13 December 2018
EMA/903773/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Trecondi

International non-proprietary name: treosulfan

Procedure No. EMEA/H/C/004751/0000

Note

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

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List of abbreviations

aGvHD	Acute graft versus host disease
ALL	Acute lymphoblastic leukaemia
alloHSCT	Allogeneic haematopoietic stem cell transplantation
ALT (GPT)	Alanine transaminase
AP	Alkaline phosphatase
AraC	Cytosine arabinoside (cytarabine)
AR	Adverse event related to the investigational product
AST (GOT)	Aspartate transaminase
ATG	Antithymocyte globulin
AUC	Area under the curve
$AUC_{0-\infty}$	Area under the concentration vs time curve from time point 0 up to infinity
autoHSCT	Autologous haematopoietic stem cell transplantation
BMF	Bone marrow failure
BSA	Body surface area
BU	Busulfan
BU/FLU/TT	Busulfan, fludarabine, thiotepa
cGy	Centi Gray
cGvHD	Chronic graft versus host disease
CI	Confidence interval
CIBMTR	Center for International Blood and Marrow Transplant Research
CIR	Cumulative incidence of relapse
CL	Clearance
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CMV	Cytomegalovirus
CR	Complete remission
CRFS	Chronic GvHD-free and relapse/progression-free survival
CsA	Cyclosporine A
CTCAE	Common terminology criteria for adverse events
CY	Cyclophosphamide
CYP	Cytochrome P450
DFS	Disease-free survival
DLI	Donor lymphocyte infusion
DNA	Desoxyribonucleic acid
EBMT	European Group for Blood and Marrow Transplantation
DSC	Differential Scanning Calorimetry
EBV	Epstein-Barr virus
EC	European Commission
ECG	Electrocardiogram
EFS	Event-free survival
ETO	Etoposide
FAS	Full-Analysis-Set
FB2	Reduced intensity conditioning regimen consisting of fludarabine plus dose-reduced busulfan
FLU	Fludarabine
FT_{10} , FT_{14}	fludarabine plus 3 × 10 g/m² or 3 × 14 g/m² treosulfan
FT_{14}/TT	fludarabine plus 3 × 14 g/m² treosulfan plus thiotepa
GCP	Good clinical practice
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte macrophage colony stimulating factor

GRFS	GvHD-free and relapse/progression-free survival
γGT	Gamma-glutamyltransferase
GvHD	Graft versus host disease
GvL	Graft versus leukaemia
GvT	Graft versus tumour
Gy	Gray
HCT-CI	Haematopoietic cell transplantation co-morbidity index
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
HR	Hazard ratio
HSCs	Haematopoietic stem cells
HSCT	Haematopoietic stem cell transplantation
HSOS	Hepatic sinusoidal obstruction syndrome
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IR	Infrared
ISCR	Individual spontaneous case report
JMML	Juvenile myelomonocytic leukaemia
LDPE	Low density polyethylene
LFS	Leukaemia-free survival
MAC	Myeloablative conditioning regimen
MDS	Myelodysplastic syndrome
MedDRA	Medical dictionary for regulatory activities
MM	Multiple myeloma
MPN	Myeloproliferative neoplasms
misMRD	Mismatched related donor
MPS	Myeloproliferative syndrome
MRD	Matched related donor
MS	Mass Spectrometry
MTD	Maximum tolerable dose
MTX	Methotrexate
MUD	Matched unrelated donor
NHL	Non-Hodgkin lymphoma
NMA	Non-myeloablative conditioning regimen
NMD	Non-malignant disorder
NMR	Nuclear Magnetic Resonance
OS	Overall survival
PBSC	Peripheral blood stem cells
PDE	Permitted Daily Exposure
PE	Polyethylene
Ph. Eur.	European Pharmacopoeia
PID	Primary immunodeficiency
PIP	Paediatric Investigation Plan
PK	Pharmacokinetic(s)
PopPK	Population pharmacokinetic model
PPS	Per-Protocol-Set
PVC	Polyvinyl chloride
RH	Relative Humidity
SAA	Severe aplastic anaemia
SCID	Severe combined immunodeficiency
SCT	Stem cell transplantation
SD	Standard deviation
SmPC	Summary of product characteristics

SOC	System organ class
SOS	Sinusoidal obstruction syndrome
$T_{1/2ß}$	Terminal half-life
TBI	Total body irradiation
TEAE	Treatment emergent adverse event
TRM	Transplant-related mortality
TREO	Treosulfan
TREO/FLU/TT	Treosulfan, fludarabine, thiotepa
TSE	Transmissible Spongiform Encephalopathy
TT	Thiotepa
UCB	Umbilical cord blood
V1	Central compartment
V2	Peripheral compartment
VOD	Veno-occlusive disease
WFI	Water for Injections
XRPD	X-Ray Powder Diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant medac Gesellschaft für klinische Spezialpräparate mbH submitted on 12 December 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Trecondi, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 23 February 2017.

Trecondi was designated as an orphan medicinal product EU/3/04/186 on 23 February 2004 in the following condition: Conditioning treatment prior to haematopoietic progenitor cell transplantation.

The applicant applied for the following indication: treosulfan is indicated as part of conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT) in patients with malignant and non-malignant diseases, in adults up to the age of 70 years and in paediatric patients older than one month.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Trecondi as an orphan medicinal product in the approved indication. This product was withdrawn from the Community Register of designated orphan medicinal products on 20 June 2019 by the European Commission. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/en/medicines/human/EPAR/trecondi.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0197/2017 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0197/2017 was not yet completed as some measures were deferred.

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 applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Applicant's request(s) for consideration

Additional Data exclusivity

The applicant requested consideration of one year data exclusivity in regards of its application for a new indication in accordance with Article 10(5) of Directive 2001/83/EC. The Applicant withdrew this request with the submission of the responses to the list of questions on 16 August 2018.

New active Substance status

The applicant indicated the active substance treosulfan contained in the above medicinal product to be considered as a known active substance.

Protocol assistance

The applicant received scientific advice/protocol assistance from the CHMP:

Scientific advice	**date**	**Area**
EMEA/H/SA/529/1/2004/PA/III	3 April 2005	quality, non-clinical, clinical development
EMEA/H/SA/529/1/2006/PA/II	28 June 2006	quality, non-clinical, clinical development
EMEA/H/SA/529/1/FU/2/2007/PA/II	22 March 2007	quality, non-clinical, clinical development
EMEA/H/SA/529/1/FU/3/2007/PA/II	24 January 2008	quality, non-clinical, clinical development
EMEA/H/SA/529/1/FU/4/2012/PA/II	17 January 2013	quality, non-clinical, clinical development

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Nithyanandan Nagercoil Co-Rapporteur: Bruno Sepodes

The application was received by the EMA on	12 December 2017
The procedure started on	1 February 2018
The Rapporteur's first Assessment Report was circulated to all CHMP members on	23 April 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	30 April 2018

The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	7 May 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	31 May 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	16 August 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	01 October 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	04 October 2018
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	18 October 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	13 November 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	28 November 2018
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Trecondi on	13 December 2018
The CHMP adopted a report on similarity of treosulfan with Tepadina on (Appendix 1)	13 December 2018

2. Scientific discussion

2.1. Problem statement

Haematopoietic stem cell transplant (HSCT) involves the intravenous infusion of autologous or allogeneic haematopoietic stem cells collected from bone marrow, peripheral blood, or umbilical cord blood to re-establish haematopoietic function in patients with damaged or defective bone marrow or immune system.

When the stem cells are collected from another person, either from relatives (identical twins, HLA-matched related, mismatched related) or unrelated donors (matched unrelated, umbilical cord blood) it is called allogeneic transplant. In 2012, more transplants were registered from unrelated donors than related.

Allogeneic HSCT has led to the cure of some forms of cancer (especially leukaemias), bone marrow failure, hereditary metabolic disorders, and severe congenital immunodeficiencies that would otherwise have been fatal.

2.1.1. Disease or condition

The proposed indication of treosulfan is as part of conditioning treatment prior to alloHSCT in adults with malignant and non-malignant diseases and in paediatric patients older than one month with malignant diseases.

2.1.2. Epidemiology

According to the latest European Society for Blood and Marrow Transplant (EBMT) report, 42 171 transplants were reported in 37 626 patients in Europe in the year 2015; of these, 17 302 HSCTs (41%) were allogeneic and 24 869 (59%) autologous. Compared with data from 2014, the total number of transplants increased by 3.3% (2.1% alloHSCT and 4.1% autoHSCT). In 2015, there were 4490 paediatric patients < 18 years of age receiving HSCT in Europe, 3338 received an allogeneic and 1152 an autologous HSCT.

The number of alloHSCTs continues to increase by 10-20% annually, and reductions in organ damage, infection, and severe acute graft versus host disease (aGvHD) seem to be contributing to improved outcomes. Total mortality among patients who underwent alloHSCT at the Fred Hutchinson Cancer Center in Seattle fell from 63% between 1993 and 1997 to 47% between 2003 and 2007. At the same time, non-relapse mortality fell from 41% to 26%. In a study by the Center for International Blood and Marrow Transplant Research (CIBMTR), the 10-year survival rate of 3788 patients who had survived at least two years after alloHSCT without relapse of their underlying disease was 85%.

Survival after transplantation is comparable among patients receiving donor stem cells from HLA-identical sibling and matched unrelated donors for several diseases.

2.1.3. Aetiology and pathogenesis

Indications for alloHSCT

Allogeneic HSCT is potentially curative for leukaemias, myelodysplastic syndromes (MDS), lymphomas and multiple myeloma (MM). It is also increasingly used in non-malignant diseases such as primary immunodeficiency, inborn errors of metabolism, haemoglobinopathies and bone marrow failure syndromes.

According to the latest EBMT report, the most common indication for an alloHSCT is acute leukaemia (55%), especially AML (39%), followed by MDS/MPS (12%). The use in CML has declined rapidly since the introduction of highly potent tyrosine kinase inhibitors.

Main indications in children are acute lymphoblastic leukaemia (ALL; 26%), primary immunodeficiency (PID; 16%), AML (14%), bone marrow failure (12%), thalassaemia (9%), and MDS/MPS (8%).

AlloHSCT is the treatment of choice in adult and paediatric patients with high-risk AML in their first complete remission (CR1). In patients with standard or good risk features, alloHSCT is reserved for their second complete remission (CR2). AlloHSCT is the only curative option for patients with primary refractory or relapsed AML.

2.1.4. Clinical presentation

Patients undergoing alloHSCT are prepared with chemotherapy alone or chemotherapy combined with radiotherapy, the so-called conditioning regimen, with three aims: to reduce the tumour burden when the disease is neoplastic, to eliminate the self-renewing capacity of the patient's own haematopoiesis, and to suppress the recipient's immune system to allow engraftment of stem cells. Exceptions are infants with severe combined immune deficiency (SCID) and patients with severe aplastic anaemia with an identical twin donor who may be grafted without conditioning.

Transplant-related mortality (TRM) after myeloablative regimens increases with increasing age, and to reduce toxicity non-myeloablative (NMA) conditioning regimens were developed.

Myeloablative conditioning does not allow autologous recovery and requires stem cell support whereas non-myeloablative regimens do not require stem cell support. Regimens that do not match these criteria have been classified as reduced-intensity conditioning (RIC), whereby the dose of total body irradiation (TBI) or the alkylating agent is usually reduced by at least 30% compared with an ablative regimen.

2.1.5. Management

Myeloablative radiation-containing conditioning regimens

High dose total body irradiation (TBI), usually 12-16 Gray (Gy) has been widely used as part of the conditioning with other chemotherapeutic agents, most commonly cyclophosphamide (CY).

Higher doses of TBI reduce the relapse risk but result in increased toxicity. Other agents such as cytarabine (AraC), etoposide (ETO), melphalan (MEL), and busulfan (BU), have been combined with TBI as conditioning regimens but there is currently no evidence suggesting that any of these combinations are superior to CY and high-dose TBI.

Myeloablative conditioning regimens without radiation

These regimens have primarily been developed for autologous transplantation, but they have also been used in the allogeneic setting. The primary advantage is reduced toxicity. Additionally, the regimen is easier to administer, and radiation can still be given to sites of prior disease following transplantation. Alkylating agents remain the mainstay of such regimens.

Commonly used regimens are based on orally or intravenously BU and other cytotoxic agents. A regimen consisting of high-dose BU (16 mg/kg total dose) and CY (200 mg/kg total dose) was developed and modified (total CY dose was decreased to 120 mg/kg) and has been widely used.

Nonmyeloablative and reduced intensity conditioning regimens

For patients with haematologic malignancies, an important contributing factor is a graft-versus-tumour (GvT) effect mediated by the allogeneic donor cells. This effect requires the permanent engraftment of donor-type immunocompetent cells, which does not necessarily require a myeloablative conditioning.

Due to its lowered toxicity, NMA transplants can be appropriate for patients older than 55 years, which is a common upper limit for standard myeloablative transplantation as well as patients with co-morbidities that would exclude them from undergoing myeloablative transplantation.

Fludarabine (FLU) has been widely used because it is highly immunosuppressive, has anti-tumour activity in haematologic malignancies and a low non-haematologic toxicity profile. Regimens that relied on FLU or lower doses of the conditioning agents are referred to as either NMA or RIC.

NMA regimens may result in minimal cytopenias that do not require stem cell support whereas RIC regimens do require stem cell support.

The optimum regimen remains to be defined and commonly used conditioning regimens are shown below.

Examples of RIC and NMA conditioning regimens

RIC regimens	**NMA regimens**
TBI ≤ 500 cGy as a single fraction or ≤ 800 cGy if fractionated	FLU + CY + antithymocyte globulin (ATG)
Total BU dose ≤ 9 mg/kg	FLU + AraC + idarubicin
Total melphalan (MEL) dose < 140 mg/m²	Cladribine + AraC
Thiotepa (TT) < 10 mg/kg	Total lymphoid irradiation + ATG
	TBI ≤ 2 Gy ± purine analogue

Complications of alloHSCT include:

- Prolonged and severe pancytopenia that requires the use of antibiotics and blood cell transfusions.
- Graft rejection where donor cells fail to regenerate within the recipient. Mechanisms include the failure of immunosuppressive agents to inactivate the host immune system, inadequate ratio of donor cells to facilitator cells infused, drug injury to marrow, or viral infections.
- Graft versus host disease (GvHD) when immunocompetent T and natural killer cells in the donor graft recognise host antigens as foreign targets and mediate a reaction. The disease may cause significant morbidity and mortality and has been divided into acute and chronic forms.

  To avoid acute GVHD, patients are given potent immunosuppressives immediately before and for many months after transplantation.

  Around 50%-70% of patients develop chronic GVHD within ten years of treatment. It may develop as a continuation of active acute GvHD, after successful clearing of acute GvHD, or may appear without antecedent acute GvHD. The target organs are more widespread than in acute GvHD. Chronic GVHD is associated with fewer relapses, indicative of a GvT effect.

- Pulmonary complications like interstitial pneumonitis, often fatal and caused by viral infection, or lung injury due to TBI or pulmonary toxins (carmustine).
- Hepatic sinusoidal obstruction syndrome, previously known as veno-occlusive disease manifests as jaundice, hepatomegaly, unexplained weight gain, or ascites. The condition usually develops by 30 days after HSCT, although it can occur later. Historically, its reported incidence ranges from approximately 5 to 60%. Risk factors for this complication include a history of previous hepatocellular disease, certain conditioning regimens, advanced age, the presence of GvHD, the type of GvHD prophylaxis, poor performance status at transplantation, and the use of matched unrelated or mismatched donor grafts.
- Late-onset problems include an increased risk of malignancy (often many years after transplant), infections (like reactivation of dormant herpes viruses), and gonadal dysfunction in up to 92% of men and 99% of women. The medications that transplant recipients need to take can impair liver function, and there is transfusion-associated haemosiderosis. Around 40% to 50% of patients suffer from lipid metabolic disturbances that increase the risk of myocardial infarction, peripheral arterial occlusive disease, and stroke. Their life expectancy is shorter than that of the overall population.

Myeloablative conditioning is associated with a high risk of mortality and morbidity and is usually contraindicated in patients with older age, with co-morbidities or patients with previous autologous SCT. Efforts have been made to reduce the toxicity of these regimens and non-myeloablative and RIC regimens expanded the patient population that could receive alloHSCT but at the expense of an increased relapse rate.

About the product

Treosulfan is a prodrug of a bifunctional alkylating agent with cytotoxic activity to haematopoietic precursor cells. The activity of treosulfan is due to the spontaneous conversion into a mono-epoxide intermediate and L-diepoxybutan. The epoxides formed alkylate nucleophilic centres of deoxyribonucleic acid (DNA) and are able to induce DNA cross-links which are considered responsible for the stem cell depleting and antineoplastic effects.

The immunosuppressive effects of treosulfan are attributed to its toxicity against primitive and committed progenitor cells, T and NK cells, reduction of cellularity of primary and secondary lymphatic organs and a preclusive effect on the ‘cytokine storm’ that precedes the development of Graft-versus-Host-Disease (GvHD) and is involved in the pathogenesis of veno-occlusive disease.

The Applicant applied for the indication:

Treosulfan in combination with fludarabine is indicated as part of conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT) in patients with malignant and non-malignant diseases, in adults up to the age of 70 years and in paediatric patients older than one month .

Following assessment the indication approved by the CHMP was:

Treosulfan in combination with fludarabine is indicated as part of conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT) in adult patients with malignant and non-malignant diseases, and in paediatric patients older than one month with malignant diseases.

Type of Application and aspects on development

Treosulfan has been granted national authorisations in several EU countries (DE, DK, IE, NL and UK) for the treatment of ovarian cancer since 1973.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as powder for solution for infusion containing 1 g or 5 g of treosulfan as active substance. The finished product contains no excipients.

The product is available in type III glass vial, with rubber stopper and aluminium cap completed with plastic bottle holder as described in section 6.5 of the SmPC.

2.2.2. Active Substance

General information

The chemical name of treosulfan is (2*S*,3*S*)-(-)-1,4-di(mesyloxy)-2,3-butanediol corresponding to the molecular formula $C_6H_{14}O_8S_2$. It has a relative molecular mass of 278.3 and the following structure:

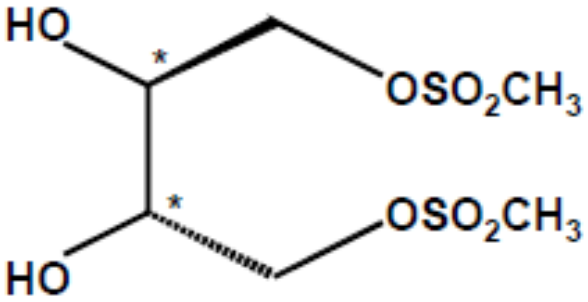


Figure 1: active substance structure

The chemical structure of treosulfan was elucidated by a combination of elemental analysis, ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS) and infrared (IR) spectroscopy.

The solid state properties of the active substance were determined by differential scanning calorimetry (DSC) and x-ray powder diffraction (XRPD).

The active substance is a non-hygroscopic white crystalline powder; it is freely soluble in acetone, soluble in water, sparingly soluble in ethanol and very slightly soluble in chloroform.

Treosulfan exhibits stereoisomerism due to the presence of two chiral centres. The chiral centres have the (*S*)-configuration and are introduced during the synthetic process with the starting material. Enantiomeric purity is controlled routinely by specific optical rotation.

Polymorphism screening was performed and two polymorphic forms were observed. The most stable polymorphic form of treosulfan is consistently produced utilizing the intended commercial manufacturing process and it was demonstrated that it does not change during storage.

Manufacture, characterisation and process controls

The active substance is obtained from a single manufacturer. It is synthesized in a convergent synthesis in 4 main steps followed by a crystallisation step from well-defined starting materials with acceptable specifications. Several synthesis intermediates are isolated.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented and are acceptable.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Treosulfan is an anti-cancer medicine indicated for the treatment of severe and life-threatening diseases. Treosulfan and its active metabolites that form *in vivo* (monoepoxide and diepoxide) are themselves genotoxic/cancerogenic substances. The mechanism of action is based on the conversion of treosulfan to the epoxides which alkylate DNA resulting in the induction of DNA cross-links (as described in section 5.1 of the SmPC). The known related impurities of treosulfan are not expected to have a higher genotoxic potential than the active substance itself.

Potential and actual impurities were well discussed with regards to their origin and characterised.

The active substance is packaged in antistatic LDPE bags which comply with the EC directive 2002/72/EC and EC 10/2011 as amended. The LDPE bags are placed into fibre drums.

Specification

The active substance specification includes tests for: description, melting point (Ph. Eur.), optical rotation (Ph. Eur.), identity (IR), assay (HPLC), clarity of the solution (Ph. Eur.), colour of the solution (Ph. Eur.), pH (Ph. Eur.), related substances (HPLC), heavy metals (Ph. Eur.), loss on drying (Ph. Eur.), sulphated ash (Ph. Eur.), residual solvents (GC), and microbiology (Ph. Eur.).

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data from 3 commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from 3 commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 60 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided.

The following parameters were tested*:* description, melting point, assay (HPLC), clarity of the solution, colour of the solution, pH, related substances, loss on drying and microbiology. The analytical methods used were the same as for release and were stability indicating.

All tested parameters were within the specifications.

Photostability testing following the ICH guideline Q1B was performed on one batch. The results of the study show that treosulfan is not photosensitive.

Results on stress conditions: high temperature study (solid state), hydrolysis study (in buffer solution used in the analytical method, in acidic conditions, in alkaline conditions) and an oxidation study (H_2O_2) were also provided on one batch. Treosulfan showed to be unstable under acidic conditions, with approximately a third of the sample being degraded into various products. Treosulfan is hydrolysed and degraded very quickly under alkaline conditions.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 60 months at 25 ºC / 60% RH in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished product is presented as white crystalline powder for solution for infusion containing 1 g or 5 g of treosulfan as active substance. The product is available in colourless type III glass vial, with rubber stopper and aluminium cap. The product is reconstituted in 0.45% sodium chloride solution. The reconstituted solution contains 50 mg treosulfan per 1 ml and appears as a clear colourless solution.

Treosulfan was developed more than two decades ago and its use is well-established (e.g. medicinal product authorised and marketed in the EU for the indication “Palliative treatment of epithelial ovarian cancer” since 1990s. The aim of pharmaceutical development was to develop a stable, sterile powder for solution for infusion formulation indicated as part of conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT) in adult patients with malignant and non-malignant diseases, and in paediatric patients older than one month with malignant diseases.

Due to its solubility in water treosulfan is suitable for intravenous infusion after reconstitution. The addition of a solubilizing agent is not necessary. The compound is intended to be marketed as a pure dried substance that should be dissolved in sterile reconstitution medium immediately before administration. Especially for use in the paediatric population, 0.45 % NaCl solution should be used instead of WFI as reconstitution medium to gain an acceptable osmolality of the resulting treosulfan solution. Stability over a period of 48 h at room temperature was confirmed by provided stability results.

The finished product contains no excipients; however a solvent and inert gas are used as excipients for production.

The same polymorphic form is consistently obtained by the commercial manufacturing process of the finished product by slow and controlled crystallisation. The polymorph is thermodynamically very stable. It was further demonstrated on three batches of the active substance and the finished product that the polymorphic form does not change during storage.

The compatibility of the active substance with excipients used for production is confirmed based on the finished product stability data. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. As the excipients are not present in the finished product on administration, they are not included in section 6.1 of the SmPC.

The sterility of the powder is achieved by sterile filtration of the dissolved active substance and aseptic processing of the subsequent precipitation/drying and filling of the sterile powder in vials which are closed with rubber stoppers and sealed with safety caps. The active substance melts at approximately 100 ºC and the re-crystallisation after cooling occurs in an uncontrolled manner. Final sterilisation could only be performed below the melting point (90-95 ºC), which is not appropriate to ensure the inactivation of some microorganism and spores. Additionally, the active substance degrades when exposed to weak X-ray radiation, therefore sterilisation via gamma irradiation was also not deemed a feasible option. The choice of sterile filtration and aseptic process is considered appropriately justified, in line with CPMP/QWP/054/98 Corr “Decision trees for selection of sterilisation methods (Annex to note for guidance on development pharmaceutics)”.

The formulation and the manufacturing process used during clinical studies is the same as that intended for marketing.

An extractables study was performed and the chosen rubber material used for stoppers meets the quality requirements with regard to leachable compounds and related safety concerns. In addition, the proposed specification for the rubber stopper complies with the requirements of the Ph. Eur. monograph and biocompatibility of the rubber formulation has been demonstrated.

The absence of leachable studies using reconstituted treosulfan with 0.45% NaCl solution or WFI in the glass vials, PVC bags and PE bags was adequately justified. Representative data on the material or polymer type (e. g. glass, PE) have been generated which have proven the (physico)-chemical compatibility and serve as recommendation and instruction for their use. As demonstrated in the in-use study the used solvents and the used packaging materials have no apparent impact on the quality of the reconstituted solution.

The primary packaging is type II glass vial, with rubber stopper and aluminium cap completed with plastic bottle holder. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of two main steps: in step 1 a sterile intermediate is produced and in step 2 the final sterile finished product is manufactured. The process is considered to be a non-standard manufacturing process.

Stability studies have been performed on the sterile intermediate finished product packed in the proposed primary container and were found satisfactory.

All manufacturing steps are well controlled by applied in-process controls which are adequate for this type of manufacturing process and pharmaceutical form.

Major steps of the manufacturing process have been validated by a number of studies on three commercial size batches of treosulfan product intermediate, which includes the preparation of the solution, sterile filtration, precipitation, drying and packing into polyethylene bags. Five different lots of the active substance were used. The sterile powder filling of treosulfan into injection vials was validated on three batches for each presentation (1000 mg and 5000 mg).

It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: description, uniformity of mass (Ph. Eur.), colour of solution (Ph. Eur.), clarity of solution (Ph. Eur.), particulate matter (visible) of solution (Ph. Eur.), particulate matter (sub-visible) of solution (Ph. Eur.), reconstitution time (in-house), pH (Ph. Eur.), identity (Ph. Eur.), related substances (HPLC), loss on drying (in-house), residual solvents (GC), sterility (Ph. Eur.), bacterial endotoxins (Ph. Eur.), assay (HPLC).

A risk assessment to evaluate the presence of elemental impurities in the finished drug product has been performed in compliance with ICH Q3D. Provided data has shown that the content of all elemental impurities remains well below the control threshold (defined as 30%) of the established permitted daily exposure (PDE).

Taking into account the provided summary of the risk assessment, additional controls for elemental impurities (i. e. in the finished product release specifications) was not required.

It was demonstrated that the wider acceptance criteria for the pH value applied to the specification for the reconstituted solution is acceptable from a safety and efficacy point of view.

A difference in the reconstitution time between the 1000 mg and 5000 mg strengths is expected and is considered acceptable. The variability of the reconstitution time is regarded as uncritical with regard to the clinical use of the product.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for 3 commercial scale batches of each strength, confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Stability data from 16 finished product batches (9 of 1 g strength and 7 of 5 g strength) of up to commercial scale stored for up to 72 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH), both in upright and inverted position, according to the ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested against tests and acceptance criteria as listed in the release specifications, with the exception of bacterial endotoxins since this parameter is not considered stability indicating. The analytical procedures used are stability indicating.

No significant changes have been observed and neither trends for increase nor for decrease of any parameter could be observed.

A finished product intermediate holding time of 12 months was supported by data.

A stability study after reconstitution of treosulfan to 50 mg/ml in 0.45 % NaCl solution (compared with reconstitution in WFI) was carried out using different containers. Over a period of 48 h storage in different containers at room temperature, for assay and impurities as well as appearance of treosulfan 50 mg/ml in 0.45 % NaCl solution no trends can be observed. The pH-value decreases; however all results comply with the specification criteria. After reconstitution of treosulfan to 50 mg/ml in WFI, analogue results can be obtained. In the context of the in-use stability studies, the assay of treosulfan did not change over the 48 hours tested and a slight increase of an unknown impurity was observed. This impurity was then identified, showing that no new toxic impurities are formed in the reconstituted solution.

The applicant has justified properly the absence of forced degradation testing and photostability study of the finished product, arguing that since the finished product is a recrystallized and sterile-filtered active substance without any additional pharmaceutical formulation, no matrix effects caused by excipients are expected. Additionally, the physical state of active substance and finished product is comparable (i. e. same polymorphism). Therefore, it is not expected that additional factors which could significantly influence

photostability exist for the finished product and consequently the data for the active substance can be extrapolated for the finished product.

Based on available stability data, the proposed shelf-life of 5 years with no special storage conditions as stated in the SmPC (section 6.3) are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on TSE safety.

2.2.6. Recommendations for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

The anti-tumour activity and safety pharmacology of treosulfan was tested against transplanted mouse and rat lymphomas/leukaemias, sarcomas and hepatomas, human tumour xenografts, human tumour biopsies, and cell lines originated from solid tumours.

The pharmacologically inactive prodrug treosulfan is transformed *in vitro* and *in vivo* to reactive intermediates with alkylating properties, in particular to a monoepoxide (EBDM) and a diepoxide (DEB) The kinetics, pH- and temperature-dependence of the transformation was studied in aqueous solutions. ADME (absorption, distribution, metabolism and excretion) properties of treosulfan and/or metabolites were determined *in vitro* and in representative animal species. The potential for pharmacokinetic drug-drug interactions was investigated *in vitro*.

Most of the toxicology information is derived from the published literature whilst other studies are presented as brief summaries of studies conducted in the 1970s and 1980s.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Mechanism of action (cytotoxicity)

Treosulfan is a water-soluble prodrug, which under physiological conditions is converted nonenzymatically into reactive intermediates. This conversion is strongly pH- and temperature dependent. The intermediates, in particular epoxides, are responsible for alkylation and crosslinking of DNA and proteins and for subsequent interference with various cellular processes including apoptosis and genotoxicity.

In treosulfan-treated cells of the human chronic myelogenous leukaemic cell line K562, DNA cross-links formed slowly, while incubation with preformed epoxides showed faster and more efficient cross-linking. Alkylation in plasmid DNA occurred at guanine bases with sequence selectivity similar to other alkylating agents such as the nitrogen mustards.

The relative potency of the three stereoisomers of the diepoxide in producing DNA interstrand cross-links was found to be: S,S-DEB > R,R-DEB > meso-DEB; whereby only S,S-DEB can be formed from S,S-treosulfan.

(2S,3S)-1,2:3,4-diepoxybutane (DEB) formed DNA-protein cross-links between cysteine thiols within proteins and the N-7 guanine positions within DNA.

DEB-mediated DNA-protein cross-linking was investigated in human fibrosarcoma (HT1080) cells. Over 150 proteins including histones, high mobility group proteins, transcription factors, splicing factors, and tubulins were found among those covalently cross-linked to chromosomal DNA in the presence of DEB. A large portion of the cross-linked proteins are known factors involved in DNA binding, transcriptional regulation, cell signaling, DNA repair, and DNA damage response.

While alkylating agents (melphalan, treosulfan) and doxorubicin demonstrated marked cytotoxicity, nucleotide analogs (gemcitabine, cytarabine) induced only limited apoptosis in human bone marrow stromal cells.

Myeloablative and immunosuppressive effects

Compared with other dimethanesulfonate compounds (related to busulfan), treosulfan exhibited relatively high *in vitro* activity, but relatively low activity *in vivo* in terms of their toxicity to different stem cell subsets.

Myeloablative and immunosuppressive properties of treosulfan were investigated in mice treated with treosulfan, cyclophosphamide, or busulfan at sublethal doses that maintained survival without bone marrow support. Treosulfan and busulfan induced a high and persisting degree of myeloablation, as compared with cyclophosphamide. Moreover, treosulfan was more effective in depletion of splenic B and T cells in comparison with busulfan and cyclophosphamide. Treatment with treosulfan induced only interleukin-2 production in spleen cells for a short time and had no significant effect on synthesis of tumor necrosis factor-alpha and/or interferon gamma as compared with that observed in splenic T cells isolated from mice treated with either busulfan or cyclophosphamide.

The immunosuppressive activity of treosulfan was investigated using human peripheral blood B and T lymphocytes and mice immunized with keyhole limpet hemocyanin. Low dose treosulfan i) induced suppression of the early immune response, probably including the proliferation/differentiation of cells repopulating lymphoid organs and ii) influenced the balance of regulatory T cell subpopulations.

Fractionated high-dose treosulfan or busulfan treatment was investigated in mice to prepare recipients for allogeneic bone marrow transplantation. In addition, treosulfan and busulfan treatment regimens including

concomitant treatment with anti-T cell antibodies and/or cyclophosphamide were assessed for induction of donor-type chimerism and tolerance to subsequent donor skin grafts after H-2 incompatible allogeneic HSCT. Concomitant treatment of the stem cell depleting agent (busulfan or treosulfan) with T cell depletion with anti-CD4 and anti-CD8 monoclonal antibodies appeared to be important for achieving immune tolerance and induction of high levels of donor-type chimerism. Cyclophosphamide was, however, effective in enhancing low levels of donor chimerism produced by treosulfan-based conditioning regimen. Permanent acceptance of donor-type skin grafts and rejection of “third party” skin grafts after low dose treosulfan-based conditioning and allogeneic HSCT was demonstrated in mice.

Anti-tumour effects (haematological malignancies)

Treosulfan was tested *in vitro* by differential staining cytotoxicity assays against 55 specimens from patients with a variety of tumour types, including acute lymphoblastic leukaemia (ALL), adult T-cell leukaemia/lymphoma (ATLL), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and non-Hodgkin’s lymphoma (NHL). Treosulfan induced dose-dependent cell death resulting in LC90 values of 2 to 512 µg/mL.

Treosulfan treatment of the myeloma cell lines NCI-H929 and U266 led to apoptosis in both cell lines in a dose- and time-dependent manner.

Chemosensitivity tests were performed in AML cell lines and primary cells from patients. All cell types displayed dose-dependent sensitivity to treosulfan.

In peripheral blood mononuclear cells (PBMCs) from patients with chronic lymphocytic leukemia (CLL) treosulfan, 4-hydroperoxy-cyclophosphamide, fludarabine or cytarabine, but not busulfan, were good inducers of apoptosis. Cell death was induced via caspase-activation. Cytotoxicity of treosulfan and busulfan was evaluated *in vitro* on two leukemia cell lines. Treosulfan was consistently more cytotoxic than busulfan.

All samples of paediatric origin were tested for *ex vivo* chemosensitivity to various drugs. Their combined drug resistance profile was analysed. Lymphoblasts at multiple relapse were comparably resistant to daunorubicin, doxorubicin, cyclophosphamide, ifosfamide, busulfan, treosulfan, fludarabine, clofarabine, and bortezomib.

The *in vivo* antileukaemic activity of treosulfan was compared with the activity of equitoxic doses of cyclophosphamide or busulfan using a human ALL xenograft mouse model. Treosulfan was more effective with regard to the numbers of complete regressions and the number of cured animals.

Secondary pharmacodynamic studies

Secondary pharmacology studies included effects of treosulfan on erythrocytes, on the migration of immunocompetent blood cells, and against solid tumour types.

Exposure of human erythrocytes to treosulfan significantly stimulated suicidal erythrocyte death or eryptosis at least in part by inducing oxidative stress and stimulating Ca2+ entry.

Treatment of PBMCs of healthy donors with treosulfan significantly inhibited the migration of immunocompetent mononuclear cells across a fibronectin layer. The effect was observed in T cells (CD4+ and CD8+ cells) as well as in CD14+ monocytes to a similar extent.

Apart from activity against haematological malignancies, treosulfan also exhibits a broad antitumour activity against numerous solid tumour types as demonstrated against human tumour xenografts in rats and mice, human tumour biopsies, and tumour cell lines.

Treosulfan was consistently more cytotoxic than busulfan when evaluated *in vitro* on four Ewing tumour, four neuroblastoma, and two osteosarcoma cell lines.

Safety pharmacology programme

Table 1: Safety Pharmacology

Organ Systems Evaluated	Species / Strain	Method of Admin.	Doses	Gender and number per Group	Noteworthy Findings	GLP Compli-ance
Cardiovascular						
Isolated intestine	Guinea pig	In vitro	0.01 mg/mL	not mentioned	No effect on the contractions elicited by acetylcholine and histamine.	no
Isolated tracheal muscle	Cat	In vitro	0.003 - 0.02 mg/mL	not mentioned	No effect on the trachea or on the dilation elicited by isoprenaline.	no
Nervous system	Mouse	p.o.	50 mg/kg	not mentioned	No analgesic effect in the "Writhing" test elicited by i.p. injection of 50 mg/kg acetic acid.	no
Central nervous system	Mouse / Ham/IRC	i.p.	150, 300 mg/kg	males	No abnormal behaviour.	no
Muscle system	Mouse	i.p.	100 mg/kg	not mentioned	Neither exertion of anticonvulsive effects against electronic stimulation, nor against convulsions elicited by the i.v. injection of pentetrazole.	no
Nervous system	Mouse	Injection (tail)	0.05 mL of a 2% solution	10 animals	No conduction anaesthesia produced on the ventrolateral tail nerves after injection on either side of the tail.	no

Cardiovascular	Rat	i.v.	Two single doses of 20 mg/kg followed by 40 mg/kg 30 min later	Females	Blood pressure recorded in the carotid artery remained unaffected.	no
Gastric secretion	Rat / SHAY	p.o.	50 mg/kg	18 animals	No significant effect on acid secretion prior administration of treosulfan 1 hour before ligation of the pylorus and 3 hours after dosing.	no
Cardiovascular	Dog / Mongrel	i.v.	Dog 1: 5, 10, 20 mg/kg Dog 2: 50, 100, 200 mg/kg	2 females	No effects on arterial or venous blood pressure or on the carotid occlusion reflex observed in anaesthetised animals. Pressure effects of norepinephrine and tyramine and the electrocardiogram were not altered.	no
Nervous system	Dog	p.o.	200 mg/kg	1 female	No signs of abnormal behaviour. Respiration,heart rate and pupil size remained unaffected.	no
Blood	Dog / Beagle	i.v. bolus for 10 to 14 days	56 to 445 mg/kg/day	1 or 2 animals	56 mg/kg and above: leukocytopenia; 111 mg/kg and above: reticulocytopenia, neutropenia; 445 mg/kg: haemoconcentration, thrombocytopenia	no
Blood	Dog / Beagle	p.o. for 5 to 19 days	56 to 1779 mg/kg/day	1 or 2 animals / group	56 mg/kg and above: leukocytopenia; reticulocytopenia, lymphopenia; 111 mg/kg and/or above: neutropenia, thrombocytopenia; 890 mg/kg: haemoconcentration	no
Blood	Monkey / Rhesus	i.v. bolus for 8 to 14 days	56 to 445 mg/kg/day	1 or 2 animals / group	56 mg/kg and above: leukocytopenia, reticulocytopenia; 111 mg/kg and/or above: neutropenia, lymphopenia; 222 mg/kg and above: haemoconcentration	no
Blood	Monkey / Rhesus	p.o. for 6 to 19 days	56 to 1779 mg/kg/day	1 or 2 animals / group	56 mg/kg and above: leukocytopenia, reticulocytopenia, lymphopenia; 222 mg/kg and above: haemoconcentration	no

Abbreviations: p.o. oral; i.p.: intraperitoneal; i.v.: intravenous; s.c.: subcutaneous

The effect of treosulfan on vital organ functions (cardiovascular system, respiratory system, central nervous system) as well as on supplemental organ functions (gastro-intestinal tract, blood) was investigated *in vitro* and *in vivo*. The orientating studies provided no evidence of adverse effects (maximum doses or concentrations tested are given in brackets):

- Cardiovascular system: blood pressure in rats (40 mg/kg i.v.), blood pressure and ECG in dogs (200 mg/kg i.v.);

- Central nervous system: general behaviour of mice (300 mg/kg i.p.) and dogs (200 mg/kg p.o.); anticonvulsive and analgesic effects in mice (100 mg/kg and 50 mg/kg i.p.);
- Respiratory system: isolated tracheal muscle preparations of the cat (2 x 10-5 g/mL);
- Gastro-intestinal tract: gastric secretion in rats (50 mg/kg p.o.), isolated small intestine preparations of the guinea pig (10-5 g/mL);
- Testing for a local anaesthetic effect in mice (tail, 0.05 mL of a 2 % treosulfan solution).

Treosulfan exhibited no potential for a pro-arhythmogenic activity in a recent (2017) GLP-compliant *in vitro* test. The whole-cell patch-clamp technique (manual patch-clamping) was used to investigate the effects of treosulfan on hERG (human-ether-à-go-go related gene) stably expressed in HEK 293 cells. Treosulfan was tested at one concentration of 1000 µM in order to determine compound effects on hERG mediated current. As the observed inhibition of the hERG tail current amplitude was less than 10%, no further concentrations were tested. Dose-dependent haematological changes were observed after i.v., i.p. or p.o. administration in mice, rats, dogs and monkeys. These effects are generally expected for alkylating agents.

Pharmacodynamic drug interactions

Treosulfan was more active against leukemic cells of 20 paediatric patients as well as against three leukemia-derived cell lines than busulfan, with increasing IC50 values from initial diagnosis (chemotherapy naive specimens) to relapse (pretreated specimens). Overall, purified stem cells were most sensitive, followed by CD56+CD3- NK and CD3+ T cells. The combination of treosulfan with fludarabine resulted in a synergistic cytotoxic effect against leukemic cells. Conditioning treatment with treosulfan/fludarabine or busulfan/fludarabine resulted in decreased severity of acute graft versus host disease (aGvHD) compared to total body irradiation (TBI). Moreover, treosulfan/fludarabine was associated with improved immune reconstitution despite early gastro-intestinal or cutaneous toxicity.

AML- or MDS-derived myeloid cell lines as well as primary marrow cells from patients with MDS and healthy donors were exposed to treosulfan, radiation or both, and the extent of apoptosis was assessed. Pre-exposure to treosulfan did not clearly enhance radiation-induced cell death.

The combination of treosulfan with total body irradiation was investigated prior to bone marrow transplantation in rats. Treosulfan was shown to possess certain characteristics of a radiosensitizer.

Low-dose treosulfan was added to an immune-suppressive regimen consisting of T cell-depleting antibodies, fludarabine, and thymic irradiation. The results indicate that low-dose treosulfan may be considered as a useful component of a truly nonmyeloablative conditioning protocol in providing for mixed haematopoietic chimerism of donor type and, consequently, in establishing a platform for adoptive immunotherapy.

Permanent mixed chimerism and donor-specific tolerance was achieved in mice conditioned, prior to a donor mouse muscle precursor cell transplantation, with a treosulfan treatment combined with a single cyclophosphamide dose, and finally donor bone marrow transplantation.

2.3.3. Pharmacokinetics

Recent validated analytical procedures replaced the methods used early in the development of treosulfan as an anti-tumour agent. For kinetic determination of treosulfan, concentrations in plasma of juvenile rats and in sodium chloride solutions (application solution) the older high performance liquid chromatography (HPLC) method with refractive index (RI) detection was validated. However, for the quantification of treosulfan and its

epoxides in plasma, cerebrospinal fluid (CSF), and brain homogenate supernatant of rats, a new HPLC method with Electrospray Ionisation (ESI)-triple quadrupole mass spectrometer (MS/MS) or UV-detection was developed. The selective and rapid HPLC-ESI-MS/MS method was also elaborated and validated for the studies of distribution of treosulfan and EBDM in rat plasma, liver, lungs, kidneys, muscle, and brain tissue.

The total radioactivity in urine, faeces and expired air of rats was calculated by means of liquid scintillation counting following [3H]-treosulfan application. In addition, whole body autoradiography was performed to measure radioactivity in tissues and carcasses.

Another HLPC-MS/MS method was optimised for the simultaneous determination of treosulfan and epoxides in plasma of children under clinical conditions.

Absorption

The route of administration of treosulfan is intravenous.

The plasma pharmacokinetics (plasma concentrations of treosulfan and basic pharmacokinetic parameters after single administration were determined in mice (i.p.), rats (i.v.), rats (i.v.; juvenile, young adult and adult rats), rabbits (i.v.) and dogs (i.v., p.o.).

Table 2: Absorption after a single dose

Species	Mouse	Rat	Rat (young adult)	Rabbit	Dog	Human
Reference	Werner et al., 2008	McEwen, 2004	LPT 27700, 2014	Romanski et al., 2016	LEO, 1978	Beelen, 2005
Test article	treosulfan	[^{3}H]-treosulfan	treosulfan	treosulfan	treosulfan	treosulfan
Gender (M/F) /Number	not known / 3	M / 1	M+F / 3	not known / 5	not known / 2	M+F / 8
Feeding condition	-	-	-	-	-	-
Vehicle, Formulation	water, solution	water, solution	water, solution	saline	water, solution	water, solution
Method of administration	i.p.	i.v. bolus	i.v. bolus	i.v. bolus	i.v. bolus	i.v. infusion (2 h)
Volume of administration			10 mL/kg			
Dose	3000 mg/kg	100 mg/kg	500 mg/kg	340 mg/kg	100 mg/kg	12 g/m^2
Sample	plasma	whole blood	plasma	plasma	plasma	plasma
Analyte	treosulfan	[^{3}H]-treosulfan	treosulfan	treosulfan	treosulfan	treosulfan
Assay	RP-HPLC	LSC	HPLC-MS/MS	HPLC	GLC	RP-HPLC
	9000 mg/m^2	600 mg/m^2	1500 mg/m^2	4080 mg/m^2	2000 m g/m^2	12000 mg/m^2
PK Parameter:						
Tmax (h)	0.21	0.08	-	-	-	-
Cmax (µg/mL or µg-eq/mL)	ca. 8000	83.8	M: 818 F: 966	1531	180 - 320	260
AUC (µg or µg-eq x h/mL) (Time for calculation – h)	-	253 (0 – 24) 471 (0 – inf)	M: 691 F: 751 (0-24)	1235 (0-inf)	-	898
T1/2 (h) (Time for calculation – h)	1.8 (terminal)	0.48 (initial) 25.8 (terminal)	0.68 (0-24)	0.14 (initial) 1.60 (terminal)	-	2.1 (terminal)

Abbreviations: i.p.: intraperitoneal; i.v.: intravenous; p.o. oral; - : no data available; M: males; F: females

A comparative pharmacokinetic study in juvenile (post-natal day 10, PND 10) and young adult rats (PND 34/35) after a single i.v. bolus application of 500 mg/kg treosulfan revealed an elimination half-life of approximately 1 hour in juvenile rats and approximately 40 minutes in young adult rats. Plasma levels of the monoepoxide EBDM were 24-fold to 44-fold lower than those of the parent compound and declined with an elimination half-life of approximately 1.3 hours for juvenile rats and approximately 40 minutes for young adult rats. Concentrations of the diepoxide DEB were below the limit of quantification.

In another study in juvenile rats (PND 10), plasma peak levels of treosulfan were noted 5 minutes after i.v. administration of 10, 50 or 250 mg/kg. Plasma concentration declined with an elimination half-life ranging from 1.1 to 1.4 hours for males and females, respectively. After daily application of treosulfan, no accumulation was noted.

Table 2: Plasma levels of treosulfan and its transformation products after single i.v. administrations to juvenile and young adult rats

Analyte	Treosulfan									
Sample (whole blood, plasma, serum etc.)	Plasma - JR		Brain - JR		Plasma - YAR		Brain - YAR		CSF#2 -YAR	
Sex (M/F)	(24/24#4)		(24/24)		(24/24)		(24/24)		(24/24)	
Assay	HPLC-MS/MS		HPLC-MS/MS		HPLC-MS/MS		HPLC-MS/MS		HPLC-MS/MS	
PK Parameters:#5	male / female		male / female		male / female		male / female		male / female	
C_{max}#1 (µmol/L)	3108	2015	133	130	2938	3471	88.2	70.3	120	118
$t_{½}$ (h)	1.04	1.15	2.40	3.07	0.68	0.68	1.99	2.21	1.42	1.34
$AUC_{0-t\ last}$ (µmol x h/L)	4846	4272	580	533	2483	2699	228	197	292	295
$AUC_{0-\infty}$ (µmol x h/L)	4929	4387	736	768	2488	2703	261	230	309	309

Analyte	S,S-EBDM (monoepoxide of treosulfan)									
Sample (whole blood, plasma, serum etc.)	Plasma - JR		Brain - JR		Plasma - YAR		Brain - YAR		CSF#2 -YAR	
Sex (M/F)	(24/24#4)		(24/24)		(24/24)		(24/24)		(24/24)	
Assay	HPLC-MS/MS		HPLC-MS/MS		HPLC-MS/MS		HPLC-MS/MS		HPLC-MS/MS	
PK Parameters:#5	male / female		male / female		male / female		male / female		male / female	
C_{max}#1 (µmol/l)	74.4	86.2	40.6	38.1	66.5	78.9	20.9	17.7	38.5	35.0
$t_{½}$ (h)	1.17	1.36	1.46	1.28	0.67	0.72	0.63	0.78	0.94	0.98
$AUC_{0-t\ last}$ (µmol x h/l)	233	226	137	128	96.3	110	24.3	21.1	60.2	68.0
$AUC_{0-\infty}$ (µmol x h/l)	241	238	149	136	97.8	112	28.0	26.1	63.8	72.4

Additional Information

All concentrations of S,S-DEB (diepoxide of treosulfan) measured in the various sample matrixes via UV detection after derivatisation and separation by HPLC were below the limit of quantification.

JR : juvenile rat; YAR : young adult rat
#1 values obtained from sample matrix analysis, all other values calculated by pharmacokinetic analysis
#2 cerebrospinal fluid (liquor)
#3 comparison to plasma value
#4 in one case blood withdrawal was not possible
#5 evaluated for male/female animals

In a pharmacokinetic study with i.v. administration, rabbits receiyved treosulfan, EBDM or DEB. The epoxides underwent a very rapid elimination with half-life of 0.069 and 0.046 hours associated with a high systemic clearance (10 and 14 L h-1 kg-1). After administration of treosulfan, the half-life of EBDM was statistically equal

to the half-life of the prodrug treosulfan (1.6 hours). These data demonstrate a formation-limited elimination of the epoxides with *in vivo* levels of EBDM and DEB being much lower than the parent compound due to the inherently high clearance of the epoxides.

Distribution

In human blood samples, the fraction unbound to plasma proteins was about 82% for the monoepoxide EBDM and for the diepoxide DEB. The unbound fraction in rat plasma was about 95% for treosulfan and about 100% for EBDM.

Distribution of treosulfan and EBDM into plasma, liver, lungs, kidneys, muscle, and brain of adult rats was demonstrated after a single intraperitoneal administration of radio-labelled [3H]-treosulfan. In comparison with the parent drug, the levels of EBDM in the plasma and tissues were much lower. The lowest quantifiable levels of both compounds were observed in the brain tissue. Low blood-brain barrier permeability of treosulfan was confirmed in an *in vitro* model.

The autoradiographic procedures may have resulted in extensive loss of volatile products (for example DEB), which may have been formed from treosulfan during sample processing. On this basis, derived data of the tissue distribution were not considered reliable. The data confirmed, however, that no covalently bound radioactivity was retained within the carcass about one week post dosing.

After a single i.v. bolus application of treosulfan (Study LPT 27700) the exposure of treosulfan in brain tissue of juvenile rats was approximately 3-fold higher and the overall elimination rate was slower compared to young adult rats. In addition exposure to S,S-EBDM in brain tissue was 5-6-fold higher in the juveniles as compared to the young adults.

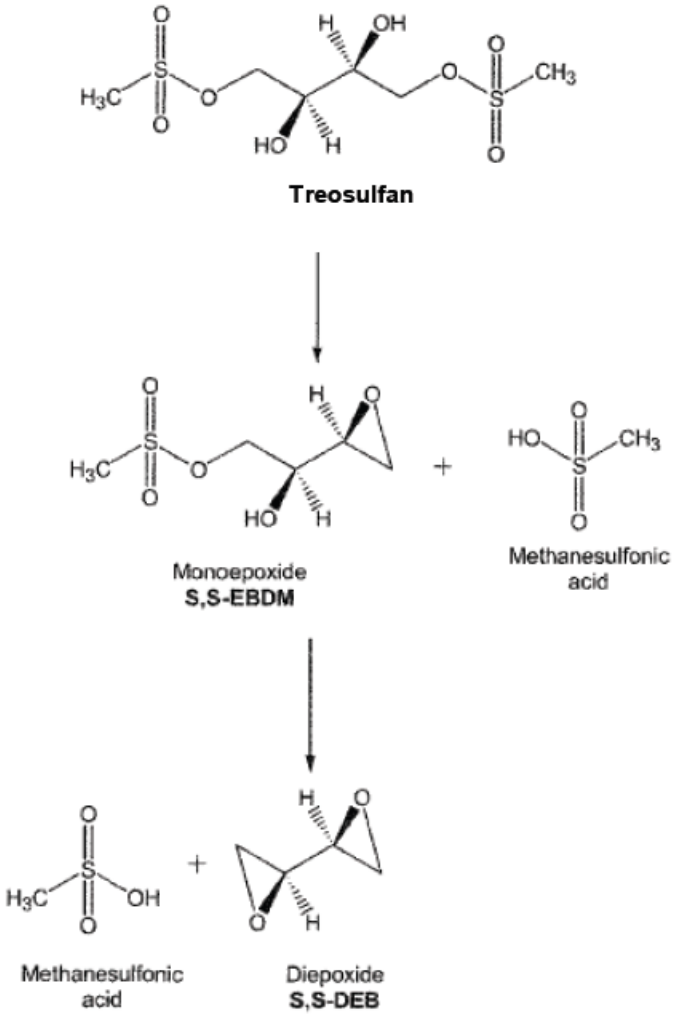
Concentrations of treosulfan and EBDM were determined in samples of plasma, liver, lungs, kidneys, muscle, and brain of adult rats after a single intraperitoneal administration of treosulfan. The two analytes could be determined in the biological matrices up to 6 hours after administration (except for EBDM in the liver); concentrations were below the lower limit of quantitation (LLOQ) in the samples collected at 24 hours. In comparison to the parent drug, the levels of EBDM in the plasma and tissues were much lower. Highest concentrations of treosulfan were observed in kidneys, and lowest quantifiable levels of treosulfan and EBDM were observed in the brain tissue.

There is no information on the distribution of treosulfan or its metabolites into breast milk.

Metabolism

The prodrug treosulfan is converted under physiological conditions in two steps by nonenzymatic intramolecular arrangement into the monoepoxide (EBDM) and the diepoxide (DEB) The expoxides react with nucleophilic centers of biomolecules (alkylation), the basis the genotoxic and carcinogenic potential. Further transformation proceeds by non-enzymatic and enzymatic processes.

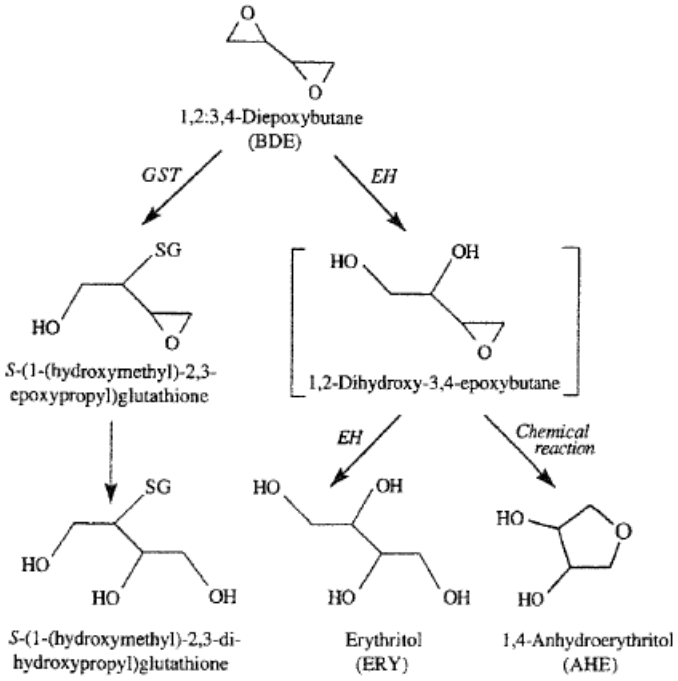
Figure 2 Conversion of treosulfan to biologically active epoxides (Source: Glowka et al., 2012)



Metabolism / detoxification processes have been thoroughly investigated for DEB since this compound is also formed *in vivo* after exposure to the industrial chemical 1,3-butadiene. Glutathione (GSH) conjugation of DEB by liver cytosol from mice, rats, and humans was evaluated *in vitro.* Analysis indicated formation of two isomeric conjugates, which were rapidly hydrolyzed to the corresponding trihydroxy conjugates. The conjugation rates in mouse and rat liver cytosol were similar and one order of magnitude higher than in human liver cytosol.

Diepoxibutane (DEB) disappeared rapidly from plasma and was only detectable up to 10 minutes after i.v. injection of DEB to one dog. Treosulfan was transformed non-enzymatically into its corresponding mono- and diepoxide (EBDM and DEB) with a half-life time of 2.2 hours.

Figure 3: Proposed scheme for the detoxification reactions of 1,2:3,4-diepoxybutane.(Boogaard and Bond, 1996) GST: glutathione S-transferase; EH: epoxide hydrolase.



The formation of glutathione conjugates and erythritol was observed in human, rat, and mouse liver fractions. The formation of anhydroerythritol was only detected in human liver microsomes.

Transformation proceeded according to a first order reaction. Hydrolysis of DEB is another important route of detoxification. Tissue preparations from rats, mice and human revealed that human liver is highly proficient in hydrolysis of DEB, while mouse liver has a relatively low hydrolytic capacity. Pulmonary hydrolysis was also most efficient in humans.

Excretion

Following i.v. injection of [3H]-treosulfan to rats, treosulfan was rapidly excreted into urine and faeces. Almost full recovery of radioactivity (91%) was found one day after dosing. Urinary recovery of treosulfan after i.v. and oral application to dogs was found to be 50% to- 60% within 8 hours. Neither EBDM nor DEB was detected in the urine.

Pharmacology studies on drug interactions

The inhibitory potency of treosulfan towards human CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4) was investigated in human liver microsomes using a standard set of CYP isoform-selective substrates and pre-incubation with pooled human liver microsomes and a NADPH regenerating system. There was no indication of an inhibitory effect of treosulfan in the dose range tested, 1 to 100 µM. Specific information on enzyme induction and inhibition of transporters by treosulfan is not available.

2.3.4. Toxicology

Single dose toxicity

Table 4 Single dose toxicity

Species and Strain	Method of Administration (Vehicle / Formulation)	Doses (mg/kg)	Gender and Number per Group	Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings
Mouse (Leo strain II)	i.v., p.o., s.c. (aqueous solution or suspension)	Treosulfan i.v., p.o., s.c.: 3500	10 animals per group, gender not provided	3500	LD_{50}: > 3500	No deaths recorded following i.v. injection of treosulfan. Treosulfan LD_{50} > 3500 mg/kg, all routes; Busulfan LD_{50} p.o. = 240 mg/kg; LD_{50} s.c. = 200 mg/kg
Mouse (CDI)	p.o. and i.v. (aqueous solution or suspension)	p.o.: 2500, 3500, 4500 i.v.: 2500	10 mice per group (5 males, 5 females)	p.o.: not mentioned; i.v.: 2500	LD_{50} p.o.: 3360 LD_{50} i.v.: >2500	p.o.: 2500 mg/kg and above resulted in lethality starting 4 to 6 days after dosing. Autopsy revealed no remarkable pathological changes. i.v.: No animal died during the observation period of 14 days.
Rat (Wistar AF/ HAN/MOL/67)	p.o. and i.p. (aqueous solution or suspension)	p.o.: 2200, 2500, 3000 i.p.: 2200, 2800, 3500	10 rats per group (5 males, 5 females)	not mentioned	p.o. LD_{50}: 2575 i.p. LD_{50}: 2860	2200 mg/kg and above resulted in lethality starting 5 to 8 days after dosing. Autopsy revealed no remarkable pathological changes.

The prodrug treosulfan was found to have much lower acute toxicity compared to the directly alkylating agent busulfan. This difference can be explained by the rapid detoxification and rapid elimination of toxic metabolites in the case of treosulfan

Repeat dose toxicity

Study results are derived from the published literature whilst other studies are presented as brief summaries of studies conducted in the 1970s and 1980s.

Following repeated administration of treosulfan to rats, dogs or monkeys, the targets of toxicity were the haematopoietic and the lymphatic systems. The observed changes in the cellularity of the bone marrow and peripherial blood and in the spleen and lymph nodes appear to be a manifestation of the target toxicity. Evidence of cytotoxicity were the haemorrhages in various tissues and functional changes in the reproductive system (gonads, sperm, ovaries). Based on overt signs of toxicity and gross pathology findings, haemorrhagic lesions appear to be dose-limiting and were most pronounced in dogs. Emesis and diarrhoea was occasionally observed in the monkey studies.

The applicant states that observations described as “CNS-depression” and “malaise” in the repeat-dose toxicity studies in dogs and monkeys are considered as signs of an impact on the general health condition, rather than signs of neurotoxicity. These studies were conducted in 1978 and these findings were not further explained. The applicant’s interpretation is corroborated by the fact that these symptoms were only noted in animals that died

or were sacrificed in a moribund condition. There was no indication for effects on CNS-functions in the safety pharmacology studies and in juvenile rats after repeated dosing.

Signs of minor toxicity occurred in the study with juvenile rats with a maximum intravenous dose of 100 mg/kg/day. A slightly decreased ALAT plasma activity (-20% to -30%) is considered toxicologically not relevant in the absence of evidence for corresponding signs of organ dysfunctions or morphological tissue lesions.

A NOAEL for treosulfan of 50 mg/kg/day was determined in the juvenile rat toxicity study. In the other repeat-dose studies, adverse effects were observed at the lowest dose tested. For both routes of administration, i.v. and p.o., the lowest dose tested was 56 mg/kg/day in dogs and monkeys in the repeat-dose toxicity studies.

The maximum tolerated dose (MTD) regarding severe toxicity including lethality after repeated intravenous administration was 100 mg/kg/day in juvenile rats, 111 mg/kg/day in dogs and monkeys.

In the 5-day rat study, an intraperitoneal dose of 278 mg/kg/day was tolerated without severe toxicity.

Overall, the toxicity following treosulfan administration seems to be similar among the animal species tested and was generally independent from the route of administration. The potential for complete reversibility of haematological effects and effects on reproductive organs within 5 weeks after repeated dosing over 3 to 4 weeks was demonstrated in the study on juvenile rats.

Genotoxicity

Table 8: Genotoxicity assays with treosulfan

Type of Study	Species / Strain	Method of Admini-stration	Duration of Dosing	Doses or Concentration	Noteworthy findings
Genotoxicity assays with treosulfan					
Interaction of treosulfan with DNA	Isolated DNA and human chronic myelogenous leukaemic K562 cells	In vitro	4 and 24 hours	0.01 to 10 mM	Treosulfan produced DNA cross-links after 4 hours incubation, but a concentration of 10 mM was required to produce 8.9% cross-linking. After 24 hours of incubation, an increase to 62% cross-links was achieved. Using 1 mM treosulfan and 24 hours incubation time, 12% cross-linking were achieved. In contrast, significant cross-linking was observed with the preformed epoxides at 4 hours, with 30% cross-linking at 10 mM, increasing to 91% with a 24-hour exposure.
Mutagenicity of treosulfan and DEB in bacteria	*Salmonella typhimurium* (strains: TA100, TA1535)	In vitro; Preincubation test, Plate test	30 minutes, 48 hours	Treosulfan: 0.12 to 30 µmol/plate; DEB: 0.06 to 3.5 µmol/plate	Maximum responses were seen at 30 and 0.88 µmol/plate for treosulfan and DEB, respectively, at pH = 6.

Mutagenicity of treosulfan and DEB in bacteria	*Salmonella typhimurium* (TA1535, TA100, TA102, Ames II™ 7000 series strains)	In vitro; Preincubation test, Plate test	30 minutes, 48 hours	Treosulfan: 0.11 to 7.5 µmol/plate; DEB: 0.11to 3.75 µmol/plate	Treosulfan (0.93 µmole/plate) induced 16.8-fold-over-background reversion or a mutagenicity ratio of 16.8 in TA1535. The response was weaker in TA100 (mutagenicity ratio of 3), and negative in strain TA102. Only two Ames II™ strains demonstrated sensitivity to treosulfan (TA7004 (CG:AT), TA7005 (GC:AT)). DEB was mutagenic in TA1535 and TA7004, but in contrast to treosulfan, DEB was mutagenic in TA102.
Mutagenicity of treosulfan and DEB in mammalian cells	CHO cells (AS52)	In vitro	3 hours	0.1 to 1.0 mM	Treosulfan (0.1 - 1.0 mM) was toxic and mutagenic at the gpt locus. A strong pH-dependency was noted. DEB was cytotoxic and mutagenic at a much lower dose (0.025 mM), but these effects were not affected by pH.
Sister chromatide exchanges induced by treosulfan in human cells	Human lymphocytes (3 donors)	In vitro	71 hours	Up to 1 µM	Treosulfan significantly increased the numbers of sister chromatide exchanges. A dose of 4.5 µg/mL increased the frequency of sister chromatide exchanges of one donor by 6 or 8 times compared with the control value.

4.3.1.2 Genotoxicity assays with metabolites

Table 9: Genotoxicity assays with treosulfan metabolites

Type of Study	Species / Strain	Method of Admini-stration	Duration of Dosing	Doses or Concentration	Noteworthy findings
Genotoxicity assays with metabolites					
Interactions of DEB with DNA	nuclear extracts from human cervical carcinoma (HeLa) cells	In vitro	3 hours	25 mM	39 human proteins were identified that form covalent DNA-protein cross-links in the presence of DEB. DNA-protein cross-linking efficiency was 2%-12%.
Interactions of DEB with DNA	human fibro-sarcoma (HT1080) cells	In vitro	3 hours incubation	0.05 to 2 µM	Over 150 proteins including histones, high mobility group proteins, transcription factors, splicing factors, and tubulins were found among those covalently cross-linked to chromosomal DNA in the presence of DEB.
Interactions of DEB stereoisomers with DNA	*Xenopus borealis* plasmids	In vitro	30 minutes	250 mM	S,S-DEB showed the highest efficiency of interstrand cross-linking consistent with its potent genotoxicity and cytotoxicity, followed by R,R-DEB, and at last meso-DEB.
Interactions of DEB stereoisomers with DNA	calf thymus DNA	In vitro	24 hours	0.01 – 0.5 mM	The 3 optical isomers of DEB exhibit different DNA cross-linking specificities.
Mutagenicity of butadiene epoxy metabolites in bacteria (Ames test)	*Salmonella typhimurium* (strain: TA100)	In vitro, Plate test	48 hours	up to 50 µmol/plate	Butadiene diolepoxide, butadiene monoepoxide and diepoxybutane, were mutagenic with and without metabolic activation (S9 mix).

4.3.2 In vivo

Table 10: In vivo genotoxicity assays

Type of Study	Species / Strain	Method of Administration	Duration of Dosing	Doses or Concentration	Noteworthy findings
Micronucleus test in mice with treosulfan	Mouse / B6CSF1	i.p.	2 days, once daily	250 to 1000 mg/kg	Treosulfan induced micronuclei in the dose range tested. A small but nonsignificant decrease in the proportion of PCE was observed, while an approximately 20-fold increase in the frequency or MN-PCE occurred.
Mutagenicity of butadiene epoxy metabolites in mice (Micronucleus test, Dominant lethal test)	Mouse / special breed and NMRI	i.p.	Single dose	up to 240 mg/kg	In the micronucleus assay, the three metabolites gave a positive response whereby the diepoxide was more effective than the monoepoxide which was more effective than the diolepoxide. In contrast to the diepoxide which was positive at a dose as low as 36 mg/kg, the monoepoxide and the diol did not show an induction of dominant lethal effects up to doses of 120 and 240 mg/kg, respectively.
Chromosomal abberations induced by DEB in mouse sperms	Mouse / B6C3F1	i.p.	Up to 3 weeks	Up to 28 mg/kg	Analysis of chromosomal aberrations in zygotic metaphases showed that late spermatids and sperm are unable to repair DEB-induced DNA damage as demonstrated by significant increases in the frequencies of zygotes with chromosomal aberrations. The dose-response study in sperm indicated a linear response for both single and repeated exposures.

Carcinogenicity

Based on its mechanism of action, treosulfan is considered as a genotoxic carcinogen. Due to sufficient evidence in humans, treosulfan was considered by International Agency for Research on Cancer (IARC) Working Groups as “carcinogenic to humans (Group 1)”. (IARC, 2012) Therefore, carcinogenicity studies in animals were deemed not necessary.

Reproduction Toxicity

A potential for an influence on reproductive performance and intrauterine development in humans can be assumed based on the cytotoxic and genotoxic properties of treosulfan. Therefore, no dedicated reproductive and developmental toxicity studies for treosulfan according to current regulatory guidance were conducted.

A number of studies specifically addressed effects of treosulfan and metabolites on male and

female reproductive organs and functions.

Toxicokinetic data

There was one dedicated toxicokinetic study as part of the study in juvenile and young adult rats with i.v. administration (described above).

Local Tolerance

Local tolerance studies were not submitted.

Other toxicity studies

N/A

2.3.5. Ecotoxicity / environmental risk assessment

Table 1. Summary of main study results

Substance (INN / Invented Name):			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107 or …		Potential PBT (Y/N)
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}		B/not B
	BCF		B/not B
Persistence	DT50 or ready biodegradability		P/not P
Toxicity	NOEC or CMR		T/not T
PBT-statement :	The compound is not considered as PBT nor vPvB The compound is considered as vPvB The compound is considered as PBT		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)		µg/L	> 0.01 threshold (Y/N)
Other concerns (e.g. chemical class)			(Y/N)
Phase II Physical-chemical properties and fate			
Study type	**Test protocol**	**Results**	**Remarks**
Adsorption-Desorption	OECD 106 or …	K_{oc} =	List all values
Ready Biodegradability Test	OECD 301		
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50, water}$ = $DT_{50, sediment}$ = $DT_{50, whole\ system}$ = % shifting to sediment =	Not required if readily biodegradable

Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**valu e**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	OECD 201	NOEC		µg/L	species
Daphnia sp. Reproduction Test	OECD 211	NOEC		µg/L	
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	NOEC		µg/L	species
Activated Sludge, Respiration Inhibition Test	OECD 209	EC		µg/L	
Phase IIb Studies					
Bioaccumulation	OECD 305	BCF		L/kg	%lipids:
Aerobic and anaerobic transformation in soil	OECD 307	DT50 $\%CO_2$			for all 4 soils
Soil Micro organisms: Nitrogen Transformation Test	OECD 216	%effect		mg/ kg	
Terrestrial Plants, Growth Test/*Species*	OECD 208	NOEC		mg/ kg	
Earthworm, Acute Toxicity Tests	OECD 207	NOEC		mg/ kg	
Collembola, Reproduction Test	ISO 11267	NOEC		mg/ kg	
Sediment dwelling organism		NOEC		mg/ kg	species

Excluding the logKow values (≤4.5), calculated separately for the pro-drug and its metabolites (EBDM or DEB), the biodegradable results as well as the refined PEC surface water value were presented regarding the treosulfan as the test material. Nevertheless, the available PK data in animals and patients indicate that EBDM or DEB are not unchanged excreted, but probably bind to the tissues proteins and DNA due to their alkylating activity. It has been suggested that the epoxides are decomposed to hydroxy-derivatives like threitol and renally excreted. Plasma concentrations of the active epoxides in humans are about 50-fold to 100-fold less compared to the parent compound.

In the context of the obligation of the MAH to take due account of technical and scientific progress, the CHMP recommends the following points for further investigation:

- Unchanged treosulfan itself is excreted to approx. 25-40% of the administered dose in urine and there is no evidence (e.g. in the SmPC) that excreta of treated patients will be collected in hospitals and consequently is expected to enter WWTs via domestic sewage. Data show that treosulfan is not readily biodegradable (elimination of 30-40% in WWT). Hence, unchanged treosulfan will enter the aquatic environment, albeit in rather low quantities. This might pose a concern if treosulfan is taken up by other organisms and converted into the active and highly toxic metabolites under physiological conditions. Therefore, the applicant should discuss the possible exposure of aquatic organisms to treosulfan and in

particular its conversion into the active metabolites in other organisms than mammals in the environment and as to whether effects are to be expected.

2.3.6. Discussion on non-clinical aspects

Treosulfan is a prodrug. The parent compound is converted under physiological conditions by non-enzymatic processes into reactive intermediates via formation of a monoepoxide and a diepoxide. The reactive intermediates alkylate DNA and create interstrand cross-links. Alkylation also affects other biological molecules and structures involved in various physiological functions and thus contributes to the general cytotoxicity.

In studies in which treosulfan was used alone or in combination with other conditioning regimens myeloablative and immunosuppressive effects were shown in various *in vitro* and *in vivo* models.

The haematopoietic stem cell toxicity against both committed and primitive haematopoietic stem cells, as well as its immunosuppressive and haematotoxic characteristics, indicated the potential usefulness of treosulfan for conditioning treatment prior to allogeneic HSCT.

The safety pharmacology studies provided no evidence of clinically relevant adverse effects.

Basic pharmacokinetic studies were performed in mice, rats, dogs, and Cynomolgus monkeys.

Dedicated toxicokinetic studies were carried out in juvenile and young adult rats. There was no evidence for an accumulation in blood or tissues after repeated exposure. The ratio of treosulfan concentrations in brain versus plasma was very low, consistent with the lack of CNS-related toxicity. In juvenile rats, the plasma half-life was slightly prolonged and the exposure was higher compared to young adults when dosed intravenously on a mg/kg body weight calculation. The applicant has provided a scientifically plausible explanation for these findings and addressed their clinical relevance.

Repeat-dose toxicity studies with intravenous treosulfan administrations were performed in dogs and monkeys. Changes in the cellularity of the bone marrow and peripheral blood (pancytopenia) as well as in the spleen and lymph nodes were a manifestation of the haematotoxic property of treosulfan. Further consequences of the cytotoxicity were haemorrhages in various tissues and functional changes in the reproductive system. Gastro-intestinal effects including emesis and diarrhoea were observed occasionally in the animal studies. The potential for complete reversibility of haematological effects and effects on reproductive organs within 5 weeks after repeated sublethal dosing over 3 to 4 weeks was demonstrated in the study on juvenile rats. Overall, the systemic toxicity upon treosulfan administration appeared to be similar among the animal species employed in the toxicity studies and generally independent from the route of administration.

Treosulfan is genotoxic. Due to the genotoxic properties, there is a risk of secondary malignancies after treatment with treosulfan. Treosulfan is classified as a human carcinogen. Therefore, no carcinogenicity studies in animals have been performed.

An adverse effect on reproductive performance and intrauterine development in humans at clinical relevant doses can be assumed based on the cytotoxic and genotoxic properties of treosulfan. Therefore, no dedicated studies were conducted. There is also a risk of an accumulation of genetic damage in sperm and heritable chromosomal aberrations of paternal origin. Pregnancy is a contraindication in section 4.3 and 4.6 of the SmPC. In section 4.6 the SmPC states that women of child bearing potential have to use effective contraception during and up to 6 months after treatment. Furthermore, the SmPC states that treosulfan might impair fertility in men and women.

In line with the Questions and Answers on “Guideline on the environmental risk assessment of medicinal products for human use” EMA/CHMP/SWP/44609/2010, an ERA for both treosulfan and/or the active metabolites was provided taking into account the specific properties of the substances. Considering the low PEC value, the available PK data and the environmental minimisation measures introduced in SmPC for this medicinal product, the ERA is accepted.

Most of the study results are derived from the published literature whilst other studies are presented as brief summaries of studies conducted in the 1970s and 1980s. Recently conducted nonclinical studies e.g. requested by the Paediatric Committee (PDCO) of the European Medicines Agency are in compliance with GLP regulations. In view of the fact that treosulfan is mutagenic, cytotoxic, carcinogenic and toxic to reproduction, and in view of its established clinical use for decades as well as the extensive experience with other alkylating agents, further non-clinical studies are not warranted.

The applicant performed a refined PEC surface water for the pro-drug and its metabolites, for which a value below the action limit of 0.01 µg/L was determined. In addition, studies regarding: 1) the log Kow for treosulfan, monoepoxide (EBDM) and diepoxide (DEB), 2) a biodegradability test and 3) PK data in animals and patients, were included in the present assessment. It has been concluded by the applicant that the active substance and both metabolites are unlikely to represent a risk for the environment. Unchanged treosulfan itself is excreted to approx. 25-40% of the administered dose in urine and there is no evidence (e.g. in the SmPC) that excreta of treated patients will be collected in hospitals and consequently is expected to enter WWTs via domestic sewage. Data show that treosulfan is not readily biodegradable (elimination of 30-40% in WWT). Hence, unchanged treosulfan will enter the aquatic environment, albeit in rather low quantities. This might pose a concern if treosulfan is taken up by other organisms and converted into the active and highly toxic metabolites under physiological conditions. The applicant discussed the possible exposure of aquatic organisms to treosulfan and in particular its conversion into the active metabolites in other organisms than mammals in the environment and as to whether effects are to be expected; this concern was resolved.

2.3.7. Conclusion on the non-clinical aspects

The non-clinical package submitted is considered adequate.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Study ID Type Status	**Objectives**	**Design** Location	**Test Product** Dosage (IV 2 h)	**Diagnosis of Patients** Number of patients (FAS) Median age (range)
Adults				
MC-FludT.6/L Phase II Efficacy Complete	Feasibility and tolerability of a conditioning therapy based on three different dose levels of treosulfan prior to alloHSCT	Non-randomised, non-controlled open-label Multicentre (DE, SE, FI, PL)	Treosulfan 10/12 or 14 g/m²/d × 3 Days -6, -5, -4	Haematological chemosensitive malignancy indicated for an alloHSCT, but with increased toxicity risk for classical (high-dose busulfan or standard-dose TBI) conditioning therapies 56 50 years (18-66)
MC-FludT.7/AML Phase II Efficacy/ Safety Complete	Evaluation of engraftment	Non-randomised, non-controlled open-label Multicentre (DE, SE, FI, PL)	Treosulfan 14 g/m²/d × 3 Days -6, -5, -4	AML 38 45 years (19-59)
MC-FludT.8/MDS Phase II Efficacy/ Safety Complete	Evaluation of engraftment	Non-randomised, non-controlled open-label Multicentre (DE, SE, FI, PL)	Treosulfan 14 g/m²/d × 3 Days -6, -5, -4	MDS 16 50 years (22-63)
MC-FludT.14/L **Trial I** Phase III Efficacy/ Safety Complete	Compare EFS within one year after transplantation between Treosulfan-based conditioning and busulfan-based conditioning.	Randomised active controlled open label non-inferiority Multicentre (DE, FR, FI, HU, IT, PL)	Treosulfan 14 g/m²/d × 3 Days -6, -5, -4	AML or MDS 320 (171 test; 159 control) 59 years (21-70)

Study ID Type Status	**Objectives**	**Design** Location	**Test Product** Dosage (IV 2 h)	**Diagnosis of Patients** Number of patients (FAS) Median age (range)
MC-FludT .14/L **Trial II** Phase III Efficacy/ Safety Complete	Compare EFS within two years after transplantation between Treosulfan-based conditioning and busulfan-based conditioning.	Randomised active controlled open label non-inferiority Multicentre (DE, FR, FI, HU, IT, PL)	Treosulfan 10 g/m²/d × 3 Days -4, -3, -2	AML or MDS 460 (220 test; 240 control) 60 years (37-70)
Paediatrics				
MC-FluT. 16/NM Phase II Efficacy/ Safety Ongoing	Safety and efficacy of treosulfan compared to conventional dose i.v. busulfan, each administered as part of a standardised fludarabine-containing conditioning regimen	Randomised active controlled open label Multicentre (Europe)	Treosulfan 10-14 g/m²/d × 3 Days -6, -5, -4	Non-malignant disease indicated for first myeloablative alloHSCT, including inborn errors of metabolism, primary immunodeficiencies, haemoglobinopathies and bone marrow failure syndromes Paediatrics (28 days, <18 years)
MC-FluT. 17/M Phase II Efficacy/ Safety Complete	Safety and efficacy of treosulfan administered as part of a standardised fludarabine-containing conditioning	Non-controlled Multicentre (Europe)	10-14 g/m²/d × 3 Days -6, -5, -4	Haematological malignant diseases (ALL, AML, MDS, JMML), requiring myeloablative conditioning for alloHSCT 70 Paediatrics (28 days, <18 years)

2.4.2. Pharmacokinetics

Absorption

Treosulfan is administered through IV route, therefore bioavailability and effect of food were not studied.

Distribution

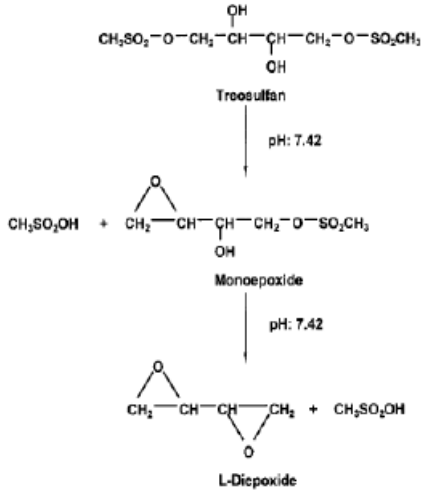
According to the literature data, treosulfan has negligible plasma protein binding [Glowka 2012; Schwarzner 2017]. The volume of distribution at steady state from different studies was reported to range from 22.1 ± 3.8 to 50.4 ± 43.9 L. According to the final population PK analysis, treosulfan volumes of distribution were 19 ± 1.7 L and 20.3 ± 1.5 L for central and peripheral volumes of distributions, respectively.

Elimination

Treosulfan is mainly excreted renally. The range of renal excretion expressed as % of total dose ranged between 14 -42% in different studies.

The metabolism of TREO was investigated by Feit et al. Treosulfan has no activity per se but needs transformation into epoxide derivatives to have a cytotoxic activity. At pH-values below 6 and a temperature of 20°C in vitro, almost no transformation of TREO occurs. However, under physiological conditions (pH 7.4, temperature 37°C), TREO is converted spontaneously (nonenzymatically) into an active monoepoxide intermediate (S,S-EBDM = (2S,3S)-1,2- epoxybutane-3,4-diol-4-methanesulfonate) and finally to L-diepoxibutane (S,S-DEB = (2S,3S)- 1,2:3,4-diepoxybutane) [Feit 1970].

Figure 4: Non-enzymatic activation pathway of treosulfan.



Pharmacokinetics of metabolites

With respect to the shape of the mean plasma concentration-time profiles of TREO and its monoepoxide metabolite, no major differences were observed between the age groups or BSA groups. Maximum TREO concentrations were reached directly after end of the infusion. The individual PK profiles were similar in shape and magnitude, independent of age group or BSA group. Decline of TREO concentrations after the end of infusion was bi-exponentially. Monoepoxide levels were about 10% of the parent compound TREO.

Dose proportionality and time dependencies

Dose proportionality

No deviations from dose proportionality or indications of time dependent PK were identified during the model development. Regression analysis of AUC versus TREO dose indicated linear pharmacokinetics (r^2 = 0.9227).
Figure 5: Correlation of TREO dose and AUC 0-∞

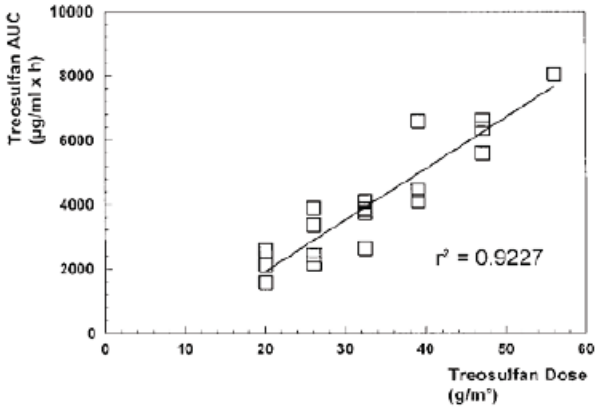


Figure 2.7.2.2.2.1-1: Correlation of TREO dose and $AUC_{0-\infty}$
(Linear regression analysis; $r^2 = 0.9277$)

Table 3: PK parameters

Table 2.7.2.2.2.1-3: PK parameters of 3 × 12 or 3 × 14 g/m² TREO prior to alloHSCT

Parameter	**3 × 12 g/m² TREO** n = 8	**3 × 14 g/m² TREO** n = 10	**P**
AUC after first dose [µg/mL × h]	898 ± 104	1104 ± 173	< 0.01
C_{max} [µg/mL]	260 ± 35	322 ± 47	< 0.01
Terminal half-life [h]	2.1 ± 0.5	2.0 ± 0.6	NS
CL_{tot} [mL/min]	225 ± 23	216 ± 32	NS
V_{ss} [L]	34 ± 5	31 ± 7	NS
Renal excretion [% of total dose]	39 ± 5	39 ± 7	NS
Data are arithmetic mean ± standard deviation (SD); multiple dose model			

AUCs and Cmax were significantly different (P < 0.01) and were clearly dose-dependent.

Time dependency

Comparison between single and repeated-dose pharmacokinetic parameters

A comparison of PK parameters obtained after the first IV infusion (2 hours) of 14 g/m² TREO versus the third infusion was done in MC-FludT.14/L Trial I.

Table 4: PK parameters of 1st vs 3rd infusion

Table 2.7.2.3.2.2-1: PK parameters of TREO first versus third infusion (median [range])

Infusion	N	C_{max} µg/mL	$AUC_{0-\infty}$ µg/mL × h	$T_{1/2}$ h	CL_{tot} mL/min	V_{ss} L
Model-independent analysis [Data source: PK Study Report, 26/03/2012; Table 10.2.1]						
1 (day -6)	22	448 [337-679]	1388 [1020-2250]	1.86 [1.12-2.56]	299 [156-458]	66.0 [37.4-94.1]
3 (day -4)	20	431 [321-599]	1482 [1037-2111]	1.93 [1.51-3.83]	286 [194-434]	66.9 [45.4-97.1]
Model-dependent analysis (2-compartment model) [Data source: PK Study Report, 26/03/2012; Table 11.1.1]						
1 (day -6)	24	475 [210-801]	1583 [877-2737]	2.02 [1.09-60.24]	265 [146-494]	39.4 [11.9-232.7]
3 (day -4)	22	434 [334-608]	1570 [1103-2806]	2.12 [1.59-119.19]	270 [149-408]	46.5 [29.1-188.4]

In the model-independent analysis, variability of values was low, all CV% were smaller than 30%. In contrast, in the model-dependent analysis CV% was high for terminal half-life (> 200%) and Vss (> 60%). Median values of all parameters were quite comparable for the first and third administration. No accumulation was observed.

Pharmacokinetics in target population

Results of the population PK

Covariate model of Treosulfan

Since the Dosing Model described historical data adequately and the current dosing scheme was based on BSA, the effect of BSA on CL, V1 and V2 was retained when additional covariate-parameter relations were tested.

Table 12: Final parameter estimates of the covariate model DMC5_finalSCM2b5.mdl.

Parameter	Value	S.E.	RSE	Low95CI	Upp95CI
V1	19	1.7	8.96	15.6	22.3
CL	17.7	0.427	2.41	16.9	18.6
V2	20.3	1.47	7.24	17.5	23.2
Q	26.1	3.75	14.3	18.8	33.5
BSA on V2	1.74	0.197	11.3	1.35	2.13
BSA on CL	1.18	0.0537	4.57	1.07	1.28
BSA on V1	1.22	0.121	9.87	0.987	1.46
BSA on Q	1.45	0.329	22.6	0.81	2.1
SHIFT bioassay	1.36	0.0658	4.83	1.23	1.49
IIV V1	0.149	0.0313	21	0.0875	0.21
IIV CL	0.0616	0.00996	16.2	0.042	0.0811
IIV V2	0.0668	0.0225	33.7	0.0227	0.111
IIV Q	0.203	0.0683	33.7	0.0687	0.337
Ω(V1,CL)	0.0544	0.0129	23.7	0.0291	0.0797
Ω(CL,V2)	0.0276	0.0122	44.1	0.00375	0.0515
σ CCV	0.0482	0.00477	9.91	0.0388	0.0575
OFV	17410.193				

Ω=covariance between between-subject variability

σ=variance of residual error ε

Distributions of predicted AUCinf displayed by BSA groups are showed that for all BSA groups, less than 1.2% of simulated AUCinf are below 760 or above 3600 µg×h/mL.

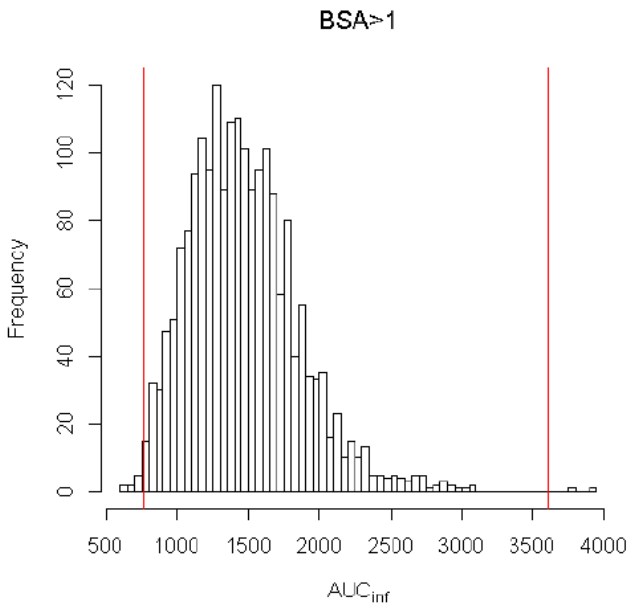


Figure 12: Distributions of AUC_{inf} stratified by BSA groups. The left and right red vertical lines indicate AUC_{inf} of 760 and 3600 µg×h/mL, respectively.

In order to investigate potential relation between exposure (AUC) and engraftment, model predicted AUC's were subjected to a graphical analysis (AUC's are calculated according historical assay results).

Visual inspection revealed no relation between AUC and time to engraftment in the investigated range of AUC's. Applying linear regression did not show a significant change of time to engraftment as function of AUC either (p-value = 0.197).

Treosulfan appears to have linear PK with dose proportional increase in AUC after IV administration.

Special populations

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
PK Trials	4	0	0

Pharmacokinetic interaction studies

- **In vitro**

The effect of treosulfan upon human CYP450 activities in vitro has been investigated by incubating TREO with pooled human liver microsomes in the presence of chemical substrates selective for CYP1A2, 2C9, 2C19, 2D6 and 3A4 catalysed reactions. To investigate whether treosulfan could be metabolically-activated, via microsomal metabolism, into an inhibitory species (metabolite) during the course of the incubations, treosulfan was pre-incubated for one hour in the presence of pooled human liver microsomes and NADPH at 37°C, prior to starting metabolic reactions. The rate of metabolic reactions with assays for CYP1A2, 2C9, 2D6 and 3A4 were

unaffected in the presence of treosulfan at concentrations up to 100 µM. With CYP2C19, some reduction in enzyme activity (ca. 25% inhibition) was observed up to 30 µM treosulfan. However, this was not reflected at the higher treosulfan concentration of 100 µM, where there was no inhibition evident.

Therefore, taking into account the magnitude of the effect seen at 30 µM treosulfan and the lack of an overall dose-response relationship, these data suggest that treosulfan does not inhibit CYP2C19 activity. In assays for CYP1A2, 2C9, 2D6 and 3A4, the respective enzyme activities (metabolite formation) were markedly reduced in the presence of known chemical inhibitors of these enzymes (furafylline, sulphaphenazole, quinidine and ketoconazole, respectively). A suitably selective chemical inhibitor of CYP2C19 enzyme is not available. These data illustrate that the incubation conditions used in this study were capable of supporting and hence demonstrating CYP-mediated inhibitory interactions. Therefore, a lack of treosulfan -derived CYP450-inhibitory interactions in this study indicates that TREO does not act as an inhibitor of human CYP450 activity in vitro [Cole 2003]. In conclusion, treosulfan is not metabolised in the liver. Therefore, pharmacokinetic interactions with medicinal products metabolised in the liver are unlikely.

2.4.3. Pharmacodynamics

Mechanism of action

Treosulfan is a "prodrug" of a bifunctional alkylating cytotoxic drug. Under physiological conditions an intra-molecular reaction occurs spontaneously (non-enzymatically), converting the pharmacologically inactive treosulfan into an active monoepoxide intermediate and finally to L-diepoxibutane. The derived epoxides react with nucleophilic centres of biological molecules, like proteins or DNA, and are responsible for antineoplastic activity. Molecular DNA-interaction of treosulfan and its transformation products show a preferential guanine-N7 alkylation, covalent inter-strand crosslinking of DNA, and potent concentration and time-dependent cross-linking activity. Irreversible DNA double-strand breaks are formed. These activities are determined by the stereo chemical structure of treosulfan (L-configuration). The D- or meso- configurated isomers are far less active.

Primary and Secondary pharmacology

Treosulfan has a broad antineoplastic and antileukaemic activity. Antineoplastic activity was demonstrated in non-clinical studies in lymphomas/leukaemias, sarcomas and hepatomas, human tumour xenografts, human tumour biopsies and cell lines. The in vitro cytotoxic effects against various human tumour cells showed it was especially active against leukaemic cells at concentrations several fold below clinically achievable plasma levels.

The safety profile of single agent treosulfan in conventional doses from its use in patients with ovarian cancer (up to 8 g/m² given every three to four weeks) is well-known and the most commonly reported undesirable effects are myelosuppression and gastrointestinal complaints (nausea, vomiting), usually mild and resolve after treatment. Safety non-clinical pharmacology studies did not show clinically relevant functional changes in the cardiovascular, respiratory or central nervous system and there were no dose-limiting effects on liver and kidneys.

Treosulfan is not able to cross the brain barrier unlike busulfan, which is known to cause undesirable effects of the nervous system.

GvHD is commonly observed after alloHSCT although it is considered not related to treosulfan but to the engraftment of the allogeneic immune system. How much the conditioning regimen influences the frequency and severity of GvHD is not known.

PD interactions

Synergistic cytotoxic effects against paediatric leukaemic cells were demonstrated for the combination of treosulfan and fludarabine.

Relationship between plasma concentration and effect

Concentration-response relationship was investigated within the Pop-PK study. The AUCs of three patients for which engraftment was not established were in the same range as AUCs of subjects for which engraftment was established. No correlation between AUC and time to engraftment in the investigated range of AUCs was seen.

2.4.4. Discussion on clinical pharmacology

All studies presented have been performed in patient populations with no studies in healthy subjects. The analytical methods used were different between different studies. The bioanalytical methods for treosulfan and its epoxide derivatives in the clinical studies are adequately validated and are suitable for their purposes. Assay performance, in terms of inter-assay precision and inter-assay relative error was considered acceptable.

The pharmacokinetics methods used for calculating PK parameters and renal clearance of treosulfan is acceptable. A population PK model was developed using FOCE with interaction estimation method using NONMEM software. The model was fitted to two compartments with linear intercompartmental clearance and elimination. The population PK objectives were clear with appropriate description of the nature of the data to be analysed. The general modelling aspects including software, estimation methods and diagnostics were properly reported. The statistical methods used are appropriate for summarising the PK data.

Treosulfan is administered through IV route, therefore bioavailability and effect of food were not studied. According to the literature data, treosulfan has negligible plasma protein binding [Glowka 2012; Schwarzner 2017]. The volume of distribution at steady state from different studies was reported to range from 22.1 ± 3.8 to 50.4 ± 43.9 L. According to the final population PK analysis, treosulfan volumes of distribution were 19 ± 1.7 L and 20.3 ± 1.5 L for central and peripheral volumes of distributions, respectively. Treosulfan is mainly excreted renally. The range of renal excretion expressed as % of total dose ranged between 14 -42% in different studies. Treosulfan does not undergo enzymatic metabolism. A pH-dependent non-enzymatic conversion to the active epoxide derivatives is responsible for the observed cytotoxic activity of treosulfan. The PK of the treosulfan metabolite, the monoepoxide metabolite is similar to the parent drug with similar t1/2 and Tmax. However, the exposure parameters (Cmax and AUC) appears to be ~30x lower than the parent drug. The consequences of possible genetic polymorphisms are not relevant as treosulfan does not appear to be substrate for any enzymatic metabolism. Treosulfan appears to have linear PK with dose proportional increase in AUC after IV administration. The PK parameters of treosulfan were similar after single and repeated dose administration indicating that treosulfan PK is not time dependent. In addition, there was no evidence of accumulation for treosulfan. The inter-individual variability was investigated using model independent and model dependent methods. The CV% was low (i.e <30%) for all the PK parameters using the model independent approach. However, the CV% was high (i.e > 80%) for t1/2 and Vss using the model dependent approach.

An updated population PK model for treosulfan was developed and fitted treosulfan PK to a two-compartment model with linear elimination and intercompartmental clearance. The covariate model tested number of covariates including BSA, data indicator, sex, age and renal function. The final model retained only BSA effect on Q, Cl, V1 and V2. The population PK model estimated all structural and random effects parameters with acceptable precision. The VPC predictions intervals showed acceptable fit with observed data, however the model under-predicted the observed concentrations in subjects with BSA larger than 1 m2. The bootstrap results

showed good agreement with the final model. The distribution of predicted AUCs showed that <1.2% are outside the target AUC range. The model results suggested a higher dose than currently planned for children with a BSA of 0.4, 0.5, 0.9 and 1.0 m2. The model predicted AUCs showed no correlation with time to engraftment.

The applicant did not provide data to describe treosulfan PK in different stages of renal impairment. However, treosulfan SmPC indicates that patients with renal impairment are generally excluded from alloHSCT which provides justification for absence of data and dose recommendation in patients with renal impairment. Treosulfan and its active epoxide metabolites are not subject to hepatic metabolism. Therefore, it is unlikely for hepatic impairment to have significant effect on treosulfan PK and the PK of its metabolites. Additionally, the treosulfan SmPC indicates that patients with hepatic impairment are generally excluded from alloHSCT which provides justification for absence of data and dose recommendation in patients with hepatic impairment. There was no observed effect for gender on treosulfan PK and it was not identified as a covariate in the pop PK model. The applicant did not provide data about the effect of race on treoosulfan PK, however, the race effect on treosulfan PK is not expected according to the applicant as treosulfan is not subject to any PK processes affected by race. Using population PK approach, body weight was found to be highly correlated with BSA, therefore only BSA was considered for the forward-backward covariate analysis. BSA was found to be a significant covariate on treosulfan clearance and volumes of distributions which supports the BSA based dosing scheme suggested by the applicant. The PK parameters appeared similar between patients < 50 years and ≥ 50 years which indicates that treosulfan PK is unlikely to be altered in old age.

The exposure parameters (Cmax and AUC) were comparable in different paediatric age and BSA groups which supports the current dose scheme proposed by the applicant. Tmax and T1/2 were comparable to the values reported in the adult population. As expected in paediatric population, volumes of distributions increased with increasing age and BSA. Based on the data provided by the applicant, the Cmax of treosulfan in adult studies after IV infusion of 10-14 g/m² ranged between 181 ± 36 and 597 ± 94 µg/mL, while the Cmax in paediatric population after IV infusion of 10-14 g/m² ranged between 566 ± 160 and 704 ± 318 µg/mL.

Treosulfan was not a time dependent inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 using midazolam and testosterone as substrates. However, using midazolam as the substrate, treosulfan was a reversible inhibitor for CYP2C19 and CYP3A4 with IC50 values of 972 and 1870 µM, respectively. These two IC50 values are within or lower than the expected Cmax of treosulfan in adult studies after IV infusion of 10-14 g/m² which ranged between 181 ± 36 and 597 ± 94 µg/mL (~ 650 – 2145 µM), while the Cmax was higher in paediatric population after IV infusion of 10-14 g/m² and ranged between 566 ± 160 and 704 ± 318 µg/mL (~2033 – 2592 µM). Therefore, the possibility of DDIs through CYP3A4 and CYP2C19 cannot be precluded.

Although treosulfan caused a statistically significant decrease in CYP3A4 mRNA expression in cryopreserved human hepatocytes. However, no induction was observed in any of the donors. Therefore, treosulfan cannot be considered as an inducer of CYP1A2, CYP2B6 and CYP3A4.

Treosulfan has been determined to be an inhibitor of probe substrate transport mediated via P-gp and MATE2-K, but not via BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, OCT1, MATE1 and BSEP.

The applicant is kindly asked to further discuss the observed inhibitory effect of treosulfan on CYP3A4, CYP2C19, P-gp and MATE2. Meanwhile, as the in vitro studies did not exclude the potential for DDIs between treosulfan and CYP3A4, CYP2C19, P-gp substrates, therefore, cautionary wording (e.g. special warnings and precautions for use) is required in the SmPC until additional evidence can be provided to exclude an interaction.

Treosulfan is a "prodrug" of a bifunctional alkylating cytotoxic drug. Under physiological conditions an intra-molecular reaction occurs spontaneously (non-enzymatically), converting the pharmacologically inactive treosulfan into an active monoepoxide intermediate and finally to L-diepoxibutane.

Treosulfan has a broad antineoplastic and antileukaemic activity. Antineoplastic activity was demonstrated in non-clinical studies in lymphomas/leukaemias, sarcomas and hepatomas, human tumour xenografts, human tumour biopsies and cell lines. The in vitro cytotoxic effects against various human tumour cells showed it was especially active against leukaemic cells at concentrations several fold below clinically achievable plasma levels.

The safety profile of single agent treosulfan in conventional doses from its use in patients with ovarian cancer (up to 8 g/m² given every three to four weeks) is well-known and the most commonly reported undesirable effects are myelosuppression and gastrointestinal complaints (nausea, vomiting), usually mild and resolve after treatment.

Treosulfan is not able to cross the brain barrier unlike busulfan, which is known to cause undesirable effects of the nervous system. Bone marrow depression and immunosuppression can lead to infections which are a major cause of morbidity and mortality of transplant patients.

Concentration-response relationship was investigated within the Pop-PK study. The AUCs of three patients for which engraftment was not established were in the same range as AUCs of subjects for which engraftment was established. No correlation between AUC and time to engraftment in the investigated range of AUCs was seen.

Treosulfan primary activity fulfils the requirements for a conditioning agent for alloHSCT. It has sufficient antineoplastic effect to eradicate the underlying disease whilst its immunosuppressive and myeloablative activities contribute to the achievement of stable transplant engraftment, complete donor-type chimerism and prevention of GvHD.

The combination of TREO/FLU/thiotepa (TT) has been proposed as an option in the paediatric indications. In paediatric patients TT is commonly used as part of conditioning to ensure engraftment, especially in non-malignant diseases that have a high risk of graft rejection. TT has no extramedullary toxicity.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology information is adequate.

The applicant has acknowledged CHMP recommendations and will submit any available clinical DDI data on CYP3A4, CYP2C19 and P-gp interactions as PAM. Due to the non-feasibility of DDI clinical studies in healthy volunteers (as the drug is highly cytotoxic), alternative approaches (e.g. PBPK and popPK modelling) might be acceptable.

2.5. Clinical efficacy

2.5.1. Dose response study(ies)

Dose response study MC-FludT.6/L

This was a non-randomised, non-controlled, dose response phase II study conducted to evaluate the feasibility and tolerability of a treosulfan-based conditioning regimen prior to allogeneic HSCT in patients with a haematological chemosensitive malignancy indicated for an allogeneic transplantation, but with an increased toxicity risk for classical (high-dose busulfan or standard-dose total body irradiation) conditioning therapies.

Study period: December 2001 – June 2005.

This study was conducted at seven centres in Germany, Finland, Poland and Sweden.

Treatment regimen

Non-clinical data had shown that a fractionated dose of TREO (up to 7.5 g/m² on three consecutive days) induce a stem-cell toxicity comparable to BU. Since TREO had a high cytotoxic effect on primitive and committed stem cells, an allogeneic transplantation was successfully performed without the need to add cyclophosphamide. Therefore, TREO was always administered on three consecutive days in all clinical trials. In addition, FLU had already been incorporated into non-myeloablative or reduced-intensity conditioning regimens because it inhibits DNA repair, has activity against lymphoid neoplasms, favourable toxicity profile and immunosuppressive properties. A previous pilot clinical study used a conditioning of 10 g/m²/d TREO (day -6 to -4) with FLU (30 mg/m²/d; day -6 to -2) in patients with various haematological malignancies and the regimen was reported to be well tolerated and effective.

Since sustained engraftment could be expected after administration of 10 g/m²/d × 3 and due to the malignant nature of the underlying disease, a dose escalation of TREO to 12 and 14 g/m²/d was used. Based on maximum tolerated dose (MTD) in the autologous setting, it was not planned to exceed the TREO dose beyond 42 g/m² (14 g/m²/d × 3).

So three doses of treosulfan (10, 12, 14 g/m²) IV infusion over 2 hours on 3 consecutive days (day –6 to day –4) were selected. In addition, fludarabine (30 mg/m²) was infused over 30 minutes after treosulfan to all patients from day -6 to day -2.

Stem cell donors were either HLA-identical siblings (MRD) or HLA-identical unrelated (MUD) or one mismatch (out of the 6 standard markers) siblings (1 misMRD).

A sample size of 15 patients per cohort was planned and patients were consecutively enrolled for each of the 3 dose group cohorts. The decision for dose escalation was based on the occurrence of pre-defined toxicities and general judgment of the safety committee.

Dose level	Evaluable patients (n)	Treosulfan (g/m²)	Fludarabine (mg/m²)
1	15	3 x 10	5 x 30
2	15	3 x 12	5 x 30
3	15	3 x 14	5 x 30

Endpoints:

- Engraftment: regeneration of granulopoiesis in peripheral blood (> 0.5 × 10^9/L neutrophilic granulocytes on the first of three consecutive days)
- Primary failure of engraftment: granulocyte count < 0.5 x 10^9/L on day +28 after transplantation
- Secondary failure of engraftment: disappearance of donor cells from the blood (granulocyte count < 0.5 x 10^9/L) after initial engraftment
- Disease status for the underlying disease according to standard criteria
- Donor-type chimerism in the recipient in BM and/or PB (complete chimerism if donor cells > 90%)

Assessment of time course and stability of donor-type haematopoiesis in the peripheral blood and bone marrow up to 6 months after transplantation.

In addition, AE, laboratory parameters, GvHD, transplant related mortality (TRM) and non- relapse mortality (NRM) were evaluated for safety assessment.

The protocol was not planned to define a MTD of treosulfan or to assess statistically significant differences of safety or efficacy parameters between the three dose groups.

Statistical methods

Descriptive analyses were based on the Full Analysis Set (FAS) stratified by treosulfan dosage and –if appropriate- by MRD vs. MUD-transplantation and CR vs. non-CR patients, respectively. Time-to-event data were analysed using Kaplan-Meier methods, log-rank tests were applied for exploratory comparisons of treatment groups. In case of competing events, nonparametric conditional and unconditional cumulative incidences were estimated, and comparisons were made using the test of Gray.

Results

Fifty six patients were enrolled, one patient was excluded from analysis (major protocol deviation) and 55 patients comprise the FAS for safety and efficacy.

Practically all patients (98 %) were considered non-eligible to standard conditioning regimens. Patients suffered from various haematological malignancies (AML: 35 %, mature B-cell neoplasms incl. MM and NHL: 36 %, MDS: 11 %, CML: 11 %, ALL: 4 %, T- and NK-cell neoplasms: 2 %) and had a median age of 50 years (range: 18 – 66 years).

Matched-related donor (47 %) and MUD (51 %) transplantations (with additional ATG-treatment in case of MUD) were allowed for patients in CR (42 %) or non-CR (51 %) at study entry.

A summary of results is shown in table below

Table 5: Efficacy results study MC-FludT.6/L (FAS)

TREO dose (g/m²/d)	**10** n=20	**12** n=18	**14** n=17
Conditional cumulative incidence reconstitution of granulopoiesis			
Day +14, n Day +28, n (%) Median time to engraftment, days	5 20 (100%) 15	7 18 (100%) 14	6 16 (94%) 14
Conditional cumulative incidence reconstitution of thrombopoiesis >20 × 10^9/L			
Day +14, n Day +28, n (%) Median time to engraftment, days	6 17 (85%) 15.5	8 15 (88%) 14	8 11 (65%) 14
Incidence of complete donor type chimerism			
Day +28 (%) Day + 100 (%) At 6 months (%) At 12 months (%)	70 95 95 95	88 94 94 94	73 93 93 93

Graft failure rates			
Primary graft failure (n) Secondary graft failure (n)	0 0	1 0	1 0
DFS			
DFS at 12 months (%) DFS at 24 months (%)	55 45	61 61	47 41
OS			
OS at 12 months (%) OS at 24 months (%)	70 60	78 78	59 53
Cumulative incidence of relapse/progression			
At 12 months (%) At 24 months (%)	30 40	29 29	24 24
Cumulative incidence of NRM			
At 12 months (%) At 24 months (%)	15 15	11 11	29 35
Cumulative incidence of TRM			
At 12 months (%) At 24 months (%)	11 23	11 11	30 30

Safety evaluations allowed no definite conclusions concerning any consistent relationship to treosulfan dose. Overall frequencies of grade III/IV AE categories mucositis/stomatitis, diarrhoea, hyperbilirubinemia and seizures were low (< 10%). No case of severe/life-threatening veno-occlusive disease (VOD) was reported. The two cases of severe/life-threatening seizures were not related to the conditioning regimen itself.

The higher incidence of NRM and TRM in 14 g/m^2 dose group should be viewed with caution, as this cohort was characterised by some poor prognostic features (e.g. comparably higher median duration of study disease, higher percentage of patients with > 2 pre-treatment lines, higher percentage of patients with MUD transplantation).

The data revealed that the conditioning treatment was tolerable and effective at all three dose levels. As the highest dosage (14 g/m²/d × 3) was safe with respect to Day +28 toxicity and to optimise efficacy against the underlying malignant disease, this dose was chosen for subsequent clinical trials.

2.5.2. Main study(ies)

MC-FludT.14/L Trial II

Methods

This was a randomised, parallel-group, open label, multicentre, group-sequential phase III non-inferiority trial to evaluate the efficacy and safety of treosulfan-based conditioning versus a busulfan-based RIC treatment prior to allogeneic HSCT in patients with AML or MDS considered ineligible to standard conditioning.

Study Participants

Inclusion criteria

1. Patients with AML or MDS per WHO 2008 indicated for allogeneic HSCT but at increased risk for standard conditioning if aged ≥ 50 years at transplant and/or had a HSCT -Comorbidity Index (HCT-CI) score > 2.
2. Availability of a HLA-identical sibling donor (MRD) or HLA-identical unrelated donor (MUD)..
3. Adult patients 18 to 70 years of age
4. Karnofsky Index ≥ 60%
5. Men capable of reproduction and women of childbearing potential had to consent to using a highly effective method of birth control while on treatment and for at least 6 months thereafter.

Exclusion Criteria

The first criterion was different in France, based on the French competent authority's request:

Applied to Germany, Hungary, Italy, Poland:

1. Patients with acute promyelocytic leukaemia with t(15;17)(q22;q12) and in CR1

Applied to France only:

- Patients with acute promyelocytic leukaemia with t(15;17)(q22;q12) and in CR1

  Patients with cytogenetic favourable AML ("low risk") and in CR1, who did not present unfavourable features like secondary or therapy-related AML or insufficient response to AML induction therapy.

  MDS patients with IPSS-R "very low risk" or "low risk" at trial entry, who did not present unfavourable clinical features during disease history like refractory severe thrombocytopaenia with severe bleeding complications, life-threatening infectious complications due to severe neutropaenia and/or very high red blood cell transfusion requirement and related complications.

Applied to all countries:

2. Patients considered contra-indicated for allogeneic HSCT due to severe concomitant illness (within 3 weeks prior to scheduled Day -6):
   - severe renal impairment (on dialysis or prior renal transplantation or S-creatinine > 3.0 x ULN or calculated creatinine-clearance < 60 ml/min)
   - severe pulmonary impairment, DLCOSB (Hb-adjusted)/or FEV1 < 50% or severe dyspnoea at rest or requiring oxygen supply
   - severe cardiac impairment diagnosed by ECG and LVEF < 40%
   - severe hepatic impairment with hyperbilirubinaemia > 3 x ULN or ALT / AST > 5 x ULN
3. Active malignant involvement of the CNS
4. HIV-positivity, active non-controlled infectious disease under treatment including active viral liver infection

5. Previous allogeneic HSCT
6. Pleural effusion or ascites > 1.0 L
7. Pregnancy or lactation
8. Known hypersensitivity to treosulfan, busulfan and/or related ingredients
9. Participation in another experimental drug trial within 4 weeks prior to Day –6 of the protocol
10. Psychiatric diseases or conditions that might compromise the ability to give informed consent.

Treatments

Test Treosulfan 10 g/m² BSA IV (2 hours infusion) on Day -4, -3, -2

(Treosulfan was given prior to fludarabine if both drugs were given on the same day)

Control Busulfan 3.2 mg/kg/d (4 x 0.8 mg/kg) IV (2 hours infusion) on Day -4, -3

All patients were to receive on Day 0 the allogeneic stem cell grafts from either a MRD or a MUD.

Mandatory for all patients:

o	Fludarabine	30 mg/m²/d	IV Day -6 to -2
o	Ciclosporin	5 mg/kg/day	PO Day -1 until day +100
	(level adapted, treatment starts IV 3 mg/kg/day)		
o	Methotrexate (MTX)	15 mg/m²	IV Day +1
		10 mg/m²	IV Day +3, +6
o	Ca-Folinate	15 mg/m²	IV Day +1
	(6 h after MTX)	10 mg/m²	IV Day +3, +6

o ATG for MUD only:

DE, IT, HU, PL:	ATG-S-Fresenius /Grafalon®	10 mg/kg IV Day -4, -3, -2
FR:	ATG-Thymoglobuline	2.5 mg/kg IV Day -2, -1

Dose modifications of fludarabine, ATG ciclosporin, MTX and Ca-folinate in accordance with the respective SmPCs were accepted if medically justified.

Mandatory for control arm:
Anticonvulsants (phenytoin/benzodiazepine).

Concomitant medication Day -6 until Day +28

It included prophylactic medication for HSOS and mucositis, and growth factors.

Interventions, which may impact on the primary objective (e.g., prophylactic or pre-emptive donor lymphocyte infusion (DLI), prophylactic/pre-emptive cytotoxic chemotherapy or radiotherapy after transplantation, but in the absence of relapse/disease progression), were not allowed.

Objectives

The primary objective was to compare EFS within 2 years after transplantation between treosulfan based conditioning and busulfan-based conditioning.

Outcomes/endpoints

Primary

- Event-free survival (EFS) within 2 years after transplantation, measured from time of end of HSCT (= day 0) to time of event. Events were defined as relapse of disease, graft failure or death (whatever occurred first).

  A primary graft failure required on Day +28 after transplant a neutrophil count ≤ 0.5 x 10^9/L and total WBC count ≤ 1 x 10^9/L (without previous engraftment or relapse/persisting disease), and donor-type chimerism of < 10% in bone marrow (BM).

  A secondary graft failure was documented in case a sustained decline of neutrophil counts ≤ 0.5 x 10^9/L and a total WBC count of ≤ 1 x 10^9/L was found in peripheral blood (PB) after initial engraftment, but in the absence of relapse or other conditions considered responsible for temporary decline of values.

Secondary

- Relapse/progression incidence (RI) within 2 years of HSCT.
- Overall survival (OS) as survival irrespective of disease status at any point in time within 2 years after HSCT.
- Non-relapse mortality (NRM) as dying without previous occurrence of a relapse or progression within 2 years after HSCT.
- Transplantation-related mortality (TRM) as all deaths due to GvHD, cardiac toxicity, pulmonary toxicity, interstitial pneumonitis, haemorrhage, hepatic sinusoidal obstruction syndrome (HSOS), skin toxicity, Epstein-Barr virus (EBV) proliferative disease, renal failure, gastrointestinal toxicity, rejection/poor graft function, CNS toxicity, multiple organ failure, infections (bacterial, viral, fungal, parasitic, unknown), or other HSCT-related causes until 2 years after transplant.
- Engraftment:
  - Granulocyte engraftment by specifying the first of 3 consecutive days with absolute neutrophilic granulocyte count > 0.5 x 10^9/L in PB.
  - Leukocyte engraftment by specifying the first of 3 consecutive days with total leukocyte count > 1 x 10^9/L in PB.
  - Platelet engraftment specifying the first of 3 consecutive days with a) platelets > 20 x 10^9/L and b) platelets > 50 x 10^9/L, in the absence of platelet transfusion.

  'Consecutive days' was defined as 3 consecutive blood samples if taken on different days.
- Donor-type chimerism: Complete donor-type chimerism was defined as when a donor to patient ratio of ≥ 95% was detected.

- GvHD-free and relapse/progression-free survival: time from HSCT to the incidence of acute GvHD of at least grade III, extensive chronic GvHD, relapse/progression, or death (whatever comes first), within 2 years after HSCT.
- Chronic GvHD-free and relapse/progression-free survival: time from HSCT to the incidence of extensive chronic GvHD, relapse/progression, or death (whatever comes first), within 2 years after HSCT.

Sample size

The sample size was calculated based on the experiment-wise one-sided type-I-error significance α = 2.5% under the hypothesis that treosulfan-based conditioning is equally effective to the comparator (HR = 1). The sample size was based on the following assumptions:

- The power of the trial was 80%.
- EFS-rate of 68.5% with busulfan-based conditioning
- Accrual rates of 10 patients per month in first six months, 15 patients per months thereafter till 24 months after re-start of the trial
- A 3% drop-out rate
- A non-inferiority margin on the hazard ratio of 1.3 (or 7.3% on absolute scale)

Based on these conditions at most 930 randomised patients, or 481 events, qualifying for the FAS were planned.

Randomisation

Randomisation in a 1:1 ratio used software SAS Version 9.4 and a permuted block technique.

Stratification factors:

- cytogenetic and/or molecular risk group for AML, or Revised International Prognostic Scoring System (IPSS-R) for MDS:
  - Risk group I: low risk and intermediate risk AML
    very low/low/intermediate IPSS-R MDS
  - Risk group II: high risk AML
    high/very high IPSS-R risk MDS
- donor type (MUD vs. MRD)
- transplantation centre

Blinding (masking)

No blinding was performed.

Statistical methods

Primary analysis

The primary objective was to demonstrate non-inferiority of treosulfan as an alternative conditioning agent to busulfan with respect to EFS. The non-inferiority margin on the hazard ratio scale was pre-specified as 1.3. If significant non-inferiority within the Per Protocol Set (PPS) could be shown, a sequential testing was to be applied starting with testing the non-inferiority within the Full Analysis Set (FAS). In case of statistical significance, superiority within the FAS with respect to the primary endpoint was to be tested based on the 'Points to Consider on Switching between Superiority and Non-inferiority (CPMP/EWP/482/99)'.

The distribution of the EFS was described using the Kaplain-Meir methods. These analyses were stratified by treatment arm and prognostic risk groups. For confirmatory analysis of non-inferiority of treosulfan-based conditioning a Cox proportional hazards regression model (stratified by centre and risk group) with donor type (MUD vs. MRD) and treatment as factors was applied for EFS.

Subgroup analyses

Exploratory data of Kaplan-Meier were summarised by means of forest plots showing EFS by donor type, risk group, combination of donor type and risk group, disease (AML and MDS), age group (< 50 years vs. ≥ 50 years), HCT-CI Score (≤ 2 vs. > 2), remission status in AML (CR1 vs. > CR1), disease status at trial entry in MDS (untreated vs. treated), risk group within AML patients, and risk group within MDS patients.

Sensitivity analyses

Sensitivity analyses were also performed to confirm the robustness of the analysis of the primary efficacy variable (EFS) within the FAS:

- Cox regression model with treatment as a factor only
- Cox regression model with treatment as a factor and site as strata variable
- Cox regression with treatment as a factor and disease (AML or MDS) as factors
- Inclusion of the disease (AML or MDS) as factor in the planned Cox regression model

Secondary analyses

For the secondary endpoints, data for the FAS and PPS were analysed.

For the endpoints OS, TRM, GvHD-free and relapse/progression-free survival, and chronic GvHD-free and relapse/progression-free survival and the exploratory endpoints time to deterioration of Karnofsky Performance Score (KPS) by at least 20 points and deterioration of KPS to less than 60 points, Kaplan-Meier estimates were calculated. A Cox proportional hazards regression model stratified by centre and risk group with donor type (MUD vs. MRD) and treatment as factors was fitted.

For the endpoints relapse/progression, and NRM, the probability over time was estimated by cumulative incidence rates. A Fine and Gray model with donor type as factor and risk group as stratum was applied to compare treatment arms.

For engraftment, the conditional cumulative incidence was estimated using conditional probability functions. The two-sided Pepe-Mori test was used to compare treatment arms.

Potential effects of covariates on the secondary endpoints were studied through subgroup analyses. As the ones described for EFS but the non-inferiority and superiority tests were omitted because the trial was designed to test non-inferiority with respect to EFS only.

Interim analyses

The trial was planned as a group-sequential trial with 3 interim analyses. The first interim was to be performed after 45 events or 220 patients to allow for a broad review of the benefits and risks of the dose reduction of the treatment regimen implemented with amendment 3. Stopping early due to proof of efficacy or futility was unlikely due to the low information. Further interim analyses were planned after 137 and 239 events occurred, or after 460 and 700 patients were randomised. The final analysis was planned after 481 events or inclusion of 930 patients at the latest.

Analysis populations

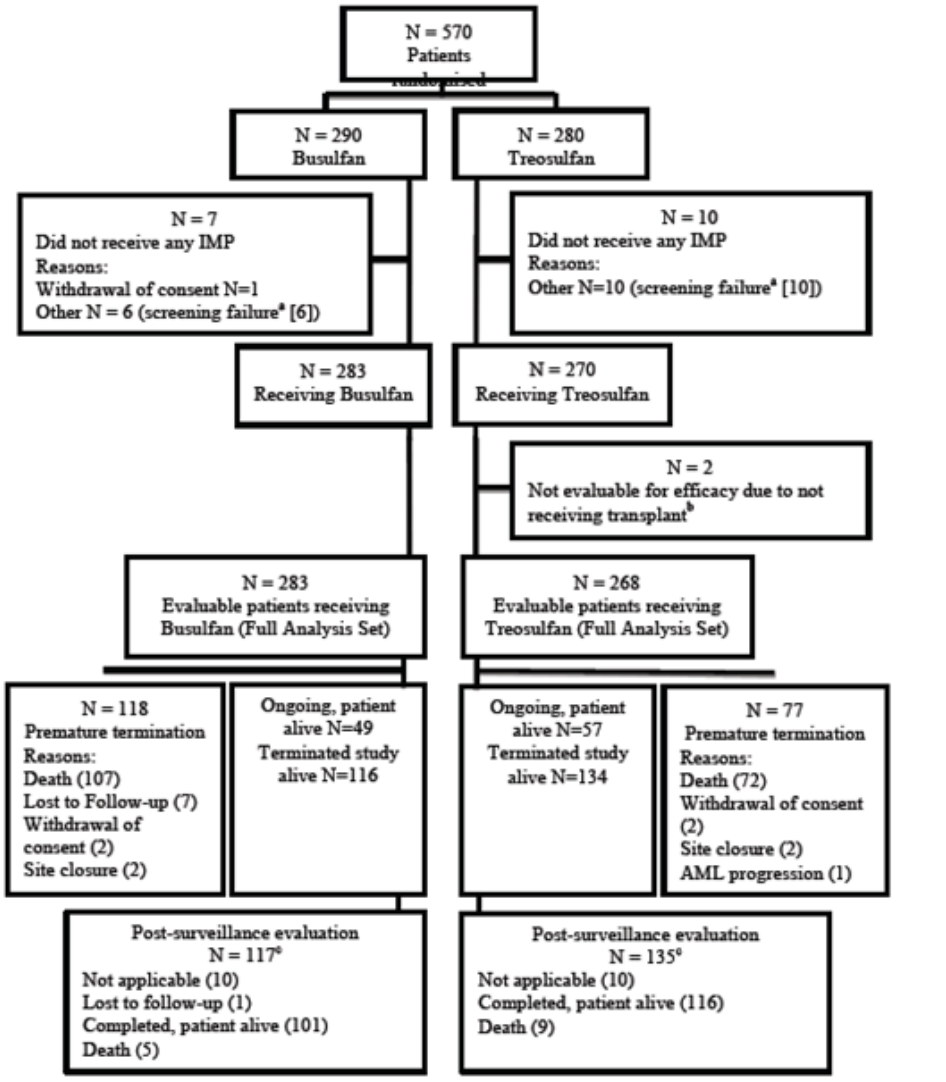
- Safety Analysis Set: all randomised patients treated at least one time with trial medication.
- Full Analysis Set (FAS): all randomised patients of the Safety Set with at least one efficacy parameter documented after baseline.
- Per Protocol Set (PPS): all patients of the FAS if they met all inclusion criteria, none of the exclusion criteria; correct allocation to treatment group; compliance with administration of trial medication; received short course MTX until Day +6 and ATG (if MUD) unless medical reasons for a deviation had been documented; lack of concomitant prophylactic/adjuvant DLI or cytotoxic therapy/radiotherapy after transplantation in the absence of relapse/disease progression.

Results

Participant flow

A total of 570 patients were randomised in Part II and a total of 476 patients subjected to statistical analysis (cut-off date 16 November 2016). A subsequent final analysis of all 570 randomised patients was conducted (cut-off date 16 March 2018).

Figure 6: Participant flow



Sources: Table 14.1.1I, Table 14.1.3A, Listing 16.2.1A, Listing 16.2.6D, Listing 16.2.3.A

[a] After randomisation, but before conditioning treatment, Investigator became aware of new information leading to patients no longer meeting the inclusion/exclusion criteria. [b] Cancellation of donor's clearance after start of conditioning treatment, no transplantation took place (1 patient); withdrew consent before HSCT (1 patient). [c] For 1 patient in each treatment group post-surveillance was filled in although the Month 24 visit was not done.

Recruitment

Multicentre study across 31 sites: Germany (20), Italy (6), France (2), Poland (2), and Hungary (1). The first patient was enrolled in 13-Jun-2013 and the last patient was enrolled on 07-Dec-2016.

Recruitment was stopped after the second interim analysis, at which point 476 of in total 570 randomised patients had been analysed.

Conduct of the study

Protocol amendments:

Inclusion of patients into part II of MC-FludT.14/L started after implementation of protocol amendment 03 (Clinical Trial Protocol V4.0, dated 20-Mar-2013).

Two further amendments were documented including the following significant changes:

- Amendment No. 04 (dated 10-Jul-2015):
    - A new PK sub-study was to be conducted due to dose reduction of treosulfan after amendment 03 and newly available bioanalytical methods.
- Amendment No.05 (dated 02-Dec-2016):
    - Clarified the DMC was to meet on a yearly basis until the last patient enrolled had been treated. Thereafter, no changes in trial conduct could result from DMC recommendations. The DMC was to meet at pre-specified time points of confirmatory efficacy analyses.
    - The range of permissible IMP dose deviation (previously < 10%) was aligned with the exclusion criteria of patients from the PPS (deviation of at most ± 20%).
    - The definition of engraftment after HSCT was amended to include that “consecutive days” was defined as 3 consecutive blood samples, if these were taken on different days. The second and third samples should have been taken on the next consecutive days whenever this was possible; exceptions were accepted.

Changes in Planned Analyses

- Two additionally secondary endpoints were included in the analysis, GvHD-free and relapse/progression-free survival (GRFS) as well as chronic GvHD-free and relapse/progression-free survival (CRFS). They were pre-specified in the SAP prior to database lock and unblinding.
- Two exploratory endpoints were included in the analysis (time to deterioration of KPS by at least 20 points and the time to deterioration of KPS to less than 60 points) as they are considered a clinically relevant description of the patient’s activity and quality of life under treatment and during follow-up.

Protocol deviations

A total of 33.8% of patients had a major protocol deviation. Protocol deviations were slightly more common in the busulfan group than in the treosulfan (37.0% vs 30.4%). “Missing evaluation at baseline” was the most common, reported for 11.0% of patients in the busulfan group and 8.3% in treosulfan.

Baseline data

Demographic baseline data in FAS was balanced across arms with more male (60.7%) than female (39.3%) patients in the study, of mean age 59.6 (SD 6.3) years and mean body weight of 80.1 (SD 17.5) Kg.

More patients with AML were included in the treosulfan arm than in control whilst more patients with MDS were included in the control than in treosulfan arm.

AML

Of the 460 patients in the FAS, 293 had AML with 138 treated with busulfan and 155 with treosulfan.

The majority of patients (93.5%) had cytogenetic marker examinations, and 39.6% had a cytogenetic marker detected. The frequency of individual cytogenetic markers was similar in each group, and no single marker was detected for more than 10% of either group.

The majority of patients had a molecular marker examination (91.8%), and just under half of the patients (49.1%) had a molecular marker detected. Slightly more patients in the treosulfan group had a molecular marker detected (53.5%) than in the busulfan (44.2%). NPM1 and FLT3-ITD were the most common markers in both groups, and the only specific markers found in more than 10% of patients.

Table 6: Summary of study disease characteristics (FAS: AML)

	Busulfan (N=138)	Treosulfan (N=155)	Total (N=293)
Time between diagnosis and HSCT [months]			
Mean (SD)	7.69 (7.99)	8.15 (7.23)	7.93 (7.59)
Median (Q1, Q3)	5.14 (3.52, 8.25)	5.32 (3.88, 9.36)	5.26 (3.68, 8.51)
Min, Max	1.2, 56.2	1.7, 46.9	1.2, 56.2
Classification of AML [n (%)]			
Any category	138 (100.0%)	155 (100.0%)	293 (100.0%)
AML with myelodysplasia-related changes	29 (21.0%)	30 (19.4%)	59 (20.1%)
AML with maturation	23 (16.7%)	21 (13.5%)	44 (15.0%)
AML with mutated NPM1	17 (12.3%)	27 (17.4%)	44 (15.0%)
AML without maturation	19 (13.8%)	21 (13.5%)	40 (13.7%)
AML, Acute myelomonocytic leukaemia	16 (11.6%)	10 (6.5%)	26 (8.9%)
AML, Acute monoblastic and monocytic leukaemia	8 (5.8%)	14 (9.0%)	22 (7.5%)
AML with minimal differentiation	7 (5.1%)	9 (5.8%)	16 (5.5%)
AML, Therapy-related myeloid neoplasms	3 (2.2%)	6 (3.9%)	9 (3.1%)
AML with t(8;21)(q22;q22); RUNX1-RUNX1T1	3 (2.2%)	3 (1.9%)	6 (2.0%)
AML, Acute erythroid leukaemia	4 (2.9%)	1 (0.6%)	5 (1.7%)
AML with mutated CEBPA	1 (0.7%)	3 (1.9%)	4 (1.4%)
AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); (CBFbeta-MYH11)	0 (0.0%)	3 (1.9%)	3 (1.0%)
AML, Acute megakaryoblastic leukaemia	1 (0.7%)	2 (1.3%)	3 (1.0%)
AML, not otherwise categorised (unspecified)	2 (1.4%)	1 (0.6%)	3 (1.0%)
AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1	1 (0.7%)	1 (0.6%)	2 (0.7%)
AML with recurrent genetic abnormalities (unspecified)	0 (0.0%)	2 (1.3%)	2 (0.7%)
AML with t(9;11)(p22;q23); MLLT3-MLL	2 (1.4%)	0 (0.0%)	2 (0.7%)
AML, Acute basophilic leukaemia	1 (0.7%)	0 (0.0%)	1 (0.3%)
AML, Blastic plasmacytoid dendritic cell neoplasm	0 (0.0%)	1 (0.6%)	1 (0.3%)
AML, Mixed phenotype acute leukaemia, T/myeloid, NOS	1 (0.7%)	0 (0.0%)	1 (0.3%)

Remission status AML [n (%)]			
CR1	117 (84.8%)	133 (85.8%)	250 (85.3%)
>CR1	21 (15.2%)	22 (14.2%)	43 (14.7%)
Blast count in bone marrow [%]			
n	138	154	292
Mean (SD)	2.12 (1.58)	1.97 (1.29)	2.04 (1.43)
Median (Q1, Q3)	2.50 (1.00, 3.00)	2.50 (1.00, 2.50)	2.50 (1.00, 3.00)
Min, Max	0.0, 11.5	0.0, 5.0	0.0, 11.5
Missing	0	1	1
AML risk group stratification [n (%)]			
Low risk	13 (9.4%)	15 (9.7%)	28 (9.6%)
Intermediate risk	61 (44.2%)	55 (35.5%)	116 (39.6%)
High risk	43 (31.2%)	63 (40.6%)	106 (36.2%)
NA if > CR1	21 (15.2%)	22 (14.2%)	43 (14.7%)

MDS

Of the 460 patients in the FAS, 167 had MDS with 102 treated with busulfan and 65 with treosulfan.

Most patients (78.4% total MDS patients) had de novo MDS and had a cytogenetic marker detected (58.7% total MDS patients). Detected markers were complex and similar for both groups. The classification of MDS disease was similar both treatment groups.

However, there were more patients in the treosulfan group (vs busulfan) with untreated MDS (52.3% vs 41.2%), dependent on RBC transfusions (64.6% vs 54.9%), very low risk (7.7% vs 1.0%) or low risk (20.0% vs 15.7%). Correspondingly, more patients rated as intermediate risk were in the busulfan group (29.4%) than the treosulfan group (16.9%).

Table 7: Summary of study disease characteristics (FAS: MDS)

	Busulfan (N=102)	**Treosulfan (N=65)**	**Total (N=167)**
Time between diagnosis and HSCT [months]			
n	100	65	165
Mean (SD)	14.17 (18.99)	15.93 (24.52)	14.86 (21.28)
Median (Q1, Q3)	7.59 (4.88, 14.09)	7.62 (4.47, 16.99)	7.62 (4.76, 16.16)
Min, Max	0.2, 128.3	0.5, 135.9	0.2, 135.9
Missing	2	0	2
Etiology [n (%)]			
De novo MDS	80 (78.4%)	51 (78.5%)	131 (78.4%)
Therapy-related MDS	22 (21.6%)	14 (21.5%)	36 (21.6%)
Classification of MDS [n (%)]			
Any category	102 (100.0%)	65 (100.0%)	167 (100.0%)
MDS, Refractory anaemia with excess blasts -2	40 (39.2%)	21 (32.3%)	61 (36.5%)
MDS, Refractory cytopenia with multilineage dysplasia (unspecified)	31 (30.4%)	22 (33.8%)	53 (31.7%)
MDS, Refractory anaemia with excess blasts -1	23 (22.5%)	19 (29.2%)	42 (25.1%)
MDS, Myelodysplastic syndrome associated with isolated del(5q)	2 (2.0%)	1 (1.5%)	3 (1.8%)
MDS, Myelodysplastic syndrome, unclassifiable	3 (2.9%)	0 (0.0%)	3 (1.8%)
MDS, Refractory cytopenia with unilineage dysplasia	1 (1.0%)	2 (3.1%)	3 (1.8%)
MDS, Refractory anaemia with ringed sideroblasts	1 (1.0%)	0 (0.0%)	1 (0.6%)
MDS, Refractory thrombocytopenia	1 (1.0%)	0 (0.0%)	1 (0.6%)

	Busulfan (N=102)	Treosulfan (N=65)	Total (N=167)
Disease status MDS [n (%)]			
Untreated	42 (41.2%)	34 (52.3%)	76 (45.5%)
Treated	60 (58.8%)	31 (47.7%)	91 (54.5%)
Blast count in bone marrow [%]			
n	101	64	165
Mean (SD)	6.29 (4.77)	5.94 (4.88)	6.16 (4.80)
Median (Q1, Q3)	5.00 (2.50, 10.00)	5.00 (2.00, 8.50)	5.00 (2.50, 9.00)
Min, Max	0.0, 19.0	0.0, 19.0	0.0, 19.0
Missing	1	1	2
RBC transfusion dependency [n (%)]			
No	46 (45.1%)	23 (35.4%)	69 (41.3%)
Yes	56 (54.9%)	42 (64.6%)	98 (58.7%)
Prognostic scoring: IPSS-R			
Median (Q1, Q3)	5.00 (3.50, 6.50)	5.00 (3.00, 6.50)	5.00 (3.50, 6.50)
Min, Max	1.0, 9.5	1.0, 9.0	1.0, 9.5
MDS risk group stratification based on IPSS-R [n (%)]			
Very low risk	1 (1.0%)	5 (7.7%)	6 (3.6%)
Low risk	16 (15.7%)	13 (20.0%)	29 (17.4%)
Intermediate risk	30 (29.4%)	11 (16.9%)	41 (24.6%)
High risk	24 (23.5%)	16 (24.6%)	40 (24.0%)
Very high risk	31 (30.4%)	20 (30.8%)	51 (30.5%)
Prognostic scoring: WPSS [n (%)]			
0	1 (1.0%)	0 (0.0%)	1 (0.6%)
1	12 (11.8%)	8 (12.3%)	20 (12.0%)
2	24 (23.5%)	14 (21.5%)	38 (22.8%)
3	28 (27.5%)	22 (33.8%)	50 (29.9%)
4	19 (18.6%)	12 (18.5%)	31 (18.6%)
5	12 (11.8%)	6 (9.2%)	18 (10.8%)
6	5 (4.9%)	2 (3.1%)	7 (4.2%)
Missing	1 (1.0%)	1 (1.5%)	2 (1.2%)

Donor type

Overall, 75.9% of patients received a HSCT from a MUD and 24.1% from a MRD, with similar frequencies in the 2 treatment groups. The majority of patients received HLA class I matched HSCT (86.5% total patients), and almost all patients received HLA class II matched HSCT (96.1% total patients). More patients with AML received MRD HSCTs (27.3%) than patients with MDS (18.6%). The frequency of HLA class I and II matched donors was similar in patients with AML and MDS, and in both treatment groups.

Concomitant illness

Overall, 82.6% of patients reported concomitant illnesses at baseline and data, including KPS and HCT-CI, were comparable for the 2 treatment groups. There were 2 noteworthy differences for MRD patients: fewer patients in the busulfan group (vs treosulfan) with HCT-CI score >2 (54.2% vs 69.2%), and with risk group I with a HCT-CI score > 2 (54.5% vs 61.6%).

Numbers analysed

Table 8: Analysis sets

Analysis set	Busulfan	Treosulfan	Total
All patients	246	230	476
Safety	240	221	461
FAS	240	220	460
PPS	234	215	449

A total of 16 patients were excluded from the FAS (2.4% in the busulfan group and 4.3% in the treosulfan group). 15/16 patients did not receive the IMP (6:9) and 1 patient in the treosulfan arm was not evaluable for efficacy due to not receiving transplant.

Outcomes and estimation

The first interim analysis was performed after 40 events had been observed. The DMC recommended to continue with the trial.

The second interim analysis was performed after enrolment of 460 patients who qualified for the FAS based on 34.1% (164) events (data cut off 19 August 2016).

All time to event variables were calculated with date of transplant (day 0) as start date.

EFS

The primary endpoint, EFS within 24 months after HSCT, was reported in the PPS for busulfan versus treosulfan as 51.1% (95% CI: 43.4%, 58.2%) vs 63.5% (95% CI: 55.4%, 70.5%), HR 0.67 (95% CI: 0.48, 0.93), one-sided p-value of 0.0000424 (adjusted for strata). This result showed statistically significant non-inferiority of treosulfan compared to busulfan with the p value far below the one-sided significance level of 0.000149 required for this interim analysis.

Confirmatory testing for non-inferiority of treosulfan versus busulfan in the FAS was demonstrated, with EFS at 24 months of 50.4% (95% CI: 42.8, 57.5) in the busulfan group, and 64.0% (95% CI: 56.0, 70.9) in the treosulfan, HR 0.65 (95% CI: 0.47, 0.90), one sided p-value 0.0000164.

Superiority testing of treosulfan vs busulfan gave a p-value of 0.0051268 (FAS, adjusted for strata) below the criteria set (nominal one-sided significance level 0.000149).

The DMC recommended to stop recruitment in November 2016 since the primary objective of non-inferiority of treosulfan versus busulfan had been achieved. This final analysis with 476 patients included in this second interim analysis, 460 patients qualifying for the FAS, constitutes the final analysis of this trial. A close out plan was implemented during which the data of subjects in the interim analysis were further cleaned. This led to database lock on 31 May 2017. That constitutes the final analysis and the amount of alpha spent was recalculated based on the updated number of 34.9% (168) of total events, per protocol.

The study continues to follow-up patients until 1 year after transplantation of the last randomised patient, with a planned analysis of all 570 randomised patients in 2018.

Table 9 EFS results – Study MC-FludT.14/L Trial II

Study population	**FAS**		**PPS**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Number of patients	220	240	215	234
Median follow-up[a], months (range)	15.4 (3.2, 26.4)	17.4 (3.0, 26.3)	15.4 (3.2, 26.4)	17.4 (3.0, 26.3)
Patients with events	30.9%	41.7%	31.2%	41.5%
Death[b]	23 (10.5%)	41 (17.1%)	22 (10.2%)	38 (16.2%)
Relapse/Progression[b]	45 (20.5%)	51 (21.3%)	45 (20.9%)	51 (21.8%)
Primary Graft failure[b]	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.4%)
Secondary Graft failure[b]	0 (0.0%)	7 (2.9%)	0 (0.0%)	7 (3.0%)
Patients without events	69.1%	58.3%	68.8%	58.5%
EFS at 12 months[b]; % (95% CI)	67.5 (60.3, 73.6)	58.5 (51.4, 64.9)	67.1 (59.8, 73.3)	58.7 (51.5, 65.2)
EFS at 24 months[b]; % (95% CI)	64.0 (56.0, 70.9)	50.4 (42.8, 57.5)	63.5 (55.4, 70.5)	51.1 (43.4, 58.2)
Hazard ratio[c] (95% CI)	0.65 (0.47, 0.90)		0.67 (0.48, 0.93)	
Hazard ratio[cde] (99.9702% CI)	0.65 (0.36, 1.19)		0.67 (0.37, 1.23)	
P-value[cde] for testing non-inferiority	0.0000164		0.0000424	
P-value[cd] for testing superiority	0.0051268		0.0090454	
P-value[c] for testing difference	0.0102535		0.0180908	

[a] Based on reverse Kaplan-Meier estimates for overall survival.
[b] Based on Kaplan-Meier estimates.
[c] Adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[d] The nominal one-sided significance level resulting from an O'Brien-Fleming type of group-sequential efficacy stopping boundary is 0.000149
[e] The non-inferiority margin for the hazard ratio is 1.3.

Table 10: Sensitivity analyses for EFS- FAS

Model	**HR (99.9702% CI for HR)**	**p-value**
Cox Regression model with treatment as factor only (1)	0.70 (0.39, 1.23)	0.0000370
Model (1) + site as strata	0.69 (0.38, 1.23)	0.0000359
Model (1) + disease (AML + MDS)	0.71 (0.40, 1.27)	0.0000835
Model (1) + site + disease (AML + MDS) + risk groups	0.67 (0.37, 1.24)	0.0000463
Model (1) + site + disease (AML + MDS) + risk groups + donor type	0.66 (0.36, 1.19)	0.0000160

Table 11: Summary results of relapse / progression (FAS)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	52 (21.7%)	45 (20.5%)
Patients without event	188 (78.3%)	175 (79.5%)
Relapse/progression at 12 months[a] [%] (95% CI)	24.9 (19.4, 31.7)	22.8 (17.3, 29.7)
Relapse/progression at 24 months[a] [%] (95% CI)	25.9 (20.2, 32.9)	26.8 (20.2, 35.0)
Hazard Ratio (Treosulfan/Busulfan)[b] (95% CI)	0.77 (0.50, 1.17)	
p-value[b]	0.2153	

Note: Relapse/progression as event, censoring at last assessment of relapse/progression status (or at date of death if no assessment of relapse/progression status is available).
[a] based on Kaplan-Meier estimates
[b] adjusted for donor type as factor, and risk group and centre as strata using Cox regression model

Table 24 Summary results of graft failures (Full analysis set)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	8 (3.3%)	0 (0.0%)
Patients without event	232 (96.7%)	220 (100.0%)
Graft failure at 12 months[a] [%] (95% CI)	4.5 (2.2, 9.1)	0.0 (0.0, 0.0)
Graft failure at 24 months[a] [%] (95% CI)	4.5 (2.2, 9.1)	0.0 (0.0, 0.0)
Hazard Ratio (Treosulfan/Busulfan)[b] (95% CI)	NA (NA, NA)	
Adjusted p-value[b]	NA	
p-value[c]	0.0056	

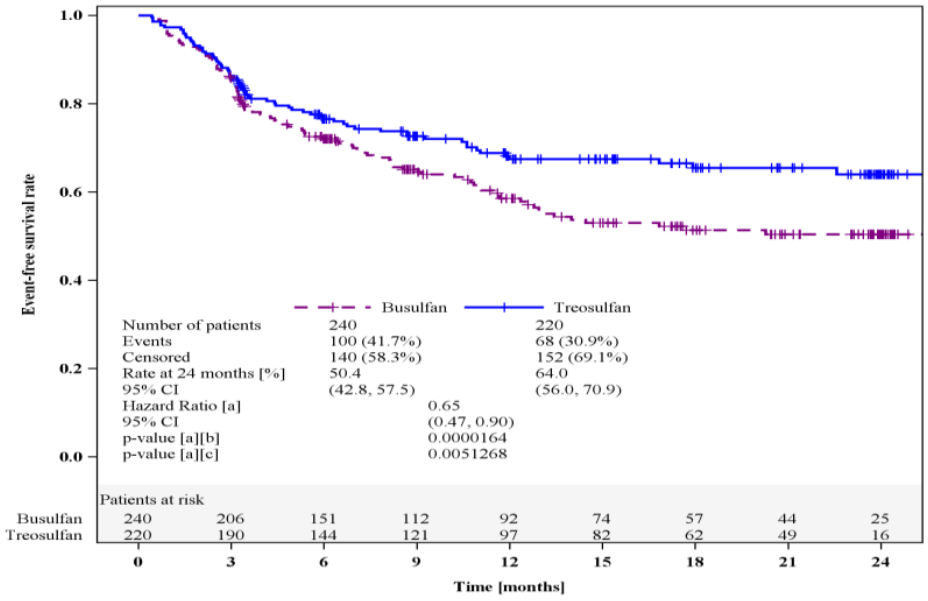
Note: Graft failure as event, censoring at last assessment of relapse/progression status (or at date of death if no assessment of relapse/progression status is available).
[a] based on Kaplan-Meier estimates
[b] adjusted for donor type as factor, and risk group and centre as strata using Cox regression model
[c] Log-rank test
[Program: Day120_CTR_IR02 / SurvivalRELGF / t_gf_fas]

Table 25 Summary of overall survival (Full analysis set)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	82 (34.2%)	52 (23.6%)
Patients without event	158 (65.8%)	168 (76.4%)
Overall survival at 12 months[a] [%] (95% CI)	67.8 (60.8, 73.8)	75.3 (68.4, 80.8)
Overall survival at 24 months[a] [%] (95% CI)	56.4 (48.4, 63.6)	72.5 (65.1, 78.6)
Hazard Ratio (Treosulfan/Busulfan)[b] (95% CI)	0.61 (0.42, 0.88)	
p-value[b]	0.0082	

Sources: Table 14.2.3A, Table 14.2.3B, Listing 16.2.6F
CI = confidence interval; N = number of patients.
[a] Based on Kaplan-Meier estimates.
[b] Adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.

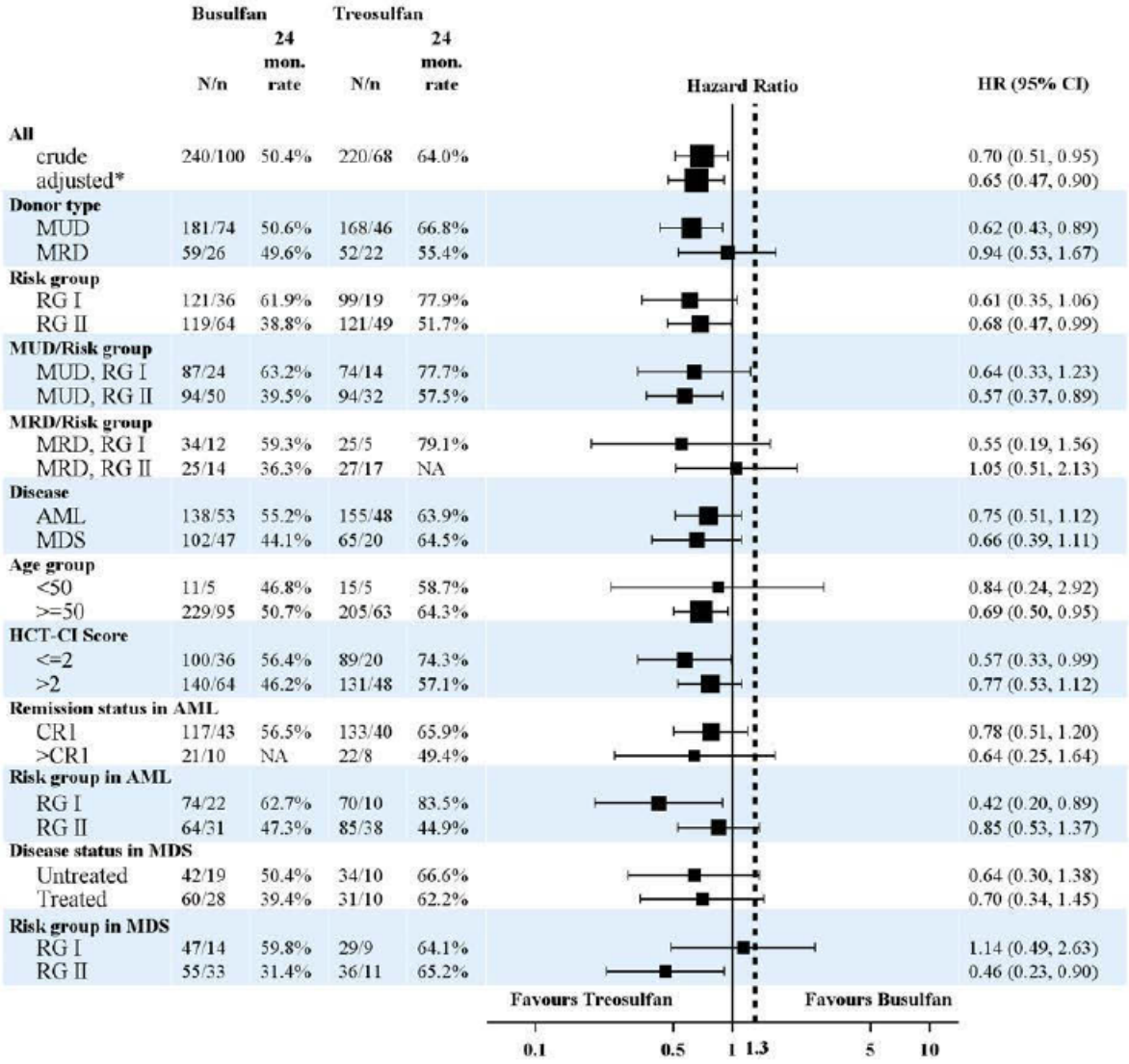
Figure 7: Kaplan-Meier estimates of EFS (FAS)



[a] Adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[b] For testing non-inferiority of Treosulfan compared to Busulfan.
[c] For testing superiority of Treosulfan compared to Busulfan.

Forest plots for EFS by prognostic factors and combinations of prognostic factors show that results of the subgroup analyses were consistent with the analysis of the total population. Other than MRD risk group II and MDS risk group I, HRs for each subgroup were in favour of treosulfan (HR < 1.0). Similar results were shown for PPS population.

Figure 8: Forest plot for EFS by prognostic factors (FAS)



Sources: Figure 14.2.1B, Table 14.2.1A, Table 14.2.1C, Table 14.2.1D, Table 14.2.1E, Table 14.2.1F, Table 14.2.1G, Table 14.2.1H, Table 14.2.1I, Listing 16.2.6F

AML = acute myeloid leukaemia; CI = confidence interval; CR = complete remission; HCT-CT = hematopoietic cell transplantation-comorbidity index; HR = hazard ratio; MDS = myelodysplastic syndrome; MRD = matched related donor; MUD = matched unrelated donor; N = number of patients; n = number of events; RG = risk group.

* adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.

Cox regression models with different prognostic subgroups as factors or strata performed as pre-planned sensitivity analyses confirmed the favourable outcome of the primary analysis.

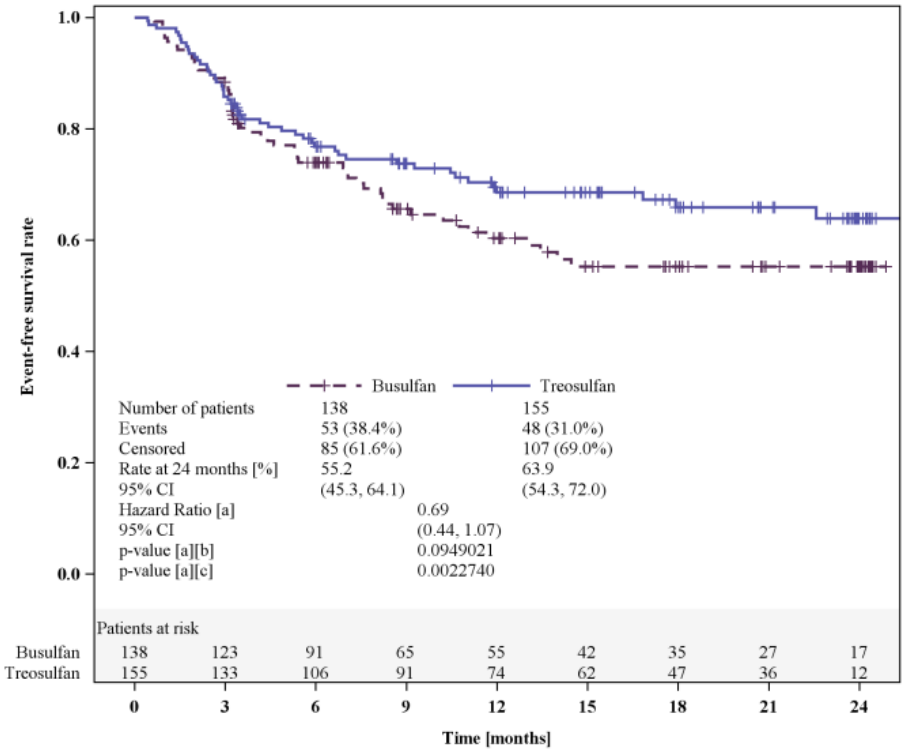
Table 12: Disease-specific EFS (FAS) - results for AML and MDS

Study	**MC-FludT.14/L Trial II**	
Treatment arm	**TREO**	**BU**
AML		
Number of patients	155 (100%)	138 (100%)
EFS at 12 months[a]; % (95% CI)	68.6 (60.1, 75.6)	60.3 (50.9, 68.5)
EFS at 24 months[a]; % (95% CI)	63.9 (54.3, 72.0)	55.2 (45.3, 64.1)
HR[b] TREO vs. BU (95% CI)	0.69 (0.44, 1.07)	
P-value[b] for testing non-inferiority	0.0022740	
P-value[b] for testing difference	0.0949021	
MDS		
Number of patients	65 (100%)	102 (100%)
EFS at 12 months[a] (%) [95% CI]	64.5 (50.0, 75.8)	56.0 (44.9, 65.8)
EFS at 24 months[a] (%) [95% CI]	64.5 (50.0, 75.8)	44.1 (32.4, 55.2)
HR[b] TREO vs. BU (95% CI)	0.59 (0.32, 1.09)	
P-value[b] for testing non-inferiority	0.0057999	
P-value[b] for testing difference	0.0915030	

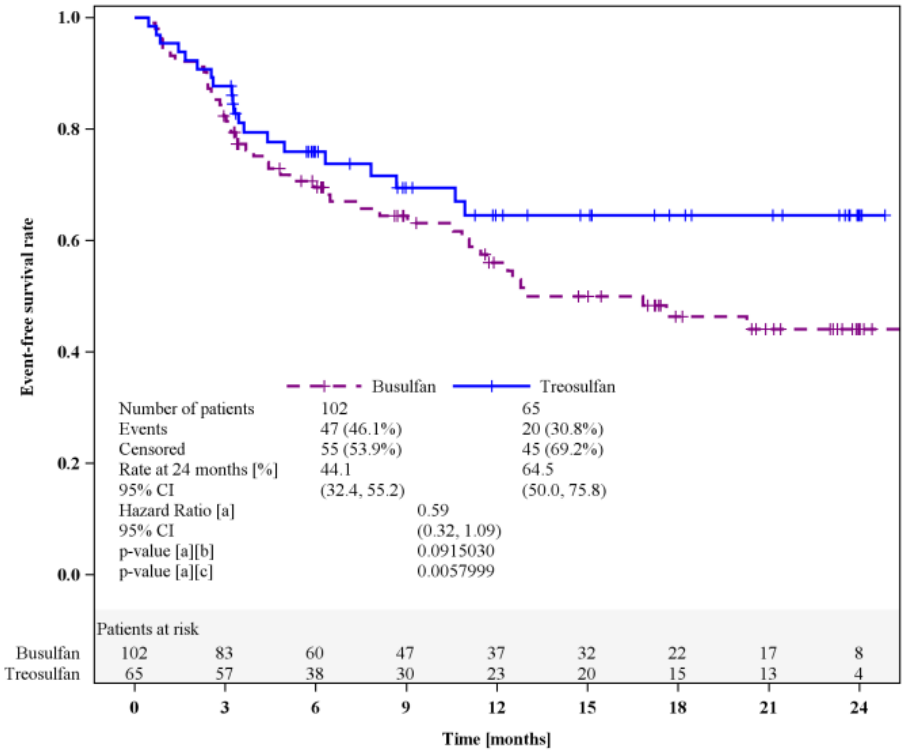
[a] based on KM estimates

[b] adjusted for donor type, risk group and centre using Cox regression model

Figure 9: Kaplan-Meier estimates of EFS per disease in MC-FludT.14/L II (Left: AML; Right: MDS)



[a] adjusted for donor type as factor, and risk group and centre as strata using Cox regression model
[b] for testing difference of Treosulfan compared to Busulfan
[c] for testing non-inferiority of Treosulfan compared to Busulfan



[a] adjusted for donor type as factor, and risk group and centre as strata using Cox regression model
[b] for testing difference of Treosulfan compared to Busulfan
[c] for testing non-inferiority of Treosulfan compared to Busulfan

Overall Survival

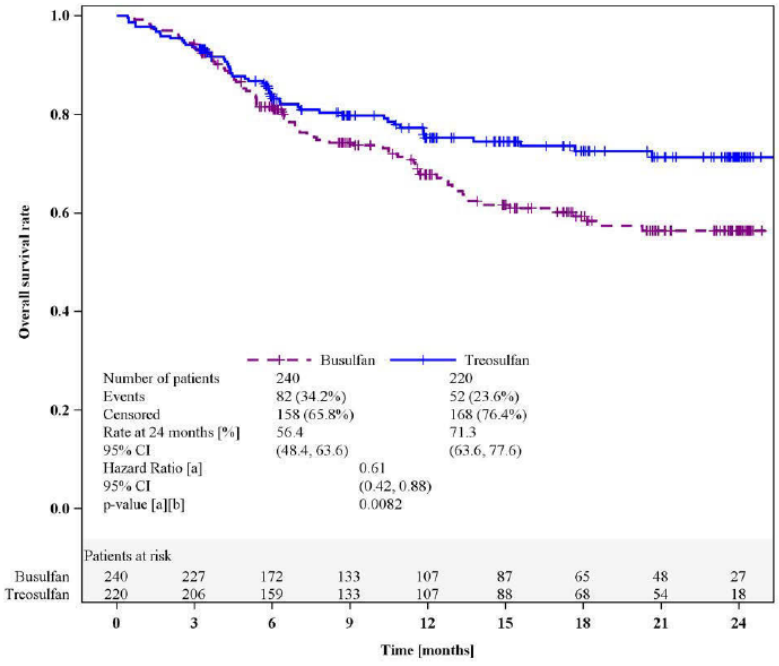
OS (at 24 months after HSCT) was statistically significantly higher in the treosulfan group compared to busulfan (HR 0.61, 95% CI: 0.42, 0.88; adjusted p-value=0.0082). The median OS for each treatment group are not available.

Table 13: Summary results of overall survival (FAS)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	82 (34.2%)	52 (23.6%)
Patients without event	158 (65.8%)	168 (76.4%)
Overall survival at 12 months[a] [%] (95% CI)	67.8 (60.8, 73.8)	75.3 (68.4, 80.8)
Overall survival at 24 months[a] [%] (95% CI)	56.4 (48.4, 63.6)	71.3 (63.6, 77.6)
Hazard Ratio (Treosulfan/Busulfan)[b] (95% CI)	0.61 (0.42, 0.88)	
Adjusted p-value[b]	0.0082	
p-value[c]	0.0186	

Sources: Table 14.2.3A, Table 14.2.3B, Listing 16.2.6F
CI = confidence interval; N = number of patients.
[a] Based on Kaplan-Meier estimates.
[b] Adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[c] Log-rank test.

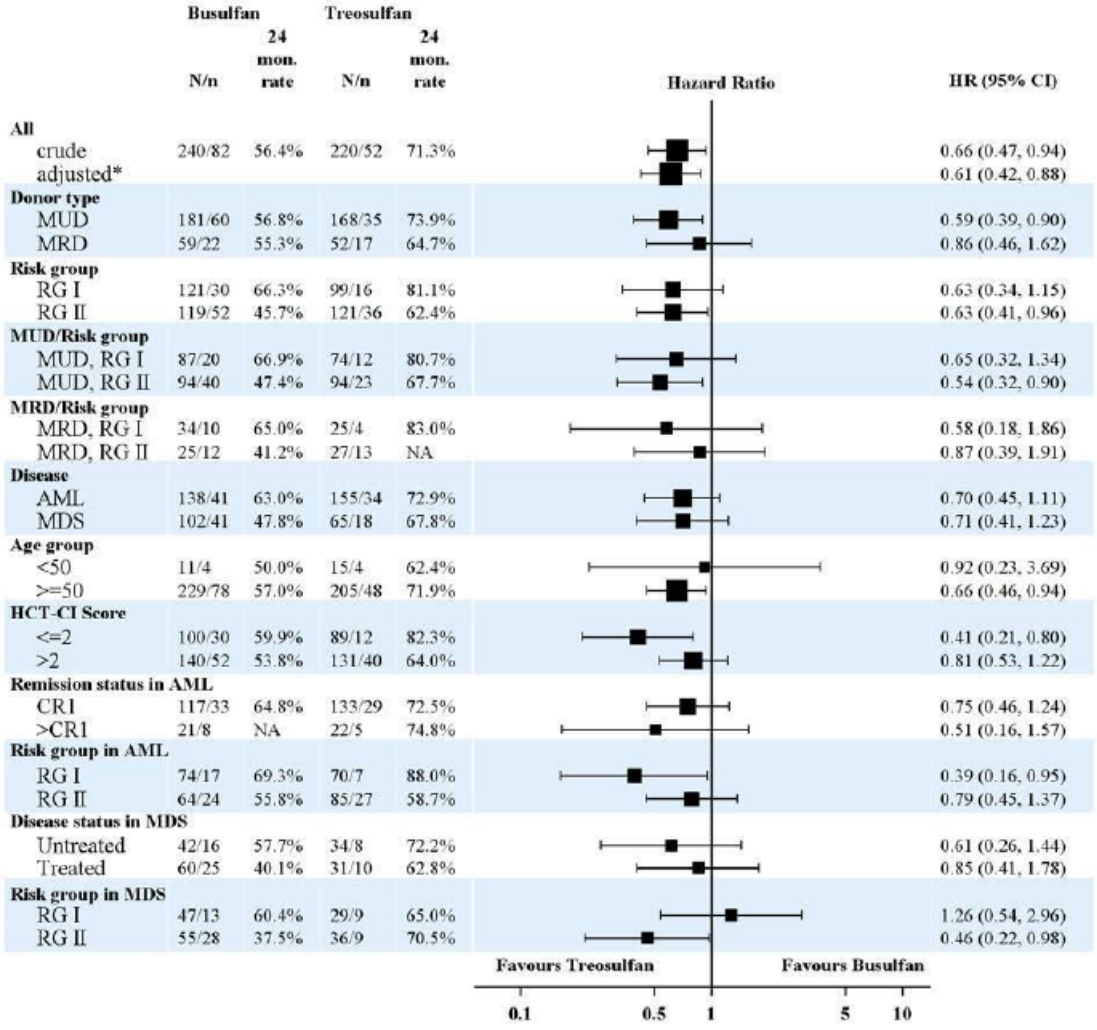
Figure 10: Kaplan-Meier estimates of overall survival (Full Analysis Set)



Sources: Figure 14.2.3A, Table 14.2.3A, Table 14.2.3B, Listing 16.2.6F
[a] adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[b] for testing the difference of Treosulfan compared to Busulfan.

Forest plots of OS by prognostic factors and combinations of prognostic factors were consistent with the analysis of the total population. Other than MDS risk group I, HRs for each subgroup were in favour of treosulfan (HR < 1.0).

Figure 4. Forest plot for OS by prognostic factors (FAS)



N = Number of patients, n = Number of events, RG = Risk group
* adjusted for donor type as factor, and risk group and centre as strata using Cox regression model
p-value adjusted for donor type as factor, and risk group and centre as strata using Cox regression model for testing difference of Treosulfan compared to Busulfan: 0.0082

The results in the PPS population are similar to the FAS.

Relapse/Progression Incidence

There was no statistical difference for relapse/progression within 24 months after HSCT between treatments (p=0.5017, adjusted for donor-type as factor, and risk group as stratum using Fine and Gray model). The HR was 0.87 (95% CI: 0.59, 1.30) in favour of treosulfan. The data for the PPS were similar.

Table 13. Summary table of relapse/progression (FAS)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	51 (21.3%)	45 (20.5%)
Patients without event (censored) or with competing event	189 (78.8%)	175 (79.5%)
Censored	140 (58.3%)	152 (69.1%)
Death[a]	41 (17.1%)	23 (10.5%)
Primary Graft Failure[a]	1 (0.4%)	0 (0.0%)
Secondary Graft Failure[a]	7 (2.9%)	0 (0.0%)
Cumulative incidence at 12 months [%] (95% CI)	22.6 (17.0, 28.2)	21.1 (15.3, 26.8)
Cumulative incidence at 24 months [%] (95% CI)	23.3 (17.6, 29.0)	24.6 (17.8, 31.3)
Hazard Ratio (Treosulfan/Busulfan)[b] (95% CI)	0.87 (0.59, 1.30)	
Adjusted p-value[b]	0.5017	
p-value[c]	0.7422	

Sources: Table 14.2.5A, Table 14.2.5B, Listing 16.2.6F
CI = confidence interval; N = number of patients.
[a] Only if this event occurred first.
[b] Adjusted for donor type as factor and risk group as stratum using Fine and Gray model.
[c] Based on test of Gray.

Graft Failure

For the FAS one patient (0.4%) in the busulfan group experienced a primary graft failure, and 7 patients (3.0%) in the busulfan group experienced a secondary graft failure. No patients in the treosulfan group experienced a primary or secondary graft failure.

The data for the PPS were similar.

Non-Relapse Mortality (NRM)

At the time of analysis, 41 patients (17.1%) in the busulfan group and 23 patients (10.5%) in the treosulfan group had died without relapse/progression. The cumulative incidence of NRM in the busulfan group was 15.2% at 12 months, and 22.6% at 24 months compared to cumulative incidence of NRM 11.4% in the treosulfan group at 12 months that did not increase further up to 24 months. The difference between the treatment groups fell just short of statistical significance (p=0.0530) with HR 0.60 (95% CI: 0.36, 1.01) in favour of treosulfan. The data for the PPS were similar.

Table 14. Summary table of non-relapse mortality (FAS)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	41 (17.1%)	23 (10.5%)
Patients without event (censored) or with competing event	199 (82.9%)	197 (89.5%)
Censored	140 (58.3%)	152 (69.1%)
Relapse/Progression[a]	51 (21.3%)	45 (20.5%)
Primary Graft Failure[a]	1 (0.4%)	0 (0.0%)
Secondary Graft Failure[a]	7 (2.9%)	0 (0.0%)
Cumulative incidence at 12 months [%] (95% CI)	15.2 (10.2, 20.3)	11.4 (7.0, 15.9)
Cumulative incidence at 24 months [%] (95% CI)	22.6 (16.2, 28.9)	11.4 (7.0, 15.9)
Hazard Ratio (Treosulfan/Busulfan)[b] (95% CI)	0.60 (0.36, 1.01)	
Adjusted p-value[b]	0.0530	
p-value[c]	0.0490	

Sources: Table 14.2.9A, Table 14.2.9B, Listing 16.2.6F
CI = confidence interval.
[a] Only if this event occurred first.
[b] Adjusted for donor type as factor and risk group as stratum using Fine and Gray model.
[c] Based on test of Gray.

Transplantation-Related Mortality (TRM)

The Kaplan-Meier estimate of TRM at 24 months was 28.2% for the busulfan group and 12.1% for the treosulfan group. As for NRM, TRM in the treosulfan group did not increase between 12 months and 24 months after transplantation whilst in the same timeframe in the busulfan treatment group, TRM increased from 18.3% to 28.2%. The difference between the treatment groups was statistically significant in favour of treosulfan (p=0.0201), HR 0.54 (95% CI: 0.32, 0.91).

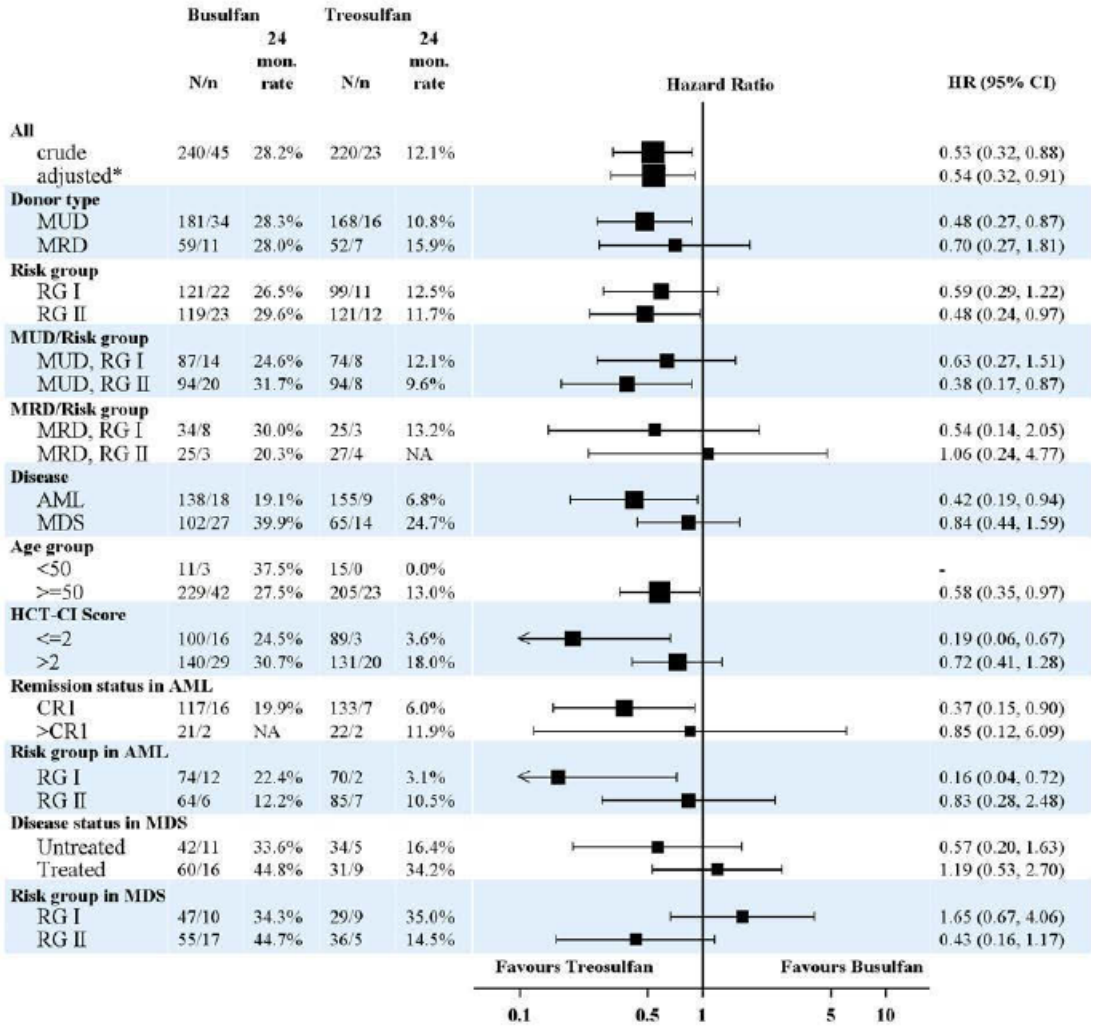
Table 15. Summary results of TRM (FAS)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	45 (18.8%)	23 (10.5%)
Patients without event	195 (81.3%)	197 (89.5%)
Transplantation-related mortality at 12 months[a] [%] (95% CI)	18.3 (13.3, 24.9)	12.1 (8.1, 17.7)
Transplantation-related mortality at 24 months[a] [%] (95% CI)	28.2 (21.4, 36.5)	12.1 (8.1, 17.7)
Hazard Ratio (Treosulfan/Busulfan)[b] (95% CI)	0.54 (0.32, 0.91)	
Adjusted p-value[b]	0.0201	
p-value[c]	0.0128	

Sources: Table 14.2.11A, Table 14.2.11B, Listing 16.2.6F
CI = confidence internal; N = number of patients.
[a] Based on Kaplan-Meier estimates.
[b] Adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[c] Log-rank test.

Forest plots by prognostic factors and combinations of prognostic factors are presented below. Results favoured treosulfan group (HR <1) except for MRD risk group II, treated MDS, and MDS risk group I.

Fig 8. Forest plot for TRM by prognostic factors (FAS)



N = Number of patients, n = Number of events, RG = Risk group
* adjusted for donor type as factor, and risk group and centre as strata using Cox regression model
p-value adjusted for donor type as factor, and risk group and centre as strata using Cox regression model for testing difference of Treosulfan compared to Busulfan: 0.0201

More patients died due to infection in the busulfan group (30 patients, 12.5%) than in the treosulfan (19 patients, 8.6%).The Kaplan-Meier estimates of TRM with infection as cause of death at 24 months were 19.8% (95% CI: 13.8%, 27.8%) for the busulfan treatment group, and 9.8% (95% CI: 6.3%, 15.0%) for treosulfan but the difference between groups was not statistically significant (p=0.2341, adjusted for strata). The HR was 0.69 (95% CI: 0.38, 1.27) in favour of treosulfan.

More patients died in the busulfan group (15 patients, 6.3%) than the treosulfan (4 patients, 1.8%) due to other causes than infection. The Kaplan-Meier estimate of non-infection TRM at 24 months was 10.5% (95% CI: 6.3%, 17.3%) for the busulfan treatment group, and 2.6% (95% CI: 0.9%, 6.8%) for the treosulfan group and the difference was statistically significant (p=0.0192, adjusted for strata). The HR was 0.26 (95% CI: 0.09, 0.80) in favour of treosulfan.

Engraftment

Engraftment at 28 days after HSCT was similar between treatment groups for all categories. The median duration of neutropenia and leukopenia was longer in the treosulfan group than the busulfan (neutropenia: 12.5 days compared to 14.0 day; leukopenia: 13.0 days compared to 14.0 days, Bu versus Treo respectively). Data for PPS was similar. A summary of results is shown below.

Table 16. Conditional cumulative incidence of reconstitution of haematopoiesis (FAS)

Treatment arm	**TREO**	**BU**
No. of patients	220	240
Granulopoiesis		
Day +14	11.1%	9.6%
Day +28	96.8%	96.2%
Maximum incidence	100%	100%
Median time to engraftment (min-max), days	18.0 (11-42)	19.0 (9-38)
Hazard ratio (95% CI) *	1.09 (0.92, 1.29)	
*Adjusted P value**	0.3378	
*P value***	0.1303	
Duration of neutropenia (days), median (Q1, Q3)	14.0 (12.0, 20.0)	12.5 (8.0, 19.0)
*P value****	0.0002	
Leukopoiesis		
Day +14	28.6%	23.4%
Day +28	99.5%	96.7%
Maximum incidence	100%	100%
Median time to engraftment (min-max), days	16.0 (10-32)	16.0 (6-39)
Hazard ratio (95% CI)*	1.14 (0.97, 1.35)	
*Adjusted P value**	0.1225	
*P value***	0.0290	
Duration of leukopenia (days), median (Q1, Q3)	14.0 (11.0, 18.0)	13.0 (8.0, 18.0)
*P value****	0.0043	
Platelets > 20 × 10^9/L		
Day +14	72.4%	77.0%
Day +28	96.8%	97.9%
Maximum incidence	99.5%	99.5%
Median time to engraftment (min-max), days	13.0 (0-34)	12.0 (0-32)
Hazard ratio (95% CI)*	0.86 (0.73, 1.02)	
*Adjusted P value**	0.0772	
*P value***	0.1127	
Platelets > 50 × 10^9/L		
Day +14	46.5%	56.9%
Day +28	92.1%	95.3%
Maximum incidence	99.3%	98.6%
Median time to engraftment (min-max), days	15.0 (10-84)	14.0 (1-33)
Hazard ratio (95% CI)*	0.84 (0.70, 0.99)	
*Adjusted P value**	0.0429	
*P value***	0.1466	

*Adjusted for donor type as factor and risk group as stratum using Fine and Gray model; **Pepe-Mori test,

***Wilcoxon-Mann-Whitney test

Results by donor type, age group, and disease were consistent across the subgroups analysed.

Complete donor chimerism

Incidence of complete donor-type chimerism was statistically significantly higher in the treosulfan group compared to busulfan at both Day +28 and Day +100 (Day +28, 93.5% compared to 82.0% [adjusted p-value=0.008]; Day +100, 86.4% compared to 78.2% [adjusted p-value=0.0205]).

Table 17. Incidence of complete donor type chimerism (FAS)

Treatment arm	TREO	BU
Patients at risk* at Day+28	215	239
Patients with documented complete chimerism at Day+28	201 (93.5%)	196 (82.0%)
Odds ratio (95% CI)	3.2113 (1.69, 6.09)	
Adjusted P-value for testing difference†	0.0080	
Patients at risk* at Day+100	206	220
Patients with documented complete chimerism at Day+100	178 (86.4%)	172 (78.2%)
Odds ratio (95% CI)	1.8850 (1.11, 3.19)	
Adjusted P-value for testing difference†	0.0205	

* Patients are at risk if they have an examination at Day +28 or Day +100 visit or if they have survived day +29 or day +107, respectively; †Stratified Cochran-Mantel-Haenszel test adjusted for donor type and risk group

The data for the PPS were similar.

GvHD-free and Relapse/Progression-free Survival

GvHD-free and relapse/progression-free survival was statistically significantly higher in the treosulfan group than the busulfan (HR 0.72, 95% CI 0.54, 0.95; adjusted p-value=0.0224). The data for the PPS were similar.

Table 18. GvHD-free and relapse/progression-free survival (FAS)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	128 (53.3%)	93 (42.3%)
Death[a]	23 (9.6%)	13 (5.9%)
Relapse/Progression[a]	49 (20.4%)	42 (19.1%)
Acute GvHD ≥ Grade III[a]	23 (9.6%)	14 (6.4%)
Extensive chronic GvHD[a]	33 (13.8%)	24 (10.9%)
Patients without event	112 (46.7%)	127 (57.7%)
GvHD-free and relapse/progression-free survival at 12 months[b] [%] (95% CI)	46.6 (39.6, 53.4)	56.5 (49.2, 63.2)
GvHD-free and relapse/progression-free survival at 24 months[b] [%] (95% CI)	38.4 (31.3, 45.5)	51.4 (43.4, 58.8)
Hazard Ratio (Treosulfan/Busulfan)[c] (95% CI)	0.72 (0.54, 0.95)	
Adjusted p-value[c]	0.0224	
p-value[d]	0.0227	

Sources: Table 14.2.23A, Table 14.2.23B, Listing 16.2.6F
CI = confidence interval; GvHD = graft-versus-host disease.
Note: GvHD-free defined as no acute GvHD of at least grade III and no extensive chronic GvHD.
[a] Only if this event occurred first.
[b] Based on Kaplan-Meier estimates.
[c] Adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[d] Log-rank test.

Chronic GvHD-free and Relapse / Progression-free Survival

Chronic GvHD-free and relapse/progression-free survival was statistically significantly higher in the treosulfan group compared to busulfan (HR 0.69, 95% CI 0.52, 0.92; adjusted p-value=0.0108). The data for the PPS were similar.

Table 19. Chronic GvHD-free and relapse/progression-free survival (FAS)

	Busulfan (N=240)	Treosulfan (N=220)
Patients with event	125 (52.1%)	90 (40.9%)
Death[a]	33 (13.8%)	19 (8.6%)
Relapse/Progression[a]	50 (20.8%)	43 (19.5%)
Extensive chronic GvHD[a]	42 (17.5%)	28 (12.7%)
Patients without event	115 (47.9%)	130 (59.1%)
Chronic GvHD-free and relapse/progression-free survival at 12 months[b] [%] (95% CI)	47.6 (40.5, 54.4)	57.5 (50.1, 64.1)
Chronic GvHD-free and relapse/progression-free survival at 24 months[b] [%] (95% CI)	38.5 (31.3, 45.6)	52.3 (44.2, 59.7)
Hazard Ratio (Treosulfan/Busulfan)[c] (95% CI)	0.69 (0.52, 0.92)	
Adjusted p-value[c]	0.0108	
p-value[d]	0.0234	

Sources: Table 14.2.25A, Table 14.2.25B, Listing 16.2.6F
CI = confidence interval; GvHD = graft-versus-host disease; N = number of patients.
Note: Chronic GvHD-free defined as no extensive chronic GvHD.
[a] only if this event occurred first.
[b] based on Kaplan-Meier estimates.
[c] adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[d] Log-rank test.

Deterioration of KPS

Fewer patients treated with TREO compared to the BU regimen deteriorated by at least 20 points (41.2% vs. 52.1%) or 60 points (5.0% vs. 14.2%) in the KPS.

Final analysis

The final clinical study report dated 18-Jul-2018 (cut off 16 March 2018) has been subsequently submitted and covers the final analysis of all 570 randomised adult patients including the post-surveillance follow-up. The results have confirmed the previously submitted efficacy data (2nd interim analysis based on 476 patients) of the TREO/FLU conditioning regimen. In the final analysis of primary endpoint EFS, hazard ratio and P values even slightly improved in favour of the TREO arm when compared to the confirmative analysis based on 476 patients (Table 6). Comparable results were obtained for the Full Analysis Set; HR 0.64 (95% CI 0.49, 0.84).

Table 6 Event-free survival in study MC-FludT.14/L Trial II (PPS)

	Interim analysis (Data source: CSR Table 11.4.1.1.A)		Final analysis (Data source: CSR Table 11.4.1.1.A)	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Number of patients	215	234	262	275
Median follow-up[a], months (range)	15.4 (3.2, 26.4)	17.4 (3.0, 26.3)	29.7 (3.0, 52.1)	29.4 (3.9, 54.3)
Patients with events	67 (31.2%)	97 (41.5%)	96 (36.6%)	134 (48.7%)
Patients without events	148 (68.8%)	137 (58.5%)	166 (63.4%)	141 (51.3%)
EFS at 12 months[b]; % (95% CI)	67.1 (59.8, 73.3)	58.7 (51.5, 65.2)	69.7 (63.7, 74.9)	60.5 (54.5, 66.0)
EFS at 24 months[b]; % (95% CI)	63.5 (55.4, 70.5)	51.1 (43.4, 58.2)	65.3 (59.0, 70.9)	51.1 (44.8, 57.0)
EFS at 36 months[b]; % (95% CI)	Not reported	Not reported	58.9 (51.5, 65.6)	49.6 (43.1, 55.7)
Hazard ratio[c] (95% CI)	0.67 (0.48, 0.93)		0.64 (0.48, 0.84)	
P-value[cd] for testing non-inferiority	0.0000424		0.0000001	
P-value[c] for testing superiority	0.0090454		0.0005777	

[a] Based on reverse Kaplan-Meier estimates for overall survival.
[b] Based on Kaplan-Meier estimates.
[c] Adjusted for donor type as factor, and risk group and centre as strata using Cox regression model.
[d] The non-inferiority margin for the hazard ratio is 1.3.

Results for overall survival are shown in Table 7, for cumulative incidence of relapse/ progression in Table 8, for graft failure in Table 9 and for cumulative incidence of non-relapse mortality in Table 10. Regarding primary and secondary efficacy parameters, the final analysis of all 570 patients, including post-surveillance data, was consistent with the confirmatory analysis based on 476 patients previously submitted to EMA in December 2017.

Table 7 Overall survival in study MC-FludT.14/L Trial II (FAS)

	Interim analysis (Data source: CSR Table 11.4.1.2.A)		Final analysis (Data source: CSR Table 11.4.1.2.A)	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Number of patients	220	240	268	283
Patients with events	52 (23.6%)	82 (34.2%)	81 (30.2%)	112 (39.6%)
Patients without events	168 (76.4%)	158 (65.8%)	187 (69.8%)	171 (60.4%)
OS at 12 months[a], % (95% CI)	75.3 (68.4, 80.8)	67.8 (60.8, 73.8)	77.8 (72.3, 82.3)	71.8 (66.1, 76.7)
OS at 24 months[a], % (95% CI)	71.3 (63.6, 77.6)	56.4 (48.4, 63.6)	72.7 (66.8, 77.8)	60.2 (54.0, 65.8)
OS at 36 months[a], % (95% CI)	Not reported	Not reported	66.8 (59.9, 72.9)	56.3 (49.6, 62.6)
Hazard ratio[b] TREO vs. BU (95% CI)	0.61 (0.42, 0.88)		0.64 (0.48, 0.87)	
P-value[b]	0.0082		0.0037	

[a]based on Kaplan-Meier estimates; [b]adjusted for donor type, risk group and centre using Cox regression model

Table 8 Cumulative incidence of relapse/progression in trial MC-FludT.14/L (FAS)

	Interim analysis (Data source: CSR Table 11.4.1.3.A)		Final analysis (Data source: CSR Table 11.4.1.3.A)	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Number of patients	220	240	268	283
Patients with events	45 (20.5%)	51 (21.3%)	61 (22.8%)	72 (25.4%)
Patients without events	175 (79.5%)	189 (78.8%)	207 (77.2%)	211 (74.6%)
At 12 months, % (95% CI)	21.1 (15.3, 26.8)	22.6 (17.0, 28.2)	19.1 (14.4, 23.8)	21.7 (16.9, 26.5)
At 24 months, % (95% CI)	24.6 (17.8, 31.3)	23.3 (17.6, 29.0)	22.0 (16.9, 27.1)	25.2 (20.0, 30.3)
At 36 months, % (95% CI)	Not reported	Not reported	25.9 (19.8, 32.1)	26.0 (20.6, 31.4)
Hazard ratio[a] TREO vs. BU (95% CI)	0.87 (0.59, 1.30)		0.82 (0.59, 1.16)	
P-value[a]	0.5017		0.2631	
[a]adjusted for donor type, risk group and centre using Cox regression model				

Table 9 Graft failure rates in MC-FludT.14/L Trial II (FAS)

	Interim analysis (Data source: CSR Table 11.4.1.4.A)		Final analysis (Data source: CSR Table 11.4.1.4.A)	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Primary graft failure	0 / 220 (0.0%)	1 / 240 (0.4%)	1/268 (0.4%)	1/283 (0.4%)
Secondary graft failure	0 / 217 (0.0%)	7 / 236 (3.0%)	0/263 (0.0%)	8/279 (2.9%)

Secondary graft failure rates are still higher in the BU-arm.

Table 10 Cumulative incidence of NRM in MC-FludT.14/L Trial II (FAS)

Study	Interim analysis (Data source: CSR Table 11.4.1.5.A)		Final analysis (Data source; CSR Table 11.4.1.5.A)	
Treatment arm	**Treosulfan**	**Busulfan**	**Treosulfan**	**Busulfan**
Number of patients	220	240	268	283
Patients with events	23 (10.5%)	41 (17.1%)	35 (13.1%)	56 (19.8%)
Patients without events	197 (89.5%)	199 (82.9%)	233 (86.9%)	227 (80.2%)
NRM at 12 months; % (95% CI)	11.4 (7.0, 15.9)	15.2 (10.2, 20.3)	10.5 (6.8, 14.2)	14.3 (10.2, 18.4)
NRM at 24 months; % (95% CI)	11.4 (7.0, 15.9)	22.6 (16.2, 28.9)	12.0 (8.0, 15.9)	20.4 (15.5, 25.2)
NRM at 36 months; % (95% CI)	Not reported	Not reported	14.2 (9.5, 18.9)	21.0 (16.1, 26.0)
Hazard ratio[a] TREO vs. BU (95% CI)	0.60 (0.36, 1.01)		0.63 (0.41, 0.97)	
P-value[a]	0.0530		0.0343	
[a]adjusted for donor type and risk group using Fine and Gray model				

In addition:

- Transplantation-related mortality (TRM) was statistically significantly lower in the treosulfan treatment group compared to the busulfan (HR=0.52; 95% CI: 0.34, 0.82; adjusted p=0.0043).
- TRM with infections as cause of death was statistically significantly lower in the treosulfan treatment group than busulfan (HR 0.57, 95% CI: 0.34, 0.97; adjusted p=0.0371). This was also the case for causes of death other than infections (HR 0.42, 95% CI: 0.18, 0.97; adjusted p=0.0423).
- Reconstitution of granulopoiesis and leukopoiesis 28 days after HSCT was similar in the treatment groups. A significant difference in favour of busulfan was observed for the reconstitution of thrombopoiesis (p=0.0036). The median duration of neutropenia was statistically significantly longer in the treosulfan

treatment group than the busulfan (14.0 days compared to 12.0 days, p <0.0001). Similar results were seen for leukopenia (14.0 days compared to 13.0 days, p=0.0007).

- Incidence of complete donor-type chimerism was statistically significantly higher in the treosulfan treatment group compared to busulfan at both Day +28 and Day +100 (Day +28, 93.2% compared to 83.3% [adjusted p <0.001]; Day +100, 86.1% compared to 80.2% [adjusted p=0.0381]).
- GvHD-free and relapse/progression-free survival was statistically significantly higher in the treosulfan treatment group than the busulfan (HR 0.73, 95% CI: 0.57, 0.92; adjusted p=0.0087).
- Chronic GvHD-free and relapse/progression-free survival was also statistically significantly higher in the treosulfan treatment group (HR 0.70, 95% CI: 0.55, 0.88; adjusted p=0.0030).
- In the post-surveillance evaluation, follow-up data for EFS, OS, relapse/progression, and NRM were obtained for a period of up to 4 years after transplantation. The analysis of these data showed a continued clinically relevant long-term advantage of treosulfan compared to busulfan for these clinically meaningful endpoints.
- For all secondary endpoints the results for the PPS were very similar to those from the FAS reported above since only 14 patients from the FAS were excluded from the PPS.

Ancillary analyses

Rationale to allow extrapolation of the data from study FluT.14/L Trial II to an indication in adults with a non-malignant disease

Patients with serious non-malignant diseases (NMD) requiring alloHSCT usually receive the transplant during childhood/adolescence (up to the age of 18 years). However, a limited number of NMD patients are also treated with alloHSCT beyond the age of 18. Main indications for an alloHSCT in adult NMD patients are severe aplastic anaemia (SAA), haemoglobinopathies (e.g. sickle cell disease [SCD], thalassaemia major [TM]) and Fanconi's anaemia [FA]). The largest study on alloHSCT in NMD patients found in the literature included 489 patients with various diseases. Age ranged from 1.5 to 51 years in 273 patients with SAA, 1.1 to 23 years in 152 patients with TM, and 6 to 26 years in 31 patients with FA [Mahmoud HK et al; Adv Res. 2015 May;6(3):449-58.]

Most of these patients are below the age of 30-40 years. Therefore, such young adult patients are usually treated within the same study protocol as paediatric patients. This is also the case with TREO-based conditioning.

In a prospective multicentre trial, TREO-based conditioning followed by alloHSCT was used in 31 NMD patients. Age of patients ranged from 0.4 to 30.5 years (median 10.7 years). The conditioning regimen consisted of TREO 14 g/m² given once daily IV on days −6 through −4 (total dose 42 g/m²), FLU 30 mg/m² given once daily IV on days −6 through −2 (total dose 150 mg/m²). This regimen is similar as used in the medac-sponsored trial in paediatric NMD patients (MC-FludT.16/NM). Due to a higher than anticipated incidence of acute grades III-IV GVHD in the first 9 patients, thymoglobulin [rabbit anti-thymocyte globulin (rATG)] was added to the regimen and given once daily IV on days −4 through −2 (total dose 6 mg/kg). Twenty-eight patients survived at a median of 26.1 (range 7-48.3) months after alloHSCT for a 2-year projected overall survival of 90%. No apparent difference in toxicity scores or mortality according to patient age or body surface area (BSA) was observed. The results demonstrate that the combination of TREO and FLU provides sufficient myeloablation to achieve engraftment in a broad range of NMDs without the potentially serious complications associated with traditional myeloablative regimens such as busulfan (BU) plus cyclophosphamide (CY). Specifically, there were

no deaths within the first 100 days, and no patient developed hepatic sinusoidal obstruction syndrome (SOS), multi-organ failure, or fatal infections [Burroughs LM et al 2014 Dec;20(12):1996-2003.]

AlloHSCT is the only curative treatment option for TM patients. A BU/CY-based regimen has been the standard myeloablative chemotherapy, but it is associated with high treatment-related toxicity, particularly in patients classified as high risk by the Pesaro criteria [Bertaina 20103; Choudhary 20138]. An Italian group treated 60 TM patients (median age 7 years; range 1-37 years) with a conditioning regimen of TREO/FLU/thiotepa. Twelve patients were adults. All patients received a similar conditioning regimen as used in the MC-FludT.16/NM trial, which included thiotepa (TT; 8 mg/kg on day -7), TREO (14 g/m²/d from days -6 to -4), and FLU (40 mg/m²/d for 4 consecutive days from days -6 to -3). All patients engrafted except one, who died on Day +11; the median time to neutrophil and platelet recovery was 20 days. Five patients (all of whom were given bone marrow cells) experienced secondary graft failure at a median of 9 months (range, 1.5-18) after HSCT; the cumulative incidence of graft failure was 9% (95% CI, 3%-19%). 4 of these 5 patients were rescued by a second allograft. The cumulative incidence of grade II-IV and grade III-IV acute GvHD was 14% (95% CI 6%-24%) and 7% (95% CI 2%- 15%), respectively. The cumulative incidence of TRM was 7% (95% CI 3%-18%). No case of hepatic SOS was recorded. With a median follow-up of 36 months (range, 4-73), the 5-year overall survival probability is excellent (93%; 95% CI 83%-97%). No difference in terms of outcome was observed between children and adults. This TREO-based conditioning regimen proved to be safe and effective for TM patients given alloHSCT. The authors propose this regimen as a suitable and appropriate option for minimising the risk of life-threatening complications in adult poor-performance status TM patients, although it is also of value for patients with good prognostic characteristics [Bernardo ME et al; Blood 2012 Jul 12;120(2):473-6] Retrospective comparison of results of alloHSCT of high-risk TM patients after conditioning of children and adults (age range 2-21 years) with standard BU/CY (139 patients) or TREO/FLU/TT (50 patients; TREO dose 14 g/m²/d × 3) revealed significant survival benefits for the TREO-based regimen. TREO/FLU/TT conditioning regimen significantly improved clinical outcomes of Pesaro Class III TM patients with regard to OS (87.4% vs. 63.6%; P = 0.011) and EFS (78.8% vs. 57.3%; P = 0.041), while BU/CY induced a higher percentage of patients with complete donor chimerism. The survival advantage of the TREO regimen was especially observed in high risk patients (OS: 86.6% vs. 39.4%, P = 0.002; EFS: 77.8%% vs. 32.4%, P = 0.003) [Mathews V et al ; PLoS One. 2013 Apr 26;8(4):61637.] A retrospective comparison of alloHSCT with conventional (non-transplant) treatment approaches for TM in 516 children and adults was published recently [Caocci 20176]. The analysis included 97 adult TM patients (age ≥ 16 years) conditioned with BU/CY-based regimens (n = 81) or TREO/FLU/TT (n = 16). Outcome data between the two groups revealed quite comparable survival rates, while risk for aGvHD was lower after TREO-based conditioning (Odds ratio 0.28, 95% CI 0.12-0.67; P = 0.004). T his finding was confirmed also in the group of adult patients (6.2% vs. 31%; P = 0.004) and unrelated donor HSCT (23.2% vs. 51%; P = 0.0012). Lowering the incidence of treatment related mortality (TRM) due to acute GvHD is an important achievement. In general, the favourable toxicity profile and the reduced TRM after TREO-based conditioning prior to MUD transplantation are highlighted by these authors. Results of this study are summarised in Table 5.

Table 5 Retrospective comparison of outcome data of adult TM patients after alloHSCT with BU/CY-based conditioning or TREO/FLU/TT [Caocci G et al; Am J Hematol. 2017 Dec;92 (12): 1303- 1310]

	All HSCT N=258			Adult HSCT N=97			UD HSCT N=85		
	BU based	Treo based	p	BU based	Treo based	p	BU based	Treo based	p
Number of patients	207	51		81	16		50	35	
OS, %	82.3±2.9	80.9±9.1	ns	69.3±5.5	70.3±15.1	ns	66.8±7.1	74.5±11.6	ns
TFS, %	78.5±3	68.7±8.8	ns	67.3±5.5	65.3±14.8	ns	59.6±7	64.4±11.1	ns
Rejection , %	5	14.9	0.02	4.1	7.1	ns	12.2	13.3	ns
Acute GvHD , %	26.5	16.1	ns	31	6.2	0.04	51	23.2	0.01
Chronic GVHD, %	12.5 (N=184)	12.7 (n=41)	ns	11.3 (N=70)	13.9 (N=14)	ns	22.9 (N=39)	11.4 (N=28)	ns
TRM, n (%)	26 (12.5)	4 (7.8)	ns	19 (23.5)	2 (12.5)	ns	13 (26)	4 (23.5)	ns

Likewise, a number of other reports describe the successful use of TREO/FLU/TT or TREO/FLU conditioning in occasional adult patients suffering from various other NMDs. Such particular cases usually are included in published manuscripts focussing on paediatric transplant patients suffering from haemoglobinopathies, lymphohistiocytosis, immunodeficiencies, or paroxysmal nocturnal haemoglobinuria who are conditioned with a TREO dosage of 42 g/m² [Markiewicz et al; Bone Marrow Transplant. 2006 Jan;37(2):231]

In general, it is recommended to transplant children with NMD as early as possible following diagnosis, prior to the development of severe disease sequelae.

These data support a treatment regimen using 14 g/m² TREO for children as well as for eligible adults with NMDs.

The pivotal study MC-FludT.14/L Trial II exclusively included adult AML and MDS patients (malignant diseases affecting bone marrow function) who were ineligible for standard high-dose, myeloablative conditioning regimens. Therefore, the TREO-based regimen had to be modified (dose reduction from 14 to 10 g/m² and start of treatment on Day -4 instead of Day -6) to reduce the duration of the neutropenic phase in order to reduce the risk for TRM like serious and life threatening infections. However, the 14 g/m² TREO regimen (starting at day -6) was shown to be highly effective and safe in two other prospective phase II trials in adult AML (MCFludT.7/AML) and MDS (MC-FludT.8/MDS) patients who were considered mainly eligible for such myeloablative regimens.

Independently of patient's age, non-malignant diseases like TM are generally associated with a higher risk of graft failure or persistent mixed donor-type chimerism, especially if reduced intensity conditioning (RIC) is used [Olsson R, et al Bone Marrow Transplant 2013l; 48(4): 537-43.]

Therefore, intensification of conditioning by additional treatment with e.g. thiotepa is the current clinical practice and – for practical reasons - requires a somewhat earlier start of treatment with TREO (day -6). This together with the encouraging efficacy and safety data discussed above substantiate the rationale to preferential use the 14 g/m²/d TREO-based regimen for eligible adult patients with NMD.

PAEDIATRICS

Efficacy in the paediatric patient population is based on the phase II MC-FludT.17/M study (malignant diseases) supported by comparison of data from Trial MC-FludT.17/M with historical paediatric engraftment data and adult trial MC-FludT.14 L, and a meta-analysis on treosulfan for conditioning in children and adolescents (Peters 2011).

Study MC-FludT.17/M

This is a multicentre, open-label, non-controlled, Phase II study with the objective to describe the safety and efficacy of treosulfan administered as part of a standardised fludarabine-containing conditioning regimen in children with haematological malignant diseases, which require myeloablative conditioning treatment for allogeneic HSCT.

The primary goal of the study is to evaluate an alternative myeloablative but with reduced toxicity, regimen in children and to contribute to PK model for age dependent dose recommendations.

Study population

Inclusion criteria

- Haematological malignant disease with an indication and a donor for allogeneic HSCT
- Age from 28 days to < 18 years.
- Patients with ALL or AML in CR (blast counts < 5% in BM) and patients with MDS or JMML with blast counts < 20% in BM at study entry
- First allogeneic HSCT or second allogeneic HSCT due to disease relapse, graft failure or secondary malignancy after previous autologous or allogeneic HSCT
- Available matched sibling (MSD), matched family (MFD) or matched unrelated donor (MUD).
- Lansky Index (less than 16 years) or Karnofsky Index (from 16 years) at least 70%

Exclusion criteria

- Impaired cardiac (left ventricular ejection fraction ≤ 35%), liver (bilirubin > three times the ULN, or AST/GOT/ALT/GPT > ten times ULN, or clinical significant coagulopathy, or active infectious hepatitis) or renal function (estimated glomerular filtration rate by Schwartz formula < 60 mL/min/1.73 m²).
- Requirement for supplementary continuous oxygen
- Fanconi anaemia and other DNA breakage repair disorders; secondary leukaemia developed from the Fanconi anaemia or other DNA breakage repair disorders.
- Treatment with cytotoxic drugs within 10 days prior to study medication administration
- Severe active infection, HIV positive
- HSCT from haploidentical or umbilical cord blood donor
- Third or later HSCT
- Concomitant involvement of central nervous system
- Obese paediatric patients with body mass index > 30 kg/m²
- Concomitant solid tumours
- Known hypersensitivity to investigational drugs

Treatments

The rationale for the selection of treatments is the same as study 16.

Treosulfan IV for 3 days on days -6 to -4 on top of pre-specified background conditioning (max 2).

The treosulfan dose per day was as follows:

10 g/m^2 in children with≤ 0.5 m^2 BSA

12 g/m^2 in children with > 0.5 and ≤ 1 m^2 BSA

14 g/m^2 in children with > 1 m^2 BSA

Fludarabine 30 mg/m2/day on days -7 to -3 (mandatory)

Thiotepa 2 x 5 mg/kg/ day on day -2 (optional decided by the investigator)

Treatment phase was 7 days including 3 days of TREO administration. Patients had an observation phase until at least day +100 (inclusive) of HSCT procedure and a follow-up phase of 1 year. A longer-term follow-up phase of at least 3 years was implemented for secondary graft failure, chronic GvHD, survival and TRM.

Sample size

The study was planned to enrol at least 70 evaluable patients from 1 month to less than 18 years including at least 30 patients aged 1 month to 10 years, 30 patients from 10 years to less than 18 years and at least 50 patients receiving a first HSCT.

Endpoints

Primary Freedom from TRM, defined as death from any transplant-related cause from the day of first administration of study medication until day +100 after HSCT

Secondary

- Engraftment defined as first of three consecutive days with ANC > 500/µl and platelet count of at least 20.000/ µl after transplant
- Donor type chimerism at day +28 and day + 100 after HSCT
- Graft failure rate
- OS until 12 months after HSCT
- Incidence of relapse/progression
- RFS/PFS
- Incidence and severity of acute GvHD
- NRM
- Rescue treatment

In addition PK data were collected to contribute to a population PK model . Final results will be provided together with data from study FludT.16/NM in a separate PK report as soon as data from study 16 become available.

Statistical methods

They were descriptive.

For the primary endpoint freedom from transplant (treatment)-related mortality until day +100 after HSCT, the rate of subjects reaching day +100 after HSCT from start of conditioning treatment without previous death due

to transplant- or treatment-related causes was presented together with its 2-sided 90% and 95% Clopper-Pearson confidence intervals (CIs).

For the endpoints TRM, OS, event-free survival (EFS), GvHD-free and relapse / PFS, and chronic GvHD-free and relapse / PFS, Kaplan-Meier estimates were calculated.

For the endpoints relapse / progression, NRM, aGvHD, and cGvHD the probability over time was estimated by cumulative incidence rates.

For engraftment, the conditional cumulative incidence was estimated using conditional probability functions. In order to reach more insight in the dose-effect of engraftment, the duration of neutropenia was analysed based on documented laboratory values and the documented dates of engraftment on an exploratory perspective.

The rate of primary graft failure was estimated as the number of subjects with primary graft failure divided by the total number of subjects receiving HSCT within the 12-months Trial Period. The rate of secondary graft failure was estimated as the number of subjects with secondary graft failure divided by the total number of subjects who have engrafted after stem cell transplantation (ie alive without documented primary graft failure) within the 12-months trial period.

The incidence of complete donor-type chimerism at visit Day +28, visit Day +100, and visit Month 12 were estimated as the number of subjects with complete chimerism divided by the total number of subjects at risk.

Relative and absolute frequencies of subjects using rescue therapies were calculated.

Subgroup analyses were summarised by means of Forest plots. The Forest plot for the subgroup analysis of the efficacy parameter (stratified by relevant subgroups disease, treosulfan dose, number of HSCT, use of Thiotepa, donor type, Clinical Trial Protocol [CTP]age group and International Council for Harmonisation [ICH] age group) included for each subgroup the associated sample size, the number of events, and the 12-months Kaplan-Meier estimates or in case of engraftment parameters the maximum conditional cumulative incidence reached and the corresponding 90% CI. The point estimate of the displayed rates or of the maximum conditional cumulative incidence reached was graphically presented by a filled square proportional to the sample size of the subgroup and a bar representing the associated 90% CI.

Study status

The first patient was enrolled on 21-Nov-2014 and the last patient was enrolled on 31-Aug-2016 across centres in Europe (Poland, Germany, UK, Italy, Czech Rep). Last patient last visit, including the 12-month follow up phase, is 14 September 2017. The study report is dated 12 March 2018. End of study, including the 3-year longer time follow-up phase, is scheduled for September 2019 with a final study report in March 2020.

Results

Recruitment of 70 patients was completed by September 2016. The majority of subjects were recruited in Poland (n= 37) followed by Germany (20), UK (10), Italy (2) and Czech Republic (1)

As of data cut off 1 November 2017 only 7 patients had premature termination (due to death) and 63 patients are ongoing and alive.

Of all subjects included in the trial, 12.9% had a major protocol deviation (9 patients). “Non-compliance” was the most reported subcategory for protocol deviations (5.7% of subjects), followed by “Inclusion / exclusion criteria violated” (2.9%), “Missing evaluations at baseline”, “Informed consent”, and “Treosulfan” treatment deviation (1.4% each)

The intensified regime with TT was given to 65 patients (92.9%) reflecting current medical practice.

Table 20. Demographic profile of paediatric patients

Study	**MC-FludT.17/M**
Study arm	**TREO**
No. of patients	70 (100%)
Gender n (%)	
Female	26 (37.1%)
Male	44 (62.9%)
Age (years)	
Mean (SD)	9.1 (5.8)
Median (Range)	9.5 (0-17)
Age group, n (%)	
28 days to < 10 years	35 (50.0%)
10 years to < 18 years	35 (50.0%)
Race, n (%)	
White	70 (100%)
Black or African American	0 (0.0%)
Asian	0 (0.0%)
Body surface area, m^2	
Mean (SD)	1.110 (0.480)
Median (Range)	1.100 (0.32-2.00)
Performance score*, n (%)	
Lansky performance score	58 (82.9%)
Karnofsky performance score	12 (17.1%)
Karnofsky/Lansky score* n (%)	
70	1 (1.4%)
80	9 (12.9%)
90	22 (31.4%)
100	38 (54.3%)
Karnofsky/Lansky score*	
Median (range)	100 (70-100)
Conditioning regimen, n (%)	
Intensified regimen (with TT)	65 (92.9%)
Standard regimen	5 (7.1%)

Table 21. Indications and donor type

Study	**MC-FludT.17/M**
Study arm	**TREO**
No. of patients	70 (100%)
Indication, n (%)	
Acute lymphoblastic leukaemia (ALL)	27 (38.6%)
Acute myeloid leukaemia (AML)	29 (41.4%)
Myelodysplastic syndrome (MDS)	10 (14.3%)
Juvenile myelomonocytic leukaemia (JMML)	4 (5.7%)
Donor type, n (%)	
Matched family donor (MFD)	1 (1.4%)
Matched sibling donor (MSD)	13 (18.6%)
Matched unrelated donor (MUD)	56 (80.0%)

Efficacy results

Results of the study MC-FludT.17/M in paediatric patients with malignant diseases are mature and cover 12 months follow-up of all patients. The final Clinical Study is dated 12-Mar-2018.

The final efficacy results are similar to the interim data submitted in December 2017 (5th DMC Interim report dated 20-Jun-2017). Minimal changes were detected and marked in the following tables comparing the submitted interim data with the data from the final CSR version 1.0.

The maximum conditional cumulative engraftment rate of 100% (90% CI 97.7, 100) is excellent and can hardly be improved. The primary endpoint (freedom from transplant (treatment)-related mortality, defined as death from any transplant(treatment)-related cause from the day of first administration of trial medication until day +100 after HSCT) is 98.6%. Only one patient died transplant (treatment)-related until Day 100.). Due to the occurrence of only 1 event, no differential effects between subgroups could be identified.

Table 14: Summary of efficacy results for trial MC-FludT.17/M. Comparison of interim and final results

	Interim data MC-FludT.17/M	Final data MC-FludT.17/M
Study arm	TREO	TREO
No. of patients	70 (100%)	70 (100%)
Conditional cumulative incidence of reconstitution of granulopoiesis		
Day +14, % (90% CI)	28.6 (18.7, 38.4)	28.6 (18.7, 38.4)
Day +28	85.1 (77.6, 92.5)	**86.9 (79.8, 93.9)**
Maximum incidence	100.0 (97.2, 100.0)	100.0 (**97.7**, 100.0)
Duration neutropenia (days)		
Median (Q1, Q3)	21.0 (16.0, 26.0)	**22.0 (17.0, 26.0)**
Conditional cumulative incidence of reconstitution of thrombopoiesis > 20×10^9/L		
Day +14, % (90% CI)	34.3 (24.5, 44.1)	34.3 (24.5, 44.1)
Day +28	78.3 (69.8, 86.7)	**78.0 (69.5, 86.5)**
Maximum incidence	100.0 (94.9, 100.0)	**94.1 (88.4, 99.9)**
Conditional cumulative incidence of reconstitution of thrombopoiesis > 50×10^9/L		
Day +14% (90% CI)	15.7 (8.4, 23.0)	15.7 (8.4, 23.0)
Day +28	60.3 (50.3, 70.3)	**62.2 (52.5, 71.9)**
Maximum incidence	100.0 (94.8, 100.0)	**91.9 (84.9, 98.8)**
Conditional cumulative incidence of reconstitution of leukopoiesis		
Day +14	30.0 (20.6, 39.4)	30.0 (20.6, 39.4)
Day +28	93.4 (87.6, 99.1)	**95.6 (90.9, 100.0)**
Maximum incidence	100.0 (97.3, 100.0)	**100.0 (97.7, 100.0)**
Incidence of complete donor type chimerism		
Patients at risk Day +28 *	69 (100%)	69 (100%)
Incidence % (90% CI)	94.2 (87.2, 98.0)	94.2 (87.2, 98.0)
Patients at risk Day +100 *	69 (100%)	69 (100%)
Incidence % (90% CI)	91.3 (83.6, 96.1)	91.3 (83.6, 96.1)
Patients at risk Months 12 *	31 (100%)	**57 (100%)**
Incidence % (90% CI)	90.3 (76.8, 97.3)	**91.2 (82.4, 96.5)**
*Patients are at risk if they have an examination at the Day +28, Day +100, or Month 12 visit or if they have survived day +29, day +107, or day +372, respectively		
Graft failure rates		
Primary graft failure	0	0
Secondary graft failure	2 / 66 (3.0%)	**1 / 70 (1.4%)****
** One event was re-assessed as disease relapse		
Overall survival (Kaplan-Meier estimates)		
Median follow-up; months (range of those surviving)	11.7 (3.4-25.9)	**12.0 (11.5, 17.7)**
Patients with event	6 (8.6%)	**7 (10.0%)**
Patients without event	64 (91.4%)	**63 (90.0%)**
OS at 12 months; % (90% CI)	88.2 (77.8, 93.9)	**91.4 (83.9, 95.5)**
Use of rescue therapies to prevent relapse or acute graft failure		
Rescue therapy	10 (14.3%)	**9 (12.9%)**
Kind of rescue therapy		
DLI	5 (7.1%)	5 (7.1%)
Stem cell boost	4 (5.7%)	4 (5.7%)
*Mesenchymal stem cells****	1 (1.4%)	0
***Use of mesenchymal stem cells was re-assessed as GvHD prevention/treatment		
Transplant(treatment)-related mortality and transplant-related mortality (TRM)		
Freedom from transplant (treatment)-related mortality until Day +100	69 (98.6%)	69 (98.6%)
TRM at 6 months; % (90% CI)	1.4 (0.3, 7.2)	1.4 (0.3, 7.2)
TRM at 12 months; % (90% CI)	3.1 (1.0, 9.7)	**2.9 (0.9, 8.9)**

Table 15: Additional efficacy results for trial MC-FludT.17/M Comparison of interim and final results

	Interim data MC-FludT.17/M	Final data MC-FludT.17/M
No. of patients	70 (100%)	70 (100%)
Conditional cumulative incidence of relapse/progression, % (90% CI)		
At 12 months	13.7 (6.2, 21.2)	**15.7 (8.6, 22.9)**
Cumulative incidence of NRM, % (90% CI)		
At 12 months	3.1 (0.0, 6.7)	**1.4 (0.0, 3.8)**

According to the interim analysis a total of 6 of the 70 patients died. Causes of death were relapse/progression for 2 patients, transplantation related, for 1 patient and "other" for 1 patient (sepsis in remission of ALL, 116 days after transplantation). For 2 patients two causes of death were reported (1 patient "relapse/progression" and "transplantation related", 1 patient "relapse/progression" and "other"). For the final analysis documentation of the two patients was cleaned regarding main cause of death, resulting in 1 patient died due to transplant related cause and one due to "other" cause. One additional death was observed after the interim analysis (transplantation related).

Comparison of engraftment data of trial MC-FludT.17M with historical data and data from the trial MC-FludT.14/L

As agreed in the PIP a comparison of engraftment data from trial MC-FludT.17/M with historical paediatric data and with data from each of the arms of the trial in adult patients with malignant diseases comparing conditioning with TREO or BU (trial MC-FludT.14/L).

For selection of historical trial data, a trial was considered relevant if the protocol matched the inclusion criteria (patients ≤ 18 years of age; malignant disease, alloHSCT, donor type, stem cell source, TREO- or BU-based conditioning) and was published rom 01-Jan-2001 to 02 May 2017.

The comparison concerned the cumulative engraftment incidence including engraftment rate, numbers of patient engrafted and not engrafted, competing events and data on descriptive statistics (confidence interval and overall survival data, if available).

A total of 6 trials investigating TREO [Peters 2011; Wachowiak 2011; Beier 2013, Strahm 2015; Nemecek 2016; Mattson 2017] and 5 publications for trials focussing on BU-based conditioning [Zahler 2016; Vicent 2002; Stancheva 2013; Skalska-Sadowska 2014; Matsuyama 1998] were selected.

In trial MC-FludT.17/M, the maximum cumulative incidence of engraftment was in the range found in historical trials using TREO-based conditioning (94% to 100%) and BU-based conditioning regimens (89 to 100%).

Limited data for 1-year OS are available from the literature. With 1-year OS of 91.4% the outcome of MC-FludT.17/M was above the range of what has been reported in the literature for TREO-based conditioning regimens (82% to 85%) or for BU-based conditioning regimens (78% to 88%).

The engraftment rate in trial MC-FludT.17/M was also within the range evaluated for trial MC-FludT.14/L (95.7% to 99.3%). The 1-year OS in trial MC-FludT.17/M was higher compared to FludT.14/L trial data: 68% in patients treated with 14 g/m²/d TREO, 75.3% in patients treated with 10 g/m²/d TREO, and 67.8% to 74.3% in patients treated with BU.

It can be concluded that the engraftment results for children treated in trial MC-FludT.17/M and results of OS are at least comparable to published data using TREO or BU-based conditioning regimens. As expected, survival outcome for the paediatric patients is higher than in adult patient populations.

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 1. Summary of Efficacy for trial MC-FludT.14/L Trial II

***Title**: A randomised, active-controlled phase III trial comparing TREO/FLU (FT_{10}) to a well-established RIC regimen consisting of BU/FLU (FB2) prior to alloHSCT in patients with AML or MDS considered ineligible to standard conditioning regimens*			
Study identifier	*MC-FludT.14/L Trial II*		
Design	*Randomised, parallel-group, open label, multicentre, international, group-sequential phase III non- inferiority trial*		
	Duration of main phase: *Duration of Run-in phase:* *Duration of Extension phase:*		*Duration of Treatment:* *Test group were treated on 3 consecutive days (Day -4 to Day -2) with treosulfan, Reference group were treated on 2 consecutive days (Day -4 to Day -3) with busulfan.* *N/A* *N/A*
Hypothesis	*Non-inferiority*		
Treatments groups	*TREO-based conditioning TREO/FLU (FT_{10})*		*TREO: 3 x 10 g/m²/d on day -4 to -2* *FLU: 30 mg/m²/d on day -6 to -2* *(FT_{10}; n = 220)*
	Busulfan- based reduced-intensity conditioning therapy (FB2)		*Busulfan 3.2 mg/kg/d on day -4, -3* *FLU: 30 mg/m²/d on day -6 to -2* *(FB2; n = 240)*
Endpoints and definitions	*Primary endpoint*	*Event –free survival*	*EFS within two years after transplantation, measured from time of end of HSCT (day 0) to time of event (defined as relapse of disease, graft failure or death)*
	Secondary endpoints	*Overall survival*	*OS at 24 months*
		Incidence of relapse/progression	*Cumulative incidence of relapse/progression at 24 months*
		Non-relapse mortality	*Probability of dying without previous occurrence of a relapse or progression within 2 years after HSCT.*
		Transplantation -related mortality	*all deaths occurring due to HSCT-related causes within 2 years after HSCT.*
		Engraftment	*Granulocyte engraftment by specifying the 1st of 3 consecutive days with absolute neutrophilic granulocyte count > 0.5 x 109/L in the peripheral blood (PB).* *Leukocyte engraftment was documented by specifying the 1st of 3 consecutive days with total leukocyte count > 1 x 109/L in the PB.* *Platelet engraftment was documented by specifying the 1st of 3 consecutive days with a) platelets > 20 x 109/L and b) platelets > 50 x 109/L, in the absence of platelet transfusion.*

		Complete donor chimerism	*Donor chimerism at day +28 and +100 after HSCT with a donor to patient ratio≥ 95%*
Database lock	*19.08.2016*		

Results and Analysis

Analysis description	***Primary Analysis***		
Analysis population and time point description	*Confirmatory statistical analysis of the primary efficacy parameter was performed within the Per-Protocol-Set (PPS) and the Full-Analysis-Set (FAS).*		
Descriptive statistics and estimate variability	*Treatment group*	*TREO/FLU (FT_{10})*	*BU/FLU (FB2)*
	Number of subject *FAS* *PPS*	 *220* *215*	 *240* *234*
	EFS (PPS) at 24 months [%]	*63.5*	*51.1*
	95% CI	*55.4, 70.5*	*43.4, 58.2*
	OS (FAS) at 24 months [%]	*71.3*	*56.4*
	95% CI	*(63.6, 77.6)*	*(48.4, 63.6)*
	Cumulative incidence relapse/ progression at 24 months (FAS) [%]	*24.6*	*23.3*
	(95% CI)	*(17.8, 31.3)*	*(17.6, 29.0)*
	Non-relapse mortality at 24 months (FAS) [%]	*11.4*	*22.6*
	95% CI	*(7.0, 15.9)*	*(16.2, 28.9)*
	Transplantation-related mortality at 24 months (FAS) [%]	*12.1*	*28.2*
	(95% CI)	*(8.1, 17.7)*	*(21.4, 36.5)*
	Engraftment-Conditional cumulative incidence at 28 days (FAS) [%]	*Granulopoiesis*	
		96.8	*96.2*
		Leukopoiesis	
		99.5	*96.7*
		Platelets > 20 x 10^9/L	
		96.8	*97.9*
	Complete donor chimerism at day 28 (FAS) [%]	*93.5*	*82.0*
Effect estimate per comparison	*Non- inferiority of EFS in PPS*	*HR*	*0.67*
		95% CI	*0.48, 0.93*
		One-sided p-value for testing non-inferiority of treosulfan compared to busulfan	*0.0000424* *(FAS, p = 0.0000164)*

		One-sided p-value for testing superiority of treosulfan compared to busulfan	*0.0090454* *(FAS, p = 0.0051268)*
	OS (FAS)	*HR*	*0.61*
		95% CI	*0.42, 0.88*
		One-sided p -value	*0.0082*
	Relapse/ progression (FAS)	*HR*	*0.87*
		95% CI	*0.59, 1.30*
		One-sided p -value	*0.50*
	Non relapse mortality	*HR*	*0.60*
		(95% CI)	*(0.36, 1.01)*
		One-sided p -value	*0.0530*
	Transplantation related mortality	*HR*	*0.54*
		95% CI	*0.32, 0.91*
		One-sided p -value	*0.0201*
	Engraftment	*HR (95% CI)*	*p value*
	Granulopoiesis *Leukopoiesis* *Platelets>20 x 10^9/L*	*1.09 (0.92, 1.29)* *1.14 (0.97, 1.35)* *0.86 (0.73, 1.02)*	*0.34* *0.0043* *0.08*
	Complete donor chimerism day 28	*OR*	*3.21*
		95% CI	*1.69, 6.09*
		One-sided p -value	*0.0080*

Summary of Efficacy for Study MC-FludT.17/M

Title: Clinical Phase 2 trial to describe the safety and efficacy of Treosulfan-based conditioning therapy prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with haematological malignancies.		
Study identifier	**MC-FludT.17/M**	
Design	Prospective, single arm, open-label, non-controlled, Phase II multicentre trial to assess safety and efficacy of Treosulfan as part of a standardised Fludarabine-containing conditioning regimen, and to contribute to a PK model.	
	Duration of main phase:	Treatment phase was 7 days with 3 days of Treosulfan administration. Observation phase until day +100 after HSCT procedure Follow-up phase of 1 year.
	Duration of Run-in phase:	N/A
	Duration of Extension phase:	Longer-term follow-up phase of at least 3 years after HSCT was implemented for secondary graft failure, chronic GvHD, survival and TRM.
Hypothesis	descriptive	

Treatments groups	Treosulfan IV for 3 days on days -6 to -4 on top of 2 pre-specified background conditioning regimens. Treosulfan dose per day: 10 g/m^2 in children with≤ 0.5 m^2 BSA 12 g/m^2 in children with > 0.5 and ≤ 1 m^2 BSA 14 g/m^2 in children with > 1 m^2 BSA Fludarabine 30 mg/m2/day on days -7 to -3 (mandatory) Thiotepa 2 x 5 mg/kg/ day on day -2 (optional decided by the investigator)		
Endpoints and definitions	Primary endpoint	Freedom from TRM	TRM as death from any transplant-related cause from the day of first administration of study medication until day +100 after HSCT from start of conditioning treatment without previous death due to transplant- or treatment-related causes
	Secondary endpoints	Engraftment	Defined as first of three consecutive days with leucocyte count of > 1 x 10^9/L, ANC > 500/µl and platelet count of at least 20.000/ µl, thrombocyte count of ≥ 20 x 109/L in the absence of platelet transfusion, thrombocyte count of ≥ 50 x 109/L in the absence of platelet transfusion
		Donor type chimerism	Donor type chimerism at day +28 and day + 100 and visit Month 12 were estimated as the number of subjects with complete chimerism divided by the total number of subjects at risk
		Graft failure rate	Primary graft failure as the number of subjects with primary graft failure divided by the total number of subjects receiving HSCT within the 12-months trial period. The rate of secondary graft failure was estimated as the number of subjects with secondary graft failure divided by the total number of subjects who have engrafted after stem cell transplantation (ie alive without documented primary graft failure) within the 12-months trial period.
		OS	OS (Kaplan- Meier estimates) until 12 months after HSCT
		relapse/ progression	Incidence of disease relapse/progression
		Rescue therapies	Relative and absolute frequencies of subjects using rescue therapies.
End of study (including 3-y follow up)	September 2019 Final study report in March 2020.		
Enrollment	The first patient was enrolled on 21-Nov-2014 and the last patient was enrolled on 31-Aug-2016 across centres in Europe. Last patient last visit, including the 12-month follow up was 14 September 2017. The study report is dated 12 March 2018.		

Results and Analysis

Analysis description	Primary Analysis	
For the primary endpoint freedom from transplant (treatment)-related mortality until day +100 after HSCT, the rate of subjects reaching day +100 after HSCT from start of conditioning treatment without previous death due to transplant- or treatment-related causes.		
Descriptive statistics and estimate variability	Treatment group	TREO
	Number of subject	N=70 (100%)
	Freedom from TRM at day +100, N % (90% CI)	69, 98.6% (93.4, 99.9)
	TRM at 6 months % (90% CI)	1.4 (0.3, 7.2)
	TRM at 12 months % (90% CI)	2.9 (0.9, 8.9)
	Engraftment	
	Conditional cumulative incidence of reconstitution of thrombopoiesis > 50 x 10^9/L	
	Day +14 (90% CI)	15.7 (8.4, 23.0)
	Day +28 (90% CI)	62.2 (52.2, 71.9)
	Max incidence	91.9 (84.9, 98.8)
	Conditional cumulative incidence of reconstitution of leukopoiesis	
	Day +14 (90% CI)	30.0 (20.6, 39.4)
	Day +28 (90% CI)	95.6 (90.9, 100.0)
	Max incidence	100.0 (97.7, 100.0)
	Conditional cumulative incidence of reconstitution of granulopoiesis	
	Day +14 (90% CI)	28.6 (18.7,38.4)
	Day +28 (90% CI)	86.9 (79.8,93.9)
	Max incidence	100.0 (97.7, 100.0)
	Conditional cumulative incidence of reconstitution of leukopoiesis	
	Day +14 (90% CI)	30.0 (20.6, 39.4)
	Day +28 (90% CI)	95.6 (90.9, 100.0)
	Max incidence	100.0 (97.7, 100.0)
	Incidence of complete donor type chimerism	
	Patients at risk day +28	69 (100%)
	Incidence % (90% CI)	94.2 (87.2, 98.0)
	Patients at risk day +100	69 (100%)
	Incidence % (90% CI)	91.3 (83.6, 96.1)
	Patients at risk month 12	57 (100%)
	Incidence % (90% CI)	91.2 (82.4, 96.5)
	Graft failure rates	
	Primary	0
	Secondary	1 / 70 (1.4%)
	OS (Kaplan Meier estimates) at 12 months % (90% CI)	91.4 (83.9, 95.5)
	Conditional cumulative incidence of relapse/ progression at 12 months % (90% CI)	15.5 (8.6, 22.9)

Clinical studies in special populations

The oldest study patients treated with TREO/FLU were 70 years old. Due to the given eligibility criteria of the prospective trials, none of the study patients was older than 70 years.

Table 16 Summary of age in adult trials by trial number (Full Analysis Set)

	Trial number					
	MC-FludT. 6/L (N=55)	MC-FludT. 7/AML (N=75)	MC-FludT. 8/MDS (N=45)	MC-FludT. 14/L (Trial 1) (N=168)	MC-FludT. 14/L (Trial 2) (N=220)	Overall (N=563)
Age [years]						
Mean (SD)	48.8 (11.8)	44.5 (10.9)	48.2 (10.6)	57.3 (8.2)	59.3 (6.6)	54.8 (10.3)
Median	50.0	45.0	50.0	59.0	60.0	57.0
Q1, Q3	41.0, 59.0	37.0, 55.0	42.0, 56.0	53.0, 63.0	55.0, 65.0	50.0, 62.0
Min, Max	18, 66	19, 59	22, 63	21, 70	37, 70	18, 70
Age group [n (%)]						
<65 years	53 (96.4%)	75 (100.0%)	45 (100.0%)	139 (82.7%)	162 (73.6%)	474 (84.2%)
65 to 74 years	2 (3.6%)	0 (0.0%)	0 (0.0%)	29 (17.3%)	58 (26.4%)	89 (15.8%)

[Program: Day120_ISS2017_CTRV1 / TableAgeDesc / t_agegr_adult_fas_by_studgr1]

Table 17 Summary of age in adult trials by trial number (Safety Set)

	Trial number					
	MC-FludT. 6/L (N=55)	MC-FludT. 7/AML (N=75)	MC-FludT. 8/MDS (N=45)	MC-FludT. 14/L (Trial 1) (N=168)	MC-FludT. 14/L (Trial 2) (N=221)	Overall (N=564)
Age [years]						
Mean (SD)	48.8 (11.8)	44.5 (10.9)	48.2 (10.6)	57.3 (8.2)	59.3 (6.6)	54.8 (10.3)
Median	50.0	45.0	50.0	59.0	60.0	57.0
Q1, Q3	41.0, 59.0	37.0, 55.0	42.0, 56.0	53.0, 63.0	55.0, 65.0	50.0, 62.0
Min, Max	18, 66	19, 59	22, 63	21, 70	37, 70	18, 70
Age group [n (%)]						
<65 years	53 (96.4%)	75 (100.0%)	45 (100.0%)	139 (82.7%)	162 (73.3%)	474 (84.0%)
65 to 74 years	2 (3.6%)	0 (0.0%)	0 (0.0%)	29 (17.3%)	59 (26.7%)	90 (16.0%)

[Program: Day120_ISS2017_CTRV1 / TableAgeDesc / t_agegr_adult_sas_by_studgr1]

Supportive studies

ADULTS

Studies FludT.7/AML & FludT.8/MDS

These two non-controlled phase II trials were similarly designed and differed only in the treated diseases, AML and MDS. The aim was to evaluate the efficacy and safety of TREO/FLU-conditioning in two indications (AML and MDS) and to include patients with increased as well as standard risk for alloHSCT with respect to toxicity.

Both studies were multicentre across four countries (Germany, Finland, Poland, Sweden) and were conducted in parallel between 2004 and 2008.

Objective

The primary objective was evaluation of engraftment.

Study population

Inclusion criteria:

- Patients with AML (FludT.7/AML) or MDS (FludT.8/MDS) indicated for allogeneic transplantation.
- Availability of a HLA-identical sibling donor (MRD) or HLA-identical unrelated donor (MUD).
- Target graft size (unmanipulated)
  - Bone marrow: 2 to 10 x 10^6 CD34+ cells/kg body weight (b.w.) recipient or ≥ 2 x 10^8 nucleated cells/kg b.w. recipient, or
  - Peripheral blood: 4 to 10 x 10^6 CD34+ cells/kg b.w. recipient.
- Age ≥ 18 and ≤ 60 years.
- Karnofsky Index (KPS) ≥ 80 %.
- Adequate contraception in female patients of child-bearing potential.

Exclusion criteria:

- FludT.7/AML: therapy related secondary AML, AML with t(8;21)(q22;q22) in CR1, acute promyelocytic leukaemia with t(15;17)(q22;q12) in CR1, secondary malignancies
- FludT.8/MDS: secondary or therapy related MDS with prior exposure to cytotoxic alkylating drugs and/or radiation therapy, previous AML-induction therapy with more than two courses (e.g. in case of blast excess)
- Previous allogeneic transplantation
- Severe concomitant illnesses / medical conditions
- Malignant involvement of the CNS
- Active infectious disease, HIV-positivity or active hepatitis infection
- Impaired liver function (bilirubin > ULN; transaminases > 3.0 x ULN)

- Impaired renal function (creatinine clearance < 60 mL/min; serum creatinine > 1.5 x ULN)
- Pleural effusion or ascites > 1 L
- Pregnancy or lactation
- Known hypersensitivity to treosulfan and/or fludarabine
- Participation in another experimental drug trial within 4 weeks before study

Treatment

Treosulfan 14 g/m² IV on Day -6 to -4 in combination with fludarabine (30 mg/m² IV Days -6 to -2).

Patients with MUD additionally received 10 mg/kg ATG-Fresenius® IV on Days -4 to Day -2.

Efficacy endpoints

- Engraftment: regeneration of granulopoiesis (> 0.5 x 10^9/L granulocytes in PB on 3 consecutive days)
- Primary failure of engraftment: granulocyte count of < 0.5 x 10^9/L in PB by Day +28
- Secondary failure of engraftment: disappearance of donor cells after initial engraftment (granulocyte count < 0.5 x 10^9/L in PB)
- Regeneration of leukocyte and platelet counts in the peripheral blood
- Donor type chimerism in BM at Days +28, +56, and +100.
- Disease status / relapse by means of differential blood count and BM puncture at Days +28, +56, and +100, thereafter every 3 months by means of clinical assessments.
- Relapse incidence
- DFS, NRM and OS: The survival status was assessed at Days +28, +56, and +100, thereafter every 3 months during the total follow-up.

Statistical methods

Data were first analysed descriptively. Statistical analysis generally consisted of calculating point- and interval estimates for the parameters of interest.

Time-to-event data were analysed by Kaplan-Meier methods when merely non-informative censoring occurred. In case of competing risks cumulative incidence and conditional probability functions were used, where appropriate.

Results

All 75 patients in AML study and 45 patients in MDS study received at least one dose of treosulfan, provided at least one efficacy evaluation after start of treatment and were included in the efficacy analysis.

Table 16 Studies FludT.7/AML & FludT.8/MDS- Demographics and baseline characteristics

Study	**FludT.7 / AML** n=75	**FludT.8 / MDS** n=45
Enrolled (n)	75	46
Analysed (n)	75	45
Demographic profile		
Age (years)		
Mean (SD)	44.5 (10.9)	48.2 (10.6)
Median (Range)	45.0 (19-59)	50 (22-63)
Gender n (%)		
Female	37 (49%)	24 (53%)
Male	38 (51%)	21 (47%)
Body weight (kg)		
Mean (SD)	74.23 (17.75)	71.66 (16.16)
Median (Range)	72.0 (45-142)	72 (45-118)
Disease characteristics		
CR1[a]	60 (80%)	
>CR1	15 (20%)	
Aetiology		
De novo		45 (100%)
Therapy related		0
Disease status		
Untreated		35 (78%)
Treated		10 (22%)
Eligible for standard conditioning		
Yes	56 (75%)	41 (91%)
No	19 (25%)	4 (9%)
Risk group		
Low	3 (4%)	3 (7%)
Intermediate	51 (68%)	
Intermediate 1		20 (44%)
Intermediate 2		14 (31%)
High	13 (17%)	8 (18%)
Other	8 (10%)	

[a] All patients with adverse risk cytogenetics were in CR1

Table 17: Studies FludT.7/AML & FludT.8/MDS- Summary of efficacy results

Study	**FludT.7 / AML** n=75	**FludT.8 / MDS** n=45
Type SC		
MRD	40%	33%
MUD	60%	67%
Conditional cumulative incidence reconstitution of granulopoiesis		

Day +14 (%)	19	24
Day +28 (%)	93	96
Maximum incidence	100	98
Median time to engraftment (min, max), days	20 (12-38)	17 (10-35)
Conditional cumulative incidence reconstitution of leukopoiesis		
Day +14 (%)	27	38
Day +28 (%)	100	96
Maximum incidence	100	98
Median time to engraftment (min, max), days	17 (10-28)	16(10, 33)
Conditional cumulative incidence reconstitution of thrombopoiesis >20 × 10^9/L		
Day +14 (%)	56	42
Day +28 (%)	93	87
Maximum incidence	96	91
Median time to engraftment (min, max), days	14 (7-31)	16 (8-71)
Conditional cumulative incidence reconstitution of thrombopoiesis >50 × 10^9/L		
Day +14 (%)	32	22
Day +28 (%)	88	78
Maximum incidence	96	89
Median time to engraftment (min, max), days	18 (10-46)	21 (10-84)
Incidence of complete donor type chimerism		
Day +28 (%)	72	78
Day + 100 (%)	92	93
At 6 months (%)	NR	NR
At 12 months (%)	NR	NR
Graft failure rates		
Primary graft failure (n)	0	1
Secondary graft failure (n)	1	1
DFS		
DFS at 12 months (%)	61	71
DFS at 24 months (%)	55	67
OS		
OS at 12 months (%)	77	82
OS at 24 months (%)	61	71
Cumulative incidence of relapse/progression		
At 12 months (%)	30	16
At 24 months (%)	34	16

Cumulative incidence of NRM		
At Day 28 (%) At Day 100 (%) At 12 months (%) At 24 months (%)	0 3 9 11	0 9 13 17
Cumulative incidence of TRM		
At 12 months (%) At 24 months (%)	12 NR	15.6 NR

Explorative analyses revealed that NRM in AML patients which was significantly in favour of patients in CR1 compared to non-CR1 (7% vs. 27% by Day +720). No other significant findings were noted in both trials.

Study MC-FludT.14/L Trial I

This was a randomised, parallel-group, open label, multicentre, group-sequential phase III non-inferiority trial to evaluate efficacy and safety of treosulfan-based conditioning versus a busulfan-based RIC treatment prior to allogeneic HSCT in patients with AML or MDS considered ineligible to standard conditioning.

Methods

The study design was nearly identical to MC-FludT.14/L Trial II (refer to main study section for details). The main differences were the following:

- The primary objective/endpoint was to compare EFS within one year after transplantation (instead of within 2 years after transplant as defined in Trial II) between treosulfan-based conditioning and busulfan-based conditioning. Events were defined as relapse of disease, graft failure, or death (whatever occurred first).
- Treosulfan was administered IV as 14 g/m²/day (1 x 14 g/m²/d) on Day -6 to -4 (instead of 10 g/m²/day (1 x 10 g/m²/d) on Day -4 to -2 in Trial II)

To stop the trial as soon as the question of non-inferiority was answered, a group-sequential approach was implemented with three confirmatory interim analyses and one final look. Interim analyses were to be performed after documentation of at least 90 events in at least 230 patients, 145 events (or latest with 345 patients) and 190 events (or latest with 440 patients) qualifying for per protocol set. An independent safety monitoring committee was implemented to supervise the trial with respect to efficacy and any potentially relevant treatment-specific differences in TRM and serious adverse events.

Results

The multicentre (n of sites) study was conducted in Finland (1), France (3), Germany (9), Hungary (1), Italy (4) and Poland (2).

Date of first enrolment: 24-Nov-2008

Date of last completed: 26-Sep-2012

At the first scheduled interim analysis, there were 279 patients enrolled and the study was amended (No 3) following recommendation by the DMC. Results of the first 330 patients included prior to implementation of Amendment No. 03 are reported below.

Table 18: Disposition of patients and selected characteristics

	Busulfan	**Treosulfan**	**Total**
Patients Randomised	159 (100%)	171 (100%)	330 (100%)
Patients in FAS	152 (95.6%)	168 (98.2%)	320 (97%)
Patients in Safety Analysis Set	152 (95.6%)	168 (98.2%)	320 (97%)
Patients in PPS	140 (88.1%)	165 (96.5%)	305 (92.4%)

Selected patient characteristics (FAS)	**Busulfan** n=152	**Treosulfan** n=168	**Total** n=320
Age (years)			
Median (Q1, Q3)	58.0 (54.0, 63.0)	59.0 (53.0, 63.0)	58.0 (54, 63.0)
Patients HCT-CI Score			
Median (Q1, Q3)	3.0 (2.0, 5.0)	3.0 (1.0, 4.0)	3.0 (2.0, 5.0)
Diagnosis			
AML	109 (71.7%)	130 (77.4%)	239 (74.7%)
MDS	43 (28.3%)	38 (22.6%)	81 (25.3%)
Donor type			
MRD	32 (21.1%)	45 (26.8%)	77 (24.1%)
MUD	120 (78.9%)	123 (73.2%)	243 (75.9%)
Remission status at study entry			
CR1	80 (73.4%)	99 (76%)	179 (74.9%)
> CR1	29 (26.6%)	31 (23.8%)	60 (25.1%)

At 12 and 24 months EFS and OS showed a favourable outcome for BU compared to the TREO regimen. There were no differences between arms with regards to the incidence of relapse but TRM was higher for the treosulfan treatment due to higher rate of infection deaths.

Summary of efficacy results (FAS)

Parameter [%] (95% C.I.)	**Busulfan n=152**	**Treosulfan n= 168**	**p-Value***
EFS			
12 months	67.5 (59.3, 74.3)	62.1 (54.3, 69.0)	0.2927
24 months	56.9 (48.2, 64.7)	51.2 (42.8, 59.0)	0.1643
OS			
12 months	74.3 (66.5, 80.5)	68.0 (60.3, 74.5)	0.2211
24 months	64.2 (55.7, 71.6)	60.2 (51.8, 67.6)	0.4118
Relapse incidence			
12 months	17.5 (11.3, 23.7)	17.4 (11.6, 23.3)	0.9151
24 months	22.1 (15.2, 29.1)	23.5 (16.6, 30.3)	0.6107
Non-relapse mortality			
12 months	14.5 (8.9, 20.1)	19.2 (13.2, 25.2)	0.3224
24 months	20.2 (13.5, 27.0)	24.0 (17.1, 30.8)	0.5591

Transplant related mortality			
Day +100	5.3 (2.7, 10.4)	10.2 (6.4, 15.9)	0.0385
12 months	14.3 (9.6, 21.1)	19.1 (13.8, 26.1)	0.1758
Transplant related mortality			
Day +100 'infection related only'	4.0 (1.8, 8.7)	7.9 (4.7, 13.3)	0.0630
12 months 'infection related only'	11.2 (7.0, 17.6)	17.1 (12.0, 23.9)	0.0637

* p-value for testing difference of treosulfan compared to busulfan

PAEDIATRICS

Meta-analysis on treosulfan-conditioning in paediatric patients with malignant and non-malignant diseases (Peters 2011)

Patients below 18 years with malignant or non-malignant disease who underwent HSCT between January 2005 and July 2010 registered in the EBMT database were eligible. To investigate a potential non-linear association between dose and outcome, fractional polynomials were used.

843 pts met the inclusion criteria and 75% could be included into the analysis (533 allogeneic, 93 autologous). The majority were male (58.9%), with non-malignant disease (56.9%), first HSCT (81.9%) and were between 1 and 12 years of age (57.2%). There were patients in all age groups including < 6 months (6.5%) , < 1 year (10.4%) and > 12 years (20.1%).

As donor 20% had a matched sibling donor. The stem cell source was bone marrow in 44% and peripheral blood in 42% of patients. For alloHSCT, the median TREO dose was 42 g/m². The majority of patients received a TREO dose between 39 and 45 mg/m² (62%). For autologous HSCT the median TREO dose was 36 g/m².

Outcome for Allogeneic HSCT

Incidence of grade III/IV acute GvHD was 10% and for limited and extensive chronic GvHD was 13% and 6%, respectively, with no correlation with age. TREO dose had no significant impact on GvHD.

Incidence of grade III/IV stomatitis, diarrhoea, and vomiting were 22%, 24%, and 14%, respectively (no correlation with TREO dose). Incidence of grade III/IV respiratory toxicity was 12%. There is a significant association between age and respiratory toxicity. Children below the age of one year (mainly NMDs) experienced more grade III/IV respiratory toxicity. Incidence of grade III/IV hyperbilirubinemia, AST increase, and mild/severe VOD was 10%, 25%, and 5%, respectively.

CNS and peripheral neurological toxicity grade III/IV was 4% and 2%, respectively. There was more severe pulmonary toxicity in the youngest age group (> 6 months) compared to other age groups.

Incidence of graft failure was 2%. There was no significant correlation of the rate of graft failure (within 100 days) with age. Dose had no significant impact on the rate of graft failure in both univariate and multivariate analysis. Furthermore, there was no significant correlation of the time to engraftment (ANC > 0.5) with age and dose.

There was a border-line significant impact of age on overall survival. The 3-year OS in children below 6 months of age is 75%, children between 6 month and 1 year have a 3-year OS of 84%. The 3-year OS of children between 1-12 years and > 12 years was 70% and 60%, respectively. This difference is mainly caused by a difference in disease related mortality (DRM). TRM is not significantly different in the different age groups. No

significant impact of dose on overall survival could be found in univariate or adjusted analysis. There was a significant impact of age on EFS and 3-year EFS decrease with increasing age. 3-year EFS in patients less than 1 year of age, 1-12 years, and > 12 years was 75%, 62%, and 53%, respectively. This difference was mainly caused by a difference in the relapse incidence.

In conclusion, TREO was shown to be highly efficient to enable engraftment without increasing the risk for severe acute or chronic GvHD. Considering that most children with malignancies were either heavily pre-treated or underwent a subsequent HSCT, the toxicity profile was surprisingly low compared to either TBI-containing regimen or other myeloablative combinations (e.g. busulfan, high dose cyclophosphamide, high dose melphalan).

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

Adults

The indication in adult patients is supported by a single Phase III clinical study (FludT.14/L Trial II) and the overall design is considered adequate.

The study population included the two diseases which are the most common indications in adults for alloHSCT, AML and MDS. Patients up to 70 years were allowed if sufficiently fit to receive a reduced intensity but still myeloablative conditioning reflecting current clinical practice. Although adults with other malignancies may also undergo allo SCT it would have implied a more heterogenous population. Non-malignant diseases are rare indications for alloHSCT in adults. Overall, the selected patient population is appropriate for an orphan indication.

The comparator is the standard busulfan based conditioning in combination with fludarabine which is a very commonly used RIC and allows a clear comparison of the effect between both regimens. The choice of endpoints with EFS at 2 years as primary is satisfactory considering both regimens achieve very high rates of engraftment. All other secondary endpoints are also considered adequate.

There is sufficient follow up for data maturity and to allow comprehensive assessment of results. The conduct of the study appears adequate and there are no concerns. Overall, the study design is in line with the main CHMP anti-cancer guideline and followed CHMP scientific advice.

The proposed dose and the regimen schedule in combination with fludarabine, as administered in the pivotal study, have been justified by an earlier dose finding study (FludT.6/L) and data from the phase III FludT.14/L Trial I.

Paediatrics

The indication in paediatric patients is based on a single arm study MC-FludT.17/M (malignant diseases of adequate design and agreed in the PIP by PDCO. A paediatric study MC FludT.16/NM conducted in a non malignant setting is currently ongoing and preliminary data have been submitted.

The dosing regimen used in the paediatrics study MC-FluT.17/M has been justified.

Efficacy data and additional analyses

Adults

In the pivotal phase III trial, adult patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) and increased risk for standard conditioning therapies because of higher age (≥ 50 years) or comorbidities (haematopoietic cell transplantation comorbidity index [HCT-CI] score > 2) were randomised to receive a conditioning regimen with 3 × 10 g/m² treosulfan combined with fludarabine (FT_{10}; n = 220) or a regimen of intravenous busulfan (total dose 6.4 mg/kg) combined with fludarabine (FB2; n = 240), followed by alloHSCT. 64% of patients had AML and 36% MDS. The median age of patients was 60 years (range 31–70 years); 25% of patients were older than 65 years.

The primary endpoint of this study was event-free survival (EFS) after 2 years. Events were defined as relapse of disease, graft failure or death (whatever occurred first). Non-inferiority of FT_{10} *versus* the reference FB2 was statistically proven Non-inferiority of TREO conditioning versus BU has been demonstrated in both analysis sets (PPS: p = 0.0000424; FAS: p = 0.0000164) with respect to the primary endpoint EFS at two years. The corresponding HR (99.9702% CI) were 0.67 (0.37, 1.23) and 0.65 (0.36, 1.19) for the PPS and FAS respectively. The sensitivity analyses are supportive of the primary analysis.

Analyses of EFS at 2 years for various pre-defined subgroups (donor type, risk group, disease, age group, HCT-CI score, remission status at study entry, and various combinations of these parameters) were always in favour of the treosulfan regimen (hazard ratio [HR] of FT_{10} *vs*. FB2 < 1), with only one exception (risk group I of MDS patients; HR 1.14 [95% CI 0.48, 2.63]).

The favourable result in EFS is supported by the outcome in secondary endpoints of overall survival (HR 0.61, 95% CI 0.42, 0.88), non-relapse mortality (HR 0.60, 95% CI 0.36, 1.01) and TRM (HR 0.54, 95% CI 0.32, 0.91). Whilst both regimens had same engraftment success rates and relapse incidence it is clear TREO regimen is less toxic than BU, evident from month 12 after transplant.

Although TREO regimen did not show superiority over BU regimen the observed favourable trend results are clinically meaningful.

Overall the subgroups analysis favoured TREO over BU and there are no concerns for the few that did not as they included small number of patients and should be viewed with caution. For those results per disease (AML or MDS) it is reassuring that TREO had a more favourable outcome than BU for EFS, OS and TRM.

These results have been confirmed in a final analysis based on 570 randomized patients which were in line or slightly better for TREO compared with the primary analysis based on 476 patients.

There is limited information available on treosulfan-based conditioning (FT_{14} regimen ± thiotepa; see section 4.2) in adult patients with non-malignant disorders (NMD). The main indications for an alloHSCT with treosulfan conditioning in adult NMD patients are haemoglobinopathies (e.g. sickle cell disease, thalassaemia major [TM]), primary immune deficiency, hemophagocytic disorder, immune dysregulatory disorder and bone marrow failure).

In one study, 31 NMD patients were treated with the FT_{14} regimen plus anti-thymocyte globulin. The age of the patients ranged from 0.4 to 30.5 years, and 29% had HCT-CI scores > 2. All patients engrafted, with a median time to neutrophil engraftment of 21 (range, 12–46) days. The two-year projected overall survival was 90%. Complete disease responses were observed in 28 patients (90%), as measured by clinical symptoms and laboratory assays (Burroughs LM et al., Biology of Blood and Marrow Transplantation 2014; 20(12):1996-2003).

An Italian group treated 60 TM patients (age range 1-37 years; including 12 adults) with the FT_{14} plus thiotepa regimen. All patients engrafted except one, who died on day +11; the median time to neutrophil and platelet recovery was 20 days. With a median follow-up of 36 months (range, 4-73), the 5-year overall survival

probability was 93% (95% CI 83-97%). No difference in terms of outcome was observed between children and adults (Bernardo ME et al.; Blood 2012; 120(2):473-6).

A retrospective comparison of treosulfan-based (n = 16) *versus* busulfan-based (n = 81) conditioning in adult patients revealed quite comparable survival rates (70.3 ± 15.1% *vs*. 69.3 ± 5.5%), while risk for acute GvHD was lower in the treosulfan group (odds ratio 0.28; 95% CI 0.12-0.67; P = 0.004) (Caocci G et al.; American Journal of Hematology 2017; 92(12):1303-1310).

A satisfactory justification to allow extrapolation of data from study FluT.14/L Trial II to non-malignant indication in adults has been presented , including the proposed dose regimen in the non-malignant setting.

Only patients up to 70 years were included in the pivotal study. Nowadays, eligibility to undergo alloHSCT is based on patient's fitness rather than age, and takes into consideration performance status, presence of co-morbidities and patient's preference. Therefore, no restriction of the indication in adults is necessary and it is up to the physician to make that decision.

Assessment of paediatric data on clinical efficacy

The clinical study report for the paediatric indication in non-malignant disease (study 16/NM) has not been submitted and there are limited data available. Most of the patients who received TREO also had TT reflecting current clinical practice.

The final analysis of the paediatric study in malignant diseases (17/M) is sufficient to support the efficacy of TREO based conditioning in the paediatric population with underlying malignant diseases which appears in line or better compared to historical paediatric data and with data from the trial in adult patients with malignant diseases.

2.5.4. Conclusions on the clinical efficacy

In conclusion, efficacy has been shown in the adult indication (malignant and non-malignant diseases) and in the paediatric indication of malignant diseases.

2.6. Clinical safety

In total, 564 adults and 88 paediatric patients have been treated with TREO- based conditioning. Across seven clinical trials (5 in adults and 2 in paediatrics) data on AE were recorded. Laboratory changes of blood count parameters within 28 days after transplant were not considered as AEs.

Patient exposure

All patients in the clinical program who received at least one dose of treatment are included in the safety analysis.

Adults

In total, 221 adult patients have been treated with the proposed TREO 10 g/m²/d IV, Day -4 to-2 given together with fludarabine within the pivotal study MC-FludT.14/L Trial II.

Table 33. Number of patients treated per TREO dose in adult patients

Dose	Day	Route	Study					Total
			6/L	7/AML	8/MDS	14/L Trial I	14/L Trial II	
3 × 10 g/m²	- 6 to - 4	IV	20	-	-	-	-	20
3 × 10 g/m²	- 4 to - 2	IV	-	-	-	-	221	221
3 × 12 g/m²	- 6 to - 4	IV	18	-	-	-	-	18
3 × 14 g/m²	- 6 to - 4	IV	17	75	45	168	-	305
Control arm*	- 4 to - 3	IV	-	-	-	152	240	392
** BU 4 x 0.8 mg/kg/d, plus FLU 5 x 30 mg/m², Day -6 to -2*								

The majority of patients suffered from AML (n = 380 [67.4%]) or MDS (n = 154 [27.3%]). A total of 30 patients suffered from other haematological malignancies, including ALL, CML, Hodgkin lymphoma, MM or NHL.

The majority of patients had an increased toxicity risk for classical conditioning therapies (n = 466 [82.6%]). Patients with severe concomitant illnesses or medical conditions had been excluded from these trials.

Paediatrics

The proposed dose regimen for TREO in children ranges from 10-14 g/m²/d, given on days -6 to -4.

Table 34. Number of patients treated per TREO dose in paediatric patients

Dose	Day	Route	Study				Total	
			16/NM		17/M			
			+ TT	- TT	+ TT	- TT	+ TT	- TT
10 g/m²/d	- 6 to - 4	IV	1	3	5	1	6	4
12 g/m²/d	- 6 to - 4	IV	11	0	23	3	34	3
14 g/m²/d	- 6 to - 4	IV	3	0	37	1	40	1
Control arm*	- 7 to - 4	IV	19	0	-	-	19	0
** BU 3.2-4.8 mg/kg/d, days -7 to -4, plus FLU 30 mg/m²/d, Day -7 to -3; TT = thiotepa 2 × 5 mg/kg day -2*								

Of the 88 paediatric patients treated with TREO-based conditioning, 18 patients had non-malignant diseases (primary immunodeficiency, haemoglobinopathy, inborn error of metabolism and bone marrow failure syndromes) and 70 patients had malignant diseases (AML, ALL, MDS, and JMML). The study in non-malignant diseases (MC-FludT.16/NM) is still ongoing.

There is supportive safety data from a registry study of the EBMT [Peters 2011] which included 626 paediatric patients with malignant and non-malignant diseases who had been treated with TREO-based conditioning.

Adverse events

Adults

The following table gives an overall summary of AEs observed in phase III studies MC-FludT.14/L Trial I/II.

Table 35. Overall summary of adverse events in trial MC-FludT.14/L

Study	**FludT.14/L Trial I**		**FludT.14/L Trial II**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Number of patients	168 (100%)	152 (100%)	221 (100%)	240 (100%)
Any adverse event				
Patients with AEs of any CTCAE Grade	100%	96.1%	93.2%	95.4%
Patients with AEs of at least CTCAE Grade III	72.6%	63.2%	53.4%	54.6%
AEs related to investigational drug (ARs)				
Patients with ARs of any CTCAE Grade	89.9%	85.5%	62.9%	70.0%
Patients with ARs of at least CTCAE Grade III	39.9%	40.1%	26.7%	30.8%
Serious adverse events				
Patients with at least one serious AE	11.3%	5.9%	8.1%	7.1%
Results in death	4.8%	1.3%	2.7%	2.1%
Life-threatening	5.4%	2.0%	3.6%	2.9%
Hospitalisation or prolongation of hospitalisation	1.8%	2.6%	2.7%	3.3%
Disability/Incapacity	0.6%	0.7%	0	0
Congenital anomaly or birth defect	0	0	0	0
Drug-related serious adverse events				
Patients with drug related serious AEs	4.8%	4.6%	2.7%	3.3%
Patients with maximum CTCAE Grade				
CTCAE Grade I	7.1%	7.9%	16.7%	15.4%
CTCAE Grade II	20.2%	25.0%	23.1%	25.4%
CTCAE Grade III	61.3%	58.6%	44.3%	48.3%
CTCAE Grade IV	6.5%	3.9%	6.3%	5.0%
CTCAE Grade V	4.8%	0.7%	2.7%	1.3%

Common AE

The following table lists the frequency of patients with treatment emergent AE (TEAEs) occurring in at least 5% of patients, with the proposed dose regimen for TREO in adults (MC-FludT.14/L Trial II), and compares it with the BU-arm. Significant differences of all grades TEAEs ($P < 0.05$; two-sided P-value) are marked with an asterisk.

In the TREO group, most frequently AEs (> 10%) included oral mucositis (34.8%), fever (32.1%), nausea/vomiting (30.3%/19.5%), infections (26.2%), oedema limbs (20.8%), headache (15.4%), febrile neutropenia (14.9%), diarrhoea (14.9%), bone/back pain (14.5%/14%), hypertension (14.9%), constipation (13.1%), and maculopapular rash (12.2%).

TEAEs which were significantly more frequent in the BU/FLU conditioning compared to the TREO regimen include eye disorders (10.4% vs. 3.6%), vertigo (7.9% vs. 3.2%), dyspnoea (8.3% vs. 3.6%), oral mucositis (47.1%

vs. 34.8%), nausea (41.3% vs. 30.3%), and increased γGT (13.3% vs. 7.2%). The only TEAEs that were significantly more frequent in the TREO group was cardiac disorders (14.9% vs. 8.3%)

Table 36. Frequency (%) of patients with TEAEs in MC-FludT.14/L Trial II, occurring in at least 5% of patients in either treatment group (Safety analysis set)

Parameter	**TEAEs, all grades**		**TEAEs, ≥ grade III**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Total number of patients	221	240	221	240
Patients with any event	93.2	95.4	53.4	54.6
Infections and infestations				
Any event	26.2	24.2	14.5	9.2
Lung infection	5.9	2.5	4.5	2.5
Other (not specified)	10.4	10.4	3.2	2.1
Blood and lymphatic system disorders				
Any event	14.9	12.1	14.9	12.1
Febrile neutropenia	14.9	12.1	14.9	12.1
Immune system disorders				
Any event	6.8	7.9	0.9	0.4
Allergic reaction	5.9	7.9	0.5	0.4
Metabolism and nutrition disorders				
Any event	20.4	18.3	7.2	5.4
Anorexia	8.6	9.6	0.9	1.3
Psychiatric disorders				
Any event	7.7	9.6	0.9	0.8
Nervous system disorders				
Any event	27.1	30.4	2.3	3.3
Headache	15.4	19.2	0.5	0.4
Dizziness	5.9	4.6		
Eye disorders				
Any event	3.6	10.4*		
Ear and labyrinth disorders				
Any event	5.4	7.9	0	0.4
Vertigo	3.2	7.9*	0	0.4
Cardiac disorders				
Any event	14.9*	8.3	3.2	2.9
Vascular disorders				
Any event	24.4	27.9	10.0	11.3
Hypertension	14.9	19.6	7.7	7.9
Hypotension	6.3	4.6	2.3	2.1
Respiratory, thoracic and mediastinal disorders				
Any event	19.0	22.9	2.7	3.8
Epistaxis	7.2	8.3	0	0.8
Dyspnoea	3.6	8.3*	0.5	1.3
Gastrointestinal disorders				
Any event	67.9	74.6	10.9	16.3

Parameter	TEAEs, all grades		TEAEs, ≥ grade III	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Mucositis oral	34.8	47.1*	4.5	7.5
Nausea	30.3	41.3*	2.7	6.7
Vomiting	19.5	21.3		
Diarrhoea	14.9	20.0	1.4	1.7
Constipation	13.1	12.1	0.5	0
Abdominal pain	9.5	10.0	1.4	0.8
Skin and subcutaneous tissue disorders				
Any event	29.4	28.3	1.8	1.7
Rash maculo-papular	12.2	9.6	1.4	1.7
Skin, other	6.8	7.9		
Pruritus	5.4	4.2	0.5	0
Purpura	5.4	3.8		
Musculoskeletal and connective tissue disorders				
Any event	37.1	27.9	4.5	2.9
Back pain	14.0	14.2	2.7	0.4
Bone pain	14.5	10.0	0.9	0.8
Arthralgia	8.6	4.2	0.9	0.4
Pain in extremity	6.3	4.2	0	1.3
Renal and urinary disorders				
Any event	9.0	9.6	1.4	0.4
General disorders and administration site conditions				
Any event	54.3	53.3	1.8	5.0
Fever	32.1	33.8	0.5	3.3
Oedema limbs	20.8	14.6	0.5	1.7
Fatigue	10.0	12.9	0.5	0
Chills	6.8	6.3		
Localised oedema	7.2	4.6	0.5	0
Pain	5.4	2.5		
Investigations				
Any event	27.6	27.5	14.9	14.6
γGT increased	7.2	13.3*	5.0	10.0
Alanine aminotransferase increased	7.7	6.7	5.0	3.8
Blood bilirubin increased	8.6	5.8	3.6	2.9
Weight gain	6.8	6.7		
Investigations - Other (not specified)	5.4	6.7	2.7	3.8
Aspartate aminotransferase increased	7.2	4.6	4.5	2.9
** P < 0.05*				

Treatment-related AE

The following table shows the frequency of AEs related to the investigational product (ARs) with the proposed dose regimen for TREO in adults (MC-FludT.14/L Trial II) , and compares it with BU-arm. Most frequent (> 5%) ARs observed with the TREO regimen include oral mucositis (30.3%), nausea (20.4%), vomiting (13.1%),

ALT/AST increased (7.2%/6.8%), infections (7.7%), fatigue (6.8%), bilirubin increased (5.9%), anorexia (5.9%), and headache (5.4%).

Drug-related TEAEs (all grades) more frequently observed with BU/FLU conditioning compared to the TREO regimen include nervous system disorders (12.1% vs. 7.2%), eye disorders (3.8% vs. 0%), ear and labyrinth disorders (4.6% vs. 0.9%), gastrointestinal disorders (55.8% vs. 45.7%; especially oral mucositis 40.0% vs. 30.3% and diarrhoea 12.1% vs. 5.0%), general disorders (20.4% vs. 13.1%; especially fever 11.7% vs. 4.5%), and increased γGT (11.3% vs. 5.0%). The only drug-related TEAEs (all grades) which were more frequently observed in the TREO group were increased transaminases (ALT: 7.2% vs. 5.4%; AST: 6.8% vs. 3.3%).

Selected (> 5% of patients) TREO-related TEAEs by donor type in all five adult trials showed the frequency of most ARs was slightly higher in the MUD group, especially with respect to infections, gastrointestinal disorders, and increased bilirubin.

Table 37. Frequency (%) of patients with ARs occurring in at least 1% of patients in either treatment group by CTCAE System Organ Class and Term in MC-FludT.14/L Trial II (Safety analysis set)

Parameter	**ARs, all grades**		**ARs, ≥ grade III**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Total number of patients	221	240	221	240
Patients with any event	62.9	70.0	26.7	30.8
Infections and infestations				
Any event	7.7	7.1	4.1	2.1
Infections and infestations – Other (not specified)	1.4	2.5	0.5	0.8
Lung infection	1.8	0.8	1.4	0.8
Catheter related infection	1.8	1.3	0.5	0.4
Sepsis	1.8	0.4	1.8	0.4
Blood and lymphatic system disorders				
Any event	4.1	5.0	4.1	5.0
Febrile neutropenia	4.1	5.0	4.1	5.0
Immune system disorders				
Any event	0	2.5	0	0.4
Allergic reaction	0	2.5	0	0.4
Metabolism and nutrition disorders				
Any event	6.3	6.7	1.4	2.5
Anorexia	5.9	5.0	0.9	1.3
Psychiatric disorders				
Any event	0.5	2.5	0	0.4
Hallucinations	0	1.3		
Nervous system disorders				
Any event	7.2	12.1	0	1.7
Headache	5.4	6.3		
Dizziness	1.8	1.3		
Tremor	0	1.3		
Syncope	0	1.7	0	1.7
Seizure	0	1.3		
Eye disorders				

Parameter	ARs, all grades		ARs, ≥ grade III	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Any event	0	3.8		
Dry eye	0	1.7		
Ear and labyrinth disorders				
Any event	0.9	4.6		
Vertigo	0.5	4.2		
Cardiac disorders				
Any event	2.7	2.5	0	1.3
Vascular disorders				
Any event	4.1	6.3	0.9	1.7
Hypertension	2.3	3.3	0.9	0.4
Haematoma	0.5	1.3	0	0.4
Respiratory, thoracic and mediastinal disorders				
Any event	7.2	9.6	0	0.4
Epistaxis	2.7	2.1		
Dyspnoea	0.9	2.9		
Cough	0.5	2.5		
Sore throat	0.5	2.5		
Pharyngeal mucositis	0.5	1.3		
Gastrointestinal disorders				
Any event	45.7	55.8	8.6	11.7
Mucositis oral	30.3	40.0	3.6	6.3
Nausea	20.4	30.0	2.3	5.4
Vomiting	13.1	13.3	0.5	1.3
Diarrhoea	5.0	12.1	0,9	0.8
Constipation	1.8	0.4		
Abdominal pain	4.1	5.0	0.9	0.8
Gastrointestinal disorders - Other, specify	1.4	0		
Stomach pain	1.4	0.8	0.5	0
Dyspepsia	0.9	1.7		
Dysphagia	0.9	2.1	0.5	0
Oral pain	1.4	0.8	0.5	0
Flatulence	0	1.3		
Gastritis	1.4	0		
Hepatobiliary disorders				
Any event	0.9	2.1	0.5	0.8
Other, specify	0.9	1.7	0.5	0.4
Skin and subcutaneous tissue disorders				
Any event	11.3	13.8	0.9	1.3
Rash maculo-papular	4.1	3.3	0.9	1.3
Other, specify	1.8	2.5		
Pruritus	2.7	1.3		

Parameter	ARs, all grades		ARs, ≥ grade III	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Purpura	1.8	2.5		
Erythema multiforme	1.4	1.3		
Palmar-plantar erythrodysesthesia syndrome	1.8	2.5		
Alopecia	0	1.3		
Musculoskeletal and connective tissue disorders				
Any event	2.3	2.9		
Bone pain	0.9	1.7		
Arthralgia	1.4	0		
Renal and urinary disorders				
Any event	3.2	3.3	0.5	0.4
Acute kidney injury	2.3	0.4	0.5	0.4
Reproductive system and breast disorders				
Any event	0	0.8	0	0.4
General disorders and administration site conditions				
Any event	13.1	20.4	0.5	0.8
Fever	4.5	11.7	0.5	0.4
Oedema limbs	3.6	2.9		
Fatigue	6.8	8.8		
Chills	0.5	1.7		
Localised oedema	1.4	0.8		
Investigations				
Any event	18.6	15.8	11.8	10.4
γGT increased	5.0	11.3	3.2	9.2
Alanine aminotransferase increased	7.2	5.4	5.0	3.3
Aspartate aminotransferase increased	6.8	3.3	4.5	2.5
Blood bilirubin increased	5.9	4.2	2.7	1.7
Creatinine increased	0	1.3		
Investigations - Other, specify	3.2	3.8	1.4	1.3
Weight gain	0.9	1.3		
Injury, poisoning and procedural complications				
Any event	0.5	0		

Paediatrics

The following table gives an overall summary of AEs observed in the two paediatric trials. No striking differences were observed between the two arms in study MC-FludT.16/NM; however, the number of patients in each arm is too small to draw any conclusions.

Table 38. Overall summary of adverse events in two paediatric trials

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Treatment arm	**TREO**	**BU**	**TREO**
Number of patients	18 (100%)	17 (100%)	70 (100%)
Any adverse event			
Patients with AEs of any CTCAE Grade	88.9%	88.2%	95.7%
Patients with AEs of at least CTCAE Grade III	72.2%	76.5%	75.7%
Drug-related adverse events			
Patients with ADRs of any CTCAE Grade	77.8%	70.6%	84.3%
Patients with ADRs of at least CTCAE Grade III	44.4%	47.1%	48.6%
Serious adverse events			
Patients with at least one serious AE	22.2%	35.3%	32.9%
Results in death	0	0	1.4%
Life-threatening	5.6%	5.9%	8.6%
Hospitalisation or prolongation of hospitalisation	16.7%	29.4%	28.6%
Disability/Incapacity	0	5.9%	1.4
Congenital anomaly or birth defect	0	0	0
Drug-related serious adverse events			
Patients with drug related serious AEs	0	0	1.4%
Patients with maximum CTCAE Grade			
CTCAE Grade I	0	5.9%	2.9%
CTCAE Grade II	16.7%	5.9%	17.1%
CTCAE Grade III	61.1%	70.6%	60.0%
CTCAE Grade IV	11.1%	5.9%	15.7%
CTCAE Grade V	0	0	0

Common AE

The most frequent TEAEs after TREO-based conditioning include gastrointestinal disorders (> 10%: stomatitis [78.4%], vomiting [68.2%], diarrhoea [63.6%], nausea [45.5%], abdominal pain [34.1%], constipation [12.5%]), pyrexia (71.6%), infections (59.1%), vascular disorders (hypertension [35.2%], haematoma [10.2%]), skin and subcutaneous tissue disorders (> 10%: maculopapular rash [29.5%], pruritus [23.9%], pain of skin [11.4%]), headache (29.5%), cough (18.2%), pain in extremity (18.2%), sinus tachycardia (12.5%), hypersensitivity (12.5%), investigations (> 10%: viral test positive [15.9%], ALT increased [10.2%]), and infusion-related reaction (10.2%).

The following table shows the frequency of patients with at least CTCAE grade III in the two paediatric trials.

Table 39. Frequency of patients with at least CTCAE grade III adverse events

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Study arm	**TREO**	**BU**	**TREO**
No. of patients	18 (100%)	17 (100%)	70 (100%)
Patients with any event	13 (72.2%)	13 (76.5%)	53 (75.7%)
Infections and infestations			
Any event	4 (22.2%)	5 (29.4%)	28 (40.0%)

Study	MC-FludT.16/NM		MC-FludT.17/M
Study arm	**TREO**	**BU**	**TREO**
Catheter related infection	1 (5.6%)	2 (11.8%)	6 (8.6%)
Sepsis	1 (5.6%)	1 (5.9%)	4 (5.7%)
Bladder infection	0	0	3 (4.3%)
Urinary tract infection	0	0	3 (4.3%)
Upper respiratory infection	0	0	2 (2.9%)
Encephalitis infection	1 (5.6%)	0	1 (1.4%)
Laryngitis	0	0	1 (1.4%)
Skin infection	0	0	1 (1.4%)
Soft tissue infection	0	0	1 (1.4%)
Enterocolitis infectious	0	1 (5.9%)	0
Oesophageal infection	1 (5.6%)	0	0
Other	0	1 (5.9%)	20 (28.6%)
Blood and lymphatic system disorders			
Any event	2 (11.1%)	0	6 (8.6%)
Febrile neutropenia	0	0	4 (5.7%)
Anaemia	0	0	1 (1.4%)
Haemolytic uremic syndrome	1 (5.6%)	0	0
Thrombotic thrombocytopenic purpura	0	0	1 (1.4%)
Other	1 (5.6%)	0	1 (1.4%)
Immune system disorders			
Any event	0	0	2 (2.9%)
Allergic reaction	0	0	2 (2.9%)
Metabolism and nutrition disorders			
Any event	0	0	9 (12.9%)
Glucose intolerance	0	0	2 (2.9%)
Hyperkalaemia	0	0	2 (2.9%)
Iron overload	0	0	2 (2.9%)
Alkalosis	0	0	1 (1.4%)
Anorexia	0	0	1 (1.4%)
Dehydration	0	0	1 (1.4%)
Hyperglycaemia	0	0	1 (1.4%)
Psychiatric disorders			
Any event	0	0	2 (2.9%)
Delirium	0	0	1 (1.4%)
Other	0	0	1 (1.4%)
Nervous system disorders			
Any event	0	0	0
Encephalopathy	0	0	0
Headache	0	0	5 (7.1%)
Paraesthesia	0	0	1 (1.4%)
Peripheral motor neuropathy	0	0	1 (1.4%)

Study	MC-FludT.16/NM		MC-FludT.17/M
Study arm	**TREO**	**BU**	**TREO**
Tremor	0	0	1 (1.4%)
Eye disorders			
Any event	0	0	1 (1.4%)
Blurred vision	0	0	1 (1.4%)
Cardiac disorders			
Any event	0	0	1 (1.4%)
Heart failure	0	0	1 (1.4%)
Vascular disorders			
Any event	5 (27.8%)	2 (11.8%)	7 (10.0%)
Hypertension	5 (27.8%)	2 (11.8%)	5 (7.1%)
Hypotension	0	0	1 (1.4%)
Capillary leak syndrome	1 (5.6%)	0	0
Other	0	0	1 (1.4%)
Respiratory, thoracic and mediastinal disorders			
Any event	1 (5.6%)	3 (17.6%)	3 (4.3%)
Pneumonitis	0	2 (11.8%)	1 (1.4%)
Hypoxia	1 (5.6%)	0	1 (1.4%)
Dyspnoea	0	0	1 (1.4%)
Pharyngeal mucositis	0	1 (5.9%)	0
Laryngeal haemorrhage	0	0	1 (1.4%)
Pulmonary oedema	0	0	1 (1.4%)
Gastrointestinal disorders			
Any event	7 (38.9%)	10 (58.8%)	39 (55.7%)
Mucositis oral	5 (27.8%)	9 (52.9%)	29 (41.4%)
Diarrhoea	3 (16.7%)	3 (17.6%)	10 (14.3%)
Nausea	2 (11.1%)	4 (23.5%)	13 (18.6%)
Vomiting	1 (5.6%)	2 (11.8%)	12 (17.1%)
Abdominal pain	1 (5.6%)	0	1 (1.4%)
Oesophageal pain	0	0	1 (1.4%)
Dysphagia	0	0	3 (4.3%)
Typhlitis	0	0	1 (1.4%)
Upper gastrointestinal haemorrhage	0	0	1 (1.4%)
Hepatobiliary disorders			
Any event	0	0	1 (1.4%)
Other	0	0	1 (1.4%)
Skin and subcutaneous tissue disorders			
Any event	0	0	8 (11.4%)
Rash maculo-papular	0	0	5 (7.1%)
Erythroderma	0	0	2 (2.9%)
Pruritus	0	0	1 (1.4%)
Other	0	0	1 (1.4%)

Study	MC-FludT.16/NM		MC-FludT.17/M
Study arm	**TREO**	**BU**	**TREO**
Musculoskeletal and connective tissue disorders			
Any event	0	0	1 (1.4%)
Chest wall pain	0	0	1 (1.4%)
Renal and urinary disorders			
Any event	0	0	5 (7.1%)
Acute kidney injury	0	0	3 (4.3%)
Cystitis noninfective	0	0	1 (1.4%)
Other	0	0	1 (1.4%)
General disorders and administration site conditions			
Any event	0	0	2 (2.9%)
Fatigue	0	0	1 (1.4%)
Fever	0	0	1 (1.4%)
Investigations			
Any event	0	0	7 (10.0%)
Blood bilirubin increased	0	0	4 (5.7%)
ALT increased	0	0	1 (1.4%)
AST increased increased	0	0	1 (1.4%)
γGT increased	0	0	1 (1.4%)
Fibrinogen	0	0	1 (1.4%)
Other	0	0	1 (1.4%)
Injury, poisoning and procedural complications			
Any event	0	0	1 (1.4%)
Burn	0	0	1 (1.4%)

Treatment related AE

The most frequent drug-related TEAEs include stomatitis (69.3%), vomiting (43.2%), diarrhoea (33.0%), nausea (30.7%), abdominal pain (15.9%), pyrexia (14.8%), infections (11.4%), and pruritus (11.4%).

Table 40. Frequency of paediatric patients with TREO-related TEAEs

[Note: Absolute and relative frequencies of patients with event relative to the total number of patients]

Primary System Organ Class Preferred Term	MC-FludT.16/NM	MC-FludT.17/M	Overall
Patients with any event	14 (77.8%)	59 (84.3%)	73 (83.0%)
Infections and infestations			
Any event	2 (11.1%)	8 (11.4%)	10 (11.4%)
Adenovirus infection	1 (5.6%)	2 (2.9%)	3 (3.4%)
Cytomegalovirus infection	0	3 (4.3%)	3 (3.4%)
Epstein-Barr virus infection	0	2 (2.9%)	2 (2.3%)
Urinary tract infection	0	2 (2.9%)	2 (2.3%)
Device related infection	0	1 (1.4%)	1 (1.1%)
Escherichia infection	0	1 (1.4%)	1 (1.1%)

Primary System Organ Class Preferred Term	MC-FludT.16/NM	MC-FludT.17/M	Overall
Infection	0	1 (1.4%)	1 (1.1%)
Mucosal infection	0	1 (1.4%)	1 (1.1%)
Penile infection	1 (5.6%)	0	1 (1.1%)
Staphylococcal infection	0	1 (1.4%)	1 (1.1%)
Blood and lymphatic system disorders			
Any event	0	1 (1.4%)	1 (1.1%)
Febrile neutropenia	0	1 (1.4%)	1 (1.1%)
Metabolism and nutrition disorders			
Any event	0	2 (2.9%)	2 (2.3%)
Alkalosis	0	1 (1.4%)	1 (1.1%)
Electrolyte imbalance	0	1 (1.4%)	1 (1.1%)
Hypomagnesaemia	0	1 (1.4%)	1 (1.1%)
Nervous system disorders			
Any event	2 (11.1%)	2 (2.9%)	4 (4.5%)
Headache	1 (5.6%)	1 (1.4%)	2 (2.3%)
Paraesthesia	0	2 (2.9%)	2 (2.3%)
Seizure	1 (5.6%)	0	1 (1.1%)
Eye disorders			
Any event	1 (5.6%)	1 (1.4%)	2 (2.3%)
Conjunctival haemorrhage	0	1 (1.4%)	1 (1.1%)
Dry eye	1 (5.6%)	0	1 (1.1%)
Vascular disorders			
Any event	1 (5.6%)	2 (2.9%)	3 (3.4%)
Capillary leak syndrome	1 (5.6%)	0	1 (1.1%)
Hypertension	0	1 (1.4%)	1 (1.1%)
Hypotension	0	1 (1.4%)	1 (1.1%)
Respiratory, thoracic and mediastinal disorders			
Any event	4 (22.2%)	4 (5.7%)	8 (9.1%)
Oropharyngeal pain	2 (11.1%)	2 (2.9%)	4 (4.5%)
Epistaxis	1 (5.6%)	2 (2.9%)	3 (3.4%)
Hypoxia	1 (5.6%)	0	1 (1.1%)
Gastrointestinal disorders			
Any event	14 (77.8%)	56 (80.0%)	70 (79.5%)
Stomatitis	13 (72.2%)	48 (68.6%)	61 (69.3%)
Vomiting	9 (50.0%)	29 (41.4%)	38 (43.2%)
Diarrhoea	9 (50.0%)	20 (28.6%)	29 (33.0%)
Nausea	4 (22.2%)	23 (32.9%)	27 (30.7%)
Abdominal pain	6 (33.3%)	8 (11.4%)	14 (15.9%)
Dysphagia	0	3 (4.3%)	3 (3.4%)
Oral pain	0	3 (4.3%)	3 (3.4%)
Anal inflammation	1 (5.6%)	1 (1.4%)	2 (2.3%)
Dyspepsia	0	2 (2.9%)	2 (2.3%)

Primary System Organ Class Preferred Term	MC-FludT.16/NM	MC-FludT.17/M	Overall
Proctitis	1 (5.6%)	1 (1.4%)	2 (2.3%)
Abdominal pain upper	0	1 (1.4%)	1 (1.1%)
Constipation	1 (5.6%)	0	1 (1.1%)
Gingival pain	1 (5.6%)	0	1 (1.1%)
Neutropenic colitis	0	1 (1.4%)	1 (1.1%)
Oesophageal pain	0	1 (1.4%)	1 (1.1%)
Hepatobiliary disorders			
Any event	1 (5.6%)	3 (4.3%)	4 (4.5%)
Venoocclusive liver disease	1 (5.6%)	1 (1.4%)	2 (2.3%)
Hepatomegaly	0	1 (1.4%)	1 (1.1%)
Hepatotoxicity	1 (5.6%)	0	1 (1.1%)
Liver disorder	0	1 (1.4%)	1 (1.1%)
Skin and subcutaneous tissue disorders			
Any event	10 (55.6%)	17 (24.3%)	27 (30.7%)
Pruritus	5 (27.8%)	5 (7.1%)	10 (11.4%)
Alopecia	7 (38.9%)	1 (1.4%)	8 (9.1%)
Rash maculo-papular	3 (16.7%)	5 (7.1%)	8 (9.1%)
Erythema	2 (11.1%)	3 (4.3%)	5 (5.7%)
Pain of skin	1 (5.6%)	3 (4.3%)	4 (4.5%)
Skin hyperpigmentation	1 (5.6%)	3 (4.3%)	4 (4.5%)
Dermatitis exfoliative	1 (5.6%)	2 (2.9%)	3 (3.4%)
Rash	0	3 (4.3%)	3 (3.4%)
Skin ulcer	1 (5.6%)	1 (1.4%)	2 (2.3%)
Urticaria	1 (5.6%)	1 (1.4%)	2 (2.3%)
Dermatitis acneiform	0	1 (1.4%)	1 (1.1%)
Dermatitis bullous	0	1 (1.4%)	1 (1.1%)
Erythema multiforme	0	1 (1.4%)	1 (1.1%)
Palmar-plantar erythrodysaesthesia syndrome	0	1 (1.4%)	1 (1.1%)
Rash erythematous	1 (5.6%)	0	1 (1.1%)
Rash generalised	0	1 (1.4%)	1 (1.1%)
Musculoskeletal and connective tissue disorders			
Any event	0	1 (1.4%)	1 (1.1%)
Pain in extremity	0	1 (1.4%)	1 (1.1%)
Renal and urinary disorders			
Any event	1 (5.6%)	3 (4.3%)	4 (4.5%)
Acute kidney injury	0	2 (2.9%)	2 (2.3%)
titis noninfective	0	1 (1.4%)	1 (1.1%)
Renal failure	1 (5.6%)	0	1 (1.1%)
Reproductive system and breast disorders			
Any event	0	1 (1.4%)	1 (1.1%)
Scrotal erythema	0	1 (1.4%)	1 (1.1%)
General disorders and administration site conditions			

Primary System Organ Class Preferred Term	MC-FludT.16/NM	MC-FludT.17/M	Overall
Any event	2 (11.1%)	12 (17.1%)	14 (15.9%)
Pyrexia	2 (11.1%)	11 (15.7%)	13 (14.8%)
Chills	1 (5.6%)	1 (1.4%)	2 (2.3%)
Fatigue	0	2 (2.9%)	2 (2.3%)
Pain	0	1 (1.4%)	1 (1.1%)
Investigations			
Any event	1 (5.6%)	13 (18.6%)	14 (15.9%)
Alanine aminotransferase increased	1 (5.6%)	7 (10.0%)	8 (9.1%)
Aspartate aminotransferase increased	0	7 (10.0%)	7 (8.0%)
Blood bilirubin increased	0	5 (7.1%)	5 (5.7%)
Gamma-glutamyltransferase increased	0	2 (2.9%)	2 (2.3%)
Aspergillus test positive	0	1 (1.4%)	1 (1.1%)
Injury, poisoning and procedural complications			
Any event	1 (5.6%)	2 (2.9%)	3 (3.4%)
Infusion related reaction	1 (5.6%)	1 (1.4%)	2 (2.3%)
Toxicity to various agents	0	1 (1.4%)	1 (1.1%)

Serious adverse events and deaths

Deaths

Adults

The overall death rate after TREO-based conditioning in the five adult trials was 31.4% (177 of 564 patients) and the major causes included transplant-related events (14.9% in the overall population; especially infections [8.2%] and GvHD [5.3%]) and relapse/progression (12.8%). Median time to death after transplantation was 5.6 months.

The death rate could be nearly halved by changing the TREO dose regimen from 14 g/m²/d (Day 6 to -4) in MC-FludT.14/L Trial I to 10 g/m²/d (Day -4 to -2) in Trial II (38.7% versus 23.5%), due to a significantly decreased rate of TRM (18.5% vs. 10.4%) and especially infection-related death (16.1% vs. 8.6%).

In the pivotal study MC-FludT.14/L Trial II, transplant-related death rate was lower in the TREO arm compared to the BU (23 patients [10.4%] versus 45 patients [18.8%] and cumulative incidence of TRM (FAS) at 24 months was 11.3% in the TREO arm and 28.2% in the BU.

Table 41. Death cases in the five adult trials

Study	6/L	7/AML	8/MDS	14/L Trial I		14/L Trial II	
Study drug	**TREO**	**TREO**	**TREO**	**TREO**	**BU**	**TREO**	**BU**
Number of patients	55 (100%)	75 (100%)	45 (100%)	168 (100%)	152 (100%)	221 (100%)	240 (100%)
alive	64%	63%	73%	68.5%	73.7%	76.5%	65.8%
dead	36%	37%	27%	31.5%	26.3%	23.5%	34.2%
Cause of death							
Relapse, progress	9%	24%	11.1%	10.7%	11.2%	11.8%	15.0%
Transplant-related	25%	12%	15.5%	18.5%	14.5%	10.4%	18.8%
Second. malignancy	-	-	-	-	0.7%	0	0.4%
Other	2%	1.3%	-	2.4%	-	0.9%	0

Unknown	-	-	-	-	-	0.5%	0
Time to death (months)							
Mean (SD)	NR	NR	NR	4.55 (2.85)	5.06 (2.76)	5.96 (4.52)	7.20 (4.73)
NR = not reported							

A detailed analysis of death cases observed in the TREO-arms of the adult trials is given below.

Table 42. Deaths cases in the TREO groups in the five adult trials

Study	**6/L** (N = 55)	**7/AML** (N = 75)	**8/MDS** (N = 45)	**14/L Trial I** (N= 168)	**14/L Trial II** (N = 221)	**Overall** (N = 564)
TREO dose (g/m²)	10-14	14	14	14	10	10-14
Survival status at study termination; n (%)						
Alive*	35 (63.6%)	47 (62.7%)	33 (73.3%)	103 (61.3%)	169 (76.5%)	387 (68.6%)
Dead	20 (36.4%)	28 (37.3%)	12 (26.7%)	65 (38.7%)	52 (23.5%)	177 (31.4%)
Cause of death; n (%)						
Relapse/progression	5 (9.1%)	18 (24.0%)	5 (11.1%)	18 (10.7%)	26 (11.8%)	72 (12.8%)
Transplantation related	14 (25.5%)	9 (12.0%)	7 (15.6%)	31 (18.5%)	23 (10.4%)	84 (14.9%)
GvHD	5 (9.1%)	4 (5.3%)	2 (4.4%)	9 (5.4%)	10 (4.5%)	30 (5.3%)
Pulmonary toxicity				1 (0.6%)		1 (0.2%)
Haemorrhage				2 (1.2%)	1 (0.5%)	3 (0.5%)
Renal failure				3 (1.8%)	5 (2.3%)	8 (1.4%)
Cardiac toxicity				1 (0.6%)	1 (0.5%)	2 (0.4%)
GI toxicity				1 (0.6%)		1 (0.2%)
Interstitial pneumonitis					1 (0.5%)	1 (0.2%)
EBV proliferative disease	2 (3.6%)	1 (1.3%)	1 (2.2%)			4 (0.7%)
Rejection/poor graft function	1 (1.8%)		1 (2.2%)			2 (0.4%)
CNS toxicity				1 (0.6%)		1 (0.2%)
Infection				27 (16.1%)	19 (8.6%)	46 (8.2%)
– Bacterial				7 (4.2%)	12 (5.4%)	19 (3.4%)
– Viral				7 (4.2%)	8 (3.6%)	15 (2.7%)
– Fungal				10 (6.0%)	2 (0.9%)	12 (2.1%)
– Unknown	7 (12.7%)	7 (9.3%)	3 (6.7%)	8 (4.8%)	2 (0.9%)	27 (4.8%)
Multiple organ failure				10 (6.0%)	5 (2.3%)	15 (2.7%)
Other	1 (1.8%)		3 (6.7%)	1 (0.6%)		5 (0.9%)
Unknown					1 (0.5%)	1 (0.2%)
Other	1 (1.8%)	1 (1.3%)		4 (2.4%)	2 (0.9%)	8 (1.4%)
Not documented				12 (7.1%)		12 (2.1%)
Time from transplantation to death (months)						
Mean (SD)	6.40 (5.58)	10.04 (6.52)	8.83 (7.21)	7.30 (7.27)	5.96 (4.52)	7.34 (6.35)
Median	4.42	9.33	6.87	5.36	4.71	5.65
Q1, Q3	1.97, 10.22	4.27, 13.77	2.41, 14.13	2.99, 8.80	2.79, 7.44	2.96, 10.32
Min, Max	0.4, 19.3	1.2, 27.9	1.7, 23.6	0.1, 36.9	0.4, 20.6	0.1, 36.9

* *The status 'alive' is displayed for all patients who did not terminate the study due to death.*

Paediatrics

Death rate in paediatric patients after TREO-based conditioning was much lower than in adult patients. Only 6 of 70 patients (8.6%) included so far into the two paediatric trial died, and only two (2.9%) from transplant-related causes.

Table 43. Death cases in the TREO group of study MC-FludT.17/M

		MC-FludT.17/M (N = 70)	
Survival status at study termination; n (%)			
Alive*		64 (91.4%)	
Dead		6 (8.6%)	
Cause of death; n (%)			
Relapse/progression		4 (5.7%)	
Transplantation related		2 (2.9%)	
GvHD		1 (1.4%)	
Pulmonary toxicity		1 (1.4%)	
Haemorrhage		1 (1.4%)	
Renal failure			
Gastrointestinal toxicity			
Interstitial pneumonitis		2 (2.9%)	
Rejection/poor graft function		1 (1.4%)	
Infection		1 (1.4%)	
– Bacterial		1 (1.4%)	
– Unknown			
Multiple organ failure		1 (1.4%)	
Other		2 (2.9%)	
Time from transplantation to death (months)			
Mean (SD)		6.56 (3.80)	
Median		7.82	
Q1, Q3		3.81, 9.66	
Min, Max		0.5, 9.7	
* *The status 'alive' is displayed for all patients who did not terminate the study due to death.*			

Adults

Most frequent serious adverse events (SAE) observed after TREO-based conditioning in 564 adult patients include infections (37 patients [6.6%]; especially sepsis and lung infection), nervous system disorders (12 [2.1%]), gastrointestinal disorders (10 [1.8%]), renal and urinary disorders (10 [1.8%]), and laboratory abnormalities (12 [2.1%]).

The overall incidence of SAEs in the TREO- and BU-arms of the two active-controlled studies was comparable. Most frequent SAEs reported in both trials included infections. Table 45 shows the frequency of patients with SAE reported in the two trials MC-FludT.14/L.

Table 45. SAEs reported in MC-FludT.14/L Trial I and II

Study	**Trial I**		**Trial II**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Total number of patients	168	152	221	240
Patients with any event	19 (11.3%)	9 (5.9%)	18 (8.1%)	17 (7.1%)
Infections and infestations				
Any event	11 (6.5%)	3 (2.0%)	11 (5.0%)	9 (3.8%)

Study	Trial I		Trial II	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Infection (documented clinically) [Blood]	6 (3.6%)	0		
Infection with normal ANC [Lung (pneumonia)]	2 (1.2%)	1 (0.7%)		
Infection with normal ANC [Brain (encephalitis, infectious)]	1 (0.6%)	0		
Infection with normal ANC [Meninges (meningitis)]	1 (0.6%)	0		
Infection with unknown ANC [Blood]	1 (0.6%)	0		
Infection with unknown ANC [Brain + Spinal cord (encephalomyelitis)]	1 (0.6%)	0		
Infection with unknown ANC [Eye NOS]	1 (0.6%)	0		
Infection (documented clinically) [Lung (pneumonia)]	1 (0.6%)	0		
Febrile neutropenia	2 (1.2%)	0		
Sepsis			5 (2.3%)	5 (2.1%)
Lung infection			6 (2.7%)	3 (1.3%)
Enterocolitis infectious			1 (0.5%)	0 (0.0%)
Other	0	2 (1.3%)	1 (0.5%)	1 (0.4%)
Blood and lymphatic system disorders				
Any event	2 (1.2%)	0		
Haemolysis	2 (1.2%)	0		
Immune system disorders				
Any event	2 (1.2%)	1 (0.7%)	1 (0.5%)	0
Cytokine release syndrome			1 (0.5%)	0
Allergic reaction	1 (0.6%)	1 (0.7%)		
Allergy, Other	1 (0.6%)	0		
Nervous system disorders				
Any event	1 (0.6%)	2 (1.3%)	1 (0.5%)	0
Ataxia	0	1 (0.7%)		
Encephalopathy	1 (0.6%)	0		
Seizure	0	1 (0.7%)		
Syncope			1 (0.5%)	0
Cardiac disorders				
Any event	5 (3.0%)	0	1 (0.5%)	1 (0.4%)
Cardiac ischaemia/infarction	1 (0.6%)	0		
Cardiopulmonary arrest	1 (0.6%)	0		
Hypotension	1 (0.6%)	0		
Supraventricular arrhythmia [Atrial fibrillation]	1 (0.6%)	0		
Supraventricular arrhythmia [Sinus arrhythmia]	1 (0.6%)	0		
Supraventricular arrhythmia [Sinus bradycardia]	1 (0.6%)	0		
Heart failure			0	1 (0.4%)
Left ventricular systolic dysfunction			1 (0.5%)	0
Right ventricular dysfunction			1 (0.5%)	0
Vascular disorders				
Any event	3 (1.8%)	0	1 (0.5%)	1 (0.4%)
Acute vascular leak syndrome	1 (0.6%)	0		
Capillary leak syndrome			0	1 (0.4%)
Haemorrhage, CNS	2 (1.2%)	0		
Hypertension			1 (0.5%)	0
Respiratory, thoracic and mediastinal disorders				
Any event	3 (1.8%)	1 (0.7%)	3 (1.4%)	3 (1.3%)
Respiratory failure			1 (0.5%)	2 (0.8%)
Aspiration			1 (0.5%)	0
Bronchopulmonary haemorrhage			1 (0.5%)	0
Dyspnoea			0	1 (0.4%)
Hypoxia	1 (0.6%)	0		

Study	Trial I		Trial II	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Pneumonitis	2 (1.2%)	0		
Other	0	1 (0.7%)		
Gastrointestinal disorders				
Any event			1 (0.5%)	3 (1.3%)
Diarrhoea			0	1 (0.4%)
Gastric haemorrhage			1 (0.5%)	0
Ileal perforation			0	1 (0.4%)
Ileus			0	1 (0.4%)
Vomiting			0	1 (0.4%)
Pancreatitis	1 (0.6%)	0		
Hepatobiliary disorders				
Any event	1 (0.6%)	2 (1.3%)	0	3 (1.3%)
Hepatic failure			0	1 (0.4%)
Liver dysfunction	0	1 (0.7%)		
Other	1 (0.6%)	1 (0.7%)	0	2 (0.8%)
Renal and urinary disorders				
Any event	3 (1.8%)	2 (1.3%)	2 (0.9%)	1 (0.4%)
Acute kidney injury			2 (0.9%)	1 (0.4%)
Renal failure	3 (1.8%)	2 (1.3%)		
General disorders and administration site conditions				
Any event			0	1 (0.4%)
Fever			0	1 (0.4%)
Injury, poisoning and procedural complications				
Any event			1 (0.5%)	0
Investigations				
Any event			1 (0.5%)	0
Bilirubin	1 (0.6%)	1 (0.7%)		
Creatinine	0	1 (0.7%)		
Acidosis	0	1 (0.7%)		

The following table shows the frequency of drug-related SAEs (SARs) reported in MC-FludT.14/L Trials.

Table 46. SARs occurring in at least 1% of patients in either treatment group reported in MC-FludT.14/L Trial I and II

Parameter	Trial I		Trial II	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Total number of patients	168	152	221	240
Patients with any event	8 (4.8%)	7 (4.6%)	6 (2.7%)	8 (3.3%)
Infections and infestations				
Any event	5 (3.0%)	2 (1.3%)	4 (1.8%)	4 (1.7%)
Infection with normal ANC [Lung (pneumonia)]	2 (1.2%)	1 (0.7%)		
Infection (documented clinically) [Blood]	2 (1.2%)	0		
Sepsis			3 (1.4%)	1 (0.4%)
Nervous system disorders				
Any event	1 (0.6%)	2 (1.3%)		
Cardiac disorders				
Any event	4 (2.4%)	0	0	1 (0.4%)
Vascular disorders				
Any event	1 (0.6%)	0		
Respiratory, thoracic and mediastinal disorders				
Any event	1 (0.6%)	1 (0.7%)	0	1 (0.4%)
Gastrointestinal disorders				

Parameter	Trial I		Trial II	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Any event			1 (0.5%)	0
Hepatobiliary disorders				
Any event	1 (0.6%)	2 (1.3%)	0	3 (1.3%)
Renal and urinary disorders				
Any event	1 (0.6%)	0		
Injury, poisoning and procedural complications				
Any event			1 (0.5%)	0
Investigations				
Any event	1 (0.6%)	3 (2.0%)	0	0

Following Day +28, only those SAEs with suspected causality to the study drug had to be reported. In trial I, three patients of the busulfan group (infections (2 patients) and late onset VOD (1 patient) and four patients of the treosulfan group (infections (all patients) and CNS bleeding (1 patient) reported SAEs. For trial II there were 3 SAEs with suspected causality reported by 2 patients in the busulfan group.

Paediatrics

A total of 27 of 88 paediatric patients (30.7%) experienced an SAE after TREO-based conditioning. Most frequent SAE was infection (19.3%).

Since start of study MC-FludT.16/NM until data snapshot 16-May-2017, 13 SAEs experienced by ten patients have been reported; seven SAEs by 4 patients with TREO and six SAEs by 6 patients with BU. No SAR has been reported so far.

In study MC-FludT.17/M 23 (32.9%) patients experienced an SAE. Most frequent SAE was infections.

Table 47. Frequency of paediatric patients with serious adverse events

Study	MC-FludT.16/NM		MC-FludT.17/M
Study arm	**TREO**	**BU**	**TREO**
No. of patients	18 (100%)	17 (100%)	70 (100%)
Patients with any event	4 (22.2%)	6 (35.3%)	23 (32.9%)
Infections and infestations			
Any event	2 (11.1%)	3 (17.6%)	15 (21.4%)
Catheter related infection		1 (5.9%)	1 (1.4%)
Sepsis	1 (5.6%)	1 (5.9%)	2 (2.9%)
Upper respiratory infection			3 (4.3%)
Encephalitis	1 (5.6%)		1 (1.4%)
Hepatitis viral			1 (1.4%)
Sinusitis			1 (1.4%)
Skin infection			1 (1.4%)
Enterocolitis		1 (5.9%)	
Cytomegalovirus infection			3 (4.3%)
Cytomegalovirus viremia			1 (1.4%)
Epstein-Barr virus infection			1 (1.4%)
Infection			1 (1.4%)
Blood and lymphatic system disorders			
Any event	1 (5.6%)		2 (2.9%)
Febrile neutropenia			2 (2.9%)
Immune thrombocytopenic purpura	1 (5.6%)		
Immune system disorders			
Any event			1 (1.4%)

Study	MC-FludT.16/NM		MC-FludT.17/M
Study arm	**TREO**	**BU**	**TREO**
Allergic reaction			1 (1.4%)
Metabolism and nutrition disorders			
Any event			1 (1.4%)
Dehydration			1 (1.4%)
Nervous system disorders			
Any event	1 (5.6%)		2 (2.9%)
Encephalopathy	1 (5.6%)		1 (1.4%)
Tremor			1 (1.4%)
Respiratory, thoracic and mediastinal disorders			
Any event	0	3 (17.6%)	2 (2.9%)
Pneumonitis	0	2 (11.8%)	0
Pneumothorax	0	1 (5.9%)	0
Laryngeal haemorrhage	0	0	1 (1.4%)
Pulmonary oedema	0	0	1 (1.4%)
Gastrointestinal disorders			
Any event	0	0	3 (4.3%)
Mucositis oral	0	0	1 (1.4%)
Enterocolitis	0	0	1 (1.4%)
Upper gastrointestinal haemorrhage	0	0	1 (1.4%)
Renal and urinary disorders			
Any event	0	0	1 (1.4%)
Acute kidney injury	0	0	1 (1.4%)
General disorders and administration site conditions			
Any event	3 (16.7%)	0	3 (4.3%)
Fatigue	3 (16.7%)	0	0
Fever	0	0	3 (4.3%)

Other Significant Adverse Events

Hyperbilirubinemia, mucositis/stomatitis, seizures, and hepatic sinusoidal obstruction syndrome (HSOS)

These AE are considered significant of conditioning treatment followed by alloHSCT.

A pooled analysis of studies MC-FludT.7/AML, 8/MDS with MC-FludT.6/L (n = 175 patients in total) revealed an incidence of 9.14% for hyperbilirubinemia grade III/IV (95% CI 5.32%, 14.42%) and 4.58% for mucositis/stomatitis grade III/IV (95% CI 1.99%, 8.81%).

In the pivotal study MC-FludT.14/L Trial II, oral mucositis (all grades) was significantly more frequent in the BU arm versus TREO arm (47.1% vs. 34.8%) but the difference between arms was smaller for grade III/IV oral mucositis (7.5% versus 4.5%).

Data from MC-FludT.14/L Trial I/II are shown in the following table. Incidences of hyperbilirubinaemia and mucositis/stomatitis were lower in Trial II. No patient treated with the reduced TREO regimen developed HSOS. No seizures related to treatment with TREO were seen in adult patients.

Table 48. Incidence of selected toxicities in trial MC-FludT.14/L (% of patients [95% CI])

Study	MC-FludT.14/L Trial I		MC-FludT.14/L Trial II	
Treatment group	**TREO**	**BU**	**TREO**	**BU**
Number of patients	168 (100%)	152 (100%)	221 (100%)	240 (100%)
Hyperbilirubinemia grade III/IV	12.5% [7.9%, 18.5%]	7.2% [3.7%, 12.6%]	3.6% (1.6%, 7.0%)	2.9% (1.2%, 5.9%)
Mucositis/stomatitis grade III/IV	10.7% [6.5%, 16.4%]	17.1% [11.5%, 24.0%]	4.5% [2.2%, 8.2%]	7.5% [4.5%, 11.6%]

Study	MC-FludT.14/L Trial I		MC-FludT.14/L Trial II	
Treatment group	**TREO**	**BU**	**TREO**	**BU**
Seizures grade III/IV	0	0.7% [0%, 3.6%]	0.5% [0.0%, 2.5%]	0.0% [0.0%, 1.5%]
HSOS grade II/III	0.6% [0%, 3.3%]	0.7% [0%, 3.6%]	0.0% [0.0%, 1.7%]	0.4% [0.0%, 2.3%]

There were no events that led to a substantial intervention (premature discontinuation of study drug, dose reduction, or substantial additional concomitant therapy) in any of the studies.

These significant AEs have not yet been analysed in the paediatric trials.

Graft versus Host disease

Adults

The cumulative incidences of acute GvHD all grades and grade III/IV as well as chronic GvHD and extensive GvHD was always numerically lower with the 10 g/m² TREO dose compared to the 14 g/m² dose.

The following tables summarise the frequency of acute and chronic GvHD observed in MC-FludT.14/L Trial I and II. No significant differences were observed between TREO and BU groups although it was numerically favourable for TREO.

Table 49.

Cumulative incidence GvHD; % [95% CI]

Study	MC-FludT.14/L Trial I				MC-FludT.14/L Trial II			
Group	**Treosulfan**		**Busulfan**		**Treosulfan**		**Busulfan**	
Day	**No. at risk**	**Incidence**	**No. at risk**	**Incidence**	**No. at risk**	**Incidence**	**No. at risk**	**Incidence**
Grade I-IV acute GvHD								
0	168	0 [0, 0]	152	0 [0, 0]	220	0 [0, 0]	240	0 [0, 0]
+14	144	19.6 [13.7,25.6]	126	21.1 [14.6, 27.5]	197	11.9 [7.6, 16.2]	212	15.8 [11.2, 20.5]
+28	88	44.6 [36.7, 52.5]	88	42.8 [34.6, 50.9]	144	32.0 [25.8, 38.1]	153	38.3 [32.2, 44.5]
+100	54	57.2 [48.7, 65.7]	58	57.2 [48.4, 66.1]	82	52.1 [45.5, 58.7]	76	58.8 [52.5, 65.0]
Median (range)	41.5 (2, 87) days		51 (1, 99) days		88 (1, 100) days		56 (8, 95) days	
Grade III/IV acute GvHD								
0	168	0 [0, 0]	152	0 [0, 0]	220	0 [0, 0]	240	0 [0, 0]
+14	164	1.2 [0.0, 2.8]	152	0 [0, 0]	217	0.5 [0.0, 1.3]	237	0.8 [0.0, 2.0]
+28	151	7.1 [3.3, 11.0]	147	3.3 [0.5, 6.1]	211	0.9 [0.0, 2.2]	228	3.3 [1.1, 5.6]
+100	122	14.3 [9.0, 19.6]	124	9.2 [4.6, 13.8]	168	6.4 [3.2, 9.6]	173	9.6 [5.9, 13.3]
Range	13 – 97 days		19 – 93 days		1 – 88 days		10 – 92 days	
Chronic GvHD								
Day +100	132	23.5 [17.2, 29.8]	127	18.1 [12.0, 24.2]	179	8.9 [4.8, 13.1]	190	8.9 [4.9, 13.0]
Month +6	66	47.7 [38.9, 56.5]	62	44.9 [35.9, 53.8]	84	39.4 [32.0, 46.8]	82	42.7 [35.4, 50.0]
Month +12	18	63.0 [53.3, 72.6]	14	61.4 [51.5, 71.3]	40	52.9 [45.2, 60.7]	41	56.6 [49.0, 64.2]
Month +24		Not reported		Not reported	7	60.1 [49.8, 70.3]	13	60.7 [53.1, 68.4]

Median (range)	6.6 (3, 12) months		6.4 (3, 12) months		272 (100, 730) days		270 (100, 735) days	
Extensive chronic GvHD								
Day +100	132	9.1 [4.4, 13.8]	126	6.3 [2.2, 10.5]	179	1.7 [0.0, 3.6]	190	4.2 [1.4, 7.1]
Month +6	102	16.7 [10.4, 22.9]	96	15.9 [9.6, 22.2]	126	11.8 [6.9, 16.6]	129	15.0 [9.8, 20.2]
Month +12	36	24.3 [17.0, 31.6]	30	24.2 [16.6, 31.8]	84	15.1 [9.6, 20.6]	75	21.9 [15.6, 28.2]
Month +24		Not reported		Not reported	13	18.4 [12.0, 24.8]	21	26.1 [19.2, 33.1]
Range	3 - 12 months		3 - 12 months		100 - 551 days		100 - 576 days	

Paediatrics

Table 50. Overview of maximum overall grades of aGvHD

Study	**MC-FludT.17/M**
Treatment arm	**TREO**
Number of patients	70
Maximum overall grade	
No aGvHD	38 (54.3%)
Grade I	12 (17.1%)
Grade II	12 (17.1%)
Grade III	5 (7.1%)
Grade IV	1 (1.4%)
Missing	2 (2.9%)
Cumulative incidence aGvHD, all grades	
At Day 14; % (90% CI)	7.2 (2.1, 12.4)
At Day 28; % (90% CI)	37.7 (28.1, 47.3)
At Day 100; % (90% CI)	43.5 (33.7, 53.3)
Cumulative incidence aGvHD, grade III/IV	
At Day 14; % (90% CI)	0.0 (0.0, 0.0)
At Day 28; % (90% CI)	7.2 (2.1, 12.4)
At Day 100; % (90% CI)	8.7 (3.1, 14.3)

Results from the two EBMT registry studies are summarised below.

Acute GvHD

The incidence of grade III/IV aGvHD after TREO-based conditioning was 10%. No significant correlation between the rate of grade III/IV aGvHD and age was found.

TREO dose had no significant impact on aGvHD in univariate and multivariate analysis adjusted for diagnoses, age, number of HSCTs, remission status, donor and conditioning regimen.

For malignant diseases, there is a borderline significant impact of age-group on the incidence of aGvHD of any grade as it decreases with age.

Chronic GvHD

The incidence of limited and extended cGvHD was 13% and 6%, respectively. No significant correlation between the rate of cGvHD and age was found. For all alloHSCT, TREO dose had no significant impact on cGvHD in both univariate analysis and multivariate analysis adjusted for diagnoses, age, number of HSCTs, remission status, donor and conditioning regimen.

However, there is a significant association between dose and the incidence of extended cGvHD in favour of the treated with higher dose. However the sample size in the lowest dose-group is small and if dose is analysed as continuous covariate, a non-significant decline of extensive cGvHD with dose has been observed.

There is a significant influence of dose and cGvHD and extensive cGvHD in the subgroup of patients with malignant diseases. With increasing doses the cGvHD-rates decline. This is especially observed in the subgroups of patients > CR1 or ALL.

Secondary malignancies

Only one of 564 adult patients treated with TREO-based conditioning developed a secondary malignancy (breast cancer). Two children in trial MC-FludT.17/M developed skin papilloma after TREO-based conditioning. However, follow up of most patients is still not long enough to allow any comparison with published data.

Two spontaneous case reports of secondary leukaemia/lymphoma after TREO-based conditioning in adult patients and five reports of secondary malignancies after TREO-based conditioning in paediatric patients (all primary immunodeficiency) are registered in medac's pharmacovigilance data base.

Laboratory findings

Adults

Haematology

Recovery of blood cells (engraftment) was an efficacy parameter in all studies with TREO-based conditioning and is described in the efficacy section. A comparable proportion of patients of both groups in adult phase III trials received growth factors (GCSF, GM-CSF) between day -6 to +28.

A summary of transfusion of erythrocytes and platelets until Day +28 allows a comparison of the degree of cytopaenias between treatment groups.

In trial I, patients in the TREO group received more erythrocyte units for a significantly longer time compared to BU but this difference disappeared in Trial II with the reduced TREO dose. More patients in the TREO groups received platelet transfusions compared to BU. The number of platelet units and duration of infusions could be reduced with the TREO regimen in Trial II.

Table 52. Transfusion of erythrocytes and platelets until Day +28 (Safety Analysis Set)

Study	**Trial I**		**Trial II**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Total number of patients	168 (100%)	152 (100%)	221 (100%)	240 (100%)
Erythrocytes				
Transfusion	155 (92.3%)	139 (91.4%)	206 (93.2%)	219 (91.3%)
No transfusion	13 (7.7%)	13 (8.6%)	15 (6.8%)	21 (8.7%)
Duration of transfusions (days)				

Study	Trial I		Trial II	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Total number of patients	168 (100%)	152 (100%)	221 (100%)	240 (100%)
Erythrocytes				
Transfusion	155 (92.3%)	139 (91.4%)	206 (93.2%)	219 (91.3%)
No transfusion	13 (7.7%)	13 (8.6%)	15 (6.8%)	21 (8.7%)
Mean (SD)	16.0 (10.6)	12.5 (9.5)	12.4 (9.8)	11.3 (9.7)
Median (Q1, Q3)	15.0 (7.0, 25.0)	10.0 (4.0, 19.0)	11.0 (4.0, 20.0)	8.0 (2.0, 17.0)
Total number of erythrocyte units				
Mean (SD)	8.3 (5.2)	7.2 (4.6)	7.0 (4.4)	6.7 (4.7)
Median (Q1, Q3)	8.0 (6.0, 10.0)	6.0 (4.0, 8.0)	6.0 (4.0, 8.0)	6.0 (4.0, 9.0)
Platelets				
Transfusion	164 (97.6%)	114 (75%)	204 (92.3%)	160 (66.7%)
No transfusion	4 (2.4%)	38 (25%)	17 (7.7%)	80 (33.3%)
Duration of transfusions (days)				
Mean (SD)	13.5 (9.2)	9.4 (7.8)	10.2 (8.3)	9.5 (7.8)
Median (Q1, Q3)	14.0 (5.0, 19.0)	9.0 (2.0, 14.0)	9.0 (3.0, 16.0)	8.0 (2.0, 15.0)
Total number of platelet units				
Mean (SD)	10.2 (12.1)	5.8 (5.8)	8.3 (11.0)	7.5 (14.7)
Median (Q1, Q3)	7.0 (4.0, 12.0)	4.0 (2.0, 8.0)	5.0 (2.0, 9.0)	4.0 (2.0, 8.0)

Clinical chemistry

The most frequent ARs were increased bilirubin and transaminases, occurring in 18.8% (bilirubin) and 5.1% (ALT)/4.4% (AST) of 564 adult patients treated with TREO-based conditioning.

Data from MC-FludT.14/L Trial I cannot be compared to Trial II, because abnormal laboratory were not registered as AE/AR in Trial I.

MC-FludT.14/L Trial II

Liver function parameters were comparable in both treatment groups at baseline. Median levels of AST and AP did not change very much up to Day +28. The highest increase in ALT, γGT, and bilirubin was usually seen on Day +6. The increase in ALT and bilirubin was more pronounced in the TREO group whereas the increase in γGT was more pronounced in the BU group and could be related to the high contents of the excipient dimethylacetamide in the intravenous BU formulation Busilvex®.

Table 53. Trial II Liver function parameters; median (Q1, Q3)

Parameter (normal value)	Treatment group	Baseline	Day -1	Day +6	Day +28
AST, U/L (M: 10-50; F: 10-35)	TREO	22.1 (16.0, 30.5)	19.7 (13.0, 27.5)	27.0 (20.0, 42.0)	24.0 (17.0, 31.7)
	BU	22.0 (17.0, 31.0)	18.0 (13.0, 26.5)	23.0 (17.0, 32.0)	23.0 (17.0, 32.0)
ALT, U/L	TREO	26.0 (16.0, 44.0)	31.7 (20.0, 57.0)	48.0 (28.5, 80.0)	24.8 (16.0, 36.0)

(M: 10-50; F: 10-35)	BU	25.0 (17.0, 40.0)	26.0 (18.0, 43.5)	38.0 (23.0, 60.0)	21.0 (15.0, 34.0)
γGT, U/L (M: 12-64; F: 9-36)	TREO	30.0 (20.2, 54.0)	47.0 (29.0, 88.0)	54.0 (34.2, 93.0)	44.0 (27.0, 82.0)
	BU	29.5 (20.0, 51.9)	56.0 (36.0, 98.0)	86.0 (49.0, 176.0)	48.0 (29.0, 84.0)
AP, U/L (M/F: 30-120)	TREO	75.0 (58.0, 94.0)	62.0 (50.0, 82.0)	67.0 (55.0, 85.0)	78.0 (61.5, 104.5)
	BU	71.0 (57.0, 97.0)	54.0 (45.0, 72.6)	64.0 (53.0, 88.0)	75.0 (58.0, 102.5)
Bilirubin, µM (M/F: 2-21)	TREO	8.6 (6.5, 12.0)	17.1 (12.0, 23.9)	18.8 (12.5, 25.7)	12.0 (8.6, 15.4)
	BU	8.7 (6.8, 13.6)	15.5 (12.0, 22.6)	13.7 (10.3, 18.8)	12.0 (8.6, 15.4)

Data for electrolytes (sodium, potassium) were comparable in both treatment groups at baseline and did not change very much during up to Day +28. Data for serum creatinine (Cr), lactate dehydrogenase (LDH), C-reactive protein (CRP), procalcitonin, and serum glucose were comparable in both groups at baseline. Serum creatinine increased after Day +6, probably due to concomitant treatment with CsA. LDH increased up to Day +28 with no differences between both groups. CRP and procalcitonin increased significantly in both groups at Day -1 but rapidly normalised. Serum glucose levels increased slightly on Day -1 in both groups but rapidly normalised.

Paediatrics

An increased bilirubin/ALT/AST in 88 paediatric TREO patients was 5.7%/9.1%/ 8.0%, respectively, and comparable to the data seen in adults.

Urinalysis

No parameters in urine were determined in any of the trials.

Vital signs

In study MC-FludT.14/L Trial II vital signs (blood pressure, pulse rate, body temperature, body weight) and KPS were comparable in the two treatment groups at baseline.

Vital signs did not change substantially during the trial.

125 patients (52.1%) in the BU group and 91 patients (41.2%) in the TREO group deteriorated by at least 20 points in the KPS. The Kaplan Meier estimate at 24 months favoured the TREO group (HR 0.71; 95% CI 0.53 0.94; P = 0.0173).

34 patients (14.2%) in the BU group and 11 patients (5.0%) in the TREO group deteriorated to less than 60 points in the KPS. The Kaplan Meier estimate at 24 months favoured the TREO group (HR 0.34; 95% CI 0.17 0.69; P = 0.0026)

Safety in special populations

Age

Table 49 Summary of TEAEs of TREO in adult trials by age group (Safety Analysis Sets, see Module 5.3.5.3.1)

MedDRA Terms	Age <65 N = 474 (84%)	Age 65-74 N = 90 (16%)	Age 75-84 N = 0	Age 85+ N = 0
Total AEs	466 (98.3%)	83 (92.2%)		
Serious AEs – Total	67 (14.1%)	11 (12.2%)		
– Fatal	11 (2.3%)	3 (3.3%)		
– Life-threatening	13 (2.7%)	4 (4.4%)		
– Hospitalization/prolong existing hospitalization	5 (1.1%)	4 (4.4%)		
– Disability/incapacity	1 (0.2%)	0 (0.0%)		
– Missing reason for seriousness	40 (8.4%)	1 (1.1%)		
AE leading to drop-out	0	0		
Psychiatric disorders	94 (19.8%)	18 (20.0%)		
Nervous system disorders	187 (39.5%)	35 (38.9%)		
Accidents and injuries (Injury, poisoning and procedural complications)	15 (3.2%)	0		
Cardiac disorders	78 (16.5%)	19 (21.1%)		
Vascular disorders	173 (36.5%)	33 (36.7%)		
Cerebrovascular disorders	0	0		
Infections and infestations	198 (41.8%)	35 (38.9%)		
Anticholinergic syndrome	0	0		
Quality of life decreased	No data	No data		
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures*	41 (8.6%)	18 (20.0%)		
Other AEs appearing more frequently in older patients (> 5% difference; see also Table 68).				
Decreased appetite	55 (11.6%)	15 (16.7%)		
Dizziness	36 (7.6%)	15 (16.7%)		
Hypertension	100 (21.1%)	24 (26.7%)		
Dyspnoea	35 (7.4%)	12 (13.3%)		
Arthralgia	42 (8.9%)	13 (14.4%)		
Oedema peripheral	111 (23.4%)	30 (33.3%)		
Blood bilirubin increased	109 (23.0%)	27 (30.0%)		
*Data source: ISS 2017, Table Q105A, Q108B; * The following MedDRA PTs were selected: Orthostatic hypotension, fall, loss of consciousness, syncope, dizziness, ataxia, fracture, spinal fracture*				

The data show that the TREO/FLU conditioning was equally well tolerated in patients above and below the age of 65 years.

In paediatric studies the number of TREO treated patients in the lowest age group (28 days- 23 months) is too small (n=15) to draw any conclusions. Age-dependent treatment related AE are shown below.

Table 54. Frequency of drug-related TEAEs occurring in more than one patient by age group

Primary System Organ Class Preferred Term	Age group		
	28 days to 23 months (N = 15)	2 to 11 years (N = 38)	12 to 17 years (N = 35)
Patients with any event	10 (66.7%)	34 (89.5%)	29 (82.9%)
Infections and infestations			
Any event	1 (6.7%)	3 (7.9%)	6 (17.1%)
Adenovirus infection		2 (5.3%)	1 (2.9%)
Cytomegalovirus infection		1 (2.6%)	2 (5.7%)
Epstein-Barr virus infection		1 (2.6%)	1 (2.9%)
Urinary tract infection			2 (5.7%)
Metabolism and nutrition disorders			
Any event		1 (2.6%)	1 (2.9%)
Nervous system disorders			
Any event	1 (6.7%)	1 (2.6%)	2 (5.7%)
Headache		1 (2.6%)	1 (2.9%)
Paraesthesia			2 (5.7%)
Eye disorders			
Any event		1 (2.6%)	1 (2.9%)
Vascular disorders			
Any event		1 (2.6%)	2 (5.7%)
Respiratory, thoracic and mediastinal disorders			
Any event	1 (6.7%)	2 (5.3%)	5 (14.3%)
Oropharyngeal pain		1 (2.6%)	3 (8.6%)
Epistaxis	1 (6.7%)		2 (5.7%)
Gastrointestinal disorders			
Any event	9 (60.0%)	33 (86.8%)	28 (80.0%)
Stomatitis	8 (53.3%)	29 (76.3%)	24 (68.6%)
Vomiting	5 (33.3%)	19 (50.0%)	14 (40.0%)
Diarrhoea	4 (26.7%)	13 (34.2%)	12 (34.3%)
Nausea	1 (6.7%)	14 (36.8%)	12 (34.3%)
Abdominal pain	1 (6.7%)	8 (21.1%)	5 (14.3%)
Dysphagia		1 (2.6%)	2 (5.7%)
Oral pain			3 (8.6%)
Anal inflammation		2 (5.3%)	
Dyspepsia			2 (5.7%)
Proctitis	1 (6.7%)	1 (2.6%)	
Skin and subcutaneous tissue disorders			
Any event	2 (13.3%)	15 (39.5%)	10 (28.6%)
Pruritus		6 (15.8%)	4 (11.4%)
Alopecia		6 (15.8%)	2 (5.7%)
Rash maculo-papular		5 (13.2%)	3 (8.6%)
Erythema		3 (7.9%)	2 (5.7%)

Primary System Organ Class Preferred Term	Age group		
	28 days to 23 months (N = 15)	2 to 11 years (N = 38)	12 to 17 years (N = 35)
Pain of skin		2 (5.3%)	2 (5.7%)
Skin hyperpigmentation		2 (5.3%)	2 (5.7%)
Dermatitis exfoliative		2 (5.3%)	1 (2.9%)
Rash		1 (2.6%)	2 (5.7%)
Skin ulcer	1 (6.7%)	1 (2.6%)	
Urticaria	1 (6.7%)	1 (2.6%)	
Renal and urinary disorders			
Any event		2 (5.3%)	2 (5.7%)
Acute kidney injury		1 (2.6%)	1 (2.9%)
General disorders and administration site conditions			
Any event	1 (6.7%)	7 (18.4%)	6 (17.1%)
Pyrexia	1 (6.7%)	6 (15.8%)	6 (17.1%)
Chills		2 (5.3%)	
Fatigue		2 (5.3%)	
Investigations			
Any event	2 (13.3%)	7 (18.4%)	5 (14.3%)
ALT increased	2 (13.3%)	4 (10.5%)	2 (5.7%)
AST increased	1 (6.7%)	4 (10.5%)	2 (5.7%)
Blood bilirubin increased		1 (2.6%)	4 (11.4%)
γGT increased		2 (5.3%)	
Injury, poisoning and procedural complications			
Any event	1 (6.7%)	1 (2.6%)	1 (2.9%)
Infusion related reaction	1 (6.7%)	1 (2.6%)	

No significant correlation between age and rate of grade III/IV aGVHD/extensive cGVHD or neurotoxicity was found.

It appears there is a correlation between age and grade III/IV stomatitis (increases with age), and grade III/IV AST elevation (increases with age), and grade III/IV respiratory toxicity and HSOS (both increase in children below 1 year with NM disease). The data is in line with the EBMT [Peters 2011].

A summary of TEAE obtained from mature data in the study FludT.17/M (CSR dated 12 March 2018, cut off data 1 Nov 2017) is included below.

Table 50 Summary of TEAEs in paediatric trials by ICH age group (Safety Analysis Set [MC-FludT.17/M])

	ICH age group			
	28 days to 23 months (N=9)	2 to 11 years (N=28)	12 to 17 years (N=33)	Overall (N=70)
Any adverse event [n (%)]				
Subjects with any adverse event	8 (88.9%)	27 (96.4%)	33 (100.0%)	68 (97.1%)
Serious adverse events [n (%)]				
Subjects with any serious adverse event	4 (44.4%)	6 (21.4%)	13 (39.4%)	23 (32.9%)
- Results in death	0 (0.0%)	0 (0.0%)	1 (3.0%)	1 (1.4%)
- Life-threatening	1 (11.1%)	0 (0.0%)	4 (12.1%)	5 (7.1%)
- Hospitalization or prolongation of hospitalization	3 (33.3%)	6 (21.4%)	12 (36.4%)	21 (30.0%)
- Disability/incapacity	0 (0.0%)	0 (0.0%)	1 (3.0%)	1 (1.4%)
- Congenital anomaly or birth defect	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
MedDRA Primary SOC				
Blood and lymphatic system disorders	0 (0.0%)	3 (10.7%)	8 (24.2%)	11 (15.7%)
Cardiac disorders	2 (22.2%)	4 (14.3%)	3 (9.1%)	9 (12.9%)
Ear and labyrinth disorders	1 (11.1%)	0 (0.0%)	1 (3.0%)	2 (2.9%)
Endocrine disorders	0 (0.0%)	1 (3.6%)	0 (0.0%)	1 (1.4%)
Eye disorders	1 (11.1%)	2 (7.1%)	8 (24.2%)	11 (15.7%)
Gastrointestinal disorders	8 (88.9%)	25 (89.3%)	32 (97.0%)	65 (92.9%)
General disorders and administration site conditions	8 (88.9%)	22 (78.6%)	25 (75.8%)	55 (78.6%)
Hepatobiliary disorders	3 (33.3%)	6 (21.4%)	16 (48.5%)	25 (35.7%)
Immune system disorders	0 (0.0%)	5 (17.9%)	7 (21.2%)	12 (17.1%)
Infections and infestations	4 (44.4%)	17 (60.7%)	24 (72.7%)	45 (64.3%)
Injury, poisoning and procedural complications	2 (22.2%)	2 (7.1%)	8 (24.2%)	12 (17.1%)
Investigations	4 (44.4%)	13 (46.4%)	13 (39.4%)	30 (42.9%)
Metabolism and nutrition disorders	1 (11.1%)	6 (21.4%)	14 (42.4%)	21 (30.0%)
Musculoskeletal and connective tissue disorders	0 (0.0%)	8 (28.6%)	15 (45.5%)	23 (32.9%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0 (0.0%)	2 (7.1%)	0 (0.0%)	2 (2.9%)
Nervous system disorders	2 (22.2%)	8 (28.6%)	14 (42.4%)	24 (34.3%)

Gender

Selected (> 5% of patients) TREO related TEAEs in all five adult trials showed higher frequency in the female group for gastrointestinal disorders (70.9% female vs 56.5% male). The other differences between both genders were of less magnitude.

No data are available in paediatric patients.

Safety related to drug-drug interactions and other interactions

No studies of specific drug-drug interactions were performed. No specific drug interaction could be identified within the clinical development programme.

Discontinuation due to AES

No patient discontinued conditioning treatment due to AEs. Only one patient (TREO group of MC-FludT.14/L Trial II) discontinued TREO treatment after the first TREO dose due to cancellation of donor's clearance after start of conditioning treatment. No transplantation took place in this patient.

Additional safety data

Adults

Final analysis MC.FludT.14/L Trial II (cut off 16 March 2018)

No relevant differences were observed between the safety results of the 2nd interim (476 patients) and the final (570 patients) analysis dated 18-Jul-2018 of study MC.FludT.14/L Trial II.

Table 34 Overall summary of AEs in study MC-FludT.14/L Trial II (Safety Analysis Set)

Analysis	**2nd Interim analysis**		**Final analysis**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Data source	*Table 12.2.1.A*		*Table 12.2.1.A*	
Number of patients	221 (100%)	240 (100%)	270 (100%)	283 (100%)
Any adverse event				
Patients with AEs of any CTCAE Grade	93.2%	95.4%	92.6%	96.1%
Patients with AEs of at least CTCAE Grade III	53.4%	54.6%	54.8%	53.4%
Drug related adverse events				
Patients with ARs of any CTCAE Grade	62.9%	70.0%	63.0%	67.8%
Patients with ARs of at least CTCAE Grade III	26.7%	30.8%	26.7%	29.0%
Serious adverse events				
Patients with at least one serious AE	8.1%	7.1%	8.5%	7.1%
Results in death	2.7%	2.1%	3.0%	2.1%
Life-threatening	3.6%	2.9%	4.8%	2.8%
Hospitalisation or prolongation of hospitalisation	2.7%	3.3%	3.0%	3.2%
Disability/Incapacity	0	0	0	0
Congenital anomaly or birth defect	0	0	0	0
Drug-related serious adverse events				
Patients with drug related serious AEs	2.7%	3.3%	3.3%	3.2%
Patients with maximum CTCAE Grade				
CTCAE Grade I	16.7%	15.4%	15.2%	16.3%
CTCAE Grade II	23.1%	25.4%	22.6%	26.5%
CTCAE Grade III	44.3%	48.3%	45.6%	47.3%
CTCAE Grade IV	6.3%	5.0%	6.7%	4.9%
CTCAE Grade V	2.7%	1.3%	2.6%	1.1%

Table 35 Frequency (%) of patients with TEAEs by SOC occurring in at least 5% of patients in either treatment group (Safety Analysis Set)

Analysis	**2nd Interim analysis**		**Final analysis**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Data source	*Table 12.2.2.1.A*		*Table 12.2.2.1.A*	
Number of patients	221 (100%)	240 (100%)	270 (100%)	283 (100%)
Patients with any event	93.2%	95.4%	92.6%	96.1%
Gastrointestinal disorders	67.9%	74.6%	68.1%	73.5%
General disorders and administration site conditions	54.3%	53.3%	56.3%	53.4%
Musculoskeletal and connective tissue disorders	37.1%	27.9%	37.8%	27.2%
Nervous system disorders	27.1%	30.4%	28.1%	30.4%
Skin and subcutaneous tissue disorders	29.4%	28.3%	29.3%	26.5%
Investigations	27.6%	27.5%	28.5%	26.5%
Vascular disorders	24.4%	27.9%	24.8%	29.7%
Infections and infestations	26.2%	24.2%	27.0%	23.7%
Metabolism and nutrition disorders	20.4%	18.3%	22.2%	19.4%
Respiratory, thoracic and mediastinal disorders	19.0%	22.9%	19.3%	21.6%
Blood and lymphatic system disorders	14.9%	12.1%	14.8%	11.0%
Cardiac disorders	14.9%	8.3%	15.2%	9.2%
Renal and urinary disorders	9.0%	9.6%	11.1%	9.2%
Psychiatric disorders	7.7%	9.6%	7.8%	8.8%
Ear and labyrinth disorders	5.4%	7.9%	6.3%	8.5%
Eye disorders	3.6%	10.4%	4.4%	10.2%
Immune system disorders	6.8%	7.9%	6.3%	7.8%

Table 36 Frequency (%) of patients with CTCAE Grade III/IV TEAEs by SOC occurring in at least 5% of patients in either treatment group (Safety Analysis Set)

Analysis	**2nd Interim analysis**		**Final analysis**	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Data source	*Table 12.2.2.1.B*		*Table 12.2.2.1.B*	
Number of patients	221 (100%)	240 (100%)	270 (100%)	283 (100%)
Patients with any event	53.4%	54.6%	54.8%	53.4%
Investigations	14.9%	14.6%	14.4%	13.4%
Gastrointestinal disorders	10.9%	16.3%	12.2%	15.5%
Blood and lymphatic system disorders	14.9%	12.1%	14.8%	11.0%
Infections and infestations	14.5%	9.2%	15.2%	9.2%
Vascular disorders	10.0%	11.3%	10.0%	12.7%
Metabolism and nutrition disorders	7.2%	5.4%	8.9%	5.7%
General disorders and administration site conditions	1.8%	5.0%	3.0%	4.6%

Table 37 Frequency (%) of patients with drug-related TEAEs by SOC occurring in at least 5% of patients in either treatment group (Safety analysis set)

Analysis	2nd Interim analysis		Final analysis	
Treatment arm	**TREO**	**BU**	**TREO**	**BU**
Data source	*Table 12.2.2.1.C*		*Table 12.2.2.1.C*	
Number of patients	221 (100%)	240 (100%)	270 (100%)	283 (100%)
Patients with any event	62.9%	70.0%	63.0%	67.8%
Gastrointestinal disorders	45.7%	55.8%	47.0%	52.7%
General disorders and administration site conditions	13.1%	20.4%	14.1%	19.8%
Nervous system disorders	7.2%	12.1%	7.4%	12.7%
Skin and subcutaneous tissue disorders	11.3%	13.8%	11.1%	13.1%
Investigations	18.6%	15.8%	18.1%	14.8%
Vascular disorders	4.1%	6.3%	4.1%	5.3%
Infections and infestations	7.7%	7.1%	8.1%	6.7%
Metabolism and nutrition disorders	6.3%	6.7%	7.0%	7.4%
Respiratory, thoracic and mediastinal disorders	7.2%	9.6%	7.0%	8.8%
Blood and lymphatic system disorders	4.1%	5.0%	4.4%	4.9%

Table 38 Cumulative incidence of grade I-IV acute GvHD (Full Analysis Set)

Analysis	2nd Interim analysis *(data source: CSR table 12.2.2.A)*		Final analysis *(data source: CSR table 12.2.2.A)*	
Group	**TREO N = 220**	**BU N = 240**	**TREO N = 268**	**BU N = 283**
Cumulative incidence at 14 days (95% CI)	11.9 (7.6, 16.2)	15.8 (11.2, 20.5)	10.5 (6.8, 14.2)	14.1 (10.1, 18.2)
Cumulative incidence at 28 days (95% CI)	32.0 (25.8, 38.1)	38.3 (32.2, 44.5)	30.0 (24.5, 35.5)	36.7 (31.1, 42.4)
Cumulative incidence at 100 days (95% CI)	52.1 (45.5, 58.7)	58.8 (52.5, 65.0)	52.8 (46.8, 58.8)	57.2 (51.5, 63.0)
Hazard ratio TREO/BU (95% CI)	0.83 (0.65, 1.06)		0.87 (0.69, 1.08)	
P value[a]	0.1276		0.2038	
[a] *based on test of Gray*				

Table 39 Cumulative incidence of grade III-IV acute GvHD (Full Analysis Set)

Analysis	2nd Interim analysis *(data source: CSR table 12.2.2.A)*		Final analysis *(data source: CSR table 12.2.2.A)*	
Group	**TREO N = 220**	**BU N = 240**	**TREO N = 268**	**BU N = 283**
Cumulative incidence at 14 days (95% CI)	0.5 (0.0, 1.3)	0.8 (0.0, 2.0)	0.7 (0.0, 1.8)	0.7 (0.0, 1.7)
Cumulative incidence at 28 days (95% CI)	0.9 (0.0, 2.2)	3.3 (1.1, 5.6)	1.1 (0.0, 2.4)	2.8 (0.9, 4.8)
Cumulative incidence at 100 days (95% CI)	6.4 (3.2, 9.6)	9.6 (5.9, 13.3)	6.4 (3.4, 9.3)	8.1 (4.9, 11.3)
Hazard ratio TREO/BU (95% CI)	0.66 (0.34, 1.27)		0.78 (0.42, 1.45)	
P value[a]	0.2099		0.4267	
[a] based on test of Gray				

Table 40 Cumulative incidence of chronic GvHD (Full Analysis Set)

Analysis	**2nd Interim analysis** *(data source: CSR table 12.2.2.B)*		**Final analysis** *(data source: CSR table 12.2.2.B)*	
Group	**TREO N = 220**	**BU N = 240**	**TREO N = 268**	**BU N = 283**
Patients at risk	179	190	229	232
Cumulative incidence at 6 month (95% CI)	39.4 (32.0, 46.8)	42.7 (35.4, 50.0)	40.3 (34.0, 46.7)	41.9 (35.5, 48.2)
Cumulative incidence at 12 month (95% CI)	52.9 (45.2, 60.7)	56.6 (49.0, 64.2)	54.8 (48.4, 61.3)	55.1 (48.7, 61.5)
Cumulative incidence at 24 month (95% CI)	60.1 (49.8, 70.3)	60.7 (53.1, 68.4)	61.7 (55.1, 68.3)	60.3 (53.8, 66.7)
Hazard ratio TREO/BU (95% CI)	0.91 (0.69, 1.20)		1.00 (0.79, 1.27)	
P value[a]	0.5236		0.9964	
[a] based on test of Gray				

Table 41 Cumulative incidence of extensive chronic GvHD (Full Analysis Set)

Analysis	**2nd Interim analysis** *(data source: CSR table 12.2.2.B)*		**Final analysis** *(data source: CSR table 12.2.2.B)*	
Group	**TREO N = 220**	**BU N = 240**	**TREO N = 268**	**BU N = 283**
Patients at risk	179	190	229	232
Cumulative incidence at 6 month (95% CI)	11.8 (6.9, 16.6)	15.0 (9.8, 20.2)	11.4 (7.3, 15.5)	13.8 (9.4, 18.2)
Cumulative incidence at 12 month (95% CI)	15.1 (9.6, 20.6)	21.9 (15.6, 28.2)	16.7 (11.9, 21.6)	20.0 (14.8, 25.2)
Cumulative incidence at 24 month (95% CI)	18.4 (12.0, 24.8)	26.1 (19.2, 33.1)	19.8 (14.5, 25.1)	28.6 (22.5, 34.7)
Hazard ratio TREO/BU (95% CI)	0.68 (0.42, 1.09)		0.71 (0.48, 1.04)	
P value[a]	0.1099		0.0750	
[a] based on test of Gray				

Table 42 Overview and cause of deaths until Month 24 (Safety Analysis Set)

Analysis	**2nd Interim analysis**		**Final analysis**	
Group	**TREO N = 221**	**BU N = 240**	**TREO N = 270**	**BU N = 283**
Data source in CSR	Table 12.3.1.1.A		Table 12.3.1.1.A	
Alive	76.5%	65.8%	73.3%	62.2%
Dead	23.5%	34.2%	26.7%	37.8%
Cause of death				
Relapse, progress	11.8%	15.0%	12.6%	16.6%
Transplant-related	10.4%	18.8%	12.2%	20.5%
Second. malignancy	0	0.4%	0.4%	0.4%
Other	0.9%	0	1.1%	0
Unknown	0.5%	0	0.4%	0.4%

Table 43 Frequency of patients with treatment emergent SAEs by CTCAE System Organ Class (Safety Analysis Set)

Analysis	2nd Interim analysis		Final analysis	
Treatment arm	**TREO N = 221**	**BU N = 240**	**TREO N = 270**	**BU N = 283**
Data source	*Table 14.3.2.2A*		*Table 12.3.1.2.A*	
Patients with any event	8.1%	7.1%	8.5%	7.1%
Infections and infestations	5.0%	3.8%	5.2%	3.5%
Respiratory, thoracic and mediastinal disorders	1.4%	1.3%	1.1%	1.4%
Gastrointestinal disorders	0.5%	1.3%	0.7%	1.1%
Hepatobiliary disorders	0	1.3%	0	1.1%
Renal and urinary disorders	0.9%	0.4%	1.1%	0.4%
Cardiac disorders	0.5%	0.4%	0.7%	0.4%
Vascular disorders	0.5%	0.4%	0.7%	0.4%
General disorders and administration site conditions	0	0.4%	0	0.4%
Immune system disorders	0.5%	0	0.4%	0
Injury, poisoning and procedural complications	0	0.4%	0	0.4%
Investigations	0.5%	0	0.4%	0
Nervous system disorders	0.5%	0	1.1%	0
Neoplasms benign, malignant and unspecified	0	0	0	0.4%

Analysis by underlying disease show that for TREO group MDS patients experience more serious adverse events than AML patients (15.3% MDS/ 5.4% AML) but this also applies to the BU-group (10.4% MDS/ 4.8% AML)). In addition, MDS patients experienced more drug related infections and general disorders for both, TREO and BU group, compared to AML patients. It can be concluded that AML patients seem to tolerate the FT10 regimen slightly better than MDS patients due to differences in underlying disease.

Adults with NM disease

The median age of adult study patients with underlying malignant disease within the medac sponsored phase II – III trials (median age 45 – 61 years) treated with TREO/FLU conditioning is much higher than the age expected in the small group of adult patients with non-malignant diseases (NMD) requiring alloHSCT (< 40 years). Adult NMD patients are usually treated with the same conditioning regimen as paediatric NMD patients. Due to a higher risk of graft failure, myeloablative or even intensified conditioning regimens are usually used [Bertaina 20103; Olsson 201319].

The two published reports on TREO-based conditioning in NMD patients which included also a few adult patients used a paediatric conditioning regimen with the higher TREO dose of 14 g/m²/d × 3 (FT14 regimen) [Bernardo 20121; Burroughs 20144]. No difference in terms of outcome was observed between children and adults who met the eligibility criteria of the treatment protocol. No apparent difference in toxicity scores or mortality according to patient age or body surface area (BSA) was reported in the US study [Burroughs 20144].

It is therefore expected that the safety data in adult NMD patients are most comparable to those observed in paediatric NMD patients treated with the FT14 regimen than to adult patients who are usually RIC-eligible and treated with FT10 accordingly. A careful benefit risk evaluation by the treating physician would be necessary before treatment.

Paediatric

For the final analysis of study 17/M one additional death was observed after the interim analysis (transplantation related) [Table 3].

The frequency of paediatric patients with serious adverse events has not been changed between interim and final results. However, one SAE was corrected from fever to febrile neutropenia.

Table 3 Causes of death in trial MC-FludT.17/M. Comparison of interim and final results

	Interim data MC-FludT.17/M	**Final data MC-FludT.17/M**
No. of patients	70 (100%)	70 (100%)
Survival status at study termination; n (%)		
Alive*	64 (91.4%)	**63 (90.0%)**
Dead	6 (8.6%)	**7 (10.0%)**
Cause of death; n (%)		
Relapse/progression	4 (5.7%)	**2 (2.9%)**
Transplantation related**	2 (2.9%)	**3 (4.3%)**
GvHD	1 (1.4%)	1 (1.4%)
Pulmonary toxicity	1 (1.4%)	1 (1.4%)
Haemorrhage	1 (1.4%)	1 (1.4%)
Renal failure	0	0
Gastrointestinal toxicity	0	0
Rejection/poor graft function	1 (1.4%)	1 (1.4%)
Multiple organ failure	0	**2 (2.9%)**
Infection	1 (1.4%)	1 (1.4%)
Bacterial	1 (1.4%)	1 (1.4%)
Interstitial pneumonitis	2 (2.9%)	2 (2.9%)
Other	2 (2.9%)	2 (2.9%)
Time from transplantation to death (months)		
Mean (SD)	6.56 (3.80)	**7.38 (4.09)**
Median (Q1, Q3)	7.82 (3.81, 9.66)	**9.46 (3.81, 9.72)**
Min, Max	0.5, 9.7	0.5, **12.3**

** The status 'alive' is displayed for all patients who did not terminate the study due to death.*
*** Multiple transplantation related causes per subject possible.*

Table 4 Frequency of paediatric patients with serious adverse events for trial MC-FludT.17/M. Comparison of interim and final results.

	Interim data MC-FludT.17/M	Final data MC-FludT.17/M
No. of patients	70 (100%)	70 (100%)
Patients with any event	23 (32.9%)	23 (32.9%)
Infections and infestations		
Any event	15 (21.4%)	15 (21.4%)
Catheter related infection	1 (1.4%)	1 (1.4%)
Sepsis	2 (2.9%)	2 (2.9%)
Upper respiratory infection	3 (4.3%)	3 (4.3%)
Encephalitis	1 (1.4%)	1 (1.4%)
Hepatitis viral	1 (1.4%)	1 (1.4%)
Sinusitis	1 (1.4%)	1 (1.4%)
Skin infection	1 (1.4%)	1 (1.4%)
Blood and lymphatic system disorders		
Any event	2 (2.9%)	**3 (4.3%)**
Febrile neutropenia	2 (2.9%)	**3 (4.3%)**
Immune thrombocytopenic purpura	0	0
Immune system disorders		
Any event	1 (1.4%)	1 (1.4%)
Allergic reaction	1 (1.4%)	1 (1.4%)
Metabolism and nutrition disorders		
Any event	1 (1.4%)	1 (1.4%)
Dehydration	1 (1.4%)	1 (1.4%)
Nervous system disorders		
Any event	2 (2.9%)	2 (2.9%)
Encephalopathy	1 (1.4%)	1 (1.4%)
Tremor	1 (1.4%)	1 (1.4%)
Respiratory, thoracic and mediastinal disorders		
Any event	2 (2.9%)	2 (2.9%)
Pneumonitis	0	0
Pneumothorax	0	0
Laryngeal haemorrhage	1 (1.4%)	1 (1.4%)
Pulmonary oedema	1 (1.4%)	1 (1.4%)
Gastrointestinal disorders		
Any event	3 (4.3%)	3 (4.3%)
Mucositis oral	1 (1.4%)	1 (1.4%)
Enterocolitis	1 (1.4%)	1 (1.4%)
Upper gastrointestinal haemorrhage	1 (1.4%)	1 (1.4%)
Renal and urinary disorders		
Any event	1 (1.4%)	1 (1.4%)
Acute kidney injury	1 (1.4%)	1 (1.4%)
General disorders and administration site conditions		
Any event	3 (4.3%)	**2 (2.9%)**
Fatigue	0	**0**
Fever	3 (4.3%)	**2 (2.9%)**

One subject reported an AE of HSOS.

The trial continues to follow-up subjects until 3 years after transplantation of the last registered subject, and according to the applicant the longer-term follow-up data will be provided within an amended report.

2.6.1. Discussion on clinical safety

Data from 221 adults treated with the proposed TREO conditioning doses and regimen with a median follow up of 15.4 months together with safety data from 341 adults exposed to different doses of TREO is considered sufficient for assessment.

AEs most frequently reported in adults with the proposed dose and regimen were gastrointestinal (oral mucositis (34.8%), nausea/vomiting (30.3%/19.5%), diarrhoea (14.9%), constipation (13.1%)), infections (26.2%), oedema limbs (20.8%), headache (15.4%), febrile neutropenia (14.9%), bone/back pain (14.5%/14%), fever (32.1%), hypertension (14.9%), and maculopapular rash (12.2%). Most frequently reported TREO-related AE included oral mucositis (30.3%), nausea (20.4%), vomiting (13.1%), ALT/AST increased (7.2%/6.8%), infections (7.7%), fatigue (6.8%), bilirubin increased (5.9%), anorexia (5.9%), and headache (5.4%).

Few TEAE were reported with a significant higher frequency in TREO arm vs BU, notably cardiac disorders (14.9% vs 8.3%) and oedema of limbs (20.8% vs 14.6%) but no significant difference for these AE appears if at least grade III or if treatment-related.

BU has a higher incidence of oral mucositis and other GI disorders compared to TREO. Whilst BU also has more frequent increases in GGT, TREO conditioning results in a more frequent higher elevation of transaminases and bilirubin. No HSOS was reported for any patient in adults receiving the proposed TREO dosage and the observed increase in transaminases/bilirubin are of no concern.

The major causes of death are transplant-related events (mainly infections followed by GVHD) and relapse/progression. Median time to death after transplantation was 5.6 months. TRM was significantly lower in the TREO arm with proposed dose compared to the BU (10.4% vs 18.8%).

Most frequent serious adverse events (SAE) after proposed TREO-based conditioning are infections (5%), especially sepsis and lung infection. The safety profile of TREO versus BU appears similar with regards to SAEs.

In a retrospective analysis in a single institution, four of 117 patients (3.4%) treated with TREO/FLU conditioning and 14 of 210 patients (6.7%) treated with dose reduced BU/FLU developed secondary malignancies [Shimoni 2013].

No relevant differences were observed between the safety results of the 2nd interim and the final analysis of study MC. FludT.14/L Trial II.

It is expected that the safety of TREO based conditioning in adults with non-malignant may be more in line with that reported for younger adults and paediatric data with NM disease treated with the FT14 regimen.

Assessment of paediatric data on clinical safety

Data from the 88 paediatric patients treated with TREO based conditioning is very limited, especially for the NM setting (n=18). The majority of patients received a dose of 12 or 14 g/m^2/day and in combination with TT reflecting current medical practice. No clinical study report has been submitted for the paediatric study 16/NM..

The paediatric data showed for NM disease TREO regimen had more frequent AE grade IV and drug related AE but a reduced incidence of serious AE compared to BU conditioning. Overall, it appears TREO regimen has not an improved safety profile compared to the BU regimen. The safety profile of TREO is more unfavourable in M compared to NM diseases, as expected with an underlying malignant disease.

The most frequent TEAEs after TREO-based conditioning in children irrespective of disease were gastrointestinal disorders (> 10%: stomatitis [78.4%], vomiting [68.2%], diarrhoea [63.6%], nausea [45.5%], abdominal pain [34.1%], constipation [12.5%]), pyrexia (71.6%), infections (59.1%), vascular disorders (hypertension [35.2%], haematoma [10.2%]), skin and subcutaneous tissue disorders (> 10%: maculopapular rash [29.5%], pruritus [23.9%], pain of skin [11.4%]), headache (29.5%), cough (18.2%), pain in extremity (18.2%), sinus tachycardia (12.5%), hypersensitivity (12.5%), investigations (> 10%: viral test positive [15.9%], ALT increased [10.2%]), and infusion-related reaction (10.2%). The most frequent drug-related TEAEs include stomatitis (69.3%), vomiting (43.2%), diarrhoea (33.0%), nausea (30.7%), abdominal pain (15.9%), pyrexia (14.8%), infections (11.4%), and pruritus (11.4%).

Mature data from the final analysis of study 17/M did not show significant differences compared to the interim analysis.

Overall, the safety profile of TREO is acceptable for a paediatric indication in malignant diseases. However, pending the clinical report for study 16/NM the data is considered at present immature to determine the safety profile of TREO based conditioning in a non-malignant indication.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

The safety profile of the proposed TREO based conditioning in adults (malignant and non-malignant disease) and in paediatrics with malignant disease is considered acceptable.

2.7. Risk Management Plan

Summary of safety concerns

Table 19: Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	Treatment-related second malignancy
Important potential risks	Seizures in small infants
Missing information	Effect on fertility Use in patients with prior alloHSCT

The safety concerns are appropriate.

Pharmacovigilance plan

The Applicant did not propose additional pharmacovigilance activities beyond routine pharmacovigilance activities.

The Applicant did not propose any additional pharmacovigilance activities to assess the effectiveness of risk minimisation measures.

Overall conclusions on the PhV Plan

Routine pharmacovigilance is sufficient to identify and characterise the risks of the product and that routine PhV remains sufficient to monitor the effectiveness of the risk minimisation measures.

Risk minimisation measures

Table 20 Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Routine risk minimisation activities
Important identified risks	
Treatment-related secondary malignancy	**Trecondi 1 g / 5 g powder for solution for infusion** Routine risk communication: SmPC sections 4.4, and 4.8; PL sections 2, and 4 Routine risk minimisation activities recommending specific clinical measures to address the risk: SmPC section 4.4: The possible risk of a second malignancy should be explained to the patient. Other routine risk minimisation measures beyond the product information: Legal status: prescription only medicine
	Treosulfan Powder for Solution for Infusion **Treosulfan 250 mg Capsule, Hard** Routine risk communication: SmPC sections 4.8, and 4.4 (only in DK, IE, and UK); PL section 4 Routine risk minimisation activities recommending specific clinical measures to address the risk: None Other routine risk minimisation measures beyond the product information: Legal status: prescription only medicine
Important potential risks	
Seizures in small infants*	Routine risk communication: SmPC section 4.4 Routine risk minimisation activities recommending specific clinical measures to address the risk: SmPC section 4.4: Children should be monitored for signs of neurological side effects. The use of clonazepam prophylaxis for children younger than 1 year might be considered. Other routine risk minimisation measures beyond the product information: Legal status: prescription only medicine
Missing information	
Effect on fertility*	Routine risk communication: SmPC sections 4.4, and 4.6; PL section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: SmPC section 4.4: Men are advised not to father a child during and up to 6 months after treatment and to seek advice on cryo-conservation of sperm prior to treatment because of the possibility of irreversible infertility. Women

Safety concern	Routine risk minimisation activities
	are informed on ovarian suppression and amenorrhoea. SmPC section 4.6: Advice on cryo-conservation of sperm prior to treatment because of the possibility of irreversible infertility. <u>Other routine risk minimisation measures beyond the product information:</u> Legal status: prescription only medicine
Use in patients with prior alloHSCT*	<u>Routine risk communication:</u> None <u>Routine risk minimisation activities recommending specific clinical measures to address the risk:</u> None <u>Other routine risk minimisation measures beyond the product information:</u> Legal status: prescription only medicine

* Safety concerns only for Trecondi 1 g / 5 g powder for solution for infusion indicated for conditioning treatment prior to alloHSCT in adult and paediatric patients with malignant and non-malignant diseases.

Overall conclusions on risk minimisation measures

The proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indications.

2.7.1. Summary of the risk management plan

The public summary of the RMP is acceptable.

2.7.2. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 0.3 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

Based on the fact that treosulfan CAP has a completely new indication compared to previously nationally authorised products and it can also be used in paediatric population, the CHMP is of the opinion that a separate entry in the EURD list for Trecondi is needed, as it cannot follow the already existing entry for treosulfan. The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request the alignment of the new PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EU birth date(EBD) to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Treosulfan is indicated as part of conditioning treatment prior to alloHSCT in adult patients with malignant and non-malignant diseases, and in paediatric patients older than one month with malignant diseases.

The aim of alloHSCT is to eradicate the underlying disease by replacing a “defective” bone marrow with a healthy donor’s marrow and to prolong survival.

3.1.2. Available therapies and unmet medical need

Patients undergoing alloHSCT are prepared with chemotherapy alone or chemotherapy combined with radiotherapy, the so-called conditioning regimen, before infusion of donor’s stem cells.

Myeloablative conditioning (with or without radiation) is associated with significant toxicity. Due to its lowered toxicity, non-myeloablative transplants can be appropriate for older or patients with co-morbidities that would otherwise exclude them from myeloablative transplantation. Fludarabine has been widely used because it is highly immunosuppressive, has anti-tumour activity in haematologic malignancies and a low non-haematologic toxicity profile. Regimens that relied on FLU or lower doses of the conditioning agents are referred to as either non-myeloablative (NMA) or reduced intensity conditioning (RIC). NMA regimens may result in minimal cytopaenias that do not require stem cell support whereas RIC regimens do require stem cell support. There are several NMA and RIC regimens available, but the optimum regimen remains to be defined. A regimen of busulfan (total dose ≤ 9 mg/kg) plus fludarabine is an example of RIC.

Treosulfan conditioning regimens can provide myeloablative and anti-leukaemic activity reducing the risk of relapse but with a reduced toxicity. The proposed regimen of treosulfan in combination with fludarabine (and thiotepa), is considered a RIC.

3.1.3. Main clinical studies

The indication in adults is based on the pivotal study MC-FludT.14/L Trial II, a Phase 3, randomized, open-label, parallel-arm, non-inferiority, controlled study that evaluated the efficacy and safety of treosulfan based regimen conditioning versus a standard busulfan based RIC prior to allogeneic HSCT in adult patients up to 70 years of age with AML (n=293) or MDS (n=167) considered ineligible to standard myeloablative conditioning. Treosulfan was administered by 2 hours IV infusion at a dose of 10g/m^2 BSA on days -4, -3 and -2 in combination with fludarabine.

The paediatrics indication is based on one open-label phase II trial, MC-FludT.17/M, supported by available data from an ongoing phase II trial (MC FludT.16/NM) and published paediatric data including transplant registries.

Study MC-FluT.16/NM is a randomised, active-controlled, parallel-group, study that evaluated the safety and efficacy of treosulfan based regimen compared to conventional dose busulfan in 38 children with non-malignant diseases, which require myeloablative conditioning treatment for allogeneic HSCT. The study is ongoing, and the study report is not available.

Study MC-FluT.17/M is a non-controlled study to describe the safety and activity of treosulfan administered as part of a standardised fludarabine-containing conditioning regimen in 70 children with haematological malignant diseases, which require myeloablative conditioning treatment for allogeneic HSCT.

3.2. Favourable effects

Adults

In adults statistically significant non-inferiority of TREO versus BU for the primary endpoint of EFS within 24 months after HSCT (PPS population 51% TREO versus 63.5% BU, HR 0.67 (95% CI 0.48, 0.93), 1-sided adjusted p =0.0000424) - confirmed by non-inferiority testing in FAS (HR 0.65, 95% CI 0.47, 0.90). A statistically significant improved rate of OS at 24 months (FAS population, 71.3 % TREO versus 56.4 % BU, HR 0.61, 95% CI = 0.42, 0.88, 1-sided adjusted p = 0.0082). No patients in TREO group experienced graft failure compared to one patient with primary failure and 7 patients with secondary failure in BU group (FAS population). The incidence of non-relapse mortality at 24 months was reduced (11.4% TREO versus 22.6% BU, HR 0.60, 95% CI 0.36, 1.01, 1-sided adjusted p = 0.05) and a statistically significant reduced transplant-related mortality at 24 months (12.1% TREO versus 28.2% BU, HR 0.54, 95% CI 0.32, 0.91, 1-sided adjusted p = 0.02) was observed. In general, consistent results for subgroup analyses, for EFS, OS and TRM, including those per disease type (AML and MDS). Statistically significantly higher incidence of complete donor-type chimerism at both Day +28 and Day +100 (Day +28, 93.5% TREO vs 82.0% BU [OR 3.21, 95% CI 1.69, 6.09, adjusted 1-sided p-value=0.008]; Day +100, 86.4% TREO vs 78.2% BU [OR 1.88, 95% CI 1.11, 3.19, adjusted 1-sided p-value=0.0205]. There was a statistically significantly higher incidence of GvHD-free and relapse/progression-free survival (HR 0.72, 95% CI 0.54, 0.95; 1-sided adjusted p-value=0.0224) and a statistically significantly higher incidence of chronic GvHD-free and relapse/progression-free survival (HR 0.69, 95% CI 0.52, 0.92; adjusted p-value=0.0108). The results were consistent in the final analysis (data cut off 16 March 2018).

Paediatrics

In Study MC-FluT.17/M the primary endpoint, freedom from transplant related mortality until day +100 post allo HSCT was 98.6%. No patient experienced a primary graft failure, but one patient experienced a secondary graft failure. The incidence of complete donor-type chimerism was 94.2% (90% CI 87.2-98.0%) at day +28 visit, 91.3% (90% CI 83.6-96.1%) at day +100 visit and 91.2% (90% CI 82.4-96.5%) at month 12 visit. Transplant-related mortality at 12 months is 2.9% (90% CI 0.9 – 8.9%).

Cumulative incidence of relapse/progression is 15.7% (90% CI 8.6-22.9%) at month +12.

3.3. Uncertainties and limitations about favourable effects

There were no uncertainties.

3.4. Unfavourable effects

AEs most frequently reported in adults with the proposed dosage are gastrointestinal (68%), infections (26.2%), oedema limbs (20.8%), headache (15.4%), febrile neutropenia (14.9%), bone/back pain (14.5%/14%), fever (32.1%), hypertension (14.9%), and maculopapular rash (12.2%).

The most frequent AEs in children irrespective of disease were gastrointestinal (stomatitis [78.4%], vomiting [68.2%], diarrhoea [63.6%], nausea [45.5%], abdominal pain [34.1%], constipation [12.5%]), pyrexia (71.6%), infections (59.1%), vascular disorders (hypertension [35.2%], haematoma [10.2%]), skin and subcutaneous tissue disorders (maculopapular rash [29.5%], pruritus [23.9%], pain of skin [11.4%]), headache (29.5%), cough (18.2%), pain in extremity (18.2%), sinus tachycardia (12.5%), hypersensitivity (12.5%), investigations (viral test positive [15.9%], ALT increased [10.2%]), and infusion-related reaction (10.2%).

The most frequent serious AE are infections (adults 5%, paediatrics non-malignant disease 11%, paediatric malignant disease 21%).

3.5. Uncertainties and limitations about unfavourable effects

There were no uncertainties.

3.6. Effects Table

Table 56. Effects Table for treosulfan as part of conditioning treatment prior to alloHSCT in adult patients with malignant and non-malignant diseases, and in paediatric patients older than one month with malignant disease (adult data cut-off: 19/08/2016).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence
Favourable Effects					
Adults					
EFS at 24m (PPS)	HR 0.67 (95% CI 0.48, 0.93) 1-sided p 0.0000424	%	63.5	51.1	• Non-inferiority TREO vs BU met in PPS and FAS
Improved OS at 24m	HR 0.61 95% CI 0.42, 0.88) 1-sided p 0.0082	%	71.3	56.4	• Secondary endpoints support primary outcome • Consistent results for subgroup analyses • Consistent results for final analyses (data cut off 16/03/2018)
Graft failure	Number patients with primary/secondary graft failure	n	0	8	
Improved NRM at 24m	HR 0.60 (95% CI 0.36, 1.01) 1-sided p 0.05	%	11.4	22.6	
Improved TRM at 24m	HR 0.54 (95% CI 0.32, 0.91) 1-sided p 0.02	%	12.1	28.2	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence
Improved complete donor-type chimerism Day +100	OR 1.88 95% CI 1.11, 3.19, 1-sided p 0.0205	%	86.4	78.2	
Improved GvHD-free relapse/PFS at 24 m	HR 0.72 95% CI 0.54, 0.95; 1-sided p 0.0224	%	51.4	38.4	
Improved chronic GvHD-free relapse/PFS at 24 m	HR 0.69 95% CI 0.52, 0.92 1-sided p 0.0108	%	52.3	38.5	
Paediatrics					
Freedom from TRM until day +100		%	98.6%	N/A	Final analysis of study 17/M in line with results interim analysis.
Primary graft failure,		n	0		
Secondary graft failure.		n	1		
Incidence complete donor-type chimerism	Day +28	(%) 90% CI	94.2% (87.2-98.0 %)		
	Day +100		91.3% (83.6-96.1 %)		
	Month 12		91.2% (82.4-96.5 %)		
TRM 12 months			2.9% (0.9 – 8.9%)		
Cumulative incidence relapse/progression month +12.			15.7% (8.6-22.9 %)		

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence
Unfavourable Effects					
Gastrointestinal disorders	Very common AE in all patients including stomatitis/mucositis, diarrhoea, nausea, vomiting, abdominal pain				None
Infections	Very common in all patients. The most common SAE.				

Abbreviations: m: months; NRM; non-relapse mortality; TRM: transplant-related mortality; SAE: serious adverse event;

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Treosulfan based conditioning has been demonstrated to be non-inferior to standard RIC with busulfan in adult patients with malignant disease undergoing alloHSCT. The proposed regimen appears well tolerated and there is no indication of a detrimental safety profile compared to busulfan.

Results in paediatric patients in the malignant setting are mature and have shown efficacy for the conditioning with TREO that outweighs the toxicity of the treatment.

3.7.2. Balance of benefits and risks

The favourable effects in the adult population outweigh the unfavourable effects especially in the context of the clinical setting of malignant diseases. The same applies to paediatric indication in malignant setting. Overall the Benefit Risk is positive in the adult indication (malignant and non-malignant diseases) and in the paediatric indication of malignant disease.

3.7.3. Additional considerations on the benefit-risk balance

A satisfactory justification to allow extrapolation to an indication in adults non-malignant disease has been presented. However extrapolation to paediatric non-malignant disease has not been possible.

Only patients up to 70 years were included in the pivotal study and there are no data in patients > 70 years from supportive studies. Nowadays, eligibility to undergo alloHSCT is based on patient's fitness rather than age, and takes into consideration performance status, presence of co-morbidities and patient's preference. Therefore, no age restriction of the indication in adults is necessary and it is up to the physician to make that decision.

3.8. Conclusions

The overall B/R of Trecondi is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Trecondi is not similar to Tepadina within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Trecondi is favourable in the following indication:

Treosulfan in combination with fludarabine is indicated as part of conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT) in adult patients with malignant and non malignant diseases and in paediatric patients older than one month with malignant diseases.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

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

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0197/2017 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.