PROTOCOLE

Prospective and multicentre evaluation of 3 different doses of IV busulfan associated with fludarabine and thymoglobuline in the conditioning of allogeneic stem cell transplantation (SCT) from a matched related or unrelated donor in patients with poor prognosis myeloïd malignancies

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1. APPROBATION AND SIGNATURES

Prospective and multicentre evaluation of 3 different doses of IV busulfan associated with fludarabine and thymoglobuline in the conditioning of allogeneic stem cell transplantation (SCT) from a matched related or unrelated donor in patients with poor risk myeloïd malignancies – AAA-IPC 2011-003

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La recherche sera conduite conformément au protocole, aux bonnes pratiques cliniques et aux dispositions législatives et réglementaires en vigueur.

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2. STUDY RATIONALE

2.1. Current and overall situation

Patients with poor prognosis or advanced AML and MDS are only curable by allogeneic hematopoietic stem cell transplantation (Allo-HSCT). Overall results remain however poor due to transplant-related mortality (TRM) due to standard myeloablative conditioning (i.e. TBI-based or oral Bu-Cy2 conditioning) and i antitumor effect that remains insufficient. In addition, peak frequency of these diseases occurring after 55 years of age, most patients represent a population for whom standard allo-HSCT is associated with the highest risk of transplant related mortality. Thus, establishing an optimal transplant procedure, combining low toxicity and high efficiency, is a major goal.

Historically, myeloablative-conditioning regimens (MAC) have been used before allogeneic stem cell transplantation, to reduce the graft failure risk, to reduce the tumor bulk, and to make "space" for donor stem cells. On the other hand, high-dose total body irradiation (TBI) [1] or high-dose oral busulfan associated to cyclophosphamide (Cy) [2] were associated with serious life threatening or lethal toxicity, limiting the use of such therapy to young (less than 45 years old) and fit patients.

More recently, these dogmas have been challenged by several studies showing that MAC was not necessary to allow engraftment, but it could be accomplished using a less intensive, or reduced-intensity conditioning regimen (RIC). Furthermore, it has been demonstrated that the therapeutic effect of allogeneic transplantation was essentially the consequence of a graft immune reaction cells against tumor cells [3-5]. This supposition has been supported by the observations of 1) a high(er) relapse rate after T-cell depletion [6], 2) by a low(er) relapse rate for patients with GVHD [7], and 3) by the tumor control observed, at least in some diseases, after infusion of donor lymphocytes (DLI) [8-12]. Thus, several reduced intensity (RIC) or nonmyeloablative conditioning regimens (NMA) have been reported to demonstrate an acceptable engraftment rate and a dramatic decrease of early non-relapse mortality (NMR) [13-16]. As a consequence, more patients can be transplanted even if older than previously accepted (up to 70 years old) [17], or suffering comorbid conditions or having been previously treated with high-dose chemotherapy [18].

However, some problems remain to be solved with RIC/NMA: notably, the relapse rate is higher than after MAC, partially linked to the inclusion of patients with more advanced, chemotherapy-refractory, disease. Indeed, when compared to MAC, the overall survival rate after allogeneic stem cell transplantation with RIC/NMA is similar, but for different reasons: a higher relapse rate, and lower NRM after RIC/NMA and higher NRM and lower relapse after MAC [19,20].

Recently, the access to cytotoxic drugs, and new drug formulations, with a more favorable toxicity profile such as intravenous busulfan (ivBU) or treosulfan, has dramatically modified the scenario. Indeed, conditioning regimens based on these agents in combination with nucleoside analogs rather than additional alkylating agents or TBI has conceptually redefined conditioning therapy and allowed the systematic investigation of a new category of myeloablative, "reduced toxicity" conditioning (RTC) regimens. Thus, the RTC regimens contain drugs at myeloablative doses but whose clinical toxicity is similar to that of RIC/NMA.

The replacement oral Bu (osBU) with ivBU is extremely interesting due to the well-known erratic bioavailability of oral formulation with a narrow therapeutic window [21].

The pharmacokinetic (PK) profile of ivBU, in association with cyclophosphamide (ivBUCy2) and administered in 4 daily doses for 4 days (total dose 12.8 mg/kg), has been extensively evaluated [22-24]. In these studies, it has been shown that:

1. IvBU single dose of 0.8 mg/kg corresponds to osBU 1 mg/kg
2. IvBU PK is constant and in 81% of patients the therapeutic window was achieved, significantly higher than osBU (50%). The mean change of PK parameters is 20%.
3. First dose AUC allows predicting the AUC at steady state
4. The solvent vehicle in the iv formulation does not modify drug (BU) metabolism
5. A close correlation between AUC, toxicity, and survival (the therapeutic range of "- window" is considered optimal between approximately 900 and 1,500 mcmol x min, when assessed as a one-dose surrogate estimate in the most commonly used (every-6-hour) 16-dose regimen, or approximately a daily AUC of 5,000 mcMol-min (range of about 3,800-6,000) when used in the once-daily 4-day regimen).

Based on these facts, the association of myeloablative dose of ivBU and Cy is well tolerated compared to osBuCy2, with a significantly reduction of liver, nervous and pulmonary toxicities and a low 1-year NRM of approximately 13% [23,25].

In the phase I and II, the ivBU dose was administered [23,24] 4 times per day like osBU. However, ivBU can be used one time per day (3.2 mg/kg/day or 130 mg/m2/day x 4 days), based on its excellent tolerance, on its activity not depending on continuous exposure (being an alkylating agent) [26-28]. A randomized study from South Korea confirmed that ivBU administered once per day for 4 days had the same toxicity and efficacy profile as compared to when it was administered 4 times a day in fractioned administration). In this study, ivBU was administered with Cy or Fludarabine (Flu), BU PK data were not different in either combination; the mean AUC were comparable (6058 vs 6457 mmol x min, respectively) [29]. Others studies are presented in annex 5.

In the standard combination of osBU plus Cy, liver toxicity is not only linked to osBU but rather to the combination of BU with Cy as demonstrated by McDonald and coworkers, who studied Cy and its activated metabolites [30], and by Hassan et al [30]. Furthermore, the activity of Cy on several hematological malignancies such as AML is questionable. Based on available experience it is likely that Cy could be replaced with a nucleoside analog with high immunosuppressive and antileukemic effects, such as Flu, which, when paired with its alternative metabolism, would improve the clinical safety of the conditioning regimen.

The Table 1 reported studies using an association of myeloablative iv BU (12.8 mg/kg or 520 mg/m²) and fludarabine. In first study, from Canada, 70 patients received ivBU, administered in a once daily schedule (3.2 mg/kg per day x 4 days) with Fludarabine (50 mg/m2/day x 5 days) and ATG (4.5 mg/kg over 3 days) (FLUBUP). GVHD prophylaxis was CyA plus short course methotrexate. Donors were HLA identical–related donors in 43 patients and MUDs for 21. The mean 100-day and 2-year NRM were 2% and 10%, respectively, and the grade II-IV aGVHD incidence was 8%.The incidence of grade II mucositis and hemorrhagic cystitis were 70% and 13%, respectively. Liver toxicity was quite typical with transaminases elevation during the first week followed by transient hyperbilirubinemia in the second week. The authors did not observe clinical veno-occlusive disease (VOD). Graft failure was observed in 3% of patients. The PK study results were similar to those reported by Ryu et al with no difference between the first and the last dose of busulfan [27].

Russell et al. compared two cohorts of patients: one conditioned with MAC without ATG and one receiving mostly FLUBUP with ATG (FLUBUP-T). Donor was HLA identical siblings in both cohorts. The use of ATG reduced significantly the NRM and cGVHD. The disease free survival (DFS) was similar between the two groups with a better overall survival in the cohort with ATG. However, the relapse rate was slightly higher in the ATG group (not statistically significant) [31].

A third study from Calgary confirmed the good tolerance of the FLUBUP-T before HLA identical sibling transplantation. In this study with 200 patients with hematological diseases, the 5-year NRM was between 4% and 6% in patients with low risk disease whatever the age, while it was 27% in patients older than 45 years and with high-risk disease. Grade 2-4 aGVHD and cGVHD incidence was 14% and 54%, respectively [32].

In a disease-specific study from the MD Anderson Cancer Center, 96 patients with AML or MDS (56% with active disease before transplantation), were grafted with a BU-Flu variant conditioning regimen, using Flu (40 mg/m²/d for 4 days), ivBU (130 mg/m²/d for 4 days). GVHD prophylaxis was performed by tacrolimus and micro-methotrexate, and low-dose ATG was added for patients having a one-antigen mismatched related or unrelated donor). The tolerance was good: severe mucositis was recorded in 13%, hemorrhagic cystitis in 3%. No neurological toxicity was observed and only 2 patients developed (reversible) VOD, again, transaminases and bilirubin elevations were observed in 18% and 9% of patients, respectively. The majority of patients with active disease before transplantation achieved complete remission, and the 1-year OS and DFS for the whole group were 65% and 52%, respectively, with no difference between patients having HLAid donors or MUDs. PK parameters were similar to those reported in the literature with a mean AUC of 4891 mmol x min [28,33].

All these studies confirmed the good tolerance of myeloablative doses of ivBU when associated with Flu, with low NRM and acute/chronic GVHD incidence limited, mainly when ATG was added to the BU-Flu variant regimens. Furthermore, the PK parameters confirmed a low inter- and intra-patient variability in BU disposition.

In spite of the favorable toxicity profile and the (supposed) synergistic antitumor effect of BU and Flu [34], some concerns have been raised as to the antitumoral efficacy of FLUBUP-T is equivalent to more standardized (primarily BU Cy2) MAC regimens [35,36]. Indeed, in a retrospective "matched-pair" analysis from the CIBMTR, comparing FLUBUP-T and osBUCy2 before allogeneic transplantation in patients with hematological malignancies, the relapse incidence was higher (42% vs 20%) and the NRM was lower in the FLUBUP T group (12% vs 34), but OS was similar. The authors concluded, that several reasons can explain these somewhat paradoxical results and in particular the low NRM in the FLUBUP-T group will expose more patients to the risk of relapse of their disease [35]. Accordingly, Russell et al. added an intermediate dose of TBI (4 Gy) to FLUBUP-T, thus achieving a reduced relapse rate without adding toxicity [37].

In a retrospective study in AML/MDS patients, the BU-Flu conditioning regimen was evaluated against ivBUCy2, using Bayesian technology [38].

In a cohort of mostly advanced patients (half of them was in more > CR1), the OS, event free survival (EFS), and DFS were significantly better in the BU-Flu group (70% vs 59%, 62% vs 37%, and 86% vs 66%, respectively). Similar results were observed in the subgroup of patients transplanted in CR. This study confirmed the efficacy of BU-Flu association in advanced AML/MDS patients. It is of particular note that there was no significant detectable loss of antileukemic efficacy in the BU-Flu group [38].

A very recent prospective study however challenged these data. Investigators from Korea found better overall survival after BUCY as compared to BUFLU mainly related to an increased relapse rate. These data are conflicting with the data we present as well as the rest of the literature. It should be pointed out that in this study 34% (BUCY) and 40% (BUFLU) of the patients were treated for acute lymphoblastic leukemia (ALL) [39]. This probably impact on the results and is likely to explain differences with previous reported results.

Authors	Study	Age	Pts	Donor	Diseases	CTX	CTX	Proph GVHD	A GVHD II-IV	C GVHD	NRM
Russell 2002	Retrospective PK studies with once daily ivBU	41	70	HLAid sib MUD	AML	FBUATG	F 250 mg/m² ivBU 3.2 mg/kg x 4d ATG 4.5 mg/kg	CsA + MTX	9%	38%	@100d: 5% @2y: 10%
De Lima 2004	Retrospective with once daily ivBU. PK studies	45	96	HLAid sib 55% MUD 38% MMUD 6%	AML, MDS	FBU	F 160 mg/m² ivBU 130 mg/m2 x 4d	FK506 + mMTX	25%	55%	@100d: 5% @1y: 3%
Chae 2007	Retrospective comparing 2 CTX	38	40 vs 55	HLAid sib 89% MUD 11%	AL 76% AL 80%	FBU vs BuCy2	F 180 mg/m² ivBU 3.2/j x 4 j	CsA + MTX	15% vs 71%	44% vs 84%	@2y: 10% vs 34%
Russell 2007	Matched pair analysis	42	54 vs 54	HLAid sib 100%	Not stated	FBUATG vs MAC no ATG	F 250 mg/m² ivBU 3.2 mg/kg x 4d ATG 4.5 mg/kg	CsA + MTX	19% vs 32%	55% vs 96%	@100d: 4% vs 17% @4y: 9% vs 34%
Chunduri 2008	Retrospective	44	36	HLAid sib 47% MUD 53%	AL 94%	FBU	F 160 mg/m² ivBU 3.2 mg/kg x 4d	FK506 + mMTX ATG MUD	19%	37%	@100d: 5%
Bredeson 2008	Matched pair analysis	46	120 vs 215	HLAid sib 100%	AL 56% AL 64%	FBUATG vs BUosCy2	F 250 mg/m² ivBU 3.2 mg/kg x 4d ATG 4.5 mg/kg	CsA + MTX	15% vs 34%	39% vs 32%	@1y: 9% vs 24% @5y: 12% vs 34%
Andersson 2008	Retrospective comparision using Bayesian method	46	148 vs 67 vs 78	HLAid sib 61% MUD 39%	AML/MDS 100%	FBU vs BUCy vs FM	F 160 mg/m² ivBU 130 mg/m² x 4d ivBU 0.8 mg/kgx 4 x 4d	FK506 + mMTX ATG MUD	15% vs 32% vs 25%	34% vs 36% vs 39%	@ 12% vs 27%
Alatrash 2011	Retrospective	58	74	HLAid sib 52% MUD 48%	AML/MDS	FBU	F 160 mg/m² ivBU 130 mg/m² x 4d	FK506 + mMTX	41%	42%	@1y: 21%

Table 1: PK : pharmacokinetic ; ivBU : intravenous busulfan ; HLAid sib : HLA identical sibling ; MUD : matched unrelated donor ; MMUD : mis-matched unrelated donor ; AL : acute leukemia ; AML : acute myeloid leukemia ; MDS : myelodysplastic syndrome ; F : fludarabine ; CTX : conditioning regimen ; MAC : myeloablative conditioning regimen ; mMTX : micro-methotrexate ; CsA : cyclosporine A ; NRM : non relapse mortality ; FM : fludarabine plus melphalan

Recently two publications have brought some new insights in this field. The MD Anderson team has analyzed 79 patients ≥ 55 years of age (median, 58 years) with AML (n = 63) or MDS (n = 16) treated with i.v. Bu-Flu conditioning regimens between 2001 and 2009 (median follow-up, 24 months). One-year transplant-related mortality (TRM) rates for patients who were in CR or who had active disease at the time of transplantation were 19% and 20%, respectively. The 2-year overall survival (OS) rates for patients in first complete remission (CR1), second CR (CR2), or refractory disease and for all patients at time of transplantation were 71%, 44%, 32%, and 46%, respectively; 2-year event-free survival (EFS) rates for patients in CR1, CR2, or refractory disease at time of transplantation and for all patients were 68%, 42%, 30%, and 44%, respectively [40]. These results show that full dose IV BU-Flu could be safely administrated to older patients.

On the other hand a retrospective study from the CIBMTR raised a warning concerning the use of ATG in the conditioning regimen [41]. Indeed, in this study, the patients receiving ATG had a lower DFS than the one who have received T-cell replete regimens. However it should be noted that the median dose of ATG received by patients was 7 mg/kg that correspond to a high dose. This confirms the critical point represented by the level of T-cell depletion. Our previous results with the dose of 7.5 or 10 mg/kg are in line showing very high relapse and infection rates [42]. Our experience with lower doses (2.5 or 5 mg/kg) did not show the same problems [16,43]. Indeed the effect of ATG may probably not be in relation with a simple threshold. We would rather suggest an optimal window: with a low dose (2.5 mg/kg) GVHD prevention is far to be optimal exposing patients to NRM. In the other hand high doses (i.e. >=7.5 mg/kg) may expose patients to high relapse rate and increased infections and thus higher NRM [44].

2.2. Location of the work in the context of the current knowledge

However, despite the widespread diffusion of RIC regimen, the question of importance of dose-intensity in the conditioning regimen for the overall efficacy of allogeneic transplantation is still unresolved [28 45].

In an attempt to address this question, our group performed a randomized study comparing a RIC regimen with a NMA regimen with the goal of identifying a regimen that could be improved upon [46]. The RIC regimen (Flu-Bu-rATG) arm consisted of Flu (30 mg/m2/day during for 5 days), oral Busulfan (Bu) (4 mg/kg/day for 2 days), and rabbit ATG (rATG) (2.5 mg/kg on day 3). CSA alone was administered for post-graft immunosuppression. The second NMAC arm (Flu-TBI) included Flu (30 mg/m²/day for 3 days) and a 2-grays TBI in one session on day 0. CSA and MMF were administered for post-graft immunosuppression. Each "regimen package" obviously differed from the other in terms of myeloablation (some with Bu vs. no Bu combined with low dose TBI) and immunosuppression (in vivo T-cell depletion in the Flu-Bu arm vs. CSA and MMF in the Flu-TBI arm). One hundred and thirty nine patients with a median age of 55 years and with various hematologic malignancies were transplanted from a matched related donor and randomized to receive NMAC or RIC. The incidence of grade II-IV acute GVHD was higher after Flu-Bu-rATG (47 vs. 27%, respectively; p = 0.01), while the incidence of c-GVHD did not differ. The Flu-Bu-rATG cohort had a higher objective decrease in measurable disease (65 vs. 46%, respectively; p = 0.05) and lower relapse rates (27 vs. 54%, respectively; p < 0.01). The NRM was higher after Flu-Bu-rATG than after Flu-TBI (38 vs. 22%, respectively; p = 0.027). At five years, the OS was 41% for the entire cohort and did not statistically differ between the 2 groups, which overall can be considered favorable, considering the patient characteristics and long follow-up. Thus, five years after transplant, the Bu regimen was associated with better disease control than the Flu-TBI regimen; however, this did not translate into better OS, because the NRM rate in the Flu-BU group was higher. The conclusion from

this trial was that both conditioning regimens had strengths and weaknesses, but were equivalent for OS.

However, following this trial, we postulated that we could improve the results after Flu-BU-rATG treatment by decreasing NRM while concomitantly retaining superior anti-tumor activity by fine-tuning the dose of r-ATG, which is an option that remains controversial. First, we confirmed our results in a large single center cohort of 100 consecutive patients with hematological malignancies undergoing allo-HSCT from an HLA-matched-related donor and treated with the same Flu-Bu-rATG RIC. With a median follow-up of 60 months, the probabilities of OS and progression-free survival (PFS) at 5 years were 60% and 54%, respectively [16]. NRM was adversely associated with acute GVHD (HR = 6; p = 0.0002) while the incidences of grade II-IV acute and extensive chronic GVHD were 43% and 69%, respectively.

Because of this more potent antitumor effect, we elicited to refine the RIC approach, associating Fludarabine-Busulfan-rabbit-ATG, with the goal to decrease TRM. Since 2000, of the 748 patients we treated with different RIC regimens, 520 were conditioned with this association. Different evolutions were performed during this period:

- Switch from high dose rATG [42,47] to one dose rATG [16];
- Switch from Oral Bu to Iv Bu.
- We then showed those 2 days instead of 1day of r-ATG conducted to lower severe acute and chronic GVHD without increasing relapse [43].
- Finally because many patients underwent transplantation while febrile in relation with the last day of rATG we decided to delayed graft infusion after one day of rest.

We recently analyzed a cohort of patients with myeloid malignancies treated with this association in Marseille and Nantes: Of the 166 patients (median age of 57 years), 106 were treated with 2 days of IV BX. 39 of these patients had CR1 AML without poor-prognosis cytogenetics (Standard-risk (SR) group) while 67 were treated for MDS, AML beyond CR1 or CR1 AML with poor-cytogenetics (poor-risk (PR) group). SR and PR groups achieved promising 2 year DFS (60% (40-76) and 46% (32-58) respectively. Although the results achieved in poor risk patients compare favorably with the ones reported after standard myeloablative conditioning [48] or less intensive regimens [49] they need further improvement.

Revisiting the CDT myeloablation intensity in the context of a limited procedure related toxicity might be one answer. Indeed the results achieved in the 60 patients with AML/MDS treated with 3 or 4 days of IV BX in Marseille and Nantes show an interesting 2-year NRM of less than10%. In addition and as previously mentioned, a recent report from the MD Anderson showed that full doses IV-BX (i.e. 4 days) could be safely administrated to older patients [40].

Thus, albeit the safety of the SCT procedure has been greatly improved, further refining the intensity of the conditioning is an important issue to explore, especially in patients with poor prognosis, the goal being to maintain the very favorable safety profile and improve the disease control. This is the goal our prospective trial; we aim to prospectively evaluate the encouraging, preliminary small scale single arm experience in a prospective multicenter trial targeting patients with high-risk myeloid malignancies. In addition, this trial will associate four ancillary studies to the main clinical objective: 1/ a prospective assessment of the quality of life of the patients over a period of 2 years 2/ an analysis of the cost effectiveness of the procedure, assessed over a period of 2 years 3/ an observational busulfan pharmacokinetic study 4/ a busulfan pharmacogenomic study

Quality of life assessment. The importance of secondary end points, such as the impact of treatment on functional status and Health Related Quality of Life (HRQL), has been recognized, particularly when alternative treatment options with similar potential for long-term survival become available [50] . The choice of the allogeneic stem cells conditioning is characterized by a relapse/toxicity arbitrage that impact the HRQL of patients. The methodological challenges posed by the assessment of HRQL are substantial. Although HRQL research is progressing, most published studies of patients with allogeneic stem cells transplantation do not include patients with a progression of their disease and do not consider the GVHD in the statistical models of HRQL comparison [51-53]. Conversely, several publications indicated the major role of GVHD in the post-allogeneic transplantation HRQL [54-57]. Attempting to address this question, our group performed a HRQL analysis alongside a randomized study comparing a RIC regimen (fludarabine, osBU, and ATG) with a NMA regimen (fludarabine plus low dose TBI). Preliminary results have been presented at ISPOR 2011[58] and the paper is under revision. We demonstrated that while evolution of HRQL across time was not different between RIC and NMA after adjustment on the baseline HRQL score and the GVHD, the global quality of Life and cognitive functioning were better in the NMA, independently of time. GVHD was a predictor of HRQL and should be included in the HRQL comparison of allogeneic transplantation conditioning regimen.
The aim of the present HRQL study alongside the phase II clinical trial is to prospectively compare the evolution of HRQL across time between BX3 and BX2 and between BX4 and BX2.

Cost effectiveness analysis. The economic impact of innovative therapeutic strategies is of major importance in our publicly funded health care system because of the rise in health care spending across the industrialized countries. However, for structural reasons, in health care the free market doesn't work. Economic evaluation was developed by economists to get round this market failure. Cost Effectiveness Analysis (CEA) is a type of economic evaluation that examines both the costs and health outcomes of alternative intervention strategies[59]. Despite the widespread diffusion of RIC regimen, the question of the economic impact of dose-intensity of the conditioning regimen is still present [60-63]. Attempting to address this question, our team performed an economic evaluation alongside a randomized study comparing a RIC regimen (fludarabine, osBU, and ATG) with a NMA regimen (fludarabine plus low dose TBI). Preliminary results have been presented at ISPOR 2011 and the paper is under revision. We demonstrated that using DFS as endpoint, the RIC was cost-effective: incremental cost-effectiveness ratio=978.64 € and using OS no differences were found between the two groups. These results highlighted that the choice of endpoints and follow-up times in the economic evaluation of cancer treatment is of major importance.
The aim of the cost-effectiveness analysis alongside the phase II clinical trial is to prospectively assess the cost and the consequences to the patients included in the randomized trial to determine the cost-effectiveness ratio of BX3 when comparing to BX2 and the cost effectiveness ratio of BX4 when comparing to BX2

Busulfan pharmacokinetic study. Marseille Pharmacokinetic laboratory will perform the pharmacokinetics for all patients.
A non-decisional, observational, PK study will be performed to evaluate the potential benefit of using "patient-individualized" dose instead of a dose standardized to body size. Indeed, it has been demonstrated that the PK variability of ivBU was lesser than osBU [21]**.** Nevertheless, it has been reported that a 2-fold degree of variability in PK parameters (clearance and AUC) was still present [33] Nath, 2008 #3505]**,** and that most PK studies of ivBU have been performed in association with Cy, with 4 daily BU administrations, most of them in pediatric populations. All these points justify the search for an individualized dosing strategy of busulfan, guided by a PK study. The results will not guide the treatment, but they will be analyzed at the end of the clinical study. Correlations will then be sought between BU PK parameters and clinical outcomes such as OS, EFS, toxicity and GvHD.

Busulfan pharmacogenomic study. A Pharmacogenomic study will be performed with collaborators at the University of Texas MD Anderson Cancer Center in Houston, Texas. Additionally, pharmacogenomic studies will be carried out on cell samples derived from volunteering patients who are transplanted with active disease.
The current collaboration has grown out from our collaboration with the Laboratory for Molecular Pharmacology and Translational Drug development at the Department of Stem cell Transplantation and Cellular Therapy at the UT MD Anderson Cancer Center (Houston, TX, USA). We have in both experimental human cell line models and in primary cell material from patients with active leukemia been working to develop an improved understanding of the (clinically active) mechanism(s) of cellular Busulfan-resistance and –sensitivity, and how to find pharmacological means for how such cellular drug resistance can be reversed/circumvented. We established human cell line models for AML and CML and have identified various genetic factors that contribute to Busulfan resistance. Changes in expression of these identified genes were confirmed in leukemia patient derived cell samples which were classified as either "clinically resistant" or "sensitive" to Busulfan. We found up-regulation of anti-apoptotic and down-regulation of pro-apoptotic genes in Busulfan-resistant ("Bu-") cells. Pro-survival HSP90 and STAT3, and various DNA repair genes were also up-regulated in drug-resistant cells. Figure 1 shows the differential expression of pro-apoptotic BNIP3 and BIK, anti-apoptotic BCL-XL and CDKN1A, and pro-survival HSP90 and STAT3 in "Bu-sensitive" and "Bu-refractory" cells derived from AML patients. We will extend the analysis of these genes using patient samples which will be collected from the currently proposed multicenter study. We have developed a collection of RT-PCR probes for these genes. In addition to the described genes we will also cellular analyze expression of PARP-1, ATM, Ku70/86 and RAD51 among DNA repair genes and expression of various GST isoforms, all of which have altered expression in Bu-resistant cell lines, but where we have yet to analyze clinical patient-derived cell material. The results from these analyses will provide us essential information on the correlation between the efficacy of Busulfan-based pretransplant conditioning therapy and expression of genes involved in Bu metabolism, DNA repair and survival/apoptosis. We anticipate that the correlative data will assist in defining the patient population who is most likely to benefit from the described treatment program.

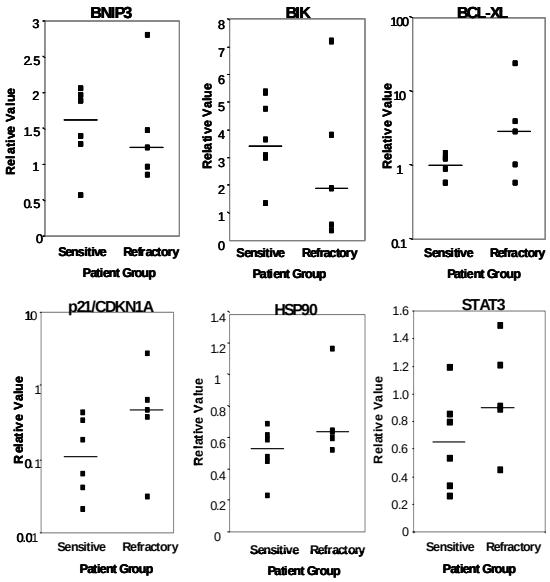

Figure 1. Gene expression analysis using samples from clinically sensitive or resistant AML patients. cDNAs were prepared using total RNA extracted from patient cell samples and expressions of specific genes were analyzed by real-time RT-PCR. The bar shows the median value for each group of patient samples

Finally, it can be anticipated that the increased understanding of cellular mechanisms underlying Busulfan resistance will assist in increasing the long-term disease control rate through more design of more effective conditioning therapy.

3. STUDY OBJECTIVES

3.1 Main objective

To assess the 2-year progression free survival rates in patients with high-risk myeloid malignancies following HSCT using different dose levels of IV Busulfan (BX3 and BX4) combined with fludarabine and thymoglobuline as conditioning therapy.

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3.2 Secondary objectives

To document the following endpoints:

- Full donor chimerism achievement, hematologic recovery and response to treatment rates
- acute GVHD, chronic GVHD, relapse, and non-relapse mortality cumulative incidences
- overall survival
- Safety

3.3 Ancillary studies

- Quality of life assessment
- Economic evaluation of the procedure
- Busulfan pharmacokinetic study
- Busulfan pharmacogenomic study

4. CONCEPTION OF THE RESEARCH

4.1 Evaluation criteria

Main Criteria

- Time to progression or death

Secondary Criteria

- Time to death and cause of death
- Time to acute and chronic GVHD according to the NIH classification and relapse
- Response to treatment
- Hematological recovery defined as the achievement 500 ANC and 50 000 platelets (without transfusion)
- Full donor chimerism achievement at M1, M2, M3
- Occurrence of grade 3-4 adverse events according the CTC AE v4.0 scale within 6 months after conditionning

Ancillary studies

- Quality of life assessment

Health Related Quality of Life (HRQL) will be measured prospectively by the EORTC QLQ-C30 and the EORT QLQ-HDC29 (high dose chemotherapy module) [64].

- Economic evaluation of the procedure
The endpoint of the cost-effectiveness analysis will be the number of quality adjusted life years gained (QALY's). The study will be done from the hospital point of view. Only direct medical costs will be included.

- Busulfan pharmacokinetic study
Plasma concentrations of busulfan will be measured (see details in annex).

- Busulfan pharmacogenomic study
The quantification of gene expression will be carried out by comparative CT methodology (i.e., threshold cycle number at which the increase in fluorescence is logarithmic).

4.2 Methodology

The objective of this prospective, randomized, multicenter clinical trial is to evaluate the efficacy of different conditioning regimens. The study is a phase II trial randomizing patients between a prospective active control arm (BX2) and two experimental arms (BX3 and BX4). A standard group was kept in this clinical trial in order to avoid the limitations induced by the comparison with historical controls in the context of continuously improving practice. Each experimental arm will be conducted in parallel according to a standard phase II trial design [67].

4.3 Description of the research

After signed inform consent and verification of eligibility criteria, patients will receive the conditioning allocated by the randomization then allograft. They will be evaluated for safety during the treatment period and up to 6 months, and for response to treatment during 2 years (see details in flow-chart chapter 7).

4.4 Duration of participation

Duration of inclusion: 3 years.
The patients will be followed during 2 years.
Study duration: 5 years.
177 patients and 10 investigators sites anticipated.

4.5 Stopping rules

For a patient: Death, lost of follow-up, consent withdrawal, patient or investigator decision
For the research: The study could be stopped prematurely on sponsor or ANSM/CPP decision.

4.6 End of research

The end of the research corresponds to the last visit of the last patient included.

5. PATIENT SELECTION

5.1 Inclusion criteria

1- Patients with poor prognosis myeloid malignancies:
   - Myelodysplasic syndrome,
   - AML beyond CR1,
   - CR1 AML with poor risk cytogenetics
2- Adult patients: aged ≥ 55 years up to 65
   or < 55 years not eligible for myeloablative conditioning regimen based on TBI or double alkylating agent combinations.
3- Availability of a HLA identical sibling or matched unrelated donor (10/10)
4- Affiliation to social security
5- Written Informed Consent

5.2 Exclusion criteria

1- History of previous Allo-HSCT
2- HIV positivity
3- Signs of chronic active hepatitis B and/or C
4- Evolutive psychiatric disease
5- Concomitant neoplasic disease
6- Pregnant or lactating woman or without contraception (for child bearing potential women)
7- Usual contra-indications for Allo-HSCT

5.3 Patient registration and randomization

After obtaining signed informed consent and validation of the results of the initial assessements, eligible patients will be registered by contacting the sponsor structure:

Département de la Recherche Clinique et de l'Innovation
Téléphone : 04.91.22.37.78
Fax: 04.91.22.36.01
e-mail : drci.up@ipc.unicancer.fr
https://www.canceropole-paca-coonline.com/crfonline/

The inclusion number will be send by mail to the investigator to confirm the inclusion as well as the result of randomization for the dose level of Busilvex (BX).

6. TREATMENTS

6.1 Conditioning regimens

<u>BX2 F5Bx2SAL2 (Reference arm)</u>

- Fludarabine (Fludara®): 30 mg/m^2 on D-6, D-5, D-4, D-3 and D-2
- Busulfan IV (Busilvex®) : 3.2 mg/kg/d on D-4 and D-3
- Thymoglobuline®: 2.5 mg/kg/d on D-3 and D-2

<u>BX3 (+ 50%): F5Bx3SAL2</u>

- Fludarabine (Fludara®): 30 mg/m² on D-6, D-5, D-4, D-3 and D-2
- Busulfan IV (Busilvex®) : 3.2 mg/kg/d on D-5, D-4 and D-3
- Thymoglobuline® : 2.5 mg/kg/d on D-3 and D-2

BX4 (+ 100%): F5Bx4SAL2

- Fludarabine (Fludara®): 30 mg/m²on D-6, D-5, D-4, D-3 and D-2
- Busulfan IV (Busilvex®) : 3.2 mg/kg/d on D-6, D-5, D-4 and D-3
- Thymoglobuline® : 2.5 mg/kg/d on D-3 and D-2

In all variant regimens the fludarabine is to be given first over about 30 min by controlled-rate infusion pump through a central line, then followed by the busulfan iv over three hours, also by pump.

6.2 GVHD Prophylaxis

Ciclosporin A: from D-3 (starting IV dose = 3 mg/kg) to D120 (then progressive reduction until D180).

6.3 Donor

- Mobilisation: GCSF (Neupogen ® or granocyte ®) during 5 to 6 days (D-5 to D-1): SC 10 µg/kg/d.
- Harvest at D0: apheresis to obtain 4 x 10^6 CD34+cells/kg. If needed the harvest will be continue on the following days for a maximum of 2 supplemental days after additional infusion of GCSF.

6.4 Cells infusion

At D0 usual premedications will be used prior to cells infusion. Number of cells infused will be recorded.

PATIENT	**D-6**	**D-5**	**D-4**	**D-3**	**D-2**	**D-1**	**D0**
Fludarabine 30 mg/m²	x	x	x	x	x		
Busulfan 3.2 mg/kg/d							
BX2			x	x			
BX3		x	x	x			
BX4	x	x	x	x			
Thymoglobuline 2.5 mg/kg/d				x	x		
cells infusion							x
Ciclosporin A 3 mg/kg				x	x	x	x

DONOR							
GCSF SC 10 µg/kg/d		**x**	**x**	**x**	**x**	**x**	
apheresis							**x**

6.5 Authorized and non-authorized concomitant medication

Medication which interfere with Busulfan pharmacokinetic during the conditioning are not allowed (such as Itraconazole, metronidazole, voriconazole, paracetamol…)
Caution should be exercised when using paracetamol prior to (less than 72 hours) or concurrently with Busilvex due to a possible decrease in the metabolism of busulfan.
After the conditioning, anti-infectious, transfusions, or groth factors should be used according to sites habits.

6.6 Other treatment

Other treatment for the disease administrated during the follow-up period will be recorded in the CRF: name, date of administration, and reason (for example, other chemotherapy, DLI, second allograft, …)

7 – DESCRIPTION OF THE RESEARCH AND EVALUATIONS

7.1 Table of evaluation

Visite	**Inclu clu-sion**	**D -7**	**D -6**	**D -5**	**D -4**	**D -3**	**D -2**	**D -1**	**D0**	**S1**	**S2**	**S3**	**S4**	**M2**	**M3**	**M4**	**M5**	**M6**	**M8**	**M10**	**M12**	**M15**	**M18**	**M21**	**M24**
Fludarabine			x	x	x	x	x																		
Busulfan BX2					x	x																			
Busulfan BX3				x	x	x																			
Busulfan BX4			x	x	x	x																			
Thymoglobuline						x	x																		
Allogreffe*									x																
Ciclosporin A						x	x	x	x	x	x	x	x	x	x	x	réduction								
PATIENT																									
[1] Consentement	x																								
[2] Critères d'inclusion/exclusion	x																								
[3] Inclusion	x																								
[4] Examen clinique	x																								
[5] NFS, plaquettes, bilan hépatique et rénal	x			x		x		x		xx	xx	xx	xx	xxxx	xxxx	x	x	x	x	x	x	x	x	x	x
[6] Myélogramme	x												x		x	si indiqué en cas de suspicion de rechute hématologique									
[7] Pharmacocinétique : 1 tube			BX4	BX3	BX2	X																			
[8] Pharmacogénomique : 7 tubes		x																							
[9] Questionnaires Qualité de vie		x											x	x				x			x		x		x
[10] Médico-économique	x																								
[11] Prise de greffe										Récupération hématologique (PNN>500 et plaquettes>20 000 sans transfusion)															
[12] Chimérisme													x	x	x										
[13] GVH, traitement GVH										x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
[14] Evaluation de la maladie, autre traitement de la maladie, statut vital													x	x	x	x	x	x	x	x	x	x	x	x	x
[15] Toxicité			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x							
[16] Ttt concomitants			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x							

[1] Free and informed written consent has to be obtained from each patient prior to any clinical examination or specific study procedure, however results from disease evaluation should be used if < 30 days before inclusion.
[2] Inclusion/Exclusion criteria should be reviewed prior to the first dose to be sure the patient is eligible for treatment.
[3] Inclusion: Demography (sex, date of birth), Characteristic of the disease (AML, MDS), date of diagnosis, disease status at inclusion, molecular biology, Karyotype, … Previous antineoplastic treatment, type of induction, date of RC1, number of treatment cycle Relevant medical/surgical history, concomitant medication Donor demography: date of birth, sex, HLA, related or not related
[4] Physical examination, weight, height, performance status (ECOG) and vital signs (pulse, blood pressure)
[5] Hematology: neutrophil, hemoglobin and platelets will be collected at the site every other day the first week, then at least twice a week until hematological recovery (neutrophil > 500 and platelet > ~~20 000~~ 50 000 without transfusion). Associated Adverse Events > grade 2 will be captured. Biochemistry (TGOP, TGOT, bilirubine, créatinine) will be collected at the site, associated Adverse Events > grade 2 will be captured.
[6] Bone marrow aspirate: in the last 14 days before start of conditioning, at M1, and M3. Then if indicated in case of suspicion of hematological relapse
[7] PK blood sampling: 1 green top/heparinized vacutainer tubes (2 ml minimum) After the first and the last dose of Busulfan: D-4 and D-3 for BX2, D-5 and D-3 for BX3, D-6 and D-3 for BX4 : T0, just before start of infusion, T end of infusion: 5 min before the end of infusion, T1h, T3h, T6h, T9h, and T11h after the end of infusion
[8] Pharmacogenomic: 7 green top/heparinized vacutainer tubes (28 mL) and blood marrow sample
[9] Quality of life : QLQ-C30, QLQ-HDC29, EQ-5D self questionnaries
[10] Number of days hospitalization in conventional unit, intensive care unit, and day clinic unit will be recorded. The date of end of initial hospitalization will be recorded. The number of consultations with ancillary specialists will be recorded.
[11] Graft enhancement and hematological recovery
[12] Chimérisme at M1, M2 and M3
[13] GVHD (according to the NIH classification) Treatment of GVH will be recorded.
[14] Disease status: The evolution of the disease (RC RP MS MP) will be evaluated according to the hemopathy and the investigators habits, the date of hematological relapse will be recorded. In case of new therapy, name, date and reason of new therapy will be recorded, but adverse event will not be longer recorded. Vital status, date of death and cause of death will be recorded
[15] Grade 3-4 Toxicities will be evaluated during treatment period until 6 months after the treatment. Specific toxicity within 30 days of infusion: mucositis, fever, infection (viral, bacterious or fungic), hepatic toxicity, renal dysfunction will be recorded
[16] Concomitant medication will be recorded (particularly immunosuppression, morphin within 30 days of infusion).

8 - SERIOUS ADVERSE EVENTS

Adverse events will be assessed according to the Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0 (annex 3). For consistency in adverse event grading, version 4.0 must be used throughout the trial regardless of any subsequent versions of the CTC that may become available.
If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, or grades 1 - 4, will be used. Adverse event monitoring should be continued for 6 months following the conditioning.

8.1 Definitions

Serious adverse event (SAE) is defined as one of the following:

- Is fatal
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Requires inpatient hospitalization ($\geq$ 24 h) or prolongation of existing hospitalization, unless hospitalization is for:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition
    - Any SAEs that are expected due to the condition being treated, including if the SAE is a primary outcome measure, and whether there has been a clear agreement with regulators not to consider these as SAEs, provided the information is collected elsewhere
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

8.2 Reporting

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until at least 30 days after the patient has received the study treatment (D30) must be reported to the DÉPARTEMENT RECHERCHE CLINIQUE & INNOVATION within 24 hours of learning of its occurrence.

Any SAEs experienced after this 30 days period should only be reported to the DÉPARTEMENT RECHERCHE CLINIQUE & INNOVATION if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Note that any follow up information provided should describe whether the event has resolved or continues, if and how it was treated and whether the patient continued or withdrew from study participation. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs.

The investigator must assess and record the relationship of each SAE to the study drug, complete the SAE Report Form, and send the completed, signed form within 24 hours to the DÉPARTEMENT RECHERCHE CLINIQUE & INNOVATION.

Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

9. STATISTICS

The main objective of this clinical trial is to evaluate if the efficacy of two doses of IV busulfan conditioning regimens (BX3 and BX4) combined with fludarabine and thymoglobuline are promising enough to warrant further clinical investigations. The present study will randomize patients between a prospective active control arm (BX2) and two experimental arms (BX3 and BX4). Each experimental arm will then be conducted in parallel according to a standard phase II trial design [67] with no planned interim analysis.

9.1 Justification of the number of patients needed

The sample size in each arm is determined to insure that the lower boundary of the 2-year PFS rate confidence interval is higher than the predefined undesirable rate p0, p0=46%. A total of 59 patients per arm (**total 177 patients**) will allow to demonstrate a 2-year PFS rate higher than 46% with 90% confidence and an error risk of 5% assuming a 2-year PFS rate of 65%.

9.2 Analysis population

Patients undergoing HSCT transplantation with no graft failure will be included in the full analysis set and considered in the treatment arm they actually received.

9.3 Analysis criteria

9.3.1 Main analysis criteria

The main analysis criteria is defined as the occurrence of progression or death within two years following HSCT transplant. In each experimental arm, a one-sided exact binomial test [70] will be used to assess the null hypothesis of a 2-year PFS rate higher than 46%. Any experimental arm (BX3 and BX4) will be considered efficient enough to warrant further study if 2-year PFS rate is declared significantly higher than 46%. In addition lack of a 10% difference or more in favour of the control arm will be tested using an exact nonparametric test with a risk of error of 5%[71].

9.3.2 Secondary analysis criteria

Secondary analysis criteria include:
- time to non-relapse mortality (NRM),
- time to acute graft versus host disease (aGVHD) of grade >=2,
- time to chronic graft versus host disease (cGVHD),
- time to progression or death,
- time to death,
- and occurrence of response to treatment and hematological recovery.

Time-to-event outcomes will be measured from the date of HSCT transplant to the date of event or first competing event if any. Patients with no of event of interest (including compet-

ing events) at the end of study visit will be censored at the date of last follow-up visit or 100 days following HSCT transplant for aGVHD.

Overall progression free survival (PFS) and survival (OS) will be estimated using the Kaplan-Meier method [68]. Cumulative incidence of non- relapse mortality (NRM), acute graft versus host disease (aGVHD) of grade >=2, chronic graft versus host disease (cGVHD) will be provided using the Prentice method taking into account the presence of competing events [69]. Pointwise estimations for survival and competing risks data will be provided with their corresponding 95% confidence interval. The proportion of patients with response and hematological recovery will be estimated with 95% confidence intervals using exact nonparametric methods for proportions.

9.2.3 Other analysis criteria

9.2.3.1 Quality of life assessment

Methods: HRQL will be measured prospectively by the EORTC QLQ-C30 and the EORT QLQ-HDC29 (high dose chemotherapy module) [64]. The transplant patients will receive self-administered questionnaire 7 days before transplant and at M1, M2, M6, M12, M18 and M24. Patients in progression of their disease will be included in the study. Linear mixed model analysis will be performed to test whether there are differences in HRQL outcomes within and between the groups over time. A group by time interaction term will be tested to explore whether any differences in the HRQL scores were a function of group, conditional of time. If this term is not significant, the main effects (treatment and time) will be evaluated for significant differences, after adjustment on the baseline score, the GVDH, and the progression of the disease. The GVHD will be coded as 1 for grade II to IV acute GVHD or extensive chronic GVHD and 0 otherwise. The GVHD (acute and chronic) will be evaluated at the time of administration of each questionnaire for all patients included in the study.

*Data collection and analysis***.** Self-administered questionnaires will be distributed in the hospital or send by mail to the patients' address. This study will be conjointly driven by the investigators of the trial and the researchers of the UMR 912 SESSTIM unit. The UMR SESSTIM will ensure the conception, and the statistical analysis. Each center will ensure the sending, the reception and the data acquisition in the electronic case report form. Interpretation of the results and redaction of reports and scientific articles will be performed by the psychosocial research team of the UMR SESSTIM in collaboration with the investigators of the trial.

9.2.3.2 Economic evaluation of the procedure

The endpoint of the cost-effectiveness analysis will be the number of quality adjusted life years gained (QALY's). The study will be done from the hospital point of view. Only direct medical costs will be included in the study. The study period is from the beginning of the conditioning regimen until 2 years after transplantation.

Cost measure. Costs evaluation in cost-effectiveness analysis consists during a first step in measuring physical quantities consumed for the treatment administration and the consequences associated with the treatment. In a second step, a monetary value is attributed to each physical quantities consumed. This monetary valorisation will be performed on the basis of French unit prices. The main cost factors included in our study are:

- The Stem cells harvest
- The hospitalization (conventional and daily clinic visits)
- The drug administration (chemotherapy, anti-infectious drugs, growth factors, GVHD treatment and prophylaxis)
- The blood products
- The main laboratory tests (including chimerism)
- The treatment of progression

Effectiveness measure. One of the main challenges faced by cost-effectiveness analysis was to develop an index including both duration and quality of life. The indicator developed was therefore based on the quality adjusted life yeargained(QALYs), which is calculated by weighting each year of lifegained with a coefficient ranging between 0 and 1. The determination of the weighting coefficient is challenging for the economists, however the standard tool called EQ-5D recently developed by the group EuroQOL can be used to weight QALYs in a way which can be easily understood by patients, while obeying the scientific conventions pertaining to the properties of coefficients of this kind [65]. In our study the utility associated with each of the health states considered in the model will be measured alongside the quality of life study, with the EQ-5D administered at day -7, day +30, day +80, day +180, day +360, day +540 and day +720. Patients in progression of their disease will be included in the analysis.

Sensitivity analysis. The statistical risk of measure and the uncertainty of some methodological choices will be covered by the sensitivity analysis.

Data collection and analysis. Physical quantities of resources consumed will be collected in the electronic CRF of the trial. EQ-5D questionnaires will be administered in the quality of life study.
This study will be conjointly driven by the investigators of the trial and the researchers of the UMR 912 SESSTIM unit. The UMR SESSTIM will ensure the conception, and the statistical analysis. Each centre will ensure the sending, the reception and the data acquisition in the electronic CRF. Interpretation of the results and redaction of reports and scientific articles will be performed by the psychosocial research team of the UMR SESSTIM in collaboration with the investigators of the trial.

10. ADMINISTRATIVE PROCEDURES

Data Monitoring Commitee

The constitution of an internal Data Monitoring Committee (DMC) will be set up by the sponsor to review and estimate the evolution of the research, in particular data relative to the security of the patients during the study.

It consists of the Investigator Coordinator, the person in charge of the pharmacovigilance, and a representative of the sponsor.

The role of this committee is consultative to the sponsor who takes the final decision of the implementation of the recommendations proposed by this committee.

Regulatory and ethical compliance

This clinical study was designed and shall be implemented and reported in accordance with the protocol, the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC), and with the ethical principles laid down in the Declaration of Helsinki.

Responsibilities of the investigator and IRB/IEC/REB

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB) before study start.

Informed consent

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC/REB-approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the patient source documents.

Amendments to the protocol

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by Health Authorities where required, and/or the IRB/IEC/REB. Only amendments that are required for patient safety may be implemented prior to IRB/IEC/REB approval. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any patient included in this study, even if this action represents a deviation from the protocol.

11. PROTOCOL ADHERENCE

Investigators ascertain they will apply due diligence to avoid protocol deviations. All significant protocol deviations will be recorded and reported.

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13. ANNEX

Annex 1 : CLASSIFICATION DE KARNOFSKY ET ECOG

ETAT GENERAL KARNOFSKY	ECHELLE	ETAT GENERAL ECOG-ZUBROD/WHO
Normal, pas de plaintes.	100 0	Activité normale, sans restriction.
Activité normale. Signes ou symptômes mineurs de la maladie. Activité normale avec efforts.	90 1 80	Restreint pour des activités physiques importantes mais patient ambulatoire et capable de fournir un travail léger.
Capable de se prendre en charge, mais incapable d'avoir une activité normale ou de travailler. Nécessite occasionnellement de l'aide, mais capable de subvenir à la plupart de ses besoins.	70 2 60	Ambulatoire et capable de se prendre en charge, mais incapable de fournir un travail pendant plus 50% de son temps.
Nécessite aide et soins médicaux fréquents. Nécessite soins médicaux et aide importante.	50 3 40	Capacité de prise en charge propre beaucoup plus limitée. Passe plus de 50 % de son temps au lit ou dans une chaise.
Sévèrement limité, grabataire. Indication d'hospitalisation, quoique la mort ne soit pas imminente. Gravement atteint. Hospitalisation nécessaire. Traitement symptomatique nécessaire	30 4 20	Complètement grabataire. Incapable de se prendre en charge. Le patient reste totalement couché au lit ou sur une chaise.

Annex 2: ECHELLE DE TOXICITE NCI CTC-AE v4.0

Se référer à l'échelle d'évaluation de la toxicité
" COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS " (CTCAE) version 4.0
que l'on peut télécharger sur le site du National Cancer Institute (NCI)

http://ctep.info.nih.gov/reporting/ctc.html

CTCAE v4.0 includes Adverse Events applicable to all oncology clinical trials regardless of chronicity or modality.

Annex 3: Busulfan pharmacokinetic study

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Blood sampling
Seven patients' blood samples will be collected for analysis in heparinized 4 ml green tubes (at least 2 ml collected) at the following times after the first and the last dose:
D-4 and D-3 for BX2,
D-5 and D-3 for BX3,
D-6 and D-3 for BX4
for a total of 14 samples for each patient:

- T0, just before start of infusion.
- Tend of infusion: 5 minutes before the end of infusion
- T1h: 1h after the end of infusion.
- T3h: 3h after the end of infusion.
- T6h: 6h after the end of infusion.
- T9h: 9h after the end of infusion
- T11h: 11h after the end of infusion

All PK-sample to be drawn from a peripheral (18G) line placed to ensure that there will be no contamination or cross-contamination between different ports on a multi-channel central line from turbulence around the tip of the catheter.

Sample preparation
When blood samples are obtained, they will immediately be placed on melting ice/wet ice, and carried to the laboratory. They will be centrifuged at **3000 x g** for 10min at +4℃, plasma will be removed and stored at -80℃ until shipping. Busulfan concentrations are stable in plasma for 2 years at -80℃ [66], samples will be batched for shipping. Busulfan in whole blood is stable for at least 6 hours if kept on wet ice (Madden T and Andersson BS, unpublished, 1995).

Sample shipping
Once a year and at the end of recruitment, samples will be shipped for analysis in dry ice to the central laboratory.

Measurement of plasma BUsulfan concentrations
Plasma concentrations of busulfan will be measured by a sensitive (limit of quantification: 50ng/ml) and specific liquid chromatography-tandem mass spectrometry (LC/MS/MS) method validated according to the FDA guidelines (11). The AUC will be then calculated by the linear trapezoidal rule.

Annex 4: Busulfan pharmacogenomic study

Collaboration with the Laboratory for Molecular Pharmacology and Translational Drug development at the Department of Stem cell Transplantation and Cellular Therapy at the UT MD Anderson Cancer Center (Houston, TX, USA).

Blood sampling: 28 ml green top/heparinized vacutainer tubes drawn

Plasma and cells frozen after separation on a Ficoll-Hypaque gradient within 1-2 hours of collection

Preparation of mononuclear cells (MNC). Peripheral blood and bone marrow samples from leukemia patients will be collected. Mononuclear cells will be purified using lymphocyte separation medium (Mediatech), pelleted and frozen at 80℃ prior to isolation of total RNA.

Extraction of total RNA. RNA will be extracted using TRIzolTM Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer, treated with DNAse, and purified using the RNeasy kit (Qiagen, Valencia, CA). The quality of the RNA will be determined on a 1% agarose-formaldehyde gel followed by ethidium bromide staining. Intact RNA will show prominent 28S and 18S rRNA bands while degraded RNA will show fast moving, diffused bands.

RReal-time PCR. The high capacity cDNA Archive kit (Applied Biosystems, Foster City, CA) will be used to synthesize cDNA. Real-time PCR amplification will be performed using either Taqman probe- or SYBR Green-based assay with the 7500 Real Time PCR System (Applied Biosystems). The quantification of gene expression will be carried out by comparative CT methodology (i.e., threshold cycle number at which the increase in fluorescence is logarithmic). The genes to be analyzed with real-time PCR will be selected based on our previously published studies as described.

Statistical Analysis:
The gene expression data will be correlated with busulfan pharmacokinetics as well as with the clinical endpoints of overall and disease-free survival, after stratification for whether patients were transplanted in complete remission or with active disease.

Annex 5: Compléments de justification de l'utilisation des produits

Busulfan

L'administration en 4 fois par jour du busulfan est issue des contraintes liées à la forme orale. En effet, la présentation du busulfan oral (Myléran) se limitait à des comprimés uniquement dosés à 2 mg. A la posologie classique de l'oral en conditionnement de greffe, soit 4 mg/kg/ jour, un patient de 70 kg avait besoin de 140 cp /jour, ce qui pouvait générer des difficultés de déglutition, d'où un fractionnement de la posologie.

Le schéma d'administration du busulfan I.V. a été initialement calqué sur celui du busulfan oral d'où les 4 administrations/jour du RCP. Cependant, des études concernant l'association du busulfan I.V. à la Fludarabine, aux mécanismes d'action différents et synergiques (Andersson 2009 page S12, Ciurea 2009 page 526, 527) ont incité les auteurs à utiliser une administration unique par jour.

L'étude de pharmacocinétique de Madden (2007) comparant l'administration de Busulfan + Cyclophosphamide (BuCy) en 4 administrations/jour au Busulfan+ Fludarabine (BuFlu) en 1 perfusion/jour a confirmé pour le busulfan I.V.
- une pharmacocinétique linéaire et reproductible
- une clearance équivalente
- une aire sous la courbe (AUC) journalière identique.

L'efficacité (prise de greffe) et la toxicité réduite (absence de maladie veino-occlusive hépatique) de ce schéma ont été validés en clinique par les études de Russell (2002), De Lima (2004) et de Chae (2007) [full papers] ainsi que Bermudez, et de la Serna (EBMT 2011 abstracts , population européenne).

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-**Bermudez** (2011) Once daily intravenous busulfan and fludarabine as conditioning for patients with significant co-morbidities and high-risk myeloid disease undergoing allogenic haematopoietic stem cell transplantation is well tolerated and effective. EBMT Congress 2011 abstract

-**de la Serna** (2011) Myeloablative conditioning with intravenous busulphan in a single daily dose and fludarabine for HLA-identical sibling allogenic HSCT in myeloid malignancies. EBMT Congress 2011 abstract

Fludarabine

La fludarabine est un médicament utilisé depuis plus de 10 ans dans le contexte des greffes allogéniques [1].

La fludarabine a été et est un composant pivotal dans le développement des conditionnements d'intensité réduite débuté en 1997 qui ont permis de réduire de façon majeure la mortalité toxique de la greffe allogénique et d'ouvrir cette thérapeutique à des populations jusque là non considérées comme les sujets de plus de 60 ans [2] [3].

Actuellement ces conditionnements représentent plus de 50% des conditionnements utilisés en France et en Europe. Il existe un grand nombre de modalités de conditionnement à intensité réduite mais tous intègrent la fludarabine [4] et il peut donc être estimé que plus de 10 000 patients par an dans le monde la reçoivent dans leur conditionnement.

Les doses prévues dans le protocole sont les doses classiques utilisées dans de nombreux conditionnements (30 mg/m^2 par jour pendant 5 jours), que nous avons utilisées chez plus de 600 patients à Marseille et qui sont largement inférieures aux doses cumulées de fludarabine utilisées dans le traitement de la leucémie lymphoïde chronique (30 mg/m^2 par jour pendant 3 jours 6 fois).

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