring of patients who are included in the protocol, but who ceased their participation in the trial, will be pursued.

Premature discontinuation of the study shall also occur in case there is:
1 – Delay or absence of neutrophil recovery (ANC <500 at day+60) in at least 10% of patients, in the absence of ongoing GVHD or antiviral treatment against cytomegalovirus or disease progression. This criteria will be studied at day 60 after inclusion of the first 15 patients (evaluation every 5 patients). The stopping rules for the absence of neutrophil recovery >10% are as follow: 2/5, 3/10, 4/15 patients (lower limit of the 90% confidence interval).

2 – Excessive transplant-related mortality at day 100 after allo-SCT >10%
This criteria will be studied at day 100 after inclusion of the first 15 patients (evaluation every 5 patients). The stopping rules for a rate of transplant related mortality >10% are as follow: 2/5, 3/10, 4/15 patients (lower limit of the 90% confidence interval).

9. STASTICAL CONSIDERATIONS

9.1 Calculation of the number of patients

An exact procedure for Ahern in one step is used to detect a maximum rate of TRM of 10% at one year post allograft, which would correspond to a difference of 15% whereas 25% as overall mortality rate for standard myeloablative conditioning in this population. With a significance level of 0.05 and a power of 0.80, 45 patients will be necessary. Anticipating that 5-10% of patients will not be evaluable for the main question (not meeting the criteria for inclusion or non-performance of allo-HSCT), 50 patients will be included in total. Every patient included in the study and who actually received the allogeneic stem cell graft will be considered in the final analysis. A descriptive analysis will be performed on the qualitative and quantitative characteristics of donors and patients as well as primary and secondary criteria. The survival analysis will be carried out by estimating the Kaplan-Meier, and by calculating the cumulative incidences of all relevant outcomes.

9.2 Dealing with missing data

Every effort will be made to keep the number of missing values for all parameters to a minimum. Missing data on overall survival is assumed to be 0 and on event free survival to be below 10 % as patient care after transplantation is very close.

9.3 Statistical analyses

Every patient included in the study and who was actually transplanted, will be taken into account at time of data analysis. A descriptive analysis will be conducted on the following parameters:
- The characteristics of donors and patients
- Primary and secondary endpoints

Qualitative data will be described in frequency and percentage and will be represented using histograms or diagrams of distribution. They will be compared using the X2 test or Fisher exact test. Quantitative data will be described using the calculations of average, standard deviation, median, and extreme values, and will be compared with the Mann & Whitney nonparametric test.
The toxicities rate will be calculated and will be given with their 95% confidence intervals.
The probabilities of survival will be estimated by the Kaplan-Meier method and by calculating the cumulative incidences for relapse/progression, GVHD and TRM incidence.
All tests will be bilateral, and the level of significance is 0.05.

10. QUALITY ASSESSMENT

10.1 Good Clinical Practice

The study will be performed according to the Guidelines for Good Clinical Practice (ICH Harmonised Tripartite Guideline for Good Clinical Practice, (17.01.1997)).

10.2 Auditing

To guarantee a high quality of treatment for the patients within the present study, the sponsor will insure that the participating centres fulfil the following criteria:

- fulfilling of legal requirements
- experience of the principle investigator of the entire study and the respective centre with respect to study conduct
- ICH/GCP knowledge and certifications

10.3 Monitoring

Regular monitoring is an essential part of the study conduct. It will be performed by the sponsor of the trial. After the initiation visit, the frequency of monitoring visits will depend on the course of the study, and recruitment. It is the monitor's responsibility to make the local investigators and all the stuff who is involved into the study or the care of the patients familiar with the protocol.
During the course of the study, the monitor will control the progress of the study, the commitment to the protocol, the documentation and careful usage of the study medication, and the maintenance of GCP guidelines and legal obligations. Problems as well as changes in reported data will be worked out in collaboration with the local investigator, who is obliged to cooperate with the monitor and to allow access to the patients' charts. Source data verification is performed by the monitor. In terms of Risk for patients, the protocol has been classified at Class C. Therefore, 100% source data verification will be performed in about 40% of the patients. In contrast, inclusion criteria will be verified in 100% of patients. The monitor will have to respect that the data she/he comes into contact with are highly confidential. A monitoring report will be provided for each visit.

11. FINAL REPORT AND PUBLICATION RULES

At the end of the study evaluation the principle investigator presents a final report, containing the clinical report, single tables, and the final conclusions.
Publication of the results is realized independently from the outcome of the trial. The study or parts of the study should be published by the writing committee only which consists of the persons in charge of the study as mentioned on the front page. According to the EBMT rules, co-authors will be offered to the local PI of participating centres, the order depending on the number of patients included by the respective centres, or depending on their contribution to the protocol or the realisation of the study. Other investigators will be mentioned in the addendum. All publications and/or communications related to this trial should at least mention the central coordinator and the PI of the trial and the sponsor.

12. REGULATORY ASPECTS

The study will be conducted according to the European Union directive (ICH Harmonised Tripartite Guideline for Good Clinical Practice, (17.01.1997)). The study has to be conducted in compliance with the protocol, GCP and all applicable regulatory requirements:

- Helsinki declaration from1964, revised in Washington in 2002,
- GCP of the International Harmonization Conference (ICH–E6, 17/07/96),
- European Directive (2001/20/CE) on the conduct of clinical trials,
- French law n° 2004-801 dated 6 August 2004,
- French bioethics law n° 2004-800 dated 6 August 2004.

Accordingly, investigating physicians, have to provide direct access to study documents to monitoring, audits, institutional internal control, external authorities, and the members of the ethical committee. Written informed consent by the patient is mandatory.

The co-ordinating investigator and all investigators will be given an up-to-date investigator's brochure containing full details of the status of the pre-clinical and clinical knowledge of the study medication. As soon as new information is obtained, an updated version will be supplied or an amendment added to the existing investigator's brochure.

12.1 Regulatory Authorities Approval (CPP and AFSSAPS)

No patient may be included in the study before the respective requirements of the national health authorities are fulfilled. The trial will begin only after the positive vote of the responsible Ethics Committee (CPP) and after the approval by the appropriate national health authority (AFSSAPS).
The protocol has to be followed strictly, protocol violations have to be documented and the reason has to be given (e.g. emergency measures). Any changes in the protocol can only be performed by the principle investigator or the protocol writing committee. Any subsequent changes will be reported or submitted for approval to the ethical committee and to local and national authorities.

12.2 Informed consent

Written informed consent is obtained by each patient before inclusion into the study. Using patient information sheets, as well as personal oral explanation by a local investigator at the patient's transplant centre, the patient will be informed of the aims and the investigational nature of the study, the exact procedures that will be done during treatment and evaluation, the possible risks and side effects, and of alternative treatment options. They will be informed as to the strict confidentiality of their patient data, but that authorised individuals other than their treating physician may review their medical records for trial purposes. Further, the patient will be informed, that their anonymized data will be scientifically analysed and published. It will be emphasised that the participation is voluntary and that consent can be withdrawn by the patient at any time without explanation of the reason. The patient is allowed to refuse further participation in the protocol, whenever he/she wants. The patient's further treatment will not be influenced by this decision.

12.3 Responsibilities

In collaboration and according to the SOP of the sponsor (CHU de Nantes), a detailed list of delegation of responsibilities has been established. It is held by the sponsor, who is finally responsible for the correct performance of delegated responsibilities by the respective persons or institutions. A copy of this list will be delivered to the participating institutions.
The investigator of each institution undertakes to conduct the trial according to the protocol which was approved by the ethics (CCP) and health authorities (AFSSAPS). The investigator must not make any changes to the protocol without the permission of the sponsor and without the CCP has given a favourable opinion on the proposed amendments.
It is the responsibility of the investigator responsible for the trial in the participating center:
- To provide his curriculum vitae as well as co-investigators,
- Identify team members, who will participate in the trial and define their responsibilities,
- To start recruiting patients after authorization of the sponsor,
- Try to include the required number of patients within the period of recruitment.

It is the responsibility of each investigator:
- To obtain the informed consent dated and signed personally by the patient before any selection process specific to the trial
- To fill in a CRF for each patient included in the trial and allow direct access to source documents to validate CRF data,
- Correct, sign and date the correction of the CRF for each patient enrolled,
- Notify the sponsor of any serious adverse events within the time required
- To accept regular monitoring visits and possibly those of auditors mandated by the sponsor or inspectors from authorities.

All documentation on the study (protocol, consents, notebooks observation, file investigator, etc.), And original documents (laboratory results, x-rays, minutes of consultations, review reports, etc..) must be kept in a safe place and considered confidential material. The data archiving will be under the responsibility of the investigator and, as required by law.

Data will be kept for a minimum of 15 years after the end of the study.

12.4 Sponsor responsabilites

It is the responsibility of the sponsor to:
- Subscribe insurance to cover its liability for the harmful consequences of research,
- Provide the investigators with all information necessary to conduct the research,
- Pay any charges in relation with the submission of this protocol to health authorities,
- Apply for authorization from a relevant CPP,
- Apply for authorization from AFSSAPS,
- Inform the Directors and Pharmacists of health facilities,
- Inform AFSSAPS, CPP and EMEA of any serious incidents that may be due to research.

12.5 Protocol amendments

Any amendment to this protocol must be agreed to by the sponsor. Written verification of EC and AFSSAPS approval will be obtained before any amendment is implemented.

12.6 Protocol deviations

When an emergency occurs that requires a deviation from the protocol for a subject, a deviation will be made only for that subject. A decision will be made as soon as possible to determine whether or not the subject (for whom the deviation from protocol was effected) is to continue in the study. The subject's medical records will completely describe the deviation from the protocol and state the reasons for such deviation. Non-emergency minor deviations from the protocol will be permitted with approval of the Principal Investigator

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14. APPENDICES (in french)

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