24 July 2014
EMA/550530/2014
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Busilvex

International non-proprietary name: BUSULFAN

Procedure No. EMEA/H/C/000472/II/0019

Note

Variation assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ABW	Actual body weight
AIBW	Adjusted ideal body weight
ALL	Acute Lymphoblastic Leukemia
Allo-HPCT	Allogeneic Hematopoietic Cell Transplantation
AML	Acute Myeloblastic Leukemia
ANC	Absolute Neutrophil Count
ASCT	Autologous Stem Cell Transplantation
ATG	Anti-Thymocyte Globulin
AUC	Area Under the Concentration versus time curve
BM	Bone Marrow
BK Virus	Virus isolated from a transplanted patient named B.K.
Bu	Busulfan
BuCy	Busilvex followed by Cyclophosphamide
BuCy2	Busilvex followed by Cyclophosphamide over 2 days
BuCy4	Busilvex followed by Cyclophosphamide (4 cycles)
CB	Cord Blood
CI	Confidence Interval
Cl	Drug clearance
CLL	Chronic Lymphocytic Leukemia
Cmax	Drug maximal concentration
CML	Chronic Myeloid Leukemia
CMML	Chronic Myelo Monocytic Leukemia
CMV	Cytomegalovirus
CNS	Central Nervous System
CP	Chronic Phase
CR	Complete remission
CR1/2/3	First/Second/Third Complete remission
CTC-NCI	Common Toxicity Criteria-National Cancer Institute
CV	Coefficient of variation
CVC	Central Venous Catheter
CVD	Corporate Vigilance Departement
Cy	Cyclophosphamide
D	Day
DAH	Diffuse Alveolar Hemorrage
DFS	Disease-Free Survival
DLCL	Diffuse Large Cell Lymphoma
EBV	Epstein Barr Virus
ECD	Electron Capture Detection
EFS	Event-Free Survival
EMA	European Medicine Agency
FB	Fludarabine in combination with Busilvex
FB1	Fludarabine and Busulfan Total dose equivalent to that delivered on one day
FB2	Fludarabine and Busulfan on two days
FB3	Fludarabine and Busulfan on three days
FB4	Fludarabine and Busulfan on four days
FBT	Fludarabine in combination with Busilvex and Thymoglobulin regimen
Flu	Fludarabine
FM	Fludarabine and Melphalan
GC	Gas Chromatography
GST	Glutathione-S-Transferase
GVHD	Graft Versus Host Disease
HD	Hodgkin's Disease
HLA	Human Leucocyte Antigen
HPCT	Hematopoietic Cell Transplantation
HPLC	High Performance Liquid Chromatography
HR	Hazard Ratio
HSC	Hematopoietic Stem Cell
IV	Intravenous
KM	Kaplan Meyer
KPS	Karnofsky Performance Score

LFS	Leukemia-Free Survival
LR	Low Risk
LLOQ	Lower limit of quantification
MAC	Myeloablative Conditioning
MDS	Myelo Dysplastic Syndrome
MedDRA	Medical Dictionnary For Regulatory Activities
Mel	Melphalan
mg/kg	Milligrams per kilogram
mL	MilliLiter
MM	Multiple Myeloma
MMUD	Mismatched Unrelated Donor
MPS	Myelo Proliferative Syndrom
MRD	Matched Related Donor
MS	Mass Spectrometry detection
MSD	Matched Sibling Donor
MUD	Matched Unrelated Donor
MTX	Methotrexate
N	Number of patients
NA	Not Applicable
NHL	Non-Hodgkin's Lymphoma
NR	Not Reported
NRM	Non-Relapse Mortality
NHL	Non Hodgkin's Lymphoma
OD	Once daily
OS	Overall Survival
PBSC	Peripheral Blood Stem Cell
PD	Pharmacodynamics
PFS	Progression-Free Survival
PNH	Paroxysmal Nocturnal Hemoglobinuria
PK	Pharmacokinetics
PTLD	Post-Transplant Lymphoproliferative Disorder
q.i.d.	Quater In Die
RA	Refractory Anemia
r-ATG	Rabbit ATG
RAEB	Refractory Anemia with Excess of Blasts
RFS	Relapse Free Survival
RI	Relapse Incidence
RIC	Reduced Intensity Conditioning régimen
RTC	Reduced Toxicity Conditioning regimen
SADRs	Serious Adverse Drug Reactions
SAA	Severe Aplastic Anemia
se	standard error
SOCs	System Organ Class
SOS	Sinusoidal Obstruction Syndrome
T1/2	Drug terminal half-life
TBI	Total Body Irradiation
TG	Thymoglobulin
TNC	Total Nucleated Cells
TRM	Treatment / Transplant Related Mortality
UCB	Umbilical Cord Blood
UCBT	Unrelated Cord Blood Transplant
UD/URD	Unrelated Donor
ULOQ	Upper limit of quantification
UV	Ultra-violet detection
Vd	Drug volume of distribution
VOD	Veno Oclusive Disease
WBC	White Blood Cells
WHO	World Health Organisation
Y	Year

1. Background information on the procedure

1.1. Type II variation

Pursuant to Article 16 of Commission Regulation (EC) No 1234/2008, Pierre Fabre Médicament submitted to the European Medicines Agency on 5 July 2013 an application for a variation.

This application concerns the following medicinal product:

Medicinal product:	**International non-proprietary name:**	**Presentations:**
Busilvex	BUSULFAN	See Annex A

The following variation was requested:

Variation requested		**Type**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	II

The MAH applied for an extension of indication for fludarabine followed by Busilvex (FB) as conditioning treatment prior to hematopoietic progenitor cell transplantation (HPCT) in adult patients when such combinations are considered the best available option. Consequently, changes were proposed to sections 4.1, 4.2, 4.5, 4.8, 5.1, 5.2 and 6.6 of the SmPC and the package leaflet. The MAH also took the opportunity to update the products information in line with QRD template version 9.0.

The variation proposed amendments to the SmPC, Annex II, Labelling and Package Leaflet.

Information on paediatric requirements

Not applicable.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the application included a critical report addressing the possible similarity with authorised orphan medicinal products.

1.2. Steps taken for the assessment of the product

The Rapporteur appointed by the CHMP was:

Rapporteur: Arantxa Sancho-Lopez

Submission date:	05/07/2013
Start of procedure:	26/07/2013
The CHMP adopted a report on similarity of Busilvex on similarity with Tepadina on (Appendix 1):	19/09/2013
Rapporteur's preliminary assessment report circulated on:	27/09/2013
Rapporteur's updated assessment report circulated on:	17/10/2013
Request for supplementary information and extension of timetable adopted by the CHMP on:	24/10/2013
MAH's responses submitted to the CHMP on:	13/01/2014
Rapporteur's preliminary assessment report on the MAH's responses circulated on:	05/03/2014
Rapporteur's revised assessment report on the MAH's responses circulated on:	14/03/2014
2nd Request for supplementary information and extension of timetable adopted by the CHMP on:	20/03/2014
MAH's responses to the 2nd RSI submitted to the CHMP on:	20/05/2014
Rapporteur's preliminary assessment report on the MAH's responses circulated on:	07/07/2014
Oral Explanation took place on:	22/07/2014
CHMP opinion:	24/07/2014

2. Scientific discussion

2.1. Introduction

Problem statement

The “conditioning” or “preparative” regimen plays a central and key role in allogeneic Hematopoietic Cell Progenitor Transplantation (HPCT). Conditioning is the major source of early toxicity and mortality, but also holds out the prospect of allo-HPCT success with long-term disease control or even cure. Allo-HPCT refers to any procedure where hematopoietic stem cells (HSC) of any donor type and any source are given to a recipient with the intention of repopulating and replacing the host hematopoietic system totally or partially. Currently, stem cells can be obtained from bone marrow (BM), from peripheral blood after mobilization with growth factors (PBSC), or from cord blood (CB). Allo-HPCT is nowadays considered as an effective and potentially curative treatment modality for different malignant and non-malignant conditions such as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), severe aplastic anemia (SAA), myeloproliferative syndromes (MPS), myelodysplastic syndromes (MDS), multiple myeloma (MM), Hodgkin’s and non-Hodgkin’s lymphomas (HD, NHL), genetic disorders (primary immunodeficiencies, metabolic disorders, hemoglobinopathies, etc.), and paroxysmal nocturnal hemoglobinuria (PNH).

The conditioning regimen is administered prior to allo-HPCT with three main objectives: creation of “marrow space”, immunosuppression of the host, and tumour load reduction or eradication. For these purposes, the conditioning can be sought to be either, fully myeloablative, little or non-myeloablative, or of reduced-intensity depending on disease and patients features [Bacigalupo A, 2009].

HPCT is a field where the practice changes fast, aiming to always improve the therapeutic benefits in patients with severe diseases for whom allogeneic transplant is the last possibility of cure. The introduction of intravenous Busulfan (IV Bu) in combination with cyclophosphamide (Cy) (BuCy2) as conditioning before HPCT has likely improved the safety of this conventional myeloablative regimen [Kashyap A, 2002], [Aggarwal C, 2006]. However, early regimen-related toxicity remains a matter of concern. Cy used at high dose in the conditioning regimen is a causal agent for hepatotoxicity, regardless of whether Bu is incorporated in the regimen. The HPCT medical community has looked for alternative regimens, to offer the possibility of an allogeneic HPCT to patients unable to receive a standard Cy-based conditioning. The objective was to give them the benefit of the immune allogeneic effect against the malignant cells (GVL effect), despite for example their age, comorbidities or a physical condition damaged by the previous therapeutic lines. Fludarabine (Flu), a purin-analog chemotherapy which has mild side effects appeared rapidly as an agent which could be combined with IV Bu [Andersson BS, 2009], [Terenzi A, 1996]. The risk of hepatotoxicity could theoretically be decreased by substituting Cy with another immunosuppressive agent like Flu [Ciurea SO, 2009], [Gandhi V, 2002]. The introduction of Flu as an immunosuppressive agent in the conditioning prior HPCT, as a first step in reduced-intensity regimens, and later in modified myeloablative regimens, has allowed extending indication of allogeneic transplantation according to patients’ need. Flu being administered once daily, IV Bu was administered immediately following Flu, so that maximum intracellular Bu levels occur concomitantly with optimal Flutriphosphate concentrations [De Lima Blood 2004], [Valdez BC, 2011].

About the product

The active substance of the medicinal product is busulfan: 1.4-butanediol-dimethane-sulfonate. Busulfan is a cytotoxic agent with bifunctional alkylating pharmacodynamic properties that interferes with DNA replication leading cells to apoptosis.

On 9 July 2003, the European Commission issued a marketing authorisation for Busilvex (IV busulfan) in the following indication:

'Busilvex followed by cyclophosphamide (BuCy2) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option.'

The recommended dose and schedule of administration in adults is:

- 0.8 mg/kg body weight (BW) of busulfan as a two-hour infusion every 6 hours over 4 consecutive days for a total of 16 doses,

- followed by cyclophosphamide at 60 mg/kg/day over 2 days initiated for at least 24 hours following the 16th dose of Busilvex.

Documentation on the safety and efficacy of Busilvex in combination with cyclophosphamide in the BuCy2 regimen prior to conventional allogeneic and/or autologous HPCT derived from two clinical trials (OMC-BUS-4 and OMC-BUS-3). These prospective, single arm, open-label, uncontrolled phase II studies were conducted in patients with haematological disease, the majority of whom had advanced disease. Patients received doses of 0.8 mg/kg busulfan every 6 hours infusion for a total 16 doses followed by cyclophosphamide at 60 mg/kg once per day for two days (BuCy2 regimen). All patients experienced a profound myelosuppression. The time to Absolute Neutrophil Count (ANC) greater than $0.5x10^9$ /l was 13 days (range 9-29 days) in allogenic patients (OMC-BUS 4), and 10 days (range 8-19 days) in autologous patients (OMC-BUS 3). All evaluable patients engrafted. There was no primary or secondary graft rejection. Overall mortality and non-relapse mortality at more than 100 days post-transplant was (8/61) 13% and (6/61) 10% in allotransplanted patients, respectively.

The indication of Bulsivex was extended on 27 October 2005 to the following paediatric indication:

'Busilvex followed by cyclophosphamide (BuCy4) or melphalan (BuMel) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation in paediatric patients.'

The recommended dose of Busilvex in paediatric patient is based on actual body weight (see section 4.2 of SmPC).

This was based on safety and efficacy data from clinical trial F60002 IN 101 G0 evaluating Busilvex in combination with cyclophosphamide in the BuCy4 or with melphalan in the BuMel regimen prior to conventional allogeneic and/or autologous HPCT. All patients experienced a profound myelosuppression. The time to Absolute Neutrophil Count (ANC) greater than $0.5x10^9$/l was 21 days (range 12-47 days) in allogenic patients, and 11 days (range 10-15 days) in autologous patients. All children engrafted. There was no primary or secondary graft rejection. 93% of allogeneic patients showed complete chimerism. There was no regimen-related death through the first 100-day post-transplant and up to one year post-transplant.

On 29 December 2001, orphan designation (EU/3/00/011) had been granted by the European Commission to Pierre Fabre Médicament, France, for busulfan (intravenous use) for the conditioning treatment prior to haematopoietic-progenitor-cell transplantation. However, this product is no longer an orphan medicine. Busilvex was withdrawn from the Community register of orphan medicinal products in July 2013 at the end of the 10-year period of market exclusivity.

With this variation, the applicant is applying for an addition of a new therapeutic indication:

'Fludarabine followed by Busilvex (FB) is indicated as conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option.'

For the above proposed indication, the following posology is proposed:

The recommended dose and schedule of administration is:

- fludarabine administered as a single daily one-hour infusion at 30 mg/m² for 5 consecutive days or 40 mg/m² for 4 consecutive days.

- Busilvex will be administered at 3.2 mg/kg as a single daily three-hour infusion immediately after fludarabine for 2 up to 4 consecutive doses.

2.2. Non-clinical aspects

No new clinical data have been submitted in this application except for the ERA (see section 2.2.1). This was considered acceptable by the CHMP.

2.2.1. Ecotoxicity / environmental risk assessment

The MAH submitted an environmental risk assessment as part of this application. The ERA included a Phase I assessment.

Table 1: Summary of main study results

Substance (INN / Invented Name): Busulfan			
CAS-number (if available): 55-98-1			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	Hansch et al 1995 EPI Suite results Hassan 1998 Westerhof 2000	-0.52 -0.68 -0.35 -0.585	Potential PBT (N)
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
Default PEC surfacewater Refined PEC surfacewater (Literature data)	0.016 1.2×10^{-6}	µg/L µg/L	< 0.01 threshold (N)

2.2.2. Discussion on non-clinical aspects

The results of the phase I of the environmental risk assessment of busulfan were submitted in this application. The Applicant provided two log Kow values of busulfan (-0.52 and -0.68) which were reported in the literature. Since the log Kow of busulfan should be determined experimentally according Guideline EMA/CHMP/SWP/44609/2010, the MAH also provided three busulfan values of log Kow determined experimentally, which were reported by Hassan 1998, Westerhof 2000 and Toropov 2010. In the publication performed by Toropov 2010, the value of log Kow reported was -0.3. However, the experimental method for its determination was not described. In the publications performed by Hassan 1998 and Westerhof 2000, a log Kow of -0.35 (in this case, log Dow = log Kow)

and of -0.585 were obtained respectively. The conducted experimental method for determining log Kow of busulfan in these two publications is considered adequate. In addition, since both values of log Kow determined experimentally are far below the prescribed limit of 4.5, testing of busulfan for Persistence, Bioaccumulation and Toxicity according to the Guideline EMEA/CHMP/SWP/4447/00 is not required.

The Applicant calculated the refined $PEC_{SURFACEWATER}$ of busulfan as 1.2 x 10^{-6} µg/L using HPCT prevalence data in Europe in 2011 and concluded that, since the refined $PEC_{SURFACEWATER}$ value of busulfan is below the action limit of 0.01 µg/L recommended in the guideline EMEA/CHMP/SWP/4447/00, the use of the medicinal product Busilvex 6 mg/ml concentrate for solution for infusion at 6 mg/ml of busulfan is unlikely to represent a risk for the environment. However, the lack of phase II assessment of busulfan was not considered justified. Since busulfan is teratogen in three mammalian species and causes sterility in rodents, it has potential to affect the reproduction of aquatic animals. Therefore, the CHMP recommended that the MAH conduct a tailored phase II assessment of busulfan according to the Guidelines EMEA/CHMP/SWP/4447/00 and EMA/CHMP/SWP/44609/2010. Results are expected by March 2016.

2.2.3. Conclusion on the non-clinical aspects

The special precautions for disposal and handling in the current SmPC of Busilvex which recommend that any unused medicinal product or waste material should be disposed of in accordance with local requirements for cytotoxic medicinal products remain applicable.

In addition, in the context of the obligation of the MAH to take due account of technical and scientific progress, the CHMP recommends the following points to be addressed:

- Conduct a tailored phase II assessment of busulfan according to the Guidelines EMEA/CHMP/SWP/4447/00 and EMA/CHMP/SWP/44609/2010.

2.3. Clinical aspects

2.3.1. Introduction

This application is supported by published scientific literature.

Among 48 publications selected further to a bibliographic search, the combination of “once daily” FB regimens was described in 33 publications (2572 patients) referred as “pivotal”. All these patients received a conditioning regimen based on IV Bu administered as a single daily administration over 3 hours. This infusion followed immediately the infusion of Flu. These 33 publications were considered as relevant for clinical efficacy of the combination providing haematological parameters (early and late parameters). Therefore, these studies were considered as pivotal studies.

- Tabular overview of clinical studies and bibliographic references

Table 2: Pivotal studies – Myeloablative Controlled regimens (MAC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Andersson, BBMT 2008	One center, USA	Retrospective & comparative.	Efficacy & Safety	FB: IV Flu 40 mg/m²/d x 4 d IVBu 130 mg/m²/d x 4 d	4	Total: 215 148	46 [19-66]	NR	OS, EFS, CHIMERISM, RELAPSE, NRRS, RFS, TRM, GVHD,
				BuCy2: IV Bu 0.8 mg/kg x 16 doses Cy 60 mg/kg IV x 2 d		67	39 [13-64]		
				FM: Flu 25 mg/m²/d x 5 d Mel 70-90 mg/m²/d x 2 d	NR	78 FM(a): 50 FM(b): 28	54 [23-66] 54 [22-74]		
Bredeson, BBMT 2008	One center, USA for FB cases and Database for controls	Matched-pair	Efficacy & Safety	FB: IV Flu 50 mg/m²/d x 5 d IV Bu 3.2mg/kg/d x 4 d IV Bu 3 h once daily	5	Total: 315 120	46 [18-65]	72/48	OS, RELAPSE, TRM, GVHD
				BuCy: Oral Bu 1 mg/kg x 16 doses Cy 120 to 180 mg/kg in 2 or 3 doses		215	44 [18-63]	127/88	

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Chunduri, BMT 2006	One center, USA	Retrospective & comparative	Efficacy & Safety	FB: IV Flu 30-40 mg/m²/d x 4 d IV Bu 3.2 mg/kg/d x 4 d	8	Total: 30 18	34 [19-61]	10/8	ENGRAFTMENT, TRM, GVHD
				FM: IV Flu 30 mg/m²/d x 5 d IV Mel 70 mg/m²/d x 2 d		12	49.5 [22-61]	4/8	
Horwitz, BBMT 2008	One center, USA	Prospective Phase II Randomized	Efficacy & Safety	IV Flu 40 mg/m²/d x 4 d IV Bu 130 mg/m²/d x 4 d	8/4	10	41 [21-54]	6/4	ENGRAFTMENT, CHIMERISM, DFS, TRM, GVHD
Lee, KJH 2010	One center, Korea	Retrospective and comparative	Efficacy & Safety	IV FB: IV Flu 40 mg/m² for 4 days, (total: 160 mg/m²), with IV Bu dose of 130 mg/m².	4	Total: 42 17	34 [17-56] 35 [18-56]	Total: 22/20 10/7	ENGRAFTMENT, CHIMERISM, OS, EFS, VOD, TRM, GVHD
				BuCy: Oral Bu 1 mg/kg or IV Bu 0.8 mg/kg x 16 doses administered every 6 hours IV Cy 60 mg/kg x 2 d		25	33 [17-48]	12/13	

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Madden, BBMT 2007	One Center, USA	Retrospective & comparative	PK & Safety	FB: Flu 40 mg/m²/d x 4 d IV Bu 130 mg/m²/d x 4 d	4	Total: 107 60	44 [13-65]	31/29	PK, Safety
				Sequential arm/Concomitant arm BuCy2 IV Bu 0.80 mg/kg over 2 hours/6 hours x 4 d Cy 60 mg/kg IV over 1 hour x 2 d	6	47	51 [18-68]	27/20	
Russell, BBMT 2010	One center, Canada	Retrospective & comparative	Efficacy & Safety	FB+TBI: IV Flu 50 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 4 d TBI 400 cGy	7	Total: 179 89	46 [18-66]	Total: 92/87 47/42	ENGRAFTMENT, OS, DFS, RELAPSE, TRM, GVHD
				FB: IV Flu 50 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 4 d		90	42 [16-65]	45/45	
Shimoni, Leuk 2006	One center, Israel	Retrospective & comparative	Efficacy & Safety	FB2: IV Flu 150-160 mg/m² IV Bu 3.2 mg/kg/d x 2 d	5	Total: 112 41	Total: 50 [17-70] 57 [18-70]	58/54 21/20	ENGRAFTMENT, OS, DFS, RELAPSE, VOD, NRM, GVHD
				FB4: IV Bu 3.2 mg/kg/d x 4		26	51 [18-64]	12/14	
				IV Bu 3.2 mg/kg/d x 4 IV BuCy2: 0.8 mg/kg x 16 doses Cy 60 mg/kg/d x 2	6	45	42 [22-58]	25/20	

Table 3: Pivotal studies – Myeloablative Uncontrolled regimens (MAC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F-	Endpoints
Alatrash, BBMT 2011	Two centers, USA-Brazil	Retrospective, single arm.	Efficacy & Safety	IV Flu 40 mg/m²/d x 4; IV Bu 130 mg/m²/d x 4 (130 mg/m² ~ 3.2 mg/kg) IV Bu once daily 3 hours.	4	79	58 [55-76]	49/30	ENGRAFTMENT, OS, EFS, TRM, GVHD
Andersson, BBMT 2011	One center, USA	Prospective phase II randomized	Efficacy & Safety	I) IV Flu 30 mg/m²/day + IV Clo 10 mg/m²/d x 4 d + IV Bu II) IV Flu 20 mg/m²/day + IV Clo 20 mg/m²/d x 4 d + IV Bu III) IV Flu 10 mg/m²/day +IV Clo 30 mg/m²/d x 4 d + IV Bu IV) IV Clo alone at 40 mg/m²/d+ IV Bu IV Bu targeted AUC x 4 d	4	51	45 [6-59]	32/19	ENGRAFTMENT, OS, EFS/PFS, RESPONSE, TRM, GVHD.
Bashir, BBMT 2011	One center, USA	Retrospective	Efficacy & Safety	IV Flu 40 mg/m²/d x 4 d IV Bu 130 mg/m²/d x 4 d or PK targeted dose (130 mg/m² ~ 3.2 mg/kg)	4	44	48 [13-63]	20/24	ENGRAFTMENT, CHIMERISM, OS, EFS, RELAPSE, TRM, GVHD
Beri, BMT 2010	One center, USA	Prospective Phase II.	PK & Safety	IV Flu 40 mg/m²/d x 4 d IV Bu one test dose (0.8 mg/kg), then targeted Bu x 4 days.	8	23	47 [15-61]	13/10	SAFETY, PK
Chunduri, BMT 2008	One center, USA	Retrospective	PK, Safety & Efficacy	IV Flu 30-40 mg/m²/d x 4 d IV Bu 3.2 mg/kg/d x 4 d	8	36	44 [18-61]	20/16	ENGRAFTMENT, OS, EFS, RELAPSE, TRM, GVHD, PK

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
De Lima, Blood 2004	Two centers, Canada, USA	Prospective Phase II	Efficacy & Safety	IV Flu 40 mg/m²/d x 4 d IV Bu 130 mg/m²/d x 4 d	4	96	49 [19-66]	48/48	ENGRAFTMENT, CHIMERISM, OS, EFS, RELAPSE, RESPONSE, VOD, TRM, GVHD, PK
Geddes, BBMT 2008	One center, Canada	Retrospective	PK/PD, Safety & Efficacy	IV Bu 3.2 mg/kg/d x 4 d IV Flu 50 mg/m²/d x 5 d	5	130* (114-16)	AUC ≤ 6000 µmol/L.min [19-66] AUC > 6000 µmol/L.min [21-63]	NR	ENGRAFTMENT, OS, PFS, RELAPSE, TRM, GVHD, PK
Kerbauy, Leuk Lymph 2011	One center, Brazil	Retrospective	Efficacy & Safety	IV Flu 40 mg/m²/d x 4 d IV Bu 130 mg/m² x 4 d	4	12	54** [29-76]	5/7	ENGRAFTMENT, OS, RELAPSE, VOD, TRM, GVHD
O'Donnell, Leuk Lymph 2010	One center, USA	Prospective phase I	PK & Safety	Test dose of Bu 0.5 mg/kg Flu 25 mg/m²/d x 5 d IV Bu: adapted dose x 4 days	5	36	55	25/11	ENGRAFTMENT, VOD, TRM, GVHD, PK

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Perkins, BMT 2011	One center, USA	Retrospective	Efficacy, Safety & PK	IV Flu 40 mg/m²/d x 4 d IV Bu for days 1 and 2: 130 mg/m²/d; following adjusted doses (total of 4 days).	4	145	48 [22-68]	83/62	ENGRAFTMENT, OS, RFS, RELAPSE, TRM , GVHD, PK,
Pidala, IJH 2011	One center, USA	Retrospective and comparative	Efficacy, Safety & PK	IV Flu 40 mg/m²/d x 4 IV Bu 75-170 mg/m²/d x 4 d with target AUC	4	FB+Ritux: 19 FB: 19	FB+Ritux: 56 [35-68] FB: 47 [27-64]	Total: 26/12 FB+Ritux: 16/3 FB: 10/19	ENGRAFTMENT, CHIMERISM, OS, TRM, GVHD, PK
Pidala, BMT, 2011	One center, USA	Retrospective	Efficacy, Safety & PK	IV Flu 40 mg/m² /d x 4 d IV Bu 130–145 mg/m²/d x 4 d	4	100	48 [22-69]	NR	ENGRAFTMENT, CHIMERISM, OS, PFS, RELAPSE, TRM, GVHD, PK
Russell, BBMT 2002	One center, Canada One center, Canada	Retrospective	Efficacy, Safety & PK	IV Bu 3.2 mg/kg/d x 4 d IV Flu 50 mg/m²/d x 5 d	4	70	41 [15-64]	NR	ENGRAFTMENT, OS, DFS, RELAPSE, RRT, TRM, GVHD, PK

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Russell, BBMT 2008	One center, Canada	Matched-pair	Efficacy, & Safety	IV Bu 3.2 mg/kg/d x 4 d IV Flu 50 mg/m²/d x 5 d TBI 400cGy	5	Total: 200 (a) 54 (b) 31 (c) 40 (d) 75	46 [18-65] (a) LR, age ≤ 45: 37 [18-45] (b) LR, age > 45: 50 [46-56] (c) HR, age ≤ 45: 48 [20-45] (d) HR, age > 45: 54 [46-65]	NR	ENGRAFTMENT, OS, DFS, RELAPSE, TRM, GVHD
Russell, BBMT 2007	One center, Canada	Retrospective	Efficacy & Safety	IV Flu 50 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 4 d TBI 400 cGy	7	Total: 64 AML: 36 ALL: 28	[18-63] AML: 46 [20-60] ALL: 31 [18-63]	40/24 23/13 17/11	ENGRAFTMENT, OS, DFS, RELAPSE, TRM, GVHD
Santarone, BBMT 2011	One center, USA	Prospective Phase II	Efficacy, Safety & PK	IV Flu 40 mg/m²/d x 4 IV Bu 130-180 mg/m²/d x 4	4	44	40 [20-57]	27/17	ENGRAFTMENT, OS, TRM, GVHD
Sanz J, Sanz MA, BBMT 2010	One center, Spain	Retrospective	Efficacy & Safety	Oral Bu 1 mg/kg/6Hx4/d x 3 d or IVBu 0.8 mg/kg/6Hx4/d x 3 d Cy 60 mg/kg/d x 2 d TTP 5 mg/kg/d x 2 d	6	Total: 49 16	Total cohort: 34 [16-52] NR	30/19	ENGRAFTMENT, RELAPSE, LFSTRM, GVHD
				IV Flu 50 mg/m²/d x 3 d TTP 5 mg/kg/d x 2 d IV Bu 3.2 mg/kg/d x 3 d	5	33	NR		

Author	N of study centers Location(s)	Study design Control type	Objective (s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Sanz J, Montesinos P, BBMT 2010	One center, Spain	Retrospective	Efficacy & Safety	Oral Bu 1 mg/kg/6Hx4/d x 3 d or IV 0.8 mg/kg/6Hx4/d x 3 d Cy 60 mg/kg/d x 2 d TTP 5 mg/kg/d x 2 d	5	Total: 26 21	Total cohort: 33 [16-48]	14/12	ENGRAFTMENT, OS, DFS, RELAPSE, TRM, GVHD
				IV Flu 50 mg/m²/d x 3 d TTP 5 mg/kg/d x 2 d IV Bu 3.2 mg/kg/d x 3 d		5			

Table 4: Pivotal studies – Reduced-Intensity Conditioning Controlled regimens (RIC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Shimoni, Leuk 2007	One center, Israel	Retrospective and comparative	Efficacy & Safety	FB: IV Flu 30 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 2 d	5	Total: 151 72	Total: 53 [16-70] 56 [23-70]	Total: 84/67 40/32	ENGRAFTMENT OS, RELAPSE VOD, TRM, GVHD
				FM: IV Flu 30 mg/m²/d x 5 d IV Mel 50-70 mg/m²/d x 2 d		79	51 [16-66]	44/35	

Table 5: Pivotal studies – Reduced-Intensity Conditioning Uncontrolled regimens (RIC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Almog, BBMT 2011	One center, Israel	Prospective phase II	Efficacy, Safety & PK	RIC (q.i.d): IV Bu 0.80 mg/kg/6h x 2 d RIC (od) IV Bu 3.2 mg/kg/d x2 d Flu: 30 mg/m²/d x 5 d	5	Total: 46 FB2: qid 15 FB2: od 13	Total: 54 [20-70] Subgroups: not available.	Total: 24/22 Subgroups: not available.	OS, RELAPSE VOD, TRM, GHVD, PK
				MAC (q.i.d):IV Bu 0.80 mg/kg x 4 doses x 4 d MAC (od): IV Bu 3.2 mg/kg/d x 4 d Cy: 120 mg/kg total dose, 2 d		BuCy: qid 9 BuCy: od 9			
Cho, IJH 2007	One center, Korea	Retrospective	Efficacy & Safety	IV Flu 30 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 2 d	5	22	43 [16-55]	16/6	ENGRAFTMENT, OS, EFS, RELAPSE, TRM, GVHD
Ho, BBMT 2011	One center, USA	Retrospective & comparative	Efficacy & Safety	IV Flu 30 mg/m²/d x 4 d IV Bu 0.8 mg/kg q12h or q24h x 4 d	4	Total: 433 MRD: 187	MRD: 56 [19-71]	Total: 276/157 MRD: 115/72	ENGRAFTMENT, OS, PFS, RELAPSE, TRM, GVHD
						URD: 246	URD: 57 [18-73]	URD: 161/85	
Lee, IJH 2010	One center, Korea	Retrospective	Efficacy & Safety	IV Flu 30 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 2 d	6	32	45 [17-65]	17/15	ENGRAFTMENT, CHIMERISM, OS, EFS, RELAPSE, TRM, GVHD

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Lee, AJH 2011	Multicentric, Korea	Prospective Phase II	Efficacy & Safety	IV Bu 3.2 mg/kg/d x 2 d IV Flu 30 mg/m²/d x 6 d	6	129*	39.5 [16-66]	75/53	ENGRAFTMENT, OS, EFS, RELAPSE, TRM, GVHD, VOD
Lee, AH 2011	One center, Korea	Prospective Phase II	Efficacy & Safety	IV Flu: 30 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 2 d + TBI 400 cGy.	6	31	39 [19-63]	23/8	ENGRAFTMENT , CHIMERISM, OS, PFS, RRT, NRM, GVHD

* 1 patient not included in the analysis due to last follow-up within 4 months.

Table 6: Supportive studies – Myeloablative Controlled regimens (MAC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of patients	Median age (years) [range]	Gender M/F	Endpoints
Chae, BMT 2007	Three centers, Korea	Retrospective comparative	Efficacy & Safety	FB: IV Flu total dose 180 mg/m² IV Bu 3.2 mg/kg/d x 4 d (total 12.8 mg/kg)	6	Total: 95 40	Total: 36 [17-54] 38.5 [21-54]	Total:54/41 21/19	ENGRAFTMENT, EFS, OS, RELAPSE, VOD, TRM, GVHD
				BuCy2: oral Bu 16 mg/kg or IV Bu total dose of 12.8 mg/kg IV Cy 120 mg/kg x 2 days		55	36 [17-49]	33/22	

Table 7: Supportive studies – Myeloablative Uncontrolled regimens (MAC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (day)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Patel, BMT 2011	Two centers, USA	Retrospective	Efficacy & Safety	IV Flu 40 mg/m²/d x 4 d IV Bu 3.2 mg/kg/d x 4 d	8	50 (52 enrolled)	L-CI: 27 [18-54] I-CI: 44 [20-59] H-CI: 46 [17-61]	4/2* 12/5 11/15	OS, RELAPSE, TRM, GVHD

L-CI= Low comorbidity index, I-CI= Intermediate comorbidity index, H-CI= High comorbidity index * data missing for 1 patient

Table 8: Supportive studies – Reduced-Intensity Conditioning Controlled regimens (RIC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Alyea, Blood 2005	One center, USA	Retrospectivean d comparative	Efficacy & Safety	FB: Flu 30 mg/m²/d x 4 IV Bu 0.8 mg/kg/d x 4	4	Total: 152 71	58 [51-70]	NR	OS, PFS, RELAPSE, TRM, GVHD
				TBI-Cy : Cy 1800 mg/m²x2; TBI 1400 cGy ; or oral Bu 16 mg/kg and Cy	NR	81	54 [51-66]		
Hamadani, HO 2011	Two centers, USA	Retrospective and comparative	Efficacy & Safety	FB2: RIC IV Flu 30 mg/m²/d x 5 d IV Bu 0.8 mg/kg/dose x 8 doses (total dose: 6.4 mg/kg).	5	Total: 112 75	58 [21-73]	Total:73/39 52/23	ENGRAFTMENT, PFS, OS, RELAPSE, NRM, GVHD
				FB4: RTC IV Flu 40 mg/m² x 4 d IV Bu 130 mg/m²/d x 4 d	4	37	53 [34-65]	21/16	
Ryu, BBMT 2007	One center, Korea	Prospective phase II randomized	PK, Efficacy & Safety	Bu1: 3.2 mg/kg once daily Bu4: 0.8 mg/kgx 4/d	6	Total: 60 Bu1:30 Bu4: 30	Total: 37.5 [16-58] Subgroups: NR	Total: 37/23 Subgroups: NR	ENGRAFTMENT, OS, RELAPSE, VOD, NRM, GVHD, PK.
				IV Bu: 12.8 mg/kg total dose Flu 30 mg/kg/d x 6		FB/Bu1: 16 FB/Bu4: 17			
				IV Bu 12.8mg/kg total dose Cy: 60 mg/kg/d x2 or IV Bu 12.8 mg/kg alone		BuCy/Bu4: 12 BuCy/Bu1: 13 Bu alone-Bu 4:1 Bu alone Bu 1:1			

Table 9: Supportive studies – Reduced-Intensity Conditioning Uncontrolled regimens (RIC)

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Armand, BBMT 2008	One center, USA	Retrospective	Efficacy & Safety	IV Bu 0.8 mg/kg/d x 4 d IV flu 30 mg/m2/d x 4 d	4	87	Total:46 [18-64] FB/NHL: 51[34-64] FB/HL: 31[18-56]	NR	ENGRAFTMENT, OS, PFS, PROGRESSION, NRM, GVHD
Bashey, BBMT 2011	Nine centers, USA	Prospective Phase II	Efficacy & Safety	IV Flu 30 mg/m²/d x 5 d IV Bu 0.8 mg/kg q 6 hours x 2 d	5	Total: 80 (82 enrolled) FB/MUD: 43 FB/MSD: 37	Total: 51 [17-70] FB/MUD: 48 [17-67] FB/MSD: 51 [24-70]	Total: 49/31 FB/MUD: 23/20 FB/MSD: 26/11	ENGRAFTMENT, OS, EFS, TRM, GVHD
Brown, BBMT 2006	One center, USA	Prospective Phase II	Efficacy & Safety	IV Bu 0.8 mg/kg/d x 4 d IV Flu 30 mg/m2/d x 4 d	4	46	53 [35-67]	34/12	ENGRAFTMENT, CHIMERISM, OS, PFS, TRM, GVHD
Hamadani, BBMT 2009	One center, USA	Retrospective	Efficacy & Safety	IV Flu 30 mg/m²/d x 5 d IV Bu 0.8 mg/kg/dose x 8 doses x 2 d	5	Total: 72 FB ATG 7.5: 39	Total: NA 56 [24-70]	Total:49/23 28/11	ENGRAFTMENT, CHIMERISM, OS, PFS, RELAPSE, NRM, GVHD
						FB ATG 6: 33	55 [24-69]	21/12	

Author	N of study centers Location(s)	Study design Control type	Objective(s) of the study	Study and control Drug(s) Dose, route, and regimens	TT (days)	N of pts	Median age (years) [range]	Gender M/F	Endpoints
Koreth, BBMT 2010	One center, USA	Retrospective	Efficacy & Safety	RIC: IV Flu 30mg/m² x 4 d IV Bu 0.8 mg/kg x 4 d once daily or twice daily	5	158	Total:63 [60-71] FB strate [60-64]: 62 [60-64] FB strate [≥ 65]: 67 [65-71]	Total: 106/52 FB strate [60-64]: 73/37 FB strate [≥ 65]: 33/15	ENGRAFTMENT, CHIMERISM, OS, PFS, RELAPSE, NRM, GVHD
Krishnamurthy, BMT 2010	One Center, UK.	Retrospective	Efficacy & Safety	IV Flu 30 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d in 4 doses daily x 2 d IV BuCy4: 3.2 mg/kg/d x 4 d IV Cy: 50 mg/kg/d x 4 d	5	18 FB: 17 BuCy: 1	54 [38-66]	12/6	ENGRAFTMENT, CHIMERISM, OS, DFS, RELAPSE, NRM, GVHD
Lee, Blood 2011	One center, Korea	Prospective Phase II	Efficacy & Safety	IV Bu 3.2 mg/kg/d x 2 d IV Flu 30 mg/m²/d x 6 d	6	83	40 [16-70]	47/36	ENGRAFTMENT, CHIMERISM, OS, EFS, RELAPSE, TRM, GVHD, PROGRESSION
Matthews, BJH 2010	One center, UK	Retrospective	Efficacy	IV Flu 30 mg/m²/d x 5 d IV Bu 3.2 mg/kg/d x 2 d	5	25	53 [34-70]	15/10	ENGRAFTMENT, RELAPSE, CHIMERISM, COMPLETE REMISSION
Sayer, BMT 2003	Multicentric, Germany	Prospective phase II	Efficacy & Safety	IV Flu 90 mg/m² 180 mg/m² total dose IV Bu total dose 6.8 mg/kg, or oral Bu 8 mg/kg total dose	NR	113	Total: 51 [16-67] FB cohort: NA	69/44	ENGRAFTMENT, CHIMERISM, OS, EFS, TRM, GVHD,
Tsirigotis, Haematologica 2006	One center, Israel	Retrospective	Efficacy & Safety	IV Flu 30 mg/m²/d x 6 d Oral Bu 4 mg/kg/d x 2 OR IV Bu 3.2 mg/kg/d x 2	6	37	Total :60 [55-69] FB cohort: NA	30/7	ENGRAFTMENT, CHIMERISM, OS, DFS, RELAPSE, RFS, NRM, GVHD

2.3.2. Pharmacokinetics

Introduction

The intravenous form of Bu was developed in order to mimic the PK profile obtained with oral Bu, and the phase I/II trials for the Busilvex clinical development were conducted with the standard q.i.d. schedule.

More recently, alternative schedules to the q.i.d. regimen have been developed with a twice or, more often, once-daily administration. The once-daily schedule represents a well-established use dosing regimen which is performed by many transplant centres. Most of the experience acquired with the once-daily regimen comes from the reduced intensity conditioning (RIC) setting when Bu is associated with Flu. Nevertheless, some experiences of once-daily IV Bu combined with other alkylating agents (i.e. Cyclophosphamide or Melphalan) in myeloablative conditioning (MAC) regimen have been investigated as well. This post-marketing experience is documented through several clinical trials reported in the literature.

The MAH provided the results of an exhaustive search through medical libraries database in order to document the relevant PK characteristics of once-daily IV Bu dosing, and to compare these with the PK background of four times daily schedule.

Additionally, a clinical trial with intravenous Bu administered once-daily and associated with cyclophosphamide in acute myelogenous leukaemia patients is ongoing and data from intermediate analyses were submitted.

Table 10: Overall design and PK methods in adult studies with once-daily IV Bu schedule

Publication	PK Study Objectives	Conditioning regimen	Schedule	Busulfan dose	Number of subject evaluable for PK /age (range)	Criteria of PK assessment (PK samples)	Number of samples and time interval of samples collection after start of infusion	Bioanalysis method	Method for PK analysis
[Madden T. 2007, see section 2.7.6]	- To confirm the linearity of IV Bu PK from 0.8 mg/kg given every 6 h up to 130 mg/m^2 given once daily - To compare the variability between Bu PK in once daily and every 6 h IV administration - To review the possible influence of concomitant administration of other drugs on the PK disposition of IV BU given once daily	IV FB IV Bu/Cy2	Two arms: - once daily - q.i.d	130 mg/m^2 (~3.2 mg/kg) 0.8 mg/kg	60 (13-65 y) 47 (18-68 y)	1st, 3rd and 4th Bu doses 1st, 5th, and 9th Bu doses	14 samples over 24h 11 samples over 6 h.	HPLC-UV	Model dependent method
[Ryu SG. 2007, see section 2.7.6]	To compare PK characteristics of IV Bu between 4-times daily and once daily administration schedule	IV FB IV Bu/Cy IV Bu alone	Randomized study with two arms: Bu once daily Bu 4 times daily	3.2 mg/kg 0.8 mg/kg	60 (16-58 y) 30 30	1st dose	6 samples over 22 h 6 samples over 6 h	LC-MS/MS	Model dependent method
[Almog S. 2011, see section 2.7.6].	- To examine the PK linearity of IV Bu between 0.8 and 3.2 mg/kg dosage. - To assess the reproducibility of IV Bu PK between first and repeated doses - To evaluate whether Bu PK is altered by co-administration of Fludarabine	Myeloablative conditioning (MAC): IV Bu/Cy Reduced intensity conditioning (RIC) IV FB	Four groups of patients: MAC once daily MAC q.i.d RIC once daily RIC q.i.d	3.2 mg/kg 0.8 mg/kg 3.2 mg/kg 0.8 mg/kg	46 (20-70 y) 13 15 9 9	Twice in each patient, once after the first infusion and again after the infusion given midway in the course of treatment	14 samples over 24 h 11 samples over 6h 14 samples over 24 h 11 samples over 6h	GC-MS method	Compartmental (one compartment) and non compartmental analysis

Publication	PK Study Objectives	Conditioning regimen	Schedule	Busulfan dose	Number of subject evaluable for PK /age (range)	Criteria of PK assessment (PK samples)	Number of samples and time interval of samples collection after start of infusion	Bioanalysis method	Method for PK analysis
[Mamlouk K. 2005, see section 2.7.6]	To evaluate the PK of IV Bu after once-daily protocol in comparison with every 6-h protocol when used in a Bu/Cy myeloablative regimen	IV or oral Bu/Cy	Three groups of patients: IV Bu once daily IV Bu q.i.d. Oral Bu q.i.d	 3.2 mg/kg 0.8 mg/kg 1mg/kg Adjustment if necessary for doses 3 and 4	 20 (25-65 y) 10 (18-52 y) 13	1st dose	 7 samples over 14 h 7 samples over 6 h 7 samples over 6 h	NR	One compartment first order elimination model for the IV Bu Non compartmental method for oral Bu
[Fernandez HF. 2002, see section 2.7.6]	To evaluate IV Bu PK in a modified IV Bu/Cy conditioning regimen: Bu administered once or twice daily	IV Bu/Cy	Group A: IV Bu twice daily Group B IV Bu once daily	1.6 mg/kg 3.2 mg/kg	6 (26-65 y) 6 (36-51 y)	First dose Peak and trough level at dose 5 in group A	11 samples over 12 h 11 samples over 18 h	HPLC-UV	Model dependent method
[Beri R. 2010, see section 2.7.6]	To evaluate whether a test dose of IV Bu at 0.8 mg/kg would allow to target a range of Bu AUC between 4000 and 6000 µM.min.	IV FB	Bu once daily after a test dose Target AUC: 4800 µM.min	Test dose 0.8 mg/kg	23 (15-61 y)	Test Dose First Dose	8 samples over 6 h	NR	NR
[O'Donnell PH. 2010, see section 2.7.6]	To determine the maximum tolerated Bu AUC in a IV FB and alemtuzumab conditioning regimen.	IV FB	Dose escalation design to target AUC levels IV Bu Flu once daily Group 1 : after a test dose Group 2: adjustment at dose 3 and 4 based on dose 1 results	 Group 1: Test dose: 0.5 mg/kg. Group 2: 1st and 2nd dose :3.2 mg/kg	56 (median 55 y) 21 15	Test Dose First Dose	6 samples over 7h	GC/MS method	One compartment, first order elimination model
[Perkins J. 2011, see section 2.7.6]	- To investigate the PK of once daily IV Bu with targeting strategy - To explore the relationship of dose 1 AUC with the transplant outcomes	IV FB	IV Bu once daily	130 mg/m2 (~3.2mg/kg) Adjustment if necessary of the 3rd and 4th dose	145 (22-68y)	1st dose	5 samples over 9 h	GC-MS method	One compartment first order elimination model

Publication	PK Study Objectives	Conditioning regimen	Schedule	Busulfan dose	Number of subject evaluable for PK /age (range)	Criteria of PK assessment (PK samples)	Number of samples and time interval of samples collection after start of infusion	Bioanalysis method	Method for PK analysis
[De Lima MJ. 2004, see section 2.7.6]	To document IV Bu PK in a once daily IV Bu and Fludarabine combination	IV FB	IV Bu once daily	130 mg/m^2 (~3.2mg/kg)	45 (19-66y)	1st, 3rd and 4th doses	15 samples over 24 h	HPLC-UV	Model dependent method
[Russell JA. 2002, see section 2.7.6]	To evaluate the PK of IV Bu given once daily in combination with Fludarabine in myeloablative conditioning protocol.	IV FB	IV Bu once daily	3.2 mg/kg	12	1st and 4th dose	13 samples over 24 h	HPLC-UV	Model dependant method
[Bonin M. 2007, see section 2.7.6]	To evaluate whether Bu could alter the PK of F-ara-A, the dephosphorylated metabolite of the pro-drug Fludarabine	Oral FB	Bu every 6h from day -5 to day -2 Flu from day -6 to day -3	Oral Bu 1 mg/kg Flu 30 mg/m^2	16 (33-66y)	F-ara A day -6 and either day -5, -4 or -3	9 samples over 12 h	HPLC-fluorescence (F-ara A)	
[Geddes M. 2008, see section 2.7.6]	To define an optimal IV Bu exposure when given once-daily with Flu and to investigate any correlation between Bu exposure and clinical outcomes	IV FB	Bu once daily	3.2 mg/kg	130 (19-66y)	3rd dose	6 samples over 7 h	HPLC-UV	Non compartmental method

Once daily Pharmacokinetic profile

Literature data

A first series of literature studies in adult patients have focused on the comparison of the IV Bu PK behaviour after once-daily (OD) schedule versus the traditional four-time daily schedule. The comparison between schedules was performed on a parallel groups design. In most of the studies, an historical cohort of control patients treated with IV Bu administered four-times daily was used for comparison purpose. In one study [Ryu SG. 2007], patients were randomly assigned according to the type of schedule. The above published works were considered as pivotal PK studies since the comparison between schedules were performed on PK data managed and analysed within the same institution and with the same bioanalytical method. These PK data were generated using the same institutional clinical practices, especially for the patients' selection, supportive care and treatment prophylaxis which might potentially impact the PK of Bu. As a consequence, the variability due to experimental conditions was considered as minimal to enable reliable comparisons in these studies.

A summary of the main PK parameters in these studies including single centre comparisons between once-daily (OD) and four-times daily (q.i.d.) schedules is reported in the following table.

Table 11: Summary of PK parameters in adult studies including a parallel group comparison between schedules

Study	Nb of pts (age range)	Schedule: Bu dose /infusion duration	PK Method	PK parameters: mean (CV%)				
				Clearance (mL/min/kg)	Volume of distribution (L/kg)	AUC (µM.min)	C_{max} (µg/mL)	$T_{1/2}$ (h)
Madden T. 2007	60 (13-65 y) 47 (18-68 y)	OD: 130 mg/m² (~ 3.2 mg/kg) over 3h q.i.d.: 32 mg/m² (~ 0.8 mg/kg) over 2h	HPLC-UV	OD: 109 mL/min/m² (26%) q.i.d.: 116 mL/min/m² (24%)	OD: 22.6 L/m² (20%) q.i.d.: 22.6 L/m² (21%)	OD: 4873 (22%) q.i.d.: 1292 (25%)	OD: 3.6 (14%) q.i.d.: 1.2 (20%)	OD: 2.7 (27%) q.i.d.: 2.9 (25%)
Ryu SG. 2007	60 (16-58 y) (randomized OD or q.i.d.)	OD: 3.2 mg/kg over 3h q.i.d.: 0.8 mg/kg over 2h	LC-MS/MS	OD (n=30): 1.90 (16%) q.i.d.. (n=30): 2.05 (18%)	OD: 0.46 (11%) q.i.d.: 0.48 (13%)	OD: 6476 (17%) q.i.d.: 1515 (18%)	OD: 4.22 (17%) q.i.d.: 1.06 (14%)	OD: 2.83 (7%) q.i.d.: 2.75 (8%)
Almog S. 2011	46 (20-70 y)	OD: 3.2 mg/kg over 3h q.i.d.: 0.8 mg/kg over 2h	GC-MS	OD FB (n=15): 2.77 (27%) q.i.d. FB (n=15): 2.90 (39%) OD BuCy2 (n=9): 3.03 (28%) q.i.d. BuCy2 (n=9): 2.87 (25%)	OD: 0.698 (20%) q.i.d.: 0.753 (33%) OD: 0.707 (14%) q.i.d.: 0.673 (14%)	OD: 4214 (27%) q.i.d.: 1111 (30%) OD: 4186 (30%) q.i.d.: 1113 (29%)	OD: 2.74 (22%) q.i.d.: 0.812 (23%) OD: 2.81 (21%) q.i.d.: 0.878 (10%)	OD: 3.0 (17%) q.i.d.: 3.1 (26%) OD: 2.8 (14%) q.i.d.: 2.9 (31%)
Mamlouk K. 2005	20 (25-65 y) 10 (18-52 y)	OD: 3.2 mg/kg over 3h q.i.d.: 0.8 mg/kg over 2h	NR	OD: 3.34 (19%) q.i.d.: 3.26 (23%)	NR	OD: 4017 (16%) q.i.d.: 1024 (19%)	NR	OD: 2.88 (12%) q.i.d.: 2.57 (26%)

OD: once-daily schedule, q.i.d.: four-times daily schedule, NR: Not reported

Within each study, PK parameters such as total body clearance, volume of distribution and terminal half-life were nearly identical regardless of the dosage schedule. The plasma exposure (AUC) measured after once-daily schedule was four-fold the value measured after q.i.d. schedule, in accordance with the dose increment from 0.80 mg/kg to 3.2 mg/kg administered for q.i.d. and OD regimen, respectively. The peak concentration (Cmax) measured after the end of infusion was about 3 times higher in OD schedule compared to q.i.d. schedule. This finding was consistent with the increasing infusion of rate (about 3 fold increase), from 0.4 mg/kg/h used in q.i.d. schedule to 1.067 mg/kg/h used in OD schedule.

The difference in the concentrations time profile between q.i.d. and once-daily schedule was illustrated in the publication of Madden et al.

Once daily schedule

Four-times daily schedule

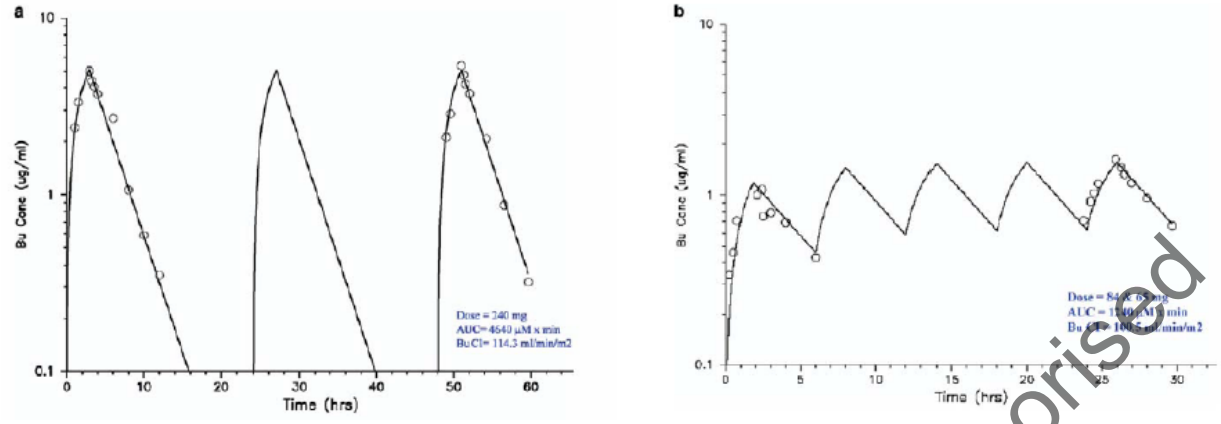


Figure 1: Plasma concentration versus time profile following IV Bu given once-daily and four times daily- From Madden T. et al.

A second series of literature data including two publications [Beri R. 2010, O'Donnell PH. 2010] compared once-daily IV Bu PK data with test dose results. In the [Beri R. 2010] study (n=23 patients), the PK parameters at the test dose of 0.8 mg/kg were found to be predictive of those obtained at the once-daily treatment dose (~ 3.5 mg/kg). This study indicated that the once-daily dose calculated from a low pre-transplant dose was accurate and that the AUC increase (from 1185 ± 400 up to 4992 ± 1100 µM.min) was proportional to the dose increment, from 0.8 up to 3.5 mg/kg. This finding contrasted with the one obtained in the [O'Donnell PH. 2010] study (n=15 patients). In this last study, the authors suggested that the test dose (0.5 mg/kg) PK parameters were not fully predictive of the once-daily treatment dose; 10 out of 15 patients had a test dose clearance that was more than 10% different from the treatment dose clearance. However, the authors recognized that inexperience of the transplant team in the PK part of the study might have contributed to unexpected results.

In a third series of publications, the PK of once-daily IV Bu was investigated as part of the clinical endpoints in FB RIC regimen and without any test dose targeting strategy or historical control (Table 12).

Table 12: Summary of PK parameters (mean and CV) across adult studies

Study	Nb of patients	Conditioning regimen	Busulfan schedule	Infusion duration	CL (mL/min/kg)	AUC (µM·min)	C_{max} (µg/mL)	T1/2 (h)
Perkins J. 2011[1]	145	FB	130 mg/m² daily	3h	2.7	4823	3.32	NR
Geddes M. 2008	130	FB	3.2 mg/kg daily	3h	13.1 L/h (31%)	4716	NR	NR
Madden T. 2007	60 47	FB Bu/Cy	130 mg/m² daily 32 mg/m² every 6h	3h 2h	109 ml/min/m² (26%) 116 ml/min/m² (24%)	4873 (22%) 1292 (25%)	3.6 1.2	2.7 2.9
De Lima MJ. 2004	45	FB	130 mg/m² daily	3h	109 ml/min/m² (26%)	4891	3.6	2.7
Ryu SG. 2007	30 30	FB Bu/Cy Bu alone	3.2 mg/kg daily 0.8 mg/kg every 6 h	3h 2h	1.90 (16%) 2.05 (18%)	6476 (17%) 1515 (18%)	4.32 1.06	2.83 2.75
Almog S. 2011	22 24	FB & Bu/Cy FB & Bu/Cy	3.2 mg/kg daily 0.8 mg/kg every 6 h	3h 2h	2.88 (33%) 2.88 (25%)	NR	NR	3.0 2.9
O'Donnell PH. 2010.[1]	15	FB	3.2 mg/kg daily 0.5 mg/kg every 6 h	3h NR	2.67 (24%) 2.90 (26%)	NR	NR	NR
Beri R. 2010.	23	FB	3.5 mg/kg daily 0.8 mg/kg every 6 h (test dose)	3h 2h	NR	4992 (22%) 1185 (34%)	NR	NR
Mamlouk K. 2005	20 10	Bu/Cy Bu/Cy	3.2 mg/kg daily 0.8 mg/kg every 6 h	3h 2h	3.34 (19%) 3.26 (23%)	4017 (16%) 1024 (19%)	NR	2.88 2.57
Russell JA. 2002	12	FB	3.2 mg/kg daily	3h	D1: 2.49 (18%) 107 mL/min/m² (16%) D4: 2.50 (20%) 107 mL/min/m² (20%)	D1: 4867 (16%) D4: 4980 (18%)	D1: 3.92 D4: 3.96	D1: 2.60 D4: 2.57

(1) median value, NR: Not reported

Except for one study [Ryu SG. 2007], consistent PK parameters were reported across studies. In the [Ryu SG. 2007] study, the mean clearance value was lower and hence the AUC and Cmax achieved with the standard dosages (either 3.2 mg/kg or 0.80 mg/kg) were higher in comparison with all the other studies. Nevertheless, the between schedules comparison in the Ryu study demonstrated a linear PK behaviour of IV Bu from 0.8 to 3.2 mg/kg dosage. After 3.2 mg/kg IV Bu dosage, the clearance values across studies (excluding the Ryu study) ranged from 2.49 to 3.34 mL/min/kg and from 107 to 109 mL/min/m². After 0.8 mg/kg dosage, the mean clearance values were between 2.67 and 3.26 mL/min/kg and 116 mL/min/m² in the [Madden T. 2007] study. These values were in the same range to those determined during the clinical development studies of Busilvex in adults and using the traditional four-time daily schedule: mean values were from 2.11 to 3.36 mL/min/kg. Average daily exposures after 3.2 mg/kg IV Bu dosage ranged from 4017 to 4992 µM.min while AUC values following 0.80 mg/kg IV Bu dosage were between 1024 and 1292 µM.min. These last values were in agreement with those observed during the clinical development of Busilvex with mean exposures ranging from 1017 to 1225 µM.min. As for AUC, consistent peak concentrations (Cmax) were reported between studies and for a same schedule of administration. Homogeneous terminal half-life values were reported across studies and whatever the dosage schedule. Mean half-life values were comprised between 2.57 and 3.0 hours after once-daily schedule in literature studies, in close agreement with the values commonly observed using the four-time daily schedule. These last values ranged from 2.57 to 2.9 h in literature studies and from 2.83 to 3.47 h in Busilvex clinical development studies.

Study F60002 IN202 G0 study

This is a Phase II trial conducted in acute myelogenous leukaemia (AML) patients conditioned with intravenous (IV) Busulfan (Bu) given once-daily and associated with Cyclophosphamide (Cy). The first patient was included in May 2010 and the last patient was enrolled in May 2012. Given that a 2 years follow-up is necessary as per protocol for mature overall survival and relapse rate assessment, the study will end by May 2014 after the follow-up period of the last ongoing patient. The final results including the two years follow-up clinical endpoints will be available by the end of 2014.

The schedule of infusion was done in 3 hours as a single daily infusion (total dose of IV Bu for one day infused as a single infusion). The study was a prospective non-randomised multicentre trial. The clinical objectives were to assess both safety and efficacy profiles including the determination of overall survival (OS) and the relapse incidence at 2 years.

The PK results from intermediate analyses are summarised below.

Thirty AML patients in CR1 were enrolled in 8 recruiting centres between May 2010 and May 2012. At the cut-off date June 2013, with a median follow-up of 12 months for each patient, all patients were evaluable for PK, safety and efficacy endpoints.

The mean daily dose (± s.d.) of IV Bu administered were 3.12 ± 0.23, 3.38 ± 0.86, 3.47 ± 1.10 and 3.28 ± 1.07 mg/kg for days 1, 2, 3 and 4, respectively. From day 2 to day 4, the mean dose increase was about 10% of dose 1. This dose increase was consistent with the higher desirable targeted exposure, shifting from 4800 µmol/L.min with a standard 3.2 mg/kg dose up to 5200 µmol/L.min with adjusted doses.

The median daily AUC after dose adjustment on each of days 2, 3 and 4 reached or was very close to the targeted AUC (i.e.: 5200 µmol/L.min). Following the first Bu dosing, individual AUCs were mostly included within the optimal 4400 – 6000 µmol/L.min AUC range.

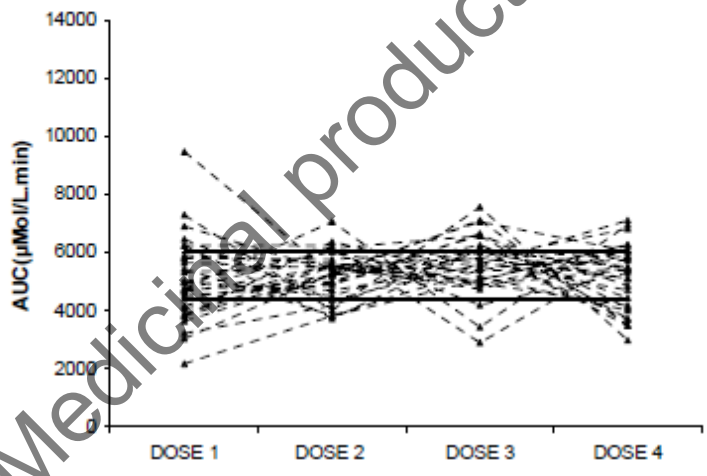


Figure 2: Individual Bu AUC per day of treatment

Although not claimed as an objective of this trial, the Bu PK data obtained following once-daily schedule from this study were compared with the Bu PK data collected according to a four-time daily schedule during the clinical development. The two populations (once-daily versus four-times daily) showed comparable patients characteristics.

Table 13: Comparison of the two populations characteristics (mean ± sd)

	Reference population (qid schedule) (n =151)	IN202G0 Study (once daily schedule) (n=30)
Age (years)	39.5±11.7	40.7±10.0
Actual Body Weight (kg)	76.6±17.9	73.3±12.7
BSA (m^2)	1.9±0.3	1.9±0.2
BMI (kg/m^2)	26.0±5.5	24.1±3.8

The comparison of IV Bu clearance was performed between the two populations. The distribution of individual clearance and the median clearance values were similar regardless the time of assessment and the schedule of administration. No statistical significance was reached by comparing the different mode of administration and the time of assessment (NS -ANOVA test).

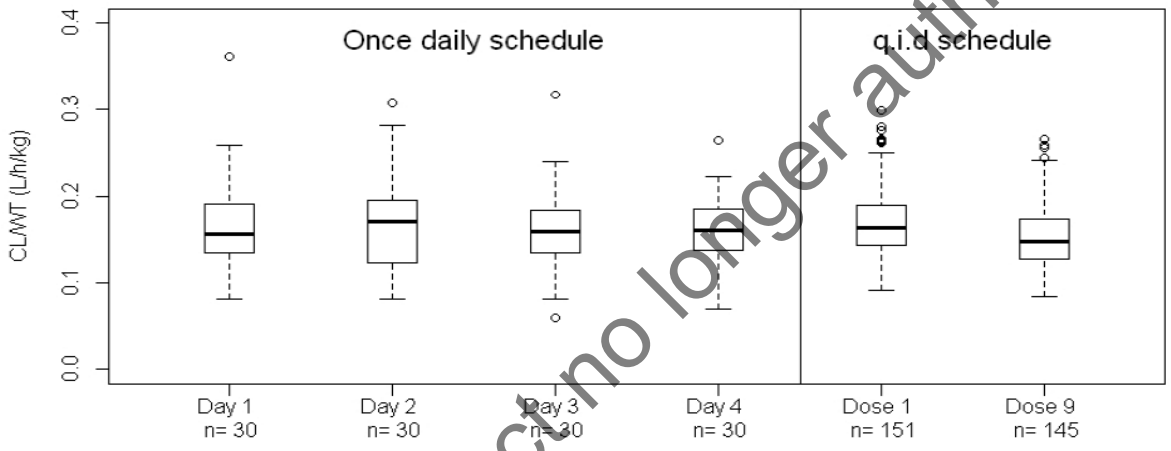


Figure 3: Comparison of clearance normalized with weight between time of assessment and schedule of administration

Intra- and inter-individual variability

Inter-patient variability

In the first set of literature studies including the comparison of parallel groups from a single institution, comparable inter-patient variability (expressed as coefficient of variation: CV) for all PK parameters (Cl, Vd, T1/2, AUC and Cmax) was reported between q.i.d. and OD schedule. There was no increase of variability after OD as compared to q.i.d. schedule. In the adult population, the CV in AUC across literature studies ranged from 16 to 22% after the OD schedule which compared well with the CV of about 20% measured after q.i.d. regimen during the clinical development program.

The literature review illustrated similar targeting performance between schedules: with 70 to 90% of patients achieving either the 900-1500 µmol/L.min range after 0.80 mg/kg q.i.d dosing or the equivalent daily exposure of 3600-6000 µmol/L.min after 3.2 mg/kg OD dosing.

Intra-patient variability

The PK evaluation over repeated once-daily administrations was documented in 6 adults' studies. Consistent results were demonstrated across studies indicating no detectable drug accumulation between daily doses and similar magnitude of intra-patient variability. In those studies, the Bu

clearance showed no statistical difference between the first daily dose and either the second, third or fourth daily doses. An intra-patient CV less than 15 to 20% was calculated in adult series. This magnitude of intra-patient variation was in agreement with the one (CV ≈ 10%) estimated during the initial clinical development from population PK analyses of adults and paediatric series after q.i.d. regimen.

Special populations

No specific studies in renal and liver impairment patients were conducted during the IV Bu development program. A population PK study indicated that the four-time daily IV Bu clearance was unaffected by elevated hepatic enzymes [Nguyen L. 2006].

In Ryu study, there were not significant differences (P=0.795) between 4-times-daily IV Bu arm (43.3%) and once-daily IV Bu arm (46.7%) with regards to the frequencies of hepatic toxicities grade III-IV (only patients with adequate hepatic functions were included).

Literature [Gibbs JP. 1999] showed that oral Bu clearance can be significantly lower in obese and severely obese patients than in normal patients. Oral Bu CL normalized to Adjusted Ideal Body Weight (AIBW) eliminated the differences in oral Bu CL among categories of weight. Using a population PK analysis, these findings were confirmed with IV Bu (four-time daily) [Nguyen L. 2006]. It was shown that IV Bu dosing should be based on AIBW for the obese and severely obese patients. The use of ABW in normal patients and the use of AIBW in obese patients respectively are expected to provide similar drug exposures (see busulfan SmPC).

In order to assess the influence of age on Bu PK disposition, a comparative analysis was carried out on both IV and oral datasets collected [Leger F, Nguyen L, Puozzo C. Institut de Recherche Pierre Fabre, Castres, France (2002)] (see busulfan EPAR). For the IV dataset, the comparison included 13 patients out of 120 above 55 years. For the oral dataset, the comparison was performed on a large dataset including 19 elderly patients above 60 years (60 to 66 years old) versus a control group of 476 younger patients (17 to 60 years old). The analysis showed similar Bu plasma exposures between elderly and control patients. This finding was confirmed by a population PK analysis showing that IV Bu clearance was not correlated with age in the adult population [Nguyen L. 2006].

Interactions

The potential influence of the associated conditioning agent or other concomitant drugs on the PK disposition of Bu administered once-daily was explored in some studies.

The effect of Flu on Bu PK was studied in the [Almog S. 2011] comparing several treatment groups: Bu/Cy (MAC) versus FB (RIC) protocols and OD versus q.i.d schedules. In the MAC protocol, Bu was administered alone over 4 days followed 24h after by 2 days of Cy whereas in the RIC protocol, Bu was given over 2 days in combination with Flu given over 5 days. Regardless of the administration schedule (OD or q.i.d.), no statistical difference was observed in all PK parameters (i.e.: CL, Vd, Cmax/dose and T1/2, all $p > 0.24$) between patients with Flu (RIC protocol) versus those without Flu (MAC protocol). The absence of effect of Flu on IV Bu PK disposition was also supported by the consistency of PK results reported across literature studies, and especially in the Madden et al. [Madden T. 2007, Ryu SG. 2007] studies comparing Bu/Cy q.i.d. protocol versus FB OD protocol.

The reverse effect of Bu on the PK disposition of Flu was investigated in the [Bonin M. 2007] study. In this study, oral Bu (1 mg/kg/6h from day-5 to -2) was given in combination with Flu (30 mg/m²/day from day -6 to -3). The PK of F-ara-A (the dephosphorylated metabolite of the pro-drug Flu) in plasma and urine was evaluated in 16 adult patients before Bu dosing (day-6) and during Bu treatment

(between days -5 to -3). No statistical significant change in plasma CL, Vd, AUC, Cmax, renal clearance and urine recovery was detected, showing no impact of Bu on the PK of Flu.

In most of the literature studies, phenytoin was used as seizure prophylaxis and was administered generally at least one day before and during the Bu therapy. The potential induction effect of phenytoin on the PK disposition of once-daily IV Bu was not specifically documented. Nevertheless, all studies with repeated doses PK investigation revealed no modification of IV Bu clearance and reproducible AUCs over the treatment days.

Other possible PK interactions were explored in the [Madden T. 2007] study where patients with concomitant drugs such as itraconazole/voriconazole or with progestin were compared to patients without these concomitant drugs. The use of concomitant drugs did not appear to alter the PK of IV Bu administered once-daily.

2.3.3. PK/PD modelling

High Busulfan exposure is associated with worse outcomes in a daily IV Busulfan and Fludarabine allogeneic transplant regimen. [Geddes M. 2008]

Objective

In adult patients treated for various haematological malignancies:

- to define an optimal Bu exposure when Bu is given once-daily with Flu.

- to investigate any correlation between Bu exposure and clinical outcomes.

Methods

Bu at 3.2 mg/kg was administered as a 3h infusion once-daily over 4 days in combination with Flu (50 mg/m²) over 5 days. One day before and the day of transplantation, total body irradiation (TBI) was given to patients with acute leukaemias.

Phenytoin was given as seizure prophylaxis 7 days before until one day after Bu therapy.

For all patients, the Bu PK samples (6 samples) were collected on the 3rd Bu dose over 7h after the end of infusion. Bu plasma concentrations were analysed by HPLC-UV method. Individual PK parameters were calculated by non-compartmental method. Patient and disease characteristics, clinical outcomes (including Non-Relapse Mortality, engraftment and Overall Survival) and treatment toxicities were compared between patients with high (> 6000 µM.min) and low (≤ 6000 µM.min) Bu exposure. Kaplan-Meier curves and log-rank tests were used to compare equality of the survival functions by Bu exposure. A Cox proportional hazards model was used to examine the individual impact of potential confounding variables on the hazard ratio for death from any cause with high versus low Bu exposure.

Results

- Patients and pharmacokinetic characteristics

One hundred and thirty (130) adult patients (19 -66 y) were enrolled: 114 patients (88%) had a Bu AUC ≤ 6000 µM.min while 16 patients (12%) had a Bu AUC > 6000 µM.min. Overall, 72% of patients achieved the 3600 – 6000 µM.min AUC range. The mean and median AUC were 4716 and 4699 µM.min (range: 2184 – 7794 µM.min). The mean clearance was 13.1 ± 4.0 L/h (CV=30%).

Various haematological malignancies were represented. No marked differences on demographics and disease status, stem cells sources and graft characteristics were seen between the two groups (AUC > 6000 µM.min vs AUC ≤ 6000 µM.min).

- PK/PD relationships

Engraftment:

The median days of stable neutrophil engraftment (first day of 3 consecutive days with neutrophils count ≥ 0.5 × 10^9/L) was 15 days and that for platelet was 18 days (platelet count > 20 × 10^9/L for 7 days without transfusion). There was no difference in both neutrophils and platelets engraftments between groups with an AUC ≤ 6000 µM.min versus > 6000 µM.min.

Overall Survival and Progression-Free survival:

The median follow-up was 29 months (ranging from 4-67 months). Estimated OS of the entire group was 70% at 12 months (95% CI: 61%-77%) and 62% at 36 months (95% CI: 52%-70%).

Significantly higher (p<0.001) OS and PFS were observed in patients with AUC ≤ 6000 µM.min than in patients with AUC > 6000 µM.min.

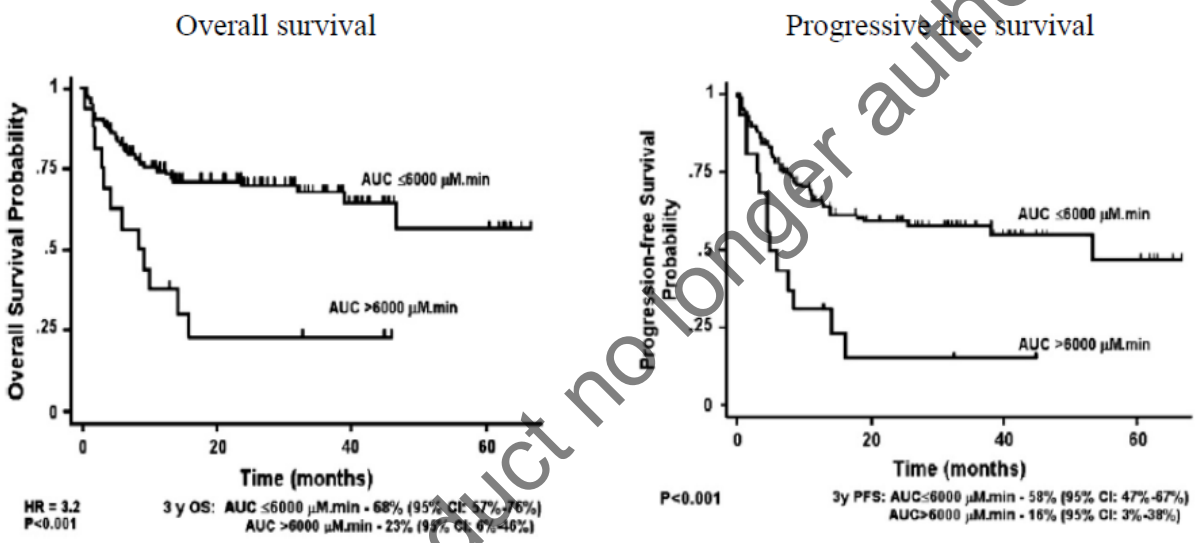


Figure 4: OS and PFS of allogeneic HSCT recipients according to the AUC groups

In a Cox proportional hazards model, the hazard ratio for the effect of high Bu exposure (AUC > 6000 µM/min versus ≤ 6000 µM/min) on OS was 3.2 (95% CI: 1.7%-6.3%). Including one at a time into the model other confounding variables such as age, CD341 cell dose, standard versus high-risk disease group, treatment with TBI, stem cell source, donor type, ABO incompatibility, the presence of at least 1 HLA mismatch and female donor-to-male recipient did not alter the hazard ratio. Adjustment for CMV positivity in either donor or recipient increased the estimated hazard ratio of high Bu exposure to 4.1 (95% CI: 2.0%-8.1%). When analysing any differences in engraftment and OS between patients with AUC < 3500 µM.min and 3500-6000 µM.min, no difference was seen between groups.

Relapse:

The relapse rate at one year was not significantly different between groups: at 31% (95% CI: 11%-54%) for high Bu exposure compared to 20% (95% CI: 13%-28%) for low Bu exposure.

Non-relapse Mortality:

There was a lower cumulative incidence of NRM in the low Bu exposure group at both 100 days and at 12 months (see Figure 5). At 12 months, the cumulative incidence of NRM remained higher in the group with Bu AUC > 6000 µM.min (38%, 95% CI: 15%-60%) compared to those with AUC ≤

6000 µM.min (14%, 95% CI: 9%-22%). Most of deaths in both groups were related to either GVHD or complications of GVHD therapy, i.e. infections / sepsis.

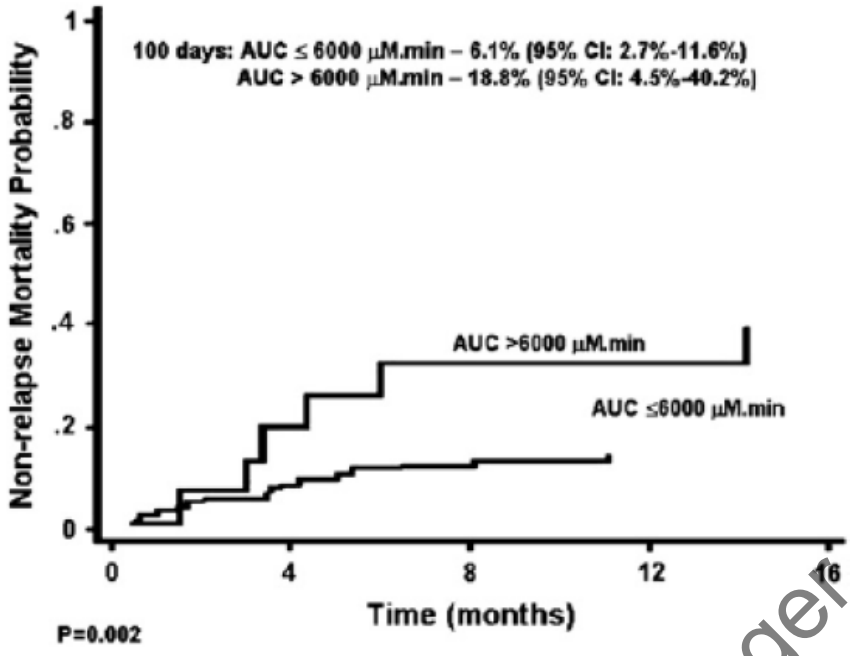


Figure 5: Cumulative incidence of non-relapse mortality of allogenic HSCT recipients according to the AUC groups

Regimen–Related Toxicities and GVHD:

No difference in organ-specific toxicities was seen between groups and no VOD was observed. Although not statistically significant, there was a trend to less severe mucositis in the low exposure group (p=0.08); all patients with Bearman grade I mucositis had an AUC < 6000 µM.min. The total incidence of a-GVHD and c-GVHD was not different between groups.

Conclusion

IV Bu once-daily PK data was evaluated in 133 adult patients and showed consistent PK results with other studies. Most of the patients (114 patients, 88%) had an AUC ≤ 6000 µM.min whereas 16 patients (12%) had an AUC > 6000 µM.min. This PK/PD study suggested that IV Bu once daily dosing given with Flu for an AUC > 6000 µM.min may be associated with increased Non Relapse Mortality and worse Overall Survival.

2.3.4. Discussion on clinical pharmacology

The PK evaluation over repeated once-daily administrations showed that Bu was cleared in less than 24h without drug accumulation between days. Stable and reproducible PK parameters over treatment days were achieved. The PK parameters were consistent throughout literature data and were in close agreement with clinical development trials.

AUC measured after one dose of once-daily schedule was four-fold the value measured after one dose of q.i.d. and the peak concentration (Cmax) measured after the end of infusion was 3 times higher in OD schedule compared to q.i.d schedule, being consistent with the dose increment (from 0.80 mg/kg to 3.2 mg/kg) and the increasing infusion of rate (from 0.4 mg/kg/h to 1.067 mg/kg/h), respectively.

Dose-independent PK parameters (i.e., Cl, Vd and T1/2) seemed to be unchanged regardless the dosage of the schedule of administration.

Apart from [Ryu BBMT 2007] (prospective comparison with a randomized parallel group design), mean PK parameters of all study were in the same range (Table 12). In Ryu study, the mean clearance value was lower and hence the AUC and Cmax achieved with the standard dosages (either 3.2 mg/kg or 0.80 mg/kg) were higher in comparison with all the other studies. Nevertheless, the comparison between schedules (once daily versus four-time daily) in this study demonstrated a linear PK behaviour of IV Bu from 0.8 to 3.2 mg/kg dosage and no statistically significant difference between the two schedules was evidenced for dose-independent PK parameters such as clearance, volume of distribution and half-life. Of note, a different method, LC-MS/Ms technique instead of HPLCUV or GC-MS, was used in this study, which may explain the higher AUC and Cmax achieved.

An intra-patient CV less than 15-20% was calculated across studies. This value was in accordance with the intra-patient variability (CV ≈ 10%) measured by population PK approach on the pooled clinical trials data performed during Busilvex development in the q.i.d. schedule [Nguyen L. 2006].

Considering that Bu metabolism is mainly hepatic and that once daily dose is four times the q.i.d. dose, the Applicant discussed whether Busilvex should be contraindicated in patients with severe hepatic impairment (see clinical safety). Based on the available safety data, the current warnings and precautions recommended in this population in the current SmPC (section 4.4) were considered adequate.

Regarding elderly and obese patients, data presented referred to oral and four-times daily IV busulfan. However, different results are not expected for once daily posology. Thus, additional warnings are not considered needed in these populations.

Regarding drug-drug interactions, Flu drug interaction could be ruled out based on Almog BBMT 2011 and Bonin BMT 2007. Additional drug interactions with itraconazole/voriconazole, progestin or phenytoin did not seem to have any influence on Bu PK parameters.

The pivotal PK/PD study by [Geddes BBMT 2008] confirmed that a toxicity limit value exists when IV Bu is given once-daily and combined with Flu. This study was based on a large series of adult patients (n=133) and indicated that a IV Bu daily AUC > 6000 µmol/L.min was associated with increased Non-Relapse Mortality (NRM) and worse Overall Survival (OS). This threshold value remained consistent with the limit of 1500 µmol/L.min per dose previously established with the four-time daily schedule in BuCy2 conditioning regimen.

It should be taken into account that except for Ryu 2007, the mean AUC for the once daily administration were all under the above mentioned limit in the publications presented. In addition, in the Ryu study, non-relapse mortality and early post-transplant toxicities were not significantly different between the once-daily and 4-times-daily regimens of intravenous Bu. Furthermore, the consistency between four time daily and once-daily schedules on PK variability and the percentage of patients inside the therapeutic window is high, thus the control of Bu exposure regardless of the schedule administration can be considered acceptable.

Regarding the results from study F60002 IN202 G0, the findings on PK for BuCy once-daily schedule were in line with the literature data for BuCy four-daily schedule.

Overall, the once daily Bu schedule and q.i.d. schedule are considered comparable and acceptable.

2.3.5. Conclusions on clinical pharmacology

In conclusion and despite the limitations of the above mentioned results (results come from several papers instead of full clinical study reports), the once daily Bu schedule and q.i.d. schedule can be considered comparable.

2.4. Clinical efficacy

Clinical efficacy is based on literature data. A systematic review was performed using the online medical library Medline and 48 publications (14 prospective, 34 retrospectives) were selected for the analysis of efficacy of the combination of Bu and Flu in adults. When the total daily dose of intravenous Bu was infused once a day instead of four doses per day, this schedule was defined as "once daily" (OD). All other schemes of administration (twice daily infusion or infusion four times a day every six hours) were defined as "other" schedule and were considered as supportive data. The combination of "once daily" FB regimens was described in 33 publications (2572 patients). These publications are referred to as "pivotal".

The analysis of the clinical efficacy was split according to the dose intensity from a modified myeloablative regimen (MAC) to reduced-intensity regimens (RIC) for pivotal. Within this classification, the clinical studies with controlled arm were reported separately from the uncontrolled studies testing the FB combination (see also tabular overview of clinical studies under 2.3.1).

2.4.1. Dose response studies

No dose response studies were submitted. The proposed dose and schedule is based on published literature.

The daily total dose of IV Bu (Busilvex) of 3.2 mg/kg/d as a single three-hour infusion proposed in this variation application is similar to the standard dose used in daily practice. This daily dose is the one used in most of the publications presented. Concerning the total dose per patient it varied according to the publications from 3.2 mg/kg to 12.8 mg/kg, depending on the choice of the dose intensity needed.

Concerning the dose of Flu, in the pivotal studies, the dose per day ranges from 30 to 50 mg/m², the duration of treatment ranged from 4 to 6 days, and the total dose used was between 120 mg/m² and 250 mg/m². The vast majority of the publications reported a total dose of 150 or 160 mg/m², with a schedule of 30 mg/m² x 5 days or 40 mg/m² x 4 days.

When it comes to sequencing of the drugs, in the list of studies collected by the Applicant, out of 47 publications reporting the schedule of fludarabine, the concomitant administration of fludarabine and busulfan was reported in 44 publications. The four-day administration was reported in 21 studies (45%) and the remaining studies reported either a five-day (32%) or six-day (13%). The sequential administration of fludarabine followed by busulfan was reported only in 5 studies (10%), with either 4 days (4 studies, 8%) or 5 days (1 study, 2%) of administration.

2.4.2. Main studies

Controlled MAC studies

There were few but reliable publications (none randomised) comparing FB in once daily with BuCy2:

- Andersson [Andersson BBMT 2008] (retrospective study) compared retrospectively over an 8-year period two sequential protocols, IV BuCy2 and IV myeloablative FB, in 215 patients. The KM

estimates for the EFS showed that the FB patients had a longer EFS, compared with the BuCy2 group (19.1 months versus 8.4 months, respectively), (p= 0.04).

• Bredeson [Bredeson BBMT 2008] (retrospective study) performed a matched-pair analysis of 120 patients treated with a myeloablative FB and ATG versus 215 patients treated with oral BuCy2. The survival rates at 1-year and 3-years were improved for the patients transplanted with intravenous FB4 regimen, at 76% [68-83] and 65% [56-73], compared to BuCy2 patients with 1-year OS and 3-year OS at 66 % [60-73] and 55% [48-62], respectively (p= 0.06 and p= 0.07).

• Lee [Lee KJH 2010] (retrospective study) retrospectively compared the efficacy of the FB4 regimen in two cohorts of patients with AML/MDS and ALL: 25 patients treated with the BuCy2 regimen and 17 with IV Flu 160 mg/m² and IV Bu at a dose of 130 mg/m^2/ day for 4 days (equivalent to a total dose of 12.8 mg/kg). The cumulative risk of relapse at 12 months and 24 months after transplantation were respectively 36% [s.e. =10%] and 36% [s.e. =10%] in the BuCy2 group and 21% [s.e.=11%] and 30% [s.e.=14%] in the FB group (p= 0.47). The FB group reported an encouraging survival with 3-year OS and EFS at 58% [s.e. =13%] and 55% [s.e. =13%] (s.e, standard error), respectively, without significant differences between the two groups.

• Shimoni [Shimoni Leuk 2006] (retrospective study) reported data collected on a cohort of consecutive 112 MDS/AML patients. The patients were included in three groups comparing intravenous BuCy2, FB4, and FB2. The once-daily FB4 regimen was safer with a NRM at 8% versus 22% in BuCy2, without difference for OS at 2 years between BuCy2 and myeloablative once daily FB (50% and 49% respectively). The 2-year OS was 47% after a reduced intensity regimen with IV FB2.

Other controlled studies were focused on early efficacy parameters [Chunduri BMT 2006], [Horwitz BBMT 2008, only controlled, prospective, randomized study for myeloablative regimen] as well as pharmacokinetics [Madden BBMT 2007], or comparisons with/without TBI [Russell BBMT 2010]. In all these experiences the FB regimen reported favourable results, in line with either the type of conditioning or the profile of the transplanted patients.

Uncontrolled MAC studies

Only two uncontrolled clinical studies in the myeloablative once-daily FB regimen did not report long-term follow-up as they were focused on early pharmacokinetic or clinical outcomes [Beri BMT 2010], [O'Donnell Leuk Lymph 2010]. In three publications the sample size of the cohort was small, making the interpretation of clinical outcomes cautious [Kerbauy Leuk Lymph 2011], [Sanz, Montesinos BBMT 2010], [Sanz, Sanz BBMT 2010]. The objective of these early clinical studies was to assess the feasibility of a conditioning based on IV FB in the setting of cord blood.

The remaining publications reported an adequate disease control. The results split by subgroups were in line with the disease-risk of the patients. The 2-year survival, when reported, ranged from 46% to 80%, depending on the type and status of the disease.

• Alatrash [Alatrash BBMT 2011] (prospective study) in MDS/AML patients aged ≥ 55 years in good general status reported a 2-year OS of 71%, 44%, 32% for patients in CR1, CR2, or refractory disease, respectively. The 2-year EFS were 68%, 42%, 30%, for patients in CR1, CR2, or refractory disease, respectively.

• Andersson [Andersson BBMT 2011] (prospective randomized study) in a prospective trial testing FB alone or in combination with clofarabine in 51 patients with CML (n=9) and MDS/AML, mainly high-risk (n=39/42), with a 2-year PFS and OS probability of 41% and 48%, respectively.

• Bashir [Bashir BBMT 2011] (prospective study)in a homogeneous cohort of 44 AML patients transplanted in CR1 reported no significant 3-year OS differences in patients above/below 55 years,

with 80%/78% (95% CI [0.59-1])/[0.64-0.94]) (p = 0.81). The 3-year EFS in patients above/below 55 years was 80%/67% (95% CI [0.59-1]/[0.53-0.86]) (p= 0.47).

- Chunduri [Chunduri BMT 2008] (retrospective study) reported in a cohort of 36 patients with mainly high risk AML (n=26) an OS of 47% after a median follow-up of 737 days [152-1,737]. The EFS/OS in the high risk AML cohort was 31%/ 35%.

- De Lima [De Lima Blood 2004] in a prospective phase II study with 96 AML/MDS CR1 patients reported a 1-year OS and EFS of 81% and 75% respectively and 65% and 52% for the entire population, respectively.

- Geddes [Geddes BBMT 2008] (retrospective study) in a retrospective study including 130 patients conditioned with FB for myeloid and lymphoid malignancies, reported an encouraging 1-year/3-year OS of 70% (95% CI [61%-77%])/62% (95% CI [52%-70%], respectively.

- Kerbauy [Kerbauy Leuk Lymph 2011] (retrospective study) reported that the one and two years OS were 53.5% and 36.3%, respectively, but the sample size of the cohort was small, making the interpretation of clinical outcomes cautious.

- Perkins [Perkins BMT 2011] (retrospective study) in haematological malignancies including 145 patients with aggressive malignant lymphoma and heavily-pretreated chronic lymphoid leukemia conditioned with the same targeted FB regimen showed a 2-year OS at 61% and 39% for patients with standard and poor risk , respectively.

- Pidala [Pidala IJH 2011] (retrospective study) reported in 38 patients transplanted for lymphoid malignancies an overall survival at 1 year of 67%.

- The same author, in a more recent publication [Pidala BMT 2011] (retrospective analysis) in 100 AML patients transplanted with targeted IV FB4 reported a 1 year OS of 66% (95% CI [46%-80%]) in CR1 patients (n= 49) and of 59% for the entire population. The PFS for this subset was 56.7% (95% CI [37.3-72.1]) at 1 year, and 53% (95% CI [33.7-69%]) at 2 years.

- Russell [Russell BBMT 2002] (retrospective study) reported a probability at 2 years of DFS of 74% ± 8% for low-risk patients and 65% ± 12% for the high-risk patients.

- The same author reported a variant with the addition of low-dose TBI in order to enhance the antileukemic activity of this regimen [Russell BBMT 2007] (retrospective study). An interesting projected DFS at 3 years of 83% ±6% for AML and 65% ±10% for ALL was reported.

- In a further publication with a large cohort of 200 patients with leukaemias and lymphomas similarly conditioned and transplanted with a matched sibling donor, the same author [Russell BBMT 2008] (retrospective study), showed an estimated 3-year survival in high risk patients > 45 years of 34% + 6%.

- Santarone [Santarone BBMT 2011] (retrospective study) in 44 ALL patients conditioned with targeted IV FB4, reported a 2 years RFS of 63% (95% CI [45%-81%]).

Controlled RIC studies

There was only one controlled study reporting favourable results in 72 FB patients who were ineligible for standard myeloablative conditioning [Shimoni Leuk 2007] (retrospective study). The control group was represented by 79 patients with lymphoid diseases conditioned with Flu and melphalan (FM). The FM regimen was more myelosuppressive and cytoreductive but significantly inferior to FB in terms of safety, with significantly higher incidence of GVHD. Among patients transplanted in remission, the 2-year OS probability was 72% (95% CI [53%- 91%]) in the FB2 group and 36% (95% CI [14%-57%]) in the FM group (p= 0.03).

Uncontrolled RIC studies

Six pivotal uncontrolled studies reported the FB experience with reduced-intensity conditioning. Five publications in different indications from US [Almog BBMT 2011] (prospective study) or/and asian centres [Cho IJH 2007] (retrospective study), [Lee AJH 2011] (prospective phase II study), [Lee IJH 2010] (retrospective study), [Lee AH 2011] (prospective phase II study) confirmed that the regimen was effective with an overall survival at 2 years ranging in MDS patients from 60% [Lee AJH 2011] to 78.7% [Cho IJH 2007] . Only one author [Almog BBMT 2011] did not report long-term outcomes, but 23 out of 46 patients were alive at a median follow-up of 421 days or 14 months. One publication [Ho BBMT 2011] reported a retrospective experience on a large cohort of high risk patients:

- Ho [Ho BBMT 2011] (retrospective study) transplanted for various haematological malignancies 433 patients conditioned with a reduced dose of IV Bu combined with intravenous Flu from matched unrelated donor (URD) (246 patients) or Matched Related Donor (MRD) (187 patients). Overall, 239 patients were considered at high-risk and 142 patients had prior myeloablative transplantation. The 2-year OS was 50% (MRD) and 56% (URD).

Analysis performed across trials (pooled analyses and meta-analysis)

The disease characteristics and patients' profile reported were various making difficult the comparison between cohorts. This explains the heterogeneity of results reported below for long-term outcomes (disease control, OS, PFS, DFS, relapse incidence).

Diseases

The entire patient population consisted of 2572 patients enrolled in 33 pivotal publications. The myeloid and lymphoid malignancies represented 73.2% and 23% of the population, respectively.

Table 14: Diagnosis of patients treated with FB regimens in pivotal publications

Diagnosis	N pts	%
AML	1277	49.7
MDS	276	10.7
AML / MDS	148	5.8
CML	146	5.7
ALL	148	5.8
Malignant Lymphoma (HL, NHL) / MM	315	12.2
CLL	126	4.9
Other Hematological Malignancies including MPD /Non Specified AL	136	5.3
TOTAL	**2572**	**100**

The efficacy of FB regimens was assessed for all type of diseases, irrespective of the proportion of lymphoid and myeloid malignancies.

Gender

The gender was calculated from 25 out of 33 pivotal publications with, overall, 1857 patients (1089 males, 768 females) treated with once daily FB schedules. The information was missing in 8 publications.

Age

Age is one of the 11 variables included in the EBMT risk score [EBMT, Handbook 2008]. The median age of patients varied across the pivotal publications, making difficult the comparison of clinical outcomes between the different FB experiences: Out of 33 pivotal publications, 11 (median age between 33 and 44 years), 17 (median age was ≥ 45 years), 5 (reported clinical efficacy in patients > 54 years), 25 (the upper age limit of patients was ≥ 60 years) and 9 (enrolled patients < 18 years).

Short-Term outcomes (myeloablation, engraftment and chimerism)

- Pivotal studies

The FB combination allowed sufficient myeloablation from severe and profound to shorter aplasia according to the publications. The variation of the median duration of neutropenia and thrombocytopenia can be modulated with the intensity of conditioning regimen through the modulation of days of IV Bu from two days (reduced-intensity or FB2) to four days (myeloablative regimen, FB4 or FB3 and thiotepa). The reduced-intensity allowed a shorter duration of aplasia (median of 12 days) and a fast haematological recovery according to the published results.

Overall, the FB combination was used to transplant 2572 patients aged from 6 to 76 years old with hematological malignancies, mainly acute leukemias, and malignant lymphoid diseases. Fast and complete engraftment rate for 86-100% of patients was reported in 23/33 clinical study reports (missing data in 10 studies).

Insufficient engraftment was seen in only one report, probably related to the absence of adequate immunosuppression in the setting of unrelated cord blood transplantation. There was no delayed engraftment. The incidence of primary graft failure was low according to these publications, in myeloablative or RIC setting.

The chimerism results according to the publications were good when the information was available, with complete donor chimerism at day +30 or day +100 in myeloablative setting >90% in the majority of publications. In the RIC setting, full donor chimerism was obtained for a majority of patients.

- Supportive studies

The FB regimens with other schedule provided effective myeloablation. Overall, the median time for neutrophil recovery ranged between 11 and 15 days in myeloablative setting according to the published data. It was mostly comprised between 11 and 15 days in the reduced-intensity regimen. Overall, the median time for platelet recovery ranged between 3 and 20 days according to published data, in myeloablative and RIC setting.

In summary, the supportive publications reported an optimal efficacy profile, with high rate of engraftment. There was a low incidence of primary graft failure, with a majority of patients with achievement of complete myeloid donor chimerism starting from day +30.

Long-term outcomes

- Pivotal studies

MAC regimens

The controlled FB studies provided positive results in terms of OS and DFS, with a median follow-up ranging from one-to 5 years post-HPCT. In these controlled studies an effective disease control was obtained in patients with various high-risk malignancies.

The large experience with pivotal uncontrolled studies reported that the FB regimens could treat various diseases and various profile of patients. The disease control was compiled in OS as well as DFS

and EFS above 60% and up to 83%, according to the type of disease, staging and previous response. The results split by subgroups were also favourable and in line with the disease-risk of the patients.

RIC regimens

A substantial number of patients received a reduced FB conditioning regimen. Most of patients were heavily pre-treated. Long-term outcomes showed that 2-year OS was superior to 50% and up to 78.5% in patients with matched sibling donors or unmatched unrelated donors.

- Supportive studies

MAC regimen

Effective disease control was achieved in all supportive studies using FB regimen with a full intensity, even in patients having comorbidities.

RIC regimen

The results allowed emphazising that encouraging survival is achieved using low-dose of IV Bu in large cohorts of patients.

Clinical studies in special populations

The analysis of IV FB literature brought no new clinical information in special population with renal impairment, hepatic impairment or obese patients. When the eligibility criteria were available, it was reported that patients could be enrolled and treated if they had adequate renal function and hepatic tests with exclusion of patients having acute or chronic hepatic, prior hepatic cirrhosis, severe renal impairment and hepatic impairment.

The available literature data showed that the FB regimen yields similar efficacy outcomes in elderly as compared to younger patients, as reported by Alatrash [Alatrash BBMT 2011], who recruited 79 patients between 55 and 76 years with the FB4 regimen.

Supportive studies

The supportive studies submitted by the MAH included 16 publications (initially 15 publications submitted on which one recent publication of M Mohty et al. was added) in 931 patients, 127 with myeloablative FB and 884 with RIC FB. In these patients the combination of IV Bu and Flu was used but with different or not reported schedules.

MAC studies

• Chae [Chae BMT 2007] (controlled retrospective study) compared the myeloablative IV FB regimen (n=40 patients) to a historical cohort treated with the standard BuCy2 (n=55 patients) with various haematological malignancies but mainly AML in CR1. The multivariate analysis confirmed that the FB regimen was an independent favorable prognostic index of OS (HR =0.168; 95% CI, [0.035-0.807]; p= 0.0026) and EFS (HR = 0.181; 95% CI [0.045–0.726]; p = 0.016).

• Patel [Patel BMT 2011] (uncontrolled retrospective study) reported a benefit for survival at 2 years from 50% (high-risk) to 85.7% (low-risk), respectively.

RIC studies

• Alyea [Alyea Blood 2005] (controlled retrospective study) assessed in 71 patients the feasibility of a non myeloablative conditioning regimen (NST) compared to a historical cohort of 81 patients conditioned with a myeloablative Cy +TBI or Bu (MAC). No difference was seen for PFS. The

cumulative incidence of relapse was 46% for NST patients and 30% for MAC patients (p = 0.052). Despite the adverse characteristics and prior organ dysfunction, the overall survival was improved in the NST group at 1 year (51% versus 39%) and at 2 years (39% versus 29%; p =0.056).

• Hamadani [Hamadani BBMT 2009] (controlled retrospective study) reported a retrospective analysis of two cohorts of patients transplanted with FB regimens: one cohort received a reduced-intensity regimen and the second group received a reduced-toxicity regimen. The outcomes reported were not comparable as the patients' profiles were different in these two groups. The 2 years expected OS rates were 52% and 40%, respectively for the group treated with a reduced-intensity regimen and for the group treated with a reduced-toxicity regimen. The 2-year PFS rates were 43% and 36%, respectively. The cumulative incidence of relapse was similar between the two groups (Gray's test p= 0.96);

• Ryu [Ryu BBMT 2007] (controlled prospective randomized phase 2 study) prospectively randomized 60 patients to two schedules of administration for IV Bu: 30 patients were randomized into the 4-times-daily IV Bu group (Bu4 arm) and 30 to the once-daily group (Bu1 arm). Thirty-three patients out of 60 patients (55.0%) were transplanted with a RIC IV FB2 regimen (IV Bu q.i.d n= 17; IV Bu once daily n= 16). Twenty-five patients (41.7%) received IV BuCy2 q.i.d. (n= 12) and IV BuCy2 once-daily (n= 13). Two patients (3.3%) received IV Bu alone: once-daily (n= 1), and q.i.d. (n= 1). The median follow-up duration of surviving patients was 511 [299-736] days. For once daily IV Bu arm (IV Bu 1 arm) the overall engraftment rate was 100% (n =30 patients). The absolute neutrophil count >500/µL was reached at a median of 14 days [10-29]. The platelet count >20,000/µL was reached at a median of 25.5 days [13-144]. The donor cell chimerism was 90.9% at day +100. The OS estimated was 70% at one year. In the Bu4 arm (classical schedule), the overall engraftment rate was 97% (n= 30 patients). The absolute neutrophil count >500/µL was reached at a median of 14 days [10-24]. The platelet count >20,000/µL was reached at a median of 26.5 days [13-114]. The donor cell chimerism was 95.7% at day +100. The 1-year estimated OS was 70% and 69% at one year in the Bu 1 arm and the Bu4, respectively (p= 0.758). Similar OS at 1 year was reported in both arms (69% and 70%, p= ns, respectively for the q.i.d. arm and the once-daily arm). The subset analyses revealed that overall survival was not significantly different between the IV Bu 1 and IV Bu 4 arms (n= 30 patients, respectively). For FB ATG patients from IV Bu 4 arm and IV Bu 1 arm, the KM OS at day +100 post-HPCT were respectively high 88.2% (95% CI [72.9%-100%]) and 81.3% (95% CI [62.2%-100%]). The KM OS at 1 year for the above FB ATG patients were respectively high 70.6% (95% CI [48.9%-92.3%] and 75.0% (95% CI [53.8%-96.2%]).

- Ten reports with consistent cohorts of patients including also elderly patients, confirmed the efficacy profile of the RIC FB regimen, offering sufficient antileukemic activity, particularly fitted to the characteristics of patients undergoing allogeneic HPCT [Armand BBMT 2008] (uncontrolled retrospective study) [Bashey BBMT 2011] (uncontrolled prospective study), [Brown BBMT 2006] (uncontrolled prospective phase II study), [Hamadani BBMT 2009] (uncontrolled retrospective study), [Koreth BBMT 2010] (uncontrolled retrospective study), [Krishnamurthy BMT 2010] (uncontrolled retrospective study), [Lee Blood 2011] (uncontrolled prospective phase II study), [Matthews BHJ 2010] (uncontrolled retrospective study), [Sayer BMT 2003] (uncontrolled retrospective study), [Tsirigotis Hematologica 2006] (uncontrolled retrospective study). The diseases and characteristics of patients varied from one report to another. A comparison of survival outcomes was difficult. Two studies were conducted using very low-dose of IV Bu [Armand BBMT 2008] and [Brown BBMT 2006] in a category of patients heavily pre-treated (relapsed NHL or HD, relapsed chronic lymphocytic leukemia), but with interesting long term results: the 1-year OS was 76% and 66% and the one year PFS was 34% and 44%, respectively.

- M Mohty et al.: Reduced-toxicity conditioning with fludarabine, once-daily intravenous busulfan and antithymocyte globulins prior to allogeneic stem cell transplantation: results of a multicenter prospective phase 2 trial

Study design and inclusion criteria

This was a prospective multicentre phase 2 study which included 80 patients diagnosed with different hematologic malignancies who underwent allo-HCT in 3 French academic transplant programs: Institut Paoli-Calmettes (Marseille, France), University Hospital of Nantes (CHU de Nantes, France) and University Hospital of Bordeaux (CHU de Bordeaux, France) between 2009 and 2011. The study was approved by each institutional review board of the participating centres, the "Comité de Protection des Personnes de Tours" and the national "Agence Française de Sécurité Sanitaire des produits de Santé". This study was registered with ClinicalTrials.gov, identifier number: NCT00841724. Informed consent from patients and donors was obtained before inclusion.

Patients aged between 18 and 65 years were eligible for the study if they had a hematologic malignancy for which an allo-HCT was indicated. Additional inclusion criteria were Karnofsky index ≥ 70% and the availability of a sibling or unrelated stem-cell donor (10-HLA match or a single HLA-Cw mismatch). Exclusion criteria were creatinine clearance less than 30 mL/min, bilirubin or aminotransferases above 3X upper normal limit, cardiac ejection fraction less than 40%, pulmonary impairment with <50% lung carbon monoxide diffusing capacity (DLCO), and pregnancy. In this protocol, eligibility criteria for a reduced intensity conditioning (RIC) allo-HCT included at least one of the following parameter: (1) patient age older than 50 years; (2) heavily pre-treated patients who received an autologous hematopoietic HCT (auto-HCT) or with more than 2 lines of chemotherapy before allo-HCT; and (3) patients with poor performance status because of significant medical comorbidities as described by Sorror et al [Sorror 2007].

Treatment protocol

In this protocol, the preparative regimen consisted of 30 mg/m^2/d Fludarabine (Flu) for 5 consecutive days (day -6 to -2), 130 mg/m2/d i.v. Busulfan (Bu) administered once daily in a 3 hours infusion for 3 consecutive days (day -5, -4 and -3; Fludarabine was administered prior to i.v. Bu infusion), and 2.5 mg/Kg/d ATG for 2 consecutive days (day -2 and -1). For GVHD prophylaxis, patients received either cyclosporine A (CSA) alone in case of an HLA-sibling donor, or CSA and mycophenolate mofetil (MMF) in case of an HLA-matched unrelated donor. All donor/recipient pairs were typed at the allelic level according to the current protocol of the European Federation for Immunogenetics (EFI) Histocompatibility Laboratory standards. A single HLA mismatch of 10 (only at HLA-Cw) was allowed at the allele level. For graft source, granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs) were recommended per protocol, but unmanipulated bone marrow (BM) was accepted when PBSC were not available.

Statistical methods

The major end point for safety was the cumulative incidence of NRM at 1 year and the major end point for efficacy was OS at 1 year. OS and DFS were calculated by the Kaplan-Meier method. Probabilities of relapse (RI), NRM, GVHD and neutrophil recovery were calculated using the cumulative incidence procedure and comparisons were performed using the Gray test. Univariate analysis was performed to compare influence of patient's age at transplantation (greater or younger than 53 years), female to male donor, diagnosis (myeloid versus lymphoid malignancies), disease status at time of transplant, type of donor, Karnofsky score, presence of comorbidities and the HCT comorbidity index on transplant outcome. Also, these parameters were evaluated in a multivariate analysis for outcome. The Cox proportional hazard regression model was used for OS and DFS, and the Fine and Gray regression model for competing risk was used for RI, NRM and cGVHD.

Patients and donors characteristics

The median age of recipients was 53 (range, 25-64 years) years. A total of 43 donor-recipient pairs (54%) were sex-mismatched. Thirty-three patients received transplant for myeloid malignancies whereas 47 patients were diagnosed with lymphoid malignancies. Fifty six patients (70%) had high-risk diseases (all patients except acute leukemia in first complete remission). Twenty-eight donors (35%) were HLA-identical siblings and 52 (65%) were MUD. The stem cell source was BM in 3 cases (4%) and G-CSF-mobilized PBSCs were used in the remaining 77 cases (96%). Fifty-four patients (68%) presented one or several comorbidities prior to allo-SCT. In 15 patients (19%) the Karnofsky score was <90%, whereas in 65 (81%) it was ≥ 90%. Finally, the HCT comorbidity index as described by Sorror et al. [Sorror 2007] was 0 for 27 patients (34%), 1 or 2 for 21 patients (26%) and > 2 for 32 patients (40%).

Table 15: Patients and donors characteristics

Characteristic (%)	Study population (N=80)
Patient age, median (range)	53 (25-64)
Sex mismatch (%)	43 (54%)
Diagnosis	
Myeloid malignancies	33 (41%)
AML	27 (34%)
MDS/MPN	6 (7%)
Lymphoid malignancies	47 (59%)
CLL	1 (1%)
T-PLL	2 (3%)
NHL	29 (25%)
HD	4 (5%)
MM	15 (19%)
ALL	5 (6%)
Disease status	
Standard risk*	24 (30%)
High risk	76 (70%)
Comorbidity	
No	26 (32%)
Yes	54 (68%)
Karnofsky score	
≥ 90%	65 (81%)
< 90%	15 (19%)
Sorror score	
0	27 (34%)
1-2	21 (26%)
>2	32 (40%)
CMV serologic status	
Seronegative donor-recipient pair	25 (31%)
Stem cell source	
Bone marrow	3 (4%)
PBSC	77 (96%)
Donor type	
HLA identical sibling	28 (35%)
HLA match unrelated donor	52 (65%)

*Standard risk disease: acute leukemia in first complete remission.

Engraftment and GVHD

All, but one, patients engrafted, at a median of 15 (range, 10-23) days after allo-HCT. The cumulative incidence of neutrophil recovery at day 28 was 98.8% (95%CI, 85.7-99.9%). Platelets engraftment occured at a median of 9 (range, 1-16) days after allo-HCT. The cumulative incidences of platelets engraftment (greater than 20×109/L at 1 month and 50×109/L at 2 months) were 97.5 % (95%CI, 89.1-99.4%) and 97.5% (95%CI, 88.4-99.5%), respectively. At day 100, the cumulative incidences of grade II-IV and of severe grade III-IV acute GVHD were 29% (n=23) and 7.5% (n=6), respectively.

The cumulative incidence of chronic GVHD (all grade) was 33.3% (95%CI, 23.1-43.9%) at 1 year and 34.9% (95%CI, 24.4-45.6%) at 2 years, with 10 patients presenting extensive chronic GVHD.

Non-relapse mortality and outcome

The overall median follow-up was 21 (range, 12-36.5) months among surviving patients. Of the 80 patients included in this study, 30 died during the follow-up period, and 50 are still alive at last follow-up [2-year OS of 61.9% (95%CI, 51.1-72.7%)]. Twenty deaths were directly attributed to disease progression or relapse, whereas 10 cases were related to allo-HCT, of whom 4 being related to acute or chronic GVHD and 3 to infections.

No deaths were related to veno-occlusive disease. At 2 years, the cumulative incidence of NRM was 11.3% (95%CI, 5.5-19.3%), and that of relapse or progression from allo-HCT was 43.8% (95CI, 31.1-55.7%). The Kaplan-Meier estimate of DFS at 2 years was 49.9% (95%CI, 32.6- 72.7%). In univariate analysis, there is a trend towards a better OS for patients with a Karnofsky score ≥90%. The Kaplan-Meier estimate of OS at 2 years was 47% (95%CI, 21-72%) for patients with a Karnofsky score <90% and 65% (95%CI, 53-77%) for those with a score ≥90% (P=0.07). In multivariate analysis, no factor was associated with OS, DFS, RI and NRM. Overall transplant-related events are summarized in the table below.

Table 16: Overall transplant-related events

Characteristic (%)	Study population (N=80)
Median follow-up, months (range)	21 (12-36.5)
OS	
At 1 years, % (95%CI)	71.3% (61.3-81.2)
At 2 years, % (95%CI)	61.9% (51.1-72.7)
DFS	
At 1 years, % (95%CI)	63.7% (53.2-74.3)
At 2 years, % (95%CI)	49.9% (32.6-57.2)
NRM	
At 1 years, (cumulative incidence, 95%CI)	10.0% (4.6-17.8)
At 2 years, (cumulative incidence, 95%CI)	11.3% (5.5-19.3)
Acute GVHD at 100 days (cumulative incidence, 95%CI)	
Grade II-IV	28.8% (19-39)
Grade III-IV	7.5% (3-15)
Chronic GVHD	
At 1 years, (cumulative incidence, 95%CI)	33.3% (23.1-43.9)
At 2 years, (cumulative incidence, 95%CI)	34.9% (25-46)
Relapse/disease progression	
At 1 years, (cumulative incidence, 95%CI)	26.3% (17.2-36.3)
At 2 years, (cumulative incidence, 95%CI)	43.8% (31.1-55.7)
Median ANC>500, days (range)	15 (10-23)
ANC>500 at day 28 (cumulative incidence)	98.8% (85.7-99.9)
Median platelet >20 $\times 10^9$/L, days (range)	9 (1-16)
Median platelet >50 $\times 10^9$/L, days (range)	11.5 (8-57)
Platelet > 20000 at 1 month (cumulative incidence)	97.5% (89.1-99.4)
Platelet > 50000 at 2 months (cumulative incidence)	97.5% (88.4-99.5)
Primary graft failure	1 (1)
Secondary graft failure	0
Cause of death	
GVHD	4 (5)
Infections	3 (4)
Other	3 (4)
Relapse/disease progression	20 (25)

This regimen successfully fulfilled its objective to achieve limited toxicity while retaining a substantial anti-tumour activity. Overall, patients included in this trial represented a common population of patients referred to allo-HCT in a majority of European transplant centres: median age was 53 years, lymphoid malignancies accounted for almost 60% of the population and 70% of patients presented high-risk features based on disease and/or patient characteristics. Moreover, 68% of patients

presented at least one comorbidity criteria prior to allo-HCT, while the HCT comorbidity index was greater than or equal to 3 in 40% of patients. Finally, 19% of patients presented an impaired performance status with a Karnofsky score below 90%. In this frail population, the cumulative incidence of NRM was as low as 10.0% at one year and 11.3% at 2 years. Furthermore, no severe extra-hematologic complications, such as veno-occlusive disease were observed in this series. The decreased toxicity in the current protocol was achieved concomitantly to a substantial disease control since OS and DFS rates at 2 years were 62% and 50% respectively in patients with a high risk malignancy for 70% of them.

Additional publications comparing Bu-Flu and Bu-Cy conditioning regimens (MAC)

- Lee at al., J Clin Oncol. 2013 Feb 20;31(6):701-9

It was a phase III randomized clinical trial to compare two myeloablative conditioning regimens for allogeneic hematopoietic cell transplantation (HCT) in patients with leukemia and myelodysplastic syndrome. After randomization, 64 patients received busulfan (3.2 mg/kg per day × 4 days) plus cyclophosphamide (60 mg/kg per day × 2 days; BuCy), and 62 patients received busulfan (same dose and schedule) plus fludarabine (30 mg/m^2 per day × 5 days; BuFlu. The median age was 41 years (range, 17 to 59 years).

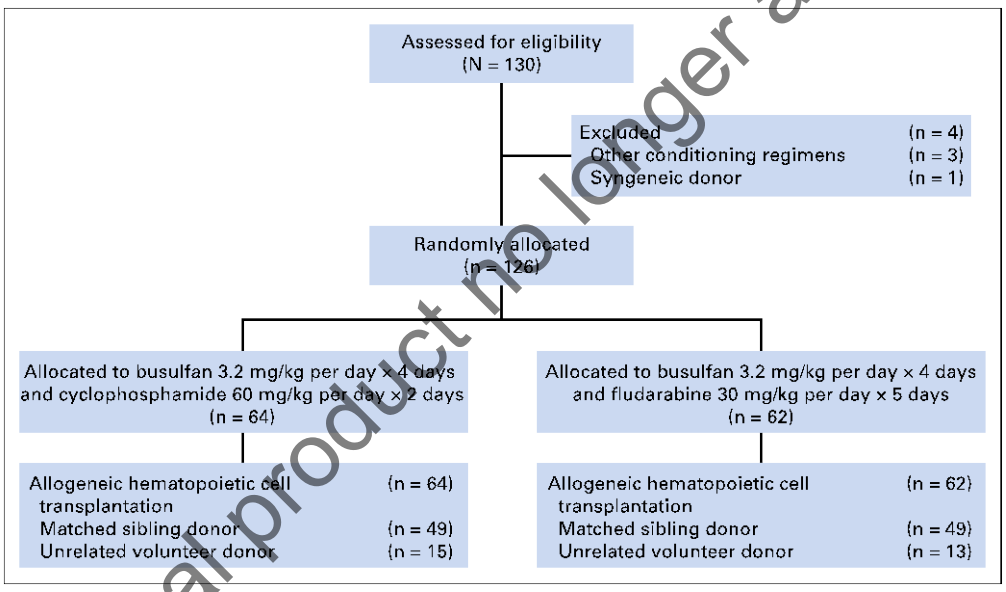
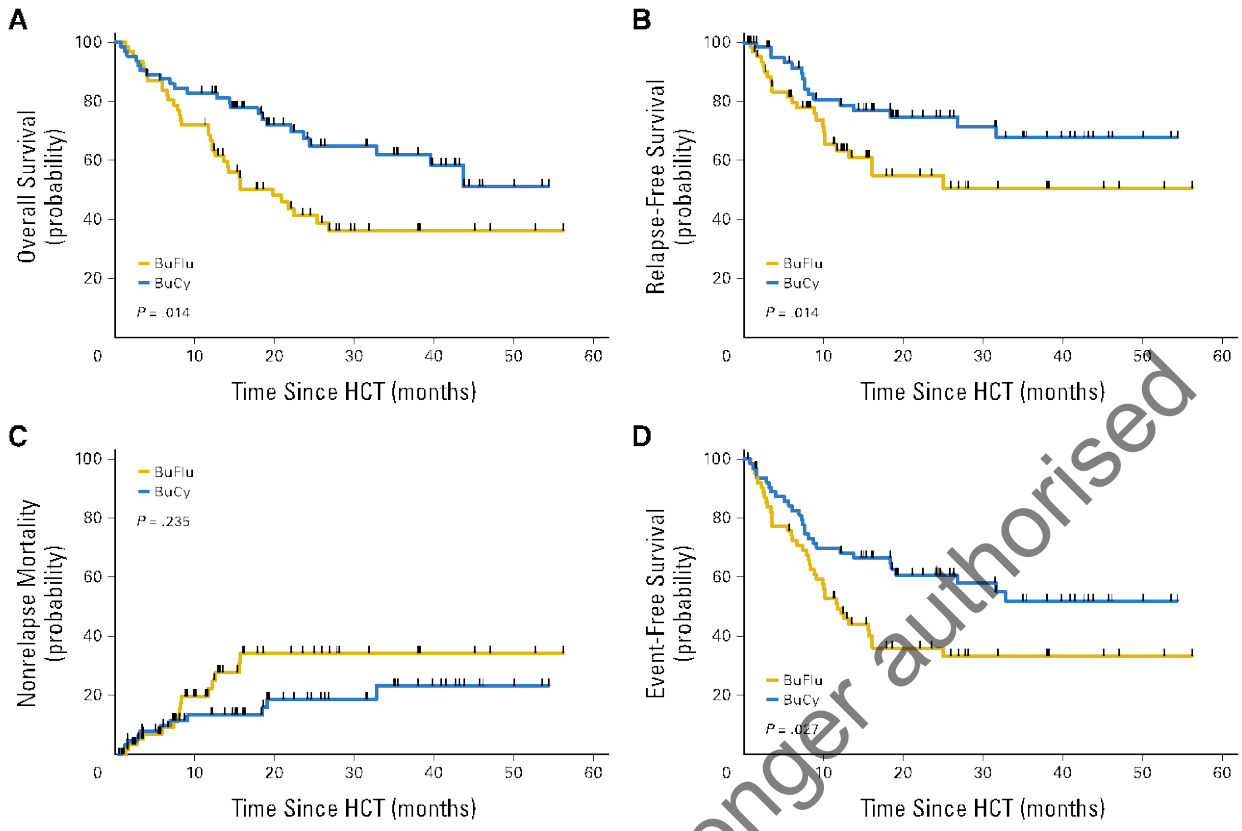


Figure 6: Flow diagram of patients

Five patients in the BuFlu arm experienced graft failure (primary, n = 1; secondary, n = 4). At 4 weeks after HCT, the median percentage of recipient hematopoietic chimerism was significantly greater in the BuFlu arm (0% v 5.5%; P < .001), and complete donor chimerism was greater in the BuCy arm (97.2% v 44.4%; P < .001). Severe (grade 3 or higher) infection and gastrointestinal adverse events were significantly more common in the BuCy arm, but the frequencies of hepatic adverse events were similar in the two arms. Non-relapse mortality was similar in the two arms, but the BuCy arm had better overall survival (OS), relapse-free survival (RFS), and event-free survival (EFS; OS at 2 years, 67.4% v 41.4%, P = .014; RFS, 74.7% v 54.9%, P = .027; EFS, 60.7% v 36.0%, P = .014)



(A) Overall survival; (B) relapse-free survival; (C) non-relapse mortality; (D) event-free survival.

HCT, hematopoietic cell transplantation

Figure 7: Survival differences between the busulfan-cyclophosphamide (BuCy) and busulfan-fludarabine (BuFlu) arms.

- Liu et al. (J Hematol Oncol. 2013 Feb 8;6:15)

It was a prospective, randomized, open-label, multicentre study to compare busulfan plus fludarabine (BuFlu) with busulfan plus cyclophosphamide (BuCy) as the conditioning regimen in allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acute myeloid leukemia (AML) in first complete remission (CR1).

A total of 108 AML-CR1 patients undergoing allo-HSCT were randomized into BuCy (busulfan 1.6 mg/kg, q12 hours, -7 ~ -4d; cyclophosphamide 60 mg/kg.d, -3 ~ -2d) or BuFlu (busulfan 1.6 mg/kg, q12 hours, -5 ~ -2d; fludarabine 30 mg/m^2.d, -6 ~ -2d) group. Hematopoietic engraftment, regimen-related toxicity (RRT), graft-versus-host disease (GVHD), transplant related mortality (TRM), and overall survival were compared between the two groups.

All patients achieved hematopoietic reconstitution except for two patients who died of RRT during conditioning. All patients obtained complete donor chimerism by day +30 post-transplantation. The incidence of total and III-IV RRT were 94.4% and 81.5% (P = 0.038), and 16.7% and 0.0% (P = 0.002), respectively, in BuCy and BuFlu group. With a median follow up of 609 (range, 3–2130) days after transplantation, the 5-year cumulative incidence of TRM were 18.8 ± 6.9% and 9.9 ± 6.3% (P = 0.104); the 5-year cumulative incidence of leukaemia relapse were 16.5 ± 5.8% and 16.2 ± 5.3% (P = 0.943); the 5-year disease-free survival and overall survival were 67.4 ± 7.6% and 75.3 ± 7.2% (P = 0.315), and 72.3 ± 7.5% and 81.9 ± 7.0% (P = 0.177), respectively in BuCy and BuFlu group.

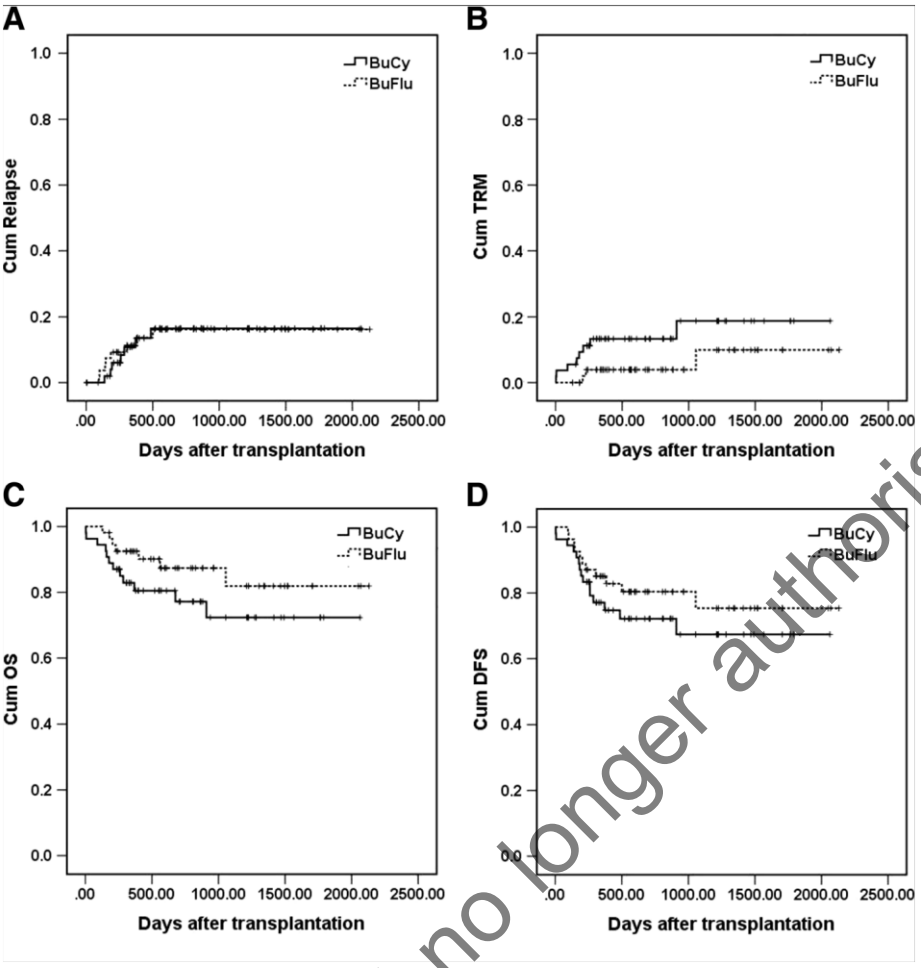


Figure 8: Cumulative incidence of relapse (A), transplant related mortality (TRM) (B), overall survival (OS) (C) and disease free survival (DFS) (D)

Table 17 Comparison of efficacy and safety from Lee and Liu studies

	Phase III - Randomized	**Lee et al (Lee 2013)**		**Liu et al (Liu 2013)**	
	Population	**AML;ALL;CML;MDS;MDS/MPN**		**AML**	
	Treatment	**BuCy2 OD**	**FB OD**	**BuCy2 q 12 hours**	**FB q 12 hours**
EFFICACY	Neutrophil engraftment	12 days	12 days	12 days	11
	Platelet engraftment	19 days	17 days	13 days	12
	1º or 2º graft failure	0	5	NA	NA
	Complete donor Chimerism	97.2 % (4 weeks)	44.4 % (4 weeks)	100% (30 days)	100% (30 days)
	Follow up time	26.7 months (range 4.0 to 56.2 months)		609 days (range 3 to 2130days)	
	X-year OS	67.4% (2 years)	41.4 % (2 years)	72.3% (5 years)	81.9% (5 years)
	X-year EFS	60.7% (2 years)	36.0 % (2 years)	NA	NA
	DFS	NA	NA	67.4% (5	75.3% (5

				years)	years)
	X-year RFS	74.7% (2 years)	54.9 % (2 years)	NA	NA
	X-year Relapse	25% (or progression)	38.7% (or progression)	16.5% (5 years)	16.2% (5 years)
SAFFETY	X-year-NRM	18.7% (2 years)	34.4 % (2 years)	NA	NA
	X-year-TRM	NA	NA	18.8% (5 years)	9.9% (5 years)
	RRT	NA	NA	94.4%	81.5%
	HVOD/HSOS	10% (no severe)	4.8% (no severe)		
	aGVHD	26.6%	32.3%	53.8% (I-II); 44.4 (III-IV)	7.7% (I-II); 0.0% (III-IV)
	cGVHD	72.2%	68.0%	47.1%	44.4%
	Deaths	35.9% [23, 11(17.2% nonrelapse)]	56,5% [35, 15 (24.2% nonrelapse)	RRD = 7.4 %	RRD=0%

2.4.3. Discussion on clinical efficacy

No formal dose-response study was submitted by the Applicant. However, the daily total dose of IV Bu of 3.2 mg/kg/d has been adequately justified based on literature data. The total dose per patient varied from 3.2 mg/kg to 12.8 mg/kg, depending on the choice of the dose intensity needed. The decision to select the IV Bu dose should be made according to the balance between risk of toxicity and risk of relapse. Considering all the criteria involved in the dose choice, it was acknowledged that only the physician will be able, on the basis of the patient's medical records and clinical status, to determine the appropriate total dose. Regarding the sequence, the importance of using the fludarabine on the same days and preceding IV busulfan is well depicted by Valdez et al in several publications [Valdez BC 2010, Valdez BC 2011 and Valdez BC 2012]. In these publications, it is indicated that nucleoside analogue (NA) facilitate access of busulfan to genomic DNA for alkylation (Valdez 2011) and that NAs can be successfully used to alter the epigenetic status of leukaemia cells resulting in chromatin remodelling, thereby making genomic DNA more susceptible to DNA alkylating agents (Valdez 2010). Regarding the timing, Russellet al [Russell 2002] reported that single daily intravenous busulfan would be more convenient and could be achieved with acceptable toxicity compared with traditional 4-times-daily dosing (see also section 2.3.2, pharmacokinetics). Additionally, according to Andersson et al [Andersson BS], the half-life of fludarabine supports a once-daily dosing, that was also extended to the intravenous formulation of busulfan with improved convenience. Therefore, the proposed dose, sequence and timing are considered adequately justified.

There seems to be a wide consensus among the scientific community (haematologist) stressing the efficacy and tolerability of this new schedule of treatment. The European Group for Blood and Marrow Transplantation (EBMT) already includes the FB combination among other possibilities, even though the oral use of busilvex instead of the IV formulation is cited in this EBMT handbook. Both, the myeloablative and the cytoreductive FB regimen as proposed by the MAH have been used for years in the clinical practice and this application is based on literature data.

Out of 33 publications initially selected for the analysis of efficacy of the combination of Bu and Flu in adults, 3 were prospective and randomised but did not allow drawing firm conclusions about the efficacy of FB administered once daily in conditioning treatment prior to HPCT:

- In the study reported in Horwith BBMT 2008 (classified as pivotal-controlled-MAC regimen), all patients were administered IV Flu 160 mg/m^2 over 4 days and IV Bu 130 mg/m^2/day for 4 days.

The patients were randomized to receive the combination of FB either sequentially or concurrently. Only 2 out of 10 patients achieve significant, yet incomplete donor hematopoietic chimerism (one sequential, the other on the concomitant treatment arms). Haematopoiesis was reconstituted in 5 patients following a second transplant. Five patients died from relapsed AML. However, this study focused on early efficacy parameters.

- In the prospective trial [Andersson BBMT 2011, (classified as pivotal-uncontrolled-MAC regimen)] FB alone or in combination with clofarabine were tested in 51 patients with CML (n=9) and MDS/AML, mainly high-risk (n=39/42). The 2-year PFS and OS probability were 41% and 48%, respectively.
- Study [Ryu BBMT 2007, (classified as supportive-controlled-RIC regimen)] was a prospective randomized phase 2 study comparing Bu administrated 4 times daily versus once daily. The 1-year estimated OS was 70% and 69% at one year in the Bu 1 arm and the Bu4, respectively (p= 0.758). Similar OS at 1 year was reported in both arms (69% and 70%, p= ns, respectively for the q.i.d. arm and the once-daily arm). Although, there were several conditionings used (FB, BuCy2 or Bu alone), the survival outcome was high in each arm, and with the same efficacy profile. In this study, the regimen was different to the proposed one since Bu (3.2 mg/kg/day x 2 days) were administer together antiymocyte globulin and Flu 30 mg/kg but for 6 days instead of 5. Furthermore, this was a phase 2 study, hence considered exploratory.

The vast majority of remaining submitted publications were uncontrolled, retrospective studies or were studies in which 'other' schedules were used (supportive publications).

Taking all 33 publications together, the FB conditioning is considered effective, allowing sufficient myeloablation, complete engraftment (rate for 86-100% of reported patients) and with a low incidence of primary graft failure. Complete donor chimerism at day +30 or day +100 in myeloablative setting >90% was observed in the majority of publications when these outcomes were reported. In the RIC setting, full donor chimerism was obtained for a majority of patients. Disease controlled was achieved, depending on the type of disease, staging and previous response, the OS, DFS and EFS ranged from 60% to 83% for MAC regimen and 2-year OS from 50% to 78.5% for RIC regimen. However, the heterogeneity of these studies together with the methodological limitations of the publications should be acknowledged and make it difficult to draw firm conclusions.

Two additional recent publications referring to prospective and randomised studies (MAC regimen) were submitted during the procedure (Lee et al., JCO 2013 and Liu et al (Liu 2013)). The study from Lee et al. was the only randomised, controlled and prospective study in the MAC setting. Although the limitations of the study and the possibility of bias against the FB arm are acknowledged, the magnitude of such interferences is unknown and the unsatisfactory results of the study cannot be disregarded. This was the only randomised study supporting the claimed indication. The other randomised controlled study (Liu, et al) can be more easily interpreted due to the homogeneity of the population included in the study (only AML). However, this study does not reflect the population and posology proposed by the applicant in this variation.

Therefore, the results of the randomised study (Lee et al), which reflects the population and posology in the proposed indication for MAC setting, raises important uncertainties at this stage on the true benefit of this treatment combination. Despite the identified limitations of Lee et al study, the level of evidence provided by other studies (not randomised, retrospectives and in some case uncontrolled) is not robust enough to overcome the uncertainties generated by Lee et al study.

Therefore, it seems that only more data derived from well-conducted studies (where bias is minimised as much as possible) will allow to better elucidate the potential differences between the FB and BuCy regimens and the true benefit of the FB combination in the MAC setting.

It is acknowledged that this combination has been used for many years, theoretically achieving comparable results with a better tolerability when compared to BuCy, and thus might be a suitable option for some patients. However, from a regulatory perspective the evidence provided at this stage cannot support the claimed indication and conditions for use for this treatment regimen in the MAC setting.

Regarding the indication in the RIC setting, the submitted evidence and clinical practice (EBMT Handbook) appears to support the indication applied for. Nevertheless, it should be taken into account that such evidence comes from non-randomised and non-controlled studies, of which the vast majority were retrospective and/or uncontrolled (only 1 retrospective controlled study was presented to support the RIC regimen). Nonetheless, the top line results from the Mohty et al (cancer 2014) study (prospective multicentre phase 2 study) in which 80 patients, aged 18 to 65 years old, diagnosed with different hematologic malignancies who underwent allo-HCT with an FB (3 days of Busilvex) reduced intensity conditioning regimen, showed that the RIC regimen of FB can be considered safe with low NRM at 2 years in high-risk patients, and efficient disease control. Therefore, the treatment with FB could be a therapeutic alternative for a subset of patients that are not candidates to receive myeloablative regimens.

The CHMP recommends the MAH to submit the final results of the multicentre prospective phase 2 trial study: “Study of a Reduced toxicity "Submyeloablative" Conditioning Regimen Prior to Allogeneic Stem Cell Transplantation in Patients With Hematological Malignancies” (NCT00841724) as soon as available.

Overall, while the evidence provided from the study of Lee et al. raises important uncertainties in the MAC setting, these worrisome results are not applicable to the RIC setting, in which all the evidence available is pointing out in the same direction. This fact along with the wide clinical use of the combination of FB in the clinical practice within the RIC setting, seem to support the approvability of the new combination in the RIC regimen.

2.4.4. Conclusions on the clinical efficacy

Due to the bibliographic nature of this application, the clinical experience using this combination is an added value. Based on the number of studies and the results, the CHMP considers the new FB schedule of treatment as effective in the RIC regimen. This combination has been widely used in the protocols for HCT for many years.

In relation to the MAC regimen, the recent publication of the study conducted by Lee at al (JCO 2013) has generated some important uncertainties which do not allow making the same recommendation as for the RIC regimen.

2.5. Clinical safety

2.5.1. Introduction

The first marketing authorisation for Busilvex was issued by the European Commission on 9 July 2003. The first launching date for Busilvex in the European Union took place in November 2003. Busilvex followed by cyclophosphamide (BuCy2) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option. In addition, Busilvex followed by cyclophosphamide (BuCy4) or melphalan (BuMel) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation in paediatric patients.

As of 8 July 2013, Busilvex has been registered in 39 countries and marketed in 32 countries by the MAH. It has been estimated that 34490 patients (including about 6500 paediatric patients) have been exposed to Busilvex since the first marketing authorisation.

The safety concerns of busulfan are as follows:

- Important identified risks: venoocclusive disease, seizure /convulsion, myelosuppression, pulmonary toxicity, reproductive toxicity, lens disorders /cataracts, secondary malignancies
- Important potential risks: cardiac tamponade
- Missing information: use in patients with hepatic impairment, use in patients with renal impairment, use in the elderly, use in obese children and adolescents.

The alternative combination, Fludarabine followed by IV Bu (FB), is widely used as a conditioning prior to allo-HPCT. The applicant provided a summary of safety data extracted from available literature on this combination in the different preparative regimens. A bibliographic search was performed using the same key words and the same database as those reported for the analysis of clinical efficacy.

The 48 publications were classified as follows:

- When the daily dose of intravenous Bu (3.2 mg/kg/day) was infused once a day instead of four doses per day, this schedule was defined as “Once daily”. These studies were defined as pivotal studies. Thirty tree (33) publications were included (total of 2570 patients).

- All other schemes of administration (twice daily infusion or infusion four times a day every six hours) were defined as “other” schedule. These studies were considered as supportive studies. The studies with a low-dose of IV Bu (0.8 mg/kg/day for 4 days), also coded “FB1” were classified in the supportive studies. Fifteen (15) publications were included (total of 931 patients).

Patient exposure

The entire FB population treated in the 48 FB studies represents 3503 patients. The patients analysed for all safety parameters were the same as those depicted for the clinical efficacy. A few paediatric patients were included in the publications. There were 25 out of 48 publications with available information for the analysis of adverse events (1532 patients) and 45 out of 48 publications for the analysis of transplant-related mortality (TRM) or NRM (non-relapse mortality).

The analysis of adverse events in the 25 studies concerned 18 pivotal studies, 14 with myeloablative and 4 with reduced doses and 7 supportive studies, 1 with myeloablative and 6 with reduced doses. In two publications [Shimoni Leuk 2006] and [Hamadani HO 2011] there were several FB subgroups of patients treated with a different dose-intensity (myeloablative or reduced-intensity). The adverse events for these subgroups were reported split according to the FB regimen and dose intensity when it was possible.

Table 18: Patients treated with FB regimens in pivotal publications (18 studies)

Author	Dose intensity	Age	Range	N pts with FB
Myeloablative				
Andersson, BBMT 2011*	FB4+Clofarabine	45	[6-59]	51
Beri, BMT 2010	FB4	47	[15-61]	23
Chunduri, BMT 2006 *	FB4	34	[19-61]	18
De Lima, Blood 2004	FB4	45	[19-66]	96
Geddes, BBMT 2008	FB4	NA	AUC</>6000 [19-66]/ [21-63]	130
Kerbauy, Leuk Lymph 2011	FB4	54	[29-76]	12
Lee, KJH 2010	FB4	35	[18-56]	17
O'Donnell, Leuk Lymph 2010	FB4	55	NA	36
Perkins, BMT 2011	FB4	48	[22-68]	145
Pidala, IJH 2011	FB4	FB+R***/FB 56/47	FB+R***/FB [35-68]/ [27-64]	38
Pidala, BMT 2011	FB4	48	[22-69]	100
Russell, BBMT 2002	FB4	41	[15-64]	70
Santarone, BBMT 2011	FB4	40	[20-57]	44
Shimoni, Leuk 2006 *	FB2/FB4	57/51	[18-70]/ [18-64]	41/ 26
Reduced Intensity				
Almog, BBMT 2011	FB2	54	[20-70]	(Total FB: 28**) FB2: 13
Cho, IJH 2007	FB2	43	[16-55]	22
Lee, AJH 2011	FB2	39.5	[19-63]	128
Shimoni, Leuk 2007 *	FB2	56	[23-70]	72
SUB-TOTAL				**1082**
Ryu, BBMT 2007 *	FB2	NR	NR	16
TOTAL				**1098**

**Controlled studies; ** N of IV FB patients; RIC other FB n= 15; ***R=Rituximab*

The 7 FB supportive myeloablative and RIC studies enrolled 419 patients, including the 15 patients enrolled in the group treated with “other regimen” in the Almog publication [Almog BBMT 2011].

Adverse events

Analysis of Adverse Events by Organ/Syndrome

Veno-Occlusive Disease (VOD)

Among all known side effects induced by alkylating agents and particularly Bu, the veno-occlusive disease is a matter of concern. It has been also reported with the use of other alkylating agents such as thiotepa, cyclophosphamide [Huitema AD, 2002], melphalan [Labidi SI, 2008] or with other treatment such as sirolimus used for the prophylaxis of GVHD [Cutler C, 2008].

21 publications reported information regarding the incidence and/or mortality of hepatic VOD in myeloablative (n=16) or reduced-intensity (n=5) FB regimens, irrespective of the schedule of administration (once-daily or other schedule).

VOD in Pivotal studies

Overall, in pivotal studies there were 1024 patients and 292 patients transplanted with myeloablative and RIC regimens, respectively. The VOD rates in myeloablative and RIC regimens were 18 (2.8%; 18/645) and 11 (3.8%; 11/292). Few publications compared the incidence among the FB recipients and control groups [Chunduri BMT 2006], [Shimoni Leuk 2006], [Almog BBMT 2011]. The myeloablative FB regimens were well tolerated with no increase of VOD.

Table 19: Hepatic VOD in pivotal studies

	Author	Dose intensity	N	Incidence of VOD n (%)	Severity of VOD	Mortality linked to VOD n (%)
Myeloablative regimens						
1	Andersson, BBMT 2011	FB4+Clo	51	1 (2)	Grade 3	0
2	Beri, BMT 2010	FB4	23	0 (0)	-	0
3	Chunduri, BMT 2006	FB4	18	0 (0)	-	0
4	De Lima, Blood 2004	FB4	96	2 (2)	Grade 1	0
5	Geddes, BBMT 2008	FB4	130	0 (0)	-	0
6	Kerbauy, Leuk Lymph 2011	FB4	12	0 (0)	-	0
7	Lee, KJH 2010	FB4	17	1 (6)	mild	0
8	O'Donnell, Leuk Lymph 2010	FB4	36	8 (22)	Severe 4 solved	2 (5.5)
9	Perkins, BMT 2011	FB4	145	6 (4)	2 mild, 4 moderate	0
10	Pidala, BMT 2011	FB4	100	0 (0)	-	0
11	Russell, BBMT 2002	FB4	70	0 (0)	–	0
12	Russell, BBMT 2008*	FB4	200	NA*	NA	1 (0.5)
13	Russell, BBMT 2010*	FB4	179	NA*	NA	1 (0.6)
14	Santarone, BBMT 2011	FB4	44	0 (0)	-	0
15	Shimoni, Leuk 2006	FB4	26	0 (0)	-	0
	Total	-	1024	18/645* (2.8)	-	4/1024 (0.4)
Reduced intensity regimens						
16	Almog, BBMT 2011***	FB2	13	2 (15.3)	2 mild, reversible	0
17	Cho, IJH 2007	FB2	22	0 (0)	-	0
18	Lee, AJH 2011	FB2	128	5 (4)	2 mild, 3 moderate	0
19	Ryu BBMT 2007	FB2	16	0	–	0
20	Shimoni, Leuk 2007	FB2	72	3 (4)	NR	NR
15bis	Shimoni, Leuk 2006**	FB2	41	1 (2.4)	Severe	
	Total	-	292	11/292 (3.8)	-	0/292 (0)

The two publications are not included in the analysis of incidence of VOD, for a total of 379 patients; **Same study with 2 different cohorts; * Cohort treated with once daily schedules in supportive study*

The mortality linked to VOD was low, with 4/1024 (0.4%) and 0/292 deaths in myeloablative and RIC regimens, respectively. Only 1 publication in the myeloablative setting reported a mortality rate linked

to VOD of 5.5%, but in patients with high Bu exposure (AUC > 6000 µmol/L.min) [O'Donnell Leuk Lymph 2010].

VOD in Supportive studies

The experience using FB with another schedule is limited, with 4 publications focusing on the early safety outcomes and regimens-related toxicities: [Almog BBMT 2011], [Chae BMT 2007], [Lee Blood 2011], [Ryu BBMT 2007]. Overall, out of 155 patients treated either with myeloablative (n=40) or RIC (n=115), 5 VOD (4.3%) were reported by Lee (1 mild, 4 moderate) [Lee Blood 2011], without VOD-related mortality.

Table 20: Hepatic VOD in supportive studies

	Author	Dose intensity	N	Incidence of VOD n (%)	Severity VOD	VOD RM* (%)°
Myeloablative regimens						
1	Chae, BMT 2007	FB4	40	0/40 (0)	-	0/40 (0)
Reduced intensity regimens						
2	Almog** (a) BBMT 2011	FB2	15	0	-	0
3	Lee, Blood 2011	FB2	83	5 (6)	1 mild, 4 moderate	0
4	Ryu BBMT 2007	FB2	17	0	-	0
	Total	-	115	5/115 (4.3)	-	0/115 (0)

** RM: Related Mortality ** Patients treated with "other" schedules; (a) Cohort treated with other schedules*

Overall, in the myeloablative and RIC settings, 34 cases of VOD out of 1471 (2.3%) evaluated patients were reported in pivotal and supportive FB publications, with a VOD-related mortality of 0.3% (n=4). The VOD incidence appeared to be lower when compared to that reported in the literature, taking into account also that the etiology of VOD is multifactorial and that several risk factors impact on the occurrence or outcomes. Coppell [Coppell BBMT 2010], across 135 studies performed between 1979 and October 2007, reported an overall mean VOD incidence of 13.7% [95% CI 13.3%-14.1%].

The total number of cases of VOD in the whole cohort remained low irrespective of the dose intensity (FB4 or FB2) and irrespective of the schedule of administration. The VOD-related mortality for the above combined studies was low.

<u>Biological hepatic tests - Liver function tests in myeloablative and RIC regimens</u>

Pivotal studies

The early abnormalities of liver function tests after HPCT are common and with multifactorial causes. The liver function tests can be altered either by the administration of concomitant treatments such as methotrexate, antibiotics, antifungal treatments, parenteral nutrition or by, hepatic infections (fungal, viral, bacterial cholangitis) and immune dysfunction. The liver function reported as dosing of transaminases/bilirubin/phosphatase alkaline was assessed in 9 publications.

Table 21: Liver toxicities – Severe adverse events (grade >2) in pivotal publications

Author	Dose intensity	N	Liver biological parameter n (%)	Severity n (%)
Myeloablative regimens				
Andersson BBMT 2011	FB4+ Clo	51	Transaminases: 1 (2) Bilirubin: 0%	Grade 3: 1 (2) Grade 4: 0 (0)
Chunduri BMT 2006	FB4	18	0	NA
De Lima, Blood 2004	FB4	96	Transaminases: 17 (17) Bilirubin: 9 (9) Phosphatases alkalines: 0	Grade 3 15 (15) Grade 4: 2 (2) Grade 3: 7 (7) Grade 4: 2 (2)
Kerbauy Leuk Lymph 2011	FB4	12	Transaminases: 2 (17) Bilirubin: 1 (8)	Grade 3: NR Grade 4: NR Grade 3: NR Grade 4: NR
Perkins, BMT 2011	FB4	145	Transaminases/Bilirubin: 15 (10)	Grade 3: 11 (7) Grade 4: 4 (3)
Pidala BMT 2011	FB4	100	Transaminases/Bilirubin: 9 (9)	Grade 3: 8 (8) Grade 4: 1 (1)
Russell BBMT 2002	FB4	70	Transaminases (ALT): 52 (74) Bilirubin (conjugated): 62 (89)	NR
Reduced intensity regimens				
Cho IJH 2007	FB2	22	Transaminases: 4 (18) Bilirubin: 2 (9) Phosphatases alkalines: 1 (5)	Grade 3: 3 (14) Grade 4: 1 (5) Grade 3: 1 (5) Grade 4: 1 (5) Grade 3: 0 (0) Grade 4: 1 (5)
Shimoni, Leuk 2007	FB2	FB2: 72	Transaminases/Bilirubin: 20 (28)	NR

Supportive studies

In the supportive publications, no data was available for myeloablative supportive studies. Only one study reported liver abnormalities in the RIC regimen [Ryu BBMT 2007].

In conclusion, the analysis of liver toxicities was done in more than 500 patients for whom transaminases and bilirubin levels were available. The total incidence of liver toxicities in the experiences with the FB regimens was in line with the profile of IV Bu. The liver abnormalities were transient and reversible most often before the 4th week after transplant. Only one publication reported 1 death related to liver failure collected in 33 publications reporting causes of death till day +100 in 2383 patients [1/2383 (0.04%)].

Central Nervous System

Seizures in pivotal and supportive studies

In pivotal studies, the impact of the IV once-daily FB combination on seizures was reported in 13 publications. Overall, there were 819 patients (myeloablative 725 patients, reduced-intensity 94 patients). In the early post-transplant period (before day+28 or until day +100), out of 13

publications, a low rate of acute seizure was reported only in 3, with a percent ranging from 1.4% to 2.3%. Four total seizures were observed in the myeloablative group and one in the reduced-intensity group. The estimated overall incidence was 5 cases out of 819 patients (0.6%).

Table 22: Seizures in pivotal studies

Author	Dose intensity	N	Incidence of seizures n (%)	Severity
Myeloablative regimens				
Andersson, BBMT 2011	FB4+Clo	51	0 (0)	NA
Beri BMT 2010	FB4	23	0 (0)	NA
Chunduri, BMT 2006	FB4	18	0 (0)	NA
De Lima, Blood 2004	FB4	96	0 (0)	NA
Geddes, BBMT 2008	FB4	130	3 (2.3)	NR
Kerbauy, Leuk Lymph 2011	FB4	12	0 (0)	NA
O'Donnell, Leuk Lymph 2010	FB4	36	0 (0)	NA
Perkins, BMT 2011	FB4	145	0 (0)	NA
Pidala, BMT 2011	FB4	100	0 (0)	NA
Russell, BBMT 2002	FB4	70	1 (1.4)	NR
Santarone, BBMT 2011	FB4	44	0 (0)	NA
Reduced intensity regimens				
Cho, IJH 2007	FB2	22	0 (0)	NA
Shimoni, Leuk 2007	FB2	72	1 (1.4)	NR

NA = Not Applicable NR=Not Reported

In supportive studies, seizures were not evaluated.

In summary, the episodes were rare and transient with no sequelae reported in these publications and no related death; the other neurological events were also of mild intensity.

Other neurologic events in pivotal and supportive studies

Other neurologic events were rarely reported. De Lima [De Lima Blood 2004] reported no grade 3-4 of other neurologic events in a cohort of 96 patients transplanted with a myeloablative once-daily FB regimen: only peripheral neuropathy grade 1 in 4 patients, grade 2 in 3 patients were reported. Peripheral neuropathy is a well-known adverse effect of Flu with a frequency evaluation as very common. Headache grade 1 in 4 patients and grade 2 in 2 patients were also reported. In one patient, a mental status change grade 2 was noticed. With IV FB2 in once-daily, an excellent neurologic tolerance was also described by Cho [Cho IJH 2007], who reported only 2 patients who developed a grade 1 headache. Among the experiences with other FB schedules, Ryu [Ryu BMT 2007] emphasized

in his prospective randomized study that there were rare neurologic events in both groups of patients transplanted with IV Bu-based regimens using a once-daily or a q.i.d schedule. These events were reported after day+28 in the follow-up period and according to the authors' opinion those events were not related to the administration of IV Bu.

There were no deaths related to neurologic events. In the TRM/NRM at day +100, the CNS was involved for other reasons with 7/197 deaths (3.6%) collected in 33 publications reporting causes of death till day +100 in 2383 patients [7/2383 (0.3%)]: CNS bleeding (n=3), encephalopathy (n=2), cerebral ischemia (n=1) and acute meningo-encephalitis (n=1) [Alatrash BBMT 2011] [Kerbauy Leuk Lymph 2011] [Lee AJH 2011] [Geddes BBMT 2008], [Russell BBMT 2010], [Bashey BBMT 2011] and [Lee Blood 2011].

In summary, the available data showed that the neurological tolerance was good with a low incidence of severe neurologic events that can be attributed to the infusion of IV Bu combined with Flu irrespective of either the dose intensity or the schedule of administration. There were no unexpected serious neurologic events related to the use of IV FB regimens.

Gastro-Intestinal tract: Mucositis/Stomatitis/Nausea/Vomiting/Diarrhoea

The gastro-intestinal events were reported in many studies. Mucositis as well as stomatitis were not differentiated among the publications. Therefore, these events were presented together.

Table 23: Gastro-intestinal toxicities in pivotal and supportive studies

Author	Dose intensity	N	Mucositis/Stomatitis n (%)	Nausea/vomiting n (%)	Diarrhea n (%)
Myeloablative regimens					
Andersson, BBMT 2011	FB4+Clo	51	Grade 3: 5 (10) Grade 4: 1 (2)	Grade 3: 1 (2) Grade 4: 0	Grade 3: 1 (2) Grade 4: 0
Chunduri, BMT 2006	FB4	18	Grade 3/4: 1 (5.5)	NR	NR
De Lima, Blood 2004	FB4	96	Grade 3: 9 (9)	Grade 3: 1 (1)	Grade 3: 2 (2)
Geddes, BBMT 2008	FB4	130	Grade 2/3: 112 (86)	NR	NR
Kerbauy, Leuk Lymph 2011	FB4	12	Grade 3/4: 0 (0)	Grade 3/4: 0 (0)	Grade 3/4: 0 (0)
Lee KJH 2010	FB4	17	Grade 2/4: 4 (24)	Grade 2/4: 10 (59)	NR
Russell, BBMT 2002	FB4	70	Grade 3/4: 0 (0)	NR	NR
Santarone, BBMT 2011	FB4	44	Grade 3/4: 26 (59)	Grade 3/4: 0 (0)	Grade 3/4: 0 (0)
RIC regimens					
Cho, IJH 2007	FB2	22	Grade 3/4: 0 (0)	Grade 3/4: 0 (0)	Grade 3/4: 0 (0)
Lee, AJH 2011	FB2	128	Grade 3/4: 14 (11)	Grade 3/4: 22 (17)	Grade 3/4: 8 (6)
Shimoni, Leuk 2007	FB2	72	Grade 3/4: 21 (29)	NR	NR

NR=Not Reported

Apart from Lee [Lee Blood 2011] in RIC regimens, all studies were pivotal studies.

Ryu [Ryu BBMT 2007] reported no differences in gastrointestinal toxicities between IV Bu administered once and four times a day in the RIC setting. Even if the analysis was not split among the cohorts including FB or BuCy2, grade 3-4 gastrointestinal toxicities were reported in 40% and 30% in IV Bu administered once or four times a day, respectively.

Overall, the FB regimens reported low rates of grades 3-4 mucositis and few other G1 adverse events whatever the type of schedule, more obvious in the RIC experience.

Pulmonary toxicity

Pivotal studies

The overall incidence of reported pulmonary events was infrequent, with 13 cases (2.8%) out of 457 patients in 5 publications.

The lung was one of the main organs involved in TRM/NRM, with 21/197 (10.7%) deaths collected in 33 publications reporting causes of death till day +100 in 2383 patients [21/2383 (0.9%)].

Table 24: Pulmonary toxicity (excluding infections) in pivotal studies with myeloablative and RIC FB regimens

Author	Dose intensity	N	Incidence of pulmonary events* n (%)	Severity
Myeloablative regimens				
De Lima, Blood 2004	FB4	96	Pleural effusion 3 (3) Pulmonary hemorrhage 1 (1) Pulmonary oedema 2 (2)	Grades 1-2 Grade 3 Grade 2
Perkins, BMT 2011	FB4	145	Interstitial pneumonia 4 (3)	NR
Pidala BMT 2011	FB4	100	Idiopathic pneumonia syndrome 1 (1)	NR
Santarone, BBMT 2011	FB4	44	0 (0)	NA
Reduced intensity regimens				
Shimoni, Leuk 2007	FB2	72	Diffuse alveolar hemorragy 2 (3)	NR

* Non infectious events. NA = Not Applicable NR=Not Reported

Supportive studies

The data reported was limited, and most of these studies were focused on other safety parameters/events.

Overall, the clinical experiences reported no unexpected severe pulmonary toxicities particularly for interstitial pneumonia (excluding infectious events), with most of the cases solved after symptomatic treatment or recovering without any treatment.

Cardiovascular

Pivotal studies

IV Bu and Flu did not induce an increase of cardiovascular toxicity for grades 1-3 without grade 4 toxicities. A good tolerance was observed in patients with various haematological malignancies and in different medical conditions [Cho IJH 2007], [De Lima Blood 2004], [Kerbauy Leuk Lymph 2011], [Shimoni Leuk 2006] and [Shimoni Leuk 2007].

Cardiovascular events were reported as TRM/NRM with 6/197 deaths (3.0%) collected in 33 publications reporting causes of death till day +100 in 2383 patients [6/2383 (0.3%)].

Table 25: Cardiac toxicity: grade 3 adverse events in myeloablative and RIC pivotal studies

Author	Dose intensity	N	Type of cardiac toxicity	n (%)
Myeloablative regimens				
De Lima, Blood 2004	FB4	96	Hypertension Hypotension Arrythmia Pericardial effusion Decreased LVEF	3 (3) 1 (1) 0 (0) 1 (1) 2 (2)
Kerbauy, Leuk Lymph 2011	FB4	12	-	0 (0)
Shimoni, Leuk 2006	FB2/FB4	FB2: 41 FB4: 26	- -	0 (0)
Reduced intensity regimens				
Shimoni, Leuk 2007	FB2	72	-	0 (0)

NA = Not Applicable

Supportive studies

Ryu [Ryu BBMT 2007] reported few toxicities in the Bu4 (IV Bu 4 times daily) and Bu1 (IV Bu once daily) arms, respectively: arrhythmia Bu4 3.3%/Bu1 0%; hypertension Bu4 3.3%/Bu1 3.3%; other Bu4 3.3%/Bu1 0%.

In conclusion, the pivotal and supportive studies in myeloablative as well as in reduced intensity FB experiences reported few cardiovascular events.

Kidney/Urinary Tract

Haemorrhagic cystitis is a complication occurring after HPCT and that may lead to prolonged hospitalization but also significant morbidity with possible sequelae. Several severe symptoms have been described, including obstruction of the urinary tract, renal failure, hydronephrosis. Early bleeding occurring up to 72h after administration of the chemotherapy are often related to known agents like Cy, ifosfamide, or Bu. The occurrence of late bleeding episodes after 2 weeks post-transplant is often associated with infectious agents (BK polyoma virus, the most common, but also CMV or adenovirus).

Overall, only one case of renal failure was reported by Bashey [Bashey BBMT 2011].

Renal failure was reported as TRM/NRM with 1/197 deaths (0.5%) collected in 33 publications reporting causes of death till day +100 in 2383 patients [1/2383 (0.04%)].

Pivotal studies

Myeloablative regimens

Chunduri et al. reported the absence of severe kidney toxicity [Chunduri BMT 2006]. The incidence of haemorrhagic cystitis in the large cohort of De Lima [De Lima Blood 2004] (96 patients) was 3% (grade 3: 3/96). In the experience of Geddes [Geddes BBMT 2008], there were a total of 18 cases of haemorrhagic cystitis out of 130 patients (13.8%). The experience of Hamadani [Hamadani HO 2011], categorized in supportive publications, reported an incidence of 18.9% (7 out of 37 patients) of haemorrhagic cystitis, without mentioning the grade.

Reversible grade 2 renal toxicity was related to tacrolimus in the publication of Kerbauy [Kerbauy Leuk Lymph 2011], showing that FB regimens were well tolerated even in elderly patients, or heavily pre-treated patients (over CR2). Lee [Lee KJH 2010] reported 3/17 cases of haemorrhagic cystitis (18%). The incidence of grade 2 haemorrhagic cystitis was 13% (9/70 patients) in the experience of Russell [Russell BBMT 2002] with no-long-term sequelae. Shimoni [Shimoni Leuk 2006] reported one grade 3 of haemorrhagic cystitis and one grade 3 of renal toxicity.

RIC regimens

In the RIC experience of Shimoni the incidence of renal toxicity related to FB regimen was 4% [Shimoni Leuk 2007].

Overall, in pivotal publications, the occurrence of haemorrhagic cystitis ranged from 3% to 18.9%.

Supportive studies

Haemorrhagic cystitis was documented in several publications and related to infections or reactivations of BK virus (13 out of 72 patients (18%)) [Hamadani BBMT 2009]. In the experience published by Ryu [Ryu BBMT 2007], there was only one case out of 30 patients treated with IV Bu once daily with grade 4 renal failure. There was no information split by conditioning but the change of schedule did not increase the known toxicity profile of IV Bu.

Infections

The infections reported in the FB publications included viral infections, bacterial infections and fungal infections with myeloablative or reduced-intensity FB regimens. The infections were very common or common in the post-transplant period [Pidala IJH 2011] [Hamadani HO 2011] [Lee AJH 2011] [Hamadani BBMT 2009] [Lee Blood 2011]. The incidences of infectious episodes varied from one publication to another, depending on several factors: dose intensity of conditioning regimens but also type of infectious prophylaxis, type and dose of the GVHD prophylaxis, poor prognostic patients with prior weak immune system.

Bacterial infections were reported up to 62% and 39% with myeloablative [Hamadani HO 2011] and RIC regimens [Hamadani BBMT 2009]. Invasive fungal infections were not reported with myeloablative regimens and achieved a maximum frequence of 6% in [Hamadani BBMT 2009]. Reactivation of viral infections CMV was common and the incidence of organ infection involving CMV reactivation in myeloablative studies ranged from 22% [Chunduri BMT 2006] to 35% [Hamadani HO 2011] and in RIC studies from 42% [Cho IJH 2007] to 80% [Lee AJH 2011]. In myeloablative studies EBV reactivation was not reported. In RIC studies, EBV reactivation ranged from 18% [Lee AJH 2011] to 61% [Lee Blood 2011]. The development of PTLD was scarce. Infections were reported as TRM/NRM with 90/197 deaths (45.7%) collected in 33 publications reporting causes of death till day +100 in 2383 patients [90/2383 (3.8%)].

Serious adverse event / deaths / other significant events

TRM/NRM

TRM/NRM corresponds to deaths that could be attributable to secondary side effects after HPCT and not related to the relapse/progression of the underlying haematological malignancies.

The results of TRM/NRM were calculated and reported by the authors using standard statistics (cumulative incidence of TRM/NRM). Additional information was extracted using the tables of cause of death issued from the publications.

In few publications, the mortality attributable to early side effects of the conditioning regimen is named as “RRM” (regimen-related mortality) and was differentiated from the TRM/NRM (side effect of the whole transplant procedure).

The median follow-up was ranging from 421 days up to 8 years post- HPCT. The analysis of the TRM/NRM results was split between the early period after transplant and the late post-transplant period. After day +100, the follow-up covered several periods: from day +100 until 6 months (6 months TRM/NRM) or until day +365 post- HPCT (1 year TRM/NRM), after day +365 until day +730 (2

year TRM/NRM). The TRM/NRM of two publications conducted with a myeloablative FB-based regimen in the setting of unrelated cord blood transplantation is reported in a specific paragraph. [Sanz, Sanz BBMT 2010] [Sanz, Montesinos BBMT 2010].

Table 26: Early and late TRM/NRM in pivotal and supportive studies with MAC regimens

Author	Day+ 30 TRM/NRM (%)	Day +100 TRM/NRM (%)	1-Year TRM/NRM (%)	Long-Term (≥ 2 years) TRM/NRM (%)	TRM/NRM by subgroups (%)
Alastrash, BBMT 2011	1	5	19	3-y 26	CR1 pts: 0 at 30 days 4 at 100 days 14 at 1 year 19 at 3 years
Andersson, BBMT 2008	-	-	-	-	CR1 pts: FB: 6 BuCy: 21 at 1 year
Andersson, BBMT 2011	0	4	15	-	-
Bashir, BBMT 2011	-	5	-	3-y 15 (95% CI : 0.75-0.97)	-
Beri, BMT 2010	-	-	-	-	-
Bredeson, BBMT 2008	-	-	FB: 9 BuCy: 24 p<0.01	3-y FB: 11 BuCy: 30 p<0.01	5-y: FB: 12 BuCy: 34 p<0.01
Chae, BMT 2007	-	-	FB: 10.4 BuCy2: 31 p=0.023	2-y FB: 10.4 BuCy2: 34.2 p = 0.039	-
Chunduri, BMT 2006	-	5.5	-	-	100 days-TRM in FM cohort: 8
Chunduri, BMT 2008	-	-	-	-	Standard-risk pts 10 High-risk pts 19
De Lima, Blood 2004	0	0	TRM: 3 RRM: 1	-	-
Geddes, BBMT 2008	-	-	-	-	Bu d-100 1-y Low exposure 6 14 High exposure: 19 38

Author	Day+ 30 TRM/NRM (%)	Day +100 TRM/NRM (%)	1-Year TRM/NRM (%)	Long-Term (≥ 2 years) TRM/NRM (%)	TRM/NRM by subgroups (%)
Horwitz, BBMT 2008	0	10	30	-	-
Kerbauy, Leuk Lymph 2011	1 pt	20	27	-	-
Lee, KJH 2010	-	-	-	17	-
Madden, BBMT 2007	-	-	-	-	-
O'Donnell*, Leuk Lymph 2010	0*	4	-	-	-
Patel, BMT 2011	-	-	-	18	2-y: Low risk pts: 14.3 Intermediate risk pts : 23.5 p = ns High risk pts: 15
Perkins, BMT 2011	-	6	21	-	-
Pidala, IJH 2011	-	-	3 Pts	-	-
Pidala, BMT 2011	-	3	15	-	-
Russell, BBMT 2002	2 Pts	5 ± 3	-	2-y: 10 ± 4	2-y MRD: 5 ± 4 AD: 19 ± 9 p= ns
Russell, BBMT 2007	0	1	2	Cumulative incidence 3 ± 2	No TRM in 17 pts ≥ 50 y
Russell, BBMT 2008	-	2.5	10	-	3-y Low Risk ≤ 45 y 4 ± 3 > 45 y 6 ± 4 High Risk ≤ 45 y 6 ± 4 > 45 y 27 ± 7 p=0.04

Author	Day+ 30 TRM/NRM (%)	Day +100 TRM/NRM (%)	1-Year TRM/NRM (%)	Long-Term (≥ 2 years) TRM/NRM (%)	TRM/NRM by subgroups (%)
Russell, BBMT 2010	-	FB+ TBI: 5.6 FB: 5.6	FB+ TBI: 13.5 FB: 15.5	-	1-y Low Risk High Risk FB+ TBI 13 27 FB: 9 ns 31 ns
Santarone, BBMT 2011	0	2	18	2-y: 18	2-y < 35 y 17 > 35 y 19 p= ns
Sanz, Sanz BBMT 2010	-	18**	-	2-y : 39 **	-
Sanz, Montesinos BBMT 2010	-	38**	50**	8-y: 59 **	-
Shimoni, Leuk 2006	-	-	-	2-y FB2 8 (95 CI, 3-23%) FB4 8 (95 CI, 2-31%)	2-y IV BuCy 22 (95 CI, 13-39%) p= 0.05 (vs FB2& FB4)

* 21 Days-TRM/NRM, ** Data for the whole cohort

Table 27: Early and late TRM/NRM in pivotal and supportive studies with RIC regimens

Author	30 Days- TRM/NRM (%)	100 Days-TRM/NRM (%)	1-Year TRM/NRM (%)	Long-Term (≥ 2 years) TRM/NRM (%)	TRM/NRM by subgroups (%)
Almog, BBMT 2011	-	-	BuFlu OD 7.7 QID 6.7	-	BuCy2 OD 44 QID 33
Alyea, Blood 2005	-	FB 6 TBI-Cy or BuCy: 30	FB: 32 p = 0.01 TBI-Cy or BuCy: 50	-	TRM for fatal pulmonary complications: FB 0 TBI-Cy 28
Armand, BBMT 2008	-	-	13 [6-20]	3-y 23 [12-33]	NR
Bashey, BBMT 2011		6 months-TRM: 8 [2-14]		2-y 23 [14-32]	6 months 2-y For MSD 3 2 For MUD 12 ns 19 ns
Brown, BBMT 2006	-	1	-	2-y 17	-
Cho, IJH 2007	0	0	-	2-y 12.6	-
Hamadani, BBMT 2009	-	r-ATG: 0 p = 0.03 R-ATG: 7.7	r-ATG: 3 p = 0.03 R-ATG: 18	-	-
Hamadani, HO 2011	-	RIC: 3 RTC: 6 p=0.03	RIC: 12 RTC: 21 p=ns	2-y RIC: 19 RTC: 32 p=ns	-
Ho, BBMT 2011	-	-	-	-	2-y URD 8 MRD 6 p=0.33
Koreth, BBMT 2010	-	-	-	2-y 10	2 y 60-64 y 10.5 ≥ 65y 8.3 p=0.84

Author	30 Days- TRM/NRM (%)	100 Days-TRM/NRM (%)	1-Year TRM/NRM (%)	Long-Term (≥ 2 years) TRM/NRM (%)	TRM/NRM by subgroups (%)
Krishnamurthy, BMT 2010	-	-	-	3-y 31 ± 14	2 y Low Risk 0 Intermediate/High Risk 71 ± 22 p<0.02
Lee, IJH 2010	-	-	-	2-y 26	CR1: 16.1 ± 8.7 CR2: 61.9 ± 19.9 p = 0.002
Lee, AJH 2011	-	-	14% (95% CI, 9%-22%)	CI: 17 (95% CI, 11%-27%)	-
Lee, Blood 2011	-	-	17% (95%CI, 11-28%)	CI : 18 (95% CI, 12-29%)	Standard-risk pts: 10 (95% CI: 4-25%) High-risk pts: 24 (95% CI: 14-41%)
Lee, AH 2011	-	-	-	3-y 20.5	
Matthews, BJH 2010	-	-	-	-	-
Ryu, BBMT 2007	-	FB QID : 11.8 [3.2-43.3] FB OD : 18.8 [6.8-52.0]	-	2 y BF QID 17.7 [6.3-49.3] BF OD 18.8 [6.8-52.0]	2 y BuCy QID 27.4 [10.3-73.0] BuCy OD 7.7 [1.2-50.6]
Sayer, BMT 2003	-	CR / PR: 12 Other: 30	-	2-y 53*	CR / PR: 33 Other: 66 p=0.029
Shimoni, Leuk 2007	-	-	-	2-y 16 [9-27]	2-y FB: 40 [30-53] p=0.003 vs FB
Tsirigotis, Haematologica 2006	-	21.6	34	At median follow-up of 22 mo 35%	-

* for the whole cohort

The FB combination was used to treat a large population of patients in pivotal (n=2572) and supportive (n=931) studies with IV FB administered either as myeloablative or reduced intensity conditioning regimens.

The myeloablative FB was well tolerated. The rate of death from acute organ failure (including mortality from VOD), related to the side effects of the conditioning was very low between day +30 and day +100, without unusual cause of death among the causes reported by the authors. The long-term follow-up confirmed the feasibility of the myeloablative FB, with one-year TRM/NRM ranging from 3% to 21%. Data were retrieved from large cohorts of patients with various disease risk, various clinical profiles and patients aged above 50 years. The 2-year TRM/NRM rates were still reliable, reflecting the safety profile of any standard myeloablative regimen.

The RIC IV FB was feasible, with TRM/NRM up to day +100 ranging from 0% to 6%. The switch of schedule from 4 times daily to once-daily administration was well tolerated, with no increase of TRM/NRM at day +100. Consistent long-term results after day +100 were reported in a large FB population older than that treated with myeloablative conditioning regimens, with acceptable TRM/NRM rates at one, two and three years, respectively. The benefit in terms of TRM reduction with the RIC IV FB regimen was still valid after several years post-transplant.

Overall, the IV FB analysis in myeloablative and RIC settings showed that:

- At day +30 till day +100 the FB regimens were well tolerated for various profiles of patients, diseases, risk and type of procedures, with low TRM/NRM rates.
- After day +100, the late TRM/NRM rates were encouraging in both myeloablative and reduced-intensity regimens especially considering that after day +100, the TRM/NRM was mainly not related to the conditioning regimen. The main causes of death included GVHD, infections or reactivations. The causality of the infections, apart the type of conditioning, was also related to the long immune suppression period and the continuation of the immunosuppressive therapy given for the prophylaxis and or the curative treatment of chronic or acute GVHD.

Adverse drug reactions reported for the combination with fludarabine (FB)

Adverse drug reactions reported in published clinical trials (RIC and MAC settings) for which the population treated with FB was clearly identified (whatever the schedules of busulfan administrations and endpoints) are presented below using the highest incidence observed.

System organ class	Very common		Common		Not known*
	MAC	RIC	MAC	RIC	-
Infections and infestations	Viral infection CMV reactivation Bacterial infection Invasive fungal infection	Viral infection CMV reactivation EBV reactivation Bacterial infection		Invasive fungal infection Pulmonary infection	Brain abscess Cellulitis Sepsis
Blood and lymphatic system disorders					Febrile neutropenia
Metabolism and nutrition disorders	Hypoalbuminaemia Electrolyte disturbance Hyperglycaemia	Hypoalbuminaemia Electrolyte disturbance Hyperglycaemia			Anorexia
Psychiatric disorders					Agitation Confusional state

					Hallucination
Nervous system disorders			Peripheral neuropathy Headache Seizure	Headache Nervous system disorders NEC	Cerebral haemorrhage Encephalo-pathy
Cardiac disorders			Arrhythmia Pericardial effusion		Atrial fibrillation
Vascular disorders			Hyper-tension Hypotension	Hyper-tension	
Respiratory thoracic and mediastinal disorders			Pleural effusion Pulmonary haemorrhage Pulmonary edema	Pulmonary haemorrhage	Respiratory failure
Gastro-intestinal disorders	Nausea Vomiting Diarrhoea Stomatitis Abdominal pain	Nausea Vomiting Diarrhoea Stomatitis			Gastro-intestinal haemorrhage
Hepato-biliary disorders	Veno occlusive liver disease	Veno occlusive liver disease		Veno occlusive liver disease	Jaundice Liver disorders
Skin and subcutaneous tissue disorders			Rash Hand and foot syndrome	Rash	
Renal and urinary disorders	Haemorrhagic cystitis**	Haemorrhagic cystitis**	Renal disorder	Renal disorder	Oliguria
General disorders and administration site conditions	Mucositis	Mucositis	Fever		Asthenia Oedema Pain
Investigations	Transaminases increased Bilirubine increased Alkaline phosphatases increased Creatinine elevated	Transaminases increased Bilirubine increased Alkaline phosphatases increased	Left Ventricular Ejection Fraction decreased	Creatinine elevated	Blood lactate dehydrogenase increased Blood uric acid increased Blood urea increased GGT increased Weight increased

* reported in post marketing experience

** include haemorrhagic cystitis induced by viral infection

Safety in special populations

Intrinsic Factors

Impact of age

Overall, the estimated total number of patients aged ≥ 55 years in the pivotal and supportive studies was > 500 patients. This number was underestimated as the precise number of patients aged ≥ 55 was not individually reported in the 48 publications. IV FB regimens enabled to treat without dose adaptation also elderly patients who would not have been treated previously.

Extrinsic Factors

No relevant safety data were described with extrinsic factors such as use of tobacco, alcohol and food habits.

Post marketing experience

Safety data presented in this section included all post-marketing cases in the adult population reported with the use of Busilvex or Busulfex in combination with Flu until 30 June 2011.

All case reports were medically confirmed or not and arose from all database in patients excluding literature case reports and cases which were not workable (e.g. cases with unknown dates of treatments).

One hundred and eight (108) post-marketing cases were received by the MAH with the use of Busilvex or Busulfex in combination with Flu until 30 June 2011.

Among these 108 cases, 29 were considered as serious and unexpected, 69 as serious and expected, 3 were assessed as non-serious and unexpected and 7 were non-serious and expected. Fifty-six (56) cases with a fatal event were reported, among which 52 were considered as serious and 4 non serious.

The following table provides all serious and non-serious cases (including death cases) reported with the use of Busilvex or Busulfex in combination with Flu in the adult population, by System Organ Class of the main event, seriousness and expectedness.

A few cases included more than one event, which were not necessarily within the same SOC or referred to a different expected/unexpected status. In addition for fatal cases, the event leading to death was not necessarily related to the main event and can have been considered as not related to Busilvex or Busulfex.

Table 28: Summary of all post-marketing cases associated to Busilvex or Busulfex in combination with Flu in the adult population

System Organ Class of the main event	Serious			Non Serious			Total
	Expected	Unexpected	Death	Expected	Unexpected	Death	
Blood and lymphatic system disorders	4	2	2	-	1	1	7
Cardiac disorders	1	1	1	-	-	-	2
Gastrointestinal disorders	1	2	2	-	-	-	3
General disorders and administration site conditions	-	-	-	1	-	-	1
Hepatobiliary disorders	2	2	2	2	-	-	6
Immune system disorders	12	4	7	1	-	-	17
Infections and infestations	30	10	24	3	-	2	43
Investigations	-	-	-	-	-	-	
Neoplasms benign, malignant and unspecified	7	2	4	-	2	1	11
Nervous system disorders	5	1	1	-	-	-	6
Psychiatric disorders	-	1	-	-	-	-	1
Renal and urinary disorders	1	3	2	-	-	-	4
Respiratory, thoracic and mediastinal disorders	5	-	5	-	-	-	5
Vascular disorders	1	1	2	-	-	-	2
Total	**69**	**29**	**52**	**7**	**3**	**4**	**108**

Serious adverse drug reactions (SADRs) occurred most frequently in the following MedDRA System Organ Classes (SOCs):

Infections and infestations

Forty (40) serious case reports were reported in this SOC, of which 10 were considered as unexpected (including 8 cases with a fatal outcome) while 30 were expected (including 16 cases with a fatal outcome) for Busilvex/Busulfex.

Immune system disorders

Sixteen (16) serious case reports referred to this SOC, of which 4 were considered as unexpected (including 2 cases with a fatal outcome) while 12 were expected (including 5 cases with a fatal outcome) for Busilvex/Busulfex.

All cases referred to patients who developed graft versus host disease or bone marrow transplant rejection. GVHD occurs when immunocompetent T-cells and Natural Killer cells in the donor graft recognize host antigens as foreign targets and mediate reaction. This reaction was considered as indirectly related to the conditioning regimens as it was a consequence of the transplant process itself and these cases were assessed by the MAH pharmacovigilance department as not related to the Bu-based conditioning regimens. However, these events were considered by the MAH's partner as suspect for causal relationship with Bu.

Neoplasms benign, malignant and unspecified

Nine (9) serious cases, referring to 2 unexpected cases (both with a fatal outcome) and 7 expected cases (including 2 cases with a fatal outcome) for Busilvex/Busulfex, involved this SOC.

The majority of cases reported in this SOC concerned patients who experienced relapse or recurrence of initial disease, which was considered as not related to the conditioning regimens. However, these cases were reported by the MAH's partner as main events and were associated to secondary events that were related to Busulfex.

Blood and lymphatic system disorders

Six (6) serious case reports involved this SOC, of which 2 were unexpected (both with a fatal outcome) and 4 were expected for Busilvex/Busulfex. They referred to patients who experienced febrile neutropenia, neutropenia, thrombotic microangiopathy (TMA) and aplasia pure red cell.

Neutropenia, thrombocytopenia, anaemia and pancytopenia are known adverse reactions of Bu as myelo-suppression and immuno-suppression are the desired therapeutic effects of the conditioning regimens.

With regards to thrombotic microangiopathy (TMA), a cumulative review of TMAs in patients undergoing haematopoietic progenitor cell transplantation (HPCT) with Bu-containing conditioning regimens was performed by MAH pharmacovigilance department in June 2008 and did not allow attributing these reactions specifically to Bu. These ADRs will continue to be closely monitored by the MAH parmacovigilance department.

Nervous system disorders

Six (6) serious case reports involved this SOC; 1 case was unexpected and 5 cases were expected (including 1 case with a fatal outcome) for Busilvex/Busulfex. These cases concerned patients who developed convulsion, encephalopathy, altered state of consciousness and ataxia.

Respiratory, thoracic and mediastinal disorders

Five (5) serious case reports referred to this SOC, all of them were expected for Busilvex/Busulfex. They concerned patients who developed interstitial lung disease (3 cases) and idiopathic pneumonia syndrome (2 cases). All these cases had a fatal outcome. Case reports referring to this SOC did not provide any new information on the pulmonary safety profile of IV Bu.

Hepatobiliary disorders

Four (4) serious cases were reported in this SOC, of which 2 were unexpected cases (1 case with fatal outcome) and 2 were expected cases (1 case with fatal outcome) for Busilvex/Busulfex.

They referred to patients who experienced veno-occlusive liver disease, hepatic function abnormal and liver disorder.

Hepatic toxicities are common problems in HPCT and are coming from chemotherapy toxicity (previous treatment administered prior HPCT), immunologic damage, infectious agents, sepsis, and liver toxicity induced by concomitant medications. Hepatic toxicity, particularly hepatic veno-occlusive disease (VOD), is recognized as a potential complication of high-dose conditioning therapy post-transplant and belongs to the major manifestations of Bu-regimens related toxicity. Furthermore, multiple treatment and patient related risk factors are identified as predisposing for the development of VOD post- HPCT, like allogenic recipients, second HPCT, prior irradiation therapy, increased liver enzymes before transplantation, concomitant treatment with methotrexate, previous chemotherapies or severity of underlying disease itself and it has to be pointed out that patients generally meet one or several of these risk factors.

2.5.2. Discussion on clinical safety

The high variability of cytogenetics, remission status, type of disease, disease characteristics, patients' characteristics (age, gender, etc) together with the fact that results were not homogeneously reported throughout the publications (the presentation of results was adapted according to the main objective of the publications), make the comparison among publications difficult. This explains the heterogeneity of results and the difficulty to draw firm conclusions based on these literature data. Nevertheless, the analysis of the clinical safety profile showed overall a very low incidence of organ toxicity/morbidity in the modified myeloablative or in the reduced intensity FB regimens. There was a low rate of mortality due to organ toxicity in the total population studied. Safety profiles for Bu in combination with Flu and Bu in combination with Cy or melphalan could be considered similar.

Although the heterogeneity of results concerning transplant-related mortality (TRM/NRM) also precludes firm conclusions, it appears that the most frequent causes of reported TRM/NRMs were infection/sepsis, GVHD, pulmonary disorders and organ failure.

The additional phase III randomised, prospective trial recently issued [Lee JCO 2013] (see also clinical efficacy) is considered of value in relation to the MAC regimen. In this study, the incidence rates of severe infection and gastrointestinal adverse events in the BuFlu arm were significantly lower than in the BuCy2 arm, but these differences were not associated with lower NRM (Non-relapsed mortality). No significant differences in other toxicities (including Hepato-toxicities) in the BuCy2 and FB were found.

Overall, the safety profile for once daily and 4 time daily schedules were similar. In the Ryu study there were no significant differences between treatment groups in regard to frequencies of severe toxicity, except nausea/vomiting and diarrhoea.

Taking into account that myelosuppression and immuno-suppression are known effects of conditioning regimens, the development of infections is very common and considered expected. In MAC, the most frequent infectious adverse reactions were: bacterial infection [range: 44.7% - 62.2%], CMV reactivation [range: 22.2% - 35.1%], fungal infection [range: 13.5% - 18.4%] and CMV infection (11.8%). In RIC, the most frequent infectious adverse reactions were Cytomegalovirus (CMV) reactivation [range: 30.7% - 80.0%], Epstein-Barr Virus (EBV) reactivation [range: 2.3% - 61%], bacterial infections [range: 32.0% - 38.9%] and viral infections [range: 1.3% - 17.2%].

Some studies suggested that the conditioning regimens containing fludarabine were associated with higher incidence of opportunistic infections such as CMV and EBV after transplantation because of the immunosuppressive effect of fludarabine [Lee JH, KJH 2010 see section 5.3], [Bainton RD, 2002], [Lazzarino M, 1999].

Late haemorrhagic cystitis occurring 2 weeks post-transplant are likely related to viral infection / reactivation. Haemorrhagic cystitis including haemorrhagic cystitis induced by viral infection were reported in MAC regimen with a range between 1.5% and 18.9% and in RIC regimen with a range between 16% and 18.1%.

No unexpected gastrointestinal adverse events were detected and the frequencies were in line with the frequencies reported in current SmPC. The highest frequency of nausea and vomiting was 59.1% and the highest frequency of stomatitis was 11%.

Frequency of veno occlusive liver disease is reported as common in relation to the currently approved indication. However, with the proposed combination, this adverse event was reported at a very common frequency. The highest incidence of 22.2% VOD was reported in a phase I dose-escalated study (dose escalation based on increased BU AUC result) and was linked mainly to patients targeted

with busulfan plasma exposure above the acceptable therapeutic limit (i.e. AUC > 6000 µmol/L .min). In the RIC regimen, VOD was reported with a range between 3.9% and 15.4%.

The treatment-related mortality/non-relapse mortality (TRM/NRM) reported until day+100 post-transplant has also been examined through a review of published data from clinical trials. It was considered as deaths that could be attributable to secondary side effects after HPCT and not related to the relapse/progression of the underlying haematological malignancies. The most frequent causes of reported TRM/NRMs were infection/sepsis, GVHD, pulmonary disorders and organ failure.

Based on the above, the SmPC was updated to reflect in section 4.8 of the SmPC as new adverse events or adverse events with a higher frequency comparing with previous information.

Considering the new dosing schedule, the fact that Bu metabolism is mainly hepatic and that safety results from several studies reported Veno occlusive liver disease (VOD) as very common and common for FB in MAC regimen and BuCy2m respectively, it was discussed whether current warning and precautions in patients with severe hepatic impairment should be strengthened. The applicant argued that a correlation between a higher peak concentration following once-daily dose and increased hepatic toxicity seems to be excluded. However, toxicity in patients with severe hepatic impairment cannot be completed excluded since a slightly higher frequency of VOD has been detected on several submitted publication following once-daily dose. Nevertheless, the risk of VOD or hepatotoxicity is considered multifactorial and the replacement of a known hepatotoxicity agent (Cy) by a drug (Flu) with a lower risk could contribute to not increase hugely the risk of VOD. Thus, the current warning in SmPC which recommends that liver enzymes and bilirubin should be monitored regularly 28 days following transplant for early detection of potential hepatoxicity, remains adequate.

With regard to specific recommendations in other special population such as patients with renal impairment as well as in obese patients, it is expected that Busilvex behaves the same way whatever the schedule of administration and the type of combination. This is supported by the fact that FB regimens, either administered once-daily or using other schedules yield similar efficacy outcomes as compared to BuCy2 regimen given on a standard schedule. In addition, FB once daily regimen did not alter the safety profile and did not contribute to additional or unexpected toxicity especially in a frail and more aged population which is unfit to receive a full myeloablative BuCy2 regimen. In summary, the reviewed data on the FB preparative regimens indicate that dosing recommendations and/or precautions currently established in the SmPC should be maintained. No dose adjustment is needed in elderly patients (see also clinical Pharmacokinetics).

In relation to post-marketing data, the safety analysis of all events reported with the use of Busilvex/Busulfex in combination with Flu was consistent with the safety analysis based on the main event of each case and was also consistent with the SmPC.

For all Bu-containing conditioning regimens, the MAH should continue to monitor very closely all ADRs, especially the following adverse reactions: thrombotic microangiopathy, veno-occlusive disease, cerebral venous sinus thrombosis, interstitial nephritis, interstitial lung diseases, Stevens-Johnson syndrome and Fanconi syndrome.

2.5.3. Conclusions on clinical safety

Although the heterogeneity and the wide range of data presented in the submitted publications hamper the evaluation of the safety of the new proposed combination, the analysis showed overall a very low incidence of organ toxicity/morbidity in the modified myeloablative or in the reduced intensity FB regimens.

There was one published phase III, randomised and prospective study that compared the combinations in the target population within the MAC setting, and showed a similar trend of safety profile of the two combinations (FB vs BuCy2) with some differences noted in severe infection or gastrointestinal events.

Regarding the safety profile in the RIC regimen, no worrisome safety issues have been identified from the published literature compared to other alternatives commonly used in this setting. Fludarabine, considering its efficacy and specially its safety/tolerability profile, can be considered as a reasonable alternative to cyclophosphamide.

Overall and up to date, it appears that the combination of fludarabine-busulfan (FB) is associated with a better safety profile than showed for BuCy, based on the publications included in this variation.

2.5.4. PSUR cycle

The PSUR cycle remains unchanged.

The marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list)) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

2.6. Update of the Product information

As a consequence of this new indication, sections 4.1, 4.2, 4.5, 4.8, 5.1, 5.2 and 6.6 of the SmPC have been updated. The Package Leaflet has been updated accordingly.

Changes were also made to the PI to bring it in line with the current Agency/QRD template which were reviewed and accepted by the CHMP.

3. Benefit-Risk Balance

Benefits

Beneficial effects

The aim of the conditioning regimen is the long-term disease control. Traditionally, BU/Cy has been one of the most used conditioning treatments prior to HCT. The MAH is seeking the extension of the indication of Busilvex to another combination also widely used in clinical practice which is the FB combination. In support of this application, the MAH has presented an extensive literature analysis.

Fludarabine, considering its efficacy and specially its safety/tolerability profile, might be considered as a reasonable alternative to cyclophosphamide. Up to date, and based on the publications provided, it appears that the combination of fludarabine-busulfan (FB) is associated with a low incidence of non-relapse mortality. In addition, FB could induce fewer regimen-related toxicities and lower non-relapse mortality than BuCy and importantly, FB seems to achieve similar or even higher disease-free survival rates than BuCy.

MAC regimen

Documentation on the efficacy of Busilvex in combination with fludarabine (FB) in the MAC setting prior to allogeneic HPCT derives from the literature review of 28 published studies. Complete donor chimerism at day +30 or day +100 in myeloablative setting >90% was observed in the majority of publications when these outcomes were reported. Disease controlled was achieved, depending on the

type of disease, staging and previous response, the OS, DFS and EFS ranged from 60% to 83% for MAC regimen.

RIC regimen

Documentation on the efficacy of Busilvex in combination with fludarabine (FB) prior to allogeneic HPCT derives from the literature review of 7 published studies involving 731 patients with myeloid and lymphoid malignancies reporting the use of intravenous busulfan infused once daily instead of four doses per day. Patients received a conditioning regimen based on the administration of fludarabine immediately followed by single daily dose of 3.2 mg/kg busulfan over 2 or 3 consecutive days. Total dose of busulfan per patient was between 6.4 mg/kg and 9.6 mg/kg.

The FB combination allowed sufficient myeloablation modulated by the intensity of conditioning regimen through the variation of number of days of busulfan infusion. Fast and complete engraftment rates in 80-100% of patients were reported in the majority of studies. A majority of publications reported a complete donor chimerism at day+30 for 90-100% of patients. The long-term outcomes confirmed that the efficacy was maintained without unexpected effects.

Data from a recently completed prospective multicentre phase 2 study including 80 patients, aged 18 to 65 years old, diagnosed with different hematologic malignancies who underwent allo-HCT with an FB (3 days of Busilvex) reduced intensity conditioning regimen became available. In this study, all, but one, patients engrafted, at a median of 15 (range, 10-23) days after allo-HCT. The cumulative incidence of neutrophil recovery at day 28 was 98.8% (95%CI, 85.7-99.9%). Platelet engraftment occurred at a median of 9 (range, 1-16) days after allo-HCT.

The 2-year OS rate was 61.9% (95%CI, 51.1-72.7%)]. At 2 years, the cumulative incidence of NRM was 11.3% (95%CI, 5.5-19.3%), and that of relapse or progression from allo-HCT was 43.8% (95CI, 31.1-55.7%). The Kaplan-Meier estimate of DFS at 2 years was 49.9% (95%CI, 32.6-72.7).

Uncertainty in the knowledge about the beneficial effects

MAC regimen

In support of the present application, Flu followed by Busilvex as conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT) in adult patients administered once daily, the company has submitted a large number of uncontrolled, retrospective publications of studies or publications in which 'other' schedules were used. The heterogeneity of the data presented and the studied population together with the exploratory or descriptive character of most of the publications make the assessment of the true benefit of this combination challenging. One of the most important biases in any bibliographic submission was the selection bias, in which only those published studies with a favourable profile were included. Additionally, the methodology followed by all these studies was not easily evaluable, with high levels of uncertainty in several aspects of the development of the studies.

Nevertheless, focusing only on those studies with the highest level of quality, the combination of FB seemed to be at least as effective as the BuCy.

However, the only phase III randomised, controlled and prospective study which evaluated the efficacy and safety of the proposed combination in the MAC setting (Lee et al. JCO 2013), unexpectedly showed that BF regimen does not appear to be a suitable replacement for the BuCy2 regimen for myeloablative conditioning therapy for allogenic HCT, especially in the subset of young adult patients. Although the limitations of the study and the possibility of bias against the FB arm are acknowledged, the magnitude

of such interferences is unknown and the unsatisfactory results of the study cannot be disregarded. Further, the optimal sequence for this combination remains to be established.

RIC regimen

The evidence provided came from non-randomised and non-controlled studies, of which the vast majority of the studies were retrospective and/or uncontrolled. Nevertheless, the submitted data and clinical practice guidelines (EBMT Handbook) is considered sufficient to support the indication applied for.

Risks

Unfavourable effects

The only published phase III, randomised and prospective study that compared the combinations FB versus BuCy2 in the MAC regimen, showed a similar trend of safety profile of the two combinations with some differences noted in severe infections or gastrointestinal events.

The safety profile of Busilvex combined with fludarabine (FB) has been examined through a review of adverse events reported in published data from clinical trials in RIC regimen. In these studies, a total of 1574 patients received FB as a reduced intensity conditioning (RIC) regimen prior to haematopoietic progenitor cell transplantation. The most frequent infectious adverse reactions were Cytomegalovirus (CMV) reactivation [range: 30.7% - 80.0%], Epstein-Barr Virus (EBV) reactivation [range: 2.3% - 61%], bacterial infections [range: 32.0% - 38.9%] and viral infections [range: 1.3% - 17.2%].

The highest frequency of nausea and vomiting was 59.1% and the highest frequency of stomatitis was 11%.

It has been suggested that conditioning regimens containing fludarabine were associated with higher incidence of opportunistic infections after transplantation because of the immunosuppressive effect of fludarabine. Late haemorrhagic cystitis occurring 2 weeks post-transplant are likely related to viral infection / reactivation. Haemorrhagic cystitis including haemorrhagic cystitis induced by viral infection was reported in a range between 16% and 18.1%.

VOD was reported with a range between 3.9% and 15.4%.

Hypoalbuminemia, Electrolyte disturbance, Hyperglycaemia, Diarrhoea, mucositis, increases in transaminases, bilirubine and alkaline phosphatases, headache, nervous system disorders, hypertension, and pulmonary haemorrhage, rash, renal disorders, have been also associated frequently to the combination of FB.

Uncertainty in the knowledge about the unfavourable effects

Uncertainty in the knowledge about the unfavourable effect of the FB combination comes from the nature of the studies, particularly from the heterogeneity and the wide range of data presented in the submitted publications. Due to the fact that the vast majority of the studies were retrospective and/or uncontrolled, it is difficult to identify and reflect the actual ADRs into the SmPC. This fact along with the patients comorbidities, pose uncertainties regarding some adverse events. However, the SmPC of Busilvex includes a review of the adverse reactions as identified in the studies conducted with the FB combination in the RIC regimen.

Benefit-risk balance

Importance of favourable and unfavourable effects

The efficacy showed in the published studies submitted in support of the current application is further substantiated by years of experience in the clinical practice. The extensive use of FB in the clinical practice reflects the aim of looking for less toxic but still efficacious therapeutic alternatives to the available treatment options.

MAC regimen

The combination of fludarabine-busulfan (FB) could be associated with low incidence of non-relapse mortality, fewer regimen-related toxicities and lower non-relapse mortality than BuCy. FB seems to achieve similar or even higher disease-free survival rates than BuCy. However, the recent publication of the RCT conducted by Lee at al raises some important uncertainties on the true benefit of this treatment combination as well as the optimal treatment sequence.

The safety profile, although in line with the known safety profile of the traditional dosing showed some differences which do not raise major concerns.

RIC regimen

All the available evidence in the RIC scenario points out in the same direction. The combination of FB seems to be efficacious in patients diagnosed with different hematologic malignancies who underwent allo-HCT. This regimen can be considered relatively safe with low NRM in high risk patients. Despite the challenges when it comes to assessing the documentation submitted, there were no contradictory data as in the MAC setting.

Benefit-risk balance

The benefit-risk balance is considered positive for the combination Busilvex following Fludarabine (FB) as conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT) in adult patients who are candidates for a reduced-intensity conditioning (RIC) regimen.

Discussion on the benefit-risk balance

MAC scenario

The evidence provided initially was limited to non-randomised mostly retrospective comparative studies, which showed rather positive results in comparison to standard therapy in this clinical setting. However, the results of the recently published paper, Lee et al., (JCO 2013), the only phase III randomised controlled trial published in the intended broad target population, pointed out just in the opposite direction raising doubts on the true benefit of this regimen, and the optimal treatment sequence, as compared to the available therapeutic alternatives. The MAH had not included this study in the submission, which is considered an important drawback. The study has some limitations, which might have impacted on the study results. The magnitude of such interferences is unknown but uncertainties remain and cannot be overcome by the results of another randomised controlled study presented during the procedure by the Cy (Liu et al) given the different posology and the differences in the studied population, limited to AML in the latest study.

Therefore, given the uncertainties raised by the Lee et al study, the efficacy of FuBu in the myeloablative conditioning setting remains to be established. Thus, a positive benefit risk balance cannot be concluded based on the evidence available at the present time. Furthermore, clinical practice guidelines do not include at present this treatment combination for the MAC setting.

RIC scenario

While in the MAC setting there are important uncertainties regarding the actual benefit of the combination of FB over the alternatives, for the RIC setting the situation is different. First, FluBu is currently recommended in the EBMT Handbook. In addition, there is a controlled study (though retrospective) and there are supportive data from various controlled/uncontrolled studies (including a very recent one yet to be published) that, contrary to the MAC setting, all point towards the fact that FluBu can be used effectively and safely in the RIC setting. Nowadays, randomised controlled trials to confirm these results are not feasible in this clinical setting. Furthermore, reduced intensity conditioning is restricted to patients not candidate to standard myeloablative conditioning regimens due to the toxicity associated to these regimens. Overall, the use of FluBu in the RIC setting is supported.

4. Recommendations

Final Outcome

Based on the review of the submitted data, the CHMP considers the following variation acceptable and therefore recommends, by consensus, the variation to the terms of the Marketing Authorisation, concerning the following changes:

Variation requested		**Type**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	II

Extension of indication for Busilvex following fludarabine (FB) as conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT) in adult patients who are candidates for a reduced-intensity conditioning (RIC) regimen. Consequently, sections 4.1, 4.2, 4.5, 4.8, 5.1, 5.2 and 6.6 of the SmPC and the package leaflet are updated. The MAH also took the opportunity to update the product information in line with QRD template version 9.0.

The variation proposed amendments to the SmPC, Labelling and Package Leaflet.

This CHMP recommendation is subject to the following conditions:

Conditions and requirements of the marketing authorisation

- Periodic Safety Update Reports

The marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list)) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk management plan (RMP)**

Not applicable.

5. EPAR changes

The EPAR will be updated following Commission Decision for this <group of> variation<s>. In particular the EPAR module 8 "*steps after the authorisation*" will be updated as follows:

Scope

Extension of indication for Busilvex following fludarabine (FB) as conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT) in adult patients who are candidates for a reduced-intensity conditioning (RIC) regimen. Consequently, sections 4.1, 4.2, 4.5, 4.8, 5.1, 5.2 and 6.6 of the SmPC and the package leaflet are updated. The MAH also took the opportunity to update the product information in line with QRD template version 9.0.

Summary

See EPAR.

Appendix

REVISED CHMP ASSESSMENT REPORT ON THE NOVELTY OF THE INDICATION/SIGNIFICANT CLINICAL BENEFIT IN COMPARISON WITH EXISTING THERAPIES

Introduction

On 5 July 2013, Pierre Fabre Médicament filed an application for the medicinal product Busilvex using the centralised procedure for the following indications:

- 'Fludarabine followed by Busilvex (FB) is indicated as conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option.'

On 29 December 2001, orphan designation (EU/3/00/011) was granted by the European Commission to Pierre Fabre Médicament, France, for busulfan (intravenous use) for the conditioning treatment prior to haematopoietic-progenitor-cell transplantation:

"Busilvex followed by cyclophosphamide (BuCy2) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option.

Busilvex followed by cyclophosphamide (BuCy4) or melphalan (BuMel) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation in paediatric patients."

This product is no longer an orphan medicine: Busilvex was withdrawn from the Community register of orphan medicinal products in July 2013 at the end of the 10-year period of market exclusivity.

Currently authorised orphan medicinal products in conditioning treatment prior to haematopoietic-progenitor-cell transplantation

1. Tepadina (thiotepa)

Potential conflict with authorised orphan-designated medicinal products protected by market exclusivity in the EU

In accordance with the Commission Communication on orphan Medicinal Products (2003/C 178/02, 29 July 2003) it is the responsibility of the CHMP to give an opinion on the similarity of products of centralised orphan indications.

This Assessment Report will focus on the potential similarity regarding Busilvex (busulfan) and Tepadina (thiotepa) for the conditioning treatment prior to haematopoietic-progenitor-cell transplantation, taking into account Commission Regulation (EC) No 847/2000 and the Commission Guideline on aspects of the application of Article 8(1) and (3) of Regulation (EC) No 141/2000: *Assessing similarity of medicinal products versus authorised orphan medicinal products benefiting from market exclusivity and applying derogations from that market exclusivity* (C(2008) 4077 final dated 19 September 2008).

II Similarity assessment

The claim of non-similarity is based on the comparison of the molecular structural features and mechanism of action, as defined in the Article 3 of Regulatory (EC) No 847/2000.

Article 3 of the Regulation (EC) No 847/2000 provides the following definition: “similar active substance means an identical active substance or an active substance

- with the same principal molecular structural features (but not necessarily all of the same molecular structural features)
- and which acts via the same mechanism of action.

The assessment of similarity therefore takes into consideration principal molecular features, mechanism of action and therapeutic indication.

II.1 Therapeutic Indication

Applicant´s position

BUSILVEX, proposed therapeutic indication
“Busilvex followed by cyclophosphamide (BuCy2) or fludarabine followed by Busilvex (FB) is indicated as conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option.
Busilvex followed by cyclophosphamide (BuCy4) or melphalan (BuMel) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation in paediatric patients.”

Of note, modifications due to the new proposed indication are underlined.

TEPADINA, therapeutic indication:
“TEPADINA is indicated, in combination with other chemotherapy medicinal products:
1) with or without total body irradiation (TBI), as conditioning treatment prior to allogeneic or autologous haematopoietic progenitor cell transplantation (HPCT) in haematological diseases in adult and paediatric patients;
2) when high dose chemotherapy with HPCT support is appropriate for the treatment of solid tumours in adult and paediatric patients.”

Conclusion
The proposed therapeutic indication of Busilvex could be considered as similar to part of approved Tepadina indication: in combination, conditioning treatment prior to haematopoietic progenitor cell transplantation in adult and paediatric patients.

CHMP comment

The CHMP agrees with the applicant's conclusion. There could be similarity as regards the therapeutic indication between Busilvex and Tepadina. The absence of similarity cannot be ruled out in view of the overlap of indication between these two medicinal products.

II.2 Mechanism of action

Applicant´s position

BUSILVEX

Pharmacotherapeutic group: Alkyl sulfonates, ATC code: L01AB01.

The primary molecular action of busulfan is alkylation of intracellular nucleophiles. Both proteins and nucleic acids are affected. With regard to DNA, busulfan reacts with guanine residues to form a four carbon di-guanine DNA cross-linkage with release of methylsulfonate.

The DNA cross-linkage causes misreading of the DNA code and single-strand breakage. The degree of DNA cross-linkage has been shown to be proportional to the dose and cytotoxicity of the compound. Busulfan-induced crosslinkages of DNA to nuclear proteins may also occur and is considered a cytotoxic mechanism. Busulfan has also been reported to esterify phosphate groups of chromosomal DNA, accounting for the fragmentation of chromosomes seen in various cell types after treatment. Chromosomal damage further contributes to the overall cytotoxic effect.

TEPADINA

Pharmacotherapeutic group: Ethylene imines, ATC code: L01AC01.

Thiotepa is a cell cycle-phase independent, non-specific alkylating antineoplastic agent, related chemically and pharmacologically to the nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals that, similar to irradiation therapy, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position that severs the linkage between the purine base and the sugar and liberates alkylated guanines.

Thiotepa undergoes complex metabolism with TEPA as the major metabolite. Both thiotepa and TEPA are active structures. TEPA is assumed to interact differently with DNA, but is also thought to produce DNA lesions.

Conclusion

Busilvex and Tepadina are both alkylating antineoplastic agents, respectively alkyl sulfonate and ethylene imine, with different mechanisms of action.

CHMP comment

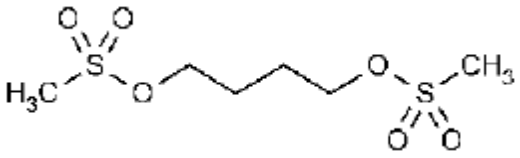
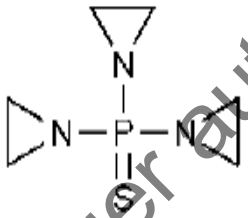
Even though both medicinal products act as alkylating agents, the different metabolic pathways of tepadina indicate different mechanism of action. However, this different pharmacodynamic effect is not shown and the same pharmacological action is expected.

In conclusion, in the absence of further data, it is not clear whether Busilvex is similar to Thiotepa or not.

II.3 Molecular Structure

Applicant´s position

In order to assess the possible structural similarity between Busilvex (busulfan) and the authorised orphan medicinal product Tepadina (thiotepa), the chemical structures, International Non-proprietary Names (INN), CAS, molecular formulae and molecular weight are detailed below:

Drug Product	Chemical Names	Chemical structure
BUSILVEX	**INN:** busulfan (Annex I: Who Chronicle, vol.13, No12, 1959) **Chemical name:** 1, 4-butanediol dimethanesulfonate **CAS:** 55-98-1 **Molecular formula:** $C_6H_{14}O_6S_2$ **Molecular weight**: 246.31 g/mol	
TEPADINA	**INN:** thiotepa (Annex II: Who Chronicle, vol.16, No3, 1962) **Chemical name:** 1,1',1''-phosphinothioylidynetris-Tris-(1-aziridinyl)phosphine sulphide **CAS:** 54-24-4 **Molecular formula:** $C_6H_{12}N_3PS$ **Molecular weight**: 189.22 g/mol	

CHMP comment

Based on the information provided, it can be concluded that busulfan and thiotepa are two different chemical entities. Busulfan is an alkyl sulfonate and thiotepa is an ethylene imine.

III Conclusion

Regarding the "therapeutic indication"**:**

There could be similarity between Busilvex and Tepadina. The absence of similarity cannot be ruled out.

Regarding the "mechanism of action"**:**

The mechanism of action seem to be partially similar, even though, it is not clear enough to what extend the differences between these two molecules and their metabolic routes can provide, in the end, a different mechanism of action.

Regarding the "molecular structure"**:**

Based on the information provided, it can be concluded that busulfan and thiotepa are two different chemical entities. Busulfan is an alkyl sulfonate and thiotepa is an ethylene imine.

Conclusion:

According to the guideline on assessing similarity of medicinal products (2008/C 242/08), "if significant differences exist within one or more of the criteria (principal molecular structure, mechanism of action and therapeutic indication as defined in the Article 3 of Regulation (EC) No 847/2000), then the product will be considered as not similar". Therefore:

Busilvex is considered not similar to Tepadina as they have different molecular structures.