EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

26 January 2023
EMA/CHMP/117245/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Trecondi

International non-proprietary name: treosulfan

Procedure No. EMEA/H/C/004751/II/0014

Note

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

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List of abbreviations

µl	Microliter
ADME	Absorption, distribution, metabolism and excretion
AE	Adverse event
aGVHD	Acute graft versus host disease
AIC	Akaike information criterion
ALAT	Alanine aminotransferase
ALL	Acute lymphoblastic leukaemia
alloHSCT	Allogeneic Haematopoietic Stem Cell Transplantation
ALT	Alanine aminotransferase
AML	acute myeloid leukaemia
ANC	Absolute neutrophil count
AP	Alkaline phosphatase
AR	Adverse reaction
AST	Aspartate aminotransferase
ATLL	Adult T-cell leukaemia/lymphoma
AUC	Area under the curve or area under the plasma concentration time curve
AUC∞	Area under the concentration time curve from time zero to infinity
b.w.	Body weight
BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein
BD	1,3-butadiene
BIC	Bayesian information criterion
BM	Bone marrow
BMSC	Bone marrow stromal cell
BMT	Bone marrow transplantation
BSA	Body surface area
BSEP	Bile salt export pump
BU	Busulfan
CAFC	Cobblestone area forming cell
CCG	Creatinine clearance or glomerular filtration rate
CD	Cluster of differentiation
CDER	Center for Drug Evaluation and Research
CHMP	Committee for Human Medicinal Products
CI	Confidence interval
CIOMS	Council for International Organisations of Medical Sciences
CiPA	Comprehensive comprehensive in vitro proarrhythmia assay
CL	Total clearance
CLL	Chronic lymphocytic leukaemia
C_{max}	Maximum plasma concentration
CNS	Central nervous system
CR	Complete remission
CRF	Case report form
CRFS	Chronic GvHD-free and relapse / progression-free survival
CRO	Contract Research Organisation
CRP	C-reactive protein

CSF	Cerebrospinal fluid
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CTD	Common technical document
CTP	Clinical Trial Protocol
CTR	Clinical trial report
CY	Cyclophosphamide
CYP	Cytochrome
DDI	Drug drug interactions
DEB	(2S,3S)-1,2:3,4-diepoxybutane
DLI	Donor-lymphocyte infusion
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
EBD, EBDM	(2S,3S)-1,2-epoxybutane-3,4-diol-4-methane-sulfonate or 3,4-epoxy-1,2-butanediol, the monoepoxide metabolite of treosulfan
EBMT	European Group for Blood and Marrow Transplantation
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ED_X	Effective dose in X% of animals
EFS	Event-free survival
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EoT	End of Trial
ESI	Electrospray ionisation
FAS	Full analysis set
FDA	Food and Drug Administration
FLU	Fludarabine
FPD	Field potential duration
GC	Gas chromatography
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transferase
GI_{50}	Drug concentration capable of 50% growth inhibition
GLC	Gas-liquid chromatography
GLP	Good laboratory practice
GMP	Good Manufacturing Practice
GOF	Goodness-of-fit
GOT	Serum glutamic oxaloacetic transaminase
GRFS	GvHD-free and relapse / progression-free survival
GSH	Glutathione
GST	Glutathione S-transferase
GvHD or GVHD	Graft-*versus*-host disease
h(s)	Hour(s)
HCT	Haematopoietic cell transplantation
hERG	Human ether à go go related gene
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen

HPLC	High-performance liquid chromatography
HSCT	Haematopoietic stem cell transplantation
HSOS	Hepatic sinusoidal obstruction syndrome
hu-Alb	Human albumin
i.p., IP	Intraperitoneal(ly)
i.v., IV	Intravenous(ly)
IARC	International Agency for Research on Cancer
IC_{50}	Half maximal (50%) inhibitory concentration
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IFN	Interferon
IgG, IgM	Immunoglobulin G, immunoglobulin M
IL	Interleukin
INN	International Non-proprietary Name
IP	Investigational product
IRB	Institutional Review Board
ISS	Integrated Summary of Safety
IUPAC	International Union of Pure and Applied Chemistry
JMML	Juvenile myelomonocytic leukaemias
KCl	Potassium chloride
$K_{e/p}$	RBC/plasma coefficient
kg	Kilogram
K_M	Michaelis constant
KPS	Karnofsky Performance Score, syn.: Karnofsky Performance Status, Karnofsky Index
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LC_X	Lowest concentration of drug observed to kill X % of cells
LDH	Lactate dehydrogenase
LD_X	Lethal dose to X % of animals
LEO	LEO Pharmaceutical Products, Denmark
LLN	Lower limit of normal
LLOQ	Lower limit of quantitation
LPS	Lansky Performance Score
LSC	Liquid scintillation counting
LVEF	Left ventricular ejection fraction
MATE	Multidrug and toxin extrusion
MDS	Myelodysplastic syndrome
MFD	Matched family donor
mg	Milligram
MHC	Major histocompatibility complex
min	Minute
mM	Millimolar
MRD	Matched related donor
mRNA	Messenger RNA
MS	Mass spectrometry
MSD	Matched sibling donor

MTD	Maximum tolerated dose
MTT	3-(4,5-dimethyl-2-tetrazolyl)-2,5-diphenyl-2H tetrazolium-bromide
MUD	Matched unrelated donor
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NCI	U.S. National Cancer Institute
NHL	Non-Hodgkin's lymphoma
NK cells	Natural killer cells
NMR	Nuclear magnetic resonance
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NRM	Non-relapse mortality
OAT	*Organic anion transporter*
OATP	Organic-anion-transporting polypeptide
OCT	*Organic cation transporter*
OECD	Organisation for Economic Co-operation and Development
OFV	Objective function value
OS	Overall survival
OS	Overall survival
p.o., PO	Per oral, orally
PB	Peripheral blood
PBMCs	Peripheral blood mononuclear cells
PBPK	Physiologically-based pharmacokinetikpharmacokinetic
PCR	Polymerase chain reaction
PCT	Procalcitonin
PDCO	Paediatric Committee
PDIP	Protocol Deviation Identification Plan
PFS	Progression-free survival
pH	Negative logarithm of H^+ concentration
PIL	Product Information Leaflet
PIP	Paediatric Investigation Plan
PK	Pharmacokinetic
PLT	Platelets
PMN	Polymorphonuclear neutrophilic leucocytes, neutrophils
PND	postnatal day
PopPK	Population pharmacokinetics
PT	Preferred Term
Q1	25%-percentile (first quartile)
Q3	75%-percentile (third quartile)
QA	Quality assurance
RAEB	Refractory anaemia with excess blasts
RBC	Red blood cells
RFS	Relapse-free survival
RNA	Ribonucleic acid
RP HPLC	Reverse phase HPLC
RT	Room temperature

S,S-DEB	(2S,3S)-1,2:3,4-diepoxybutane
S,S-EBDM	(2S,3S)-1,2-epoxybutane-3,4-diol-4-methanesulfonate
s.c., SC	Subcutaneous(ly)
s.d.	standard deviation
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAR	Serious adverse reaction
SB	Serious breaches
SCD	Sickle cell disease
SCID	Severe combined immunodeficient
SCT	Stem cell transplantation
SD	Standard deviation
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
$t_{½}$	Plasma elimination half-life
TBI	Total body irradiation
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
t_{max}	Time to reach C_{max}
TMF	Trial Master File
TNF	Tumour necrosis factor
TREO	Treosulfan
TRM	Transplantation-related mortality
TRM	Transplant-related mortality
TTCB	Conditioning regimen, comprising donor specific transfusion, treosulfan and cyclophosphamide injections and BMT
ULN	Upper limit of normal
USA	United States of America
versus, vs.	As the alternative to or in contrast with
V_{max}	Maximum reaction rate, described in the Michaelis-Menton kinetics
VPC	Visual predictive checks
WBC	White blood cells, leucocytes
WHO	World Health Organisation

1. Background information on the procedure

1.1. Type II variation

Pursuant to Article 16 of Commission Regulation (EC) No 1234/2008, medac Gesellschaft fur klinische Spezialpraparate mbH submitted to the European Medicines Agency on 7 March 2022 an application for a variation.

The following variation was requested:

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

Extension of indication to include additional non-malignant transplant indications (non-malignant diseases in the paediatric population) for Trecondi 1 g/5 g powder for solution for infusion based on final 12-months follow-up results of study MC-FludT.16/NM; a randomised phase II interventional study aimed to compare Treosulfan-based conditioning therapy with Busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with non-malignant diseases.
Further, the MAH proposes to amend an existing warning on skin toxicity based on new literature data. Moreover, the MAH proposes to introduce a slightly modified dosing regimen according to the patient's body surface based on long-term follow-up data of paediatric study MC-FludT.17/M, a Phase II 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, as well as a final analysis of the population pharmacokinetics of treosulfan in paediatric patients. As a consequence, sections 4.1, 4.2, 4.4, 4.8 and 5.1 of the SmPC are updated. The Package Leaflet is updated in accordance. Version 1.0 of the RMP has also been submitted.

The variation requested amendments to the Summary of Product Characteristics and Package Leaflet and to the Risk Management Plan (RMP).

Information relating to orphan designation

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

At the time of the granting of the Marketing Authoirisation, the Commission decided that on the basis of the COMP's final opinion Trecondi no longer met the criteria for designation (established in Article 3 of Regulation No 141/2000) and that, therefore, it could not be designated as an orphan medicinal product. However, following the judgement of the General Court of 23-Sep-2020 the orphan designation was re-established by the Commission Implementing Decision C(2020)8389 (final), dated 24-Nov-2020.

Information on paediatric requirements

Pursuant to Article 8 of Regulation (EC) No 1901/2006, the application included EMA Decision P/0346/2020 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP was completed.

The PDCO issued an opinion on compliance for the PIP P/0346/2020.

Information relating to orphan market exclusivity

The orphan market exclusivity for "Conditioning treatment prior to haematopoietic progenitor cell transplantation" (based on designation EU/3/04/186) started on 24 Jun 2019. This orphan market exclusivity will expire on 24 Jun 2029.

There is no authorised orphan medicinal product for a condition related to the proposed indication.

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the MAH did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Protocol assistance

The MAH did not seek Protocol Assistance at the CHMP.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Fátima Ventura Co-Rapporteur: <N/A>

Timetable	Actual dates
Submission date	7 March 2022
Start of procedure:	23 April 2022
CHMP Rapporteur Assessment Report	29 June 2022
PRAC members comments	29 June 2022
Updated PRAC Rapporteur Assessment Report	30 June 2022
PRAC Outcome	7 July 2022
CHMP members comments	11 July 2022
Updated CHMP Rapporteur(s) (Joint) Assessment Report	14 July 2022
Request for supplementary information (RSI)	21 July 2022
CHMP Rapporteur Assessment Report	11 October 2022
PRAC Rapporteur Assessment Report	14 October 2022
PRAC Outcome	27 October 2022
CHMP members comments	28 October 2022
Updated CHMP Rapporteur Assessment Report	3 November 2022
Request for supplementary information (RSI)	10 November 2022

Timetable	Actual dates
CHMP Rapporteur Assessment Report	22 December 2022
PRAC members comments	N/A
CHMP members comments	16 January 2023
Updated CHMP Rapporteur Assessment Report	19 January 2023
CHMP Opinion	26 January 2023

2. Scientific discussion

2.1. Introduction

2.1.1. Problem statement

Disease or condition

The MAH is now requesting an extension of the indication to include additional non-malignant transplant indications (**non-malignant diseases** in the paediatric population based on final 12-months follow-up results of study **MC-FludT.16/NM**.

State the claimed the therapeutic 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 and non-malignant diseases.

Epidemiology

According to the Worldwide Network of Blood and Marrow Transplantation (WBMT), 82 718 HSCTs were conducted worldwide by 1 662 teams from 86 countries in 2016. Of those transplants, on average 53.5% are autologous and 46.5% are allogeneic; 45.2% occur in Europe, 24.4% in North America, 22.7% in the South East Asia/Western Pacific Region, 5.1% in Latin-America and 2.7% in the African/East Mediterranean Region. Absolute numbers of alloHSCTs had increased from 20 333 in 2006 to 38 425 in 2016.

In 2019, there were 5 189 paediatric patients < 18 years of age receiving HSCT in Europe, 3 990 received an allogeneic and 1 199 an autologous HSCT [Passweg 2021].

Change in HSCT numbers is different for each indication with novel indications emerging. The number of alloHSCTs continues to increase annually, and reductions in organ damage, infection, and severe acute graft versus host disease (aGvHD) seem to be contributing to improved outcomes.

Biologic features, Aetiology and pathogenesis

The use of the product reflects the current clinical practice of transplantation in the European Union.

Clinical presentation

In paediatric patients, alloHSCT has become a therapeutic option with curative potential for treatment of otherwise incurable hematological malignancies and non-malignant diseases, e.g., immunodeficiencies, haemoglobinopathies (Hb-pathies), bone marrow failure (BMF) syndromes, or metabolic diseases.

In non-malignant diseases such as primary immunodeficiencies (PIDs) alloHSCT has become increasingly successful.

Management

Patients undergoing an alloHSCT are prepared with chemotherapy alone or chemotherapy combined with radiotherapy, the so-called conditioning or preparative 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 in order to allow engraftment of donor stem cells. Exceptions to this rule are infants with severe combined immune deficiency (SCID) and patients with severe aplastic anaemia (SAA) with an identical twin donor who may be grafted without conditioning.

The purine analogue fludarabine (FLU) has been widely incorporated into such regimens. It is highly immunosuppressive, producing profound lymphopenia, which has been shown to facilitate allogeneic stem cell engraftment. It has the additional advantages of having anti-tumour activity in haematologic malignancies and a low non-haematologic toxicity profile

Efficacy, safety and PK of treosulfan-based conditioning regimens in paediatric subjects prior to alloHSCT have already been demonstrated in several published studies. It has been shown that treosulfan is highly effective enabling sustained engraftment without increasing the risk for severe acute or chronic graft-versus-host disease (aGvHD / cGvHD). Considering that most children and adolescents with malignancies or non-malignant diseases have either been heavily pre-treated or have already undergone a previous HSCT, the toxicity profile is obviously low compared with either TBI containing regimens or other myeloablative treatments (e.g., busulfan, cyclophosphamide, etoposide or melphalan). However, children > 4 years of age undergoing alloHSCT for acute lymphoblastic leukaemia should preferentially be conditioned with TBI-based regimens. Results of the medac-sponsored phase 2 trial MC-FludT.17/M in 70 children with malignant transplantation indications support this observation.

2.1.2. About the product

Treosulfan, a prodrug of a bifunctional alkylating agent and a broad antineoplastic and potent anti-leukemic medicine fulfils the criteria for a perfect cytotoxic agent for conditioning regimens: sufficient stem cell toxicity (with respect to primitive as well as committed stem cells) and immunosuppression, to enable rapid and stable engraftment, low organ toxicity, especially with respect to the liver, kidneys, lung, and the nervous system, sufficient cytotoxicity to guarantee an effective treatment of the underlying hematological malignancy, and predictive pharmacokinetics (IV administration, linear pharmacokinetics, low inter-individual variability, no enzyme-dependent drug activation).

Under physiological conditions (pH 7.4, temperature 37°C) the pharmacologically inactive treosulfan converts spontaneously (non-enzymatically) into a reactive monoepoxide intermediate and finally to L-diepoxybutan. These epoxides are able to react with neutrophilic centres of biological molecules like proteins or DNA and are considered to be responsible for the antineoplastic activity via secondary biological mechanisms.

Due to its pronounced toxicity against committed and primitive hematopoietic stem cells as well as its proven immunosuppressive and haematotoxic characteristics, treosulfan is currently developed as an alternative conditioning agent to busulfan or TBI.

Based on the given clinical experience in adults, treosulfan has been widely used as therapeutic alternative in paediatric HSCT conditioning regimens in the past years.

Patients, who undergo HSCT for non-malignant diseases, especially immunodeficiencies and metabolic diseases, are mostly infants, toddlers, and small children. The patterns of acute and especially late effects are different compared to adult transplantation patients and demand special attention. Mental development, growth and hormonal disorders, fertility, and risk of secondary cancer (particularly after TBI) are of special interest and are followed-up by national and international registries (e.g., within EBMT). Since the first successful allogeneic HSCT, more than 50,000 paediatric patients have undergone this procedure in Europe, and more than 2500 children are transplanted annually in Europe.

The MAH is now requesting an extension of the indication to include additional non-malignant transplant indications (non-malignant diseases in the paediatric population based on final 12-months follow-up results of study MC-FludT.16/NM.

Further, the MAH proposes to amend an existing warning on skin toxicity based on new literature data and to introduce a slightly modified dosing regimen according to the patient's body surface based on long-term follow-up data of paediatric study MC-FludT.17/M in paediatric patients with haematological malignancies, as well as a final analysis of the population pharmacokinetics of treosulfan in paediatric patients.

2.1.3. The development programme/ compliance with CHMP guidance/ scientific advice

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 following relevant guidelines were followed:

- Evaluation of Anticancer Medicinal Products in Man (CPMP/EWP/205/95 Rev. 3)
- Appendix 1 to the Guideline on the Evaluation of Anticancer Medicinal Products in Man (CHMP/EWP/267575/06)
- Addendum on Paediatric Oncology (CPMP/EWP/569/02)
- ICH E11 Clinical investigation of medicinal products in the paediatric population (CPMP/ICH/2711/99)
- Guideline on clinical trials in small populations (CHMP/EWP/83561/05)
- Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population (EMEA/CHMP/EWP/147013/2004/Corr)

Treosulfan based conditioning in adult patients:

Three medac sponsored Phase 1 / 2 trials investigated the conditioning regimen treosulfan / fludarabine in adult subjects. Efficacy and safety parameters of treosulfan-based conditioning were analysed in

subjects with advanced haematological malignancies prior to alloHSCT. Furthermore, a prospective, comparative pivotal Phase 3 clinical trial (medac protocol code **MC-FludT.14/L**) in adult acute myeloid leukaemia and myeloid dysplastic syndrome (MDS) subjects, who were non-eligible for standard busulfan- or TBI-based myeloablative conditioning, started in Nov-2008. After dose- and regimen-adjustment for treosulfan to 10 g/m² on day -4, -3, -2, the trial restarted in June 2013 and was completed in Jan-2018. The trial demonstrated statistically significant non-inferiority regarding event-free survival (EFS) as well as improved overall survival (OS) and non-relapse mortality (NRM) in favour of treosulfan.

Treosulfan based conditioning in paediatric patients:

As a requirement within the paediatric investigational plan (PIP; PIP number EMEA-000883- PIP01-10, decision number P/122/2011) for treosulfan and subsequent modifications, the paediatric committee of EMA (PDCO) requested medac to conduct a retrospective evaluation (meta-analysis) on safety and efficacy data of treosulfan-based conditioning in paediatric subjects registered within the EBMT database. Within this registry study, data of 521 allogeneic and 83 autologous paediatric subjects transplanted between Jan-2005 and Jul-2010 were evaluated.

The median treosulfan dose administered for alloHSCT was in total 42 g/m^2 within 3 consecutive days. Treatment was effective and well tolerated in children of all age groups with malignant as well as non-malignant diseases. The dose of treosulfan was without significant impact on all analysed safety and efficacy parameters. In addition, medac was requested within the PIP to conduct 2 transplantation trials in paediatric subjects, one in subjects with malignant diseases (**MC-FludT.17/M**) and another trial in subjects with non-malignant diseases (**MC-FludT.16/NM**).

The **MC-FludT.17/M** trial started on 21-Nov-2014 and was completed on 30-Sep-2019 (last subject completed longer term follow-up).

In non-malignant disorders with a high risk of graft rejection, intensified regimens with additional thiotepa combined with either treosulfan / fludarabine or busulfan / fludarabine are recommended by EBMT / ESID guidelines to ensure engraftment.

In order to evaluate the current clinical practice of the use of thiotepa in combination with either treosulfan / fludarabine-based or busulfan / fludarabine-based conditioning regimens, another retrospective analysis of the EBMT database in children with non-malignant diseases, who underwent allogeneic HSCT between the years 2010 and 2014 was performed. A large variety of non-malignant diseases comprising data from 2187 patients were included in the EBMT registry analysis. In total, 895 out of the 2187 patients received a preparative regimen consisting of treosulfan / fludarabine either in combination with thiotepa (473 patients) or without thiotepa (422 patients). Thiotepa was most frequently added in Hb-pathies (374 out of 590 patients), affecting about half of the patients treated with treosulfan / fludarabine or busulfan / fludarabine. The number of patients who received thiotepa in addition to treosulfan / fludarabine increased significantly from 44% (66 out of 149 patients) in the year 2010 to 65% of patients (140 out of 217 patients) in the year 2014. Several retrospective studies covering the years 2009 to 2015 also indicate the more frequent use of thiotepa in treosulfan / fludarabine-based conditioning regimens in paediatric patients with nonmalignant diseases. Depending on the underlying disease, thiotepa was administered in up to 74% of patients.

The more frequent use of thiotepa reflects the current clinical practice of most transplantation centres in the European Union. The trial, **MC-FludT.16/NM**, was designed based on the modified PIP number EMEA-000883-PIP01-10-M01, decision number P/0104/2013. As the initial trial design did not reflect the more frequent use of thiotepa, but required the vast majority of subjects (at least 85 out of 100 subjects) to be treated without additional thiotepa, a PIP modification was submitted to the PDCO of EMA in Nov-2016 in order to permit the additional use of thiotepa in all qualified subjects at the discretion of the

Investigator. The PIP modification number EMEA-000883-PIP01-10-M03 was approved by the PDCO of EMA on 17-Mar-2017 (Decision number P/0059/2017). Details of trial design and conduct were predefined by the PDCO. The most recent CTP version complies with the most recent PIP modification (EMEA-000883-PIP01-10-M05; Decision number P/0346/2020).

MC-FludT.16/NM: Clinical phase II trial to compare safety and efficacy of treosulfan-based conditioning therapy with busulfan-based conditioning therapy in children of different age groups with non-malignant diseases requiring myeloablative conditioning treatment prior to alloHSCT. To describe the TREO compared to the conventional dose BU (control), each product was administered as part of a standardised FLU-containing conditioning regimen and also contributed to a pharmacokinetic model.

Analysis of treosulfan and monoepoxide plasma levels in a subgroup of subjects allocated to the test arm of the trial contributes to a separately reported population PK evaluation for treosulfan.

The clinical trial report (CTR) focusses mainly on the complete and final 12-month data, but also contains longer-term follow-up data that were available by data cut-off on 07-Jun-2021.

MC-FludT.17/M: Clinical phase II trial to describe the safety and efficacy of TREO-based conditioning therapy prior to alloHSCT in paediatric patients with haematological malignancies; To describe the safety and efficacy of TREO as part of a standardised FLU-containing conditioning and to contribute to a pharmacokinetic model.

The final analysis of **MC-FludT.14/L** Trial II has confirmed the non-inferiority of TREO-based conditioning compared to reduced-intensity conditioning therapy based on intravenous BU. Additionally, superiority of TREO versus BU could now be shown with the final data set.

Longer-term follow-up data will be collected until the last recruited subject has completed visit Month 36 and presented in an updated version of the CTR expected in 2023. Joint PK and Pop-PK evaluations of treosulfan and monoepoxide drug concentration measurements of both trials **MCFludT. 16/NM** and **MC-FludT.17/M** were reported by Venn Life Sciences.

Furthermore, both paediatric studies have been completed. Final CSRs are now available, with 3-year follow-up survival data for study MC-FludT.17/M and 1-year follow-up data for study 16/NM.

Moreover, the PopPK model for TREO was updated and the BSA-adapted dose regimen of TREO in paediatric patients slightly modified.

2.1.4. General comments on compliance with GLP, GCP

GLP

Most of the early study results on treosulfan are available as brief summaries of LEO contracted studies conducted in the 1970s and 1980s to support the use of treosulfan in clinical trials and application for marketing authorisation as an anti-cancer agent. The corresponding data as well as other supportive information is also published in the literature. Accordingly, the historical information of the nonclinical dossier regarding study design and reporting of results does not comply with current regulatory quality standards like Good Laboratory Practice (GLP) and guidelines for e.g. the conduct of safety studies (OECD and ICH guidelines). However, the studies were generally carried out state of the art at the time of performance and were accepted by the European authorities. Other, more recently conducted nonclinical studies (e.g. a 4-week subchronic toxicity and toxicokinetic study in adult rats as well as a toxicity study in juvenile rats) are in full compliance with GLP regulations.

GCP

All 7 clinical studies submitted by medac have been designed, conducted, and analysed according to the principles of Good Clinical Practice.

2.2. Non-clinical aspects

2.2.1. Introduction

The investigational drug substance, treosulfan, was already registered in the 70ies and 80ies of the last century by LEO Pharmaceutical Products in Denmark (LEO) for the chemotherapeutic treatment of patients with ovarian cancer in several European countries. Pharmacological and toxicological characteristics of treosulfan were initially assessed by the U.S. National Cancer Institute (NCI) under the compound identifier NSC-39069.

In 1990 medac GmbH took over the product with all legal rights and obligations. Later on, chemistry, manufacturing and control data, as well as new nonclinical and clinical data have been generated by medac to support the clinical development of a treosulfan-based conditioning therapy prior to autologous or allogeneic haematopoietic stem cell transplantation (HSCT).

The early nonclinical development of treosulfan as an anti-cancer agent started in the 1960s in the research laboratories of LEO. By order of LEO, treosulfan (NSC-39069) was extensively tested in vitro and in vivo by the Cancer Chemotherapy National Service Center in collaboration with the NCI. The corresponding study reports were compiled by LEO within a nonclinical dossier for application of marketing authorisation of treosulfan for treatment of ovarian cancer in several European countries (LEO, 1978b). The nonclinical study results of NSC-39069 and various other structures were published as special issue of Cancer Chemotherapy Reports (Schmidt et al., 1965).

Later on, an extensive nonclinical programme was performed to evaluate the pharmacodynamics, pharmacokinetics and the toxicological properties of treosulfan. In some studies, other alkylating agents, including busulfan, or reactive metabolites of treosulfan were applied for comparison and elucidation of mechanistic aspects.

Most of the early study results on treosulfan are available as brief summaries of LEO contracted studies conducted in the 1970s and 1980s to support the use of treosulfan in clinical trials and application for marketing authorisation as an anti-cancer agent. The corresponding data as well as other supportive information is also published in the literature.

Accordingly, the historical information of the nonclinical dossier regarding study design and reporting of results does not comply with current regulatory quality standards like Good Laboratory Practice (GLP) and guidelines for e.g. the conduct of safety studies (OECD and ICH guidelines). However, the studies were generally carried out state of the art at the time of performance and were accepted by the European authorities.

Other, more recently conducted nonclinical studies (e.g. a 4-week subchronic toxicity and toxicokinetic study in adult rats as well as a toxicity study in juvenile rats) are in full compliance with GLP regulations. The most recent 4-week toxicity study in rats also used the new lyophilised drug product formulation and covers, therefore, the only three days treatment period recommended for clinical use in the indication of conditioning prior to HSCT. In view of the alkylating properties and the toxicological characteristics of treosulfan as well as the clinical use for life-threatening conditions indicated for HSCT,

further specific toxicological qualification procedures, in particular regarding potential genotoxic properties of impurities, metabolites and decomposition products, are deemed not necessary. Moreover, its established clinical use in Europe for decades and the corresponding pharmacovigilance information is in line with the overall conclusions from the nonclinical safety studies drawn by the MAH.

In June 2019, the European Commission approved treosulfan for marketing authorisation with the following label: "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" (see SmPC TRECONDI®).

Meanwhile, medac also established a new production processes and a lyophilized formulation of the drug product.

Treosulfan is a prodrug. The parent compound is water soluble and converts under physiological conditions by non-enzymatic intra-molecular substitution into a reactive monoepoxide intermediate (S,S-EBDM) and subsequently to diepoxybutane (S,S-DEB) (Figure 2.4-4). 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 stem cell toxicity as well as generally broad cytotoxicity of treosulfan.

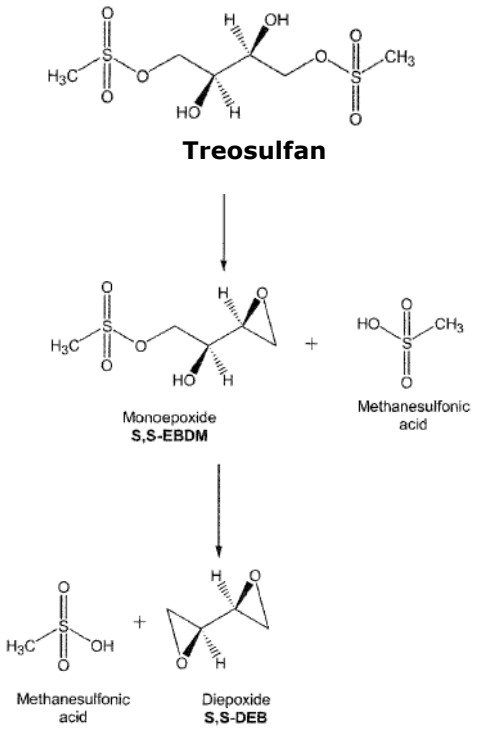


Figure 2.4-1: Conversion of treosulfan to biologically active epoxides (Glowka et al., 2012)

The two hydroxyl groups in position 2 and 3 of the treosulfan molecule are responsible for significant differences between treosulfan and busulfan (1,4-butanediol-dimethylsulfonate), which is currently approved for conditioning treatment prior to HSCT. These differences include physico-chemical, pharmacological, pharmacokinetic and toxicological characteristics.

The directly alkylating agent busulfan was used as positive control treatment in a number of pharmacological and toxicological tests and demonstrated a higher potential to induce hepatotoxicity, lung toxicity, CNS toxicity and effects on fertility than treosulfan.

Due to its long-established clinical use for treatment of European patients with ovarian cancer and the well-known clinical as well as nonclinical safety profile, pharmacokinetics and efficacy, treosulfan was more recently evaluated in high-dose treatment regimens with subsequent autologous HSCT. The proven haematopoietic stem cell toxicity against both, primitive and committed haematopoietic stem cells, as well as its immunosuppressive and haematotoxic characteristics, qualify treosulfan in particular as a developmental candidate for conditioning treatment of adult and paediatric patients with malignant as well as non-malignant diseases indicated for allogeneic HSCT.

For conditioning treatment, preferentially the combination of treosulfan with fludarabine (for pre-transplant immunosuppression) is applied. The drug product is used for intravenous infusion after dissolution in 20 mL (1.0 g strength) or 100 mL (5.0 g strength) 0.45% sterile saline. A single daily dose of 10 g/m² to 14 g/m^2 body surface area of treosulfan is to be administered on 3 consecutive days.

The MAH is now requesting an extension of the indication to include additional non-malignant transplant indications (**non-malignant diseases** in the paediatric population based on final 12-months follow-up results of study **MC-FludT.16/NM**.

Further, the MAH proposes to amend an existing warning on skin toxicity based on new literature data and to introduce a slightly modified dosing regimen according to the patient's body surface based on long-term follow-up data of paediatric study MC-FludT.17/M in paediatric patients with haematological malignancies, as well as a final analysis of the population pharmacokinetics of treosulfan in paediatric patients.

2.2.2. Pharmacology

Introduction

An overview of relevant studies to evaluate the pharmacodynamic profile of treosulfan is shown:

Table 1: Overview of pharmacodynamic studies

Type of Study	Test System	Administration	Reference
PRIMARY PHARMACODYNAMICS			
Mechanism of action (cytotoxicity)			
Interaction of treosulfan and epoxides with DNA	Isolated DNA and human chronic myelogenous leukaemic K562 cells	in vitro	Hartley et al., 1999
Interaction of EBDM and DEB with DNA	Isolated calf thymus DNA	in vitro	Romanski et al., 2019
Interactions of DEB stereoisomers with DNA	*Xenopus borealis* plasmids	in vitro	Millard et al., 2006
Interactions of DEB with DNA and proteins	Nuclear extracts from human cervical carcinoma (HeLa) cells	in vitro	Michaelson-Richie et al., 2010
Interactions of DEB with DNA	Human fibrosarcoma (HT1080) cells	in vitro	Gherezghiher et al., 2013
Chemosensitivity of human bone marrow stromal cells	Bone marrow stromal cells line HS-5 and primary cells	in vitro	Schmidmaier et al., 2006
Myeloablative and immunosuppressive effects			
Sensitivity of murine bone marrow towards busulfan and treosulfan *in vitro* and *in vivo*	Murine bone marrow cells	in vitro, i.p.	Westerhof et al., 2000
Sensitivity of treosulfan compared to busulfan against leukaemia and different cells types	Primary lymphoblastic and myeloid leukaemia cells	in vitro, ex vivo	Munkelt et al, 2008
Sensitivity of murine bone marrow towards single dose and fractionated treosulfan compared to busulfan *in vitro* and *in vivo*	Murine bone marrow cells	in vitro, ip.	Ploemacher [report], 2000
Single and fractionated treosulfan treatment on the femoral CAFC content	Mouse	i.p.	Ploemacher [report], 2003
Induction of donor-specific tolerance for skin grafts across full MHC barriers	Mouse	in vitro, i.v.	van Pel., et al., 2003
Induction of specific skin graft tolerance across haploidentical MHC barriers	Mouse	in vitro, i.v.	van Pel et al., 2004
Nonmyeloablative conditioning in a murine sickle cell disease model	Mouse	i.p.	Devadasan et al., 2018

Type of Study	Test System	Administration	Reference
Myeloablative and immunosuppressive properties of treosulfan in mice	Mouse	i.p.	Sjöö et al., 2006
Immunosuppressive activity of low-dose treosulfan in mice	Mouse	i.p.	Melchers et al., 2000
Use of high-dose treosulfan or busulfan to prepare recipients for allogeneic BMT in mice	Mouse	i.p.	BioTransplant, 2000
Permanent acceptance of skin grafts after low-dose treosulfan and BMT in mice	Mouse	i.p.	Andersson et al., 2003
Anti-tumour effects (haematological malignancies)			
Efficacy against fresh human tumour cells	Several tumour types (ALL, ATLL, AML, MDS, NHL)	in vitro	Bath Analytical, 1994
Cytotoxic effects of treosulfan and busulfan against paediatric leukaemic cells	Primary lymphoblastic and myeloid leukaemia cells	in vitro, ex vivo	Munkelt et al., 2008
Cytotoxicity on paediatric tumou cells	leukaemia cell lines	in vitro	Lanvers-Kaminsky et al., 2006
Cell death in myeloma cells	NCI-H929 and U266 cell lines from patients	in vitro	Meinhardt et al., 2003
Treosulfan-induced apoptosis, chemosensivity tests	U937, THP-1, HL-60, TUR, and primary AML cells from patients	in vitro	Schmidmaier et al., 2004
Apoptosis induction by treosulfan and other cytotoxic agents	PBMCs from patients with CLL	in vitro	Ristovska et al., 2009
Drug-resistance profile of treosulfan	ALL samples, paediatric patients	in vitro	Pogorzala et al., 2015
Antileukaemic activity of treosulfan in xenografted human ALL	Mouse	i.p.	Fichtner et al., 2003
Antileukaemic activity of treosulfan in xennografted Dunning leukaemia	Rats	i.p., p.o.	Jones et al., 1960, Schmidt et al., 1965

SECONDARY PHARMACODYNAMICS			
Eryptosis following treosulfan treatment	Human erythrocytes	in vitro	Peter et al., 2015
Treosulfan impedes the migration of immunocompetent cells	Human venous blood	in vitro	Kopadze et al., 2007
In vitro sensitivity of human ovarian tumours	Human ovarian tumours	in vitro	Wilson and Neal, 1981
Efficacy of treosulfan against fresh human tumour cells *in vitro*	Human tumour cells	in vitro	Bath Analytical, 1994
Sensitisation of glioma cells to treosulfan	Human glioma cells	in vitro	Reber et al., 1998
Hyperthermia enhanced chemosensitivity of human malignant glioma cells	Human glioma cells	in vitro	Hermisson and Weller, 2000
Potentiation of treosulfan cytotoxicity by BSO in malignant glioma cells *in vitro* and *in vivo*	Human glioma cells, and rats	in vitro, i.p.	Wick et al., 2002
Ex vivo chemosensitivity profile of choroidal melanoma	Human primary uveal melanoma specimens	ex vivo	Myatt et al., 1997
Ex vivo chemosensitivity assessment of choroidal melanoma	Human primary uveal melanoma specimens	ex vivo	Saakyan et al., 2020
Ex vivo sensitivity of choroidal melanoma by combination of treosulfan with gemcitabine or cytosine arabinoside	Human primary uveal melanoma specimens	ex vivo	Neale et al., 1999
Treosulfan for malignant melanoma *in vitro* and *in vivo*	Melanoma cell lines, and human primary melanoma cells	in vitro and ex vivo	Neuber et al., 1999
Chemosensitivity testing in malignant melanoma	Human metastatic melanoma tissue specimens	ex vivo	Ugurel et al., 2003
In vitro and *in vivo* activity of treosulfan towards human renal-cell carcinoma	Renal tumour cells, and mouse	in vitro,.i.v.	Köpf-Maier, 1998
Cytotoxic effect of treosulfan on spheroids from primary cell cultures of kidney cell carcinomas	Primary renal cell carcinoma cells.	ex vivo	Kugler et al., 1998

Antitumour activity of treosulfan in human lung carcinomas	Mouse	i.p.	Köpf-Maier and Saß, 1996
Treosulfan demonstrate potent activity in Ewing's sarcoma	Mouse	i.p.	Werner et al., 2008
Effects of treosulfan in several xennografted tumours	Mouse and Rats	i.p., p.o.	White, 1962
SAFETY PHARMACOLOGY			
Electrophysiological examination on the hERG-mediated potassium current	HEK 293 cells	in vitro	B'SYS, 2017
Effect on hERG, hNav1.5, hCav1.2, hKir2.1 and hKCNQ1/minK cardiac ion channels	HEK293 cells (partly transfected / recombinant), CHO-K1 cell line	in vitro	CYP1269 R11-R12, 2020
Effect on beat period and field potential duration (FPD) in cardiomyocytes	fCDI hiPSC-derived cardiomyocytes	in vitro	CYP1269 R10, 2020
Effects on smooth muscle	Guinea pig intestine, cat trachea	in vitro	LEO, 1978b
Effects on central nervous system, cardiovascular function, gastric secretion, blood	Mouse, rat, dog, monkey	p.o., i.p., i.v.	LEO, 1978b
PHARMACODYNAMIC DRUG INTERACTIONS			
Synergistic effects against leukaemia cells after combination with fludarabine	Primary lymphoblastic and myeloid leukaemia cells	in vitro, ex vivo	Munkelt et al., 2008
Interaction of radiation and treosulfan	AML-and MDS-derived myeloid cell lines and primary marrow cells from patients and healthy donors	in vitro	Zang et al., 2015
Improved immune reconstitution after cytostatic conditioning with treosulfan/ fludarabine combination	Mouse	i.p.	Bouazzaoui et al., 2014
Addition of treosulfan to a nonmyeloablative conditioning regimen	Mouse	i.p.	Ploemacher et al., 2004b
Induction of tolerance across fully mismatched barriers by a nonmyeloablative treatment excluding antibodies of irradiation use	Mouse	i.p.	Stephan et al., 2006
Influence of time interval between the last treosulfan injection and total body irradiation on toxicity in rats	Rat	i.p.	Sender et al., 2009

Abbreviations: p.o.: oral; i.p.: intraperitoneal; i.v.: intravenous

Primary pharmacodynamic studies

Primary pharmacodynamic properties of treosulfan comprehend cytotoxicity, the main mechanism of action, myeloablative and immunosuppressive effects, and anti-tumour effects with focus on haematological malignancies considered relevant for the proposed use as conditioning treatment prior to HSCT.

In treosulfan-treated cells of the human chronic myelogenous leukemic 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 (Hartley et al., 1999).

DEB formed DNA-protein cross-links between cysteine thiols within proteins and the N-7 guanine positions within DNA (Michaelson-Richie et al., 2010). 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 signalling, DNA repair, and DNA damage response (Gherezghiher et al., 2013).

While alkylating agents (melphalan, treosulfan) and doxorubicin demonstrated marked cytotoxicity, nucleotide analogs (gemcitabine, cytarabine) induced only limited apoptosis in human bone marrow stromal cells (Schmidmaier et al., 2006).

Cytotoxicity of treosulfan and busulfan was evaluated in vitro in two human leukaemia cell lines and revealed a consistently higher cytotoxic potential than busulfan (Lanvers-Kaminsky et al., 2006). ALL samples of paediatric origin were tested for ex vivo chemosensitivity to various cytotoxic drugs. Their combined drug resistance profile was analysed. Lymphoblasts from patients who experienced multiple relapses were comparably resistant to daunorubicin, doxorubicin, cyclophosphamide, ifosfamide, busulfan, treosulfan, fludarabine, clofarabine, and bortezomib (Pogorzala et al., 2015).

Compared with other dimethanesulfonate compounds (related to busulfan), treosulfan exhibited comparably high in vitro activity, but relatively low activity in vivo (in case of only one single dose treatment) in terms of their toxicity to different haematopoietic stem cell subsets (Westerhof et al., 2000). Treosulfan was more active against 20 leukemic cell specimens of paediatric origin and against 3 leukaemia-derived cell lines than busulfan. Beyond that, purified haematopoietic CD34+ stem cells were most sensitive against treosulfan, followed by CD56+CD3- NK and CD3+T cells. In the lymphocyte subsets, treosulfan was threefold and in stem cells 20fold more effective than busulfan (Munkelt et al., 2008).

Using dose fractionation and repeated administrations of treosulfan, profound stem cell depleting properties in all stem cell subsets were shown in vitro and in vivo. This is in contrast to busulfan, which is predominantly toxic for long-term repopulating stem cells which might explain its low immunosuppressive potential (Ploemacher [report], 2000; Ploemacher [report], 2003).

Conditioning with non-myeloablative doses of treosulfan, followed by transplantation with syngeneic bone marrow (BM) donor cells, permitted long-term mixed-donor chimerism in a murine model of sickle cell disease. The induced level of chimerism correlated with improvement in sickle cell disease (SCD) related haematologic parameters, normalisation of urine osmolality, and improvement in liver and spleen pathology (Devadasan et al., 2018).

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 than busulfan or cyclophosphamide in depletion of splenic B and T cells. Treatment with treosulfan induced only interleukin-2 production in spleen cells of mice for a short time only and had no significant effect on synthesis of tumour necrosis factor-alpha and/or interferon-gamma as compared with either busulfan or cyclophosphamide (Sjöö et al., 2006).

The immunosuppressive activity of treosulfan was investigated using human peripheral blood B and T lymphocytes and mice immunized with keyhole limpet haemocyanin. Low dose treosulfan induced suppression of the early immune response, probably including the proliferation/differentiation of cells repopulating lymphoid organs and influenced the balance of regulatory T cell subpopulations (Melchers et al., 2000).

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 (van Pel et al., 2003, 2004). 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 (BioTransplant, 2000).

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 and confirmed functional aspects of the established donor-related immune system (Andersson et al.,2003).

Anti-haematological malignancies effects of Treosulfan were tested in vitro by differential staining cytotoxicity assays against 55 tissue specimens from patients with acute lymphoblastic leukaemia (ALL), adult T-cell leukaemia/lymphoma (ATLL), acute myeloid leukaemia (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 (Bath Analytical, 1994). Treosulfan was also more active than busulfan against leukaemic cells isolated from 20 paediatric ALL patients as well as against three leukaemia-derived cell lines with increasing IC50 values from initial diagnosis (chemotherapy naive specimens) to relapse (pretreated specimens; Munkelt et al., 2008). 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 (Meinhardt et al., 2003).

Chemosensitivity tests were performed in AML cell lines and primary cells isolated from patients. All cell types displayed dose-dependent sensitivity to treosulfan (Schmidmaier et al.,2004). In peripheral blood mononuclear cells (PBMCs) from patients with chronic lymphocytic leukaemia (CLL) treosulfan, 4-hydroperoxy-cyclophosphamide, fludarabine or cytarabine, but not busulfan, were effective inducers of apoptosis. Cell death was induced via caspase-activation (Ristovska et al., 2009).

The in vivo antileukaemic activity of treosulfan was compared with the activity of equitoxic doses of cyclophosphamide or busulfan using human ALL cells established in a xenotransplant mouse model in immunocompromised animals. Treosulfan was more effective with regard to the numbers of complete ALL regressions and the number of cured animals (Fichtner et al., 2003). In Fisher rats with Dunning leukaemia the chemotherapeutic activity of treosulfan was very much greater than that of busulfan, and was fully equivalent to the effect of 6-mercaptopurine (Jones et al., 1960; Schmidt et al., 1965).

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 in vitro significantly stimulated suicidal erythrocyte death (eryptosis), at least in part by inducing oxidative stress and stimulating Ca2+ entry. This effect presumably contributes to the anaemia observed after treosulfan treatment in the animal studies (Peter et al., 2015).

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 (Kopadze et al., 2007).

Apart from activity against haematological malignancies, treosulfan also exhibits a broad anti-tumour 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 (at lower IC90 values) than busulfan when evaluated in vitro on four human Ewing tumour, four human neuroblastoma, and two human osteosarcoma cell lines (Lanvers-Kaminsky et al., 2006).

Safety pharmacology programme

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 historical studies (White, 1962; LEO, 1978b) provided no evidence of adverse effects regarding (maximum doses 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.), dogs (222 mg/kg i.v and monkey (222 mg/kg i.v.) 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).

- Local anaesthetic effects: mice (tail, injection of 0.05 mL of a 2% treosulfan solution).

A recently conducted in vitro hERG K+ channel assay exhibited no potential for an arrhythmogenic effect at a treosulfan concentration of 1000 µM (B'SYS, 2017), which corresponds approximately to the clinically relevant C_{max} of 300 µg/ml in adult patient treated with 10 g/m^2 BSA (Hilger et al., 1998). Up to a five-time higher treosulfan concentration (5000 µM) was assessed on an array of key cardiac ion channels proposed by CiPA, in particular hERG, hNav1.5 (both peak and late), hCav1.2, hKir2.1, and hKCNQ1/minK ion channels. In these assays, the % inhibitions at the highest concentration tested were < 50%.

In cardiomyocytes, only the treosulfan top concentration of 5000 µM caused a transient increase in the beat period as well as a nominal shortening of the field potential duration (FPD), while the sodium channel endpoints were not affected at any of the concentrations tested in this assay. The change in the beat period at 5000 µM treosulfan may result from the instability of the test compound and the given closed cell culture system (CYP1269 R10, 2020).

Pharmacodynamic drug interactions

The combination of treosulfan with fludarabine resulted in a synergistic cytotoxic effect against human leukaemic cells isolated from 20 paediatric patients (Munkelt et al., 2008). 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 (Bouazzaoui et al., 2014).

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. In vitro pre-exposure to treosulfan did not clearly enhance radiation induced cell death (Zang et al., 2015).

The combination of treosulfan with TBI prior to bone marrow transplantation in rats showed that treosulfan possesses certain characteristics of a radio-sensitiser in vivo (Sender et al., 2009). Results of low-dose treosulfan added to an immune-suppressive regimen consisting of T cell depleting antibodies, fludarabine, and thymic irradiation indicate that low-dose treosulfan may be considered as a useful component of a truly nonmyeloablative conditioning protocol in providing for sustained mixed

haematopoietic chimerism of donor type and, consequently, in establishing a platform for adoptive immunotherapy as well as donor-derived organ transplantation (Ploemacher et al., 2004b).

Permanent mixed chimerism and donor-specific tolerance was achieved in mice conditioned, prior to donor mouse muscle precursor cell transplantation, with a treosulfan treatment combined with a single cyclophosphamide dose, and finally a donor bone marrow transplantation (Stephan et al., 2006).

Profound stem cell toxic, myeloablative and immunosuppressive effects were demonstrated in various in vitro and in vivo models employing treosulfan alone or in combination with other clinically established conditioning components. The strong anti-leukemic activity of treosulfan potentially reduces post-transplant relapse of underlying malignancies in a clinical setting of allogeneic HSCT.

2.2.3. Pharmacokinetics

An overview of relevant studies conducted to evaluate the absorption, distribution, metabolism, and excretion (ADME) of treosulfan is shown:

Table 2.4-2: Overview of ADME and *in vitro* pharmacokinetic studies

Type of Study	Test System	Admini-stration	Reference
Absorption / Bioavailability			
Plasma levels after single dose	Dog	i.v., p.o.	LEO, 1978a
Absorption in a single rat after a single dose	Rat	i.v.	MDC/01, 2004
Plasma levels after single dose	Mouse	i.p.	Werner et al., 2008
Plasma levels after repeated dose	Juvenile rat	i.v.	LPT 26054, 2011
Plasma levels after repeated dose	Rat	i.v.	LPT 37259, 2020
Plasma levels of treosulfan and its transformation products after single dose	Juvenile and young adult rat	i.v.	LPT 27700, 2014
Plasma levels of treosulfan and its transformation products after single dose	Rabbit	i.v.	Romanski et al., 2016
Distribution			
Plasma protein binding of treosulfan and epoxide metabolites	Rat and human blood samples	in vitro	Glowka et al., 2012 Romanski et al., 2015a
Red blood cell/plasma partition coefficient	Rat	i.p.	Romanski et al., 2018
Tissue distribution	Rat	i.v.	MDC/01, 2004
Transport of treosulfan across blood-brain barrier	In vitro	n.a.	Linz et al., 2015
Blood-brain barrier penetration of treosulfan and its transformation products after single dose	Rat, juvenile and young adult	i.v.	LPT 27700, 2014 Romanski et al., 2015a
Determination of treosulfan and its monoepoxide in plasma, liver, lungs, kidneys, muscle, and brain	Rat	i.p.	Romanski et al., 2017b
Disposition of treosulfan and its monoepoxide in plasma, bone marrow, liver, lungs, brain, and quadriceps femoris	Rat	i.p,.	Romanski et al., 2017c
Metabolism			
Glutathione conjugation of treosulfan	Human liver cytosol	in vitro	Romanski and Glowka, 2019
Glutathione conjugation of DEB	Liver and lung cytosol of mouse and rat, human liver cytosol	in vitro	Boogaard et al., 1996
Role of hydrolysis in detoxification of DEB	Liver and lung microsomes of mouse, rat, and human	in vitro	Boogaard and Bond, 1996

Transformation of treosulfan to DEB (dependence on pH and temperature)	Aqueous solution, human plasma	in vitro	Feit, 1997
Activation of treosulfan at pH 7.4 and 37°C accompanied by hydrolysis of the active epoxides	Aqueous solutions/ buffer	in vitro	Romanski et al., 2015b
Metabolism of treosulfan and its transformation products after single dose	Rabbit	i.v.	Romanski et al., 2016
Kinetic and mechanistic study of the pH-dependent epoxidation of treosulfan including the reaction inhibition in a borate buffer	Aqueous solutions/ buffer	in vitro	Romanski et al., 2017a
Distribution of treosulfan and its monoepoxide in, bone marrow, liver, lungs, brain, and muscle	Rat	i.p.	Romanski et al., 2017c
Excretion			
Excretion into urine	Rat	i.p.	Romanski et al., 2017c
Excretion into urine	Dog	i.v., p.o.	LEO, 1978a
Excretion into faeces, urine and expired air	Rat	i.v.	MDC/01, 2004
Pharmacokinetic Drug Interactions			
CYP inhibitory potential	human liver micro-somes	in vitro	MDC/02, 2003
Microsomal metabolic stability with CYP reaction phenotyping of treosulfan	human liver microsomes	in vitro	CYP1269 R13, 2021
CYP time dependent inhibition (IC50 shift) potential of treosuflan	human liver microsomes	in vitro	CYP1269 R9, 2018a
Inhibitory potential of treosulfan on human transporters	cell test systems and membrane vesicles	in vitro	CYP1269 R9, 2018b
CYP induction potential of treosulfan	human hepatocytes	in vitro	CYP1269 R9, 2018c
CYP3A1, CYP3A2, CYP2C6 and CYP2C11 mRNA expression and activity	Rat liver tissue	ex vivo	2019MED001, 2020

Abbreviations: i.p.: intraperitoneal; i.v.: intravenous; p.o.: oral

Various bioanalytical procedures were validated and applied to detect concentrations of treosulfan and related epoxides in plasma, urine and tissues of different species.

Treosulfan and its alkylating metabolites were rapidly transformed, partly metabolised and excreted if not bound to biological macromolecules. Treosulfan as well as the epoxides distribute well into the bone marrow, reaching 70-80% of plasma concentration. Accordingly, it is observed a preferred exposure of the target tissue facilitating the intended action of conditioning treatment, namely myeloablation as well as immunosuppression of recipients of HSCT. There is no indication for accumulation in blood or tissues after repeated exposure.

In contrast to busulfan, treosulfan has a very low potential to cross the blood-brain-barrier which is in line with the clinical experience that anti-convulsive co-medication is not necessary for treosulfan based conditioning treatment.

In opposition to gender, age does influence the pharmacokinetic parameters of treosulfan and its monoepoxide transformation product as demonstrated in experiments in juvenile rats. Half-life of treosulfan in plasma of juvenile rats was somewhat longer than in young adults and accordingly, a higher exposure can be expected in juvenile animals. This observation does also fit to clinical PK data in paediatric patients and is reflected in a population pharmacokinetic modelling. The low SS-EBDM exposure of liver, lungs and brain, may contribute to comparably low toxicity of treosulfan with regard to vital organ functions of experimental animals.

As treosulfan does not undergo either relevant spontaneous or GST-medicated conjugation with GSH, the interactions with other GSH-conjugated drugs or competition are not expected. However, metabolic phenotyping identified treosulfan as a weak substrate of CYP2D6, and in vitro and ex vivo studies as well as PBBK modelling of clinical data did not exclude a potential low to moderate risk for DDIs between treosulfan and CYP3A4 and CYP2C19 substrates.

2.2.4. Toxicology

An overview of toxicological studies considered for the nonclinical safety assessment of treosulfan is provided.

Table 2.4-3: Toxicology Programme

Study type and duration	Administration	Species	Compound administered	Reference
Single-dose toxicity	p.o., s.c., i.v.	Mouse	treosulfan, busulfan	LEO, 1978b
	p.o., i.p.	Rat	treosulfan	
Repeat-dose toxicity				
5 days	p.o., i.p.	Rat	treosulfan	White, 1962, LEO, 1978b
26 days in juvenile animals *	i.v.	Rat	treosulfan	LPT 26054, 2011
28 days *	i.v.	Rat	trosulfan	LPT 37259, 2020
14 to 17 days	p.o., i.p.	Dog, monkey	treosulfan	LEO, 1978b
4 weeks	i.p.	Mouse, rat	DEB	Doerr et al., 1996
7 months	p.o.	Rat	treosulfan, busulfan	LEO, 1978b
Genotoxicity				
Interaction with DNA	in vitro	isolated DNA or fragments	treosulfan, metabolites	Gherezghiher et al., 2013; Hartley et al., 1999; Michaelson-Richie et al., 2010; Millard et al., 2006; Park et al., 2005
Mutagenicity in bacteria	in vitro	*S. typhi-murium* strains, *E. coli strain*	treosulfan, metabolites	Abu-Shakra et al., 2000, Zeiger and Pagano, 1989, Provivo 38665, 2022
Mutagenicity in mammalian cell systems	in vitro	Chinese hamster ovary cells	treosulfan, DEB	Zhu and Zeiger, 1993
Increase of sister chromatide exchanges *	in vitro	Human lymphocytes	treosulfan	Clare et al., 1982
Clastogenic activity (chromosomal aberrations)	in vitro	Human lymphocytes	treosulfan	Provivo 38666, 2022
Micronucleus test	i.p.	Mouse	treosulfan, DEB	Adler et al., 1997; Shelby et al., 1989

Carcinogenicity				
No studies performed				
Reproductive and developmental toxicity				
Effects on male and female fertility	p.o., i.p.	Mouse, rat	treosulfan, DEB	LEO, 1978b
Study on gonadal and ovarial toxicity	i.p.	Mouse	treosulfan, busulfan	Levi et al., 2018
Subchronic (26 days) toxicity in juvenile animals *	i.v.	Rat	treosulfan	LPT 26054, 2011
Effects on spermatogenesis	i.p.	Mouse	DEB	Spanò et al.,1996
Local tolerance				
Effects of local administration (ear)	intraarterial, perivenous	Rabbit	treosulfan	LEO, 1985
Other Toxicity				
not applicable				
Toxicokinetics				
Subchronic (26 days) toxicity in juvenile animals *	i.v.	Rat	treosulfan	LPT 26054, 2011
Subchrnic toxicity, 28 days *	i.v.	Rat	trosulfan	LPT 37259, 2020

* Study conducted according to GLP

Abbreviations: p.o.: oral; i.p.: intraperitoneal; i.v.: intravenous; s.c.: subcutaneous; DEB: 1,2:3,4-diepoxybutane

Single dose toxicity

Treosulfan demonstrated low acute toxicity when administered intravenously (i.v.), orally (p.o.) or subcutaneously (s.c.) to mice and rats. No lethality was induced in mice following an i.v. injection of up to 3500 mg/kg of treosulfan (LEO, 1978b). A comparison of the p.o. and s.c. LD50 values of treosulfan (> 3500 mg/kg) and busulfan (240 and 200 mg/kg, respectively) substantiates that treosulfan exhibits a considerably lower acute toxicity. Death of rats used in acute toxicity tests occurred within 4 to 6 days after dosing. Details on symptoms or pathological findings were not reported (LEO, 1978b).

Repeat dose toxicity

Repeat-dose toxicity studies for treosulfan with intravenous administration, the intended clinical route, were performed in rats, dogs and monkeys, while limited information on tolerability in mice can be deduced from the primary pharmacology programme.

Dose-dependent haematological changes were observed after repeated i.v., i.p. or p.o. administration in mice, rats, dogs, and monkeys. These effects are generally expected for alkylating agents (White, 1962; LEO, 1978b; LPT 26054, 2011; LPT 37259, 2020).

Within historical toxicity studies, with lethality reporting only, treosulfan was administered i.p. once daily over 5 days to rats of two different strains. In Holtzman rats, the highest non-lethal dose was 1113 mg/kg, in Fischer rats 278 mg/kg. The LD50 were calculated with approximately 1364 and 696 mg/kg, respectively (LEO, 1978b).

Results of a more recent GLP-compliant study on tolerability of subchronic daily intravenous treosulfan administrations in juvenile rats starting on PND 10 are summarised. The 26-day treatment period was

followed by a post-treatment observation / recovery period of 5 weeks. A complete reversibility was noted for all systemic changes observed during the treatment period for female rats. Only a slight delay of the physical development of the high dosed juvenile male rats (100 mg treosulfan/kg b.w./day) was noted (LPT 26054, 2011).

Within a recently conducted GLP compliant 4-week subchronic toxicity study, rats were treated with 10, 50, and 150 mg treosulfan. Haematological changes, related to the pharmacodynamic properties of the cytostatic drug treosulfan were observed starting at the low dose levels of 10 mg/kg b.w./day. At the end of the treatment period, a high number of erythrocytes and/or a high haemoglobin concentration were analysed in the urine of male animals treated.

Animals in high dose groups (150 mg treosulfan/kg b.w./day) presented additional signs of systemic toxicity like decreased body weights and decreased absolute and relative spleen and thymus weights. The weight decrease of the spleen correlated to a lymphoid atrophy. No histopathological correlate was found for the thymus weight decrease. The histopathological examination revealed test item related lymphohistiocytic infiltration in the skeletal muscle starting at 10 mg treosulfan/kg b.w./day, urothelial hypertrophy with atypical cells in the urinary bladder starting at 50 mg/kg b.w./day; increased fatty bone marrow and decreased red bone marrow; lymphoid atrophy of the spleen and decreased incidence of lymphoid hyperplasia of lymph nodes at 150 mg/kg b.w./day.

All changes noted for the clinical chemistry parameters and the organ weights had completely subsided at the end of the 2 week treatment-free recovery period. The body weights of the high dosed animals were still slightly reduced but revealed a tendency towards normalisation.

Changes in haematological parameters were still noted and urinalysis still revealed a high number of erythrocytes and/or a high haemoglobin concentration in the urine of the male animals at the intermediate and high dose levels. Histopathology still revealed a lymphohistiocytic infiltration in the skeletal muscle starting at 10 mg/kg b.w./day and urothelial hypertrophy with atypical cells in the urinary bladder at 50 and 150 mg/kg b.w./day. The changes in the bone marrow, spleen and lymph nodes were reversible at the end of the 2 week treatment-free recovery period. In addition, a necrosis of myofibers in the skeletal muscle was noted at the end of the recovery period.

Considering the afore mentioned findings, the no observed effect level (NOEL) was below 10 mg/kg b.w./day.

In a historical chronic toxicity study in Leo Wistar rats, treosulfan (5 and 50 mg/kg/day) or busulfan (1 mg/kg/day) were administered orally by gavage 6 days per week over 7 months. Treatment resulted in reduced body weight in the high-dose treosulfan group. Body weights and the number of neutrophilic granulocytes in peripheral blood were reduced in the busulfan group.

Findings regarding organ weights, gross pathology or histopathology revealed effects on the male and female reproductive organs (gonads and ovaries) in all treatment groups. No abnormalities were observed for liver, kidney, lung, heart, spleen, adrenals and prostate or uterus compared to controls. Overall, toxicity was less pronounced in treosulfan-treated compared to busulfan-treated rats. A No Observed Adverse Effect Level (NOAEL) was not established for both compounds in this study. Treatment was tolerated without lethality (LEO, 1978b).

Limited information on tolerability in mice can be deduced from the primary pharmacology programme. Tolerability in Beagle dogs was investigated following daily i.v. (56, 111, 222 or 445 mg/kg) or p.o. (56, 111, 222, 445, 890 or 1779 mg/kg) administrations of treosulfan for 5 to 19 consecutive days. The duration of treatment was dependent on survival. All three animals dosed i.v. with 222 or 445 mg/kg/day developed general signs of systemic intolerance, described as CNS-depression, malaise, weight loss, anorexia, and collapse of peripheral circulation, leading to death on Day 11 or Day 12. The

haematopoietic system was affected at all dose levels as evidenced by leukopenia, neutropenia, and bone marrow depression. Reticulocytopenia, thrombocytopenia and lymphopenia were observed at the dose 111 mg/kg/day or higher. Post-mortal findings included decreased cellularity of bone marrow and signs of hyperaemia starting at 56 mg/kg/day. Animals dosed with 222 mg/kg/day or higher showed severe haemorrhagic lesions in several organs and atrophy of the spleen (LEO, 1978b). A NOAEL was not established in the dog studies. The maximum tolerated dose (MTD) based on lethality was 111 mg/kg/day for i.v. (LEO, 1978b).

Rhesus monkeys received treosulfan i.v. (56, 111, 222 or 445 mg/kg) or p.o. (56, 111, 222, 445, 890 or 1779 mg/kg) once daily for 6 to 19 consecutive days. Duration of treatment was dependent on survival. Starting at 222 mg/kg/day of i.v. treatment, the monkeys showed signs of intolerability including a symptom described as CNS-depression, malaise, emesis, severe diarrhoea, anorexia, and collapse of peripheral circulation. These animals died on Day 8 to Day 13. Monkeys in all dose groups developed reticulocytopenia, leukopenia, and bone marrow depression. Weight loss and lymphopenia were observed in all animals starting at 111 mg/kg. Haemoconcentration was seen after 222 and 445 mg/kg/day. At necropsy, decrease in cellularity of bone marrow (all groups), atrophy of spleen and lymph nodes (at 111 mg/kg/day and above), and haemorrhagic lesions, especially in the gastro-intestinal tract, were noted (LEO, 1978b). Monkeys dosed with 111 or 222 mg/kg/day were sacrificed in moribund condition on Days 18 and 13, respectively. Monkeys dosed with 445 mg/kg/day or higher died on Days 6 to 10. Animals in all dose groups developed reticulocytopenia, leukopenia, lymphopenia, and bone marrow depression. At 222 mg/kg/day or higher, haemoconcentration was noted. Higher doses induced atrophy of lymph nodes and haemorrhages in the adrenal cortex, bladder mucosa and lung (LEO, 1978b).

A NOAEL was not established in the monkey studies. The MTD based on lethality was 111 mg/kg/day for the i.v. route (LEO, 1978b).

Genotoxicity

Transformation of treosulfan to the reactive epoxides EBDM and DEB is a prerequisite for the genotoxic properties of treosulfan. Due to the presence of two versus one nucleophilic centres, reactions of DEB with DNA is much more efficient compared to EBDM (Gherezghiher et al., 2013; Hartley et al., 1999; Michaelson-Richie et al., 2010; Millard et al., 2006; Park et al., 2005).

Treosulfan up to the 3160 µg per plate was tested with and without metabolic activation in the Ames reverse mutation test, using four *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and in one *Escherichia coli* strain WP2 uvrA. Treosulfan caused with and without metabolic activation a concentration-related base-pair substitution mutagenic effect in the *S. typhimurium* strains TA100 and TA1535 (Provivo 38665, 2022). These results are supported by literature, where, treosulfan and DEB showed a pH-dependent spectrum of mutagenic responses in the Ames test (*S. typhimurium* strains TA100, TA102, TA1535, TA7004, TA7005; Abu-Shakra et al., 2000; Zeiger and Pagano, 1989). Both compounds were mutagenic at the gpt locus in Chinese hamster ovary cells (Zhu and Zeiger, 1993).

A potential to induce sister chromatid exchanges in human lymphocytes was demonstrated with treosulfan in vitro (Clare et al., 1982).

In an in vitro cytogenetic study using human lymphocyte cultures treosulfan tested up to 50 µg/ml revealed mutagenic properties with respect to chromosomal or chromatid damage in the absence as well as presence of metabolic activation (Provivo 38666, 2022). The results are in accordance with literature data, where induction of micronuclei or chromosomal aberrations in bone marrow cells of mice was shown for treosulfan doses of 250 to 1000 mg/kg administered i.p. Similarily, DEB gave a positive

response in the bone marrow micronucleus test and dominant lethal test (effect on implantation rate) in mice treated i.p. with of 36 mg/kg (Adler et al., 1997; Shelby et al., 1989).

Carcinogenicity

According to the ICH S9 and ICH S1A guidelines unequivocally genotoxic compounds implying a hazard to humans are not to be subjected to long-term carcinogenicity studies (ICH guidelines). Therefore, specific carcinogenicity studies in animals were not performed with treosulfan.

Reproduction toxicity

According to the ICH guideline S9 on nonclinical evaluation for anticancer pharmaceuticals, reproductive and developmental toxicity studies are not considered essential for pharmaceuticals that are genotoxic, target rapidly dividing cells in general toxicity studies (e.g., crypt cells, bone marrow), or in case they belong to a class that has been well characterized as causing developmental toxicity. All of these conditions apply to treosulfan. Therefore, no dedicated reproductive and developmental toxicity studies for treosulfan addressing the fertility, early embryonic or embryo-fetal, pre- and postnatal development including maternal function were conducted.

Nevertheless, subchronic toxicity of treosulfan was investigated in juvenile and young adult rats. The effect of intravenously administered treosulfan on development of juvenile rats was investigated in a more recent GLP-compliant subchronic study with juvenile Sprague-Dawley rats. Ten animals per sex were treated i.v. with daily doses of 10, 50 or 100 mg/kg of treosulfan from PND 10 to 35. Body weights of high-dose males and females were markedly reduced. Haematological changes included reduced numbers of leucocytes and neutrophile granulocytes in all dose groups. Urinalysis, ophthalmological examination, auditory examination, and macroscopic post mortem findings revealed no effects attributable to treosulfan treatment. Relative to body weight, weights of gonads, prostate, spleen, and thymus were reduced in high dosed rats and a slightly delayed time-point of vaginal opening was noted for the high dosed female animals. After a 5-week post-treatment observation period, only a slightly decreased body weight and the correlating reduced organ weights in high dosed males indicated a slight delay of physical development. Ten of 60 satellite animals of a 250 mg/kg/day dose group dedicated for pharmacokinetic investigations died prematurely on PND 19 to 30. The cause of death was not further examined. Most likely, a combined effect of treosulfan toxicity and weakening by frequent blood drawings resulted in a moribund state of the young adult rats (LPT 26054, 2011).

Studies with the transformation product DEB in mice and rats confirmed the potential of treosulfan to affect uterine-ovarian development and implantation as well as sperm development.

In pregnant rats, DEB reduced foetal growth and viability and induced implantation losses and foetal resorption (Chi et al., 2002; Doerr et al., 1996; Marchetti and Wyrobek, 2008). DEB also induced depletion of spermatids, reduction of the secondary spermatocyte layers, and altered chromatin packaging in sperms of mice treated with a single i.p. dose of up to 78 mg/kg and followed up for up to 4 weeks (Spanò et al., 1996).

In another study with i.p. doses of up to 85 mg/kg on Day 1, 3, and 5, DEB induced signs of male reproductive toxicity including abnormal forms of sperms, reduced sperm motility, and accumulation of testicular cells in the G2/M phase. In a mouse premeiotic spermatocyte-derived cell line, DEB induced signs of genotoxic effects after incubation with 100 to 500 µM DEB (Dong et al., 2015).

Considering the substance class as well as the mode of action, treosulfan must be assumed a drug possessing all aspects of reproductive and developmental toxicity.

Toxicokinetic data

Three dedicated GLP compliant toxicokinetic studies in rats were performed using i.v. administration of treosulfan. One study was conducted as part of a 4-week toxicity study in adult rats (LPT 37259, 2020), a second one in juvenile rats treated from PND 10 to 35 (LPT 26054, 2011). The third study was performed as single-dose toxicity study in juvenile and young adult rats (LPT 27700, 2014). For results of toxicokinetic evaluations of treosulfan after single dose and repeat-dose treatment refer to CTD Section 2.4.4 and 2.6.5.3.2 and 2.6.5.4. These data reveal a linear dose relationship with AUC in adult rats. Accordingly, toxicity findings including laboratory and histopathological changes are exposure related.

Local tolerance

Intraarterial or perivenous administration of a clinical-grade formulation of treosulfan to the ears of rabbits were tolerated without signs of local lesions. No remarkable local findings were reported regarding the injection sites in the animal studies with i.v. or i.p. administration (LEO, 1985).

Other toxicity studies

Photosafety

No nonclinical photosafety studies were performed because treosulfan exhibits no significant light absorption at wave lengths in the range of 290 nm to 700 nm.

Antigenicity

Antigenicity has not been described for treosulfan.

Immunogenicity

No formal non-clinical immunogenicity studies have been conducted.

Dependence

Dependence on treosulfan has not been studied, and there is no evidence of treosulfan dependence. But given the indications for treatment with treosulfan as conditioning regimen prior to HSCT, the mode of action and the adverse drug reactions of treosulfan, dependence and abuse can be ruled out.

Studies on Metabolites

Studies with the treosulfan metabolite DEB confirmed the expected cytotoxicity and genotoxicity in vitro and in vivo. Furthermore, effects of DEB on male and female reproductive organs and functions corroborate that the alkylating activity of the treosulfan related epoxides are responsible for both the pharmacodynamic activity and toxicities including mutagenicity, carcinogenicity and reproductive and developmental toxicity.

Maximum Tolerated Doses (MTD) and Systemic Exposure in Animals versus Humans

The maximum tolerated dose (MTD) regarding severe toxicity including lethality after single dose treosulfan administration in mouse and rats was about 10,000 and 15,000 mg/m^2, respectively. Repeated, subchronic intravenous administration to rats was tested up to a maximum dose of 150 mg/kg/day, corresponding to 900 mg/m^2/day, only. In dogs and monkeys 111 mg/kg/day were tolerated when treated i.v. over a period of 10 to 14 and 8 to 19 days. In the Table below 2.4-4, the corresponding

animal doses in terms of mg/m² body surface area (BSA) are compared with the MTD in adult patients of 12.5 mg/m² (single dose without subsequent HSCT; refer to Harstrick et al., 1996). For conversion of dose in mg/kg body weight to dose in mg/m² body surface area standard factors according to FDA guidance were used (FDA, 2005).

Apparently much higher doses are tolerated by humans than by animal species. However, in case the treosulfan MTD of 47 g/m² of human exposure is considered with subsequent HSCT the ratio MTD animal/human is about 0.2.

Table 2.4-4: Comparison of the Maximum Tolerated Dose (MTD) for treosulfan in toxicology studies and in humans

Species and exposure conditions	**MTD** (mg/kg/day)	**MTD** (mg/m²/day) [a]	**MTD animal/ human**
Mouse *Leo strain II* (single dose, i.v., p.o.,s.c)[a]	> 3,500	> 10,500	**0.84**
Mouse *Charles River CDI* (single dose, p.o.) [b]	3,360 [d]	10,080 [d]	**0.81**
Mouse *Charles River CDI* (single dose, i.v.)	> 2,500	> 7,500	**0.60**
Rat *Wistar* (single dose p.o.)[c]	2575 [d]	15,450 [d]	**1.24**
Rat *Wistar* (single dose i.p.)[c]	2860 [d]	17,160 [d]	**1.37**
Rat *Holzman* (5 days, i.p.)	1113 / 1364 [d]	6,678 / 8,184 [d]	**0.53 / 0.65**
Rat *Fischer* (5 days, i.p.)	278 / 696 [d]	1668 / 4,176 [d].	**0.13 / 0.33**
Rat (28 days, i.v.)	150 [e]	900 [e]	**0.07**
Dog (10-14 days, i.v. bolus)	111	2220	**0.18**
Monkey (8-19 days, i.v. bolus)	111	1332	**0.17**
Human (adult, single dose, i.v. 2 h infusion) [f]	-	**12500**	-
Mouse (single dose, i.p., prior to HSCT) [g]	3000	9,000	**0.19**
Mouse (3 days, i.p., prior to HSCT) [g]	2500	7,500	**0.16**
Human (adult, single dose, i.v., prior to HSCT) [h]		**47000**	

[a] Animals were observed 96 hours after treatment; [b] Animals died on days 4 to 9 after dosing; [c] Animals died between 5 to 8 days post dosing; [d] LD_{50}; [e] Highest dose tested LPT 37259, 2020; [f] Harstrick et al., 1996; [g] Ploemacher [report], 2000; [h] Scheulen et al., 2000

The maximum tolerated single dose of i.v. applied treosulfan in humans is 12.5 g/m². When overcoming this pronounced haematotoxicity of higher doses by HSCT, the treosulfan dose can be increased 4- to 5-fold to a MTD of 47 g/m2. Considering the proposed indication of treosulfan "conditioning prior to HSCT" which is characterised by the administration of an otherwise lethal treosulfan dose, the assessment of a usual safety margin might be somewhat misleading.

2.2.5. Ecotoxicity/environmental risk assessment

An ERA report was prepared in accordance with the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use, EMEA/CHMP/SWP/4447/00 corr 2, 2006 and Questions and answers document on the Guideline, EMA/CHMP/SWP/44609/2010 Rev.1, 2016.

A Phase I ERA has been provided for the extension of indication to include additional non-malignant transplant indications (non-malignant diseases in the paediatric population) for Trecondi 1 g/5 g powder for solution for infusion.

1. The Phase I of the environmental risk assessment requires the calculation of the predicted environmental concentration of treosulfan in surface water. For PEC calculation, the MAH referred to

the formulas given in the guidelines EMEA/CHMP/SWP/4447/00 corr 2, 2006 and EMA/CHMP/SWP/44609/2010 Rev.1, 2016.

Fpen and PECsurfacewater refined values, in line with the Guidelines are endorsed.

Since PECsurfacewater of 0.003 µg/L is evidently below the action limit of 0.01 µg/L, the environmental risk assessment can stop in Phase I, in agreement with EMA Guideline EMEA/CHMP/SWP/4447/00 corr 2, 2006.

2. Experimental log Kow values for treosulfan and its epoxides, of -1.58, -1.18 and -0.40, were far below the trigger value of 4.5, for further screening of persistence, bioaccumulation and toxicity (PBT). Log Kow values were experimentally determined using the shake-flask method (OCDE 107), according to EMA/CHMP/SWP/44609/2010 Rev. 1, 2016, and the original study was provided by the MAH (Glówka, Romanski and Siemiatkowska, 2013). Thus, no formal PBT assessment is required.

According to EMA guidelines, if the PECsurfacewater value is below 0.01 µg/L, and no other environmental concerns are apparent, it is assumed that the medicinal product is unlikely to represent a risk for the environment following its prescribed usage. Furthermore, the octanol-water partition coefficient (log Kow) far below 4.5 indicating that the risk of bioaccumulation in aquatic organisms is low. Phase II ERA is deemed not necessary, in agreement with EMA Guideline.

In summary, Trecondi 1 g/5 g powder for solution for infusion is not predicted to present a risk to the environment, following its prescribed usage.

3. The precautionary and safety measures taken to reduce any risk to the environment on the SmPC and PL have been applied and are considerable acceptable.

2.2.6. Discussion on non-clinical aspects

Pharmacology

Treosulfan has known cytotoxic, myeloablative and immunesuppressive effects whichconstitute the mechanism of action of this prodrug supporting the approved and the extended indication, i.e. conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT).

Under physiological conditions, treosulfan is converted to reactive intermediates which alkylate DNA and create inter-strand cross-links besides affecting also other biological molecules and structures involved in various physiological functions. Its myeloablative and immunosuppressive effects were shown in various in vitro and in vivo models with treosulfan alone or combined with other conditioning regimens such as fludarabine or cyclophosphamide, total body (thymus in particular) irradiation (TBI) or anti-T cell antibodies. The clinical development of treosulfan-based conditioning, however, is focused on the combination with fludarabine for additional pre-transplant immunosuppression.

Treosulfan has a high potential to eliminate a broad range of haematopoietic progenitor cell subsets when administered in repeat-dose treatment regimens, which facilitates the induction of high levels of engraftment and donor-type chimerism after allogeneic HSCT. It also exhibits a strong immunosuppressive potential which is required to suppress acute recipient mediated allograft rejection.

From the information provided the conclusion that the safety pharmacology studies on treosulfan provided no evidence of clinically relevant adverse effects is supported.

Pharmacokinetics

Appropriate analytical methods were developed and validated for the detection of treosulfan and its transformation products EBDM and DEB in aqueous solutions, biological fluids and tissues. As treosulfan does not undergo conjugation with GSH, it prevents interpatient variability of drug clearance due to GST activity, GSH depletion, and interactions or competition with GSH conjugated drugs. Nevertheles, metabolic phenotyping identified treosulfan as a substrate of CYP2D6, and in vitro and ex-vivo studies did not exclude a potential DDI between treosulfan and CYP3A4 and CYP2C19 substrates; this cautionary wording is already included in PI and was not changed in the scope of this variation.

Treosulfan and its active metabolites were rapidly metabolised and eliminated, mainly via renal excretion. The kinetics of biotransformation is strongly dependent on the pH which is highly regulated in the in vivo system. No relevant sex differences have been observed and there is no indication for an accumulation in blood or any tissues after repeated exposure. The predominant distribution of treosulfan in primary lymphatic organs probably facilitates its myeloablative potential.

Toxicology

Toxicity studies revealed occasional gastro-intestinal effects including emesis and diarrhoea as well as haematuria and lymphohistiocytic infiltration in the skeletal muscle. However, exposure of different animal species to treosulfan did not reveal dose-limiting functional or structural changes regarding liver, kidneys, the cardiovascular, respiratory, or central nervous system. Some of these observations are also reflected in the list of adverse reactions included in the SmPC for both adult and paediatric population.

Overall, systemic toxicity upon treosulfan administration seems to be similar among the animal species employed in the nonclinical toxicological program and is independent of the route of administration. The observed haematotoxic and immunotoxic effects of treosulfan, at least, should be overcome after subsequent HSCT as well known from clinical experience.

Treosulfan, as other alkylating drugs, is mutagenic and provides a carcinogenic potential in animals and humans. Accordingly, a warning of the risk of secondary malignancies after treatment with treosulfan is already included in the approved SmPC and was not changed in the scope of this variation.

Ecotoxicity/environmental risk assessment

The MAH submitted an ERA Phase I arising from the use of Trecondi 1g/5g powder for solution for infusion, according to on the guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA/CHMP/SWP/4447/00, June 2006) and the Questions and Answers on Guideline on the environmental risk assessment of medicinal products for human use (EMA/CHMP/SWP/44609/2010 Rev. 1, 2016).

Fpen has been refined based on prevalence data, and the PECsurfacewater value of 0.003 µg/L is far below the action limit of 0.01 µg/l. Regarding screening on persistence, bioaccumulation and toxicity, the provided experimental log Kow values for treosulfan and its epoxides, of -1.58, -1.18 and -0.40, are far below the trigger value of 4.5. Thus, there is no need for further assessment of potential persistence, bioaccumulation and toxicity (PBT).

The provided data are acceptable and the risk assessment stopped in Phase I of the procedure. Trecondi 1g/5g powder for solution for infusion, following its prescribed usage, is unlikely to represent a risk for the environment.

The precautionary and safety measures taken to reduce any risk to the environment on the SmPC and PL have been applied.

Assessment of paediatric data on non-clinical aspects

Histopathological examinations on juvenile or adult rats revealed no necrotic or inflammatory lesions in the liver, kidneys, heart, lungs or adrenals. Complete reversibility of haematological effects and delay of post-natal development was demonstrated in the study in juvenile rats with only a slight delay of the physical development.

Minor signs of toxicity were noted in the subchronic toxicity study when juvenile rats were treated 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.

Functional differences of maturing BBB, which results in a higher permeability of BBB in juvenile animals compared to adults, induced a higher ratio of treosulfan concentrations in brain versus plasma in juvenile animals. However, penetration of BBB is overall at a low level in both juvenile as well as adult rats. This finding is consistent with the comparably low frequency of clinical observations on CNS-related toxicities. In juvenile rats, treosulfan plasma half-life was slightly prolonged and the exposure is higher compared to young adults when dosed intravenously on a mg/kg body weight calculation. This information is already included in the approved PI and was not changed in the scope of this variation. Moreover, the clinical relevance of this finding is adequately addressed by the established BSA related dose calculation for treosulfan in children, which based on PK data was updated in PI.

2.2.7. Conclusion on the non-clinical aspects

The nonclinical safety, efficacy and pharmacokinetic data provided for treosulfan is considered adequate to support the application for the extension of the indication to conditioning treatment prior to haematopoietic progenitor cell transplantation in paediatric patients with non-malignant diseases. Relevant information is already reflected in the current SmPC and no changes have been introduced within this variation.

Based on the updated data submitted in this application, the extended indication, to include additional non-malignant transplant indications (non-malignant diseases in the paediatric population), for Trecondi 1 g/5 g powder for solution for infusion does not lead to a significant increase in environmental exposure further to the use of treosulfan. Treosulfan should be used according to the precautions stated in the SmPC in order to minimize any potential risks to the environment.

2.3. Clinical aspects

2.3.1. Introduction

In the scope of this variation application the MAH intends to extent the indication to include additional non-malignant transplant indications (non-malignant diseases in the paediatric population) for Trecondi 1 g/5 g powder for solution for infusion based on final 12-months follow-up results of the study MC-FludT.16/NM; a randomised phase II interventional study aimed to compare Treosulfan-based conditioning therapy with Busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with non-malignant diseases.

Further, the MAH proposes to amend an existing warning on skin toxicity based on new literature data. In a recent publication from Even-Or et al. 2020 [1] regarding skin toxicities (e.g. rash, dermatitis) following TREO-thiotepa-FLU-based conditioning regimen in non-malignant paediatric patients, an increase of skin disorders was observed when patients received sodium bicarbonate-containing hydration in the course of TREO infusion. The MAH postulated that this effect could be due to the acceleration of the pH-dependent formation of alkylating epoxides. The effect may be prevented by keeping the skin clean and dry on days of treosulfan infusion. Therefore, the MAH suggested to include this information in medac's SmPC as follows:

Current text	Proposed text
Dermatitis diaper	~~*Dermatitis diaper*~~ ***Skin disorders***
	An increase of skin disorders (e.g. rash, dermatitis) was observed when patients received sodium bicarbonate-containing hydration in the course of treosulfan infusion, potentially because of acceleration of the pH-dependent formation of alkylating epoxides. Keep skin clean and dry on days of treosulfan infusion.

Moreover, the MAH proposes to introduce a slightly modified dosing regimen according to the patient's body surface based on long-term follow-up data of **paediatric study MC-FludT.17/M**; a Phase II 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, as well as a final analysis of the population pharmacokinetics of Treosulfan in paediatric patients. As a consequence, sections 4.1, 4.2, 4.4, 4.8 and 5.1 of the SmPC are proposed to be updated.

Treosulfan (TREO) is an analogue of busulfan (BU) differing by two additional hydroxyl groups in positions 2 and 3. These two additional hydroxyl groups confer water-soluble properties allowing TREO dissolution in aqueous media in the contrary to BU. Though resembling BU in chemical formula, there are a number of important differences. In contrast to BU, which directly ("primarily") alkylates thiols, TREO has to be converted non-enzymatically, but pH-dependent to alkylating monoepoxide intermediates and L-diepoxybutane, which are considered responsible for the alkylating and cytotoxic effects [Fennelly 1979].

Treosulfan is a bifunctional alkylating agent which has been shown to possess antineoplastic activity in the animal tumour screen and in clinical trials. Since many years the clinical usefulness of TREO in the treatment of ovarian cancer has been proved within several Phase II and Phase III studies.

Currently, TREO is approved in several European countries (DE, DK, IE, NL, UK) for the palliative treatment of epithelial ovarian cancer. It is available as powder for solution for injection or infusion as well as capsules for oral use. The usual intravenous (IV) dose in this indication is 8 g/m2 given every 3-4 weeks if used as a single agent and 5 g/m2 if combined with cisplatin.

Furthermore, TREO has been tested in phase II trials for several other tumour types, including small cell lung cancer, breast cancer, malignant melanoma, and as a stem cell toxic agent within a conditioning regimen before autologous or allogeneic haematopoietic stem cell transplantation.

On 20 June 2019, TREO (trade name Trecondi®) was approved in the European Union for the conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT). In Switzerland, Trecondi® was approved on 10 August 2020. In Canada, TREO (trade name Trecondyv®) was approved on July 28, 2021.

Treosulfan is indicated as part of conditioning treatment prior to alloHSCT in malignant and non-malignant diseases in adults up to the age of 70 years and paediatric patients older than one month in non-malignant diseases. HSCT involves the intravenous infusion of autologous or allogeneic haematopoietic stem cells (HSCs) 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. This procedure is often performed as part of therapy to eliminate a bone marrow infiltrative process such as leukaemia, or to correct congenital immunodeficiency disorders. In addition, HSCT is used to allow patients with cancer to receive higher doses of chemotherapy than bone marrow can usually tolerate; bone marrow function is then salvaged by replacing the marrow with previously harvested HSCs (autoHSCT). Allogeneic HSCT can be a curative option for some malignant conditions, bone marrow failure, hereditary metabolic disorders, and severe congenital immunodeficiencies that would otherwise have been fatal.

GCP

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

- Tabular overview of clinical studies

Table 1: Tabular listings of all clinical studies

CTD Section	Type of study	Study code / Reference	Study title	Report location
5.3.1	**Reports of Biopharmaceutic Studies**			
5.3.1.1	Bioavailability (BA) Study Reports	-	-	
5.3.1.2	Comparative BA and Bioequivalence (BE) Study Reports	-	-	
5.3.1.3	*In vitro-In vivo* Correlation Study Reports	-	-	
5.3.1.4	Reports of Bioanalytical and Analytical Methods for Human Studies	CRS Study No.: 106/09-05.TN	Validation of a chromatographic method for determination of the concentrations of treosulfan in human plasma and in human urine Part I: Assay validation (plus amendment to this report) Determination of the concentrations of treosulfan in human plasma and in human urine of the clinical study MC-FludT.14/L Part II: Routine analysis	5.3.1.4
		Celerion Study No.: CA12939	Validation of an LC-MS/MS Method for the Determination of Treosulfan and its Monoepoxide Metabolite in Diluted Human Plasma (Citrate) [26-Mar-2015]	5.3.1.4
		Celerion Study No.: CA13526	Validation of an LC-MS/MS Method for the Determination of Treosulfan Diepoxide in Diluted Human Plasma (Citrate) [09-Nov-2016]	5.3.1.4
5.3.2.	**Reports of Studies Pertinent to Pharmacokinetics using Human Biomaterials**			
5.3.2.1	Plasma Protein Binding Study Reports	Schwarzner 2017	Determination of the binding properties of treosulfan to human serum albumin by microscale thermophoresis.	5.3.2.1

CTD Section	Type of study	Study code / Reference	Study title	Report location
		Feit et al. 1970	Feit PW, Rastrup-Andersen N, Matagne R. Studies on epoxide formation from (2S,3S)-threitol 1,4-bismethanesulfonate. The preparation and biological activity of (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate. J Med Chem. 1970 Nov; 13(6):1173-5. PMID: 547985	5.4
5.3.2.2	Reports of Hepatic Metabolism and Drug Interaction Studies	BioDynamics study no: MDC/02 (Cole 2003)	Treosulfan: Investigation of the potential to inhibit human CYP450-mediated metabolism *in vitro*	4.2.2.4
5.3.2.3	Reports of Studies Using Other Human Biomaterials	Bath Analytical Report No. 94.23 (Bosanquet 1994)	Efficacy of treosulfan against fresh human tumour cells *in vitro*	4.2.1.2
		Meinhardt 2003	Treosulfan is an effective inducer of cell death in myeloma cell lines and primary myeloma cells from patients	5.4
		Lanvers-Kaminsky 2006	Cytotoxicity of treosulfan and busulfan on pediatric tumor cell lines	5.4
		Ristovska 2009	Apoptosis induction by treosulfan is superior to busulfan in risk-defined human CLL cells	5.4
		Schmidmaier 2004	Treosulfan-induced apoptosis in acute myeloid leukemia cells is accompanied by translocation of protein kinase C delta and enhanced by bryostatin-1	5.4
		Nitsch 2014	Synergistic cytotoxic activity of treosulfan and gemcitabine in pancreatic cancer cell lines	5.4
		Linz 2014	Can in-vitro chemoresponse assays help find new treatment regimens for malignant gliomas?	5.4
		Munkelt 2008	Cytotoxic effects of treosulfan and busulfan against leukemic cells of pediatric patients	5.4
		Kopadze 2007	Treosulfan impedes the migration of immunocompetent cells.	5.4

CTD Section	Type of study	Study code / Reference	Study title	Report location
		Glowka 2012	HPLC method for determination of biologically active epoxy-transformers of treosulfan in human plasma	5.4
5.3.3	**Reports of Human Pharmacokinetic (PK) Studies**			
5.3.3.1	Healthy Subject PK and Initial Tolerability Study Reports	-	-	
5.3.3.2	Patient PK and Initial Tolerability Study Reports	PK-substudy of MC-FludT.14/L Trial I	Pharmacokinetic sub-study for patients randomised into the test arm "treosulfan-based conditioning" of study MC-FludT.14/L	5.3.3.2
		Beelen 2005	Dose-escalated treosulphan in combination with cyclophosphamide as a new preparative regimen for allogeneic haematopoietic stem cell transplantation in patients with an increased risk for regimen-related complications.	5.4
		Hilger 1998	Clinical pharmacokinetics of intravenous treosulfan in patients with advanced solid tumors.	5.4
		Nemecek 2011	Conditioning with treosulfan and fludarabine followed by allogeneic hematopoietic cell transplantation for high-risk hematologic malignancies.	5.4
		Scheulen 2000	Clinical phase I dose escalation and pharmacokinetic study of high-dose chemotherapy with treosulfan and autologous peripheral blood stem cell transplantation in patients with advanced malignancies.	5.4
		Główka 2008	Pharmacokinetics of high-dose i.v. treosulfan in children undergoing treosulfan-based preparative regimen for allogeneic haematopoietic SCT.	5.4
		Główka 2015	Pharmacokinetics of treosulfan and its active monoepoxide in pediatric patients after intravenous infusion of high-dose treosulfan prior to HSCT.	5.4

CTD Section	Type of study	Study code / Reference	Study title	Report location
		Koyyalamudi 2016	Development and Validation of a High Pressure Liquid Chromatography-UV Method for the Determination of Treosulfan and Its Epoxy Metabolites in Human Plasma and Its Application in Pharmacokinetic Studies.	5.4
		Ten Brink 2014	Pharmacokinetics of treosulfan in pediatric patients undergoing hematopoietic stem cell transplantation.	5.4
5.3.3.3	Intrinsic Factor PK Study Reports	Kinesis Reference CD140025	Non-compartmental analysis of treosulfan and monoepoxide in paediatric patients based on studies FludT.16/NM and FludT.17/M (pharmacokinetic analysis). Clinical pharmacokinetic interim report Version 1.0 dated 09 Jun 2017.	5.3.3.3
		Venn Life Sciences Report CD140025	Non-compartmental analysis of Treosulfan and monoepoxide in paediatric patients based on studies MC-FludT.16/NM and MC-FludT.17/M (pharmacokinetic analysis). [21-Apr-2020]	5.3.3.3
5.3.3.4	Extrinsic Factor PK Study Reports	-	-	
5.3.3.5	Population PK Study Reports	Kinesis 2011	Kinesis. Population PK modelling of treosulfan: a covariate analysis. Version 1.0 dated 30-12-2011.	5.3.3.5
		Kinesis 2012	Addendum I to "Population PK modelling of treosulfan: a covariate analysis. Version 1.0 dated 30-12-2011". Version 2.0 dated 14-09-2012.	5.3.3.5
		Kinesis 2017	Kinesis. Population pharmacokinetic modelling of treosulfan in paediatric patients – Interim analysis report. Version 1.0 dated 12-June-2017.	5.3.3.5
		Venn Life Sciences Report CD140025	Population Pharmacokinetic Modelling of Treosulfan in Paediatric Patients – Final Analysis Report. [02-Apr-2020]	5.3.3.5
5.3.4	**Reports of Human Pharmacodynamic (PD) Studies**			

CTD Section	Type of study	Study code / Reference	Study title	Report location
5.3.4.1	Healthy Subject PD and PK/PD Study Reports	-	-	
5.3.4.2	Patient PD and PK/PD Study Reports	MC-FludT.14/L	[PD: For study titles please refer to CTD section 5.3.5.1 and 5.3.5.2.]	5.3.5.1
		MC-FludT.6 /L		5.3.5.2
		MC-FludT.7 /AML		5.3.5.2
		MC-FludT.8 /MDS		5.3.5.2
		MC-FludT.16/NM		5.3.5.2
		MC-FludT.17/M		5.3.5.2
		MC-FludT.6 /L	[PK/PD: For study title please refer to CTD section 5.3.5.2.]	5.3.5.2
5.3.5	**Reports of Efficacy and Safety Studies**			
5.3.5.1	Study Reports of Controlled Clinical Studies Pertinent to the Claimed	MC-FludT.14/L Trial I	Clinical phase III trial to compare TREO-based conditioning therapy with busulfan-based reduced intensity conditioning prior to alloHSCT in patients with AML or MDS considered ineligible to standard conditioning regimens [TREO regimen: 14 g/m²/d; day -6 to -4]	5.3.5.1

CTD Section	Type of study	Study code / Reference	Study title	Report location
	Indication		Additional Engraftment Analyses to "Clinical phase III trial to compare TREO-based conditioning therapy with busulfan-based reduced intensity conditioning prior to alloHSCT in patients with AML or MDS considered ineligible to standard conditioning regimens [TREO regimen: 14 g/m²/d; day -6 to -4]"	
		MC-FludT.14/L Trial II	Clinical phase III trial to compare TREO-based conditioning therapy with busulfan-based reduced intensity conditioning prior to alloHSCT in patients with AML or MDS considered ineligible to standard conditioning regimens [TREO regimen: 10 g/m²/d; day -4 to -2]	5.3.5.1
5.3.5.2	Study Reports of Uncontrolled Clinical Studies	MC-FludT.6 /L	Allogeneic haematopoietic stem cell transplantation in patients with advanced haematological malignancies after treosulfan-based conditioning therapy	5.3.5.2
		MC-FludT.7 /AML	Clinical phase II trial to evaluate the safety and efficacy of treosulfan based conditioning prior to allogeneic haematopoietic stem cell transplantation in patients with acute myeloid leukaemia	5.3.5.2
		MC-FludT.8 /MDS	Clinical phase II trial to evaluate the safety and efficacy of treosulfan based conditioning prior to allogeneic haematopoietic stem cell transplantation in patients with myelodysplastic syndrome (MDS)	5.3.5.2
5.3.5.2	Study Reports of Uncontrolled Clinical Studies	MC-FludT.16/NM	Clinical phase II trial to compare Treosulfan-based conditioning therapy with Busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation (HSCT) in paediatric patients with non-malignant diseases	5.3.5.2
		MC-FludT.16/NM Final CTR	Clinical phase II trial to compare Treosulfan-based conditioning therapy with Busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation (HSCT) in paediatric patients with non-malignant diseases. [CTR V 1.0; 02-Dec-2021]	5.3.5.2
		MC-FludT.17/M	Clinical phase II trial to describe the safety and efficacy of Treosulfan-based conditionning therapy prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with haematological malignancies	5.3.5.2
			Additional Engraftment Analyses to "Clinical phase II trial to describe the safety and efficacy of Treosulfan-based conditionning therapy prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with haematological malignancies"	
		MC-FudT.17/M Final CTR	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. [CTR V 2.0; 24-Mar-2020]	5.3.5.2
5.3.5.3	Reports of Analyses of Data from More Than One Study	Baumgart 2017	Comparison of engraftment data: Paediatric treosulfan phase II conditioning trial MC-FludT.17/M with historical paediatric engraftment data and data from the adult treosulfan conditioning trial MC-FludT.14/L. V1.0 dated 25-Jul-2017.	5.3.5.3
		ISS	Integrated summary of safety on treosulfan [11-Aug-2017]	5.3.5.3
5.3.5.4	Other Clinical Study Reports	Peters 2011	Meta-analysis on treosulfan for conditioning in children and adolescents before haematopoietic stem cell transplantation.	5.3.5.4
		Peters 2017	Retrospective EBMT-PD/IE-WP analysis: Treosulfan or Busulfan based conditioning before allogeneic haematopoietic stem cell transplantation in paediatric patients with non-malignant diseases.	5.3.5.4

Table 2: Listing of clinical studies in adult patients

Type of Study	Study Identifier (code)	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product; Dosage Regimen; Route of Administration	Number of Subjects (FAS)	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase II efficacy	MC-FludT.6/L	5.3.5.2	Feasibility and tolerability of a conditioning therapy based on three different dose levels of treosulfan prior to alloHSCT	Non-randomised, non-controlled, open label	Treosulfan 10/12 or 14 g/m²/d × 3 Days -6, -5, and -4 IV (2 h)	56	Patients with a haematological chemosensitive malignancy indicated for an alloHSCT, but presenting an increased toxicity risk for classical (high-dose busulfan or standard-dose TBI) conditioning therapies	3 days	Complete Final study report
Phase II efficacy safety	MC-FludT.7/AML	5.3.5.2	Evaluation of engraftment	Non-randomised, non-controlled open-label	Treosulfan 14 g/m²/d × 3 Days -6, -5, and -4 IV (2 h)	38	Patients with AML	3 days	Complete Final study report
Phase II efficacy safety	MC-FludT.8/MDS	5.3.5.2	Evaluation of engraftment	Non-randomised, non-controlled, open-label	Treosulfan 14 g/m²/d × 3 Days -6, -5, and -4 IV (2 h)	16	Patients with MDS	3 days	Complete Final study report
Phase III efficacy safety	MC-FludT.14/L Trial I	5.3.5.1	To compare event-free survival within one year after transplantation between Treosulfan-based conditioning and Busulfan-based conditioning.	Randomised active controlled, open label, non-inferiority trial	Treosulfan 14 g/m²/d × 3 Days -6, -5, and -4 IV (2 h)	320 (171 test drug; 159 active control)	Patients with AML or MDS	3 days	Complete Final study report
Phase III efficacy safety	MC-FludT.14/L Trial II	5.3.5.1	To compare event-free survival within one year after transplantation between Treosulfan-based conditioning and Busulfan-based conditioning.	Randomised active controlled, open label, non-inferiority trial	Treosulfan 10 g/m²/d × 3 Days -4, -3, and -2 IV (2 h)	460 (220 test drug; 240 active control)	Patients with AML or MDS	3 days	Complete Final study report

Table 3: Listing of clinical studies in paediatric patients

Type of Study	Study Identifier (code)	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product; Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase II efficacy safety	MC-FludT.16/NM	5.3.5.2	Safety and efficacy of treosulfan (test) compared to the conventional dose i.v. busulfan (control), each administered as part of a standardised fludarabine-containing conditioning regimen. Population PK analysis	Randomised active controlled, open-label study	Treosulfan 10 - 14 g/m²/d × 3 Days -6, -5, and -4 IV (2 hours)	101 (51 test drug; 50 active control)	Children with non-malignant disease indicated for first myeloablative alloHSCT, including inborn errors of metabolism, primary immunodeficiencies, haemoglobinopathies and bone marrow failure syndromes	3 days	Complete Final study report 5.3.5.2
Phase II efficacy safety	MC-FludT.17/M	5.3.5.2	Safety and efficacy of treosulfan administered as part of a standardised fludarabine-containing conditioning. Population PK analysis	Non-controlled study	Treosulfan 10 - 14 g/m²/d × 3 Days -6, -5, and -4 IV (2 hours)	70	Children with haematological malignant diseases (ALL, AML, MDS, JMML), requiring myeloablative conditioning treatment followed by alloHSCT	3 days	Complete Final study report 5.3.5.2

2.3.2. Pharmacokinetics

As a requirement of the EMA/PDCO approved paediatric investigational plan (PIP) for Treosulfan, the MAH had to analyse all available PK data on paediatric uses of Treosulfan and to conduct two clinical transplantation studies in paediatric patient populations.

Study MC-FludT.16/NM was conducted in children of different age groups with non-malignant diseases requiring myeloablative conditioning treatment prior to AlloHSCT to describe the safety and efficacy of Treosulfan-based conditioning therapy compared to Busulfan-based conditioning therapy.

Study MC-FludT.17/M was conducted in children of different age groups with haematological malignancies requiring myeloablative conditioning treatment prior AlloHSCT to describe the safety and efficacy of Treosulfan-based conditioning therapy.

PK data were analyzed in a sufficient sized subset of subjects enrolled in studies **MC-FludT.16/NM and MC-FludT.17/M**. Further details about these studies can be found in the clinical trial protocols.

Both medac-sponsored trials contributed to the initially built population PK model (December 2011) for Treosulfan. This model was based on PK data acquired in 7 different historical studies from mostly adults and only a limited number of children. The model was used to evaluate and predict the pharmacokinetics of Treosulfan in children and is described in Pop PK report. By this model, disposition of Treosulfan was described by two compartments and linear kinetics. A covariate analysis was performed and detected body surface area (BSA) as the only clinically relevant covariate for clearance and volumes of distribution of Treosulfan. The model was used to establish dose recommendations for Treosulfan in children based on BSA. Using the final PK data from both new paediatric studies, the current dose recommendation based on BSA will be validated or updated, if necessary.

Data used

For the final analysis, data of 24 subjects from study MC-FludT.16/NM and of 59 subjects from study MC-FludT.17/M were available for analysis and their stratification is visible in table 2:

Table 2: Number of subjects for PK analyses in studies MC-FludT.16/NM and MC-FludT.17/M stratified by BSA and age groups.

	MC-FludT.16/NM	MC-FludT.17/M
BSA group		
≤0.5 m^2	9	7
>0.5 – 1.0 m^2	13	25
>1.0 m^2	2	27
Age group		
<1 yr	6	5
≥1 – <2 yr	7	4
≥2 – <4 yr	2	11
≥4 – <12 yr	7	17
≥12 – <18 yr	2	22
Total	24	59

Covariate data were provided by the sponsor according to the DTS. In the two novel studies BSA was calculated using the formula from Mosteller. The GFR was estimated according to the Schwartz formula using serum creatinine and patient's body height.

For subjects with missing dosing date or dosing time, the affected records were flagged and dosing time was imputed using scheduled dosing date or time. For subjects with missing sampling date or sampling time, the affected records were flagged and excluded from the analysis. By default, missing covariates were imputed by the last observation carried forward (LOCF) method, except for baseline values for which backward propagation was used, if feasible. If for a covariate no information was available for a subject, the median value of the missing covariate was imputed for continuous covariates, while for discrete covariates the most frequent value was used. Extreme and unexpected individual data points ("outliers") were excluded if they had a considerable impact on the modelling results. Decisions were based on model-free exploratory outlier analysis of individual concentration curves and/or on conditional weighted residuals and combined profile plots per treatment from intermediate modelling results. Reasons for exclusion are described in the report. Excluded data are re-included in the final model to evaluate the impact of exclusion(s) on the parameter estimates and model performance.

METHODS

Non-linear mixed effects modelling was performed using the software NONMEMxvi. Results were analysed and graphically displayed by means of the statistical software package Rxvii (version 3.2.2). Compilation of NONMEM executable files were performed by using the FORTRAN compiler gfortran (version 4.5.0). The same software environment was used for conducting visual predictive checks (VPC) and simulations. For simulation, the random seed in NONMEM were set to an arbitrary 8-digits number. SAS and R for Windows were used for data handling and additional statistics. Throughout model development, the first-order conditional estimate approximation with interaction (FOCE INTERACTION) was used for estimation.

A Pop PK or mixed-effects model was previously developed to describe blood concentrations of Treosulfan, and to perform a covariate analysis. The initially developed Pop PK model describing disposition of Treosulfan comprises two compartments (with dosing in the central compartment) with linear intercompartmental clearance and elimination. A diagram of its structure is displayed in Figure 1. This structure was unchanged for the updated model, unless there were strong indications that the structure should be changed.

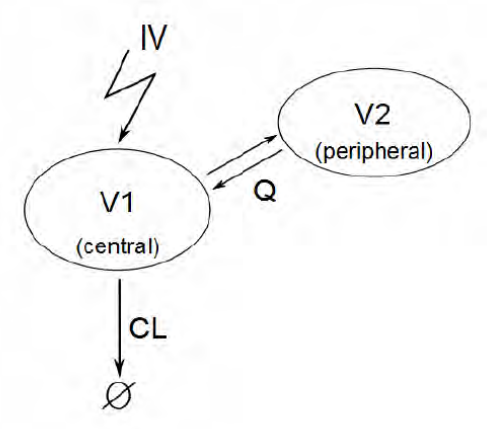


Figure 1: Two compartmental model describing disposition of Treosulfan. After IV administration into the central compartment V1, Treosulfan is distributed into the peripheral compartment V2 and eliminated. Both processes have linear kinetics determined by inter-compartmental clearance Q and clearance CL.

In the initially developed Pop PK model, inter-individual variability on pharmacokinetic parameters was assumed to be log-normally distributed. IIV was investigated for each parameter and was included in the model if it led to an improvement in OFV after a successful minimization and covariance step. Furthermore, additional criteria like condition number, goodness-of-fit plots, and IIV of remaining parameters were considered as well. The residual error, which accounts for unexplained errors (e.g. measurement and experimental errors, or model misspecification to some extent) in the plasma concentrations, was described by the constant CV + constant additive error model.

In the initially developed Pop PK model, BSA was implemented as a covariate on volume of distribution of the central compartment (V1), volume of distribution of the peripheral compartment (V2), and clearance (CL). Unless during model development strong indications were present that this needed to be changed, these covariate relations were kept in the model. To determine the final covariate model, the following covariates were considered for testing: age, body weight, body height, BSA, GFR (CCG), gender, background conditioning regimen, study (site ID), data indicator (historical or new data). The Stepwise Covariate Model (SCM) building tool of PsN was used for covariate selection. Before running the SCM method, correlations between covariates were analysed. For highly correlated covariates, e.g. body weight with BSA, only one was considered for the first forward-backward analysis. The other was reconsidered in a second analysis. During the forward inclusion stage, covariates were added until no significant relations at the 0.05 level remain; during the backward deletion stage, significance was tested at the 0.01 level. The impact of covariates was also judged by the decrease in inter-subject variability, general appearance of goodness-of-fit plots, effect size of the covariate on the particular parameter, and physiologic plausibility.

The model's ability to describe and predict observed data was assessed by a variety of numerical, statistical, and graphical methods. Among them were evaluation of OFV, goodness-of-fit plots, analysis of residuals, VPCs, and scientific plausibility of parameter estimates. As much as possible standard methods available in PsN were used.

Simulations using the two types of models (Dosing Model (the initial model) and Updated Model) were used for model evaluations and dose recommendations. The following rationale was given for dosing, with an objective AUC of 1355 ug×h/mL.

- No relation between AUC and (time to) engraftment was observed in the AUC range of 760-3600 ug×h/mL.
- Dosing according to an objective AUC < 1355 ug×h/mL increases the risk that AUC for some subjects will be lower than 760 yg×h/mL (i.e., outside the range for which time to engraftment versus AUC information is available).

RESULTS

The initial Dosing Model was finalised with data derived from studies MC-FludT.16/NM and MC-FludT.17/M. To compensate for the different bioanalytical methods used for acquiring the historical and newly obtained data an additional model parameter was estimated to allow a proportional shift of the data derived from studies MC-FludT.16/NM and MC-FludT.17/M towards the historical data. Using the same statistical model (IIV on all model parameters), minimization was successful with successful covariance step. Diagnostic plots on pooled data revealed no relevant bias. Hence, it was decided to retain its deterministic and statistical structure, and the model was chosen as base model. Inclusion of a sigmoidal age function scaling clearance in the base model had a modest effect on the model fit. The OFV dropped only by 5 points while the estimated maturation parameters (a TM50 value of 49 weeks (assuming a gestational age of 40 weeks) and a Hill coefficient of 6.6) indicated a rather abrupt effect appearing at a relatively young age. For most subjects the age function approached 1 and for those subjects with lower values the effect on the AUC was below 5%. As a consequence, no maturation function was included at this stage of modelling. The base Pop PK model for Treosulfan on historical data and data from studies MCFludT.16/NM and MC-FludT.17/N is a two compartmental model with zero order infusion, and linear elimination and inter-compartmental clearance including an effect of BSA on CL, V1 and V2.

An exploratory covariate analysis was performed to get a general overview of the covariate data. The evaluated categorical covariates were gender (SEX), study identifier (STID), data indicator (STFLAG, historical or new data), background conditioning regimen (CREG). The continuous covariates were body surface area (BSA), height (HGT), body weight (WGT), age (AGE) and glomerular filtration rate (CCG, creatinine clearance or glomerular filtration rate).

Table 2: Summary of the continuous covariates

Table 6: Summary of continuous covariates for the subjects included in input file nmpk_treosul_v16.csv.

	BSA [m^2]	AGE [yr]	WGT [kg]	HGT [cm]	CCG [ml/min/1.73m^2]
n	198	198	198	198	198
Mean	1.35	27.03	49.12	140.90	137.30
SD	0.60	23.83	29.49	37.60	54.27
Min-Max	0.30-2.38	0.33-77	5.60-114.0	57.0-189.0	47.0-403.7
Median	1.62	16.0	54.6	161.0	126.4
5% perc	0.41	0.69	8.00	69.85	68.91
95% perc	2.11	65.15	97.01	178.3	231.44

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. The following covariate-model parameter relations were tested in a full (forward inclusion and backward deletion) SCM analysis:

- V1: SEX, STFLAG, AGE, CCG; CL: SEX, STFLAG, AGE, CCG ; V2: SEX, STFLAG, AGE, CCG; Q: SEX, STFLAG, AGE, CCG, BSA

Note that CCG contains the normalized GFR values for the new studies and the creatinine clearance or (normalized) GFR values for the historical studies. The SCM analysis was performed with p=0.05 for forward inclusion and p=0.01 for backward deletion. The final backward model described an allometric effect of BSA on Q, CL, V1 and V2 and an effect of CCG on CL. Minimization of the final backward model exited successfully with successful covariance step. However, the effect of CCG on CL could not precisely be estimated. It was estimated with a rather large RSE value of 33%. Removing CCG on CL had a minimal effect on the resulting AUC so it was decided also to remove this model-parameter-covariate relation from the final SCM model.

The final Pop PK model for Treosulfan based on historical data, and data derived from the studies MC-FludT.16/NM and MC-FludT.17/M is a two compartmental model (with dosing in the central compartment) with linear elimination and intercompartmental clearance, see Figure 1. IIV was included on CL, V1, V2, and Q. The model describes an effect of BSA on CL, V1, V2 and Q (PK=TVPK*(BSA/1.75)theta where PK represents CL, V1, V2 or Q). Unexplained residual error is described by a constant CV. To compensate for bioassay differences between historical and new data, a SHIFT model parameter was included describing this difference. Final model parameters and Numerical diagnostic measures are presented are listed in Table 12. Standard Goodness-of-Fit plots are presented in Figure 6. These plots revealed no model bias based on the pooled data. ETA shrinkage (shη) of V1, V2 and Q were high (between 27 and 44%). shη of CL and epsilon (shε) shrinkage were low (less than 10%) as can be seen in Table 22. A representative selection of individual fits and visual predictive checks (VPC) are presented below.

Table 12: Final parameter estimates of the covariate model Final_finalSCMa5.mdl.

Parameter	Value	S.E.	RSE	Low95CI	Upp95CI
V1 [L]	18.9	1.77	9.38	15.4	22.3
CL [L/h]	17.7	0.427	2.41	16.9	18.6
V2 [L]	20.3	1.55	7.61	17.3	23.4
Q [L/h]	26.0	3.97	154.2	18.2	33.8
BSA on V1	1.17	0.116	9.96	0.939	1.39
BSA on CL	1.19	0.0458	3.85	1.10	1.28
BSA on V2	1.82	0.205	11.3	1.42	2.22
BSA on Q	1.57	0.355	22.6	0.874	2.27
SHIFT bioassay	1.36	0.0654	4.81	1.23	1.49
IIV V1	0.146	0.0317	21.7	0.0839	0.208
IIV CL	0.0557	0.00725	13.0	0.0415	0.0699
IIV V2	0.0578	0.0189	32.6	0.0208	0.0948
IIV Q	0.196	0.0613	31.2	0.0761	0.316
Ω(V1,CL)	0.0566	0.0112	19.7	0.0348	0.0785
Ω(CL,V2)	0.019	0.00717	37.8	0.00489	0.033
σ CCV	0.0475	0.00473	9.95	0.0382	0.0567
OFV	17627.255				

IIV=variance of between-subject variability

Ω=covariance between between-subject variability

σ=variance of residual error ε

Table 22: Eta (sh_ε) and epsilon (sh_η) shrinkage in the final model.

Random Effect	Description	Shrinkage (%)
ETA1	IIV on V1	27.150
ETA2	IIV on CL	6.9712
ETA3	IIV on V2	34.152
ETA4	IIV on Q	43.729
EPS1	CCV residual error	9.3396

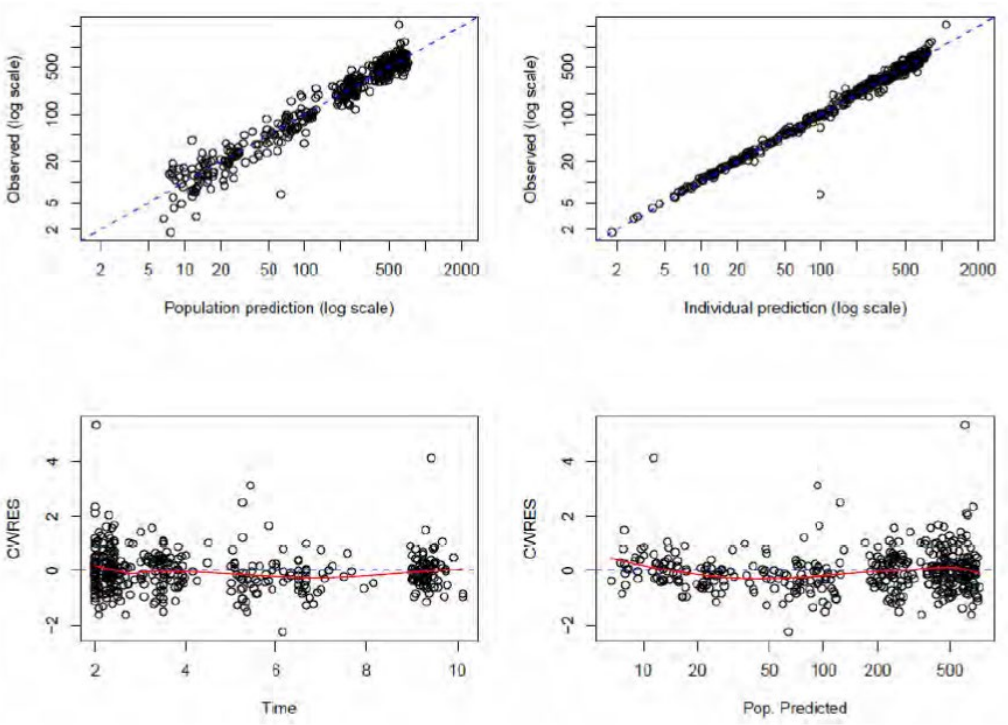
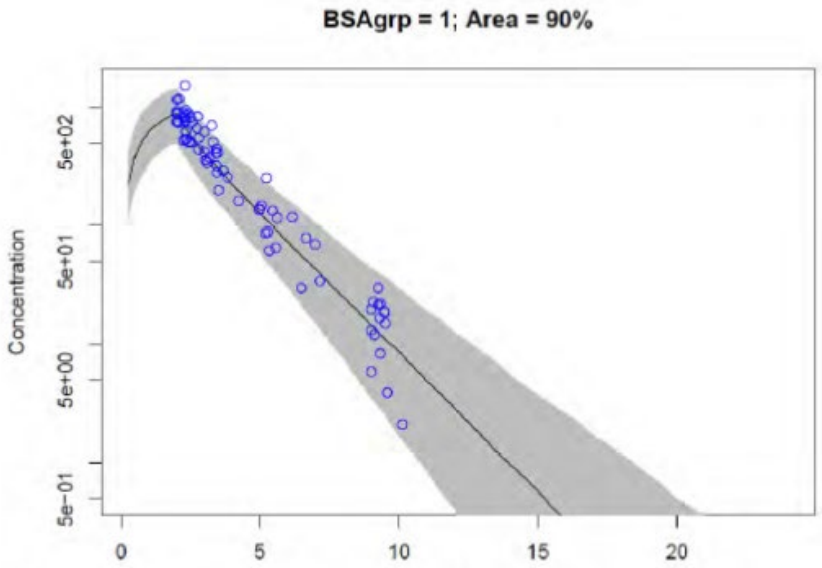


Figure 6: Standard diagnostic plots on new data. Upper row displays observed versus population and individual prediction [µg/mL]. Lower row displays CWRES versus time [h] and population prediction.



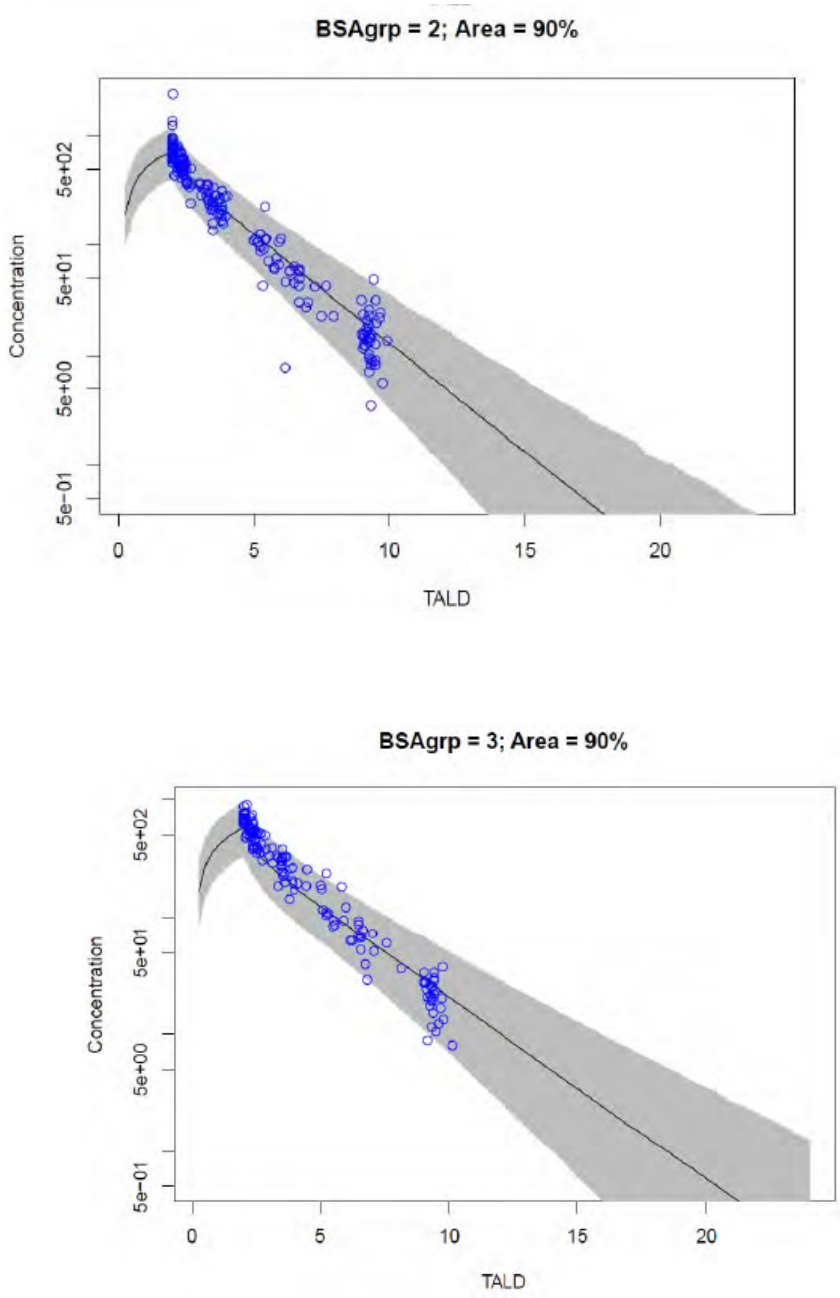


Figure 11: VPCs of the Updated Model on new data displayed by BSA groups. BSA group 1: BSA≤0.5m²; BSA group 2: 0.5<BSA≤1m²; BSA group 3: BSA>1m². Concentration refers to treosulfan concentration in µg/mL and TALD refers to time after last dose in hours.

In general, prediction of the Final Model improved compared to the prediction of the Dosing Model but the prediction of observed concentrations in subjects with BSA larger than 1 m2 (BSAgrp = 3) is still biased. Concentrations between 2 and 3 h after start of infusion are within the prediction range but almost all concentrations are above the median of the simulated concentrations. Concentrations at later timepoints are all centered around the median.

SIMULATIONS

Simulations were made in order to calculate the geometric mean and 95% prediction interval of AUCs (AUC∞ based on analytical formula) by use of the Updated Model. For that 500 concentration-time profiles were simulated and distribution of simulated AUC∞ based on posterior estimates of CL was

calculated. Distributions of predicted AUCinf displayed by BSA groups are shown in Figure 12. For all BSA groups, 0.77% of simulated AUCinf are below 760 and 0.03% above 3600 µg×h/mL. Since this is below the maximum of 2.5%, dose adjustment criterion is passed. For each separate BSA group the dose adjustment criterium is passed as well.

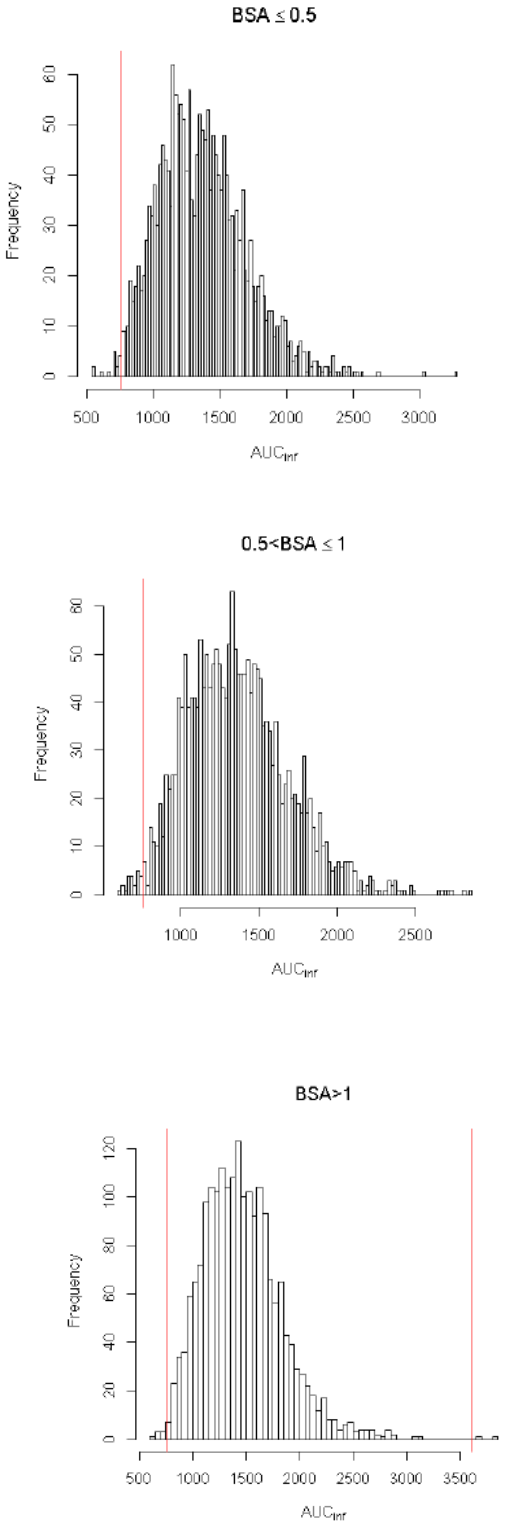


Figure 12: Distributions of AUC_{inf} stratified by BSA groups. The left and right red vertical lines indicate AUC_{inf} of 760 and 3600 µg×h/mL, respectively.

Dosing scheme based on the initial Dosing Model and Final Model are given in Table 14. According to the final dosing scheme children with a BSA of 0.4, 0.5, 0.9 and 1.0 m2 would receive a higher dose from a PK perspective.

Table 14: Initial and final dosing schemes.

BSA	Dosing Scheme by initial protocol MC-FludT16/NM and FludT17/M (2011)		Dosing scheme based on Final Model		Final Dosing Scheme	
(m^2)	(g)	(g/m^2)	(g)	(g/m^2)	(g)	(g/m^2)
0.3	3.0	10	2.94	9.8	3.0	10
0.4	4.0	10	4.14	10.3	4.8	12
0.5	5.0	10	5. 40	10.8	6.0	12
0.6	6.6	12	6.71	11.2	7.2	12
0.7	7.7	12	8.06	11.5	8.4	12
0.8	8.8	12	9.45	11.8	9.6	12
0.9	10.8	12	10.9	12.1	12.6	14
1.0	12.0	12	12.3	12.3	14.0	14
1.1	15.4	14	13.8	12.5	15.4	14
1.2	16.8	14	15.3	12.8	16.8	14
1.3	18.2	14	16.8	12.9	18.2	14
1.4	19.6	14	18.4	13.1	19.6	14
1.5	21.0	14	20.0	13.3	21.0	14
1.6	22.4	14	21.6	13.5	22.4	14
1.7	23.8	14	23.2	13.6	23.8	14
1.8	25.2	14	24.8	13.8	25.2	14
1.9	26.6	14	26.4	13.9	26.6	14
2.0	28.0	14	28.1	14.0	28.0	14

The following observations were made:

- In VPCs using the Dosing Model (the initially developed modeliii), C_{max} was underestimated and median of simulated concentrations were not centered on observed new data in the largest BSA group;
- In VPCs using the Updated Model, observed concentrations were covered by the predicted region but were above the median of simulated concentrations between 2 and 3 hrs in subjects with a BSA > 1.0 m^2;
- 0.03% of simulated AUCinf was higher than 3600 µg×h/mL and 0.77% of simulated AUCinf were below 760 µg×h/mL;
- According to predictions by the Updated Model, from a PK perspective, the updated dosing scheme indicated that children with a BSA of 0.4, 0.5, 0.9 and 1.0 m2 should receive a higher dose than currently indicated by the study protocol to obtain the target exposure of 1355 µg×h/mL. Deviations ranged between 9% for BSA levels 0.6, 0.7 and 0.8 m^2, up to 20% for BSA levels 0.4 and 0.5 m^2 and 17% for BSA levels 0.9 and 1.0 m^2.

Based on these observations at this final analysis, an increase of the current dose levels for children with a BSA of 0.4, 0.5, 0.9 and 1.0 m^2 might be considered from a PK perspective. Paediatric subjects with a BSA < 0.4 m^2 should receive a daily dose of 10 g/m^2 while paediatric subjects with a BSA ≥ 0.4 m^2 and a BSA < 0.9 m^2 should receive a daily dose of 12 g/m^2 while children with a BSA ≥ 0.9 m^2 should receive a daily dose of 14 g/m^2.

2.3.3. Pharmacodynamics

Mechanism of action

TREO is considered a "prodrug" of a bifunctional alkylating cytotoxic drug. The introduction of two hydroxyl groups in position 2 and 3 of the molecule is responsible for the striking differences between TREO and BU with respect to physico-chemical, pharmacological and toxicological characteristics, but also with respect to the mode of activation and mechanism of alkylation [Brookes 196112; Feit 197036; Hartley 199950; Köpf-Maier 1996]. TREO has to be activated by transformation into epoxide species, which are considered responsible for alkylation and cross-linking of macromolecules like DNA. Cytotoxic effects are predominantly, but not exclusively, affecting rapidly proliferating cells and tissues like malignant cells or e.g. normal haematopoietic cells [Feit 1970; Hartley 1999; Munkelt 2008].

TREO presents end-standing methanesulfonyloxy groups in the neighborhood of hydroxyl groups. In consequence, an intramolecular alkylation and formation of epoxide rings occurs. Afterwards, these epoxides are able to alkylate nucleophilic centers. In contrast, BU directly alkylates nucleophilic centers. Under physiological conditions (pH 7.4, temperature 37°C), this intra- molecular reaction occurs spontaneously (non-enzymatically), converting the pharmacologically inactive TREO into an active monoepoxide intermediate and finally to L-diepoxibutane as shown in Figure 1:

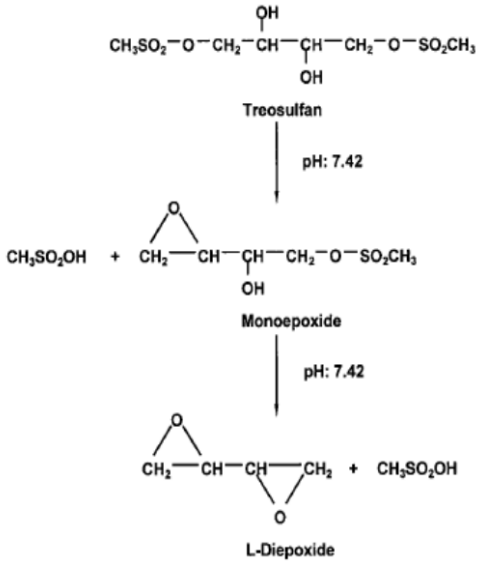


Figure 1: Non-enzymatic activation pathway of treosulfan [Feit 1970]

Given the known mechanism of action of treosulfan, it is pharmacologically plausible and expect that it can also act as preconditioning agent for non-malignant diseases. Therefore, from a mechanistic point of view, the extension of indication can be supported.

Primary and secondary pharmacology

Regarding the relevant pharmacodynamic data to support this variation, namely the extension of indication to non-malignant disease, it can be considered that the MAH has presented data to support it.

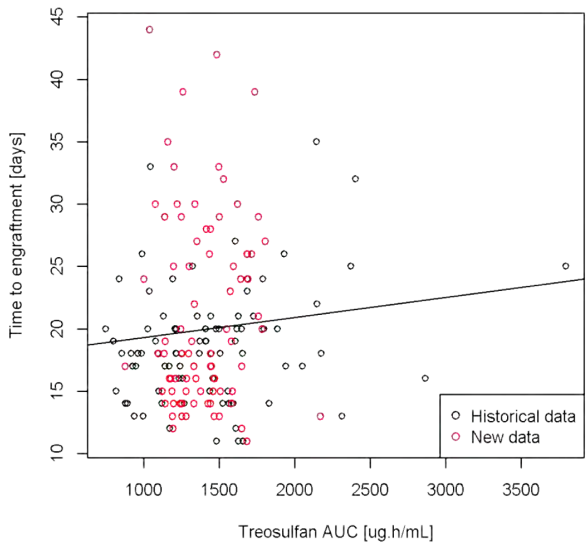
Sufficient toxicity against haematopoietic stem cells (HSCs) is a requirement for a conditioning agent. Preclinical in vitro and in vivo studies have clearly shown that TREO is evidently cytotoxic to all HSC subsets, both primitive and committed. HSC toxicity is most pronounced when TREO is given in fractionated doses instead of a single dose administration [Ploemacher 2004; Sjöö 2006; Van Pel 2003,

Westerhof 2000]. A study with non-malignant HSCs from 5 healthy donors has shown that purified CD34+ HSCs were most sensitive to TREO, followed by CD56+CD3- NK and CD3+ T cells [Munkelt 2008].

When used in doses higher than conventional ones, TREO demonstrates also immunosuppressive action, e.g. inhibition of T cell proliferation, induction of apoptosis of immunocompetent cells (T cells, B cells, NK cells, monocytes) and reduced expression of proinflammatory cytokines [Kopadze 2007; Munkelt 2008; Sjöö 2006]. Immunosuppressive properties of TREO are considered to be of great importance with regard to its application in a conditioning regimen for alloHSCT. Together with the myeloablative activity, they contribute to the achievement of stable transplant engraftment, complete donor-type chimerism as well as prevention of GvHD.

Engraftment of donor stem cells and time to 100% donor cell engraftment are parameters which characterise the pharmacodynamic effects of TREO-based conditioning. Concentration-response relationship was investigated within the PopPK 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. Since engraftment is reached in nearly all patients, time to 100% donor cell engraftment was considered as a better suited parameter to study this concentration-response relationship. However, no correlation between AUC and time to engraftment in the investigated range of AUCs was seen.

Figure 13 Relation between time to engraftment and AUC of TREO



(Data source: Venn Life Sciences 2020, PopPK Report, Figure 13)

2.3.4. PK/PD modelling

All available data on PK of TREO in children of different age groups and adults were used to develop a first PopPK model [Kinesis 2011]. PK data from trial MC-FludT.14/L [PK Study Report, 26/03/2012] and published data in adults [Beelen 2005; Hilger 1998; Scheulen 2000; Nemecek 2011] and paediatric patients [Beier 2012; Główka 2008] were used. This model revealed that conventional dosing simply based on body surface area (BSA) results in a significantly higher exposure (AUC) of smaller children and infants with low BSA compared to adolescents or adults. Various paediatric transplant working groups published their results of PK evaluation of TREO in children and reported similar observations [Beier 2012; Chiesa 2014; Główka 2015; Koyyalamudi 2016; Ten Brink 2014].

The developed model consists of two compartments with first order distribution and elimination processes.

The model estimated relation between clearance and BSA suggests that for dosing the following formula should be used:

$Dose_{model}$ (g) = Target AUC (µg/mL × h)/1000 × 17.8 × (BSA/1.75)$^{1.29}$

No relation between time of engraftment and AUC was observed in the range of 760-3600 µg/mL × h. Therefore, considering the variability between subjects, aiming for a dose to reach a target AUC of about 1300 µg/mL × h (as observed in adults after administration of 14 g/m² TREO) should be sufficient. Higher dosing would not decrease time to engraftment while there is not enough data to imply that lower dosing would not increase time to engraftment. For an AUC of 1300 µg/mL × h, the following dosing scheme was presented:

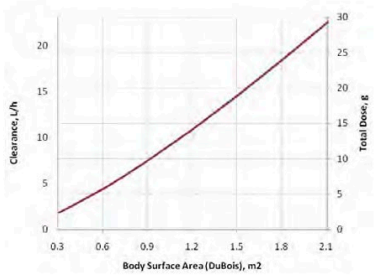


Figure 9 Relation between BSA and recommended dose

The initial modelling report [Kinesis 2011] was amended in 2012 [Kinesis 2012]. The MAH aimed for an exposure level (AUC0-∞) in children comparable to that observed with a dose of 14 g/m² in adult patients. Aimed AUC for a dose of 14 g/m² was calculated by ordinary least squares regression analysis, including now only adult data for all dose groups. This was done on log transformed data, due to log normality of the calculated AUC's. Based on the regression equation, the calculated geometric mean AUC after administration of 14 g/m² was 1355 (90% CI 1308 – 1404) µg/mL × h. This target AUC was chosen for the paediatric studies. Conventional dosing (based on g/m² BSA only) results in significantly higher exposure for smaller children with lower BSA, while exposure in adolescents/adults is more or less equal. In addition, no clear differences in exposure for adults with high BSA (> 2.0 m²) compared to adults with lower BSA were observed; however, the available data for adults with BSA > 2.0 m² was relatively limited. Table 21 shows a comparison of exposure simulations (n = 200) for conventional dosing (14 g/m²) and model-based dosing, aiming for an AUC of 1355 µg/mL × h, indicating the reduced variability in C_{max} and AUC between BSA levels.

Based on this PopPK report, a dose recommendation was derived for the paediatric transplant trials MC-FludT.17/M and MC-FludT.16/NM aiming a target AUC for TREO in all paediatric age groups of 1355 µg/mL × h corresponding to an exposure of 14 g/m² in adult patients [Table 22].

Table 22 Recommended dosing scheme

BSA (m²)	Conventional dosing scheme	Model-based dosing scheme (Target AUC = 1355 µg/mL × h)		Recommended dosing schedule	
	14 g/m²	Final model (g)	Final model (g) excl. 2 outliers	g	g/m²
0.3	4.2	2.5	2.8	3.0	10
0.4	5.6	3.6	3.9	4.0	10
0.5	7.0	4.8	5.2	5.0	10
0.6	8.4	6.1	6.5	6.6	11
0.7	9.8	7.4	7.9	7.7	11
0.8					
0.9					
1.0					
1.1					
1.2					
1.3					
1.4	19.6	18.1	18.4	18.2	13
1.5	21.0	19.8	20.1	19.5	13
1.6	22.4	21.5	21.7	20.8	13
1.7	23.8	23.2	23.4	23.8	14
1.8	25.2	25.0	25.1	25.2	14
1.9	26.6	26.8	26.8	26.6	14
2.0	28.0	28.7	28.6	28.0	14
2.1	29.4	30.5	30.4	29.4	14
2.2	30.8	32.4	32.1	30.8	14
2.3	32.2	34.3	33.9	32.2	14
2.4	33.6	36.3	35.8	33.6	14

Data source: Kinesis 2012 (addendum to population PK modelling), table 4

Table 23 BSA-dependent dosing for children based on 14 g/m² TREO dose in adults

Body surface area (m²)	Treosulfan dose (g/m²)
≤ 0.5	10
> 0.5 – 1.0	12
> 1.0	14

Data source: Final study protocol MC-FludT.16/NM and MC-FludT.17/M

At the end, the following more simplified BSA-adjusted dosing table was used in trials FludT.16/NM (n = 17) and MC-FludT.17/M [Table 23].

Pharmacokinetic data for TREO and its monoepoxide metabolite from two paediatric studies were analysed. For this final analysis, data of 24 subjects from study MC-FludT.16/NM and of 59 subjects from study MC-FludT.17/M were available. Patient's ages were less than 1 year (n = 11), 1 to < 2 years (n = 11), 2 to < 4 years (n = 13), 4 to < 12 years (n = 24), and 12 to < 18 years (n = 24). Patients with a body surface area (BSA) ≤ 0.5 m² had received 10 g/m² TREO, > 0.5 to 1.0 m² had received 12 g/m² TREO, and patients > 1.0 m² BSA had received 14 g/m² TREO. Pharmacokinetic and statistical analysis were done by Venn Life Sciences ED BV [former name Kinesis Pharma BV] (Breda, The Netherlands) using the validated computer program Phoenix™ WinNonlin® (version 6.2.1).

Non-compartmental analysis model 202 (constant infusion input, plasma data) was applied for the PK analysis of TREO, model 200 (extravascular input, plasma data) was applied for monoepoxide. All blood samples taken to determine TREO and monoepoxide plasma concentrations were available for the PK analysis. Treosulfan and monoepoxide concentrations were reported as 'corrected' (corrected for dilution and hematocrit). For the PK analysis of TREO and monoepoxide 'corrected' concentrations were used. In the report, TREO and monoepoxide refer to the 'corrected' values. The lower limit of quantification of

TREO and monoepoxide was 500 ng/mL and 10 ng/mL, respectively. The actual individual infusion duration ranged between 1.97 h and 2.50 h. 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, except for one subject with a lower profile compared to other subjects in the lower age/BSA groups. Decline of TREO concentrations after the end of infusion was bi-exponentially. Inter-subject variability of plasma concentrations by time-point based on percent of geometric coefficient of variation ranged from 10.2% to 96.4% in the different age and BSA groups. Maximum plasma concentration of TREO ranged between 118 and 2,060 µg/mL and almost all were reached directly after end of infusion. AUC0-∞ values ranged between 274 and 3,212 µg/mL × h. The AUC0-∞ of one subject (i.e., 274 µg/mL × h) was below the range what is considered safe and effective (760 – 3,600 µg/mL × h), for all other subjects AUC0-∞ was within this range. No major differences in mean C_{max} were observed. Mean AUClast and AUC0-∞ values of TREO were comparable for the lower three age groups and appeared slightly higher with increasing age group for the two higher age groups. However, the ranges for C_{max} and AUC0-∞ were overlapping and therefore C_{max} and AUC0-∞ can be considered comparable for all age groups. Mean values of Cl and Vd were comparable between age groups 1 (1 month to < 1 year), 2 (1 to < 2 years) and 3 (2 to < 4 years) and thereafter increased with increasing age group. Mean apparent terminal half-life was comparable between the different age groups and ranged between 1.3 and 1.6 h, due to CL and Vd increasing in a similar extent.

Mean C_{max} values were comparable between the different BSA groups. Mean AUClast and AUC0-∞ values of TREO were slightly higher with increasing BSA group. Based on mean values, AUCs were approximately 20% higher in the BSA group ≥ 1.0 m² compared to the BSA group ≤ 0.5 m². However, the ranges for C_{max} and AUC∞ were overlapping and therefore C_{max} and AUC∞ can be considered comparable for all BSA groups. Mean values of Cl and Vd were increased with increasing BSA group. Mean apparent terminal half-life was comparable between the different BSA groups and ranged between 1.3 and 1.6 h, due to CL and Vd increasing in a similar extent. With respect to the shape of the mean plasma concentration-time profiles of the monoepoxide, no major differences were observed between the age groups or BSA groups, except for the lowest age and BSA group where a large variability in the peak concentrations was observed. Maximum monoepoxide 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, except for two subjects in the lower age/BSA groups and one subject in the 2 to <4 years and >0.5 to ≤ 1.0 m² group. No major differences in Cmax, AUClast and AUC0-∞ of monoepoxide were observed between the different age and BSA groups. Mean apparent terminal half-life is comparable between the different age groups and ranged between 1.3 and 1.7 h.

The PopPK model was updated in 2018 with the inclusion of new PK data from studies MCFludT.16/NM (n = 21) and MC-FludT.17/M (n = 59) [Kinesis 2018]. Since no relation between AUC and time to engraftment was previously observed in the AUC range of 760-3600 µg/mL × h, an AUC value in this exposure interval was considered as safe and effective. In a first step, prediction of the dosing model was evaluated by means of dose normalized visual predictive checks (VPCs) and distributions of AUClast. The VPCs predicted historical concentrations adequately for all three BSA groups. The updated PopPK model is a two-compartment model with linear elimination and intercompartmental clearance. After IV administration into the central compartment (V1), TREO is distributed into the peripheral compartment (V2) and eliminated. Both processes have linear kinetics determined by intercompartmental clearance (Q) and clearance (CL). The model described an effect of BSA on CL, V1, V2 and Q.

The following observations were made: – In VPCs using the initially developed dosing model, C_{max} was underestimated and median of simulated concentrations were not centered on observed new data in the largest BSA group. – In VPCs using the updated model, observed concentrations were covered by the

predicted region but were above the median of simulated concentrations in the largest BSA group. – 0.03% of simulated AUC0-∞ was higher than 3600 µg/mL × h and less or equal than 1.13% of simulated AUC0-∞ were below 760 µg/mL × h. – According to predictions by the updated model, from a PK perspective, the updated dosing scheme indicated that children with a BSA of 0.4, 0.5, 0.9 and 1.0 m² would receive a higher dose than currently indicated by the study protocol to obtain the target exposure of 1355 µg/mL × h. Deviations ranged between 9% for BSA levels 0.6, 0.7 and 0.8 m², up to 20% for BSA levels 0.4 and 0.5 m² and 17% for BSA levels 0.9 and 1.0 m². This new BSA-adapted dosing regimen was implemented in the ongoing trial MC-FludT.16/NM (protocol version 5.1, dated 15-May-2019). Since then up to the end of recruitment the following schedule was used.

The final PopPK model was reported to medac in 2020 [Venn Life Sciences 2020].

Distributions of predicted AUC0-∞ displayed by BSA groups are shown in Figure 12. For all BSA groups, 0.77% of simulated AUC0-∞ are below 760 and 0.03% above 3,600 µg/mL × h.

Figure 12 Distributions of $AUC_{0-\infty}$ stratified by BSA groups

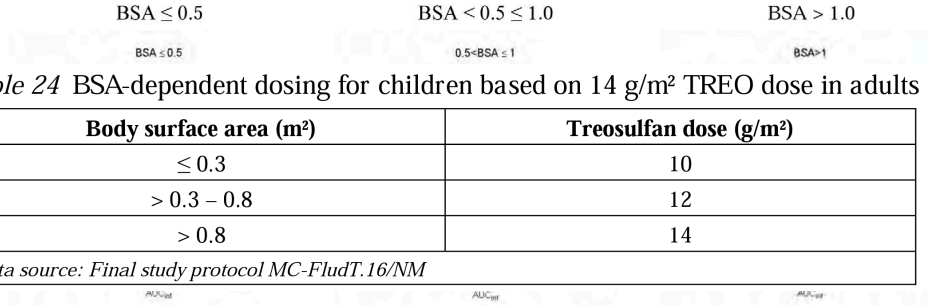


Table 24 BSA-dependent dosing for children based on 14 g/m² TREO dose in adults

Body surface area (m²)	Treosulfan dose (g/m²)
≤ 0.3	10
> 0.3 – 0.8	12
> 0.8	14
Data source: Final study protocol MC-FludT.16/NM	

The left and right red vertical lines indicate AUCinf of 760 and 3600 µg× h/mL, respectively.
(Data source: Venn Life Sciences 2020, PopPK Report, Figure 12)

Based on these observations at this final analysis, an increase of the current dose levels for children with a BSA of 0.4, 0.5, 0.9 and 1.0 m² might be considered from a PK perspective [Table 24].

2.3.5. Discussion on clinical pharmacology

Pharmacokinetics

An initial PopPK model was develop by the MAH in order to predict the PK of Treosulfan in children. This model was based on published data, and studies MC-FludT.16/NM and MC-FludT.17/M were undertaken after a PIP, in order to validate the initial PopPK model but only included a partial subset of subjects for whom data was available. This interim PopPK model was evaluated at the initial submission and the company is now submitting the updated one with the full data available. In practice, no major changes are expected as the number of included subjects is only slightly increased from the 2017 PopPK model with the inclusion of new PK data from study MCFludT.16/NM (n = 24 an increase in 7 subjects). In general, the procedure for data management, covariates considered, and data removal is the same as previously undertaken by the MAH. Out of the 2505 data records, 139 data records (5,5%) were flagged and excluded. Of these 139 data records, 111 occurred at exactly the same time as a dosing event, 8 showed a positive concentration before the first dosing was applied and 20 (0.8%) records showed suspicious behaviour as observed in exploratory data analysis prior to modelling. These are acceptable.

The nonlinear mixed effects modelling approach as implemented in NONMEM version 7.1.0 was used for model estimation, with first-order conditional estimation method with interaction (FOCEI). The previous developed model was used as starting model and a final covariate model was then developed by forward-backward analysis. The descriptive and predictive performance of the developed population PK model

was investigated using commonly used methods. Overall, the modelling strategy is considered acceptable.

The starting model, updated with the new available data, consisted in a 2-compartmental mammillary model with first order distribution and elimination processes with BSA as a covariate associated to all the model parameters. This model was considered sufficient to explain the data, as the introduction of a maturation function did not result in a significant improvement of the model fitting characteristics.

The introduction of covariates by forward-backward selection resulted in the inclusion of the CCG (creatinine clearance or glomerular filtration rate) in the CL parameter. The MAH considered that this inclusion was not supported due to a low precision of the estimated parameter, with an RSM = 33%.

Based on the provided model, CL of Treosulfan was found to be 17.7 L/h at the recommended 14 g/m^2 dose and the volume of distribution (sum of V1 and V2) was 39.2 L for a subject with a BSA of 1.75 m^2. These values are basically similar to the ones previously determined with a reduced data.

Goodness-of-Fit plots revealed no model bias based on the pooled data. ETA shrinkage (shη) of V1, V2 and Q were high (between 27 and 44%). This is not much problematic due to the covariate selection process that was independent of the EBE of the model.

Based on the VPC, prediction of the Final Model improved compared to the prediction of the initial model but the prediction of observed concentrations in subjects with BSA larger than 1 m^2 (BSAgrp = 3) is still biased. This should be further explored and the reason for the under prediction of the initial concentrations for the BSAgrp 3 properly discussed. Also, the VPC of the two studies and by BSAgrp should be provided showing also the median, 5th & 95th confidence intervals of the observed and simulated data for better assessment of the model according to Bergstrand et al 2011 (Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models). VPCs stratified on age groups are considered relevant given the submission and should, therefore, be presented. Finally, the estimated exponents of the BSA covariate (1.17 for V1, 1.19 for CL, 1.82 for V2 and 1.57 for Q) indicate that the central compartment scales almost proportionally with BSA, however for the peripheral compartment, the effects are stronger and scale more than proportionally with BSA. The MAH evaluated whether bodyweight-based scaling (with conventional exponents of 0.75 for CL and Q, and 1.0 for V1 and V2) as compared to BSA-based scaling is not a better alternative to describe the pharmacokinetics of treosulfan.

Simulations under the final model and for the current drug administration regime for the 3 BSA groups showed that 0.77% of simulated AUCinf are below 760 and 0.03% above 3600 µg×h/mL. This indicates that the current regime is resulting in AUC inside the target interval of 760 – 3600 ugxh/mL and since the defined dosing criteria were considered fulfilled, the original dosing schedule was to be kept. The MAH was asked to justify why a new dosing schedule is still proposed. Regarding the new proposed dosing schedule, the MAH provided simulations on the resulting AUCs for the newly proposed BSAgrp dosing with particular relevance for the newly proposed children with a BSA of 0.4, 0.5, 0.9 and 1.0 m^2 that would receive a higher dose from a PK perspective. The detailed description of the maturation function explored during the PopPK model development and discussion of its non-inclusion in the final model was requested to the MAH.

After receiving responses to the issues raised above two other concerns remained to be solved. These were related with: a) the newly proposed regime and the potential risk of increased safety issues; and b) the fact that subsequent to observations of some cases of secondary graft failure in the Treosulfan arm during the MC-FludT.16/NM study some patients received higher doses than the ones in the new proposed dosage regimen. With the responses to the second RSI, the MAH provided sufficient information that indicate that the proposed final BSA-categories for dose calculation seem adequate. In addition, the MAH included in section 5.1 of the SmPC, as requested, information related with the two subjects that

in MCFludT.16/NM trial were effectively dosed with higher doses that the original posology proposed. Overall, all the issues raised in the assessment were resolved.

Pharmacodynamics

Given the known mechanism of action of treosulfan, it is pharmacologically plausible and expected that it can also act as preconditioning agent for non-malignant diseases. Therefore, from a mechanistic point of view, the extension of indication can be supported.

Given the absence of a clear relationship of the AUC of treosulfan with the primary endpoint chosen, no direct relation can be performed regarding a PK/PD relationship. Further support to this indication should be performed with efficacy rather than PD data.

The proposed TREO dose regimen for adult patients is 10 g/m²/d, given on three consecutive days from Day -4 to -2 before HSCT. All 268 patients treated with this regimen within the pivotal study MC-FludT.14/L Trial II achieved engraftment of donor HSCTs. Only one patient in the TREO group experienced a primary graft failure. Complete donor-type chimerism at Day + 28 was achieved in a similar proportion of patients compared to the higher dose regimen used in MC-FludT.14/L Trial I. The duration of neutropenia was reduced with the modified regimen compared to the formerly used TREO regimen of 14 g/m²/d × 3 (Day -6 to -4) used in study MC-FludT.14/L Trial I (14.0 vs. 17.5 days). As a result of this regimen change, the incidence of infections (27.0% vs. 43.5%; TREO-related: 8.1% vs. 11.9%) and infection-related death (9.3% vs. 16.1%) could be significantly reduced. Additionally, event-free (EFS) and overall survival (OS) at 24 months increased significantly (EFS: 65.7% vs. 51.2%; OS: 72.7% vs. 60.2%).

Paediatric patients are usually treated with MAC instead of RIC regimens to avoid graft failure or disease relapse [Algeri 20211; Ali 20202, Lum 2019]. This is justified because children usually do not have comorbidities and tolerate higher doses of chemotherapy. Therefore, the paediatric dose regimen includes a TREO dose that corresponds to a 14 g/m² exposure in adult patients and most patients additionally received thiotepa. Results of the two paediatric studies support this strategy as OS is much better and non-relapse mortality (NRM) lower compared to the two studies 14/L in adults. Based on the resulting PopPK model the MAH recommended a BSA-dependent dose calculation for children. However, based on the data obtained from this PopPK model the proposed slightly modified dosing regimen needs to be further justified as outlined above.

After receiving responses to the request for supplementary information raised above two other concerns remained to be solved. These were related with the newly proposed regime and the potential risk of increased safety issues. In addition, the fact that subsequent to observations of some cases of secondary graft failure in the Treosulfan arm during the MC-FludT.16/NM study some patients with BSA from >0.3 to <0.4 m^2 and from > 0.8 to < 0.9 m^2 received higher doses than the ones in the new proposed dosage regimen, was considered that should be reflected in section 5.1 of the SmPC (with indication of the number of patients involved).

With the responses to the second request for supplementary information the MAH provided sufficient arguments to support that an increased safety risk by application of the proposed final BSA categories for dose calculation of treosulfan is not expected despite the absence of historical values of treosulfan with AUCinf>3600 mg.h/L observed in the paediatric trials MC-FludT.16NM and MC-FludT.17/M, that prevents the establishment of a correlation between AUCinf and the number of related adverse events. This conclusion is instead supported by historical values of Treosulfan AUC as high as 6302 mg.h/L in adult patients which were considered safe. In this line of reasoning, the higher simulated values for the critical BSA levels are well below this value. Taking this in consideration, as well as the increased risk of secondary graft failures after treosulfan-based conditioning, the proposed final BSA-categories for

dose calculation seem adequate. In addition, the MAH also clarified that after the protocol amendment, two subjects were effectively dosed with higher doses that the original posology proposed. These presented a BSA value of 0.5 m^2 and 0.9 m^2 having received 12 g/m^2/day and 14 g/m^2/day. These values would be the same under the final proposed regime. Both patients did not experience graft failure and survived while censored at day 366. This information will be included in section 5.1 of the SmPC.

2.3.6. Conclusions on clinical pharmacology

The clinical pharmacology documentation is in overall sufficient to support the proposed changes. The MAH provided additional information that indicate that the proposed final BSA-categories for dose calculation seem adequate. In addition, the MAH included in section 5.1 of the SmPC, as requested, information related with the two subjects that in MCFludT.16/NM trial were effectively dosed with higher doses that the original posology proposed.

2.4. Clinical efficacy

Seven studies (5 in adult patients and 2 in paediatric patients) were performed by the MAH which provide important information on the efficacy of TREO as part of a conditioning regimen before alloHSCT:

- One dose-response study (MC-FludT.6/L)
- Two non-randomised phase II studies in two specific haematological malignancies (MC-FludT.7/AML; MC-FludT.8/MDS), and
- Two randomised, active-controlled trials (MC-FludT.14/L Trial I and Trial II), all using a conditioning regimen consisting of treosulfan (TREO) plus fludarabine (FLU) in adult patients have been performed by the MAH.
- One completed non-controlled trial **(MC-FludT.17/M)** with TREO-based conditioning in paediatric patients with haematological malignancies (incl. ALL, AML, MDS and JMML).
- One completed randomised active-controlled trial in paediatric patients with non-malignant diseases **(MC-FludT.16/NM)** has also been.

A deferral for these two last studies had been granted by the Paediatric Committee of the EMA (see Paediatric Investigation Plan [PIP]). According to the PIP, one meta-analysis of engraftment data [Baumgart 2017] and an EBMT registry study [Peters 2011] on the use of TREO-based conditioning in paediatric patients with malignant and non-malignant diseases were performed and integrated into the data package.

Additionally, another EBMT registry study [Peters 2017] on the use of TREO- or BU-based conditioning in paediatric patients with non-malignant diseases was performed and is part of the documentation.

2.4.1. Dose response studies

MC-FludT.6/L

This international, non-controlled, multicentre dose-response phase II study investigated a conditioning regimen of TREO in combination with FLU. Patients with a haematological chemosensitive malignancy indicated for alloHSCT (CML, NHL, CLL, HL, MM, AML, ALL, MDS), but presenting an increased toxicity

risk for classical (high-dose busulfan [BU] or standard-dose total body irradiation [TBI]) conditioning therapies were recruited into this trial. Age > 50 years (51%), previous high-dose chemotherapy and autologous HSCT (38%), previous infectious complications (29%) and previous intensive chemotherapy (20%) were the most frequent risk categories, homogeneously distributed between the different dose groups. 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). This study was initiated at seven study centres in Germany, Finland, Poland and Sweden.

2.4.2. Main studies

MC-FludT.16/NM

Title of Study

Clinical Phase II trial to compare treosulfan-based conditioning therapy with busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation (HSCT) in paediatric patients with non-malignant diseases.

Methods

Objectives

To describe the safety and efficacy of TREO compared to the conventional dose BU (control), each administered as part of a standardised FLU-containing conditioning regimen and to contribute to a pharmacokinetic model.

Primary Objective

The primary objective of this trial was the comparative evaluation of freedom from transplantation (treatment)-related mortality, defined as death from any transplantation (treatment)-related cause from start of conditioning treatment (visit Day - 7) until day +100 after HSCT.

This multicentre study was conducted in Germany, Poland, the Czech Republic and Italy.

Secondary Objectives

1. Comparative evaluation of engraftment after HSCT, defined as the first of 3 consecutive days for each of the following 4 criteria:
    - A leucocyte count of > 1 x 10^9/L
    - An absolute neutrophil count of > 0.5 x 10^9/L
    - A platelet (PLT) count of at least 20 x 10^9/L
    - A PLT count of at least 50 x 10^9/L
2. Comparative evaluation of safety including early toxicity (defined as toxicities occurring until day +28) based on the Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) until day +100 after HSCT, serious adverse reactions (SARs) until the end of the longer-term follow-up phase.

3. Comparative evaluation of "Hepatic sinusoidal obstruction syndrome" ("HSOS", according to Jones et al [1], "Lung toxicity" (CTCAE term "Pulmonary fibrosis"), "Hepatic toxicity" (according to Bearman [2]), and "Infections of any CTCAE grade" (non-serious and serious) until day +100.

4. Comparative evaluation of donor-type chimerism on day +28, day +100, and 12 months after HSCT.

5. Comparative evaluation of overall survival (OS) until 12 months after HSCT.

6. Comparative evaluation of primary and secondary graft failure until 12 months after HSCT.

7. Comparative evaluation of incidence and severity of acute (a) graft-versus-host disease (GvHD) (until day +100) and chronic GvHD (cGvHD) (until 12 months after HSCT).

8. Comparative evaluation of use of rescue therapies including donor-lymphocyte infusions (DLIs), stem cell infusions with or without further conditioning regimens, re-occurrence of transfusion dependence (ie, necessity of regular transfusions of red blood cells or PLTs.

9. Evaluation of pharmacokinetic (PK) parameters of treosulfan and its epoxides and to develop a PK model for assessing relevant covariates.

10. Comparative evaluation of secondary graft failure, cGvHD, donor-type chimerism, OS and transplantation-related mortality (TRM) during the longer-term follow-up phase.

Methodology

Prospective, randomised (1:1), open-label, multicentre, active-controlled, parallel-group Phase 2 clinical trial to describe the safety and efficacy of intravenous (i.v.) treosulfan compared to the conventional (myeloablative) dose of i.v. busulfan, each administered as part of a standardised fludarabine-containing conditioning regimen, and to contribute to a PK model.

Subjects were randomised to receive conditioning treatment with treosulfan on day -6, -5, and -4 (body surface area [BSA] adapted dosing), or busulfan on day -7, -6, -5, and -4 (actual body weight adapted dosing) followed by allogeneic HSCT on day 0. Randomisation was stratified by 2 pre-specified background conditioning regimens:

• Stratum A: conditioning therapy with additional thiotepa.

• Stratum B: conditioning therapy without additional thiotepa.

The trial duration per subject consisted of 4 phases:

• Treatment phase: 7 days with either 3 days of treosulfan administration or 4 days of busulfan administration (completed)

• Observation phase: until day +100 after HSCT (according to the PIP, this is defined until at least visit Day +100 [inclusive] of HSCT procedure) (completed)

• Follow-up phase: until 12 months after HSCT (completed)

• Longer-term follow-up phase (after completion of PIP): a minimum of 3 years of HSCT (ongoing).

The current clinical trial report (CTR) focusses mainly on the complete and final 12-month data, but also contains longer-term follow-up data that were available by data cut-off on 07-Jun-2021. Longer-term follow-up data will be collected until the last recruited subject has completed visit Month 36 and presented in an updated version of the CTR expected in 2023.

Test product, Dose, Mode of Administration:

Intravenous treosulfan 1 g or 5 g: 10 or 12 or 14 g/m²/day (BSA-adapted) on day -6 to day -4 before HSCT

Busulfan 60 mg: 3.2 to 4.8 mg/kg/day (actual body weight adapted) on day -7 to day -4 before HSCT

Duration of Treatment:

Subjects within the test arm received treosulfan on 3 consecutive days (day -6 to day -4), while subjects in the reference arm received busulfan on 4 consecutive days (day -7 to day -4).

Subjects within both treatment arms received i.v. fludarabine (30 mg/m²/d) on 5 consecutive days (day -7 to -3) as mandatory non-investigational product. On Investigator's discretion, subjects could receive i.v. thiotepa in 2 single doses of 5 mg/kg given on day -2.

Allogeneic HSCT was performed on day 0.

Criteria for Evaluation:

Efficacy:

Freedom from transplantation (treatment)-related mortality: Freedom from transplantation (treatment)-related mortality was the primary endpoint of the trial and defined as death from any transplantation (treatment)-related cause from start of conditioning treatment (ie, visit Day -7) until day +100 after HSCT. The associated time span of TRM (see endpoint TRM) was defined as the interval from end of HSCT to death due to transplantation-related cause whereas the time span of treatment related mortality was defined as interval from start of conditioning treatment, ie, visit Day -7, until end of HSCT.

Transplantation-related mortality: TRM was defined as the probability of dying from GvHD, interstitial pneumonitis, pulmonary toxicity, infection (bacterial, viral, fungal, parasitic, unknown), Epstein Barr Virus (EBV) proliferative disease, rejection / poor graft function, HSOS, haemorrhage, cardiac toxicity, central nervous system toxicity, gastrointestinal toxicity, skin toxicity, renal failure, multiple organ failure, other HSCT-related cause. The associated time span was defined as the interval from end of HSCT to death due to transplantation-related cause. TRM was evaluated from the end of HSCT until the end of the longer-term follow-up phase.

Overall survival: Overall survival (OS) was defined as the probability of surviving. Survival time was defined as the time length between end of HSCT and the day of death due to any cause. OS was evaluated from the end of HSCT until the end of the longer-term follow-up phase.

Graft failure: The incidence of graft failure was defined as the probability of having a graft failure (primary or secondary) and being alive without using "stem cell infusion (re-transplant) with conditioning" rescue therapy (ie, second allogenic transplantations) between the end of HSCT and the end of the longer-term follow-up phase. In addition, the rate of primary and secondary graft failures was assessed.

Engraftment: Neutrophilic granulocytes engraftment was defined as the first of 3 consecutive days with an granulocyte count > 0.5 x 10^9/L in PB, leucocyte engraftment was defined as the first of 3 consecutive days with a total leucocyte count > 1 x 10^9/L in PB, PLT engraftment was defined as the first of 3 consecutive days with a) PLTs > 20 x 10^9/L or b) PLTs > 50 x 10^9/L in PB in the absence of PLT transfusion. Time to engraftment was defined as the time span between end of HSCT and neutrophil granulocyte / leucocyte / PLT engraftment. In addition, the duration of neutropenia (neutrophilic granulocytes ≤ 0.5 x 10^9/L) and leukopenia (leucocytes granulocytes ≤ 1.0 x 10^9/L) was analysed based on documented laboratory values.

Quantification of donor type chimerism: Complete donor-type chimerism was defined if a value of ≥ 95% donor-type was detected. Mixed chimerism was defined as having a recipient fraction > 5% (to 94%).

Incidences of complete donor-type chimerism were estimated as the number of subjects with complete chimerism divided by the total number of subjects at risk. To investigate the mixed donor-type chimerism the frequency of subjects with at least 20% or 50% donor-type chimerism was calculated. Chimerism was evaluated on visit Day +28, +100 and Month 12, and during longer-term follow-up.

Event-free survival: Event-free survival (EFS) was defined as the time length between end of HSCT and the date of graft failure or "stem cell infusion (re-transplant) with conditioning" rescue therapy (ie, second allogenic transplantations) or death (whatever occurred first). EFS was evaluated from the end of HSCT until the end of the longer-term follow-up phase.

GvHD-free survival: GvHD-free survival was measured from end of HSCT to time of event. The associated time span was defined as the interval from end of HSCT to aGvHD of at least grade III, moderate or severe cGvHD, or death (whatever occurred first). GvHD-free survival was evaluated from the end of HSCT until the end of the longer-term follow-up phase.

Chronic GvHD-free survival: cGvHD-free survival was measured from end of HSCT to time of event. The associated time span was defined as the interval from end of HSCT to moderate or severe cGvHD or death (whatever occurred first). cGvHD-free survival was evaluated from the end of HSCT until the end of the longer-term follow-up phase.

Rescue therapies: The use of and duration of using rescue therapies like DLIs, stem cell boost, stem cell infusion (retransplantation) with conditioning, stem cell infusion (re-transplantation) without conditioning, transfusion dependence for red blood cells, transfusion dependence for PLTs, and other was described from end of HSCT until the end of the longer-term follow-up phase.

Pharmacokinetics:

Pharmacokinetic (PK) data were collected to contribute to a population model and to assess covariates. On day -6, blood samples were collected from a subset of subjects allocated to treosulfan treatment. Bioanalytical methods applied and results of PK and population pharmacokinetic analysis are reported by Celerion and Venn Life Science.

Study participants

In total, 101 subjects (67 male, 34 female) have been analysed in this clinical trial including:

- 44 subjects from 28 days to < 4 years of age
- 41 subjects from 4 years to < 12 years of age.

The number of subjects in each analysis set was: All subjects 54 (Bu) 52 (Treo) 106 (Total); Safety set 50 (Bu) 51 (Treo) 101 (Total); Full analysis set 50 (Bu) 51 (Treo) 101 (Total)

Inclusion criteria

1. Non-malignant disease indicated for first myeloablative allogeneic HSCT: inborn errors of metabolism, primary immunodeficiencies (PIDs), haemoglobinopathies (Hb-pathies), and bone marrow failure syndromes.
2. First allogeneic HSCT.
3. Available matched sibling donor, matched family donor or matched unrelated donor. For bone marrow (BM) and peripheral blood (PB) match was defined as at least 9/10 allele matches after four digit typing in human leucocyte antigen (HLA)-A, -B, -C, -DRB1 and –DQB1 antigens. For umbilical cord blood match was defined as at least 5/6 matches after 2 digit typing in HLA-A and –B and four digit typing in –DRB1 antigens.

4. Age at time of registration from 28 days to less than 18 years of age.
5. Lansky (subjects < 16 years of age) or Karnofsky (subjects ≥ 16 years of age) performance score of at least 70%.
6. Written informed consent of the parents/legal guardian and subject's assent/consent according to national regulations.
7. Female subjects of child-bearing potential or partner of male subjects with child-bearing potential must use a highly effective method of contraception (pearl index < 1%) such as complete sexual abstinence, combined oral contraceptive, hormone intrauterine contraceptive device, vaginal hormone ring, transdermal contraceptive patch, contraceptive implant or depot contraceptive injection in combination with a second method of contraception like a condom or a cervical cap / diaphragm with spermicide or surgical sterilisation (vasectomy) in male subjects or male partners during the trial and at least 6 months thereafter. For female subjects on the trial, the vasectomised male partner should be the sole partner for that subject.
8. Negative pregnancy test for females of child-bearing potential.

Results

The CSR of study MC-FludT.16/NM focusses on completed 12 month follow-up data for all subjects, but also contains follow-up data available as of the data cut-off on 07-Jun-2021. Longer-term follow-up data will be collected until the last recruited subject has completed visit Month 36 and presented in an updated version of the CSR expected in 2023. The study is based on an approved PIP, incl. four agreed modifications.

The results of the primary endpoint were: In the Full analysis set (FAS), the proportion of subjects which had died from transplantation- or treatment-related cause until day +100 was higher in the busulfan arm (5 of 50 subjects; 10.0%) than the treosulfan arm (0 of 51 subjects, 0.0%). The incidence of freedom from transplantation (treatment)-related mortality until day +100 was 90.0% (90% CI: 80.1%, 96.0%) in the busulfan arm and 100.0% (90% CI: 94.3%, 100.0%) in the treosulfan arm (p=0.0528), thus in favour of treosulfan.

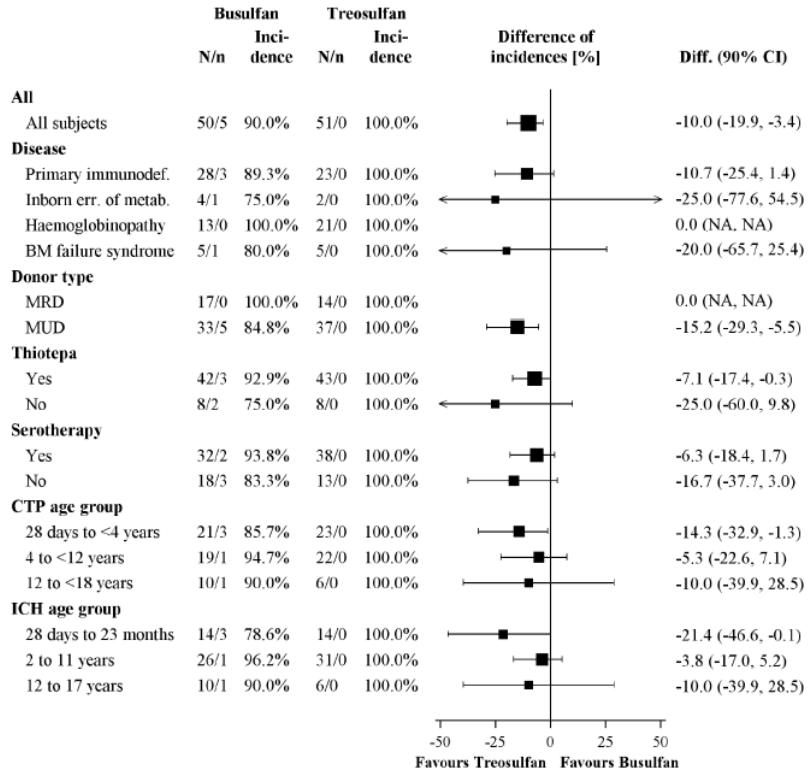
Table 11.4.1.1.A Incidence of freedom from transplantation (treatment)-related mortality until day +100 (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Freedom from transplantation (treatment)-related mortality until day +100		
Subjects with event	5 (10.0%)	0 (0.0%)
Subjects without event	45 (90.0%)	51 (100.0%)
Incidence [%] (90% CI) (95% CI)	90.0 (80.1, 96.0) (78.2, 96.7)	100.0 (94.3, 100.0) (93.0, 100.0)
Diff. incidences [%] (90% CI) (95% CI)		-10.0 (-19.9, -3.4) (-21.8, -2.0)
Odds ratio[a] (90% CI) (95% CI)		<.0001 (NA) (NA)
p-value[ab]		0.0528
Unadjusted p-value[c]		0.0267

Source: Table 14.2.1A
[a] Adjusted for thiotepa and disease; [b] Stratified Cochran-Mantel-Haenszel test; [c] Fisher's exact test
Abbreviations: CI = confidence interval; N = total number of subjects.

Results from exploratory subgroup analyses (incidences of transplantation (treatment)-related mortality until day +100 by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. The forest plot for transplantation (treatment)-related mortality until day +100 by subgroups is given in Figure 11.4.1.1.A. The results of the subgroup analyses were consistent with the main analysis.

Figure 11.4.1.1.A **Forest plot for freedom from transplantation (treatment)-related mortality until day +100 by subgroups (Full Analysis Set)**



N = Number of subjects, n = Number of events

Source: Figure 14.2.1A

Abbreviations: BM = bone marrow; CI = confidence interval; CTP = clinical trial protocol; Diff. = difference; ICH = International Council for Harmonization; Inborn err.of metab. = Inborn error of metabolism; MRD = matched related donor; MUD = matched unrelated donor.

The key results of the exploratory analysis of the secondary endpoints were:

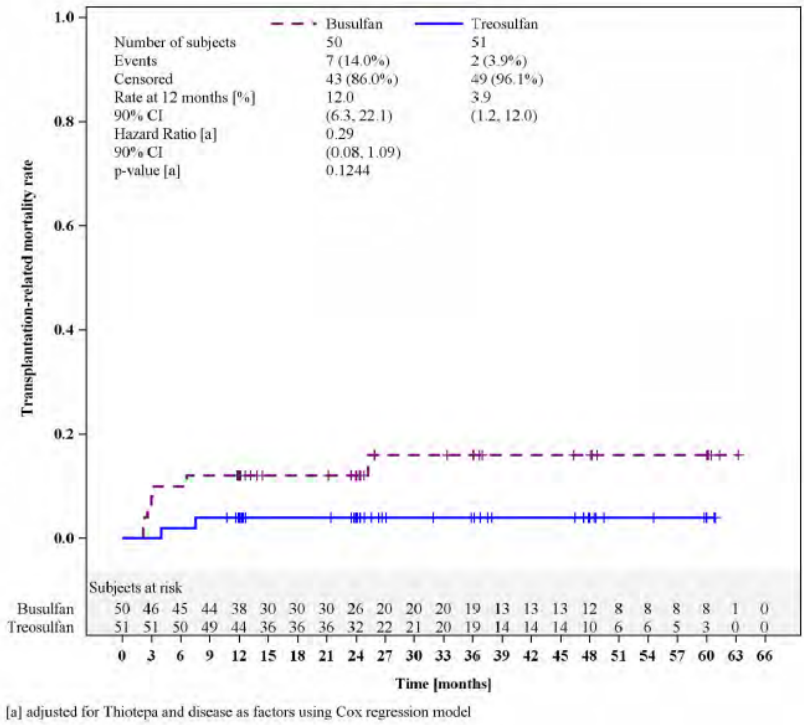
- TRM at visit Month 12 was 12.0% (90% CI: 6.3%, 22.1%) in the busulfan arm and 3.9% (90% CI: 1.2%, 12.0%) in the treosulfan arm (HR 0.29, 90% CI: 0.08, 1.09; p=0.1244), thus in favour of treosulfan.

Table 11.4.1.2.A Summary results of transplantation-related mortality (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	7 (14.0%)	2 (3.9%)
Subjects without event	43 (86.0%)	49 (96.1%)
Transplantation-related mortality at 12 months[a] [%] (90% CI)	12.0 (6.3, 22.1)	3.9 (1.2, 12.0)
Transplantation-related mortality at 24 months[a] [%] (90% CI)	12.0 (6.3, 22.1)	3.9 (1.2, 12.0)
Transplantation-related mortality at 36 months[a] [%] (90% CI)	16.0 (8.6, 28.6)	3.9 (1.2, 12.0)
Hazard Ratio (Treosulfan/Busulfan)[b] (90% CI)	0.29 (0.08, 1.09)	
p-value[b]	0.1244	
Unadjusted p-value[c]	0.0718	

Sources: Table 14.2.2A
[a] based on Kaplan-Meier estimates
[b] adjusted for thiotepa and disease as factors using Cox regression model
[c] Log-rank test
Abbreviations: CI = confidence interval; N = total number of subjects.

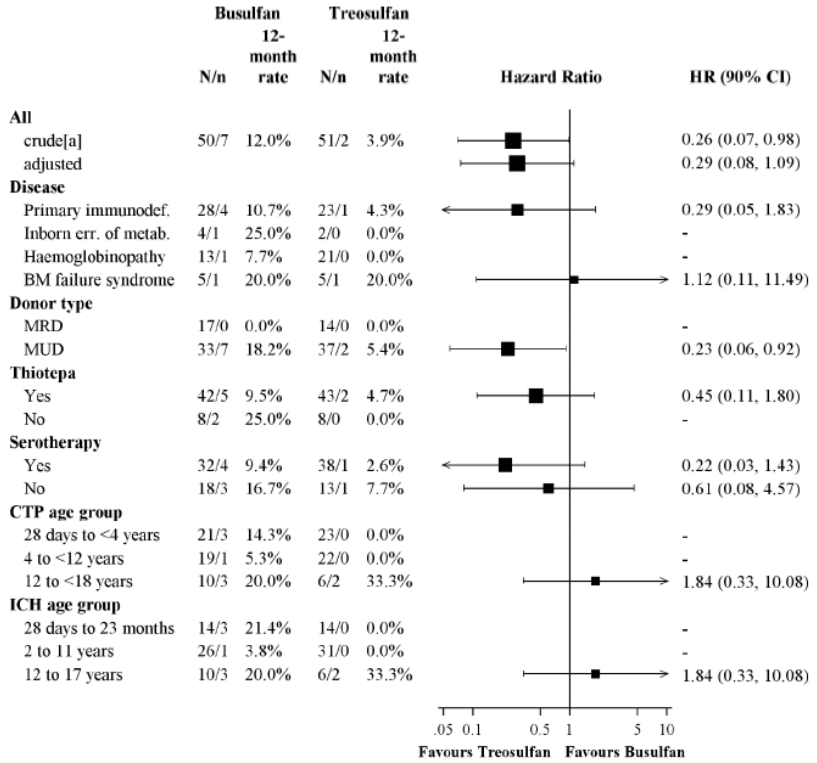
Figure 11.4.1.2.A Kaplan-Meier estimates of transplantation-related mortality (Full Analysis Set)



Sources: Figure 14.2.2A
Abbreviations: CI = confidence interval

Results from exploratory subgroup analyses (Kaplan-Meier estimates for TRM by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for TRM displaying 12-month rates by subgroups for the FAS is given in Figure 11.4.1.2.B. The results of the subgroup analyses were consistent with the main analysis.

Figure 11.4.1.2.B **Forest plot for transplantation-related mortality displaying 12-month rates by subgroups (Full Analysis Set)**



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Cox regression model

Sources: Figure 14.2.2B

Abbreviations: BM = bone marrow; CI = confidence interval; CTP = clinical trial protocol; HR = hazard ratio; ICH = International Council for Harmonization; Inborn err.of metab. = Inborn error of metabolism; MRD = matched related donor; MUD = matched unrelated donor.

In the 14 g/m²/day dose group, 2 of 10 subjects (20%) died from transplantation–related cause; no event (0.0%) occurred in the 2 lower dose groups. The Kaplan-Meier estimate of TRM at 12 months was 20.0% (90% CI: 6.7%, 51.1%) in the 14 g/m²/day dose group and 0% (90% CI: 0.0%, 0.0%) in the other dose groups. This apparent dose-dependency has to be seen in the context of PK data, which showed a comparable drug exposure for all 3 dose groups. Thus, other prognostic factors (like age, disease, donor type) have to be taken into account when interpreting the observed effect between dose groups.

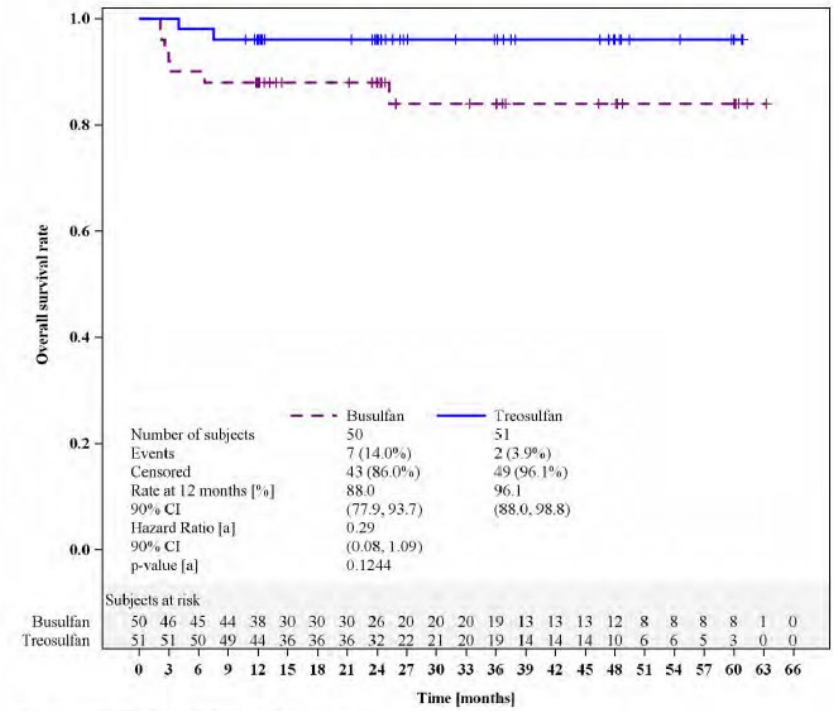
- OS at visit Month 12 was 88.0% (90% CI: 77.9%, 93.7%) in the busulfan arm and 96.1% (90% CI: 88.0%, 98.8%) in the treosulfan arm (HR 0.29, 90%CI: 0.08, 1.09, p=0.1244), thus in favour of treosulfan.

Table 11.4.1.3.A Summary results of overall survival (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Median follow-up[a] [months] (range of those surviving)	25.4 (11.7, 63.3)	25.6 (10.7, 60.9)
Subjects with event	7 (14.0%)	2 (3.9%)
Subjects without event	43 (86.0%)	49 (96.1%)
Overall survival at 12 months[b] [%] (90% CI)	88.0 (77.9, 93.7)	96.1 (88.0, 98.8)
Overall survival at 24 months[b] [%] (90% CI)	88.0 (77.9, 93.7)	96.1 (88.0, 98.8)
Overall survival at 36 months[b] [%] (90% CI)	84.0 (71.4, 91.4)	96.1 (88.0, 98.8)
Hazard Ratio (Treosulfan/Busulfan)[c] (90% CI)	0.29 (0.08, 1.09)	
p-value[c]	0.1244	
Unadjusted p-value[d]	0.0718	

Sources: Table 14.2.3A
[a] based on reverse Kaplan-Meier estimates
[b] based on Kaplan-Meier estimates
[c] adjusted for thiotepa and disease as factors using Cox regression model
[d] Log-rank test
Abbreviations: CI = confidence interval; N = total number of subjects.

Figure 11.4.1.3.A Kaplan-Meier estimates of overall survival (Full Analysis Set)

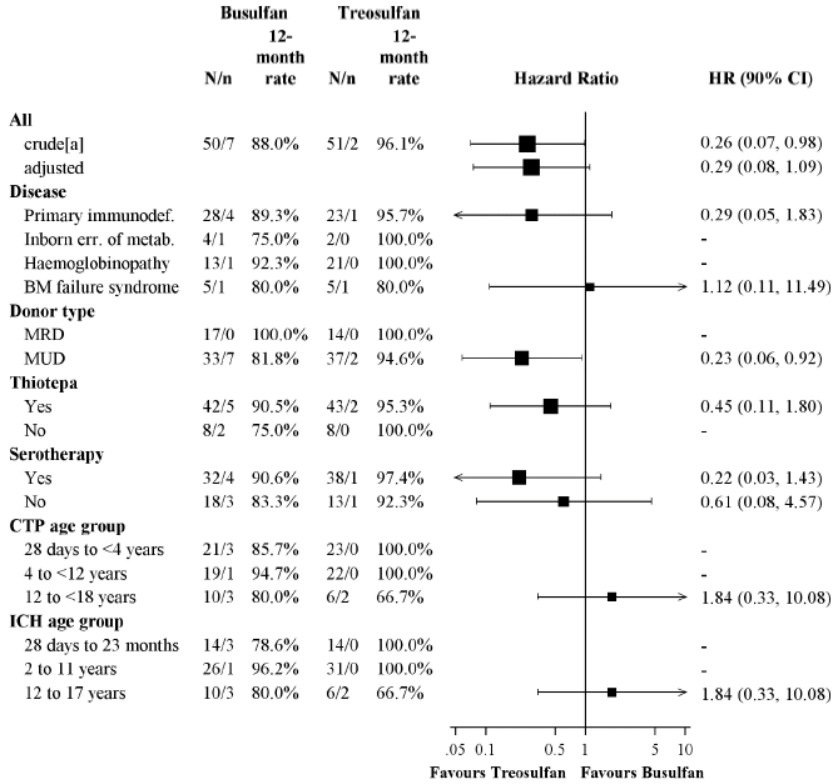


[a] adjusted for Thiotepa and disease as factors using Cox regression model

Source: Figure 14.2.3A

Results from exploratory subgroup analyses (Kaplan-Meier estimates for OS by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for OS displaying 12-month rates by subgroups for the FAS is presented. The results of the subgroup analyses were consistent with the main analysis.

Figure 11.4.1.3.B Forest plot for overall survival displaying 12-month rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Cox regression model

Source: Figure 14.2.3B

Abbreviations: BM = bone marrow; CI = confidence interval; CTP = clinical trial protocol; HR = hazard ratio; ICH = International Council for Harmonization; Inborn err.of metab. = Inborn error of metabolism; MRD = matched related donor; MUD = matched unrelated donor.

In the 14 g/m²/day dose group, 2 of 10 subjects (20%) died; no event occurred in the 2 lower dose groups. The Kaplan-Meier estimate of OS at 12 months was 80.0% (90% CI: 48.9%, 93.3%) in the 14 g/m²/day dose group, and 100% (90% CI: 100.0%, 100.0%) in the other dose groups. This apparent dose-dependency has to be seen in the context of PK data, which showed a comparable drug-exposure for all 3 dose groups. Thus, other prognostic factors (like age, disease, or donor type) have to be taken into account when interpreting the observed difference between dose groups.

- The rate of graft failures at visit Month 12 was 4.0% (90% CI: 0.0%, 8.6%) in the busulfan arm and 15.8% (90% CI: 7.4%, 24.3%) in the treosulfan arm (HR 5.48, 90% CI: 1.44, 20.91, p=0.0366), thus statistically significant in favour of busulfan. Primary and secondary graft failures were evaluated as secondary endpoints.

Table 11.4.1.4.A Rates of primary and secondary graft failure (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Primary graft failure	2 / 50 (4.0%)	2 / 51 (3.9%)	4 / 101 (4.0%)
Secondary graft failure	0 / 48 (0.0%)	9 / 49 (18.4%)	9 / 97 (9.3%)

Source: Table 14.2.4A
Note: Rate of primary/secondary graft failure calculated as number of subjects with graft failure by the number of subjects at risk.
- At risk for primary graft failure: Subjects with HSCT
- At risk for secondary graft failure: Subjects whose neutrophilic granulocytes engrafted after HSCT or were never below the required level

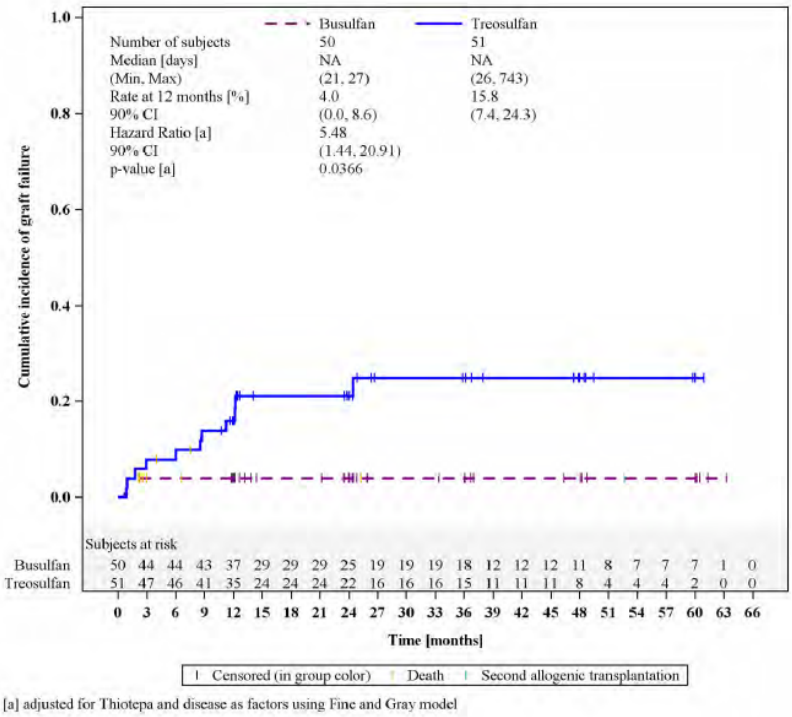
Abbreviations: N = total number of subjects.

Table 11.4.1.4.B Summary results of graft failure (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	2 (4.0%)	11 (21.6%)
Subjects without event (censored) or with competing event	48 (96.0%)	40 (78.4%)
Censored	41 (82.0%)	38 (74.5%)
Death[a]	6 (12.0%)	2 (3.9%)
Second allogenic transplantation[a]	1 (2.0%)	0 (0.0%)
Cumulative incidence at 12 months [%] (90% CI)	4.0 (0.0, 8.6)	15.8 (7.4, 24.3)
Cumulative incidence at 24 months [%] (90% CI)	4.0 (0.0, 8.6)	21.0 (11.2, 30.9)
Cumulative incidence at 36 months [%] (90% CI)	4.0 (0.0, 8.6)	24.8 (13.6, 35.9)
Hazard Ratio (Treosulfan/Busulfan)[b] (90% CI)	5.48 (1.44, 20.91)	
p-value[b]	0.0366	
Unadjusted p-value[c]	0.0097	

Sources: Table 14.2.4B
[a] only if this event occurred first
[b] adjusted for thiotepa and disease as factors using Fine and Gray model
[c] based on Gray test
Abbreviations: CI = confidence interval; N = total number of subjects.

Figure 11.4.1.4.A Cumulative incidence of graft failure (Full Analysis Set)



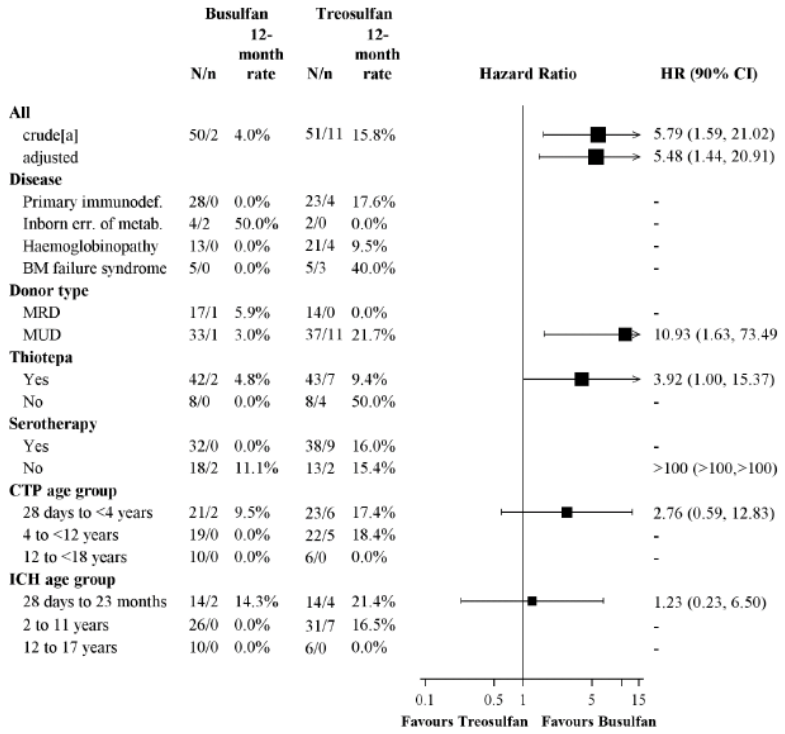
Source: Figure 14.2.4A
[a] minimum and maximum of observed event times
[b] adjusted for thiotepa and disease as factors using Fine and Gray model
[c] based on Gray test

Abbreviations: CI = confidence interval; Max = maximum ; Min = minimum.

Results from exploratory subgroup analyses (cumulative incidences of graft failure by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for graft failure displaying 12-month rates by subgroups is presented. The results of the subgroup analyses were

consistent with the main analysis. All graft failures reported for subjects treated with treosulfan were in the subgroup of MUD, and 9 out of 11 subjects (81.8%) with graft failure received serotherapy.

Figure 11.4.1.4.B Forest plot for graft failure displaying 12-month rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Fine and Gray model

Source: Figure 14.2.4B
Abbreviations: BM = bone marrow; CI = confidence interval; CTP = clinical trial protocol; HR = hazard ratio; ICH = International Council for Harmonization; Inborn err.of metab. = Inborn error of metabolism; MRD = matched related donor; MUD = matched unrelated donor.

The cumulative incidences of graft failure at 12 months were 22.2% (90% CI: 0.0%, 45.0%) in the 10 g/m²/day dose group, 19.1% (90% CI: 7.5%, 30.7%) in the 12 g/m²/day dose group, and 0.0% (90% CI: 0.0%, 0.0%) in the 14 g/m²/day dose group. This apparent dose-dependency has to be seen in the context of PK data, which showed a comparable drug-exposure for all 3 dose groups. Thus, other prognostic factors (like age, disease, or donor type) have to be taken into account when interpreting the observed difference between dose groups.

- Reconstitution of granulopoiesis, leukopoiesis, and thrombopoiesis was similar in the treatment arms. However, the median duration of CTCAE grade IV neutropenia was statistically significantly longer in the treosulfan arm than the busulfan arm (20.0 days compared to 14.5 days, p=0.0108). Similar results were seen for duration of CTCAE grade IV leukopenia (19.0 days in the treosulfan arm compared to 14.5 days in the busulfan arm, p=0.0087).

Results from exploratory subgroup analyses (conditional cumulative incidences of reconstitution of thrombopoiesis > 50 x10^9/L by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. Subgroup analyses were generally consistent with the main analysis.

- The fraction of subjects with complete donor type chimerism decreased between visit Day +28 and visit Month 12 in both treatment arms, however, the incidence of complete donor-type chimerism was comparable between the treatment arms (visit Day +100: p=0.1196; visit Month 12: p=0.2445). The fraction of subjects with mixed donor type chimerism ≥ 20% and ≥ 50% decreased between visit Day +28 and visit Month 12 in the treosulfan arm whereas it remained nearly

unchanged in the busulfan arm. However, the incidences of mixed donor-type chimerism ≥ 20% and ≥ 50% were comparable between the treatment arms, apart from visit Month 12, when the incidence of mixed donor type chimerism ≥ 50% was statistically significantly higher in the busulfan arm (p=0.0189).

Table 11.4.1.6.A Incidence of complete donor type chimerism until Month 12 visit (Full Analysis Set)

	Busulfan	Treosulfan
Subjects at risk at Day +28 visit[a]	**N=50**	**N=51**
Subjects with complete chimerism	41 (82.0%)	43 (84.3%)
Subjects without complete chimerism	9 (18.0%)	7 (13.7%)
Subjects without information	0 (0.0%)	1 (2.0%)
Incidence [%] (90% CI) (95% CI)	82.0 (70.7, 90.3) (68.6, 91.4)	84.3 (73.5, 91.9) (71.4, 93.0)
Odds ratio[bc] (90% CI) (95% CI)		1.5824 (0.61, 4.08) (0.51, 4.89)
p-value[bcd]		0.4250
Unadjusted p-value[be]		0.7858
Subjects at risk at Day +100 visit[a]	**N=46**	**N=51**
Subjects with complete chimerism	39 (84.8%)	34 (66.7%)
Subjects without complete chimerism	5 (10.9%)	14 (27.5%)
Subjects without information	2 (4.3%)	3 (5.9%)
Incidence [%] (90% CI) (95% CI)	84.8 (73.3, 92.6) (71.1, 93.7)	66.7 (54.3, 77.5) (52.1, 79.2)
Odds ratio[bc] (90% CI) (95% CI)		0.3972 (0.15, 1.06) (0.12, 1.28)
p-value[bcd]		0.1196
Unadjusted p-value[be]		0.0418
Subjects at risk at Month 12 visit[a]	**N=43**	**N=49**
Subjects with complete chimerism	33 (76.7%)	24 (49.0%)
Subjects without complete chimerism	9 (20.9%)	14 (28.6%)
Subjects without information	1 (2.3%)	11 (22.4%)
Incidence [%] (90% CI) (95% CI)	76.7 (63.8, 86.8) (61.4, 88.2)	49.0 (36.5, 61.5) (34.4, 63.7)
Odds ratio[bc] (90% CI) (95% CI)		0.5429 (0.23, 1.28) (0.20, 1.51)
p-value[bcd]		0.2445
Unadjusted p-value[be]		0.1455

Source: Table 14.2.9A

[a] Subjects are at risk if they have an examination at the Day +28, Day +100, Month 12 visit or if they have survived day +30, +107, +379, respectively.

[b] Missing values are excluded for odds ratio calculation and tests.

[c] Adjusted for thiotepa and disease; [d] Stratified Cochran-Mantel-Haenszel test; [e] Fisher's exact test

Abbreviations: CI = confidence interval; N = total number of subjects.

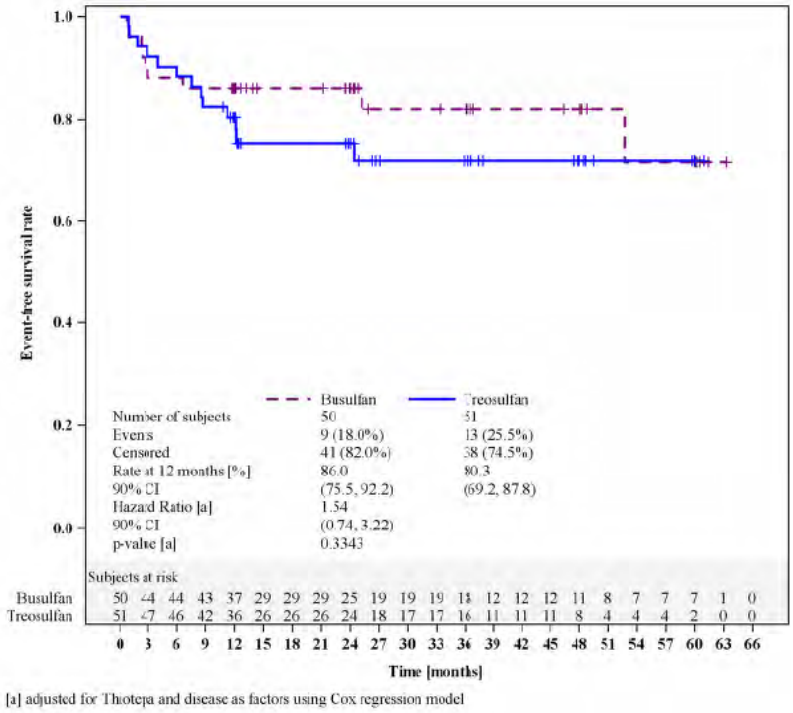
- EFS at visit Month 12 was 86.0% (90% CI: 75.5%, 92.2%) in the busulfan arm and 80.3% (90% CI: 69.2%, 87.8%) in the treosulfan arm (p=0.3343), thus in favour of busulfan. As graft failure is included in the event definition, the lower EFS observed in the treosulfan arm reflects the higher rate of secondary graft failures in the treosulfan arm, while death was more frequently reported in the busulfan arm.

Table 11.4.1.7.A Summary results of event-free survival (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	9 (18.0%)	13 (25.5%)
Death[a]	6 (12.0%)	2 (3.9%)
Primary graft failure[a]	2 (4.0%)	2 (3.9%)
Secondary graft failure[a]	0 (0.0%)	9 (17.6%)
Second allogeneic transplantation[a]	1 (2.0%)	0 (0.0%)
Subjects without event	41 (82.0%)	38 (74.5%)
Event-free survival at 12 months[b] [%] (90% CI)	86.0 (75.5, 92.2)	80.3 (69.2, 87.8)
Event-free survival at 24 months[b] [%] (90% CI)	86.0 (75.5, 92.2)	75.3 (63.2, 83.9)
Event-free survival at 36 months[b] [%] (90% CI)	81.9 (69.0, 89.8)	71.9 (58.8, 81.4)
Hazard Ratio (Treosulfan/Busulfan)[c] (90% CI)	1.54 (0.74, 3.22)	
p-value[c]	0.3343	
Unadjusted p-value[d]	0.3683	

Source: Table 14.2.10A
[a] only if this event occurred first
[b] based on Kaplan-Meier estimates
[c] adjusted for thiotepa and disease as factors using Cox regression model
[d] Log-rank test
Abbreviations: CI = confidence interval; N = total number of subjects.

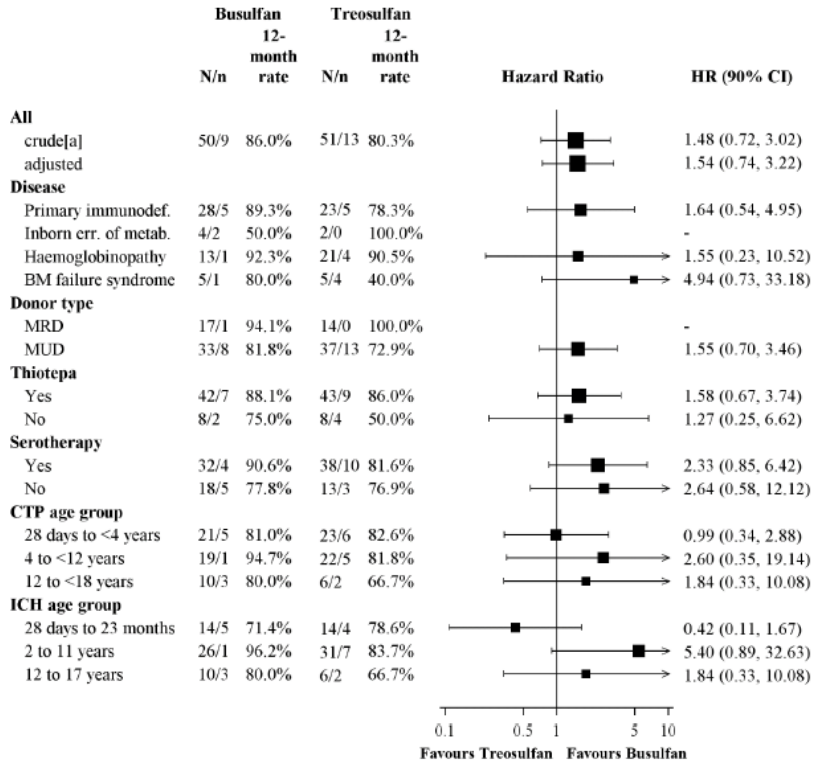
Figure 11.4.1.7.A Kaplan-Meier estimates of event-free survival (Full Analysis Set)



Source: Figure 14.2.10A
[a] minimum and maximum of observed event times
[b] adjusted for thiotepa and disease as factors using Cox regression model
[c] Log-rank test
[d] stratified by thiotepa and disease
Abbreviations: CI = confidence interval.

Results from exploratory subgroup analyses (Kaplan-Meier estimates for EFS by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for EFS displaying 12-month rates by subgroups is given in Figure 11.4.1.7.B. The results of the subgroup analyses were consistent with the main analysis.

Figure 11.4.1.7.B Forest plot for event-free survival displaying 12-month rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Cox regression model

Source: Figure 14.2.10B
Abbreviations: BM = bone marrow; CI = confidence interval; CTP = clinical trial protocol; HR = hazard ratio; ICH = International Council for Harmonization; Inborn err.of metab. = Inborn error of metabolism; MRD = matched related donor; MUD = matched unrelated donor.

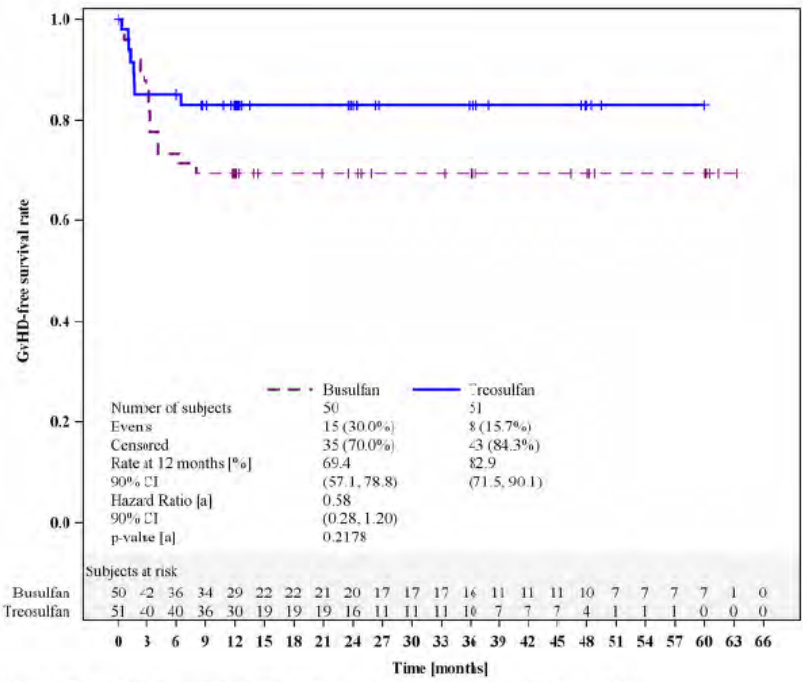
- cGvHD-free survival at visit Month 12 was 69.4% (90% CI: 57.1%, 78.8%) in the busulfan arm and 89.3% (90% CI: 79.0%, 94.7%) in the treosulfan arm (p=0.0308), thus statistically significant in favour of treosulfan.

Table 11.4.1.8.A Summary results of GvHD-free survival (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	15 (30.0%)	8 (15.7%)
Death[a]	4 (8.0%)	0 (0.0%)
Acute GvHD of at least Grade III[a]	4 (8.0%)	7 (13.7%)
Moderate/severe chronic GvHD[a]	7 (14.0%)	1 (2.0%)
Subjects without event	35 (70.0%)	43 (84.3%)
GvHD-free survival at 12 months[b] [%] (90% CI)	69.4 (57.1, 78.8)	82.9 (71.5, 90.1)
GvHD-free survival at 24 months[b] [%] (90% CI)	69.4 (57.1, 78.8)	82.9 (71.5, 90.1)
GvHD-free survival at 36 months[b] [%] (90% CI)	69.4 (57.1, 78.8)	82.9 (71.5, 90.1)
Hazard Ratio (Treosulfan/Busulfan)[c] (90% CI)	0.58 (0.28, 1.20)	
p-value[c]	0.2178	
Unadjusted p-value[d]	0.1610	

Source: Table 14.2.11A
Note: GvHD-free defined as no acute GvHD of at least grade III and no moderate/severe chronic GvHD
[a] only if this event occurred first
[b] based on Kaplan-Meier estimates
[c] adjusted for thiotepa and disease as factors using Cox regression model
[d] Log-rank test
Abbreviations: CI = confidence interval; GvHD = graft-versus-host disease; N = total number of subjects.

Figure 11.4.1.8.A Kaplan-Meier estimates of GvHD-free survival (Full Analysis Set)

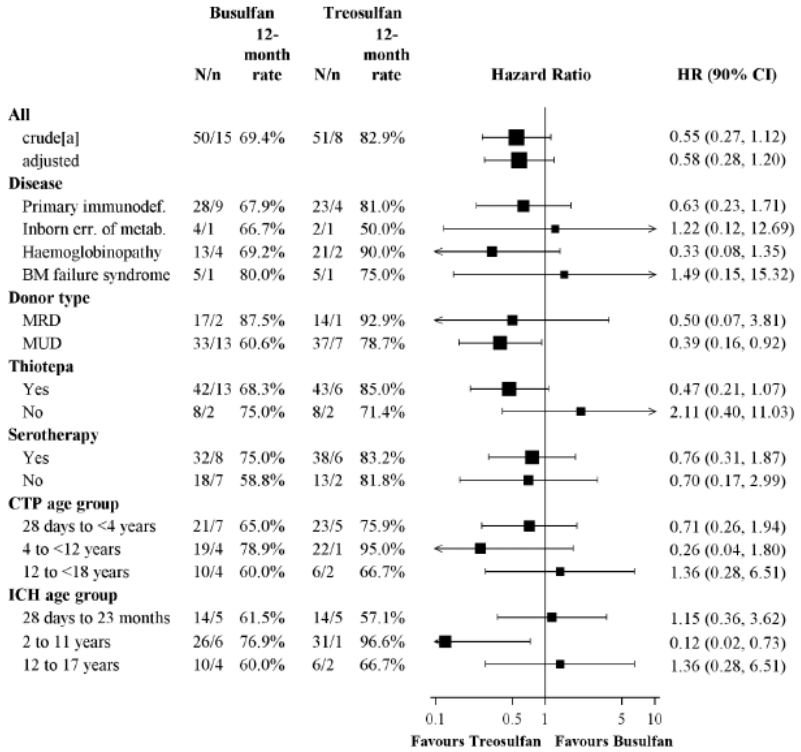


Note: GvHD-free defined as no acute GvHD of at least grade III and no moderate/severe chronic GvHD
[a] adjusted for Thiotepa and disease as factors using Cox regression model

Source: Figure 14.2.11A
Abbreviations: CI = confidence interval; GvHD = graft-versus-host disease.

Results from exploratory subgroup analyses (Kaplan-Meier estimates for GvHD-free survival by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for GvHD-free survival displaying 12-month rates by subgroups is presented. The results of the subgroup analyses were consistent with the main analysis.

Figure 11.4.1.8.B Forest plot for GvHD-free survival displaying 12-month rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Cox regression model

Source: Figure 14.2.11B
Abbreviations: BM = bone marrow; CI = confidence interval; CTP = clinical trial protocol; GvHD = graft-versus-host disease; HR = hazard ratio; ICH = International Council for Harmonization; Inborn err.of metab. = Inborn error of metabolism; MRD = matched related donor; MUD = matched unrelated donor.

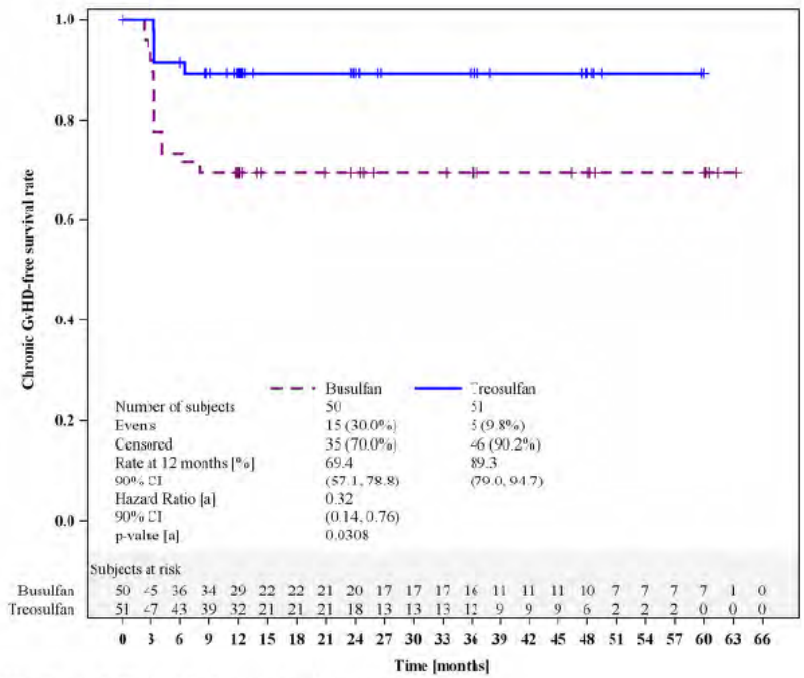
- cGvHD-free survival at 12 months was 69.4% (90% CI:57.1%, 78.8%) in the busulfan arm and 89.3% (90% CI: 79.0%, 94.7%) in the treosulfan arm (p=0.0308, adjusted for thiotepa and disease as factors using the Cox regression model). This observation has to be considered in the context of reported incidence of cGvHD and OS. The HR for cGvHD-free survival of 0.32 (90% CI: 0.14, 0.76) was statistically significant in favour of treosulfan.

Table 11.4.1.9.A Summary results of cGvHD-free survival (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	15 (30.0%)	5 (9.8%)
Death[a]	5 (10.0%)	0 (0.0%)
Moderate/severe chronic GvHD[a]	10 (20.0%)	5 (9.8%)
Subjects without event	35 (70.0%)	46 (90.2%)
Chronic GvHD-free survival at 12 months[b] [%] (90% CI)	69.4 (57.1, 78.8)	89.3 (79.0, 94.7)
Chronic GvHD-free survival at 24 months[b] [%] (90% CI)	69.4 (57.1, 78.8)	89.3 (79.0, 94.7)
Chronic GvHD-free survival at 36 months[b] [%] (90% CI)	69.4 (57.1, 78.8)	89.3 (79.0, 94.7)
Hazard Ratio (Treosulfan/Busulfan)[c] (90% CI)	0.32 (0.14, 0.76)	
p-value[c]	0.0308	
Unadjusted p-value[d]	0.0147	

Source: Table 14.2.12A
Note: Chronic GvHD-free defined as no moderate/severe chronic GvHD
[a] only if this event occurred first
[b] based on Kaplan-Meier estimates
[c] adjusted for thiotepa and disease as factors using Cox regression model
[d] Log-rank test
Abbreviations: CI = confidence interval; (c)GvHD = (chronic) graft-versus-host disease; N = total number of subjects.

Figure 11.4.1.9.A Kaplan-Meier estimates of chronic GvHD-free survival (Full Analysis Set)

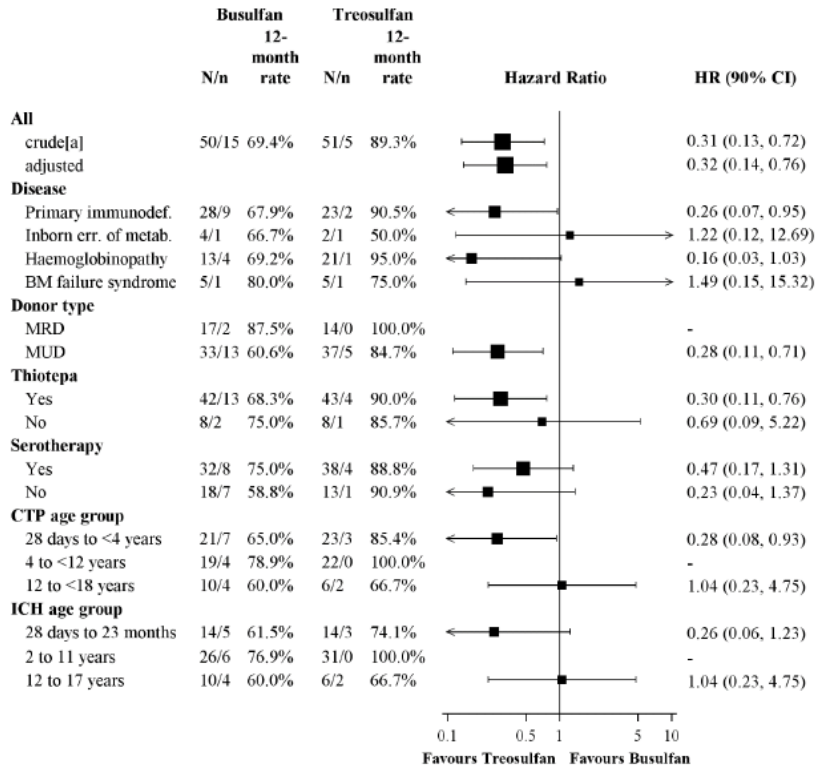


Note: Chronic GvHD-free defined as no moderate/severe chronic GvHD
[a] adjusted for Thiotepa and disease as factors using Cox regression model

Source: Figure 14.2.12A
Abbreviations: CI = confidence interval; GvHD = graft-versus-host disease.

Results from exploratory subgroup analyses (Kaplan-Meier estimates for cGvHD-free survival by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for cGvHD-free survival displaying 12-month rates by subgroups is presented. The results of the subgroup analyses were consistent with the main analysis.

Figure 11.4.1.9.B **Forest plot for cGvHD-free survival displaying 12-month rates by subgroups (Full Analysis Set)**



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Cox regression model

Source: Figure 14.2.12B

- The frequency of subjects with rescue therapies is given. In total, 21 subjects (42.0%) in the busulfan arm and 21 subjects (41.2%) in the treosulfan arm received any rescue therapy, mostly red blood cell or PLT transfusions. No differences were observed between the treatment arms. One subject (ID163112) in the busulfan arm received a re-transplantation with conditioning treatment as rescue therapy because of recurrent haemolytic episodes and recurrent CMV re-activations. The subject did not experience graft failure, but presented a 50% mixed chimerism.

Table 11.4.1.10.A Frequency of subjects with rescue therapies (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Any rescue therapy		
Subjects with rescue therapy	21 (42.0%)	21 (41.2%)
Subjects without rescue therapy	29 (58.0%)	30 (58.8%)
Incidence [%] (90% CI) (95% CI)	42.0 (30.1, 54.6) (28.2, 56.8)	41.2 (29.5, 53.7) (27.6, 55.8)
Odds ratio[a] (90% CI) (95% CI)	0.7592 (0.38, 1.54) (0.33, 1.76)	
p-value[ab]	0.5264	
Unadjusted p-value[c]	1.0000	
DLIs		
Subjects with rescue therapy	2 (4.0%)	5 (9.8%)
Subjects without rescue therapy	48 (96.0%)	46 (90.2%)
Incidence [%] (90% CI) (95% CI)	4.0 (0.7, 12.1) (0.5, 13.7)	9.8 (3.9, 19.5) (3.3, 21.4)
Odds ratio[a] (90% CI) (95% CI)	1.6932 (0.37, 7.68) (0.28, 10.25)	
p-value[ab]	0.5716	
Unadjusted p-value[c]	0.4364	
Stem cell boost		
Subjects with rescue therapy	1 (2.0%)	2 (3.9%)
Subjects without rescue therapy	49 (98.0%)	49 (96.1%)
Incidence [%] (90% CI) (95% CI)	2.0 (0.1, 9.1) (0.1, 10.6)	3.9 (0.7, 11.8) (0.5, 13.5)
Odds ratio[a] (90% CI) (95% CI)	0.8574 (0.07, 10.65) (0.04, 17.26)	
p-value[ab]	0.9252	
Unadjusted p-value[c]	1.0000	
Stem cell infusion (re-transplant) with conditioning		
Subjects with rescue therapy	1 (2.0%)	0 (0.0%)
Subjects without rescue therapy	49 (98.0%)	51 (100.0%)
Incidence [%] (90% CI) (95% CI)	2.0 (0.1, 9.1) (0.1, 10.6)	0.0 (0.0, 5.7) (0.0, 7.0)
Odds ratio[a] (90% CI) (95% CI)	<.0001 (NA) (NA)	
p-value[ab]	0.3566	
Unadjusted p-value[c]	0.4950	
Stem cell infusion (re-transplant) without conditioning		
Subjects with rescue therapy	0 (0.0%)	0 (0.0%)
Subjects without rescue therapy	50 (100.0%)	51 (100.0%)
Incidence [%] (90% CI) (95% CI)	0.0 (0.0, 5.8) (0.0, 7.1)	0.0 (0.0, 5.7) (0.0, 7.0)
Transfusion dependence for red blood cells		
Subjects with rescue therapy	17 (34.0%)	17 (33.3%)
Subjects without rescue therapy	33 (66.0%)	34 (66.7%)
Incidence [%] (90% CI) (95% CI)	34.0 (23.0, 46.5) (21.2, 48.8)	33.3 (22.5, 45.7) (20.8, 47.9)
Odds ratio[a] (90% CI) (95% CI)	0.7725 (0.37, 1.62) (0.32, 1.86)	
p-value[ab]	0.5741	
Unadjusted p-value[c]	1.0000	
Transfusion dependence for platelets		
Subjects with rescue therapy	14 (28.0%)	14 (27.5%)
Subjects without rescue therapy	36 (72.0%)	37 (72.5%)
Incidence [%] (90% CI) (95% CI)	28.0 (17.8, 40.3) (16.2, 42.5)	27.5 (17.4, 39.5) (15.9, 41.7)
Odds ratio[a] (90% CI) (95% CI)	0.8631 (0.40, 1.86) (0.35, 2.16)	
p-value[ab]	0.7566	
Unadjusted p-value[c]	1.0000	
Other rescue therapies		
Subjects with rescue therapy	2 (4.0%)	4 (7.8%)
Subjects without rescue therapy	48 (96.0%)	47 (92.2%)
Incidence [%] (90% CI) (95% CI)	4.0 (0.7, 12.1) (0.5, 13.7)	7.8 (2.7, 17.1) (2.2, 18.9)
Odds ratio[a] (90% CI) (95% CI)	1.4174 (0.31, 6.58) (0.23, 8.83)	
p-value[ab]	0.7045	
Unadjusted p-value[c]	0.6779	

Source: Table 14.2.13A (2 of 2)

[a] Adjusted for thiotepa and disease; [b] Stratified Cochran-Mantel-Haenszel test; [c] Fisher's exact test

Abbreviations: CI = confidence interval; DLIs = ; donor lymphocyte infusions; N = total number of subjects

Results from exploratory subgroup analyses (frequency of subjects with any rescue therapy by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for GvHD-free survival displaying 12-month incidences by subgroups is presented. The results of the subgroup analyses were consistent with the main analysis. The treatment effect on rescue therapy was not relevantly influenced by other factors as shown in the logistic regression models.

MC-FludT.17/M

Title of Study

Clinical phase II trial to describe the safety and efficacy of TREO-based conditioning therapy prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with haematological malignancies

Methods

The overall objective of this Phase 2 trial was to describe the safety and efficacy of i.v. Treosulfan administered as part of a standardised Fludarabine containing conditioning and to contribute to a PK model which permits to extend the use of Treosulfan in the paediatric population by extrapolating efficacy.

Primary Objective

The primary objective of this trial was to estimate the freedom from transplant (treatment)-related mortality until 100 days after HSCT.

The patient recruitment in this study was successfully completed within the planned recruitment period until end of September 2016. In total, 18 of the 24 centres initiated have enrolled 70 patients.

The trial was conducted in centres for stem cell transplantations in 5 countries: 10 sites in Germany, 4 sites in Poland, 1 site in the UK, 2 sites in Italy, and 1 site in the Czech Republic.

Secondary Objectives

1. Evaluation of engraftment after HSCT, defined as the first of 3 consecutive days for each of the following 4 criteria:
    - A leucocyte count of > 1 x 10^9/L
    - An absolute neutrophil count (ANC) of > 0.5 x 10^9/L
    - A platelet count of ≥ 20 x 10^9/L in the absence of platelet transfusion
    - A platelet count of ≥ 50 x 10^9/L in the absence of platelet transfusion
2. Evaluation of safety including early toxicity based on the Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) until day +100 after HSCT, serious adverse reactions (SARs) until the end of the longer-term follow-up phase
3. Evaluation of hepatic sinusoidal obstruction syndrome (HSOS, according to Jones et al [23]), lung toxicity (CTCAE term "Pulmonary Fibrosis"), hepatic toxicity (according to Bearman [6]), and infections of any CTCAE grade (non-serious and serious) until day +100
4. Evaluation of donor-type chimerism on day +28, day +100, and 12 months after HSCT
5. Evaluation of non-relapse mortality (NRM), transplant-related mortality (TRM), graft failure rate, incidence of relapse / progression, relapse-free survival (RFS) / progression free survival (PFS), and overall survival (OS) until 12 months after HSCT
6. Evaluation of incidence and severity of aGvHD (until day +100) and cGvHD (until 12 months after HSCT)
7. Evaluation of use of rescue therapies including donor-lymphocyte infusions (DLIs) and further conditioning regimens

8. Evaluation of PK parameters of Treosulfan and its epoxides and to develop a PK model for assessing relevant covariates

9. Evaluation of NRM, TRM, secondary graft failures, relapse / progression, RFS / PFS, OS, and GvHD during the longer-term follow-up phase

Methodology

Prospective, single arm, open-label, multicentre, non-controlled, Phase 2 clinical trial with trial duration per subject consisting of 4 phases:

- Treatment phase: 7 days with 3 days of Treosulfan administration
- Observation phase: until day +100 after HSCT (according to the PIP, this is defined until at least visit Day +100 [inclusive] of HSCT procedure)
- Follow-up phase: until 12 months after HSCT
- Longer-term follow-up phase (after completion of PIP): a minimum of 3 years after HSCT

Treatments Administered

The required total dose of Treosulfan was calculated on the basis of the subject's BSA. In a retrospective meta-analysis of the EBMT, data of 521 allogeneic and 83 autologous transplanted paediatric subjects were evaluated. Subjects received 3 doses of Treosulfan. The administered total dose ranged from 30 g/m^2 to 42 g/m^2. Efficacy and safety analysis revealed that the conditioning treatment using Treosulfan was tolerable and effective in children of all age groups.

A PK analysis was conducted with all available data on any paediatric use of Treosulfan and a population PK model was developed using data of 23 children and 93 adults. A BSA dependant, tabulated model-based dose recommendation was derived aimed at a Treosulfan area under the curve (AUC) of 1355 µg/h*ml, corresponding to an exposure of 3 x 14 g/m^2 in adult subjects.

Within this clinical trial, paediatric subjects were treated with Treosulfan according to the simplified BSA-adjusted dosing table. This was implemented to avoid substantial over-exposure of the smallest infants (with BSA 0.3 to 0.5 m^2) and accounted for biological differences in the diverse paediatric age (BSA) groups.

$$BSA = (\text{weight [kg]} \times \text{height [cm]} / 3600)\ ½$$

BSA (m^2)	Treosulfan dose (g/m^2/day)
≤ 0.5	10
> 0.5 – 1.0	12
> 1.0	14

In general, the administered dose of Treosulfan was not to be differed by > 10% from the calculated dose.

Treosulfan intravenous (i.v.): At dose levels 10 g/m², 12 g/m², and 14 g/m² over 2 hours on 3 consecutive days on visit Days -6, -5, and -4 before alloHSCT.

This trial allowed administration of 2 different background conditioning regimens with Treosulfan for the treatment of ALL, AML, MDS, and JMML: one background conditioning regimen consisted of a standardised Fludarabine-containing regimen (regimen A) whereas the other consisted of an intensified

regimen with Fludarabine and Thiotepa (regimen B). The Investigator decided for each individual subject whether to treat the subject with regimen A or with regimen B.

- Fludarabine i.v. as single doses of 30 mg/m2 on 5 consecutive days (from visit Day -7 to -3).
- Subjects may receive Thiotepa i.v. in 2 single doses of 5 mg/kg given on visit Day -2 given at the discretion of the investigator

Regimen A ("standard regimen"):

Day	-7	-6	-5	-4	-3	-2	-1	0	+1	+3	+6
Treosulfan i.v. (trial medication) (BSA-adapted: 10, 12, or 14 g/m²/day over 120 min, to be administered prior to Fludarabine)		X	X	X							
Fludarabine i.v. (30 mg/m²/day)	X	X	X	X	X						
Allogeneic stem cell transplantation								X			

Regimen B ("intensified regimen"):

Day	-7	-6	-5	-4	-3	-2	-1	0	+1	+3	+6
Treosulfan i.v. (trial medication) (BSA-adapted: 10, 12, or 14 g/m²/day over 120 min, to be administered prior to Fludarabine)		X	X	X							
Fludarabine i.v. (30 mg/m²/day)	X	X	X	X	X						
Thiotepa i.v. (2 x 5 mg/kg/day)						XX					
Allogeneic stem cell transplantation								X			

All subjects with ALL (38.6%), AML (41.4%), MDS (14.3%), or JMML (5.7%) received Treosulfan i.v. at a dose of 10 g/m2/day (8.6%), 12 g/m2/day (37.1%), or 14 g/m2/day (54.3%) according to their individual BSA. In addition, majority of the subjects (92.9%) received an intensified regimen with i.v. Thiotepa.

Criteria for Evaluation:

Efficacy:

Freedom from transplantation (treatment)-related mortality: Freedom from transplant (treatment)-related mortality until 100 days after HSCT was assessed as the primary endpoint of the trial, and was defined as death from any transplant (treatment)-related cause from the day of first administration of conditioning treatment until 100 days after HSCT. This endpoint is a combination of TRM and treatment-related mortality. Treatment-related deaths were defined as any death prior to HSCT. Treatment-related mortality was planned to be evaluated from the day of the first administration of trial medication, ie, visit Day -6, until HSCT but was finally evaluated from the day of the first administration of conditioning treatment, ie, visit Day -7, until HSCT. For TRM refer to the related endpoint "transplant-related mortality"

Transplantation-related mortality: TRM after HSCT was defined as the probability of dying from a transplant-related cause, i.e. which could not be attributed to disease relapse / progression or by deaths without previous relapse / progression. TRM was evaluated from end of HSCT to visit 12 months after HSCT. TRM was continuously assessed during the longer term follow up phase.

Overall survival: OS after HSCT was defined as the probability of surviving and was evaluated from the end of HSCT up to the visit 12 months after HSCT. OS was continuously assessed during the longer term follow up phase.

Relapse / Progression Incidence: The incidence of relapse / progression after HSCT was defined as the probability of having relapse / progression of the underlying disease, death due to any cause, or End of Trial, whatever comes first. RFS / PFS was continuously assessed during the longer term follow up phase.

Relapse-free / Progression-free Survival: RFS / PFS until 12 months after HSCT was defined as the time length between end of HSCT and the date of relapse / progression of the underlying disease or death due to any cause, or End of Trial, whatever comes first. RFS / PFS was continuously assessed during the longer term follow up phase.

Graft Failures: All subjects were continuously assessed for primary or secondary graft failure from end of the HST up to visit 12 months after HSCT. Secondary graft failure was continuously assessed during the longer term follow up phase.

Non-relapse Mortality: NRM after HSCT was defined as the probability of dying in the absence of persisting disease or previous occurrence of relapse / progression or graft failure. NRM was evaluated from end of the HSCT to visit 12 months after HSCT. NRM was continuously assessed during the longer term follow up phase.

Engraftment: Engraftment was defined as neutrophil count > 0.5 x 10^9/L, leucocyte count > 1 x 10^9/L, and platelet counts > 20 x 10^9/L or > 50 x10^9/L) and assessed up to 100 days after HSCT.

Donor-type Chimerism: Complete donor-type chimerism was defined as ≥ 95% donor cells detected and was evaluated on visit Day +28, +100 and the visit 12 months after HSCT.

Event-free Survival: EFS was assessed as an additional exploratory endpoint of the trial and was defined as the length of time between end of HSCT and the date of relapse / progression, graft failure, or death (whatever occurs first).

GvHD-free Relapse-free / Progression-free Survival: GvHD-free and RFS / PFS (GRFS) was assessed as an additional exploratory endpoint of the trial.

Chronic GvHD-free and Relapse-free / Progression-free Survival: Chronic GvHD-free and RFS / PFS was also assessed as an additional exploratory endpoint of the trial.

Rescue Therapies: Use of rescue therapies in order to prevent acute graft failure or disease relapse / progression was also assessed.

Pharmacokinetics:

Pharmacokinetics: Pharmacokinetic (PK) data were collected to contribute to a population model and to assess covariates. On day -6, blood samples were collected from a subset of subjects.

Study participants

This trial included paediatric subjects (infants / toddlers, children, and adolescents) with haematological malignant disease, who require myeloablative conditioning treatment followed by alloHSCT (first HSCT or second HSCT due to disease relapse, graft failure or secondary malignancy after previous autologous or alloHSCT).

There was no gender-specific effects or adverse reactions (ARs) known or to be expected. Thus, male and female subjects were to be included into the trial.

At least 70 evaluable male and female paediatric subjects from 28 days to < 18 years of age were to be included in this clinical trial and in the analysis. However, at least:

• 30 subjects from 28 days to < 10 years of age

• 30 subjects from 10 years to < 18 years of age

• 50 subjects receiving a first HSCT were to be included.

A maximum of 30 subjects with second alloHSCT were to be included in the trial. The recruitment for a specific age group was to be closed when a maximum of 40 subjects had been included in this age group.

Subjects were equally distributed between the CTP age groups of 28 days to < 10 years and 10 years to < 18 years (50% each), and more subjects were in the ICH age group of 12 to 17 years (47.1%) and 2 to 11 years (40.0%) than 28 days to 23 months (12.9%).

The intensified regime with TT was given to 65 patients (92.9%).

The donor type was matched unrelated donor (MUD) for 56 patients (80.0%), matched sibling donor (MSD) for 13 patients (18.6%) and matched family donor (MFD) for 1 patient (1.4%).

Baseline disease characteristics included ALL (n = 27), AML (n = 29), MDS (n = 10), and JMML (n = 4).

Inclusion criteria

1. Haematological malignant disease ie, ALL, AML, MDS, or JMML, indicated for alloHSCT
2. Indication for first alloHSCT or second alloHSCT due to disease relapse, graft failure, or secondary malignancy after previous HSCT
3. Available matched sibling donor (MSD), matched family donor (MFD), or matched unrelated donor (MUD). For BM and PB match was defined as 9 / 10 or 10 / 10 allele matches after 4 digit typing in human leucocyte antigens (HLAs)-A, B, C, and DRB1 and DQB1
4. Subjects with ALL or AML in complete morphologic remission (blast counts < 5% in BM) and subjects with MDS or JMML with blast counts < 20% in BM at trial entry
5. Age at time of registration from 28 days to < 18 years of age
6. Lansky (subjects aged < 16 years) or Karnofsky (subjects aged ≥ 16 years) performance score of at least 70%
7. Written informed consent of the parents / legal guardians and subject's assent / consent according to national regulations
8. Females of child-bearing potential or male subjects' partners with child-bearing potential had to have been using a highly effective method of contraception (pearl index < 1%) such as complete sexual abstinence, combined oral contraceptive, hormone intrauterine contraceptive device, vaginal hormone ring, transdermal contraceptive patch, contraceptive implant or depot contraceptive injection in combination with a second method of contraception like a condom, or a cervical cap / diaphragm with spermicide, or surgical sterilisation (vasectomy) in male subjects or male partners during the trial and at least 6 months thereafter
9. Negative pregnancy test for females of child bearing potential
10. All inclusion criteria had to be checked within 3 weeks before start of conditioning treatment on day -7. In case of repeated examinations due to hospital standards, the most current result was applicable (e.g., laboratory results)

Results

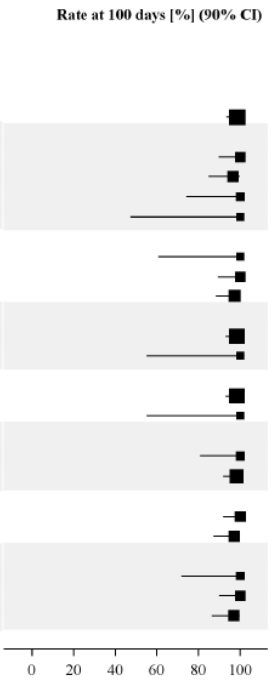
The results of the primary objective and the results of secondary objectives until Visit M 12 have been described in version 1.0 of this CTR. The longer-term follow-up efficacy results from subjects until 3

years after transplantation of the last registered subject are provided in this final CTR (version 2.0). The study is based on an approved PIP, incl. four agreed modifications. The agreed PIP includes a comparison of engraftment data (cumulative incidence) 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 (MC-FludT.14/L). Results comparing the month 12 data of MC-FludT.17/M trial with historical engraftment data are given.

The results of the primary endpoint were: Overall, the rate for freedom from transplant (treatment)-related mortality until 100 days after HSCT was 98.6% (90% CI: 93.4, 99.9). Due to the occurrence of only 1 event, no differential effects between subgroups could be identified. The respective subgroups in which the event appeared are shown in the forest plot.

Figure 11.4.1.1.A Forest plot for freedom from transplant (treatment)-related mortality until day +100 by subgroups (Full Analysis Set)

	N	n	Rate at 100 days [%] (90% CI)
Overall	70	1	98.6 (93.4, 99.9)
Disease			
ALL	27	0	100.0 (89.5, 100.0)
AML	29	1	96.6 (84.7, 99.8)
MDS	10	0	100.0 (74.1, 100.0)
JMML	4	0	100.0 (47.3, 100.0)
Treosulfan dose			
10 g/m2/day	6	0	100.0 (60.7, 100.0)
12 g/m2/day	26	0	100.0 (89.1, 100.0)
14 g/m2/day	38	1	97.4 (88.1, 99.9)
Number of HSCT			
1st	65	1	98.5 (92.9, 99.9)
2nd	5	0	100.0 (54.9, 100.0)
Thiotepa			
yes	65	1	98.5 (92.9, 99.9)
no	5	0	100.0 (54.9, 100.0)
Donor type			
MRD	14	0	100.0 (80.7, 100.0)
MUD	56	1	98.2 (91.8, 99.9)
CTP age group			
28 days to less than 10 years	35	0	100.0 (91.8, 100.0)
10 years to less than 18 years	35	1	97.1 (87.1, 99.9)
ICH age group			
28 days to 23 months	9	0	100.0 (71.7, 100.0)
2 to 11 years	28	0	100.0 (89.9, 100.0)
12 to 17 years	33	1	97.0 (86.4, 99.8)



N = Number of subjects, n = Number of events until 100 days

Sources: Figure 14.2.1A. Table 14.2.1A, Listing 16.2.6F.
Abbreviations: ALL = acute lymphoblastic leukaemia; AML = acute myeloid leukaemia; CI = confidence interval; CTP = Clinical Trial Protocol; HSCT = haematopoietic stem cell transplantation; ICH = International Council of Harmonisation; JMML = juvenile myelomonocytic leukaemia; MDS = myelodysplastic syndrome; MRD = matched related donor; MUD = matched unrelated donor;

The key results of the exploratory analysis of the secondary endpoints were:

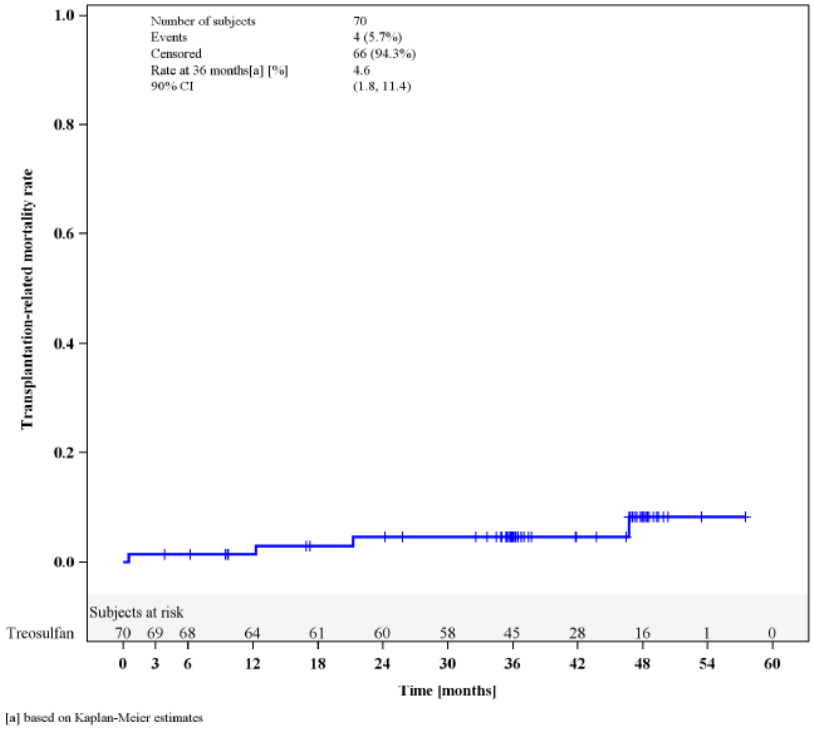
1. TRM: At the end of the longer-term follow-up period, 4 subjects (5.7%) had died from a transplant-related cause. The Kaplan-Meier estimate of TRM at 12 months was 1.4% (90% CI: 0.3, 7.2) and increased to 4.6% (90% CI: 1.8, 11.4) at 36 months.

Table 11.4.1.2.A Summary results of transplant-related mortality (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	4 (5.7)
Subjects without event [n (%)]	66 (94.3)
Transplantation-related mortality at 100 days[a] [%]	1.4
90% CI	(0.3, 7.2)
Transplantation-related mortality at 12 months[a] [%]	1.4
90% CI	(0.3, 7.2)
Transplantation-related mortality at 24 months[a] [%]	4.6
90% CI	(1.8, 11.4)
Transplantation-related mortality at 36 months[a] [%]	4.6
90% CI	(1.8, 11.4)

Sources: Table 14.2.2A, Table 14.2.2B, Listing 16.2.6F
[a] Based on Kaplan-Meier estimates
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

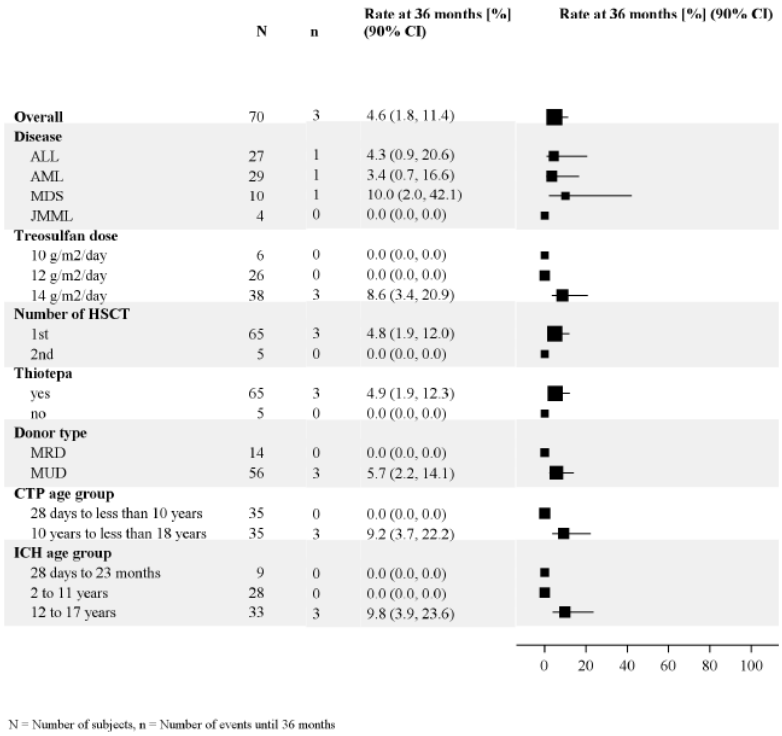
Figure 11.4.1.2.A Kaplan-Meier estimates of transplant-related mortality (Full Analysi Set)



Sources: Figure 14.2.2A, Table 14.2.2A, Table 14.2.2B, Listing 16.2.6F.
Abbreviations: CI = confidence interval.

A statistically significant difference in TRM was recorded between CTP age groups; however, only few events were observed and small numbers of subjects were in these groups, which also cover different diseases and risk groups.

Figure 11.4.1.2.B Forest plot for transplant-related mortality displaying 36-months rates by subgroups (Full Analysis Set)



	N	n	Rate at 36 months [%] (90% CI)
Overall	70	3	4.6 (1.8, 11.4)
Disease			
ALL	27	1	4.3 (0.9, 20.6)
AML	29	1	3.4 (0.7, 16.6)
MDS	10	1	10.0 (2.0, 42.1)
JMML	4	0	0.0 (0.0, 0.0)
Treosulfan dose			
10 g/m2/day	6	0	0.0 (0.0, 0.0)
12 g/m2/day	26	0	0.0 (0.0, 0.0)
14 g/m2/day	38	3	8.6 (3.4, 20.9)
Number of HSCT			
1st	65	3	4.8 (1.9, 12.0)
2nd	5	0	0.0 (0.0, 0.0)
Thiotepa			
yes	65	3	4.9 (1.9, 12.3)
no	5	0	0.0 (0.0, 0.0)
Donor type			
MRD	14	0	0.0 (0.0, 0.0)
MUD	56	3	5.7 (2.2, 14.1)
CTP age group			
28 days to less than 10 years	35	0	0.0 (0.0, 0.0)
10 years to less than 18 years	35	3	9.2 (3.7, 22.2)
ICH age group			
28 days to 23 months	9	0	0.0 (0.0, 0.0)
2 to 11 years	28	0	0.0 (0.0, 0.0)
12 to 17 years	33	3	9.8 (3.9, 23.6)

N = Number of subjects, n = Number of events until 36 months

Sources: Figure 14.2.2C, Table 14.2.2A, Table 14.2.2D, Table 14.2.2E, Table 14.2.2F, Table 14.2.2G, Table 14.2.2H, Table 14.2.2I, Table 14.2.2J, Listing 16.2.6F.

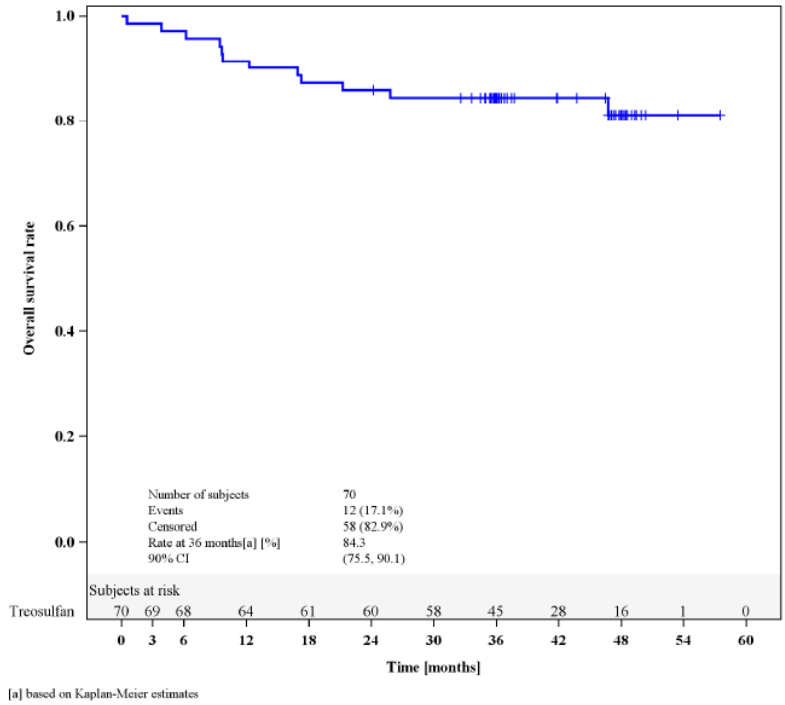
2. OS: The median duration of follow-up based on reverse Kaplan-Meier estimate was 41.8 months (range of those surviving: 24.2 months to 57.5 months). At the end of the longer-term follow-up period, 58 subjects (82.9%) were alive. The Kaplan-Meier estimate of OS at 12 months after HSCT was 91.4% (90% CI: 83.9, 95.5) and was 84.3% (90% CI: 75.5, 90.1) at 36 months after HSCT.

Table 11.4.1.3.A Summary results of overall survival (Full Analysis Set)

	Treosulfan (N=70)
Median follow-up[a] [months] (range of those surviving)	41.8 (24.2, 57.5)
Subjects with event [n (%)]	12 (17.1)
Subjects without event [n (%)]	58 (82.9)
Overall survival at 12 months[b] [%]	91.4
90% CI	(83.9, 95.5)
Overall survival at 24 months[b] [%]	85.7
90% CI	(77.1, 91.2)
Overall survival at 36 months[b] [%]	84.3
90% CI	(75.5, 90.1)

Sources: Table 14.2.3A, Table 14.2.3B, Listing 16.2.6F
[a] Based on reverse Kaplan-Meier estimates
[b] Based on Kaplan-Meier estimates
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.3.A Kaplan-Meier estimates of overall survival (Full Analysis Set)



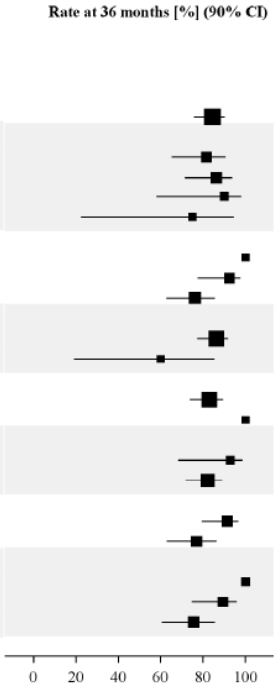
[a] based on Kaplan-Meier estimates

Sources: Figure 14.2.3A, Table 14.2.3A, Table 14.2.3B, Listing 16.2.6F.
Abbreviations: CI = confidence interval.

OS at 36 months was comparable for the disease groups, ie, 81.5% for ALL subjects, 86.2% for AML subjects, 90.0% for MDS subjects, and 75.0% for JMML subjects. Due to the individual BSA-related dose calculation, OS was 100% in the 10 g/m^2/day Treosulfan group, however with only 6 subjects, and 76.2% in the 14 g/m^2/day Treosulfan group. As expected, a statistically significant difference in OS was recorded between subjects in first HSCT (36-month rate: 86.1%) and subjects in second HSCT (36-month rate: 60.0%); however, only few events were observed and only 5 subjects were in the latter group. A statistically significant difference in OS was also observed between the CTP age groups.

Figure 11.4.1.3.B Forest plot for overall survival displaying 36-months rates by subgroups (Full Analysis Set)

	N	n	Rate at 36 months [%] (90% CI)
Overall	70	11	84.3 (75.5, 90.1)
Disease			
ALL	27	5	81.5 (65.2, 90.7)
AML	29	4	86.2 (71.3, 93.7)
MDS	10	1	90.0 (57.9, 98.0)
JMML	4	1	75.0 (22.3, 94.6)
Treosulfan dose			
10 g/m2/day	6	0	100.0 (100.0, 100.0)
12 g/m2/day	26	2	92.3 (77.4, 97.5)
14 g/m2/day	38	9	76.2 (62.5, 85.5)
Number of HSCT			
1st	65	9	86.1 (77.2, 91.7)
2nd	5	2	60.0 (19.1, 85.4)
Thiotepa			
yes	65	11	83.0 (73.7, 89.3)
no	5	0	100.0 (100.0, 100.0)
Donor type			
MRD	14	1	92.9 (68.1, 98.6)
MUD	56	10	82.1 (71.7, 88.9)
CTP age group			
28 days to less than 10 years	35	3	91.4 (79.3, 96.6)
10 years to less than 18 years	35	8	77.0 (62.6, 86.5)
ICH age group			
28 days to 23 months	9	0	100.0 (100.0, 100.0)
2 to 11 years	28	3	89.3 (74.6, 95.7)
12 to 17 years	33	8	75.6 (60.6, 85.6)



N = Number of subjects, n = Number of events until 36 months

Sources: Figure 14.2.3B, Table 14.2.3A, Table 14.2.3C, Table 14.2.3D, Table 14.2.3E, Table 14.2.3F, Table 14.2.3G, Table 14.2.3H, Table 14.2.3I, Listing 16.2.6F.

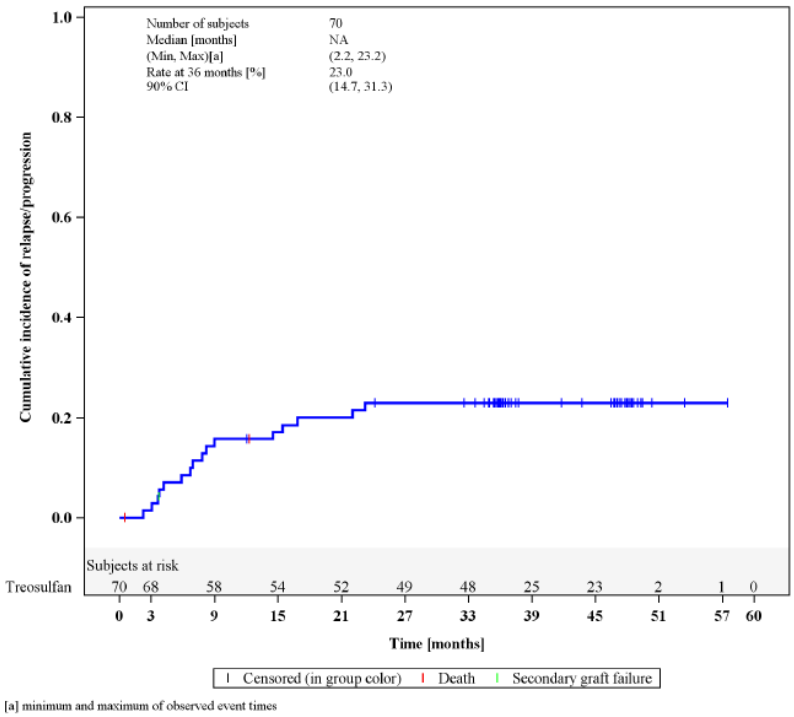
3. Relapse / Progression Incidence: At the end of the longer-term follow-up period, 16 subjects (22.9%) had experienced disease relapse / progression. The cumulative incidence of relapse / progression at 12 months was 15.7% (90% CI: 8.6, 22.9) and at 36 months was 23.0% (90% CI: 14.7, 31.3). No relapse was observed beyond 24 months after HSCT.

Table 11.4.1.4.A Summary results of relapse / progression (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	16 (22.9)
Subjects without event (censored) or with competing event [n (%)]	54 (77.1)
Censored	51 (72.9)
Death[a]	2 (2.9)
Primary graft failure[a]	0 (0.0)
Secondary graft failure[a]	1 (1.4)
Cumulative incidence of relapse/progression at 12 months (%)	15.7
90% CI	(8.6, 22.9)
Cumulative incidence of relapse/progression at 24 months (%)	23.0
90% CI	(14.7, 31.3)
Cumulative incidence of relapse/progression at 36 months (%)	23.0
90% CI	(14.7, 31.3)

Sources: Table 14.2.4A, Table 14.2.4B, Listing 16.2.6F

Figure 11.4.1.4.A Cumulative incidence of relapse / progression (Full Analysis Set)

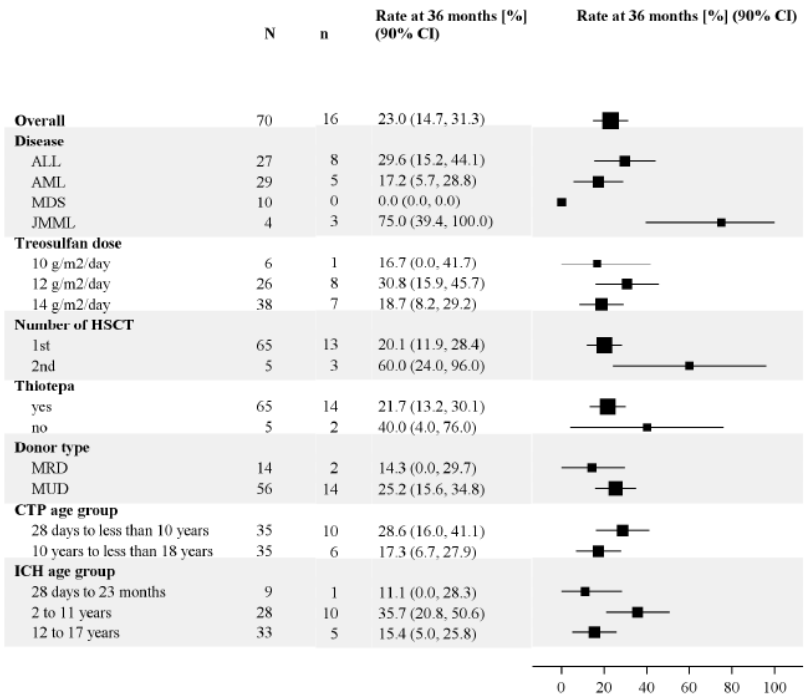


[a] minimum and maximum of observed event times

Sources: Figure 14.2.4A, Table 14.2.4A, Table 14.2.4B, Listing 16.2.6F.

In the disease subgroups, a statistically significant difference in relapse / progression was recorded between JMML (75%), ALL (29.6%), AML (17.2%), and MDS (0.0%) subgroups; however, only small numbers of JMML and MDS subjects were in these groups. A statistically significant difference in relapse / progression was also recorded between subjects in first HSCT (20.1%) and subjects in second HSCT (60.0%); however, only 5 subjects were in the second transplant group reflecting their poor prognosis in general.

Figure 11.4.1.4.B Forest plot for relapse / progression displaying 36-months rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events until 36 months

Sources: Figure 14.2.4B, Table 14.2.4B, Table 14.2.4C, Table 14.2.4D, Table 14.2.4E, Table 14.2.4F, Table 14.2.4G, Table 14.2.4H, Table 14.2.4I, Listing 16.2.6F.

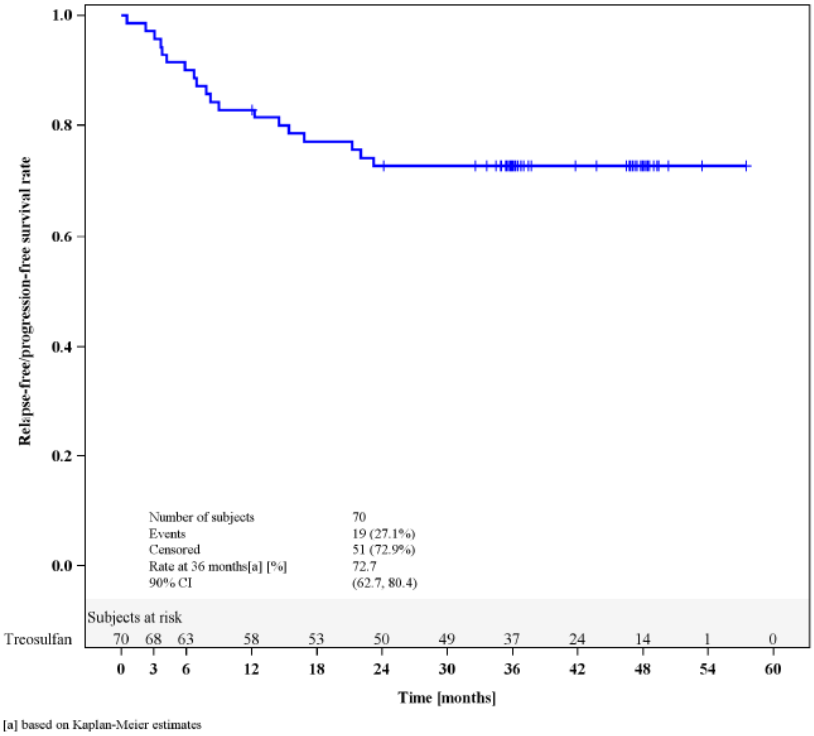
4. RFS / PFS until 12 months after HSCT: At the end of the longer-term follow-up period, 16 subjects (22.9%) had experienced disease relapse / progression and 3 subjects (4.3%) had died without previous relapse / progression. The Kaplan-Meier estimate of RFS / PFS at 12 months was 82.9% (90% CI: 73.9, 89.0), reached a plateau at 24 months and remained 72.7% (90% CI: 62.7, 80.4) at 36 months after HSCT.

Table 11.4.1.5.A Summary results of relapse-free / progression-free survival (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	19 (27.1)
Death[a]	3 (4.3)
Relapse/Progression[a]	16 (22.9)
Subjects without event [n (%)]	51 (72.9)
Relapse-free/progression-free survival at 12 months[b] (%)	82.9
90% CI	(73.9, 89.0)
Relapse-free/progression-free survival at 24 months[b] (%)	72.7
90% CI	(62.7, 80.4)
Relapse-free/progression-free survival at 36 months[b] (%)	72.7
90% CI	(62.7, 80.4)

Sources: Table 14.2.5A, Table 14.2.5B, Listing 16.2.6F
[a] Only if this event occurred first
[b] Based on Kaplan-Meier estimates
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.5.A Kaplan-Meier estimates of relapse-free / progression-free survival (Ful Analysis Set)

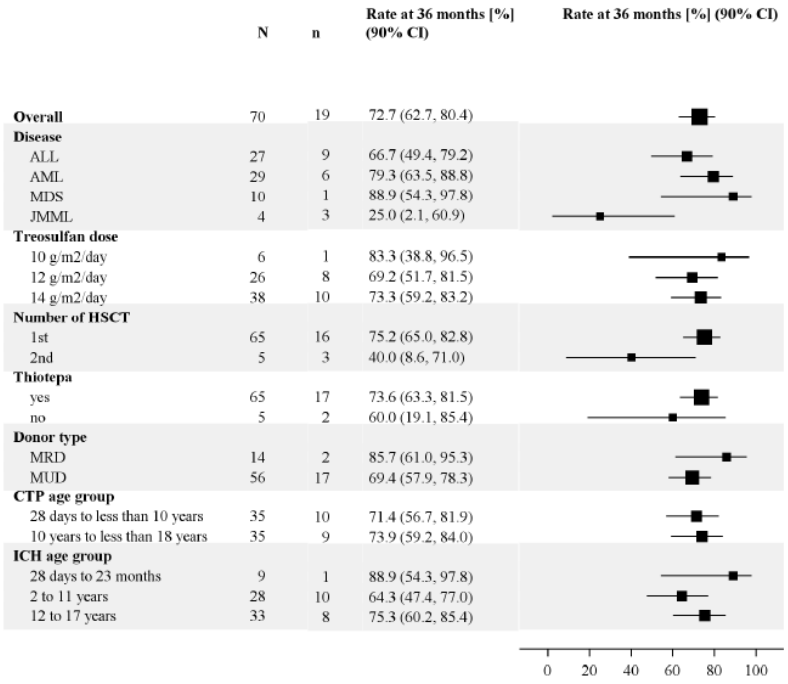


[a] based on Kaplan-Meier estimates

Sources: Figure 14.2.5A, Table 14.2.5A, Table 14.2.5B, Listing 16.2.6F

In the disease subgroups, a statistically significant difference in RFS / PFS was recorded between JMML (25.0%), ALL (66.7%), AML (79.3%), and MDS (88.9%) subgroups; however, only small numbers JMML and MDS subjects were in these groups. As expected, a statistically significant difference in RFS / PFS was also recorded between subjects in first HSCT (75.2%) and subjects in second HSCT (40.0%); however, only 5 subjects were in the second HSCT group. In contrast, no significant differences were observed between the different dose groups.

Figure 11.4.1.5.B Forest plot for relapse-free / progression-free survival displaying 36 months rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events until 36 months

Sources: Figure 14.2.5B, Table 14.2.5A, Table 14.2.5C, Table 14.2.5D, Table 14.2.5E, Table 14.2.5F, Table 14.2.5G, Table 14.2.5H, Table 14.2.5I, Listing 16.2.6F.

5. Graft failure after HSCT: No subject experienced a primary graft failure and only 1 subject with ALL experienced a secondary graft failure.

Table 11.4.1.6.A Results of primary and secondary graft failure (Full Analysis Set)

	n/N (%) [a]	90% CI
Primary graft failure	0/70 (0.0)	(0.0, 4.2)
Secondary graft failure	1/69 (1.4)	(0.1, 6.7)

Source: Table 14.2.6A, Table 14.2.6B, Listing 16.2.6B
[a] No. of events (n) / No. at risk (N), where No. of events = No. of subjects with graft failure
Note: At risk for primary graft failure: subjects with HSCT. At risk for secondary graft failure: Subjects whose neutrophilic granulocytes engrafted after HSCT or were never below the required level
Abbreviations: CI = confidence interval; HSCT = haematopoietic stem cell transplantation; N = number of subjects; n = number of subjects in category.

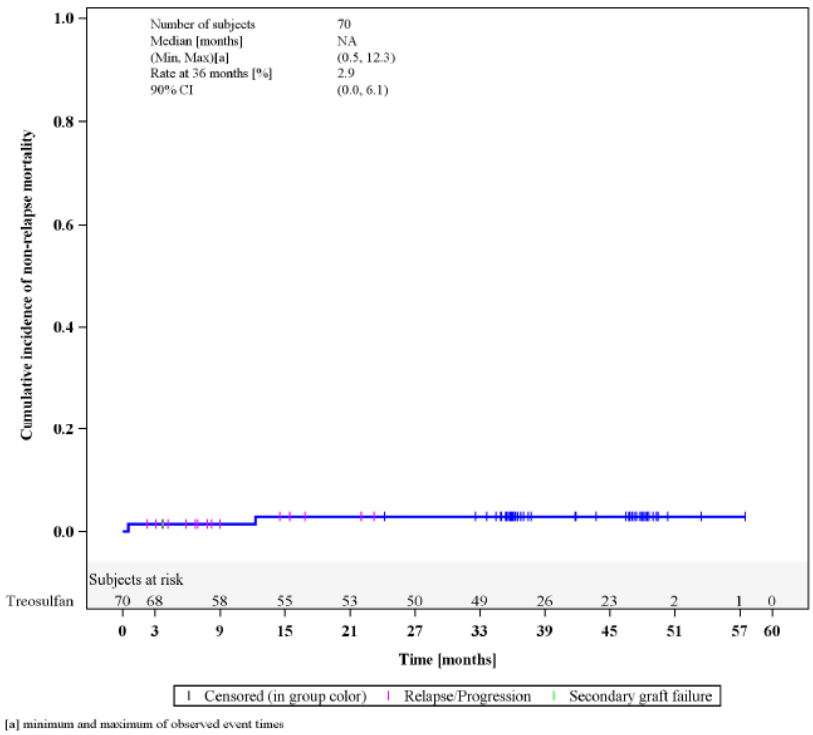
6. NRM after HSCT: At the end of the longer-term follow-up period, 2 subjects (2.9%) had died without relapse or graft failure. The cumulative incidence of NRM at 12 months was 1.4% (90% CI: 0.0, 3.8) and was only 2.9% (90% CI: 0.0, 6.1) at 36 months after HSCT.

Table 11.4.1.7.A Summary results of non-relapse mortality (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	2 (2.9)
Subjects without event (censored) or with competing event [n (%)]	68 (97.1)
Censored	51 (72.9)
Relapse/Progression[a]	16 (22.9)
Primary graft failure[a]	0 (0.0)
Secondary graft failure[a]	1 (1.4)
Cumulative incidence of non-relapse mortality at 12 months (%)	1.4
90% CI	(0.0, 3.8)
Cumulative incidence of non-relapse mortality at 24 months (%)	2.9
90% CI	(0.0, 6.1)
Cumulative incidence of non-relapse mortality at 36 months (%)	2.9
90% CI	(0.0, 6.1)

Sources: Table 14.2.7A, Table 14.2.7B, Listing 16.2.6F
[a] Only if this event occurred first
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.7.A Cumulative incidence of non-relapse mortality (Full Analysis Set)



[a] minimum and maximum of observed event times

Sources: Figure 14.2.7A, Table 14.2.7A, Table 14.2.7B, Listing 16.2.6F.

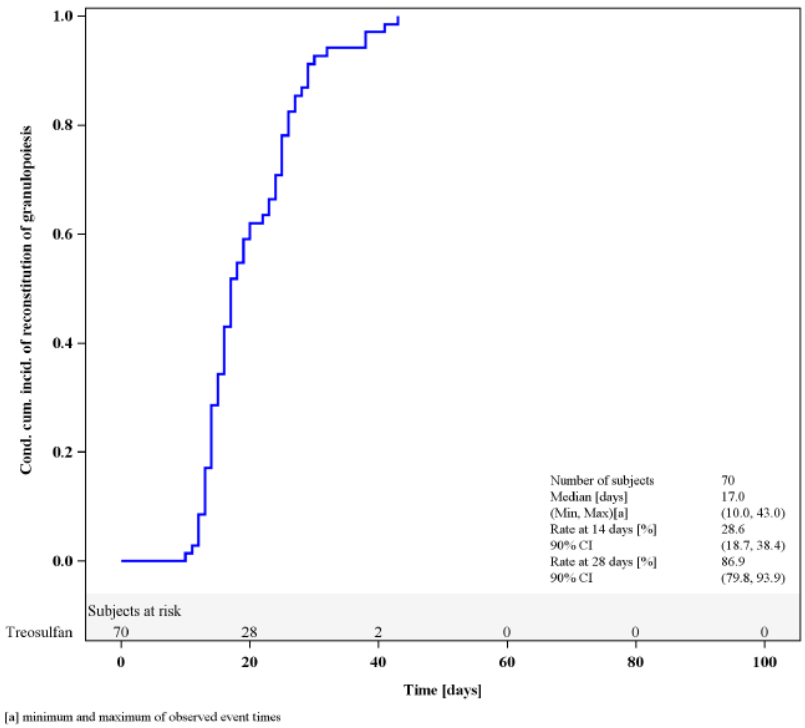
7. Engraftment: The number of subjects with reconstitution of granulopoiesis was 69 (98.6%). The conditional cumulative incidence at 14 days after HSCT was 28.6% (90% CI: 18.7, 38.4) and increased to 86.9% (90% CI: 79.8, 93.9) at 28 days after HSCT. The maximum conditional cumulative incidence reached was 100.0% (90% CI: 97.7, 100.0).

Table 11.4.1.8.A Summary results of reconstitution of granulopoiesis (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	69 (98.6)
Subjects without event (censored) or with competing event [n (%)]	1 (1.4)
Censored	1 (1.4)
Death[a]	0 (0.0)
Relapse/Progression[a]	0 (0.0)
Rescue therapies[a]	0 (0.0)
Conditional cumulative incidence at 14 days (%)	28.6
90% CI	(18.7, 38.4)
Conditional cumulative incidence at 28 days (%)	86.9
90% CI	(79.8, 93.9)
Maximum conditional cumulative incidence reached (%)	100.0
90% CI	(97.7, 100.0)

Sources: Table 14.2.8A, Table 14.2.8B, Listing 16.2.6E
[a]Only if this event occurred first.
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.8.A Conditional cumulative incidence of reconstitution of granulopoiesis (Full Analysis Set)



Sources: Figure 14.2.8A, Table 14.2.8A, Table 14.2.8B, Listing 16.2.6E

All subjects experienced neutropenia. Despite the excellent engraftment rate, the median duration of neutropenia was long (22 days; range 7 days to 44 days).

Table 11.4.1.8.B Descriptive analysis of duration of neutropenia (Full Analysis Set)

	Treosulfan (N=70)
Neutropenia [n (%)]	
Yes[a]	70 (100.0)
Duration of neutropenia [days][b]	
N	69
Mean (SD)	22.3 (7.7)
Median (Q1, Q3)	22.0 (17.0, 26.0)
Min, Max	7, 44

Source: Table 14.2.8K, Listing 16.2.6E
[a] Neutrophilic granulocytes <= 0.5 G/L at least once between Day -7 and Day +28
[b] First date with neutropenia until date of engraftment (subjects at risk = subjects with neutropenia and neutrophilic granulopoesis).
Abbreviations: Max = maximum; Min = minimum; N = number of subjects; n = number of subjects in category; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.

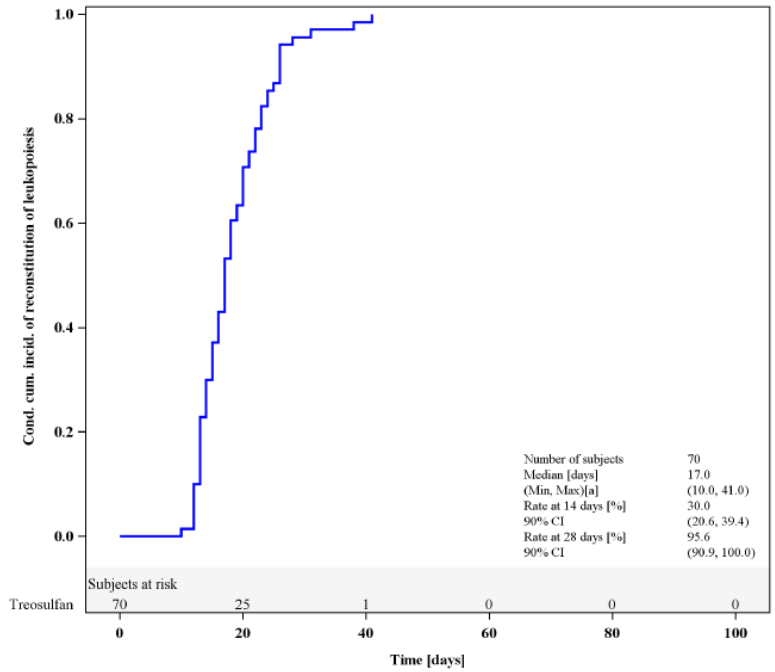
The number of subjects with reconstitution of leukopoiesis was 69 (98.6%). The conditional cumulative incidence at 14 days after HSCT was 30.0% (90% CI: 20.6, 39.4) and increased to 95.6% (90% CI: 90.9, 100.0) at 28 days after HSCT. The maximum conditional cumulative incidence reached was 100.0% (90% CI: 97.7, 100.0).

Table 11.4.1.8.C Summary results of reconstitution of leukopoiesis (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	69 (98.6)
Subjects without event (censored) or with competing event [n (%)]	1 (1.4)
Censored	1 (1.4)
Death[a]	0 (0.0)
Relapse/Progression[a]	0 (0.0)
Rescue therapies[a]	0 (0.0)
Conditional cumulative incidence at 14 days (%)	30.0
90% CI	(20.6, 39.4)
Conditional cumulative incidence at 28 days (%)	95.6
90% CI	(90.9, 100.0)
Maximum conditional cumulative incidence reached (%)	100.0
90% CI	(97.7, 100.0)

Sources: Table 14.2.9A, Table 14.2.9B, Listing 16.2.6E
[a] Only if this event occurred first
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.8.C Conditional cumulative incidence of reconstitution of leukopoiesis (Full Analysis Set)



[a] minimum and maximum of observed event times

Sources: Figure 14.2.9A, Table 14.2.9A, Table 14.2.9B, Listing 16.2.6E.

All subjects experienced leukopenia. Despite the excellent engraftment rate, the median duration of leukopenia was long (20 days; range 11 days to 42 days).

Table 11.4.1.8.D Descriptive analysis of duration of leukopenia (Full Analysis Set)

	Treosulfan (N=70)
Leukopenia [n (%)]	
Yes[a]	70 (100.0)
Duration of leukopenia [days][b]	
N	69
Mean (SD)	20.5 (6.1)
Median (Q1, Q3)	20.0 (16.0, 24.0)
Min, Max	11, 42

Source: Table 14.2.9K, Listing 16.2.6E

[a] Leucocytes ≤ 1 G/L at least once between Day -7 and Day +28.

[b] First date with leukopenia until date of engraftment (subjects at risk = subjects with leukopenia and leucopoesis).

Abbreviations: Max = maximum; Min = minimum; N = number of subjects; n = number of subjects in category; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.

The number of subjects with reconstitution of thrombopoiesis > 20 x 109/L was 65 (92.9%). The conditional cumulative incidence at 14 days after HSCT was 34.3% (90% CI: 24.5, 44.1) and increased to 78.0% (90% CI: 69.5, 86.5) at 28 days after HSCT. The maximum conditional cumulative incidence reached was 94.1% (90% CI: 88.4, 99.9). In the disease subgroups, a statistically significant difference in reconstitution of thrombopoiesis > 20 x 109/L was recorded between AML (100.0%), ALL (92.6%), MDS (90.0%), and JMML (75.0%) subgroups; however, only small number of MDS and JMML subjects were in these groups.

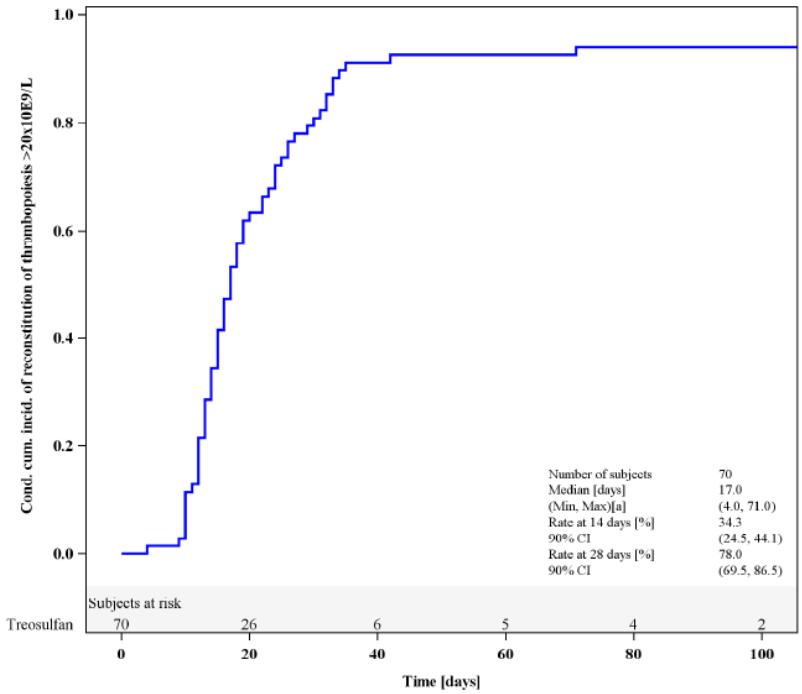
Table 11.4.1.8.E Summary results of reconstitution of thrombopoiesis > 20 x 10^9/L (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	65 (92.9)
Subjects without event (censored) or with competing event	5 (7.1)
Censored	5 (7.1)
Death[a]	0 (0.0)
Relapse/Progression[a]	0 (0.0)
Rescue therapies[a]	0 (0.0)
Conditional cumulative incidence at 14 days (%)	34.3
90% CI	(24.5, 44.1)
Conditional cumulative incidence at 28 days (%)	78.0
90% CI	(69.5, 86.5)
Maximum conditional cumulative incidence reached (%)	94.1
90% CI	(88.4, 99.9)

Sources: Table 14.2.10A, Table 14.2.10B, Listing 16.2.6E.

[a] Only if this event occurred first.
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.8.E Conditional cumulative incidence of reconstitution of thrombopoiesis > 20 x 10^9/L (Full Analysis Set)



[a] minimum and maximum of observed event times

Sources: Figure 14.2.10A, Table 14.2.10A, Table 14.2.10B, Listing 16.2.6E

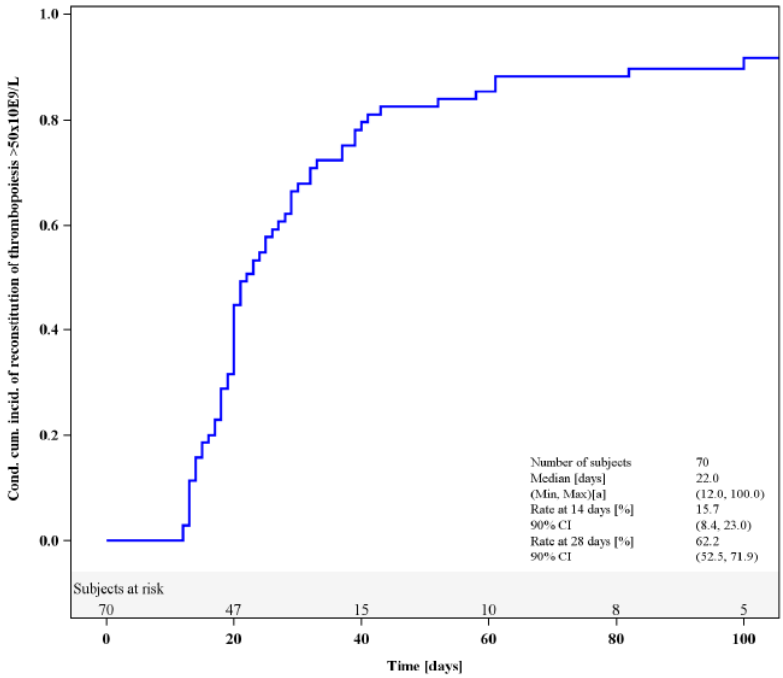
The number of subjects with reconstitution of thrombopoiesis > 50 x 10^9/L was 63 (90.0%). The conditional cumulative incidence at 14 days after HSCT was 15.7% (90% CI: 8.4, 23.0) and increased to 62.2% (90% CI: 52.5, 71.9) at 28 days after HSCT. The maximum conditional cumulative incidence reached was 91.9% (90% CI: 84.9, 98.8). In the disease subgroups, a statistically significant difference in reconstitution of thrombopoiesis > 50 x 10^9/L was recorded between AML (100.0%) and ALL (90.1%) subgroups; however, only small numbers of subjects were in these groups.

Table 11.4.1.8.F Summary results of reconstitution of thrombopoiesis > 50 x 10^9/L (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	63 (90.0)
Subjects without event (censored) or with competing event [n (%)]	7 (10.0)
Censored	7 (10.0)
Death[a]	0 (0.0)
Relapse/Progression[a]	0 (0.0)
Rescue therapies[a]	0 (0.0)
Conditional cumulative incidence at 14 days (%)	15.7
90% CI	(8.4, 23.0)
Conditional cumulative incidence at 28 days (%)	62.2
90% CI	(52.5, 71.9)
Maximum conditional cumulative incidence reached (%)	91.9
90% CI	(84.9, 98.8)

Sources: Table 14.2.11A, Table 14.2.11B, Listing 16.2.6E
[a] Only if this event occurred first
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.8.F Conditional cumulative incidence of reconstitution of thrombopoiesis > 50 x 10^9/L (Full Analysis Set)



[a] minimum and maximum of observed event times

Sources: Figure 14.2.11A, Table 14.2.11A, Table 14.2.11B, Listing 16.2.6E

8. Donor-type Chimerism: At visit Day +28, the incidence of complete donor-type chimerism was 94.2% (90% CI: 87.2, 98.0), at visit Day +100 the incidence was 91.3% (90% CI: 83.6, 96.1), and at visit Month 12 the incidence was 91.2% (90% CI: 82.4, 96.5). In the subgroups, a statistically significant difference was recorded for disease at visit Day +100 and at visit Month 12, and for the use of Thiotepa at visit Day +28. An incomplete donor-type chimerism was detected for 3 subjects at visit Day +28. However, 2 of them developed a complete donor-type chimerism by visit Day +100. Only one subject with JMML was assessed with a mixed donor-type chimerism of only 12% on visit Day +28, further decreasing to 2% on visit Month 12 suggesting an autologous recovery of haematopoiesis. The detected Treosulfan AUC∞ (ie, 271 µg/h*mL) of this subject was far below the expected range considered safe and effective (760 to 3600 µg.h/mL). No relapse was reported for this subject until 12 months after HSCT.

Table 11.4.1.9.A Incidence of complete donor-type chimerism (Full Analysis Set)

	Treosulfan
Subjects at risk at Day +28 visit[a]	**N=69**
Subjects with complete chimerism at Day +28 visit [n (%)]	65 (94.2)
Subjects without complete chimerism at Day +28 visit [n (%)]	3 (4.3)
Subjects without information at Day +28 visit [n (%)]	1 (1.4)
Incidence of complete chimerism at Day +28 visit (%)	94.2
90% CI	(87.2, 98.0)
Subjects at risk at Day +100 visit[a]	**N=69**
Subjects with complete chimerism at Day +100 visit [n (%)]	63 (91.3)
Subjects without complete chimerism at Day +100 visit [n (%)]	6 (8.7)
Incidence of complete chimerism at Day +100 visit (%)	91.3
90% CI	(83.6, 96.1)
Subjects at risk at Month 12 visit[a]	**N=57**
Subjects with complete chimerism at Month 12 visit [n (%)]	52 (91.2)
Subjects without complete chimerism at Month 12 visit [n (%)]	3 (5.3)
Subjects without information at Month 12 visit [n (%)]	2 (3.5)
Incidence of complete chimerism at Month 12 visit (%)	91.2
90% CI	(82.4, 96.5)

Source: Table 14.2.12A, Listing 16.2.6C
[a] Subjects are at risk if they have a chimerism examination at the Day +28, Day +100, or Month 12 visit or if they have survived day +30, day +107, or day +379, and did not experience any relapse/progression or graft failure until the start of the visit that is, day +28, day +100 or day +365 respectively.
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

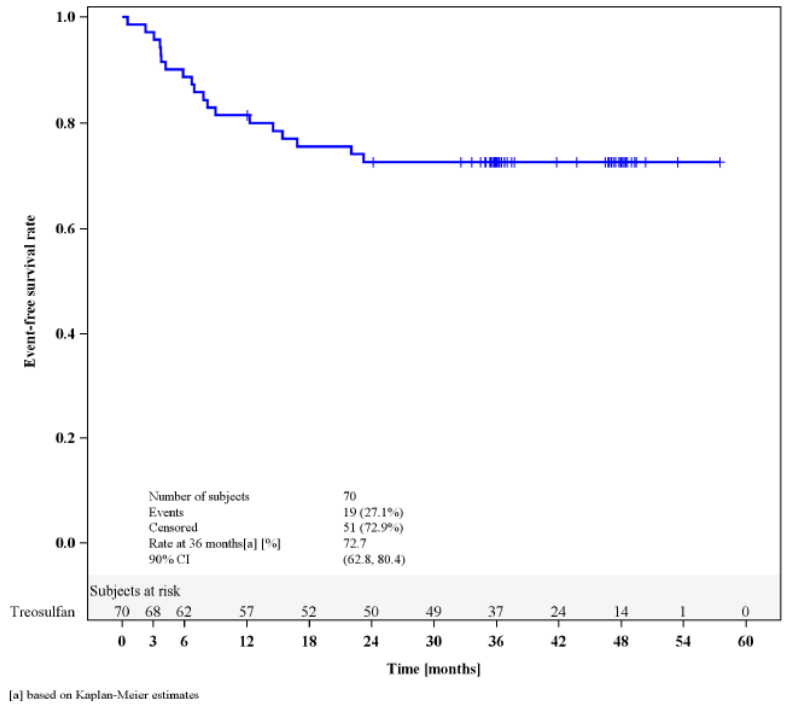
9. Event-free Survival: At the end of the longer-term follow-up period, 19 subjects (27.1%) experienced an event. The Kaplan-Meier estimate of EFS at 12 months was 81.4% (90% CI: 72.3, 87.8), reached a plateau after 24 months and was 72.7% (62.8, 80.4) at 36 months.

Table 11.4.1.10.A Summary results of event-free survival (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	19 (27.1)
Death[a]	2 (2.9)
Relapse/Progression[a]	16 (22.9)
Primary graft failure[a]	0 (0.0)
Secondary graft failure[a]	1 (1.4)
Subjects without event [n (%)]	51 (72.9)
Event-free survival at 12 months[b] (%)	81.4
90% CI	(72.3, 87.8)
Event-free survival at 24 months[b] (%)	72.7
90% CI	(62.8, 80.4)
Event-free survival at 36 months[b] (%)	72.7
90% CI	(62.8, 80.4)

Sources: Table 14.2.13A, Table 14.2.13B, Listing 16.2.6G
[a] Only if this event occurred first
[b] Based on Kaplan-Meier estimates
Abbreviations: CI = confidence interval; N = number of subjects; n = number of subjects in category.

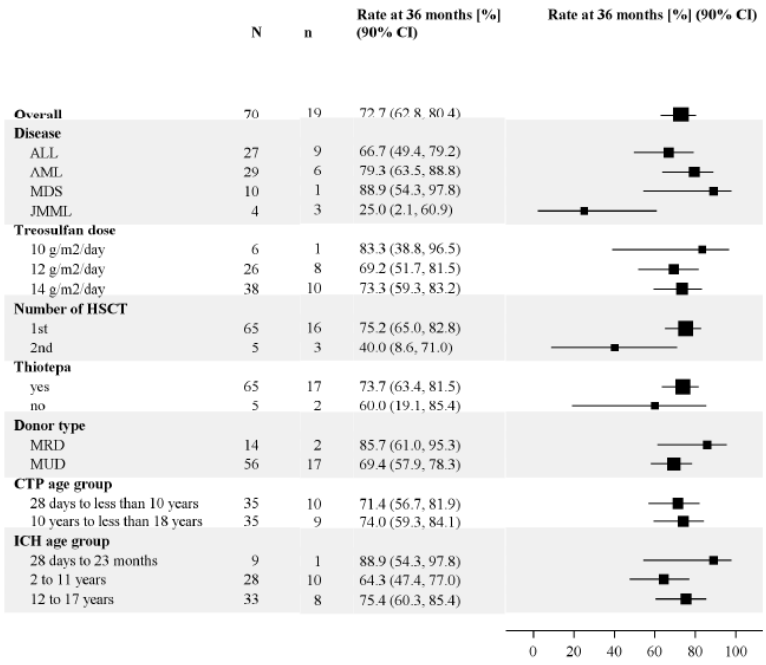
Figure 11.4.1.10.A Kaplan-Meier estimates of event-free survival (Full Analysis Set)



Sources: Figure 14.2.13A, Table 14.2.13A, Table 14.2.13B, Listing 16.2.6G.

A statistically significant difference in EFS was recorded between JMML (25.0%), ALL (66.7%), AML (79.3%), or MDS (88.9%) disease subgroups, however, only small numbers of subjects were in the JMML and MDS groups. No statistically significant difference was recorded between the age subgroups and the dose subgroups. As expected, a statistically difference in EFS was also recorded between subjects in first HSCT (75.2%) and subjects in second HSCT (40.0%), however, only 5 subjects were in the second HSCT group.

Figure 11.4.1.10.B Forest plot for event-free survival displaying 36-months rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events until 36 months

Sources: Figure 14.2.13B, Table 14.2.13A, Table 14.2.13C, Table 14.2.13D, Table 14.2.13E, Table 14.2.13F, Table 14.2.13G, Table 14.2.13H, Table 14.2.13I, Listing 16.2.6G.

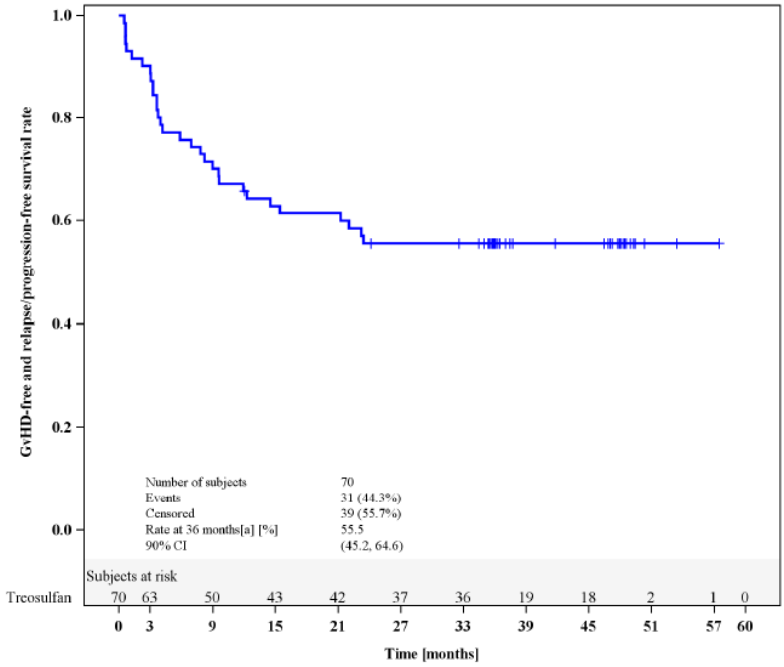
10. GvHD-free Relapse-free / Progression-free Survival: At the end of the longer-term follow-up period, 39 subjects (55.7%) were alive and had not experienced GvHD (ie, aGvHD ≥ Grade III or moderate / severe cGvHD) or relapse / progression. The Kaplan-Meier estimate of GRFS at 12 months was 65.7% (90% CI: 55.5, 74.1), reached a plateau at 24 months, and was 55.5% (90% CI: 45.2, 64.6) at 36 months.

Table 11.4.1.11.A Summary results of GvHD-free and relapse / progression-free survival (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event [n (%)]	31 (44.3)
Death[a]	3 (4.3)
Relapse/Progression[a]	14 (20.0)
Acute GvHD of at least Grade III[a]	6 (8.6)
Moderate/severe chronic GvHD[a]	8 (11.4)
Subjects without event [n (%)]	39 (55.7)
GvHD-free and relapse/progression-free survival at 12 months[b] (%)	65.7
90% CI	(55.5, 74.1)
GvHD-free and relapse/progression-free survival at 24 months[b] (%)	55.5
90% CI	(45.2, 64.6)
GvHD-free and relapse/progression-free survival at 36 months[b] (%)	55.5
90% CI	(45.2, 64.6)

Sources: Table 14.2.14A, Table 14.2.14B, Listing 16.2.6G
[a] Only if this event occurred first
[b] Based on Kaplan-Meier estimates
Note: GvHD-free defined as no acute GvHD of at least Grade III and no moderate/severe chronic GvHD
Abbreviations: CI = confidence interval; GvHD = graft-versus-host disease; N = number of subjects; n = number of subjects in category.

Figure 11.4.1.11.A Kaplan-Meier estimates of GvHD-free and relapse / progression-free survival (Full Analysis Set)

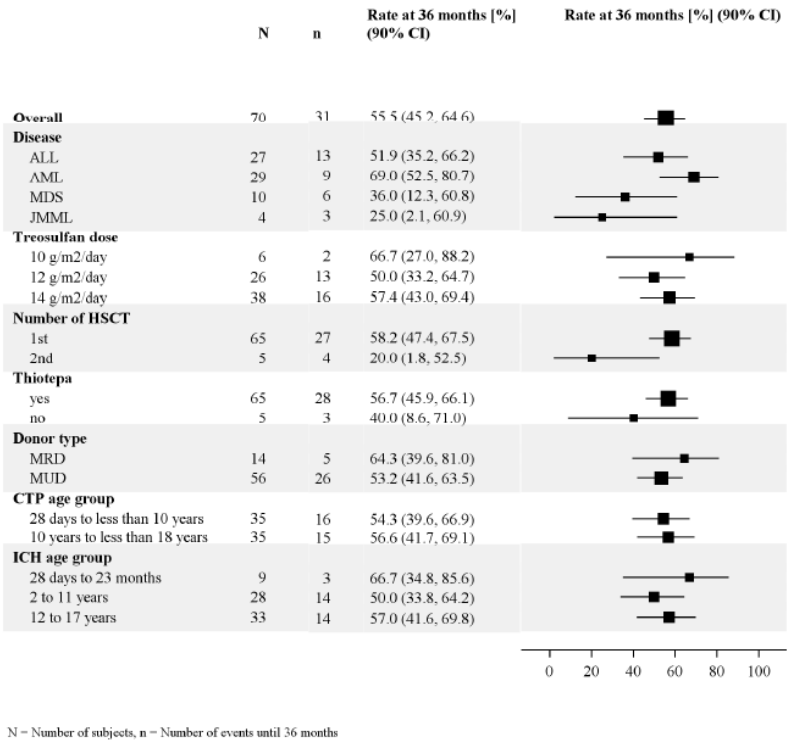


Note: GvHD-free defined as no acute GvHD of at least grade III and no moderate/severe chronic GvHD
[a] based on Kaplan-Meier estimates

Sources: Figure 14.2.14A, Table 14.2.14A, Table 14.2.14B, Listing 16.2.6G.

In the disease subgroups, GRFS at 36 months was 51.9% for ALL subjects, 69.0% for AML subjects, 36.0% for MDS subjects, and 25.0% for JMML subjects. A statistically significant difference in GRFS was recorded between subjects in first HSCT (58.2%) and subjects in second HSCT (20.0%), however, only five subjects were in the second HSCT group. No difference was obvious when dose and age groups were compared.

Figure 11.4.1.11.B Forest plot for GvHD-free and relapse / progression-free survival displaying 36-months rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events until 36 months

Sources: Figure 14.2.14B, Table 14.2.14A, Table 14.2.14C, Table 14.2.14D, Table 14.2.14E, Table 14.2.14F, Table 14.2.14G, Table 14.2.14H, Table 14.2.14I, Listing 16.2.6G.

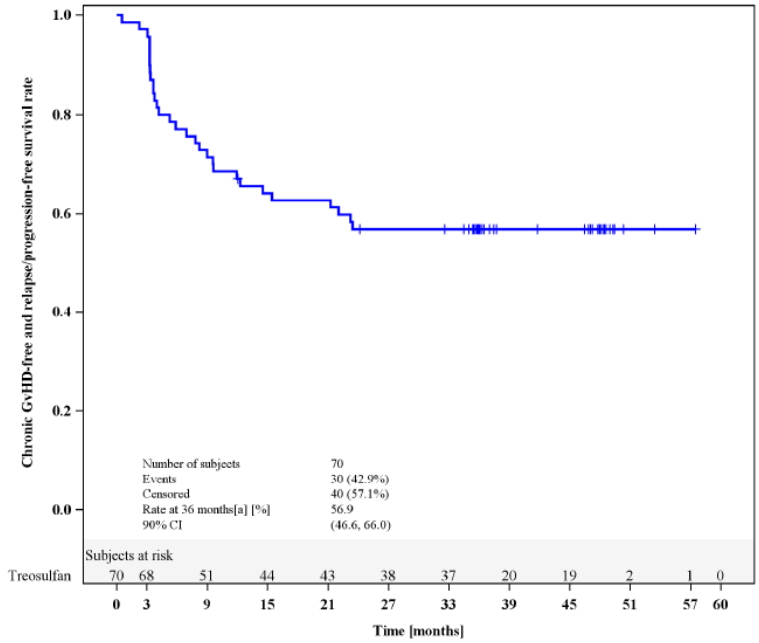
11. Chronic GvHD-free and Relapse-free / Progression-free Survival: At the end of the longer-term follow-up period, 40 subjects (57.1%) were alive and had not experienced moderate / severe cGvHD or relapse / progression. The Kaplan-Meier estimate of CRFS at 12 months was 67.1% (90% CI: 57, 75.4), reached a plateau at 24 months and finally resulted in 56.9% (90% CI: 46.6, 66.0) at 36 months.

Table 11.4.1.12.A Summary results of chronic GvHD-free and relapse / progression-free survival (Full Analysis Set)

	Treosulfan (N=70)
Subjects with event	30 (42.9%)
Death[a]	3 (4.3%)
Relapse/Progression[a]	14 (20.0%)
Moderate/severe chronic GvHD[a]	13 (18.6%)
Subjects without event	40 (57.1%)
Chronic GvHD-free and relapse/progression-free survival at 12 months[b] [%]	67.1
90% CI	(57.0, 75.4)
Chronic GvHD-free and relapse/progression-free survival at 24 months[b] [%]	56.9
90% CI	(46.6, 66.0)
Chronic GvHD-free and relapse/progression-free survival at 36 months[b] [%]	56.9
90% CI	(46.6, 66.0)

Sources: Table 14.2.15A, Table 14.2.15B, Listing 16.2.6G.
[a] Only if this event occurred first.
[b] Based on Kaplan-Meier estimates.
Note: Chronic GvHD-free defined as no moderate/severe chronic GvHD.
Abbreviations: CI = confidence interval; GvHD = graft-versus-host disease; N = number of subject; n = number of subjects in category.

Figure 11.4.1.12.A Kaplan-Meier estimates of chronic GvHD-free and relapse-free / progression-free survival (Full Analysis Set)

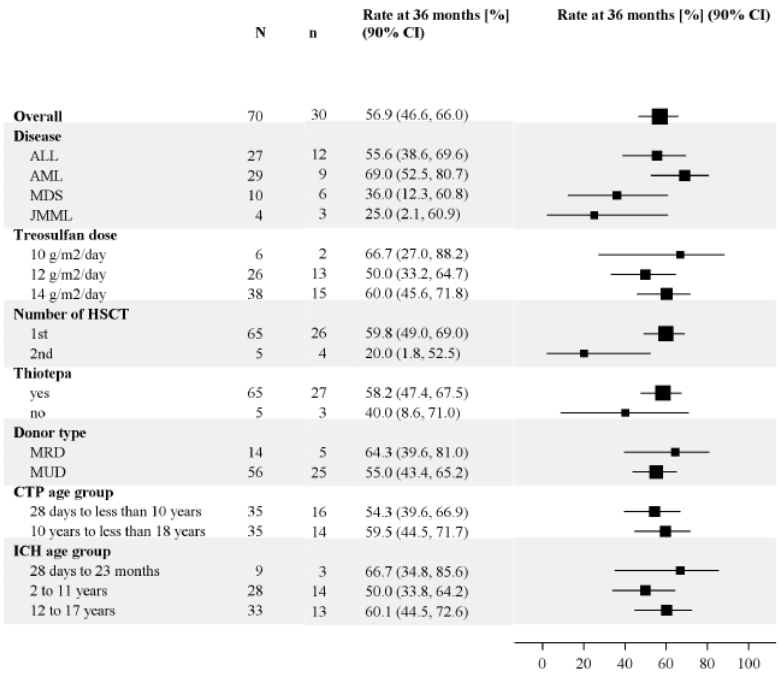


Note: Chronic GvHD-free defined as no moderate/severe chronic GvHD
[a] based on Kaplan-Meier estimates

Sources: Figure 14.2.15A, Table 14.2.15A, Table 14.2.15B, Listing 16.2.6G.

In the disease subgroups, CRFS at 36-months was 55.6% for ALL subjects, 69.0% for AML subjects, 36.0% for MDS subjects, and 25.0% for JMML subjects. A statistically significant difference in CRFS was recorded between subjects in first HSCT (59.8%) and subjects in second HSCT (20.0%), however, only 5 subjects were in the second HSCT group (Table 14.2.15J). No difference was obvious when dose and age groups were compared.

Figure 11.4.1.12.B Forest plot for chronic GvHD-free and relapse-free / progression-free survival displaying 36-months rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events until 36 months

Sources: Figure 14.2.15B, Table 14.2.15A, Table 14.2.15C, Table 14.2.15D, Table 14.2.15E, Table 14.2.15F, Table 14.2.15G, Table 14.2.15H, Table 14.2.15I, Listing 16.2.6G.

12. Rescue Therapies: Five subjects (7.1%) were treated with DLIs (2 subjects thereof in the context of documented relapse events), 4 subjects (5.7%) were treated with stem cell boost (1 subject thereof in the context of documented relapse event and 1 subject in the context of graft failure event). At the end of the longer-term follow-up period, 11 subjects (15.7%) had used rescue therapies. The 2 additional subjects (Subject ID 170403 and Subject ID 173204) received a rescue therapy of chemotherapy (both in the context of documented relapse events).

Table 11.4.1.13.A Frequency of subjects with rescue therapies (Full Analysis Set)

	Treosulfan (N=70)
Any rescue therapies [n (%)]	
No	58 (82.9)
Yes	11 (15.7)
Missing	1 (1.4)
Use of DLIs [n (%)]	
No	64 (91.4)
Yes	5 (7.1)
Missing	1 (1.4)
Use of stem cell boost [n (%)]	
No	65 (92.9)
Yes	4 (5.7)
Missing	1 (1.4)
Use of chemotherapy treatment [n (%)]	
No	67 (95.7)
Yes	2 (2.9)
Missing	1 (1.4)
Use of further conditioning treatment [n (%)]	
No	69 (98.6)
Missing	1 (1.4)
Use of other rescue therapies [n (%)]	
No	66 (94.3)
Yes	3 (4.3)
Missing	1 (1.4)

Source: Table 14.2.16A, Listing 16.2.6H
Note: Multiple use of rescue therapies per subject possible
Abbreviations: DLI = donor-lymphocyte infusions; N = number of subjects; n = number of subjects in category.

Registry study on treosulfan-conditioning in paediatric patients with malignant and non-malignant diseases

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 analysed.

Selection of the treatment regimen

Any TREO-based conditioning followed by allogeneic or autologous HSCT was analysed.

Study endpoints

The following endpoints were analysed: overall survival (OS) and causes of death, event-free survival (EFS), relapse incidence, disease-related mortality (DRM), transplantation-related mortality (TRM), engraftment, graft failure, acute and chronic graft versus host disease (aGvHD, cGvHD), specific adverse events (stomatitis, diarrhoea, vomiting, respiratory toxicity, liver toxicity, neurological toxicity.

Study status

843 patients met the inclusion criteria and 75% could be included into the analysis (533 allogeneic, 93 autologous). In a supplemental analysis, data from 41 children below the age of 6 months were analysed for toxicity.

Efficacy Results - Allogeneic HSCT

Incidence of grade III/IV acute GvHD was 10% with no correlation with age. Incidence of limited and extensive chronic GvHD was 13% and 6%, respectively, again 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 were 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). The transplant related mortality 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.

Registry study on treosulfan-conditioning in paediatric patients with non-malignant diseases

The MAH asked the EBMT Paediatric and Inborn Error Working Party to have a look on the use of TREO and BU for the conditioning of children and adolescents who recently underwent HSCT for non-malignant diseases (NMD). The EBMT registry is the most comprehensive registry for HSCT in Europe, including 536 transplant centres in 57 different countries including also non-EU-countries.

The EBMT Paediatric and the EBMT Inborn Working party performed a retrospective EBMT registry study for the time period January 2010 to December 2014 according to EBMT guidelines. NMDs include huge varieties on congenital or acquired disorders of early and later childhood and are divided in the subgroups primary immunodeficiency (PID) - severe combined immune deficiency (SCID) [PID-SCID], PID-chronic granulomatous disease [PID-CGD)], PID-other, haemoglobinopathies, bone marrow failure syndromes, histiocytic disorders, and inherited disorders-other.

Selection of the treatment regimen

In the past five years, data from several hundreds of children with NMD who underwent either TREO-based or BU-based conditioning therapy in combination with FLU were reported and included in the EBMT registry data base. Moreover, the additional use of TT as part of the conditioning regimen was of particular interest for the analysis.

Study endpoints

The following endpoints were analysed: overall survival (OS), disease-free survival (DFS), incidence of disease recurrence (IDR), transplantation-related mortality (TRM), engraftment, and acute and chronic graft versus host disease (aGvHD, cGvHD).

Study status

The centre survey was performed in February 2016 and the retrospective EBMT-analysis started in September 2016 and was finished in May 2017.

Efficacy Results

Patients with NMD who are eligible for HSCT present different challenges compared to those with malignant diseases: children with inherited disorders such as SCID often come to transplant as infants

under one year of age with organ damage and co-morbidities. GvHD which may be associated with a beneficial graft-versus-leukaemia effect in patients with high risk haematological diseases, is of no added value in controlling the underlying genetic illness and may adversely affect subsequent immune reconstitution and have an unnecessarily negative impact on HSCT-related morbidity and quality of life in the short and long term outcome of patients with NMDs. On the other hand, graft rejection is a known severe complication.

Data from several hundreds of children with NMD who underwent either TREO-based or BU-based conditioning therapy in combination with FLU were reported and included in the EBMT registry data base. Moreover, the additional use of TT as part of the conditioning regimen was of particular interest for the analysis. TT is increasingly used with TREO-and BU-based conditioning because it has shown added value for preventing graft failure in patients with haemoglobinopathies and other diseases with high rejection risk. Additionally, TT is penetrating the brain which is supportive in correcting metabolic diseases with neurological impairment.

This registry study included patients with PID-SCID (n = 320), PID-CGD (n = 202), PID-Wiskott (n = 82), PID-other (n = 242), haemoglobinopathies (n = 590), bone marrow failure syndromes (n = 191), and histiocytic disorders (n = 183).

The patients were grouped according to the given conditioning regimen, i.e. a combination of TREO/FLU (n = 422), BU/FLU (n = 1063), TREO/FLU/TT (n = 473) or BU/FLU/TT (n = 220). The choice of the conditioning regimen before alloHSCT was under discretion of the treating physician. The median age at transplantation was 3.7 years (range 0-18 years) and was significantly different for the conditioning groups (1.5 vs. 4 vs. 4.8 vs. 4.5, respectively; $P < 0.001$). Fifty-two percent of children were transplanted below 4 years of age, 489 patients were less than 12 months old, and 61.5% were male. Most patients received a stem cell graft from unrelated donors (52.3%), followed by transplantation from an HLA-identical sibling or twin donor: 33.1% and 8.4% were grafted from a HLA-phenotypically identical family member or an HLA-mismatched family member. The stem cell source was bone marrow (59.4%), peripheral blood stem cells (21.4%) or cord blood (17.9%), respectively.

The use of TREO-based conditioning has significantly increased during the years, especially in children with inherited disorders and haemoglobinopathies.

The primary outcome parameters of this retrospective analysis are in favour for the combination of TREO/FLU/TT with significantly better results in 1-year OS (89.5%; 95% CI 86.5-92.6%), day 100 TRM (4.8%; 95% CI 2.8-6.7%) and 1-year TRM (8.3%; 95% CI 5.5-11%). This translated also for DFS which was significantly best after TREO/FLU/TT conditioning (86%; 95% CI 82.6-89.5%).

When testing for univariate Cox-regression, TREO/FLU/TT remained superior for OS, TRM and DFS compared to the three other conditioning regimens. However, when adjusted for age at transplant, diagnosis, year of transplant, stem cell sources and donor type, the significances were not reached.

Most patients with thalassaemia major (TM) and sickle cell disease (SCD) are nowadays transplanted after a conditioning regimen with TREO/FLU/TT and for TM-patients 1 year TRM is best after this drug combination. In contrast, only few patients with SCID received a TT-containing regimen because rejection risk is not as high as in haemoglobinopathies and there is no need to cross the blood-brain-barrier.

Outcome of patients with chronic granulomatous disease who were transplanted below 4 years of age or older than 12 years was excellent with no TRM and 100% OS when conditioned with a TREO-containing regimen. The group of patients with the highest treatment failures due to TRM are children above 4 years of age who suffer from histiocytic disorders. Unfortunately, so far, none of the applied conditioning regimen resulted in superior outcome.

When looking to other influencing factors in a multivariate Cox model, the underlying disease, age at transplantation and stem cell source was highly influencing OS, TRM and DFS with the worst prognosis for older patients, histiocytic disorders and cord blood as stem cell source. Year of transplantation did not significantly influence the primary outcome. TREO/FLU ± TT showed a trend for better outcome compared to BU/FLU ± TT and was never worse.

Summary of main studies

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.16/NM

Title: Clinical Phase 2 trial to compare treosulfan-based conditioning therapy with busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with non-malignant diseases		
Study identifier	MC-FludT.16/NM (EudraCT number 2013-005508-33; Clinicaltrials.gov Identifier NCT02349906)	
Design	Prospective, randomised (1:1), multicentre, open-label, active-controlled, parallel-group trial	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	Treatment phase: 7 days with either 3 days of treosulfan administration or 4 days of busulfan administration (completed) Observation phase: until day +100 after HSCT (according to the Paediatric Investigational Plan [PIP], this is defined until at least visit Day +100 [inclusive] of HSCT procedure) (completed) Follow-up phase: until 12 months after HSCT (completed) Longer-term follow-up phase (after completion of PIP): a minimum of 3 years of HSCT (ongoing).
Hypothesis	Non-inferiority	
Treatments groups	Treosulfan (i.v.) 1 g or 5 g + Fludarabine (i.v)	10 or 12 or 14 g/m²/day (BSA-adapted) on day -6 to day -4 before HSCT 30 mg/m²/d) on 5 consecutive days (day -7 to -3 before HSCT
	Busulfan (i.v.) 60 mg + Fludarabine (i.v)	3.2 to 4.8 mg/kg/day (actual body weight adapted) on day -7 to day -4 before HSCT 30 mg/m²/d) on 5 consecutive days (day -7 to -3 before HSCT
	+/- Thiotepa (i.v.) (Investigator's discretion)	2 single doses of 5 mg/kg given on day -2 before HSCT

Endpoints and definitions	Primary endpoint	Freedom from transplantation (treatment) -related mortality	Comparative evaluation of Freedom from transplantation (treatment)-related mortality, defined as death from any transplantation (treatment)-related cause from start of conditioning treatment (visit Day -7) until day +100 after HSCT
	Secondary endpoint	Transplantation-related mortality	Probability of dying from GvHD, interstitial pneumonitis, pulmonary toxicity, infection (bacterial, viral, fungal, parasitic, unknown), Epstein Barr Virus (EBV) proliferative disease, rejection / poor graft function, HSOS, haemorrhage, cardiac toxicity, central nervous system toxicity, gastrointestinal toxicity, skin toxicity, renal failure, multiple organ failure, other HSCT-related cause
	Secondary endpoint	Overall survival	Probability of surviving. Survival time was defined as the time length between end of HSCT and the day of death due to any cause. Evaluated from the end of HSCT until the end of the longer-term follow-up phase
	Secondary endpoint	Graft failure	Probability of having a graft failure (primary or secondary) and being alive without using "stem cell infusion (re-transplant) with conditioning" rescue therapy (ie, second allogenic transplantations) between the end of HSCT and the end of the longer-term follow-up phase. In addition, the rate of primary and secondary graft failures was assessed
	Secondary endpoint	Engraftment	Neutrophilic granulocytes engraftment was defined as the first of 3 consecutive days with an granulocyte count > 0.5 x 10^9/L in PB, leucocyte engraftment was defined as the first of 3 consecutive days with a total leucocyte count > 1 x 10^9/L in PB, PLT engraftment was defined as the first of 3 consecutive days with a) PLTs > 20 x 10^9/L or b) PLTs > 50 x 10^9/L in PB in the absence of PLT transfusion. Time to engraftment was defined as the time span between end of HSCT and neutrophil granulocyte / leucocyte / PLT engraftment. In addition, the duration of neutropenia (neutrophilic granulocytes $\leqslant$ 0.5 x 10^9/L) and leukopenia (leucocytes granulocytes $\leqslant$ 1.0 x 10^9/L) was analysed based on documented laboratory values
	Secondary endpoint	Quantification of donor type chimerism	If a value of $\geqslant$ 95% donor-type was detected. Mixed chimerism was defined as having a recipient fraction > 5% (to 94%). Incidences of complete donor-type chimerism were estimated as the number of subjects with complete chimerism divided by the total number of subjects at risk. To investigate the mixed donor-type chimerism the frequency of subjects with at least 20% or 50% donor-type chimerism was calculated. Chimerism was evaluated on visit Day +28, +100 and Month 12, and during longer-term follow-up

	Exploratory endpoint	Event-free survival	Time length between end of HSCT and the date of graft failure or "stem cell infusion (re-transplant) with conditioning" rescue therapy (ie, second allogenic transplantations) or death (whatever occurred first). EFS was evaluated from the end of HSCT until the end of the longer-term follow-up phase.
	Secondary endpoint	GvHD-free survival	Measured from end of HSCT to time of event. The associated time span was defined as the interval from end of HSCT to aGvHD of at least grade III, moderate or severe cGvHD, or death (whatever occurred first). GvHD-free survival was evaluated from the end of HSCT until the end of the longer-term follow-up phase.
	Secondary endpoint	Chronic GvHD-free survival	Measured from end of HSCT to time of event. The associated time span was defined as the interval from end of HSCT to moderate or severe cGvHD or death (whatever occurred first). cGvHD-free survival was evaluated from the end of HSCT until the end of the longer-term follow-up phase.
	Secondary endpoint	Rescue therapies	Use of and duration of using rescue therapies like DLIs, stem cell boost, stem cell infusion (retransplantation) with conditioning, stem cell infusion (re-transplantation) without conditioning, transfusion dependence for red blood cells, transfusion dependence for PLTs, and other was described from end of HSCT until the end of the longer-term follow-up phase
Database lock	07-Jun-2021		
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 (EudraCT number 2013-003604-39)		
Design	Prospective, single arm, open-label, multicentre, non-controlled, Phase 2 clinical trial		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Treatment phase: 7 days with 3 days of Treosulfan administration Observation phase: until day + 100 after HSCT (according to the Paediatric Investigation Plan [PIP], this is defined until at least visit Day +100 (inclusive) of HSCT procedure) Follow-up phase: until 12 months after HSCT Longer-term follow-up phase (after completion of PIP): a minimum of 3 years after HSCT
Hypothesis	Superiority		
Treatments groups	Treosulfan (i.v.) 1 g or 5 g + Fludarabine (i.v)		At dose levels 10 g/m², 12 g/m², and 14 g/m² over 2 hours on 3 consecutive days on visit Days -6, -5, and -4 before alloHSCT single doses of 30 mg/m2 on 5 consecutive days (from visit Day -7 to -3)
	+/- Thiotepa (i.v.) (Investigator's discretion)		in 2 single doses of 5 mg/kg given on visit Day -2 before HSCT

Endpoints and definitions	Co-Primary endpoint	Freedom from transplantation (treatment) -related mortality	Death from any transplant (treatment)-related cause from the day of first administration of conditioning treatment until 100 days after HSCT. This endpoint is a combination of TRM and treatment-related mortality
	Co-Primary endpoint	Transplantation-related mortality	probability of dying from a transplant-related cause, i.e. which could not be attributed to disease relapse / progression or by deaths without previous relapse / progression
	Secondary endpoint	Overall survival	Probability of surviving and was evaluated from the end of HSCT up to the visit 12 months after HSCT. OS was continuously assessed during the longer term follow up phase
	Secondary endpoint	Relapse / Progression Incidence	Probability of having relapse / progression of the underlying disease, death due to any cause, or End of Trial, whatever comes first. RFS / PFS was continuously assessed during the longer term follow up phase
	Secondary endpoint	Relapse-free / Progression -free Survival	Time length between end of HSCT and the date of relapse / progression of the underlying disease or death due to any cause, or End of Trial, whatever comes first. RFS / PFS was continuously assessed during the longer term follow up phase
	Secondary endpoint	Graft failure	Probability of having a graft failure (primary or secondary) from end of the HST up to visit 12 months after HSCT. Secondary graft failure was continuously assessed during the longer term follow up phase
	Secondary endpoint	Non-relapse Mortality	Probability of dying in the absence of persisting disease or previous occurrence of relapse / progression or graft failure. NRM was evaluated from end of the HSCT to visit 12 months after HSCT. NRM was continuously assessed during the longer term follow up phase
	Secondary endpoint	Engraftment	Neutrophil count > 0.5 x 10^9/L, leucocyte count > 1 x 10^9/L, and platelet counts > 20 x 10^9/L or > 50 x10^9/L) and assessed up to 100 days after HSCT
	Secondary endpoint	Quantification of donor type chimerism	$\geqslant$ 95% donor cells detected and was evaluated on visit Day +28, +100 and the visit 12 months after HSCT
	Exploratory endpoint	Event-free survival	Length of time between end of HSCT and the date of relapse / progression, graft failure, or death (whatever occurs first)..
	Exploratory endpoint	GvHD-free Relapse-free / Progression -free Survival	

	Exploratory endpoint	Chronic GvHD-free and Relapse-free / Progression-free Survival	
	Secondary endpoint	Rescue therapies	Use of and duration of using rescue therapies
Database lock	07-Jun-2021		
Results and Analysis			

Table 2.7.3.3.2.3-13: Freedom from TRM until Day +100 (Kaplan-Meier estimates)

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source in CSR	*Table 11.4.1.1.A*		*Table 14.2.1A*
Study arm	**TREO**	**BU**	**TREO**
Total number of patients	51 (100%)	50 (100%)	70 (100%)
Patients with event	0 (0%)	5 (10%)	1 (1.4%)
Patients without event	51 (100%)	45 (90%)	69 (98.6%)
Freedom from TRM until Day +100 (90% CI)	100% (94.3, 100)	90% (80.1, 96.0)	98.6% (93.4, 99.9)
Odds ratio[a] (90% CI)	< 0.0001 (NA)		
P value[b]	0.0528		
Unadjusted P value[c]	0.0267		
[a] Adjusted for thiotepa and disease; [b] Stratified Cochran-Mantel-Haenszel test; [c] Fisher's exact test			

Table 2.7.3.3.2.3-12: Transplant-related mortality (Kaplan-Meier estimates)

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source	*Table 11.4.1.2.A*		*Tables 11.4.1.2.A*
Study arm	**TREO**	**BU**	**TREO**
Total number of patients	51 (100%)	50 (100%)	70 (100%)
Median follow-up; months (range of those surviving)	25.6 (10.7, 60.9)	25.4 (11.7, 63.3)	41.8 (24.2, 57.9)
Patients with event	2 (3.9%)	7 (14.0%)	4 (5.7%)
Patients without event	49 (96.1%)	43 (86.0%)	66 (94.3%)
TRM at 12 months; % (90% CI)	3.9 (1.2, 12.0)	12.0 (6.3, 22.1)	1.4 (0.3, 7.2)
TRM at 24 months; % (90% CI)	3.9 (1.2, 12.0)	12.0 (6.3, 22.1)	4.6 (1.8, 11.4)
TRM at 36 months; % (90% CI)	3.9 (1.2, 12.0)	16.0 (8.6, 28.6)	4.6 (1.8, 11.4)

Hazard ratio (TREO/BU)[b]; 90% CI[a]	0.29 (0.08, 1.09)	
P value[b]	0.1244	
Unadjusted P value[c]	0.0718	
[a] based on Kaplan-Meier estimates; [b] adjusted for TT and disease as factors using Cox regression model; [c] Log-rank test		

Table 2.7.3.3.2.3-9: Overall survival (Kaplan-Meier estimates)

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source	*Table 11.4.1.3.A*		*Table 11.4.1.3.A*
Study arm	**TREO**	**BU**	**TREO**
Total number of patients	51 (100%)	50 (100%)	70 (100%)
Median follow-up; months (range of those surviving)	25.6 (10.7-60.9)	25.4 (11.7, 63.3)	41.8 (24.2-57.5)
Patients with event	2 (3.9%)	7 (86.0%)	12 (17.1%)
Patients without event	49 (96.1%)	43 (100%)	58 (82.9%)
OS at 12 months; % (90% CI)	96.1 (88.0, 98.8)	88.0 (77.9, 93.7)	91.4 (83.9, 95.5)
OS at 24 months; % (90% CI)	96.1 (88.0, 98.8)	88.0 (77.9, 93.7)	85.7 (77.1, 91.2)
OS at 36 months; % (90% CI)	96.1 (88.0, 98.8)	84.0 (71.4, 91.4)	84.3 (75.5, 90.1)
HR (TREO/BU)[c] (90% CI)	0.29 (0.08, 1.09)		
P value[*]	0.1244		
[*] *adjusted for TT and disease as factors using Cox regression model*			

Table 2.7.3.3.2.3-7: Graft failure rates

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source	*Table 11.4.1.4.A*		*Table 11.4.1.6.A*
Study arm	**TREO**	**BU**	**TREO**
Total number of patients	51	50	70
Primary graft failure	2 (3.9%)	2 (4.0%)	0
Secondary graft failure	9 (18.4%)	0 (0.0%)	1 (1.4%)

Table 2.7.3.3.2.3-1: Conditional cumulative incidence of reconstitution of granulopoiesis

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source in CSR	*Tables 11.4.1.5.A/-B*		*Tables 11.4.1.8.A/-B*
Study arm	**TREO**	**BU**	**TREO**
No. of patients	51 (100%)	50 (100%)	70 (100%)
Patients with event	40 78.4%)	36 (72.0%)	69 (98.6%)
Patients without event (censored) or with competing event	11 (21.6%)	14 (28.0%)	1 (1.4%)
Censored	2 (3.9%)	2 (4.0%)	1 (1.4%)
Death[*]	0	0	0
Rescue therapy[*]	9 (17.6%)	12 (24.0%)	0
Conditional cumulative incidence, % (90% CI)			
Day +14	23.8 (13.0, 34.6)	47.4 33.1, 61.6)	28.6 (18.7, 38.4)
Day +28	81.0 (68.2, 93.7)	88.5 (77.9, 99.1)	86.9 (79.8, 93.9)
Maximum incidence	97.3 (88.7, 100.0)	100.0 (94.1, 100.0)	100.0 (97.7, 100.0)
Duration of neutropenia (days)[†]			
n	49	48	69
Mean (SD)	19.9 (7.7)	15.9 (7.3)	22.3 (7.7)
Median (Q1, Q3)	20.0 (15.0, 25.0)	14.5 (10.0, 21.0)	22.0 (17.0, 26.0)
Min, Max	8, 43	5, 34	7, 44
P value[‡]	0.0108		
**only if this event occurred first; [†]First date with neutropenia until date of engraftment (patients at risk = patients with neutropenia and neutrophilic granulopoiesis); [‡]based on the Wilcoxon-Mann-Whitney test*			

Table 2.7.3.3.2.3-3: Conditional cumulative incidence of reconstitution of leukopoiesis

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source in CSR	*Table 11.4.1.5.C*		*Table 11.4.1.8.C*
Study arm	**TREO**	**BU**	**TREO**
No. of patients	51 (100%)	50 (100%)	70 (100%)
Patients with event	40 (78.4%)	36 (72.0%)	69 (98.6%)
Patients without event (censored) or with competing even	11 (21.6%)	14 (28.0%)	1 (1.4%)
Censored	2 (3.9%)	2 (4.0%)	1 (1.4%)
Death[*]	0	0	0
Rescue therapy[*]	9 (17.6%)	12 (24.0%)	0
Conditional cumulative incidence, % (90% CI)			
Day +14	23.8 (13.0, 34.6)	50.0 (35.8, 64.2)	30.0 (20.6, 39.4)
Day +28	90.5 (83.5, 97.4)	88.5 (77.7, 99.3)	95.6 (90.9, 100.0)
Maximum incidence	96.8 (87.1, 100.0)	100.0 (94.1, 100.0)	100.0 (97.7, 100.0)
P value (Pepe-Mori test)	0.2469		
**only if this event occurred first*			

Table 2.7.3.3.2.3-4: Conditional cumulative incidence of reconstitution of thrombopoiesis > 20 × 10^9/L

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source in CSR	*Table 11.4.1.5.E*		*Table 11.4.1.8.E*
Study arm	**TREO**	**BU**	**TREO**
No. of patients	51 (100%)	50 (100%)	70 (100%)
Patients with event	40 (78.4%)	35 (70.0%)	65 (92.9%)
Patients without event (censored) or with competing even	11 (21.6%)	15 (30.0%)	5 (7.1%)
Censored	2 (3.9%)	3 (6.0%)	5 (7.1%)
Death[*]	0	0	0
Rescue therapy[*]	9 (17.6%)	12 (24.0%)	0
Conditional cumulative incidence, % (90% CI)			
Day +14	33.3 (20.5, 46.1)	34.2 (21.3, 47.1)	34.3 (24.5, 44.1)
Day +28	85.7 (77.3, 94.2)	77.6 (64.0, 91.3)	78.0 (69.5, 86.5)
Maximum incidence	100.0 (93.8, 100.0)	96.8 (86.6, 100.0)	94.1 (88.4, 99.9)
P value (Pepe-Mori test)	0.8595		
**only if this event occurred first*			

Table 2.7.3.3.2.3-5: Conditional cumulative incidence of reconstitution of thrombopoiesis > 50 × 10^9/L

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source in CSR	*Table 11.4.1.5.F*		*Table 11.4.1.8.F*
Study arm	**TREO**	**BU**	**TREO**
No. of patients	51 (100%)	50 (100%)	70 (100%)
Patients with event	39 (76.5%)	35 (70.0%)	63 (90.0)
Patients without event (censored) or with competing even	12 (23.5%)	15 (30.0%)	7 (10.0)
Censored	3 (5.9%)	3 (6.0%)	7 (10.0)
Death[*]	0	0	0
Rescue therapy[*]	9 (17.6%)	12 (24.0%)	0
Conditional cumulative incidence, % (90% CI)			
Day +14	19.0 (8.4, 29.7)	28.9 (16.3, 41.6)	15.7 (8.4, 23.0)
Day +28	73.8 (61.9, 85.7)	50.4 (36.4, 64.4)	62.2 (52.5, 71.9)
Maximum incidence	94.8 (86.3, 100.0)	97.1 (87.9, 100.0)	91.9 (84.9, 98.8)
P value (Pepe-Mori test)	0.3635		
**only if this event occurred first*			

Table 2.7.3.3.2.3-6: Incidence of complete donor type chimerism

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source	*Table 14.2.9A*		*Table 11.4.1.9.A*
Study arm	**TREO**	**BU**	**TREO**
Patients at risk Day +28 visit[*]	51 (100%)	50 (100%)	69 (100%)
Complete chimerism	43 (84.3%)	41 (82.0%)	65 (94.2%)
No complete chimerism	7 (13.7%)	9 (18.0%)	3 (4.3%)
No information	1 (2.0%)	0	1 (1.4%)
Incidence of complete chimerism; % (90% CI)	84.3 (73.5, 91.9)	82.0 (70.7, 90.3)	94.2 (87.2, 98.0)
Adjusted P value	0.425		
Patients at risk Day +100 visit[*]	51 (100%)	46 (100%)	69 (100%)
Complete chimerism	34 (66.7%)	39 (84.8%)	63 (91.3%)
No complete chimerism	14 (27.5%)	5 (10.9%)	6 (8.7%)
No information	3 (5.9%)	2 (4.3%)	0
Incidence of complete chimerism; % (90% CI)	66.7 (54.3, 77.5)	84.8 (73.3, 92.6)	91.3 (83.6, 96.1)
Adjusted P value	0.1196		
Patients at risk Months 12 visit[*]	49 (100%)	43 (100%)	57 (100%)
Complete chimerism	24 (49.0%)	33 (76.6%)	52 (91.2%)
No complete chimerism	14 (28.6%)	9 (20.9%)	3 (5.3%)
No information	11 (22.4%)	1 (2.3%)	2 (3.5%)
Incidence of complete chimerism; % (90% CI)	49.0 (36.5, 61.5)	76.7 (57.0, 86.0)	91.2 (82.4, 96.5)
Adjusted P value	0.2445		
Patients at risk Months 24 visit[*]	31	30	no data
Complete chimerism	12 (38.7%)	22 (73.3%)	
No complete chimerism	11 (35.5%)	6 (20.0%)	
No information	8 (25.8%)	2 (6.7%)	
Incidence of complete chimerism; % (90% CI)	38.7 (24.1, 55.0)	73.3 (57.0, 86.0)	
Adjusted P value	0.1058		
[*]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 +29/30, day +107, day +372/379, or day +760, respectively			

Table 2.7.3.3.2.3-8: Event-free survival (Kaplan-Meier estimates)

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source in CSR	*Tables 11.4.1.7.A*		*Table 11.4.1.3.A / -10.A*
Study arm	**TREO**	**BU**	**TREO**
Total number of patients	51 (100%)	50(100%)	70 (100%)
Median follow-up; months (range of those surviving)	25.6 (10.7, 60.9)	25.4 (11.7, 63.3)	41.8 (24.2, 57.5)
Event-free survival			
Patients with event	13 (25.5%)	9 (18.0%)	19 (27.1%)
Death[a]	2 (3.9%)	6 (12.0%)	2 (2.9%)
Relapse/progression	-	-	16 (22.9%)
Primary graft failure[a]	2 (3.9%)	2 (4.0%)	0
Secondary graft failure[a]	9 (17.6%)	0	1 (1.4)
Second allogeneic transplant[a]	0	1 (2.0%)	-
Patients without event	38 (74.5%)	41 (82.0%)	51 (72.9%)
EFS at 12 months; (90% CI)[b]	80.3 (69.2, 87.8)	86.0 (75.5, 92.2)	81.4% (72.3; 87.8)
EFS at 24 months; (90% CI)[b]	75.3 (63.2, 83.9)	86.0 (75.5, 92.2)	72.7 (62.8, 80.4)
EFS at 36 months; (90% CI)[b]	71.9 (58.8, 81.4)	81.9 (69.0, 89.8)	72.7 (62.8, 80.4)
Hazard Ratio (TREO/BU)[c] (90% CI)	1.54 (0.74, 3.22)		
P-value[c]	0.3343		

[a] *only if this event occurred first*
[b] *based on Kaplan-Meier estimates*
[c] *adjusted for TT and disease as factors using Cox regression model*

Table 2.7.3.3.2.3-10: Summary results of relapse/remission in trial MC-FludT.17/M

Study	**MC-FludT.17/M**
Data source	*Table 11.4.1.4.A*
No. of patients	70 (100%)
Patients with event	16 (22.9%)
Patients without event (censored) or with competing even	54 (77.1%)
Censored	51 (72.9%)
*Death**	2 (2.9%)
*Primary graft failure**	0
*Secondary graft failure**	1 (1.4%)
Conditional cumulative incidence, % (90% CI)	
At 12 months	15.7 (8.6, 22.9)
At 24 months	23.0 (14.7, 31.3)
At 36 months	23.0 (14.7, 31.3)

**only if this event occurred first*

Table 2.7.3.3.2.3-14: Cumulative incidence of NRM in trial MC-FludT.17/M

Study	**MC-FludT.17/M**
Data source	*Table 11.4.1.7.A*
No. of patients	70 (100%)
Patients with event	2 (2.9%)
Patients without event (censored) or with competing even	68 (97.1%)
Censored	51 (72.9%)
*Relapse/progression**	16 (22.9%)
*Primary graft failure**	0
*Secondary graft failure**	1 (1.4%)
Cumulative incidence at 12 months, % (90% CI)	1.4 (0.0, 3.8)
Cumulative incidence at 24 months, % (90% CI)	2.9 (0.0, 6.1)
Cumulative incidence at 36 months, % (90% CI)	2.9 (0.0, 6.1)

**only if this event occurred first*

Table 2.7.3.3.2.3-11: Rescue therapies

Study	MC-FludT.16/NM		MC-FludT.17/M
Data source	*Table 11.4.1.10.A*		*Listing 11.4.13.A*
Study arm	**TREO**	**BU**	**TREO**
Total number of patients	51 (100%)	50 (100%)	70 (100%)
Rescue therapy	21 (41.2%)	21 (42.0%)	11 (15.7%)
Kind of rescue therapy			
Transfusion dependent for RBCs	17 (33.3%)	17 (34.0%)	
Transfusion dependent for platelets	14 (27.5%)	14 (28.0%)	
DLI	5 (9.8%)	2 (4.0%)	5 (7.1%)
Stem cell boost	2 (3.9%)	1 (2.0%)	4 (5.7%)
Re-transplant with conditioning	0	1 (2.0%)	
Chemotherapy			2 (2.9%)
Other	4 (7.8%)	2 (4.0%)	3 (4.3%)

2.4.3. Discussion on clinical efficacy

The current variation aims to include an additional non-malignant transplant indication in the paediatric population for Trecondi 1 g/5 g powder for solution for infusion based on final 12-months follow-up results of study MC-FludT.16/NM; a randomised phase II interventional study aimed to compare Treosulfan-based conditioning therapy with Busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with non-malignant diseases.

Moreover, the MAH proposes to introduce a slightly modified dosing regimen according to the patient's body surface based on long-term follow-up data of paediatric Phase II study MC-FludT.17/M, on the efficacy of Treosulfan based conditioning therapy prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with haematological malignancies, as well as a final analysis of the population pharmacokinetics of treosulfan in paediatric patients. Additionally, one meta-analysis of engraftment data [Baumgart 2017] and two EBMT registry studies [Peters 2011, Peters 2017] on the use of TREO-based conditioning in paediatric patients with malignant and non-malignant diseases were performed and integrated into the data package.

Considering the aim of the current variation the discussion of clinical efficacy is focused in the two paediatric clinical trials **MC-FludT.16/NM and MC-FludT.17/M**.

A non-controlled trial (MC-FludT.17/M) with treosulfan (TREO)-based conditioning in combination with fludarabine (FLU) and with or without Thiotepa (TT) has been performed in paediatric patients with haematological malignancies (MD). Another randomised active-controlled trial in paediatric patients with non-malignant diseases (NMD) and where TREO/FLU/TT is compared with busulfan (BU)/FLU/TT has been completed recently (MC-FludT.16/NM). Efficacy data with TREO-based conditioning from 70 patients with malignant and 51 patients with various non-malignant diseases have been assessed.

Main indications for alloHSCT in children are haematological malignancies such as ALL (26%), AML (14%), and MDS/MPS (8%), but also non-malignant disorders (NMD) like primary immunodeficiency (PID; 16%), bone marrow failure (BMF; 12%), and thalassaemia (9%) [Passweg 2014]. However, the use of tresosulfan as part of the conditioning-regimen prior alloHSCT is only currently approved for paediatric patients with malignant disease.

All such patients were included in the two above-mentioned paediatric trials with treosulfan-based conditioning besides published reports.

AlloHSCT is a very complex treatment method which consists of the conditioning regimen, the infusion of allogeneic HSCs, and pre/post-transplant immunosuppressive measures (GvHD prevention). The efficacy of the conditioning regimen can therefore be measured only in the context of the whole transplant procedure. Engraftment (as conditional cumulative incidence of engraftment) of the donor HSCs is the only parameter which is generally considered directly dependent on the efficacy of a conditioning therapy and as such it was selected as the primary endpoint in the three initial non-controlled trials in adult patients and included as a secondary endpoint in all other studies, including MC-FludT.16/NM and MC-FludT.17/M.

Almost all patients achieve engraftment after conditioning with TREO/FL or BU/FL and as such it is accepted that it was not the primary endpoint in the assessed trials. Other typical efficacy endpoints used in clinical trials with alloHSCT include cumulative incidence of relapse (CIR), non-relapse mortality (NRM) or transplant-related mortality (TRM), and Graft versus Host disease (GvHD) [Kim 2013]; considering the curative potential of this treatment also survival endpoints such as disease-free survival (DFS), event-free survival (EFS), and overall survival (OS) were adequately selected as secondary endpoint.

For the MC-FludT.16/NM and MC-FludT.17/M trials and upon request from PDCO, the selected primary endpoint was Freedom from transplant (treatment) related mortality until day +100 after HSCT, defined as death from any transplant-related cause from start of conditioning treatment (day -7) until day +100 after HSCT. The analysis of the estimates from Kaplan-Meier for freedom from TRM until Day+100 after alloHSCT indicates that both treosulfan and busulfan give good results but the treosulfan behaves better with 0% (.16/NM) or 1.4% subjects (.17/M) with event for treosulfan vs 10% for busulfan (unadjusted p-value 0.0267; adjusted p-value 0.0528).

Transplant-related mortality (KM estimates) after TREO-based conditioning was very low in patients with both non-malignant (3.9%) as well as malignant diseases (5.7%) but somewhat higher with BU in no-malignant diseases (14.0%) (HR (TREO/BU) 0.29 (90% CI 0.0, 1.09); p value 0.1244). This was also seen in the EBMT registry analysis with no influence of age or dose of treosulfan and significantly lower TRM for TREO/FLU/TT than for BU/FLU/TT conditioning [Peters 2017]. In both treatments for non-malignant diseases the TRM was maintained over time however it increases after 2 years in malignant disease with treosulfan.

TREO dosing in the paediatric studies aimed to reach TREO plasma levels in the range observed with the 14 g/m² dose in adults. The data from both studies suggest that paediatric patients tolerate TREO-based conditioning better than adult patients and justify that there was no need to reduce the TREO dose in this patient population. Even so some adjustment of the TREO dose according with patient's BSA is being proposed in the current variation and justification for that proposal and clarification of some other PK-related issues were provided (see above Discussion on Clinical Pharmacology).

Overall survival at 3 years after TREO-based conditioning was 84.3% (95% CI 75.5-90.1%) in patients with malignant diseases and similar to BU-based conditioning in patients with non-malignant disease (84.0%; 95% CI 75.5-90.1%). The later patients when treated with TREO presented a numerically (p value 0.1244) better OS (96.1%) than BU with an HR (TREO/BU) of 0.29 (90% CI 0.08, 1.09). This is also seen from the data available from the EBMT registry specially after a first HSCT [Peters 2011] and statistically significance is seen in the case of non-malignant diseases at 1 year (89.5% for TREO/FLU/TT vs 81.3%; for BU/FLU/TT (log rank test P = 0.012)[Peters 2017].

The maximum conditional cumulative incidence of neutrophil engraftment was 97.3% in patients with non-malignant diseases for TREO and 100% for BU and for TREO in patients with malignant diseases.

Median duration of neutropenia was significantly longer with TREO compared to BU in trial MC-FludT.16/NM (20 days vs. 14.5 days) (p value 0.0108). The MAH justifies this difference with an imbalance in the underlying diseases of both treatment arms but the same duration is seen in the case of TREO treatment in paediatric patients with malignant diseases which may require more close monitoring of these patients when under treatment with TREO. Similar data were obtained for the reconstitution of leukopoiesis (96.8% TREO-NMD, 100% BU-NMD and 100% TREO-MD; p value 0.2469) and thrombopoiesis (>20x10^9/L: 100% TREO-NMD, 96.8% BU-NMD and 94.1% TREO-MD – p value 0.8595; >50x10^9/L: 94.8% TREO-NMD, 97.1% BU-NMD and 91.9% TREO-MD – p value 0.3635). These data are also in line with the meta-analysis of engraftment data from trial MC-FludT.17/M, published paediatric data, and data from each of the arms of the two active-controlled studies in adult patients (MC-FludT.14/L Trials I/II), as per the PIP [Baumgart 2017] and with the date from the two meta-analyses of the EBMT [Peters 2011]. However, in patients with non-malignant diseases [2017 EBMT; Peters 2017], a significant correlation between conditioning treatment and neutrophil engraftment was observed for those patients who additionally received thiotepa (TT) with at day +100 after alloHSCT only 83.5% of patients reached neutrophil engraftment in the BU/FLU/TT group compared to 96.1% in the TREO/FLU/TT cohort. Only 2 of 121 paediatric patients (NMD and MD) (1.7%) treated with TREO-based or with BU-based conditioning had a primary graft failure; however in the case of NMD treatment with TREO seems to lead to higher rate of secondary graft failure (18.4% TREO-NMD vs 0% BU-NMD vs 1.4% TREO-MD) but all the graft failures in the TREO-NMD occurred in patients who had received a transplant from a matched unrelated donor which may explain the outcome.

The incidence of complete donor-type chimerism was lower in the patients treated with TREO with non-malignant diseases compared to the patients with malignant diseases up to 24 months and also lower than with BU-treatment from Day+100 onwards.

Event-free survival was assessed as an additional exploratory endpoint in both paediatric trials. Overall EFS rates are similar for the TREO treatment in both NMD and MD patients and decreases with time for NMD ad reaches a plateau at 2 years for MD being higher (although non-significantly, p value 0.3343) for TREO than for BU-treatment (HR 1.54 (90% CI 0.74, 3.22). Nevertheless, this is due to mostly due to secondary graft failure and no on death number, where BU treatment has higher rate (3.9% TREO vs 12.0% BU). This follows the data available from the EBMT registry analysis [Peters 2011] which also indicates some influence of age likely related with difference in relapse incidence.

The cumulative incidence of relapse/progression in the 70 patients with MD treated with TREO-based conditioning in trial MC-FludT.17/M was 23.0% at 24 and 36 months with 15.7% (n=11) of these patients requiring rescue therapy mainly of donor lymphocyte infusion (DLI) or stem cell boost. Patients with non-malignant diseases required more rescue therapies (41.2%; especially transfusions for RBCs [33%] and platelets [27.5%]) than patients with malignant diseases. Nevertheless, no difference is observed regarding rescue therapies between the two treatment arms of the MC-FludT.16/NM trial. This is aligned with data on incidence of disease relapse (IDR) in these patients available from the EBMT registry [Peters 2017]. Of note, in the disease subgroups of the MC-FludT.17/M trial, a statistically significant difference in relapse/progression was recorded between JMML (75%), ALL (29.6%), AML (17.2%), and MDS (0.0%) subgroups; however, these results may be due to the small number of subjects with these pathologies included in the study as well with the general poor prognosis of these pathologies.

Overall the efficacy results of the first randomised Phase II alloHSCT trial (MC-FludT.16/NM) in paediatric patients with non-malignant diseases demonstrated a benefit for the treosulfan conditioning regimen over busulfan conditioning regimen in the selected subject population regarding Freedom from transplant (treatment) related mortality until day +100 after HSCT, TRM, OS, GvHD-free and cGvHD-free survival and EFS despite a higher rate of secondary graft failures and lower complete donor-type chimerism.

Therefore, in children with non-malignant diseases indicated for alloHSCT the risk of graft failure must be carefully weighed against the risk of TRM when choosing the conditioning regimen.

The second Phase II trial in paediatric patients with malignant haematological disease (MC-FludT.17/M) has shown that a BSA-adapted i.v. dose of 10 g/m^2, 12 g/m^2, or 14 g/m^2 Treosulfan given on Days -6, -5, and 4 can be successfully used as conditioning treatment before alloHSCT in the selected paediatric population. After two rounds of assessment, the selected BSA-adapted dosing and additional PK issues were adequately justified/clarified (see above Discussion on Clinical Pharmacology).

Most of the subjects (92.9%) were treated with the intensified conditioning regimen (Thiotepa in addition to Fludarabine). The primary endpoint of the trial in paediatric patients with malignant diseases and of the trial in paediatric population with non-malignant disease treated with treosulfan, the rate for freedom from transplant (treatment)-related mortality until 100 days after HSCT, was 98.6% (90% CI: 93.4, 99.9) and 100% (90% CI: 94.3, 100), respectively indicating the tolerability and safety of this regimen in both conditions with favourable outcome when compared with busulfan-containing regimen (90%; 90% CI: 80.1, 96.0). The detailed safety analysis along with the Kaplan Meier estimation of NRM at 12 months and 36 months supports this observation.

Based on the engraftment and chimerism data approaching 100% and > 90%, respectively, efficacy parameters like EFS, OS, and GvHD-free and relapse-free survival confirm the effectiveness of this conditioning treatment. Statistically significant unfavourable results for JMML and second HSCT subgroups with regard to relapse / progression as well as the survival parameters were noted. However, these results may be due to the small number of subjects with the pathologies included in the study as well with the general poor prognosis of these pathologies.

In overall, the efficacy of treosulfan as part of the conditioning treatment conditioning treatment prior to alloHSCT in paediatric patients with non-malignant diseases is based on the following considerations:

- The final analysis of MC-FludT.14/L Trial II has confirmed the non-inferiority of TREO-based conditioning compared to reduced-intensity conditioning therapy based on intravenous BU. Additionally, superiority of TREO versus BU could now be shown with the final data set.
- The final analysis of MC-FludT.14/L Trial II has confirmed the non-inferiority of TREO-based conditioning compared to reduced-intensity conditioning therapy based on intravenous BU. Additionally, superiority of TREO versus BU could now be shown with the final data set.
- Furthermore, both paediatric studies have been completed. Final CSRs are now available, with 3-year follow-up survival data for study MC-FludT.17/M and 1-year follow-up data for study MC-FludT.16/NM.
- Moreover, the PopPK model for TREO was updated and the BSA-adapted dose regimen of TREO in paediatric patients slightly modified.

2.4.4. Conclusions on the clinical efficacy

Overall, the reported efficacy results of these two Phase II allo-HSCT trials resulted in a benefit for the Treosulfan-based conditioning regimen used in paediatric patients with selected malignant diseases, confirming the approved indication as well as in paediatric patients with selected non-malignant diseases in respect to a Busulfan-based condition regimen and thus to allow to support the extension of the use of Treosulfan to this population.

As a consequence, sections 4.1, 4.2, 4.4, 4.8 and 5.1 of the SmPC are updated as well as the Package Leaflet.

The MAH agreed to a CHMP recommendation to submit the final study report of study MC-FludT.16/NM when available. This is of interest as allo-HSCT will be applied in the treatment for non-malignant diseases with the expectation of long-term treatment benefit.

2.5. Clinical safety

Introduction

The most commonly reported undesirable effects of treosulfan are myelosuppression (leukocytopenia, thrombocytopenia, anaemia) and gastrointestinal complaints (nausea, vomiting). They are usually mild and resolve after treatment.

Bone marrow suppression is the dose-limiting side effect. However, it is this toxicity that supported the development of TREO for conditioning treatment prior to HSCT, especially in the allogenic setting. For this indication, TREO is always combined with FLU. Most paediatric patients receive additionally thiotepa (TT). This conditioning regimen is followed by infusion of haematopoietic stem cells. Furthermore, other immunosuppressive agents are usually given concomitantly or shortly thereafter.

Bone marrow depression (neutropenia, leukocytopenia, thrombocytopenia, anaemia) and immunosuppression are therefore desired therapeutic effects of the conditioning regimen and consequently cannot be considered as undesirable effects. Therefore, any changes in blood counts and differential blood counts occurring between Day -6 and Day +28 had not to be documented as AEs. Especially during the time of bone marrow aplasia induced by the conditioning regimen, infections may develop and are a major source of morbidity and mortality of patients.

Hyperbilirubinemia, mucositis/stomatitis, seizures, and HSOS (formerly designated as veno-occlusive disease/VOD) are considered as significant adverse events of conditioning treatment followed by alloHSCT. These significant AEs were of special interest in all clinical studies with TREO-based conditioning.

Graft versus host disease (GvHD) is commonly observed after alloHSCT. GvHD is considered not related to TREO but to the engraftment of the allogeneic immune system. How much the conditioning regimen influences the frequency and severity of GvHD is a matter of debate. Therefore, incidences of acute and chronic GvHD were intensively monitored in all studies.

Facing the above, the adverse events (AEs) and adverse reactions (ARs) observed with TREO-based conditioning followed by alloHSCT are not only due to TREO alone but relate to the whole complex treatment procedure of alloHSCT.

Study **MC-FludT.16/NM** was performed against busulfan (BU)-containing regimen. Busulfan is also used for conditioning prior to alloHSCT, mostly together with FLU or CY. Important ARs of conditioning regimens with BU/FLU followed by alloHSCT include infections or reactivation of opportunistic infectious pathogens, nervous system disorders, eye disorders, cardiac disorders, vascular disorders, respiratory thoracic and mediastinal disorders , gastrointestinal disorders, hepato-biliary disorders, skin and subcutaneous tissue disorders, musculoskeletal and connective tissue disorders, renal and urinary disorders, reproductive system and breast disorders , general disorders and administration site conditions and investigations. Such ARs could potentially also be observed with TREO.

In order to support pooled analyses of medical coded terms across all TREO trials, AEs of all studies were recoded according to MedDRA Version 20.0.

The results are summarised in an integrated summary of safety (ISS 2021; Report location: CTD Section 5.3.5.3). The primary objective of this ISS was to characterise the safety profile of TREO in the transplant

setting, for adult and paediatric population. The characterisation of TREO's undesirable effects and respective frequencies is reflected in the Summary of Product Characteristics (SmPC) which has been updated in the current variation in respect to Summary of the safety profile, as well as the Tabulated list of adverse reactions for Paediatric population and Description of selected adverse reactions.

Patient exposure

A total 121 paediatric patients have been treated with TREO-based conditioning, including 51 patients with non-malignant diseases (primary immunodeficiency, haemoglobinopathy, inborn error of metabolism and bone marrow failure syndromes) and 70 patients with malignant diseases (AML, ALL, MDS, and JMML).

The study in non-malignant diseases (MC-FludT.16/NM) also included an active-control group with 50 evaluable patients treated with the reference conditioning regimen BU/FLU ± TT.

This safety data set is supplemented by safety data derived from two registry studies of the EBMT [Peters 201193; Peters 2017] which included a total of 1 521 paediatric patients with malignant and non-malignant diseases who had been treated with TREO-based conditioning. Therefore, available safety data cover all paediatric patients which are currently treated with alloHSCT.

Table 2.7.4.1.3-6: Demographic profile of paediatric patients

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source	*Tables 11.2.1.A and 11.2.4.B*		*Tables 11.2.A and 11.2.3.B*
Study arm	**TREO**	**BU**	**TREO**
No. of patients	51 (100%)	50 (100%)	70 (100%)
Gender n (%)			
Female	15 (29.4%)	19 (38.0%)	26 (37.1%)
Male	36 (70.6%)	31 (62.0%)	44 (62.9%)
Age (years)			
Mean (SD)	5.0 (4.4)	6.0 (5.3)	9.1 (5.8)
Median (Range)	4 (0, 17)	5 (0, 17)	9.5 (0-17)
ICH age group, n (%)			
28 days to 23 months	14 (27.5%)	14 (28.0%)	9 (12.9%)
2 years to 11 years	31 (60.8%)	26 (52.0%)	28 (40.0%)
12 years to 17 years	6 (11.8%)	10 (20.0%)	33 (47.1%)
Race, n (%)			
White	41 (80.4%)	43 (86.0%)	70 (100%)
Black or African American	2 (3.9%)	2 (4.0%)	0 (0.0%)
Asian	6 (11.8%)	1 (2.0%)	0 (0.0%)
Arabian	1 (2.0%)	1 (2.0%)	0 (0.0%)
Arabic	0	2 (4.0%)	0 (0.0%)
Turkish	1 (2.0%)	1 (2.0%)	0 (0.0%)
Body surface area, m^2			
Mean (SD)	0.746 (0.297)	0.836 (0.396)	1.110 (0.480)
Median (Range)	0.67 (0.30, 1.50)	0.71 (0.35, 1.97)	1.100 (0.32-2.00)
Applied performance score*, n (%)			
Lansky performance score	50 (98.0%)	48 (96.0%)	58 (82.9%)
Karnofsky performance score	1 (2.0%)	2 (4.0%)	12 (17.1%)
Karnofsky/Lansky score*, n (%)			
70	1 (2.0%)	2 (4.0%)	1 (1.4%)
80	0 (0.0%)	1 (2.0%)	9 (12.9%)
90	7 (13.7%)	9 (18.0%)	22 (31.4%)
100	43 (84.3%)	38 (76.0%)	38 (54.3%)
Karnofsky/Lansky score*			
Median (range)	100 (70-100)	100 (70-100)	100 (70-100)

*Karnofsky score if age ≥ 16 years at registration, Lansky score if age < 16 years at registration

The majority of patients received the intensified regimen with thiotepa and a transplant from a matched unrelated donor.

Table 2.7.4.1.3-7: Diseases, donor type, and conditioning regimen in the two paediatric studies

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Data source	*Tables 11.2.1.A, -4.B, 14.1.1D*		*Tables 11.2.A, 14.1.4C/-P*
Study arm	**TREO**	**BU**	**TREO**
No. of patients	50 (100%)	51 (100%)	70 (100%)
Disease, n (%)			
AML			29 (41.4%)
ALL			27 (38.6%)
MDS			10 (14.3%)
JMML			4 (5.7%)
Haemoglobinopathy	23 (45.1%)	28 (56.0%)	
Primary immunodeficiency	21 (41.2%)	13 (26.0%)	
Bone marrow failure syndrome	5 (9.8%)	5 (10.0%)	
Inborn error of metabolism	2 (3.9%)	4 (8.0%)	
Donor type, n (%)			
MRD	14 (27.4%)	17 (34.0%)	14 (20.0%)
MUD	37 (72.6%)	33 (66.0%)	56 (80.0%)
Conditioning regimen, n (%)			
Intensified regimen (with TT)	43 (84.3%)	42 (84.0%)	65 (92.9%)
Standard regimen	8 (15.7%)	8 (16.0%)	5 (7.1%)

For treatment of infants and smaller children consideration a population-pharmacokinetic model for dose calculation was developed. According to this model, body surface area was the only clinically relevant covariate for plasma clearance and volume of distribution of TREO (**see above section 5.3.2. Pharmacokinetics and LoQ**). The proposed dose regimen for TREO in children therefore ranges from 10-14 g/m²/d, given on days -6 to -4.

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Study arm	**Treosulfan**	**Busulfan**	**Treosulfan**
Data source in CSR	Table 14.3.6.1A/B/C/E		Table 12.1.A/B
Number of patients	51	50	70
Dose regimen treosulfan			
10 g/m²/d × 3	9 (17.6%)		6 (8.6%) patients
12 g/m²/d × 3	32 (62.7%)		26 (37.1%) patients
14 g/m²/d × 3	10 (19.6%)		38 (54.3%) patients
Mean total dose (SD); g/m²	36.00 (3.82)		38.65 (3.95)
Mean total dose (SD); g	27.77 (13.67)		44.53 (21.98)
Dose regimen busulfan			Not applicable
Regimen		3.2-4.8 mg/kg/d, Days -7, -6, -5, -4	-
Mean total dose (SD); mg/kg		16.63 (2.88)	-
Mean total dose (SD); mg		363.0 (194.7)	-
Dose regimen fludarabine			
Regimen	30 mg/m²/d, Days -7, -6, -5, -4, -3	30 mg/m²/d, Days -7, -6, -5, -4, -3	30 mg/m²/d, Days -7, -6, -5, -4, -3
Dose regimen thiotepa			
Number of patients	43 (84.3%)	42 (84.0%)	65 (92.6%)
Regimen	2 × 5 mg/kg, Day -2	2 × 5 mg/kg, Day -2	2 × 5 mg/kg, Day -2
Mean total dose [SD]; mg			349.6 (198.8)
SD = standard deviation			

Adverse events (overall)

The following table gives an overall summary of AEs observed in the two paediatric trials. In the BU group, more patients than in the TREO group experienced life-threatening SAEs and SAEs resulting in death as well as drug-related SAEs. In the TREO group, more patients than in the BU group experienced SAEs that required hospitalisation or prolongation of hospitalisation.

Table 2.7.4.2.1.1-6: Overall summary of adverse events in two paediatric trials

Study	MC-FludT.16/NM		MC-FludT.17/M
Treatment arm	**TREO**	**BU**	**TREO**
Data source	*Table 12.2.1.A*		*Table 12.2.1.A*
Number of patients	51 (100%)	50 (100%)	70 (100%)
Any adverse event			
Patients with AEs of any CTCAE Grade	96.1%	96.0%	97.1%
Patients with AEs of at least CTCAE Grade III	80.4%	82.0%	75.7%
Drug-related adverse events			
Patients with ADRs of any CTCAE Grade	80.4%	74.0%	90.0%
Patients with ADRs of at least CTCAE Grade III	51.0%	50.0%	48.6%
Serious adverse events			
Patients with at least one serious AE	35.3%	32.0%	32.9%
Results in death	0%	8.0%	1.4%
Life-threatening	5.9%	8.0%	8.6%
Hospitalisation or prolongation of hospitalisation	31.4%	16.0%	28.6%
Disability/Incapacity	0%	2.0%	1.4%
Congenital anomaly or birth defect	0%	0%	0%
Drug-related serious adverse events			
Patients with drug related serious AEs	3.9%	6.0%	1.4%
Patients with maximum CTCAE Grade			
CTCAE Grade I	0%	2.0%	4.3%
CTCAE Grade II	15.7%	12.0%	17.1%
CTCAE Grade III	66.7%	60.0%	60.0%
CTCAE Grade IV	13.7%	16.0%	15.7%
CTCAE Grade V	0%	6.0%	0%

Treatment-emergent adverse events

The most frequently observed TEAEs after TREO-based conditioning include: gastrointestinal disorders (90.9%; includes stomatitis [74.4%], vomiting [67.8%], diarrhoea [62.8%], nausea [38.8%], abdominal pain [37.2%], and constipation [13.2%]), pyrexia (71.9%), infections (63.6%), skin and subcutaneous tissue disorders (58.7%; includes maculopapular rash [27.3%], pruritus [22.3%], and alopecia [9.9%]; Dermatitis diaper may occur in small children because of excretion of TREO in the urine. Therefore, diapers should be changed frequently up to 6-8 hours after each infusion of TREO), hepatotoxicity (41.3%), investigations (37.2%; includes increased ALT [13.2%], positive viral test [11.6%], increased AST [9.9%], and increased bilirubin [9.9%]), hypertension (33.9%), headache (27.3%), pain in extremity (18.2%), cough (19.0%), hypokalaemia (11.6%), hypersensitivity (10.7%), and sinus tachycardia (10.7%).

The most frequent (> 10%) severe at least CTCAE grade III TEAEs in the two paediatric trials include infections (38.0%), stomatitis (35.5%), diarrhoea (14.0%), nausea (12.4%), vomiting (12.4%), and hypertension (11.6%). There was a significant association between age and respiratory toxicity in paediatric patients in the EBMT registry [Peters 2011]. Children below the age of one year (mainly non-malignant diseases) experienced more respiratory grade III/IV toxicity. This observation could be explained by the fact that it is likely that babies underwent HSCT for immunodeficiencies, i.e. diseases which are often associated with severe pulmonary infections even before HSCT.

More patients (> 5% difference) transplanted for malignant versus non-malignant diseases experienced viremia (20.0% vs. 2.0%), device-related infection (11.4% vs. 3.9%), hypersensitivity reactions (17.1% vs. 2.0%), hypokalaemia (15.7% vs. 5.9%), psychiatric disorders (7.1% vs. 0%), nausea (45.7% vs. 29.4%), bone pain (10.0% vs. 0%), positive viral test (20% vs. 0%), and increased blood bilirubin (12.9% vs. 5.9%).

Epistaxis (2.9% vs. 15.7%), abdominal pain (31.4% vs. 45.1%), hepatotoxicity (34.3% vs. 51.0%), pruritus (18.6% vs. 27.5%), alopecia (1.4% vs. 21.6%), increased CRP (0% vs. 11.8%), and infusion-related reaction (7.1% vs. 17.6%) were more frequently seen in patients transplanted for non-malignant diseases.

The frequency of TEAEs was broadly similar in the 2 treatment arms of **study 16/NM**, with a few exceptions. Differences ≥ 10% for TREO vs. BU were seen for diarrhoea (58.8% vs. 46.0%), abdominal pain (45.1% vs. 30.0%), lung infection (2.0% vs. 12.0%), dry skin (0% vs. 10.0%) and maculo-papular rash (25.5% vs. 14.0%) while more patients in the BU arm compared to the TREO arm experienced (BU vs TREO) oral mucositis (48.0% vs. 27.5%), nausea (18.0% vs. 5.9%). Differences ≥ 5% TREO vs. BU were seen for infections (39.2% vs. 34.0%), febrile neutropenia (7.8% vs. 0%), and vascular events (19.6% vs. 12.0%), whereas more patients in the BU vs TREO presented vomiting (14.0% vs. 7.8%) and respiratory disorders (18.0% vs. 7.8%).

Drug-related TEAEs

The most frequently (≥ 5%) observed TREO-related TEAEs include stomatitis (66.1%), vomiting (42.1%), diarrhoea (33.1%), nausea (26.4%), hepatotoxicity (26.4%), abdominal pain (16.5%), pyrexia (13.2%), infections (11.6%), ALT increased (10.7%), and pruritus (10.7%). Most frequent (≥ 5%) severe ARs (≥ Grade III) after TREO-based conditioning include stomatitis (32.2%), nausea (8.3%), diarrhoea (7.4%), and infections (5.0%). The overall incidence of drug-related infections (11.6%) in 121 paediatric patients was slightly higher to that seen in adults and a frequency higher in the paediatric age group 12-17 years (6 of 39 [15.4%]) compared to infants < 2 years of age (1 of 23 [4.3%]) but the limited number of patients included into the two paediatric studies is insufficient to reach to final conclusions.

Drug-related TEAEs were reported by 74.0% of subjects in the BU arm, and 80.4% of subjects in the TREO arm. Substantial differences (> 10% difference) between the 2 treatment arms (BU vs. TREO) were seen in the incidence of diarrhoea (22.0% vs. 39.2%), nausea (30.0% vs. 17.6%), abdominal pain (12.0% vs. 23.5%), other hepatobiliary disorders (46.0% vs. 33.3%), and pruritus (4.0% vs. 15.7%).

Drug-related TEAEs with at least CTCAE grade III TEAEs were reported by 50.0% of patients in the BU arm, and 51.0% of patients in the TREO arm. There was a substantial difference (> 10% difference) between the 2 treatment arms in the incidence of oral mucositis (BU vs. TREO: 36.0% vs. 25.5%).

The most frequent AR reported in blood and lymphatic system disorders SOC category was febrile neutropenia, which was recorded in only 2 of 121 paediatric patients (1.7%) treated with TREO-based conditioning.

In respect to endocrine disorders, data from a retrospective, multicentre study of 137 children undergoing alloHSCT, indicate that the frequency of gonadal damage associated with TREO was significantly lower than that observed after BU [Faraci 2019]. This is a small study where very few TREO-based treated patients were included (19) versus BU-based treated patients (118), but this observation is corroborated by data from a more recent study [Leiper 2020] where the fertility in survivors of HSCT after three chemotherapy-conditioning regimens of different intensity which included TREO-based, BU/cyclophosphamide (BU/Cy) and Fludarabine/melphalan (Flu-Mel) containing regimens was assessed by measuring serum concentrations of Anti-Müllerian hormone (AMH) and Inhibin B. The MAH data obtained suggested less ovarian reserve impairment after TREO and Flu-Mel than after Bu-Cy and the mean serum AMH concentration was significantly better with treosulfan (>1.0 µg/L) than with Flu-Mel or Bu-Cy. The same more favourable trend for TREO-based conditioning regimen is seen in males regarding Inhibin B data with the Flu-Mel group suffering greatest impairment. These authors reach the same conclusion, that a TREO-based regimen confers a more favourable gonadal compromise than Flu-Mel or Bu-Cy in both sexes.

Only 5 paediatric patients developed neurological ARs (3 x headache, 2 x paraesthesia, 1 x seizure) after TREO-based conditioning. Only two paediatric patients developed ARs belonging to Eye disorders SOC category. Only three vascular disorders related ARs (Capillary leak syndrome, hypertension, hypotension) were seen in paediatric patients. No paediatric patient developed ARs belonging to ear and

labyrinth disorders or cardiac disorders. Two cases of acute kidney injury (AKI) were seen in the paediatric studies (1.7%).

Serious adverse event/deaths/other significant events

The following table summarises the **death** cases in the two paediatric trials. Death rate in paediatric patients after TREO-based conditioning was much lower than that observed in adult patients. Only 14 of 121 patients (11.6%) included so far into the two paediatric trials died, and only 6 (5.0%) from transplant-related causes. In the study in patients with non-malignant diseases more patients died transplant-related in the BU group compared to the TREO group (7 vs. 2). Only two patients in the TREO group died in MU-FludT.16/NM trial so far.

Table 2.7.4.2.1.2-3: Death cases in the two paediatric trials

	MC-FludT.16/NM		MC-FludT.17/M	Overall
Treatment	**Busulfan**	**Treosulfan**	**Treosulfan**	**Treosulfan**
Total number of patients	50	51	70	121
Survival status at study termination; n (%)				
Alive[a]	43 (86.0%)	49 (96.1%)	58 (82.9%)	107 (88.4%)
Dead	7 (14.0%)	2 (3.9%)	12 (17.1%)	14 (11.6%)
Cause of death; n (%)				
Relapse/progression	0	0	8 (11.4%)	8 (6.6%)
Transplantation related	7 (14.0%)	2 (3.9%)	4 (5.7%)	6 (5.0%)

	MC-FludT.16/NM		MC-FludT.17/M	Overall
Treatment	**Busulfan**	**Treosulfan**	**Treosulfan**	**Treosulfan**
GvHD	2 (4.0%)	2 (3.9%)	1 (1.4%)	3 (2.5%)
Pulmonary toxicity	1 (2.0%)	0 (0.0%)	1 (1.4%)	1 (0.8%)
Haemorrhage	1 (2.0%)	0 (0.0%)	1 (1.4%)	1 (0.8%)
Renal failure	0 (0.0%)	1 (2.0%)	0 (0.0%)	1 (0.8%)
Gastrointestinal toxicity	0 (0.0%)	1 (2.0%)	0 (0.0%)	1 (0.8%)
Interstitial pneumonia	1 (2.0%)	0 (0.0%)	2 (2.9%)	2 (1.7%)
EBV proliferative disease	0 (0.0%)	1 (2.0%)	0 (0.0%)	1 (0.8%)
Infection	5 (10.0%)	1 (2.0%)	3 (4.3%)	4 (3.3%)
Multiple organ failure	1 (2.0%)	2 (3.9%)	4 (3.3%)	4 (3.3%)
Other	1 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Time from transplantation to death (months)				
Mean (SD)	6.44 (8.44)	5.73 (2.49)	14.97 (12.35)	13.65 (11.87)
Median (Range)	2.96 (2.2, 25.3)	5.73 (4.0, 7.5)	11.01 (0.5, 46.7)	9.69 (0.5, 46.7)

Data source: ISS 2021, Table 4.1.2A; CSR Table 12.3.1.1B; [a] The status 'alive' is displayed for all patients who did not die during the trial.

Data in paediatric patients with non-malignant diseases are also available from the 2017 EBMT registry [Peters 2017] and also show a lower number of death cases with TREO/FLU/TT versus BU/FLU/TT (10.1% vs. 17.0%). The most common causes of death after HSCT for non-malignant diseases were infection, graft versus host disease, the original disease and transplant associated organ complications. Deaths due to graft failure or due to secondary malignancies were rare events.

Table 2.7.4.2.1.2-4: Causes of death in children with non-malignant diseases; n (%)

Regimen	**TREO/FLU**	**BU/FLU**	**TREO/FLU/TT**	**BU/FLU/TT**
Number of patients in the registry	422	1063	473	229
Total number of deaths	69 (16.4%)	182 (17.1%)	48 (10.1%)	39 (17.0%)
Cardiac toxicity	0	4 (2.5%)	2 (4.5%)	2 (6.2%)
Haemorrhage	3 (4.6%)	6 (3.8%)	2 (4.5%)	2 (6.2%)
Failure/rejection	1 (1.5%)	4 (2.5%)	1 (2.3%)	2 (6.2%)
Infection	28 (43.1%)	45 (28.1%)	13 (29.5%)	11 (34.4%)
Interstitial pneumonitis	8 (12.3%)	11 (6.9%)	3 (6.8%)	1 (3.1%)
GvHD	8 (12.3%)	43 (26.9%)	8 (18.2%)	5 (15.6%)
Original disease	7 (10.8%)	21 (13.1%)	9 (20.5%)	5 (15.6%)
Second malignancy	1 (1.5%)	1 (0.6%)	0	0
Other	2 (3.1%)	10 (6.2%)	2 (4.5%)	1 (3.1%)
Transplant-related	7 (10.8%)	15 (9.4%)	4 (9.1%)	3 (9.4%)
Not reported/unknown	4	22	4	7

Data source: Peters 2017 (Table 12)

Table 2.7.4.2.1.4.3-1 shows the incidence of selected significant AEs observed in the two paediatric trials. No pulmonary fibrosis was seen. In trial MC-FludT.16/NM, more patients in the BU arm developed HSOS (5 versus 1). All other parameters were comparable in both groups.

Table 2.7.4.2.1.4.3-1: Incidence of selected toxicities in two paediatric trials (Safety Set)

Adverse event	MC-FludT.16/NM		MC-FludT.17/M
Treatment arm	**BU**	**TREO**	**TREO**
Number of patients	50	51	70
HSOS [Jones 1987]			
Patients with event	5 (10.0%)	1 (2.0%)	1 (1.4%)
Incidence of event [%] (95% CI)	10.0 (3.3, 21.8)	2.0 (0.0, 10.4)	1.4 (0.0, 7.7)
P value	0.1120		
Early toxicity (until Day +28)			
Patients with event	48 (96.0%)	48 (94.1%)	68 (97.1%)
Incidence of event [%] (95% CI)	96.0 (86.3, 99.5)	94.1 (83.8, 98.8)	97.1 (90.1, 99.7)
P value	1.0000		
Hepatic toxicity [Bearman 1988]			
Patients with event	27 (54.0%)	26 (51.0%)	24 (34.3%)
Incidence of event [%] (95% CI)	54.0 (39.3, 68.2)	51.0 (36.6, 65.2)	34.3 (23.3, 46.6)
P value	0.8429		
Infections			
Patients with event	35 (70.0%)	31 (60.8%)	50 (71.4%)
Incidence of event [%] (95% CI)	70.0 (55.4, 82.1)	60.8 (46.1, 74.2)	71.4 (59.4, 81.6)
P value	0.4044		
Data source in CSR	*Table 12.3.1.3.A*		*Table 12.3.1.3.A*

Other Serious Adverse Events

The frequency of SAEs occurring in at least 2 of the total 121 patients treated with TREO within two clinical studies in paediatric patients is shown in the table below. Results are comparable in both trials. A total of 41 of 121 paediatric patients (33.9%) experienced an SAE after TREO-based conditioning. Most frequent SAEs were infection (22.3%), pyrexia (6.6%) and febrile neutropenia (2.5%).

Table 2.7.4.2.1.3-7: Frequency of SAEs occurring in ≥ 2 patients treated with TREO (Safety Set)

	MC-FludT.16/NM	MC-FludT.17/M	Overall
Total number of patients	51	70	121
Patients with any event	18 (35.3%)	23 (32.9%)	41 (33.9%)
Infections and infestations			
Any event	12 (23.5%)	15 (21.4%)	27 (22.3%)
Cytomegalovirus infection	3 (5.9%)	3 (4.3%)	6 (5.0%)
Sepsis	2 (3.9%)	2 (2.9%)	4 (3.3%)
Upper respiratory tract infection	1 (2.0%)	3 (4.3%)	4 (3.3%)
Device related infection	1 (2.0%)	1 (1.4%)	2 (1.7%)
Encephalitis	1 (2.0%)	1 (1.4%)	2 (1.7%)
Epstein-Barr virus infection	1 (2.0%)	1 (1.4%)	2 (1.7%)
Blood and lymphatic system disorders			
Any event	2 (3.9%)	3 (4.3%)	5 (4.1%)
Febrile neutropenia	0 (0.0%)	3 (4.3%)	3 (2.5%)
Nervous system disorders			
Any event	1 (2.0%)	2 (2.9%)	3 (2.5%)
Encephalopathy	1 (2.0%)	1 (1.4%)	2 (1.7%)
Respiratory, thoracic and mediastinal disorders			
Any event	0 (0.0%)	2 (2.9%)	2 (1.7%)
Gastrointestinal disorders			
Any event	1 (2.0%)	3 (4.3%)	4 (3.3%)
General disorders			
Any event	6 (11.8%)	2 (2.9%)	8 (6.6%)
Pyrexia	6 (11.8%)	2 (2.9%)	8 (6.6%)
Note: Absolute and relative frequencies of patients with event relative to the total number of patients (N).			
Data source: ISS 2021, Table 4.2.2A			

Frequency of SAEs was also comparable in both groups of trial MC-FludT.16/NM, with the occurrence of more infections and more general disorders (all cases were fever) in the TREO group.

Table 2.7.4.2.1.3-8: Frequency of patients with SAEs by SOC in trial MC-FludT.16/NM

	MC-FludT.16/NM	
Treatment	**Treosulfan**	**Busulfan**
Total number of patients	51	50
Patients with any event	18 (35.3%)	16 (32.0%)
Infections and infestations	12 (23.5%)	7 (14.0%)
Neoplasms	1 (2.0%)	0
Blood and lymphatic system disorders	1 (2.0%)	4 (8.0%)
Immune system disorders	1 (2.0%)	0
Nervous system disorders	1 (2.0%)	1 (2.0%)
Respiratory disorders	0	4 (8.0%)
Gastrointestinal disorders	1 (2.0%)	0
Hepatobiliary disorders	0	2 (4.0%)
General disorders	6 (11.8%)	1 (2.0%)
Investigations	1 (2.0%)	0
Injury, poisoning and procedural complications	1 (2.0%)	0
Data source: CSR Table 12.3.1.2.A		

The following table summarises the results of aGvHD observed in the two paediatric trials. Incidences were slightly higher in patients with non-malignant diseases. The cumulative incidence of aGvHD after TREO-based conditioning in the two paediatric studies was 54.9% in patients with non-malignant diseases and 43.5% in patients with malignant diseases. This incidence is comparable to that observed in adult patients.

Table 2.7.4.2.1.5.1-6: Summary table of aGvHD

Study	**MC-FludT.16/NM**		**MC-FludT.17/M**
Treatment arm	**TREO**	**BU**	**TREO**
Number of patients	51	50	70
Cumulative incidence aGvHD, all grades			
At Day 14; % (90% CI)	13.7 (5.8, 21.7)	2.0 (0.0, 5.3)	7.2 (2.1, 12.4)
At Day 28; % (90% CI)	37.3 (26.1, 48.4)	30.0 (19.3, 40.7)	37.7 (28.1, 47.3)
At Day 100; % (90% CI)	54.9 (43.4, 66.4)	42.0 (30.5, 53.5)	43.5 (33.7, 53.3)
HR TREO/BU* (90% CI)	1.65 (1.02, 2.67)		
P value*	0.0889		
Cumulative incidence aGvHD, grade III/IV			
At Day 14; % (90% CI)	2.0 (0.0, 5.2)	2.0 (0.0, 5.3)	0.0 (0.0, 0.0)
At Day 28; % (90% CI)	2.0 (0.0, 5.2)	4.0 (0.0, 8.6)	5.8 (1.2, 10.4)
At Day 100; % (90% CI)	13.7 (5.8, 21.7)	8.0 (1.7, 14.3)	8.7 (3.1, 14.3)
HR TREO/BU* (90% CI)	1.63 (0.55, 4.81)		
P value*	0.4598		
Data source	*Tables 14.3.5.1A; 14.3.5.3A*		*Table 14.3.5B*
** adjusted for TT and disease as factors using Fine and Gray model*			

Results of GvHD from the two EBMT registry studies indicate that the incidence of grade III/IV aGvHD after TREO-based conditioning was 10% with no significant correlation with age. Also, the TREO dose had no significant impact on aGvHD in both univariate and multivariate analysis adjusted for diagnoses, age, number of HSCTs, remission status, donor and conditioning regimen. For malignant diseases, there was a borderline significant impact of age-group on the incidence of aGvHD of any grade (P = 0.045), the aGvHD-incidence is monotonously decreasing with age [Peters 2011 (chapter 3.1.1)]. For non-malignant diseases, there is no significant association between grade III/IV toxicity and dose-group in the subgroups of patients according to donor type, and diagnoses [Peters 2011].

Table 2.7.4.2.1.5.1-8: Acute GvHD and age group

Patient group	Age group	N	aGvHD all grades	P*	aGvHD grade III/IV	P*
All	< 0.5 years	41	28%	1.000	5%	0.464
	0.5-1 year	65	50%		9%	
	1-12 years	314	46%		11%	
	12 years	101	35%		10%	
Malignant	< 0.5 years	0	-	0.045	-	1.000
	0.5-1 year	7	71%		0	
	1-12 years	100	54%		14%	
	12 years	58	40%		12%	
Non-malignant	< 0.5 years	41	28%	0.947	5%	0.737
	0.5-1 year	58	47%		11%	
	1-12 years	214	43%		10%	
	12 years	43	28%		7%	
First HSCT	< 0.5 years	40	28%	0.953	5%	0.315
	0.5-1 year	62	52%		10%	
	1-12 years	254	48%		13%	
	12 years	81	36%		11%	
*Mantel-Haenszel test: tests the alternative-hypothesis that there is a monotone relationship between dose group and toxicity rates						

Cumulative incidence of cGvHD was higher in the BU arm of trial MC-FludT.16/NM.

Table 2.7.4.2.1.5.1-7: Summary table of cGvHD

Study	MC-FludT.16/NM		MC-FludT.17/M
Treatment arm	**TREO**	**BU**	**TREO**
Number of patients	51	50	70
Cumulative incidence of cGvHD			
At 12 months; % (90% CI)	12.8 (4.8, 20.8)	38.6 (26.6, 50.7)	23.9 (15.3, 32.4)
At 24 months; % (90% CI)	12.8 (4.8, 20.8)	38.6 (26.6, 50.7)	25.4 (16.6, 34.1)
At 36 months; % (90% CI)	12.8 (4.8, 20.8)	38.6 (26.6, 50.7)	25.4 (16.6, 34.1)
HR TREO/BU* (90% CI)	0.31 (0.14, 0.69)		
P value*	0.0168		
Cumulative incidence of moderate/severe cGvHD			
At 12 months; % (90% CI)	10.6 (3.2, 18.0)	22.7 (12.3, 33.1)	17.9 (10.2, 25.6)
At 24 months; % (90% CI)	10.6 (3.2, 18.0)	22.7 (12.3, 33.1)	19.4 (11.5, 27.4)
At 36 months; % (90% CI)	10.6 (3.2, 18.0)	22.7 (12.3, 33.1)	19.4 (11.5, 27.4)
HR TREO/BU* (90% CI)	0.46 (0.18, 1.15)		
P value*	0.1611		
Data source	*Tables 14.3.5.4A; 14.3.5.5A*		*Table 14.3.5ZA*
* adjusted for TT and disease as factors using Fine and Gray model			

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.

Secondary malignancies are well established complications in long-term survivors after alloHSCT. Two children treated in trial MC-FludT.17/M developed skin papilloma after TREO-based conditioning. One patient treated in the TREO arm of trial MC-FludT.16/NM developed MDS. In a retrospective analysis of 944 children who underwent HSCT for PID at two UK centres, 12 patients (1.27%) developed a non-posttransplant lymphoproliferative disorder malignancy. Three of these patients had received a TREO-based conditioning regimen [Unni 2018]. Primary immunodeficiencies are diseases associated with an increased risk for neoplasias per se.

Laboratory findings

Haematology: In trial MC-FludT.16/NM, the median level of WBCs at baseline was similar in the two treatment arms. The median level of WBC fell severely after Day -3. The lowest median value was recorded at Day +6 for both treatment arms, 0.08 G/L in the BU arm and 0.02 G/L in the TREO arm. The median value in both arms rose steadily from this point, and at Day +100 the median value was 3.23 G/L for the BU arm, and 4.40 G/L for the TREO arm. In trial MC-FludT.17/M, the median WBC level at baseline was 1.99 G/L (Q1 1.42; Q3 3.34). The level of WBC fell severely at visit Day -1 and continued to fall further with continuation of conditioning treatment. The lowest median value was recorded at visit

Day +6, 0.02 G/L (Q1: 0.01; Q3: 0.05). The median value rose steadily from this point, and at visit Day +100 the median value was 3.57 G/L (Q1: 2.30; Q3: 5.33).

Clinical chemistry: Incidence of increased bilirubin/ALT/AST in 121 paediatric TREO patients was 6.6%/10.7%/ 6.6%, comparable to the data seen in adults. In trial MC-FludT.16/NM no significant differences between both treatment groups were seen. Median levels of liver parameters were always below the upper limit of normal (ULN) with the exception of ALT which was slightly elevated at Day +6; median levels of electrolytes (sodium, potassium) did not much change from baseline in both treatment groups; median levels of CRP and procalcitonin increased after conditioning treatment but values normalized up to Day +28. The other parameters were relatively unaffected. In trial MC-FludT.17/M, baseline values of the liver function parameters (ALT, AST, γGT, AP, bilirubin) showed a considerable variability. Only γGT and bilirubin showed a significant increase of values compared to baseline while the other parameters were always below ULN. No SAE related to a change in laboratory values was reported; median levels of electrolytes did not much change from baseline; median values of LDH, CRP and procalcitonin increased to some extent after baseline but CRP and procalcitonin rapidly returned to normal values.

Vital signs, physical findings, and other observations related to safety

In trial MC-FludT.16/NM, treatment with TREO did not remarkably influence systolic or diastolic blood pressure, pulse rate and body temperature. Six patients in the BU arm (12.0%) and 8 patients in the TREO arm (15.7%) experienced CTCAE grade III "Hypertension". No patient in the BU arm but one patient in the TREO arm experienced CTCAE grade III "Hypotension". No CTCAE grade IV event was reported. The median Karnofsky/Lansky Performance Score (KPS/LPS) did not change significantly and usually ranged between 90 and 100. The Kaplan Meier estimate at 12 months for subjects showing a deterioration to less than 60 points was 8.3% in the TREO group and 12.7% in the BU group.

In trial MC-FludT.17/M, treatment with TREO did not remarkably influence systolic or diastolic blood pressure, pulse rate and body temperature. Five patients reported AE of CTCAE Grade III Term "Hypertension" (all resolved, except for 1 patient with not recovered/not resolved), 1 patient reported AE for each CTCAE Grade III Term "Hypotension" and "Fever" (both resolved). The median Karnofsky/Lansky Performance Score (KPS/LPS) did not change significantly and usually ranged between 90 and 100. The overall Kaplan-Meier estimate at 12 months for subjects showing a deterioration to less than 60 points was 13.6% (90% CI 7.3, 24.5).

Adverse events (per trial)

MC-FludT.16/NM

Criteria for Evaluation:

Safety:

Freedom from transplantation (treatment)-related mortality & Transplantation-related mortality: refer to efficacy evaluation.

Acute GvHD: Time to aGvHD was defined as the time between end of HSCT and the date of first occurrence of aGvHD. Acute GvHD was evaluated from the end of HSCT until 100 days after transplantation.

Chronic GvHD: Time to cGvHD was defined as the time between 100 days after end of HSCT and the first episode of cGvHD. Chronic GvHD was evaluated from 100 days after transplantation until the end of the longer-term follow-up.

Adverse events: All adverse events (AEs) (serious and non-serious) occurring between day -7 and day +100 were recorded continuously. After day +100, only serious AEs with suspected relatedness (serious adverse reactions [SAR]) to the investigational medicinal product (IMP) were documented up to the end of the longer-term follow-up phase.

Clinical laboratory tests: The subject's viral status was characterised by HIV, cytomegalovirus, EBV, hepatitis A, B, C, and herpes tests within 3 weeks prior to day -7. Pregnancy was assessed routinely in the subject's urine or serum within 3 weeks prior to day -7, in any female who had experienced menarche. The standard laboratory parameters (total blood count, differential blood count, serum chemistry, were documented once within 3 weeks prior to Day -7 (also for calculated GFR), at given time points between visit Day -7 and Day +28, and at visit Day +100 (total and differential blood count only).

Vital signs, physical examinations: Blood pressure, pulse, height, weight, and body temperature were assessed between day - 10 and day -8, blood pressure, pulse, and body temperature were assessed in addition at visit Day 0, +28, +100, Month 6, 9, and 12. Karnofsky Performance Score (KPS) or Lansky Performance Score (LPS) were assessed within 3 weeks prior to Day - 7, and at visit Day 0, Day +28, Day +100, Month 6, 9, and 12.

Results

SAFETY:

- The incidences of total Treatment-emergent adverse events (TEAEs) (busulfan: 96.0%, treosulfan: 96.1%) and subcategories of TEAEs were broadly similar in the 2 treatment arms. Differences of ⩾ 10% between the treatment arms were noted only in a few cases.

More subjects in the treosulfan arm than the busulfan arm had events in the SOCs "Skin and subcutaneous tissue disorders" and "Nervous system disorders".

Table 12.2.1.A Overall summary of treatment emergent adverse events (Safety Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Any adverse event [n (%)]			
Subjects with any adverse event	48 (96.0%)	49 (96.1%)	97 (96.0%)
Subjects with AEs of at least CTCAE grade III	41 (82.0%)	41 (80.4%)	82 (81.2%)
Drug-related adverse events [n (%)]			
Subjects with any drug-related adverse event	37 (74.0%)	41 (80.4%)	78 (77.2%)
Subjects with drug-related AEs of at least CTCAE grade III	25 (50.0%)	26 (51.0%)	51 (50.5%)
Serious adverse events [n (%)]			
Subjects with any serious adverse event	16 (32.0%)	18 (35.3%)	34 (33.7%)
- Results in death	4 (8.0%)	0 (0.0%)	4 (4.0%)
- Life-threatening	4 (8.0%)	3 (5.9%)	7 (6.9%)
- Hospitalization or prolongation of hospitalization	8 (16.0%)	16 (31.4%)	24 (23.8%)
- Disability/incapacity	1 (2.0%)	0 (0.0%)	1 (1.0%)
- Congenital anomaly or birth defect	0 (0.0%)	0 (0.0%)	0 (0.0%)
Drug-related serious adverse events [n (%)]			
Subjects with any drug-related serious adverse event	3 (6.0%)	2 (3.9%)	5 (5.0%)
Maximum CTCAE grade of adverse events [n (%)]			
Subjects with AEs of a maximum CTCAE grade I	1 (2.0%)	0 (0.0%)	1 (1.0%)
Subjects with AEs of a maximum CTCAE grade II	6 (12.0%)	8 (15.7%)	14 (13.9%)
Subjects with AEs of a maximum CTCAE grade III	30 (60.0%)	34 (66.7%)	64 (63.4%)
Subjects with AEs of a maximum CTCAE grade IV	8 (16.0%)	7 (13.7%)	15 (14.9%)
Subjects with AEs of a maximum CTCAE grade V	3 (6.0%)	0 (0.0%)	3 (3.0%)

Source: Table 14.3.1A

Abbreviations: AE = adverse event; CTCAE: Common Terminology of Adverse Events; N = total number of subjects; n = number of subjects in each category.

- The incidences of TEAEs related to IMP, were broadly similar in the 2 treatment arms (busulfan: 74.0%, treosulfan: 80.4%). Differences of ⩾ 10% between the treatment arms were noted only in a few cases.

More subjects in the busulfan arm than the treosulfan arm reported events in the SOC "Hepatobiliary disorders", while more subjects in the treosulfan arm than the busulfan arm reported events in the SOC "Skin and subcutaneous tissue disorders".

- The incidences of TEAEs of at least CTCAE grade III were broadly similar in the 2 treatment arms (busulfan: 82.0%, treosulfan: 80.4%). Differences of ⩾ 10% between the treatment arms were noted only in a few cases.

More subjects in the busulfan arm than the treosulfan arm had events in the SOCs "Gastrointestinal disorders" and "Respiratory, thoracic and mediastinal disorders". At the Term level, more subjects in the busulfan arm than the treosulfan arm reported "Mucositis oral" and "Nausea" but more subjects in the treosulfan arm reported diarrhoea and abdominal pain.

- The incidences of TEAEs of at least CTCAE grade III related to IMP were broadly similar in the 2 treatment arms (busulfan: 50.0%, treosulfan: 51.0%).

Differences of ⩾ 10% between the treatment arms were noted for the Term "Mucositis oral" which occurred more frequently in the busulfan arm than the treosulfan arm.

Table 12.2.2.1.A Frequency of subjects with treatment emergent adverse events by CTCAE System Organ Class and Term occurring in at least 5% of subjects in either treatment arm (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Subjects with any event	48 (96.0%)	49 (96.1%)	97 (96.0%)
Gastrointestinal disorders			
Any event	47 (94.0%)	46 (90.2%)	93 (92.1%)
Mucositis oral	40 (80.0%)	36 (70.6%)	76 (75.2%)
Vomiting	32 (64.0%)	34 (66.7%)	66 (65.3%)
Diarrhea	23 (46.0%)	30 (58.8%)	53 (52.5%)
Abdominal pain	15 (30.0%)	23 (45.1%)	38 (37.6%)
Nausea	19 (38.0%)	15 (29.4%)	34 (33.7%)
Constipation	7 (14.0%)	8 (15.7%)	15 (14.9%)
Gastrointestinal disorders - Other, specify	4 (8.0%)	3 (5.9%)	7 (6.9%)
Gastritis	2 (4.0%)	3 (5.9%)	5 (5.0%)
Stomach pain	2 (4.0%)	3 (5.9%)	5 (5.0%)
Anal mucositis	1 (2.0%)	3 (5.9%)	4 (4.0%)
General disorders and administration site conditions			
Any event	39 (78.0%)	39 (76.5%)	78 (77.2%)
Fever	36 (72.0%)	36 (70.6%)	72 (71.3%)
Infusion related reaction	6 (12.0%)	9 (17.6%)	15 (14.9%)
Chills	3 (6.0%)	7 (13.7%)	10 (9.9%)
Localized edema	4 (8.0%)	4 (7.8%)	8 (7.9%)
Fatigue	4 (8.0%)	3 (5.9%)	7 (6.9%)
Pain	3 (6.0%)	4 (7.8%)	7 (6.9%)
Infections and infestations			
Any event	35 (70.0%)	31 (60.8%)	66 (65.3%)
Infections and infestations - Other, specify	21 (42.0%)	23 (45.1%)	44 (43.6%)
Rhinitis infective	6 (12.0%)	4 (7.8%)	10 (9.9%)
Sepsis	5 (10.0%)	3 (5.9%)	8 (7.9%)
Lung infection	6 (12.0%)	1 (2.0%)	7 (6.9%)
Catheter related infection	4 (8.0%)	2 (3.9%)	6 (5.9%)
Enterocolitis infectious	2 (4.0%)	3 (5.9%)	5 (5.0%)
Skin infection	1 (2.0%)	3 (5.9%)	4 (4.0%)

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Hepatobiliary disorders			
Any event	29 (58.0%)	27 (52.9%)	56 (55.4%)
Hepatobiliary disorders - Other, specify	29 (58.0%)	26 (51.0%)	55 (54.5%)
Skin and subcutaneous tissue disorders			
Any event	23 (46.0%)	30 (58.8%)	53 (52.5%)
Pruritus	9 (18.0%)	14 (27.5%)	23 (22.8%)
Skin and subcutaneous tissue disorders - Other, specify	9 (18.0%)	13 (25.5%)	22 (21.8%)
Rash maculo-papular	7 (14.0%)	13 (25.5%)	20 (19.8%)
Alopecia	6 (12.0%)	11 (21.6%)	17 (16.8%)
Dry skin	5 (10.0%)	0 (0.0%)	5 (5.0%)
Skin hyperpigmentation	3 (6.0%)	2 (3.9%)	5 (5.0%)
Urticaria	1 (2.0%)	3 (5.9%)	4 (4.0%)
Hypertrichosis	0 (0.0%)	3 (5.9%)	3 (3.0%)
Respiratory, thoracic and mediastinal disorders			
Any event	21 (42.0%)	22 (43.1%)	43 (42.6%)
Cough	13 (26.0%)	10 (19.6%)	23 (22.8%)
Epistaxis	7 (14.0%)	8 (15.7%)	15 (14.9%)
Hypoxia	3 (6.0%)	4 (7.8%)	7 (6.9%)
Sore throat	2 (4.0%)	5 (9.8%)	7 (6.9%)
Respiratory, thoracic and mediastinal disorders - Other, specify	3 (6.0%)	1 (2.0%)	4 (4.0%)
Pharyngeal mucositis	3 (6.0%)	0 (0.0%)	3 (3.0%)
Vascular disorders			
Any event	21 (42.0%)	21 (41.2%)	42 (41.6%)
Hypertension	18 (36.0%)	19 (37.3%)	37 (36.6%)
Hematoma	6 (12.0%)	3 (5.9%)	9 (8.9%)
Hypotension	2 (4.0%)	3 (5.9%)	5 (5.0%)
Capillary leak syndrome	0 (0.0%)	3 (5.9%)	3 (3.0%)
Nervous system disorders			
Any event	14 (28.0%)	21 (41.2%)	35 (34.7%)
Headache	12 (24.0%)	14 (27.5%)	26 (25.7%)
Dizziness	6 (12.0%)	3 (5.9%)	9 (8.9%)
CTCAE System Organ Class CTCAE Term	**Busulfan (N=50)**	**Treosulfan (N=51)**	**Total (N=101)**
Investigations			
Any event	17 (34.0%)	16 (31.4%)	33 (32.7%)
Alanine aminotransferase increased	10 (20.0%)	8 (15.7%)	18 (17.8%)
Investigations - Other, specify	4 (8.0%)	8 (15.7%)	12 (11.9%)
Aspartate aminotransferase increased	7 (14.0%)	4 (7.8%)	11 (10.9%)
GGT increased	6 (12.0%)	2 (3.9%)	8 (7.9%)
Blood bilirubin increased	3 (6.0%)	3 (5.9%)	6 (5.9%)
Metabolism and nutrition disorders			
Any event	15 (30.0%)	14 (27.5%)	29 (28.7%)
Anorexia	3 (6.0%)	3 (5.9%)	6 (5.9%)
Iron overload	3 (6.0%)	3 (5.9%)	6 (5.9%)
Hyperkalemia	2 (4.0%)	3 (5.9%)	5 (5.0%)
Hypokalemia	2 (4.0%)	3 (5.9%)	5 (5.0%)
Musculoskeletal and connective tissue disorders			
Any event	11 (22.0%)	14 (27.5%)	25 (24.8%)
Pain in extremity	6 (12.0%)	9 (17.6%)	15 (14.9%)
Back pain	2 (4.0%)	3 (5.9%)	5 (5.0%)
Renal and urinary disorders			
Any event	11 (22.0%)	14 (27.5%)	25 (24.8%)
Hematuria	5 (10.0%)	5 (9.8%)	10 (9.9%)
Urinary frequency	1 (2.0%)	4 (7.8%)	5 (5.0%)
Cardiac disorders			
Any event	9 (18.0%)	8 (15.7%)	17 (16.8%)
Sinus tachycardia	7 (14.0%)	5 (9.8%)	12 (11.9%)
Sinus bradycardia	4 (8.0%)	1 (2.0%)	5 (5.0%)
Blood and lymphatic system disorders			
Any event	5 (10.0%)	10 (19.6%)	15 (14.9%)
Blood and lymphatic system disorders - Other, specify	3 (6.0%)	2 (3.9%)	5 (5.0%)
Febrile neutropenia	0 (0.0%)	4 (7.8%)	4 (4.0%)
Hemolysis	1 (2.0%)	3 (5.9%)	4 (4.0%)

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Eye disorders			
Any event	7 (14.0%)	5 (9.8%)	12 (11.9%)
Eye disorders - Other, specify	3 (6.0%)	1 (2.0%)	4 (4.0%)
Reproductive system and breast disorders			
Any event	5 (10.0%)	2 (3.9%)	7 (6.9%)
Injury, poisoning and procedural complications			
Any event	2 (4.0%)	3 (5.9%)	5 (5.0%)
Endocrine disorders			
Any event	1 (2.0%)	3 (5.9%)	4 (4.0%)
Immune system disorders			
Any event	0 (0.0%)	3 (5.9%)	3 (3.0%)
Psychiatric disorders			
Any event	3 (6.0%)	0 (0.0%)	3 (3.0%)

Source: Table 14.3.1C
Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects (N) (4 of 4)
Abbreviations: CTCAE: Common Terminology of Adverse Events; N = number of subjects.

Table 12.2.2.1.B Frequency of subjects with treatment emergent adverse events of at least CTCAE grade III by CTCAE System Organ Class and Term occurring in at least 5% of subjects in either treatment arm (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Subjects with any event	41 (82.0%)	41 (80.4%)	82 (81.2%)
Gastrointestinal disorders			
Any event	30 (60.0%)	25 (49.0%)	55 (54.5%)
Mucositis oral	24 (48.0%)	14 (27.5%)	38 (37.6%)
Diarrhea	7 (14.0%)	7 (13.7%)	14 (13.9%)
Nausea	9 (18.0%)	3 (5.9%)	12 (11.9%)
Vomiting	7 (14.0%)	4 (7.8%)	11 (10.9%)
Infections and infestations			
Any event	17 (34.0%)	20 (39.2%)	37 (36.6%)
Infections and infestations - Other, specify	4 (8.0%)	14 (27.5%)	18 (17.8%)
Sepsis	5 (10.0%)	3 (5.9%)	8 (7.9%)
Catheter related infection	4 (8.0%)	2 (3.9%)	6 (5.9%)
Lung infection	5 (10.0%)	1 (2.0%)	6 (5.9%)
Vascular disorders			
Any event	6 (12.0%)	10 (19.6%)	16 (15.8%)
Hypertension	6 (12.0%)	8 (15.7%)	14 (13.9%)
Investigations			
Any event	6 (12.0%)	7 (13.7%)	13 (12.9%)
Alanine aminotransferase increased	4 (8.0%)	4 (7.8%)	8 (7.9%)
Aspartate aminotransferase increased	2 (4.0%)	3 (5.9%)	5 (5.0%)
Investigations - Other, specify	2 (4.0%)	3 (5.9%)	5 (5.0%)
Respiratory, thoracic and mediastinal disorders			
Any event	9 (18.0%)	4 (7.8%)	13 (12.9%)
Hypoxia	2 (4.0%)	3 (5.9%)	5 (5.0%)
Blood and lymphatic system disorders			
Any event	4 (8.0%)	7 (13.7%)	11 (10.9%)
Febrile neutropenia	0 (0.0%)	4 (7.8%)	4 (4.0%)
General disorders and administration site conditions			
Any event	7 (14.0%)	4 (7.8%)	11 (10.9%)
Fever	1 (2.0%)	3 (5.9%)	4 (4.0%)
Metabolism and nutrition disorders			
Any event	6 (12.0%)	3 (5.9%)	9 (8.9%)
Renal and urinary disorders			
Any event	3 (6.0%)	3 (5.9%)	6 (5.9%)
Hematuria	3 (6.0%)	1 (2.0%)	4 (4.0%)
Skin and subcutaneous tissue disorders			
Any event	1 (2.0%)	3 (5.9%)	4 (4.0%)

Source: Table 14.3.1E
Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects (N) (2 of 2)
Abbreviations: CTCAE: Common Terminology of Adverse Events; N = number of subjects.

Table 12.2.2.1.C **Frequency of subjects with treatment emergent drug-related adverse events by CTCAE System Organ Class and Term occurring in at least 5% of subjects in either treatment arm (Safety Analysis Set)**

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Subjects with any event	37 (74.0%)	41 (80.4%)	78 (77.2%)
Gastrointestinal disorders			
Any event	34 (68.0%)	36 (70.6%)	70 (69.3%)
Mucositis oral	29 (58.0%)	32 (62.7%)	61 (60.4%)
Vomiting	19 (38.0%)	22 (43.1%)	41 (40.6%)
Diarrhea	11 (22.0%)	20 (39.2%)	31 (30.7%)
Nausea	15 (30.0%)	9 (17.6%)	24 (23.8%)
Abdominal pain	6 (12.0%)	12 (23.5%)	18 (17.8%)
Anal mucositis	1 (2.0%)	3 (5.9%)	4 (4.0%)
Hepatobiliary disorders			
Any event	23 (46.0%)	17 (33.3%)	40 (39.6%)
Hepatobiliary disorders - Other, specify	23 (46.0%)	16 (31.4%)	39 (38.6%)
Skin and subcutaneous tissue disorders			
Any event	11 (22.0%)	18 (35.3%)	29 (28.7%)
Alopecia	6 (12.0%)	11 (21.6%)	17 (16.8%)
Pruritus	2 (4.0%)	8 (15.7%)	10 (9.9%)
Skin and subcutaneous tissue disorders - Other, specify	3 (6.0%)	6 (11.8%)	9 (8.9%)
Rash maculo-papular	2 (4.0%)	4 (7.8%)	6 (5.9%)
Dry skin	4 (8.0%)	0 (0.0%)	4 (4.0%)
Investigations			
Any event	11 (22.0%)	11 (21.6%)	22 (21.8%)
Alanine aminotransferase increased	6 (12.0%)	6 (11.8%)	12 (11.9%)
Aspartate aminotransferase increased	3 (6.0%)	3 (5.9%)	6 (5.9%)
Blood bilirubin increased	2 (4.0%)	3 (5.9%)	5 (5.0%)
GGT increased	4 (8.0%)	1 (2.0%)	5 (5.0%)
Investigations - Other, specify	2 (4.0%)	3 (5.9%)	5 (5.0%)
General disorders and administration site conditions			
Any event	9 (18.0%)	7 (13.7%)	16 (15.8%)
Fever	8 (16.0%)	5 (9.8%)	13 (12.9%)
Respiratory, thoracic and mediastinal disorders			
Any event	8 (16.0%)	6 (11.8%)	14 (13.9%)
Epistaxis	2 (4.0%)	3 (5.9%)	5 (5.0%)
Sore throat	2 (4.0%)	3 (5.9%)	5 (5.0%)
Cough	3 (6.0%)	1 (2.0%)	4 (4.0%)
Infections and infestations			
Any event	5 (10.0%)	6 (11.8%)	11 (10.9%)
Infections and infestations - Other, specify	3 (6.0%)	5 (9.8%)	8 (7.9%)
Metabolism and nutrition disorders			
Any event	5 (10.0%)	1 (2.0%)	6 (5.9%)
Nervous system disorders			
Any event	3 (6.0%)	3 (5.9%)	6 (5.9%)

Source: Table 14.3.1G

Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects (N) (2 of 2)

Abbreviations: CTCAE: Common Terminology of Adverse Events; N = number of subjects.

Table 12.2.2.1.D Frequency of subjects with treatment emergent drug-related adverse events of at least CTCAE grade III by CTCAE System Organ Class and Term occurring in at least 5% of subjects in either treatment arm (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Subjects with any event	25 (50.0%)	26 (51.0%)	51 (50.5%)
Gastrointestinal disorders			
Any event	22 (44.0%)	19 (37.3%)	41 (40.6%)
Mucositis oral	18 (36.0%)	13 (25.5%)	31 (30.7%)
Nausea	7 (14.0%)	3 (5.9%)	10 (9.9%)
Diarrhea	3 (6.0%)	6 (11.8%)	9 (8.9%)
Vomiting	4 (8.0%)	1 (2.0%)	5 (5.0%)
Investigations			
Any event	1 (2.0%)	6 (11.8%)	7 (6.9%)
Alanine aminotransferase increased	1 (2.0%)	3 (5.9%)	4 (4.0%)
Respiratory, thoracic and mediastinal disorders			
Any event	4 (8.0%)	1 (2.0%)	5 (5.0%)
Infections and infestations			
Any event	3 (6.0%)	1 (2.0%)	4 (4.0%)
Metabolism and nutrition disorders			
Any event	3 (6.0%)	0 (0.0%)	3 (3.0%)

Source: Table 14.3.1I
Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects (N)
Abbreviations: CTCAE: Common Terminology of Adverse Events; N = number of subjects.

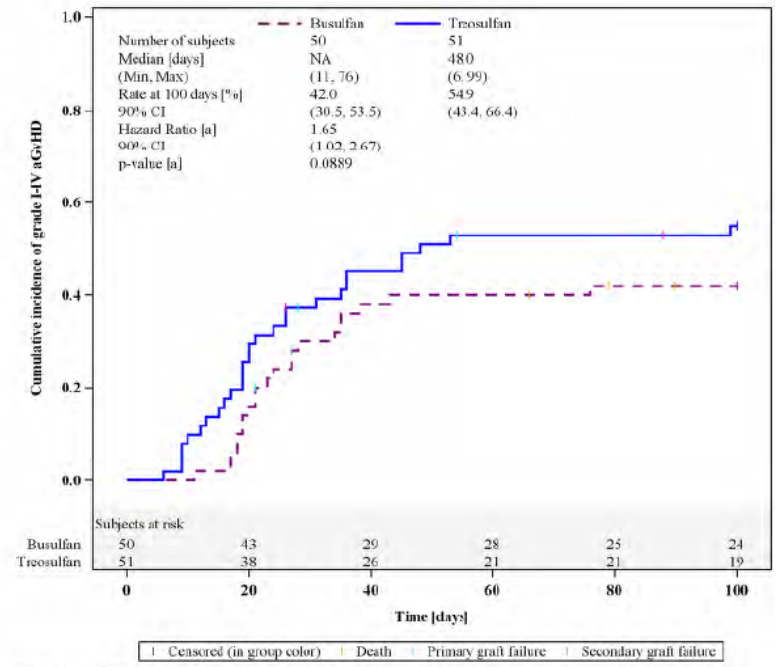
- The incidence of aGvHD grade I-IV was similar in the 2 treatment arms (busulfan: 42.0%, treosulfan: 54.9%, p=0.0889) as was the incidence of aGvHD Grade II-IV (busulfan: 26.0%, treosulfan: 27.5%, p=0.6407), and Grade III-IV (busulfan: 8.0%, treosulfan: 13.7%, p=0.4598).

Table 12.2.2.2.A Summary results of grade I-IV aGvHD (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	21 (42.0%)	28 (54.9%)
Subjects without event (censored) or with competing event	29 (58.0%)	23 (45.1%)
Censored	24 (48.0%)	19 (37.3%)
Death[a]	3 (6.0%)	0 (0.0%)
Primary graft failure[a]	2 (4.0%)	2 (3.9%)
Secondary graft failure[a]	0 (0.0%)	2 (3.9%)
Cumulative incidence at 14 days [%] (90% CI)	2.0 (0.0, 5.3)	13.7 (5.8, 21.7)
Cumulative incidence at 28 days [%] (90% CI)	30.0 (19.3, 40.7)	37.3 (26.1, 48.4)
Cumulative incidence at 100 days [%] (90% CI)	42.0 (30.5, 53.5)	54.9 (43.4, 66.4)
Hazard Ratio (Treosulfan/Busulfan)[b] (90% CI)	1.65 (1.02, 2.67)	
p-value[b]	0.0889	
Unadjusted p-value[c]	0.1641	

Source: Table 14.3.5.1A
[a] only if this event occurred first
[b] adjusted for thiotepa and disease as factors using Fine and Gray model
[c] based on Gray test
Abbreviations: aGvHD = acute graft-versus-host disease; CI = confidence interval; N = total number of subjects.

Figure 12.2.2.2.A Cumulative incidence of grade I-IV aGvHD (Full Analysis Set)



[a] adjusted for Thiotepa and disease as factors using Fine and Gray model

Source: Figure 14.3.5.1A

Abbreviations: aGvHD = acute graft-versus-host disease; CI = confidence interval; Max = maximum; Min = minimum; NA = not applicable.

Table 12.2.2.2.B Summary results of grade II-IV aGvHD (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	13 (26.0%)	14 (27.5%)
Subjects without event (censored) or with competing event	37 (74.0%)	37 (72.5%)
Censored	32 (64.0%)	33 (64.7%)
Death[a]	3 (6.0%)	0 (0.0%)
Primary graft failure[a]	2 (4.0%)	2 (3.9%)
Secondary graft failure[a]	0 (0.0%)	2 (3.9%)
Cumulative incidence at 14 days [%] (90% CI)	2.0 (0.0, 5.3)	9.8 (3.0, 16.7)
Cumulative incidence at 28 days [%] (90% CI)	18.0 (9.1, 26.9)	13.7 (5.8, 21.7)
Cumulative incidence at 100 days [%] (90% CI)	26.0 (15.8, 36.2)	27.5 (17.2, 37.7)
Hazard Ratio (Treosulfan/Busulfan)[b] (90% CI)	1.21 (0.62, 2.34)	
p-value[b]	0.6407	
Unadjusted p-value[c]	0.8745	

Source: Table 14.3.5.2A

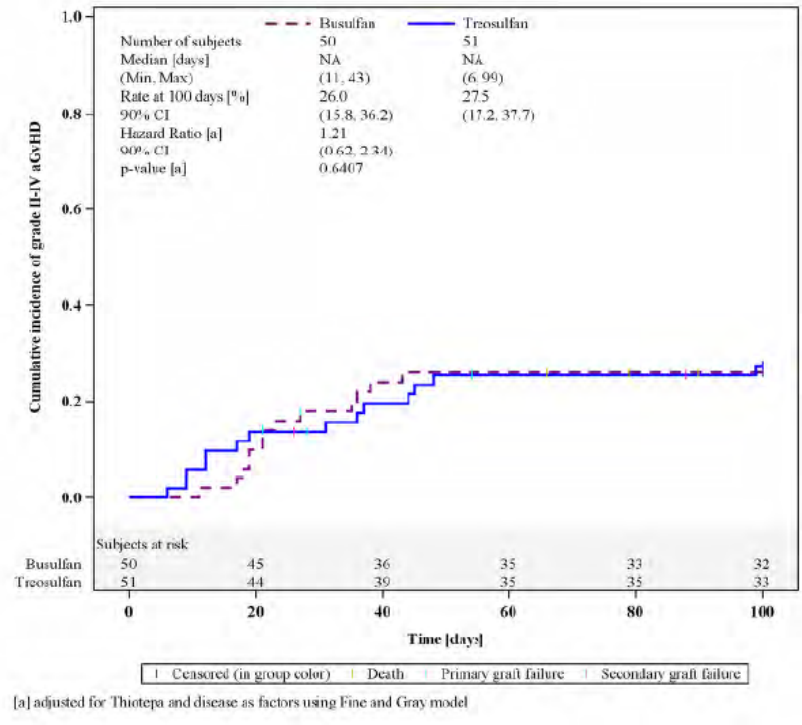
[a] only if this event occurred first

[b] adjusted for Thiotepa and disease as factors using Fine and Gray model

[c] based on Gray test

Abbreviations: aGvHD = acute graft-versus-host disease; CI = confidence interval; N = total number of subjects.

Figure 12.2.2.2.B Cumulative incidence of grade II-IV aGvHD (Full Analysis Set)



[a] adjusted for Thiotepa and disease as factors using Fine and Gray model

Source: Figure 14.3.5.2A

Abbreviations: aGvHD = acute graft-versus-host disease; CI = confidence interval; Max = maximum; Min = minimum; NA = not applicable.

Table 12.2.2.2.C Summary results of grade III-IV aGvHD (Full Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Subjects with event	4 (8.0%)	7 (13.7%)
Subjects without event (censored) or with competing event	46 (92.0%)	44 (86.3%)
Censored	41 (82.0%)	40 (78.4%)
Death[a]	3 (6.0%)	0 (0.0%)
Primary graft failure[a]	2 (4.0%)	2 (3.9%)
Secondary graft failure[a]	0 (0.0%)	2 (3.9%)
Cumulative incidence at 14 days [%] (90% CI)	2.0 (0.0, 5.3)	2.0 (0.0, 5.2)
Cumulative incidence at 28 days [%] (90% CI)	4.0 (0.0, 8.6)	2.0 (0.0, 5.2)
Cumulative incidence at 100 days [%] (90% CI)	8.0 (1.7, 14.3)	13.7 (5.8, 21.7)
Hazard Ratio (Treosulfan/Busulfan)[b] (90% CI)	1.63 (0.55, 4.81)	
p-value[b]	0.4598	
Unadjusted p-value[c]	0.3734	

Source: Table 14.3.5.3A

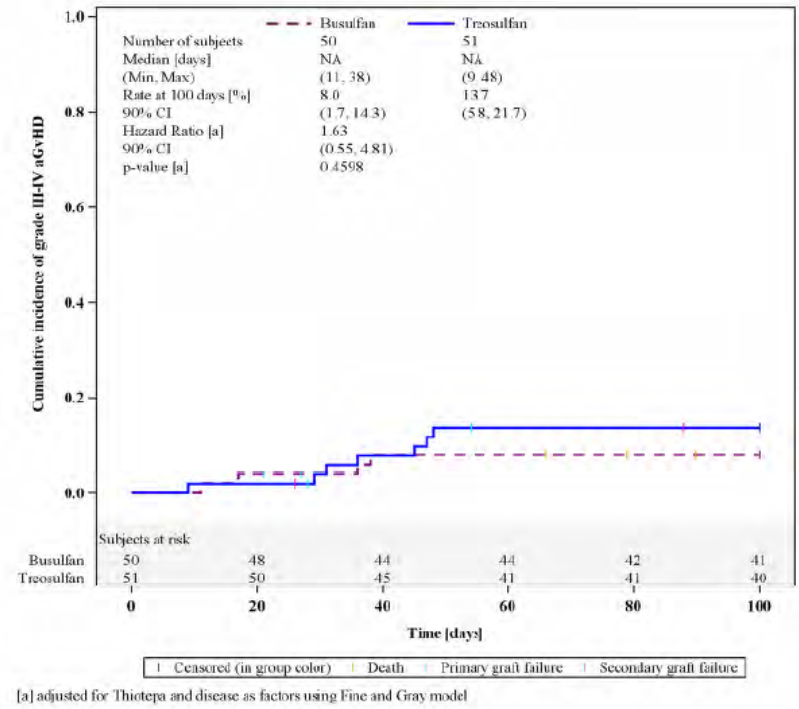
[a] only if this event occurred first

[b] adjusted for Thiotepa and disease as factors using Fine and Gray model

[c] based on Gray test

Abbreviations: aGvHD = acute graft-versus-host disease; CI = confidence interval; N = total number of subjects.

Figure 12.2.2.2.C Cumulative incidence of grade III-IV aGvHD (Full Analysis Set)



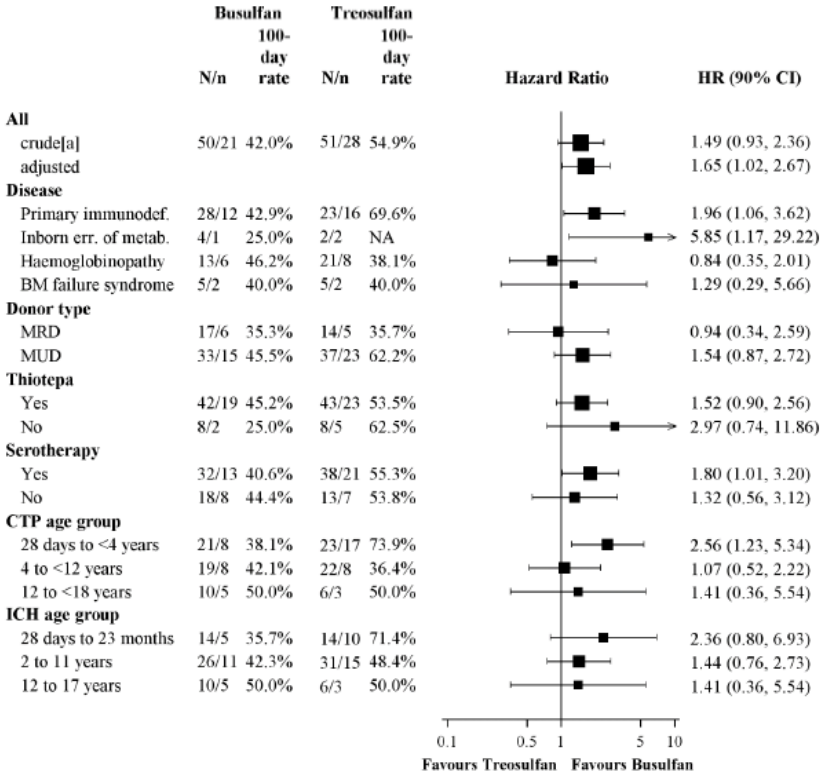
[a] adjusted for Thiotepa and disease as factors using Fine and Gray model

Source: Figure 14.3.5.3A

Abbreviations: aGvHD = acute graft-versus-host disease; CI = confidence interval; Max = maximum; Min = minimum; NA = not applicable.

Results from exploratory subgroup analyses (100-day rates of aGvHD by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for 100-day rates of grade I-IV aGvHD by subgroups is given in Figure 11.4.1.1.A. The results of the subgroup analyses were consistent with the main analysis.

Figure 12.2.2.2.D **Forest plot for grade I-IV aGvHD displaying 100-day rates by subgroups (Full Analysis Set)**



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Fine and Gray model

Source: Figure 14.3.5.1B

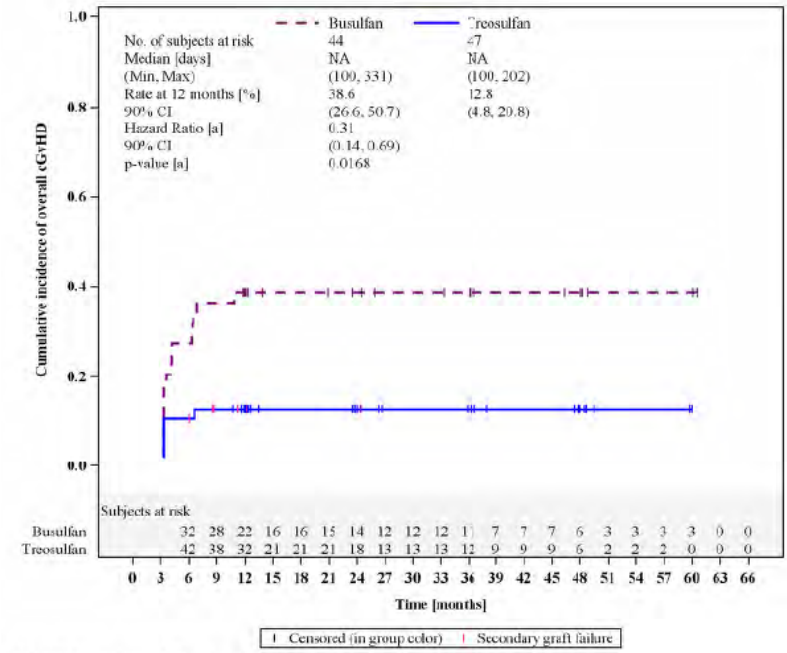
- The incidence of overall cGvHD was significantly higher in the busulfan arm than the treosulfan arm (busulfan: 38.6%, treosulfan: 12.8%, p=0.0168). There was an advantage in favour of treosulfan for moderate / severe cGvHD (busulfan: 22.7%, treosulfan: 10.6%, p=0.1611).

Table 12.2.2.2.D **Summary results of overall cGvHD (Full Analysis Set)**

	Busulfan (N=50)	Treosulfan (N=51)
Subjects at risk[a]	44	47
Subjects with event	17 (38.6%)	6 (12.8%)
Subjects without event (censored) or with competing event	27 (61.4%)	41 (87.2%)
Censored	27 (61.4%)	34 (72.3%)
Death[b]	0 (0.0%)	0 (0.0%)
Primary graft failure[b]	0 (0.0%)	0 (0.0%)
Secondary graft failure[b]	0 (0.0%)	7 (14.9%)
Cumulative incidence at 12 months [%] (90% CI)	38.6 (26.6, 50.7)	12.8 (4.8, 20.8)
Cumulative incidence at 24 months [%] (90% CI)	38.6 (26.6, 50.7)	12.8 (4.8, 20.8)
Cumulative incidence at 36 months [%] (90% CI)	38.6 (26.6, 50.7)	12.8 (4.8, 20.8)
Hazard Ratio (Treosulfan/Busulfan)[c] (90% CI)	0.31 (0.14, 0.69)	
p-value[c]	0.0168	
Unadjusted p-value[d]	0.0059	

Source: Table 14.3.5.4A
[a] Subjects are at risk if they have survived 100 days after end of HSCT without graft failure.
[b] only if this event occurred first
[c] adjusted for thiotepa and disease as factors using Fine and Gray model
[d] based on Gray test
Abbreviations: cGvHD = chronic graft-versus-host disease; CI = confidence interval; N = total number of subjects.

Figure 12.2.2.2.E Cumulative incidence of overall cGvHD (Full Analysis Set)

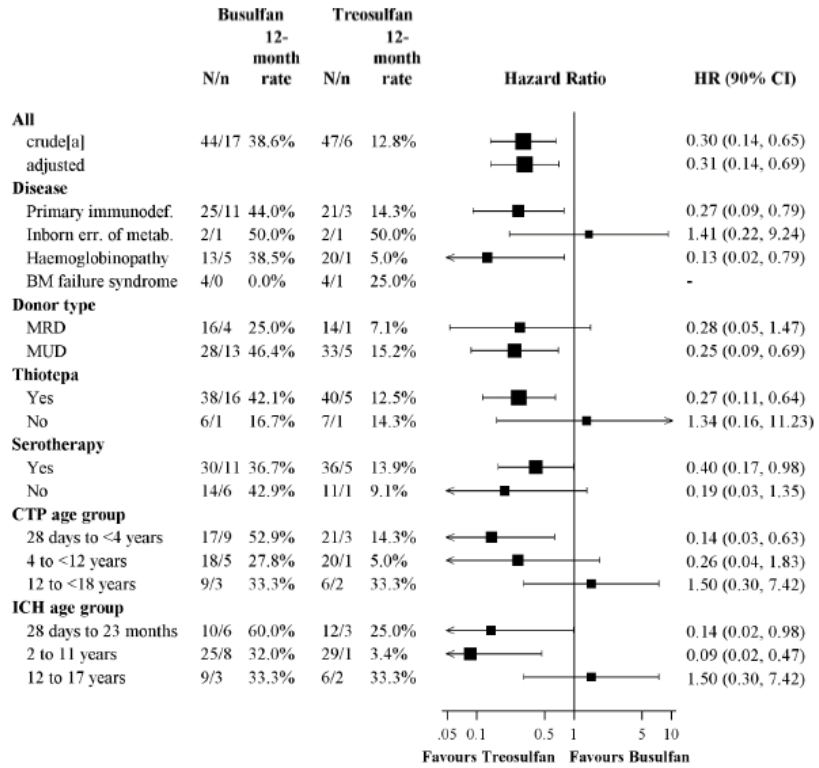


Note: Subjects are at risk if they have survived 100 days after end of HSCT without graft failure.
[a] adjusted for Thiotepa and disease as factors using Fine and Gray model

Source: Figure 14.3.5.4A

Results from exploratory subgroup analyses (overall cGvHD by disease, donor type, thiotepa, serotherapy, CTP age group, and ICH age group) are presented. A forest plot for 12-month rates of overall cGvHD by subgroups is given below. The results of the subgroup analyses were consistent with the main analysis.

Figure 12.2.2.2.F Forest plot for overall cGvHD displaying 12-month rates by subgroups (Full Analysis Set)



N = Number of subjects, n = Number of events
[a] not adjusted, all other HRs are adjusted for Thiotepa and disease as factor using Fine and Gray model

Source: Figure 14.3.5.4B

- Overall, 9 of 101 subjects (8.9%) died until data cut-off, 7 of 50 subjects (14.0%) in the busulfan arm and 2 of 51 subjects (3.9%) in the treosulfan arm. All deaths were transplantation-related. Most common cause of death was infection in the busulfan arm and GvHD associated with multiple organ failure in the treosulfan arm.

Table 12.3.1.1.A Overview and cause of deaths (Safety Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Survival status at trial termination [n (%)]			
Alive[a]	43 (86.0%)	49 (96.1%)	92 (91.1%)
Dead	7 (14.0%)	2 (3.9%)	9 (8.9%)
- If dead, cause of death [n (%)]			
Transplantation related	7 (14.0%)	2 (3.9%)	9 (8.9%)
- If dead, time from transplantation to death [months]			
n	7	2	9
Mean (SD)	6.44 (8.44)	5.73 (2.49)	6.29 (7.37)
Median (Q1, Q3)	2.96 (2.27, 6.64)	5.73 (3.98, 7.49)	3.22 (2.60, 6.64)
Min, Max	2.2, 25.3	4.0, 7.5	2.2, 25.3

Source: Table 14.3.2.1A
[a] The status 'alive' is displayed for all subjects who did not terminate the trial due to death.
Abbreviations: Max = maximum; Min = minimum; N = total number of subjects; n = number of subjects in each category; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.

Table 12.3.1.1.B Summary of detailed causes of deaths (Safety Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Survival status at trial termination [n (%)]			
Alive[a]	43 (86.0%)	49 (96.1%)	92 (91.1%)
Dead	7 (14.0%)	2 (3.9%)	9 (8.9%)
- If dead, cause of death [n (%)][b]			
Transplantation related[c]	7 (14.0%)	2 (3.9%)	9 (8.9%)
GvHD	2 (4.0%)	2 (3.9%)	4 (4.0%)
Pulmonary toxicity	1 (2.0%)	0 (0.0%)	1 (1.0%)
Haemorrhage	1 (2.0%)	0 (0.0%)	1 (1.0%)
Renal failure	0 (0.0%)	1 (2.0%)	1 (1.0%)
Multiple organ failure	1 (2.0%)	2 (3.9%)	3 (3.0%)
Infection	5 (10.0%)	1 (2.0%)	6 (5.9%)
Bacterial	2 (4.0%)	1 (2.0%)	3 (3.0%)
Viral	2 (4.0%)	0 (0.0%)	2 (2.0%)
Fungal	1 (2.0%)	0 (0.0%)	1 (1.0%)
Unspecified	0 (0.0%)	1 (2.0%)	1 (1.0%)
Interstitial pneumonitis	1 (2.0%)	0 (0.0%)	1 (1.0%)
EBV proliferative disease	0 (0.0%)	1 (2.0%)	1 (1.0%)
Gastrointestinal toxicity	0 (0.0%)	1 (2.0%)	1 (1.0%)
Other transplant related cause	1 (2.0%)	0 (0.0%)	1 (1.0%)

Source: Table 14.3.2.1B
[a] The status 'alive' is displayed for all subjects who did not terminate the trial due to death.
[b] Causes answered 'Unknown' are not counted. Please refer to data listing.
[c] Multiple transplantation related causes per subject possible
Abbreviations: EBV = Epstein-Barr virus; GvHD = graft-versus-host disease; N = total number of subjects; n = number of subjects in each category.

- The incidence of TESAEs was also similar in the 2 treatment arms (busulfan: 32.0%, treosulfan: 35.3%). TESAEs were most commonly reported in the SOCs "Infections and infestations", "General disorders and administration site conditions, and "Blood and lymphatic system disorders " .

Differences of ⩾ 5% between the treatment arms were noted only in a few cases. More subjects in the busulfan arm than the treosulfan arm had events in the SOCs "Blood and lymphatic system disorders" and "Respiratory, thoracic and mediastinal disorders", while more subjects in the treosulfan arm than the busulfan arm had events in the SOC "Infections and infestations" and "General disorders and administration site conditions".

At the Term level, more subjects in the busulfan arm than the treosulfan arm had "Lung infection", while more subjects in the treosulfan arm than the busulfan arm had "Infections and infestations - Other" and "Fever".

Table 12.3.1.2.A Frequency of subjects with treatment emergent-serious adverse events by CTCAE System Organ Class and Term (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Subjects with any event	16 (32.0%)	18 (35.3%)	34 (33.7%)
Infections and infestations			
Any event	7 (14.0%)	12 (23.5%)	19 (18.8%)
Infections and infestations - Other, specify	0 (0.0%)	5 (9.8%)	5 (5.0%)
Sepsis	2 (4.0%)	2 (3.9%)	4 (4.0%)
Lung infection	3 (6.0%)	0 (0.0%)	3 (3.0%)
Catheter related infection	1 (2.0%)	1 (2.0%)	2 (2.0%)
Enterocolitis infectious	1 (2.0%)	1 (2.0%)	2 (2.0%)
Bronchial infection	0 (0.0%)	1 (2.0%)	1 (1.0%)
Encephalitis infection	0 (0.0%)	1 (2.0%)	1 (1.0%)
Upper respiratory infection	0 (0.0%)	1 (2.0%)	1 (1.0%)
General disorders and administration site conditions			
Any event	1 (2.0%)	6 (11.8%)	7 (6.9%)
Fever	0 (0.0%)	6 (11.8%)	6 (5.9%)
Multi-organ failure	1 (2.0%)	0 (0.0%)	1 (1.0%)
Blood and lymphatic system disorders			
Any event	4 (8.0%)	1 (2.0%)	5 (5.0%)
Blood and lymphatic system disorders - Other, specify	2 (4.0%)	1 (2.0%)	3 (3.0%)
Anemia	2 (4.0%)	0 (0.0%)	2 (2.0%)
Respiratory, thoracic and mediastinal disorders			
Any event	4 (8.0%)	0 (0.0%)	4 (4.0%)
Bronchopulmonary hemorrhage	1 (2.0%)	0 (0.0%)	1 (1.0%)
Pneumonitis	1 (2.0%)	0 (0.0%)	1 (1.0%)
Pneumothorax	1 (2.0%)	0 (0.0%)	1 (1.0%)
Respiratory, thoracic and mediastinal disorders - Other, specify	1 (2.0%)	0 (0.0%)	1 (1.0%)
Hepatobiliary disorders			
Any event	2 (4.0%)	0 (0.0%)	2 (2.0%)
Hepatobiliary disorders - Other, specify	2 (4.0%)	0 (0.0%)	2 (2.0%)
Nervous system disorders			
Any event	1 (2.0%)	1 (2.0%)	2 (2.0%)
Encephalopathy	1 (2.0%)	1 (2.0%)	2 (2.0%)
Gastrointestinal disorders			
Any event	0 (0.0%)	1 (2.0%)	1 (1.0%)
Vomiting	0 (0.0%)	1 (2.0%)	1 (1.0%)
Immune system disorders			
Any event	0 (0.0%)	1 (2.0%)	1 (1.0%)
Immune system disorders - Other, specify	0 (0.0%)	1 (2.0%)	1 (1.0%)
Injury, poisoning and procedural complications			
Any event	0 (0.0%)	1 (2.0%)	1 (1.0%)
Injury, poisoning and procedural complications - Other, specify	0 (0.0%)	1 (2.0%)	1 (1.0%)
Investigations			
Any event	0 (0.0%)	1 (2.0%)	1 (1.0%)
Investigations - Other, specify	0 (0.0%)	1 (2.0%)	1 (1.0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)			
Any event	0 (0.0%)	1 (2.0%)	1 (1.0%)
Myelodysplastic syndrome	0 (0.0%)	1 (2.0%)	1 (1.0%)

Source: Table 14.3.2.2A
Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects (N) (2 of 2)
Abbreviations; CTCAE: Common Terminology of Adverse Events; N = total number of subjects.

- In the busulfan arm, 5 TESAEs had a fatal outcome while no fatal TESAE was reported for the treosulfan arm. None of the fatal TESAEs was assessed to be IMP-related.
- The incidence of TESAEs related to the IMP was also similar in the 2 treatment arms (busulfan: 6.0%, treosulfan: 3.9%).

Table 12.3.1.2.B Frequency of subjects with treatment emergent drug-related serious adverse events by CTCAE System Organ Class and Term (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Busulfan (N=50)	Treosulfan (N=51)	Total (N=101)
Subjects with any event	3 (6.0%)	2 (3.9%)	5 (5.0%)
Hepatobiliary disorders			
Any event	2 (4.0%)	0 (0.0%)	2 (2.0%)
Hepatobiliary disorders - Other, specify	2 (4.0%)	0 (0.0%)	2 (2.0%)
Blood and lymphatic system disorders			
Any event	1 (2.0%)	0 (0.0%)	1 (1.0%)
Blood and lymphatic system disorders - Other, specify	1 (2.0%)	0 (0.0%)	1 (1.0%)
Injury, poisoning and procedural complications			
Any event	0 (0.0%)	1 (2.0%)	1 (1.0%)
Injury, poisoning and procedural complications - Other, specify	0 (0.0%)	1 (2.0%)	1 (1.0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)			
Any event	0 (0.0%)	1 (2.0%)	1 (1.0%)
Myelodysplastic syndrome	0 (0.0%)	1 (2.0%)	1 (1.0%)
Respiratory, thoracic and mediastinal disorders			
Any event	1 (2.0%)	0 (0.0%)	1 (1.0%)
Bronchopulmonary hemorrhage	1 (2.0%)	0 (0.0%)	1 (1.0%)

Sources: Table 14.3.2.2B
Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects (N)
Abbreviations: CTCAE: Common Terminology of Adverse Events, N = total number of subjects.

- The incidences of significant AEs were similar in the 2 treatment arms: "Early toxicity" (busulfan: 96.0%, treosulfan: 94.1%, p=1.000), "Hepatic toxicity" (busulfan: 54.0%, treosulfan: 51.0%, p=0.8429), and "Infections" (busulfan: 70.0%, treosulfan: 60.8%, p=0.4044). However, the incidence of "HSOS" was slightly higher in the busulfan arm (all grades: busulfan: 10.0%, treosulfan: 2.0%, p=0.1120; ⩾ grade III according to Jones: busulfan 4.0%, treosulfan 0.0%, p=0.2426). No case of "Lung toxicity" (CTCAE Term "Pulmonary Fibrosis") was recorded.

Table 12.3.1.3.A Incidence of all treatment emergent significant adverse events (Safety Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
HSOS according to Jones (1987)		
Subjects with HSOS	5 (10.0%)	1 (2.0%)
Subjects without HSOS	45 (90.0%)	50 (98.0%)
Incidence of HSOS [%]	10.0	2.0
90% CI	(4.0, 19.9)	(0.1, 9.0)
95% CI	(3.3, 21.8)	(0.0, 10.4)
p-value[a]	0.1120	
Early toxicity defined as any AE occurring until day +28		
Subjects with Early toxicity	48 (96.0%)	48 (94.1%)
Subjects without Early toxicity	2 (4.0%)	3 (5.9%)
Incidence of Early toxicity [%]	96.0	94.1
90% CI	(87.9, 99.3)	(85.5, 98.4)
95% CI	(86.3, 99.5)	(83.8, 98.8)
p-value[a]	1.0000	
Hepatic toxicity according to Bearman (1988)		
Subjects with Hepatic toxicity	27 (54.0%)	26 (51.0%)
Subjects without Hepatic toxicity	23 (46.0%)	25 (49.0%)
Incidence of Hepatic toxicity [%]	54.0	51.0
90% CI	(41.5, 66.2)	(38.7, 63.2)
95% CI	(39.3, 68.2)	(36.6, 65.2)
p-value[a]	0.8429	
Lung toxicity (CTCAE term "Pulmonary fibrosis")		
Subjects with Lung toxicity	0 (0.0%)	0 (0.0%)
Subjects without Lung toxicity	50 (100.0%)	51 (100.0%)
Incidence of Lung toxicity [%]	0.0	0.0
90% CI	(0.0, 5.8)	(0.0, 5.7)
95% CI	(0.0, 7.1)	(0.0, 7.0)

Table 12.3.1.3.A Incidence of all treatment emergent significant adverse events (Safety Analysis Set)

	Busulfan (N=50)	Treosulfan (N=51)
Infections (SOC "Infections and infestations")		
Subjects with Infections	35 (70.0%)	31 (60.8%)
Subjects without Infections	15 (30.0%)	20 (39.2%)
Incidence of Infections [%]	70.0	60.8
90% CI	(57.6, 80.5)	(48.3, 72.3)
95% CI	(55.4, 82.1)	(46.1, 74.2)
p-value[a]	0.4044	

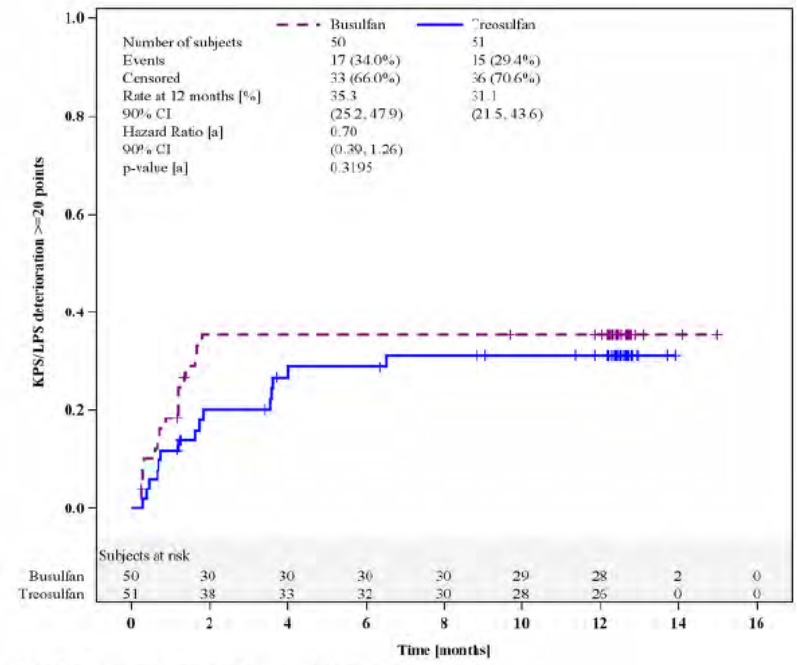
Source: Table 14.3.2.3A

[a] Fisher's exact test (2 of 2)

Abbreviations: AE = adverse event; CI = confidence interval; CTCAE = Common Terminology of Adverse Events; HSOS = hepatic sinusoidal obstruction syndrome; N = total number of subjects; SOC = system organ class.

- No unknown risks were identified.
- Laboratory parameters were largely comparable for the 2 treatment arms throughout the trial. The durations of leukopenia and neutropenia were significantly longer in the treosulfan arm than the busulfan arm.
- Vital signs were largely comparable for the 2 treatment arms throughout the trial.
- The median KPS following transplantation and clinically relevant exploratory endpoints "time to deterioration of KPS by at least 20 points" as well as "deterioration of the KPS to less than 60 points" were comparable for the 2 treatment arms.

Figure 12.4.2.3.A Kaplan-Meier estimates for time to deterioration of Karnofsky / Lansky Performance Score by at least 20 points (Safety Analysis Set)

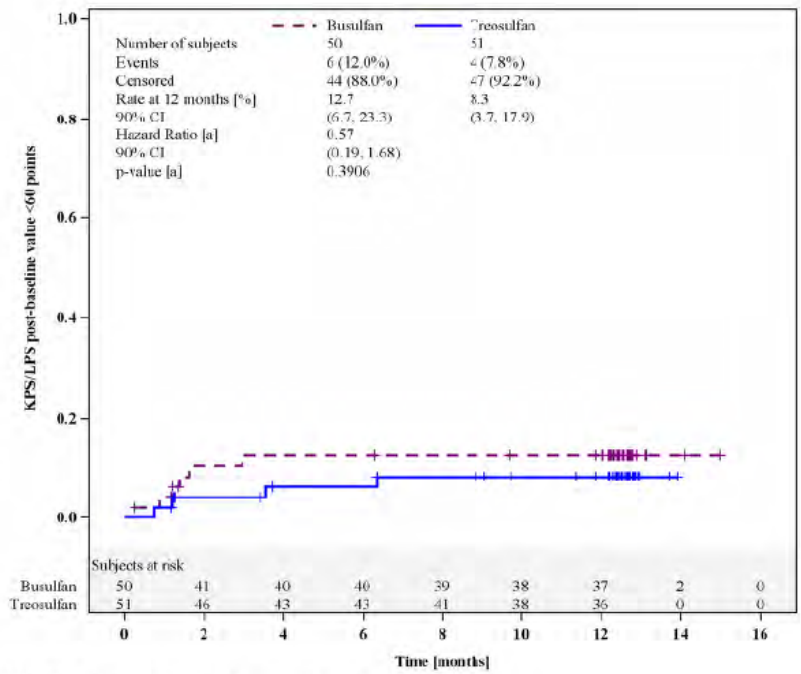


Note: Months are months from baseline visit to first deterioration >=20 points.
[a] adjusted for Thiotepa and disease as factors using Cox regression model

Sources: Figure 14.3.6.6F

Abbreviations: CI = confidence interval; KPS = Karnofsky Performance Score; LPS = Lansky Performance Score.

Figure 12.4.2.3.B Kaplan-Meier estimates for time to Karnofsky/Lansky Performance Score less than 60 points (Safety Analysis Set)



Note: Months are months from baseline visit to first post-baseline value <60 points.
[a] adjusted for Thiotepa and disease as factors using Cox regression model

Source: Figure 14.3.6.6G
Abbreviations: CI = confidence interval; KPS = Karnofsky Performance Score; LPS = Lansky Performance Score.

MC-FludT.17/M

Criteria for Evaluation:

Safety:

Adverse events (AEs): All AEs (serious and non serious) occurring between day -7 and day +100 were recorded continuously. After day +100, only serious AEs with suspected relatedness (SAR) to the IP were documented up to visit 12 months after the HSCT. SARs were continuously assessed during the longer term follow up phase and results were reported.

Acute (a) and chronic (c) GvHD: aGvHD was classified as GvHD up until 100 days after HSCT. Time to cGvHD was defined as the time between 100 days after end of HSCT and the first episode of cGvHD and evaluated up to visit 12 months after HSCT. cGvHD episodes were continuously assessed during the longer term follow up phase and results were reported.

Clinical laboratory tests: The subject's viral status was characterised by HIV, cytomegalovirus, Epstein Barr virus (EBV), hepatitis A, B, C, and herpes tests within 3 weeks prior to day -7. Pregnancy was assessed routinely in the subject's urine or serum within 3 weeks prior to day -7, in any female who had experienced menarche. The standard laboratory parameters (total blood count, differential blood count, blood chemistry, and the computed GFR) were documented once within 3 weeks prior to Day -7 and until visit Day +28, and on visit Day +100, if applicable.

Vital signs, physical examinations: Blood pressure, pulse, height, weight, and body temperature were assessed between day -10 and day -8, and on visit Day 0, +28, +100, and on visit Month 6, 9, and 12. Lansky Performance Score (LPS), or Karnofsky Performance Score (KPS) were assessed within 3 weeks prior to Day -7, and at visit Day 0, visit Day +28, visit Day +100, visit Month 6, 9, and 12.

Results

SAFETY:

1. The incidences of total Treatment-emergent adverse events (TEAEs) were reported by 97.1% of subjects, of these 90% of subjects reported drug-related TEAEs. This could be expected with an intensive treatment procedure as alloHSCT.

Table 12.2.1.A Overall summary of treatment-emergent adverse events (Safety Analysis Set)

	Treosulfan (N=70)
Any adverse event [n (%)]	
Subjects with any adverse event	68 (97.1)
Subjects with AEs of at least CTCAE grade III	53 (75.7)
Drug-related adverse events [n (%)]	
Subjects with any drug-related adverse event	63 (90.0)
Subjects with drug-related AEs of at least CTCAE grade III	34 (48.6)
Serious adverse events [n (%)]	
Subjects with any serious adverse event	23 (32.9)
- Results in death	1 (1.4)
- Life-threatening	6 (8.6)
- Hospitalization or prolongation of hospitalization	20 (28.6)
- Disability/incapacity	1 (1.4)
- Congenital anomaly or birth defect	0 (0.0)
Drug-related serious adverse events [n (%)]	
Subjects with any drug-related serious adverse event	1 (1.4)
Maximum CTCAE grade of adverse events [n (%)]	
Subjects with AEs of a maximum CTCAE grade I	3 (4.3)
Subjects with AEs of a maximum CTCAE grade II	12 (17.1)
Subjects with AEs of a maximum CTCAE grade III	42 (60.0)
Subjects with AEs of a maximum CTCAE grade IV	11 (15.7)
Subjects with AEs of a maximum CTCAE grade V	0 (0.0)

Sources: Table 14.3.1.A, Listing 16.2.7A, Listing 16.2.7B
Abbreviations: AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events; N = number of subjects; n = number of subjects in category.

TEAEs were most commonly reported in the System Organ Classes (SOCs) "Gastrointestinal disorders", "General disorder and administration site conditions", and "Infections and infestations" (TEAEs reported by 92.9%, 78.6%, and 71.4% of subjects, respectively). The most frequently reported AEs by preferred term (PT) were "Mucositis oral" (77.1%), "Fever" (72.9%), "Vomiting (68.6%), and Diarrhoea" (65.7%), as expected considering the nature of the drug. However, the frequent use of the intensified conditioning regimen (65 out of 70 subjects received additional treatment with Thiotepa) might have negatively affected this result.

Table 12.2.2.1.A Frequency of subjects with treatment-emergent adverse events by CTCAE System Organ Class and Term occurring in at least 5% of subjects (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Subjects with any event [n (%)]	68 (97.1)
Gastrointestinal disorders [n (%)]	
Any event	65 (92.9)
Mucositis oral	54 (77.1)
Vomiting	48 (68.6)
Diarrhoea	46 (65.7)
Nausea	32 (45.7)
Abdominal pain	22 (31.4)
Constipation	8 (11.4)
Oral pain	5 (7.1)
Dyspepsia	4 (5.7)
General disorders and administration site conditions [n (%)]	
Any event	55 (78.6)
Fever	51 (72.9)
Fatigue	6 (8.6)
Oedema face	5 (7.1)
Infusion related reaction	5 (7.1)
Chills	4 (5.7)
Oedema limbs	4 (5.7)
Pain	4 (5.7)
Infections and infestations [n (%)]	
Any event	50 (71.4)
Infections and infestations - Other, specify	43 (61.4)
Catheter related infection	7 (10.0)
Urinary tract infection	6 (8.6)
Upper respiratory infection	5 (7.1)
Sepsis	4 (5.7)
Skin and subcutaneous tissue disorders [n (%)]	
Any event	41 (58.6)
Rash maculo-papular	20 (28.6)
Pruritus	13 (18.6)

Sources: Table 14.3.1B, Table 14.3.1C, Listing 16.2.7A, Listing 16.2.7B.

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Skin and subcutaneous tissue disorders - Other, specify	11 (15.7)
Erythema multiforme	8 (11.4)
Pain of skin	8 (11.4)
Respiratory, thoracic and mediastinal disorders [n (%)]	
Any event	28 (40.0)
Cough	13 (18.6)
Nasal congestion	7 (10.0)
Dyspnoea	5 (7.1)
Pneumonitis	4 (5.7)
Sore throat	4 (5.7)
Vascular disorders [n (%)]	
Any event	26 (37.1)
Hypertension	21 (30.0)
Hematoma	7 (10.0)
Hypotension	4 (5.7)
Hepatobiliary disorders [n (%)]	
Any event	25 (35.7)
Hepatobiliary disorders - Other, specify	25 (35.7)
Nervous system disorders [n (%)]	
Any event	24 (34.3)
Headache	19 (27.1)
Lethargy	4 (5.7)
Tremor	4 (5.7)
Musculoskeletal and connective tissue disorders [n (%)]	
Any event	23 (32.9)
Pain in extremity	13 (18.6)
Bone pain	7 (10.0)
Back pain	6 (8.6)
Investigations [n (%)]	
Any event	21 (30.0)
Blood bilirubin increased	9 (12.9)
Alanine aminotransferase increased	8 (11.4)
Aspartate aminotransferase increased	8 (11.4)
Investigations - Other, specify	4 (5.7)
Metabolism and nutrition disorders [n (%)]	
Any event	21 (30.0)

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Hypokalemia	11 (15.7)
Hypomagnesemia	4 (5.7)
Renal and urinary disorders [n (%)]	
Any event	14 (20.0)
Acute kidney injury	4 (5.7)
Haematuria	4 (5.7)
Urinary tract pain	4 (5.7)
Immune system disorders [n (%)]	
Any event	12 (17.1)
Allergic reaction	12 (17.1)
Eye disorders [n (%)]	
Any event	11 (15.7)
Blurred vision	4 (5.7)
Eye pain	4 (5.7)
Blood and lymphatic system disorders [n (%)]	
Any event	10 (14.3)
Febrile neutropenia	5 (7.1)
Cardiac disorders [n (%)]	
Any event	9 (12.9)
Sinus tachycardia	8 (11.4)
Reproductive system and breast disorders [n (%)]	
Any event	7 (10.0)
Injury, poisoning and procedural complications [n (%)]	
Any event	6 (8.6)
Psychiatric disorders [n (%)]	
Any event	5 (7.1)

- TEAEs of at least CTCAE grade III were reported by 75.5% of subjects, and most commonly reported in the SOCs "Gastrointestinal disorders", "Infections and infestations", and "Metabolism and nutrition disorders" (TEAEs reported by 55.7%, 41.4%, and 24.3% of subjects, respectively).

Table 12.2.2.1.B Frequency of subjects with treatment-emergent adverse events of at least CTCAE Grade III by CTCAE System Organ Class and Term occurring in at least 5% of subjects (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Subjects with any event [n (%)]	53 (75.7)
Gastrointestinal disorders [n (%)]	
Any event	39 (55.7)
Mucositis oral	29 (41.4)
Nausea	12 (17.1)
Vomiting	11 (15.7)
Diarrhoea	10 (14.3)
Infections and infestations [n (%)]	
Any event	29 (41.4)
Infections and infestations - Other, specify	20 (28.6)
Catheter related infection	6 (8.6)
Sepsis	4 (5.7)
Metabolism and nutrition disorders [n (%)]	
Any event	17 (24.3)
Hypokalemia	10 (14.3)
Investigations [n (%)]	
Any event	8 (11.4)
Blood bilirubin increased	5 (7.1)
Skin and subcutaneous tissue disorders [n (%)]	
Any event	8 (11.4)
Rash maculo-papular	5 (7.1)
Blood and lymphatic system disorders [n (%)]	
Any event	7 (10.0)
Febrile neutropenia	5 (7.1)
Vascular disorders [n (%)]	
Any event	7 (10.0)
Hypertension	5 (7.1)
Nervous system disorders [n (%)]	
Any event	5 (7.1)
Renal and urinary disorders [n (%)]	
Any event	5 (7.1)

Sources: Table 14.3.1D, Table 14.3.1E, Listing 16.2.7A, Listing 16.2.7B.

- TEAEs related to IP were reported by 90.0% of subjects. Such TEAEs were most commonly reported in the SOCs of "Gastrointestinal disorder", "Hepatobiliary disorders", and "Skin and subcutaneous tissue disorders" (TEAEs reported by 80.0% and 24.3% of subjects each for "Hepatobiliary disorders" and "Skin and subcutaneous tissue disorders"). The most commonly reported TEAEs by PT were "Mucositis oral" (68.6%), "Vomiting" (41.4%), "Nausea" (32.9%), and "Diarrhoea" (28.6%).

Table 12.2.2.1.C Frequency of subjects with drug-related adverse events by CTCAE System Organ Class and Term occurring in at least 5% of subjects (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Subjects with any event [n (%)]	63 (90.0)
Gastrointestinal disorders [n (%)]	
Any event	56 (80.0)
Mucositis oral	48 (68.6)
Vomiting	29 (41.4)
Nausea	23 (32.9)
Diarrhoea	20 (28.6)
Abdominal pain	8 (11.4)
Hepatobiliary disorders [n (%)]	
Any event	17 (24.3)
Hepatobiliary disorders - Other, specify	17 (24.3)
Skin and subcutaneous tissue disorders [n (%)]	
Any event	17 (24.3)
Pruritus	5 (7.1)
Rash maculo-papular	5 (7.1)
Skin and subcutaneous tissue disorders - Other, specify	5 (7.1)
General disorders and administration site conditions [n (%)]	
Any event	12 (17.1)
Fever	11 (15.7)
Investigations [n (%)]	
Any event	12 (17.1)
Alanine aminotransferase increased	7 (10.0)
Aspartate aminotransferase increased	5 (7.1)
Blood bilirubin increased	5 (7.1)
Infections and infestations [n (%)]	
Any event	8 (11.4)
Infections and infestations - Other, specify	6 (8.6)
Respiratory, thoracic and mediastinal disorders [n (%)]	
Any event	4 (5.7)

Sources: Table 14.3.1F, Table 14.3.1G, Listing 16.2.7A, Listing 16.2.7B.

- TEAEs of at least CTCAE Grade III related to IP were reported by 48.6% of subjects. Such events were only reported by > 5% of subjects for 4 SOCs: "Gastrointestinal disorders" (42.9% of subjects), "Infections and infestations" (7.1%), "Skin and subcutaneous tissue disorders" (7.1%), and "Investigations" (5.7%) with "Mucositis oral" and "Nausea" as the most common Terms. The frequent use of the intensified conditioning regimen B (65 out of 70 subjects received additional treatment with Thiotepa) might have negatively affected this result. In paediatric population, "Mucosal inflammation" and "Nausea" are very common ARs considered at least possibly related to Thiotepa.

Table 12.2.2.1.D Frequency of subjects with drug-related adverse events of at least CTCAE Grade III by CTCAE System Organ Class and Term occurring in at least 5% of subjects (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Subjects with any event [n (%)]	34 (48.6)
Gastrointestinal disorders [n (%)]	
Any event	30 (42.9)
Mucositis oral	26 (37.1)
Nausea	7 (10.0)
Vomiting	4 (5.7)
Infections and infestations [n (%)]	
Any event	5 (7.1)
Infections and infestations - Other, specify	5 (7.1)
Skin and subcutaneous tissue disorders [n (%)]	
Any event	5 (7.1)
Investigations [n (%)]	
Any event	4 (5.7)

Sources: Table 14.3.1H, Table 14.3.1I, Listing 16.2.7A, Listing 16.2.7B
Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; N = number of subjects.

- The incidence of Treatment-emergent serious adverse events (TESAEs) were reported by 32.9% of subjects (n=23), of these only 1 subject (1.4%) reported any drug-related serious adverse event (SAE) (Grade III "Mucositis oral"). TESAEs were reported by > 2 subjects for only 3 CTCAE Terms; "Infections and infestations-Other" (6 subjects), "Upper respiratory infection" (3 subjects), and "Febrile neutropenia" (3 subjects).

Table 12.3.1.2.A Frequency of subjects with treatment-emergent serious adverse events by CTCAE System Organ Class and Term (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Subjects with any event [n (%)]	23 (32.9)
Infections and infestations [n (%)]	
Any event	15 (21.4)
Infections and infestations - Other, specify	6 (8.6)
Upper respiratory infection	3 (4.3)
Sepsis	2 (2.9)
Catheter related infection	1 (1.4)
Encephalitis infection	1 (1.4)
Hepatitis viral	1 (1.4)
Sinusitis	1 (1.4)
Skin infection	1 (1.4)
Blood and lymphatic system disorders [n (%)]	
Any event	3 (4.3)
Febrile neutropenia	3 (4.3)
Gastrointestinal disorders [n (%)]	
Any event	3 (4.3)
Enterocolitis	1 (1.4)
Mucositis oral	1 (1.4)
Upper gastrointestinal hemorrhage	1 (1.4)
General disorders and administration site conditions [n (%)]	
Any event	2 (2.9)
Fever	2 (2.9)
Nervous system disorders [n (%)]	
Any event	2 (2.9)
Encephalopathy	1 (1.4)
Tremor	1 (1.4)
Respiratory, thoracic and mediastinal disorders [n (%)]	
Any event	2 (2.9)
Laryngeal haemorrhage	1 (1.4)
Pulmonary oedema	1 (1.4)
Immune system disorders [n (%)]	
Any event	1 (1.4)
Allergic reaction	1 (1.4)
Metabolism and nutrition disorders [n (%)]	
Any event	1 (1.4)
Dehydration	1 (1.4)
Renal and urinary disorders [n (%)]	
Any event	1 (1.4)
Acute kidney injury	1 (1.4)

Sources: Table 14.3.2.2A, Listing 14.3.2.2F
Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects (N)
Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; N = number of subjects (2 of 2)

Table 12.3.1.2.B Frequency of subjects with drug-related treatment-emergent serious adverse events by CTCAE System Organ Class and Term (Safety Analysis Set)

CTCAE System Organ Class CTCAE Term	Treosulfan (N=70)
Subjects with any event [n (%)]	1 (1.4)
Gastrointestinal disorders [n (%)]	
Any event	1 (1.4)
Mucositis oral	1 (1.4)

Sources: Table 14.3.2.2B, Listing 14.3.2.2E, Listing 14.3.2.2F
Note: Absolute and relative frequencies of subjects with event relative to the total number of subjects
Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; N = number of subjects; n = number of subjects in category.

- No unknown risks were identified in the trial. No suspected unexpected serious adverse reactions (SUSARs) occurred during the trial.
- At the end of the trial, 14 subjects out of the 70 registered subjects terminated the trial prematurely. Death was the predominant reason for premature termination for a total of 12 subjects (17.1%). The causes of these deaths were relapse / progression (8 subjects) and transplantation-related (4 subjects). Seven subjects (10.0%) died within 12 months after HSCT. Furthermore, 1 subject was lost to follow-up and 1 subject (1.4%) withdrew his/her consent from participating in the trial. The mean (standard deviation [SD]) time from transplantation to death was 14.97 (12.35) months.

Table 12.3.1.1.A Overview and detailed causes of deaths (Safety Analysis Set)

	Treosulfan (N=70)
Survival status at trial termination [n (%)]	
Alive[a]	58 (82.9)
Dead	12 (17.1)
- If dead, cause of death [n (%)][b]	
Relapse/progression	8 (11.4)
Transplantation related	4 (5.7)
- If dead, time from transplantation to death [months]	
n	12
Mean (SD)	14.97 (12.35)
Median (Q1, Q3)	11.01 (7.82, 19.25)
Min, Max	0.5, 46.7
- If dead, cause of death [n (%)][b]	
Relapse/progression	8 (11.4)
Transplantation related[c]	4 (5.7)
GvHD	1 (1.4)
Pulmonary toxicity	1 (1.4)
Haemorrhage	1 (1.4)
Multiple organ failure	2 (2.9)
Infection	3 (4.3)
Bacterial	2 (2.9)
Unspecified	1 (1.4)
Interstitial pneumonitis	2 (2.9)

Sources: Table 14.3.2.1A, Table 14.3.2.1B, Listing 14.3.2.1C
[a] The status 'alive' is displayed for all subjects who did not terminate the trial due to death.
[b] Causes answered 'Unknown' are not counted. Please refer to data listing.
[c] Multiple transplantation related causes per subject possible.
Abbreviations: GvHD = graft-versus-host disease; Max = maximum; Min = minimum; N = number of subjects; n = number of subjects in category; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.

- The vital signs did not change substantially during the trial with an exception of diastolic blood pressure. The median diastolic blood pressure at baseline was 59.0 mmHg (Q1 [25%-percentile] 55.0, Q3 [75%-percentile] 67.0) with considerable variability (range from 43 to 81). At visit Month 12, median change from baseline was 5.0 (Q1 2.0, Q3 11) mmHg (range from -23 to 34).
- A total of 7 subjects (12.1%) deteriorated to less than 60 points in the LPS and 2 subjects (16.7%) deteriorated to less than 60 points in the KPS.
- None of the subjects reported any lung toxicity ("Pulmonary Fibrosis") during the trial. Only 1 subject reported an AE of HSOS Grade II (according to Jones et al). Hepatic toxicity of any grade (according to Bearmann) was reported by 34.3% of subjects. Infections of any CTCAE grade belonging to SOC "Infections and Infestations" were reported by 71.4% of subjects.

Table 12.3.1.3.A Incidence of all treatment-emergent significant adverse events (Safety Analysis Set)

	Treosulfan (N=70)
HSOS according to Jones (1987)	
Subjects with HSOS according to Jones (1987) [n (%)]	1 (1.4)
Subjects without HSOS according to Jones (1987) [n (%)]	69 (98.6)
Incidence of HSOS according to Jones (1987) (%)	1.4
90% CI	(0.1, 6.6)
Early toxicity defined as any AE occurring until day +28	
Subjects with Early toxicity defined as any AE occurring until day +28 [n (%)]	68 (97.1)
Subjects without Early toxicity defined as any AE occurring until day +28 [n (%)]	2 (2.9)
Incidence of Early toxicity defined as any AE occurring until day +28 (%)	97.1
90% CI	(91.3, 99.5)
Hepatic toxicity according to Bearman (1988)	
Subjects with Hepatic toxicity according to Bearman (1988) [n (%)]	24 (34.3)
Subjects without Hepatic toxicity according to Bearman (1988) [n (%)]	46 (65.7)
Incidence of Hepatic toxicity according to Bearman (1988) (%)	34.3
90% CI	(24.9, 44.7)
Lung toxicity (CTCAE term "Pulmonary fibrosis")	
Subjects with Lung toxicity (CTCAE term "Pulmonary fibrosis") [n (%)]	0 (0.0)
Subjects without Lung toxicity (CTCAE term "Pulmonary fibrosis") [n (%)]	70 (100.0)
Incidence of Lung toxicity (CTCAE term "Pulmonary fibrosis") (%)	0.0
90% CI	(0.0, 4.2)
Infections (SOC "Infections and infestations")	
Subjects with Infections (SOC "Infections and infestations") [n (%)]	50 (71.4)
Subjects without Infections (SOC "Infections and infestations") [n (%)]	20 (28.6)
Incidence of Infections (SOC "Infections and infestations") (%)	71.4
90% CI	(61.2, 80.2)

Sources: Table 14.3.2.3A, Listing 16.2.7C
Abbreviations: AE = adverse event; CI = confidence interval; CTCAE = Common Terminology Criteria for Adverse Events; N = number of subjects; n = number of subjects in category; SOC = System Organ Class.

- Acute GvHD Grade I-IV was reported by 42.9% of subjects (n=30), with a cumulative incidence at 100 days of 43.5% (90% CI: 33.7, 53.3), and overall chronic GvHD by 25.4% subjects (n=17), with a cumulative incidence of chronic GvHD at 12 months was 23.9% (90% CI: 15.3,32.4), reached a plateau before 24 months and was subsequently maintained at 36 months with 25.4% (90% CI: 16.6, 34.1). Acute GvHD Grade III-IV was reported by 8.6% of subjects, with a cumulative incidence at 100 days of 8.7% (90% CI: 3.1, 14.3). A total of 19.4% of subjects (n=13) experienced moderate / severe chronic GvHD. The cumulative incidence of moderate / severe chronic GvHD at 12 months was 17.9% (90% CI: 10.2, 25.6), reached a plateau at 24 months and was subsequently maintained at 36 months with 19.4% (90% CI: 11.5, 27.4).

Table 12.2.2.2.A Summary table of aGvHD (Full Analysis Set)

	Treosulfan (N=70)
Grade I-IV	
Subjects with event [n (%)]	30 (42.9)
Subjects without event (censored) or with competing event	40 (57.1)
Censored	38 (54.3)
Death[a]	1 (1.4)
Relapse/Progression[a]	1 (1.4)
Primary graft failure[a]	0 (0.0)
Secondary graft failure[a]	0 (0.0)
Cumulative incidence of acute GvHD at 100 days (%)	43.5
90% CI	(33.7, 53.3)
Grade II-IV	
Subjects with event [n (%)]	18 (25.7)
Subjects without event (censored) or with competing event	52 (74.3)
Censored	49 (70.0)
Death[a]	1 (1.4)
Relapse/Progression[a]	2 (2.9)
Primary graft failure[a]	0 (0.0)
Secondary graft failure[a]	0 (0.0)
Cumulative incidence of acute GvHD at 100 days (%)	26.1
90% CI	(17.4, 34.8)
Grade III-IV	
Subjects with event [n (%)]	6 (8.6)
Subjects without event (censored) or with competing event	64 (91.4)
Censored	61 (87.1)
Death[a]	1 (1.4)
Relapse/Progression[a]	2 (2.9)
Primary graft failure[a]	0 (0.0)
Secondary graft failure[a]	0 (0.0)
Cumulative incidence of acute GvHD at 100 days (%)	8.7
90% CI	(3.1, 14.3)

Sources: Table 14.3.5A, Table 14.3.5B, Listing 16.2.7G
[a] Only if this event occurred first.
Abbreviations: CI = confidence interval; aGvHD = acute graft-versus-host disease; N = number of subjects; n = number of subjects in category.

Table 12.2.2.2.B Summary table of cGvHD (Full Analysis Set)

	Treosulfan (N=70)
Overall	
Subjects at risk[a]	67
Subjects with event [n (%)]	17 (25.4)
Subjects without event (censored) or with competing event [n (%)]	50 (74.6)
Censored	37 (55.2)
Death[b]	1 (1.5)
Relapse/Progression[b]	11 (16.4)
Primary graft failure[b]	0 (0.0)
Secondary graft failure[b]	1 (1.5)
Cumulative incidence of chronic GvHD at 12 months (%)	23.9
90% CI	(15.3, 32.4)
Cumulative incidence of chronic GvHD at 24 months (%)	25.4
90% CI	(16.6, 34.1)
Cumulative incidence of chronic GvHD at 36 months (%)	25.4
90% CI	(16.6, 34.1)
Moderate/severe	
Subjects at risk[a]	67
Subjects with event [n (%)]	13 (19.4)
Subjects without event (censored) or with competing event [n (%)]	54 (80.6)
Censored	40 (59.7)
Death[b]	1 (1.5)
Relapse/Progression[b]	12 (17.9)
Primary graft failure[b]	0 (0.0)
Secondary graft failure[b]	1 (1.5)
Cumulative incidence of chronic GvHD at 12 months (%)	17.9
90% CI	(10.2, 25.6)
Cumulative incidence of chronic GvHD at 24 months (%)	19.4
90% CI	(11.5, 27.4)
Cumulative incidence of chronic GvHD at 36 months (%)	19.4
90% CI	(11.5, 27.4)

Sources: Table 14.3.5ZA, Table 14.3.5ZB, Listing 16.2.7G

Safety in special populations

Age of patients

Age- and BSA-dependent pharmacokinetics of TREO in children was investigated in the PK sub-study of trials MC-FludT.16/NM and MC-FludT.17/M [Venn Life Sciences 2020]. 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. However, a covariate analysis revealed that BSA was the only clinically relevant covariate for clearance and volumes of distribution. Therefore, patients with a BSA of < 1 m² received a lower dose of TREO in the clinical trials (≤ 0.5 m²: 10 g/m²; > 0.5 to 1 m²: 12 g/m²; > 1 m²: full dose of 14 g/m²) to reach a similar exposure.

A general trend to higher incidence of TEAEs was observed in patients receiving the highest dose group of TREO (14 g/m²) which refers also to the older patient group. Seizures were observed only in the 10 g/m² dose group (n = 2 [8.7%]), i.e. the lowest age group (both children received the transplant for non-malignant diseases). Furthermore, there is a trend of higher incidence of severe TEAEs with higher doses of TREO and older age of the patients, especially with respect to blood and lymphatic system disorders (e.g. febrile neutropenia) and gastrointestinal disorders (e.g. stomatitis, nausea/vomiting).

Age-dependent frequency of drug-related TEAEs was lower in the youngest age group. Influence of age on several toxicity parameters were also explored in paediatric patients within a registry study performed by the EBMT [Peters 2011].

No significant correlation between age groups and the rate of grade III/IV aGvHD and extensive cGvHD, hyperbilirubinemia, diarrhoea, vomiting, CNS toxicity or peripheral neurological toxicity was found. However, considering all AE grades, more CNS toxicity was seen in the youngest age group. There was a significant increase of stomatitis and AST elevation grade III/IV with age. There was a significant association between age and respiratory toxicity. Children below the age of one year (mainly non-malignant diseases) experienced more respiratory grade III/IV toxicity. However, this could be explained by the fact that it is likely that these small children underwent HSCT for immunodeficiencies which are often associated with severe pulmonary infections even before HSCT.

Gender of patients

TEAEs were more frequently observed in female patients with only a few AEs being more frequently observed in male patients (CMV infection, cough, chills, and peripheral oedema). The same is observed for the incidence of severe (≥ grade III) TEAEs which was slightly higher in female patients, especially with respect to blood and lymphatic system disorders (17.1% vs. 10.0%), nervous system disorders (12.2% vs. 2.5%), gastrointestinal disorders (56.1% vs. 50.0%), and investigations (26.8% vs. 12.5%).

With respect to drug-related TEAEs, more (> 5% difference) female patients experienced vomiting (female vs. male: 48.8% vs. 38.8%), nausea (31.7% vs. 26.3%), abdominal pain (22.0% vs. 13.8%), hepatobiliary disorders (31.7% vs. 26.3%), increased ALT (14.6% vs. 8.8%), and increased AST (12.2% vs. 3.8%), whereas more male patients experienced infections (male vs. female: 15.0% vs. 4.9%), anal inflammation (5.0% vs. 0%), and general disorders (17.5% vs. 9.8%).

Concomitant use of thiotepa in paediatric patients

The majority of paediatric patients received the intensified regimen with thiotepa, which is associated with a trend of more TEAEs; however, the number of patients not receiving thiotepa is too small to draw final conclusions. TEAEs which have only been recorded in the thiotepa group (only those AEs considered with a frequency ≥ 5%) include viremia (n = 15 [13.9%]), EBV infection (10 [9.3%]), device-related infection (10 [9.3]%), adenovirus infection (8 [7.4%]), sepsis (7 [6.5%]), febrile neutropenia (9

[8.3%]), iron overload (6 [5.6%]), tremor (6 [5.6%]), eye disorders (15 [13.9%]), haematoma (10 [9.3%]), hypotension (7 [6.5%]), epistaxis (10 [9.3%]), oropharyngeal pain (9 [8.3%]), nasal congestion (7 [6.5%]), dyspnoea (6 [5.6%]), constipation (16 [14.8%]), oral pain (6 [5.6%]), pruritus (27 [25.0%]), pain of skin (10 [9.3%]), erythema (11 [10.2%]) and erythema multiforme (9 [8.3%]), pain in extremity (22 [20.4%]), back pain (9 [8.3%]), bone pain (7 [6.5%]), haematuria (9 [8.3%], reproductive system and breast disorders (11 [10.2%]), face oedema (7 [6.5%]), and blood bilirubin increased (12 [11.1%]).

Safety related to drug-drug interactions and other interactions

As per the SmPC, no interaction of treosulfan was observed in high dose chemotherapy. Moreover, detailed in vitro studies did not completely exclude potential interactions between high plasma concentrations of treosulfan and CYP3A4, CYP2C19, or P-gp substrates. Therefore, medicinal products with a narrow therapeutic index (e.g. digoxin) that are substrates for CYP3A4, CYP2C19 or P-gp should avoided during treatment with treosulfan.

The effect of treosulfan on the pharmacokinetics of fludarabine is not known.

No studies of specific drug-drug interactions were performed because TREO is given for three days only. No specific drug interaction could be identified within the clinical development programme.

Gonadal toxicity

Gonadal impairment is an important late effect with a significant impact on quality of life of transplanted patients. Faraci et al. [Faraci 2019] compared gonadal function after BU-based or TREO-based conditioning regimens in pre- and postpubertal children. This retrospective, multicentre study included children transplanted in paediatric EBMT centres between 1992 and 2012 who did not receive gonadotoxic chemoradiotherapy before the transplant. 137 patients transplanted in 25 paediatric EBMT centres were evaluated. Median age at transplant was 11.04 years (range, 5 to 18); 89 patients were boys and 48 girls. Eighty-nine patients were prepubertal at transplant and 48 postpubertal. One hundred eighteen children received BU and 19 TREO. A higher proportion of girls treated with TREO in the prepubertal stage reached spontaneous puberty compared with those treated with BU ($P = 0.02$). Spontaneous menarche was more frequent after TREO than after BU ($P < 0.001$). Postpubertal boys and girls treated with TREO had significantly lower luteinizing hormone levels ($P = 0.03$ and $P = 0.04$, respectively) compared with the BU group. This study suggests that the frequency of gonadal damage associated with TREO is significantly lower than that observed after BU.

Although this is a small study where very few TREO-based treated patients were included (19) versus BU-based treated patients (118), this observation is corroborated by data from a more recent study [Leiper 2020] where the fertility in 121 survivors of all ages of myeloid leukaemia and other haematological and immunodeficiency disorders who have undergone HSCT after three chemotherapy-conditioning regimens of different intensity which included TREO-based (25 males and 16 females), BU/cyclophosphamide (BU/Cy) (32 males and 23 females) and Fludarabine/melphalan (Flu-Mel) (13 males and 12 females) containing regimens was assessed by measuring serum concentrations of Anti-Müllerian hormone (AMH) and Inhibin B. Normal age-matched control data were used to convert the measurements of AMH and lnhibin B to standard deviation scores (SDS). The AMH data obtained suggested less ovarian reserve impairment after TREO and Flu-Mel than after Bu-Cy and the mean serum AMH concentration was significantly better with treosulfan (>1.0 µg/l) than with Flu-Mel or Bu-Cy. The same more favourable trend for TREO-based conditioning regimen is seen in males regarding Inhibin B data with the Flu-Mel group suffering greatest impairment. These authors reach the same conclusion,

that a TREO-based regimen confers a more favourable gonadal compromise than Flu-Mel or Bu-Cy in both sexes.

Gonadal toxicity was investigated in one pre-clinical study [Levi 2018] that concludes that treosulfan induces distinctive gonadal toxicity compared with busulfan. The rationale for this study was the knowledge that busulfan is considered highly gonadotoxic but the gonadal toxicity profile of treosulfan was not yet clear. In this pre-clinical study, pubertal and prepubertal male and female mice were injected with treosulfan or busulfan and sacrificed one week, one month or six months later. The assessment of testicular and ovarian functions indicated that treosulfan testicular toxicity was milder than that of busulfan toxicity and the ovarian toxicity of both treosulfan and busulfan was severe and permanent.

In the RMP, impaired fertility (male/female) is recognized as not yet reported in clinical studies and therefore classified within the safety concerns list as a missing information with the need for long-term follow up data after TREO-based conditioning (especially in paediatric patients)..

Discontinuation due to adverse events

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

Post marketing experience

Not applicable

2.5.1. Discussion on clinical safety

All seven trials also provide important safety data for the use of TREO. In total, the submitted data package includes safety data from 613 adult patients and 121 paediatric patients who have been treated with TREO-based conditioning.

Considering the aim of the current variation the discussion of clinical safety is focused in the two paediatric clinical trials **MC-FludT.16/NM and MC-FludT.17/M**.

The most commonly reported undesirable effects of TREO are myelosuppression (leukocytopenia, thrombocytopenia, anaemia) and gastrointestinal complaints (nausea, vomiting). They are usually mild and resolve after treatment.

Bone marrow suppression (neutropenia, leukocytopenia, thrombocytopenia, anaemia) is the dose-limiting side effect of TREO. However, it is this toxicity that supported the development of TREO for conditioning treatment prior to HSCT, especially in the allogenic setting. For this indication, TREO is always combined with FLU. Most paediatric patients receive additionally thiotepa (TT). This conditioning regimen is followed by infusion of haematopoietic stem cells.

Bone marrow depression and immunosuppression are therefore desired therapeutic effects of the conditioning regimen and consequently cannot be considered as undesirable effects. Therefore, any changes in blood counts and differential blood counts occurring between Day -6 and Day +28 were not documented as AEs. Especially during the time of bone marrow aplasia induced by the conditioning regimen, infections may develop and are a major source of morbidity and mortality of patients.

Facing the above, the adverse events (AEs) and adverse reactions (ARs) observed with TREO-based conditioning followed by alloHSCT are not only due to TREO alone but relate to the whole complex treatment procedure of alloHSCT.

A total 121 paediatric patients have been treated with TREO-based conditioning, including 51 patients with non-malignant diseases (primary immunodeficiency, haemoglobinopathy, inborn error of metabolism and bone marrow failure syndromes) and 70 patients with malignant diseases (AML, ALL, MDS, and JMML).

The study in non-malignant diseases (MC-FludT.16/NM) also included an active-control group with 50 evaluable patients treated with the reference conditioning regimen BU/FLU ± TT.

This safety data set is supplemented by safety data derived from two registry studies of the EBMT [Peters 201193; Peters 2017] which included a total of 1 521 paediatric patients with malignant and non-malignant diseases who had been treated with TREO-based conditioning. Therefore, available safety data cover all paediatric patients which are currently treated with alloHSCT.

Two comparisons can be made based on the safety data submitted: a) a comparison of the safety between **treosulfan**- and **busulfan**-based conditioning regimens prior HSCT in paediatric patients with non-malignant diseases as gathered within the **MC-FludT.16/NM** trial; b) and a comparison of the safety of the treosulfan-based conditioning regime prior HSCT between paediatric patients with **non-malignant diseases** (from **MC-FludT.16/NM**) and with **malignant diseases** as gathered within the **MC-FludT.17/M**.

a) From the data gathered in patients with **non-malignant diseases** it is possible to observe that frequency of TEAEs, including TEAEs with at least CTCAE grade III TEAEs, was broadly similar in the 2 treatment arms with some exceptions. The TREO group showed higher frequency of TEAEs including drug-related TEAEs, with differences ≥ 10%, for diarrhoea, abdominal pain, maculo-papular rash and pruritus but lower frequency for lung infection, dry skin, oral mucositis, nausea and hepatobiliary disorders; Higher frequency, with differences ≥ 5%, was observed with TREO for infections, febrile neutropenia and vascular events but lower frequency for vomiting and respiratory disorders. The frequency of gonadal damage and HSOS associated with TREO was lower than that observed after BU.

Frequency of SAEs was also comparable in both groups of trial MC-FludT.16/NM however the TREO group of subjects experienced less life-threatening SAEs and SAEs resulting in death as well as drug-related SAEs than the BU group but more SAEs, such as infections and more general disorders (fever) and conditions that required hospitalisation or prolongation of hospitalisation. This was confirmed by data from EBMT registry. The most common causes of death after HSCT for non-malignant diseases were infection, graft versus host disease, the original disease and transplant associated organ complications.

Cumulative incidence of aGvHD of all grades as well as of grade III/IV was higher for the TREO group up to Day +100 of the HSCT but on the contrary the cumulative incidence of cGvHD was higher in the BU arm

Laboratory parameters were largely comparable for the 2 treatment arms throughout the trial. The durations of leukopenia and neutropenia were significantly longer in the treosulfan arm than the busulfan arm. In trial MC-FludT.16/NM, the median level of WBCs at baseline, incidence of increased bilirubin/ALT/AST and median change levels from baseline of electrolytes (sodium, potassium) had no significant differences between both treatment groups. The same is largely verified for vital signs. The Kaplan Meier estimate at 12 months for subjects showing a deterioration to less than 60 points was slightly more favourable for the TREO group (8.3%) than for the BU group (12.7%).

b) When comparing the use of TREO prior HSCT in paediatric patients with **malignant and non-malignant diseases** similar rate of AEs of any CTCAE grade and drug-related ADRs of at least CTCAE Grade III was seen although the rate of AEs of at least CTCAE Grade III was somewhat lower (difference <5%) and the drug-related ADRS of any CTCAE grade (difference >5%) higher

in the case of patients with malignant diseases. Patients with at least one SAE were comparable between the two classes of diseases when treated with TREO, with life-threatening AEs slightly more associated with the treatment of malignant diseases (difference <5%). Subjects with non-malignant disease also presented slightly higher drug-related SAE (3.9% vs 1.4%).

It is possible to see that more patients (> 5% difference) with malignant diseases experienced viremia, device-related infection, hypersensitivity reactions, hypokalaemia, psychiatric disorders, nausea, bone pain, positive viral test, and increased blood bilirubin while epistaxis, abdominal pain, hepatoxicity, puritus, alopecia, increased CRP and infusion-related reactions were found in patients transplanted for non-malignant diseases.

Number of death cases were considerable higher in the trial MC-FludT.17/M (17.1%, n=12) than in the TREO arm of the MC-FludT.16/NM (3.9%, n=2) being largely due to relapse/progression in the case of the former trial. However, the mean time from transplantation to death was longer for the patients with malignant diseases treated with TREO than for the patients with non-malignant (14.97 months vs 5.75 months). Incidences of hepatic toxicity and infections were higher (but no significant) for the patients with malignant diseases than for patients with non-malignant diseases. Results were comparable in both trials regarding the frequency of SAEs.

Incidences of aGvHD were slightly higher in patients with non-malignant diseases (54.9%) than with malignant diseases (43.5%). However, for malignant diseases, there was a borderline significant impact of age-group on the incidence of aGvHD of any grade (P = 0.045). Mainly in non-malignant diseases children below the age of one year experienced more respiratory grade III/IV toxicity, which could be explained by the fact that it is likely these small children usually underwent HSCT for immunodeficiencies, that are often associated with severe pulmonary infections even before HSCT. Also, for non-malignant diseases, EBMT registry studies data indicate that there is no significant association between grade III/IV toxicity and dose-group in the subgroups of patients according to donor type, and diagnoses [Peters 2011]. Cumulative incidence of cGvHD is maintained over time for patients with both malignant and non-malignant diseases and was considerably higher (difference >10%) when TREO is used in the patients with malignant diseases. The same (although with a difference >5%) was observed for the cumulative incidence of moderate/severe cGvHD. The overall Kaplan Meier estimate at 12 months for subjects showing a deterioration to less than 60 points was higher for patients with malignant diseases (13.6%) than with non-malignant diseases (8.3%). Acute GvHD Grade III-IV was reported in less than 9% of the subjects and was maintained at 100 days. Also, for chronic GvHD cumulative incidence did not change up to the end of the duration of the trial's follow-up, i.e. 36 months.

The MAH proposes to amend an existing warning on section 4.4 of the SmPC and corresponding section of PL on skin toxicity based on new literature data. In a recent publication from Even-Or et al. 2020 [1] regarding skin toxicities following TREO-thiotepa-FLU-based conditioning regimen in non-malignant paediatric patients, an increase of skin disorders was observed when patients received sodium bicarbonate-containing hydration in the course of TREO infusion. The MAH postulated that this effect could be due to the acceleration of the pH-dependent formation of alkylating epoxides. The effect may be prevented by keeping the skin clean and dry on days of treosulfan infusion. Rash and dermatitis are given as examples of such skin toxicities. The MAH specified the exact ADRs and their severity (see SmPC sections 4.4 and 4.8).

With the responses to the second request for supplementary information comparative results on graft failures in line with the final Clinical Trial Report of the MCFludT.16/NM trial were reintroduced in section 5.1 of the SmPC.

2.5.2. Conclusions on clinical safety

As expected considering the target indication of intensive treatment conditioning-based treatment in paediatric population with malignant diseases prior alloHSCT and confirming the previous safety data almost all subjects treated with treosulfan in this disease setting presented TEAEs (68/70). Likewise, the combination treatment with Thiotepa in the majority of the subjects (65/70) also explains the frequency of TEAEs and TEAEs of at least CTCAE grade III related or not with the drug. These adverse events are already included in section 4.8 of the SmPC related with paediatric population. Among the TESAEs, upper respiratory infection and febrile neutropenia are reported, the former is not clearly included in SmPC and the latter is included with unknown frequency. No unknown risks were identified and there was no occurrence of any SUSARs. At the end of the trial where treosulfan was included in the conditioning regime for patients with malignant diseases, 20% subjects (n=14) terminated the trial prematurely, being death from relapse/progression and transplantation-related the main cause in 17.1% of the subjects (n=12). Laboratory parameters and vital signs did not change and performance scores were as expected.

Overall, safety data gathered from the MU-FludT.17/M trial is in line with the adverse reactions listed for adults and paediatric patients already included in the approved PI and can be explained by the intensive treatment prior alloHSCT of treosulfan on top of standardized fludarabine, and in the majority of the cases also with thiotepa, as well due to the underlying malignant disease (ALL, AML, MDS, and JMML) and transplantation-procedure per se. The safety profile is comparable to those reported for treosulfan in other trials and comparable or even favourable to those reported for BU- or TBI-based conditioning regimens.

No unknown risks were identified in the trial and there was no occurrence of any SUSARs. Comparable incidences of aGvHD were observed between the treatment arms however a significantly and clinically meaningful lower incidence of overall cGvHD was found with treosulfan. More deaths occurred in the busulfan arm than the treosulfan arm. Overall, safety results favour the treatment with treosulfan in comparison with busulfan in for conditioning prior to alloHSCT in paediatric patients with non-malignant diseases.

An existing warning on section 4.4 of the SmPC and corresponding section of PL on skin toxicity (e.g. rash, dermatitis) was revised based on new literature data.

Further safety data will be provided as the MAH agreed to a CHMP recommendation to submit the final study report of study MC-FludT.16/NM when available.

2.5.3. PSUR cycle

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.

2.6. Risk management plan

The MAH submitted/was requested to submit an updated RMP version with this application.

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

The PRAC considered that the risk management plan version 1.4 is acceptable.

The CHMP endorsed this advice without changes.

The CHMP endorsed the Risk Management Plan version 1.4 with the following content:

Safety concerns

Summary of 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*

*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.

Pharmacovigilance plan

No pharmacovigilance studies are planned, on-going or have been completed by medac GmbH.

Routine pharmacovigilance activities beyond ADR reporting and signal detection:

Specific adverse reaction follow-up questionnaires: None

Other forms of routine pharmacovigilance activities: None

No additional pharmacovigilance activities are performed or planned for TREO.

Risk minimisation measures

Description of routine risk minimisation measures 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; PL section 4 Routine risk minimisation activities recommending specific clinical measures to address the risk: None

Safety concern	Routine risk minimisation activities
	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, and 4.8; PL sections 2, and 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 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. Other routine risk minimisation measures beyond the product information: Legal status: prescription only medicine
Use in patients with prior alloHSCT*	Routine risk communication: None 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

* 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.

2.7. Update of the Product information

As a consequence of this new indication, sections 4.1, 4.2, 4.4, 4.8, 5.1 and 5.2 of the SmPC have been updated. The Package Leaflet has been updated accordingly.

2.7.1. User consultation

A justification for not performing a full user consultation with target patient groups on the package leaflet has been submitted by the MAH and has been found acceptable for the following reasons:

-this new indication does not introduce any major amendments to the Package Leaflet which affects the readability and requires to conduct a new user test. The introduced changes are the new indication, the amendment of an existing warning on skin toxicity based on new literature data and the update of side effects, being the majority of the changes related to rearrangements in terms of frequency, only a few

new effects were introduced. This does not impact the readability already tested for this PL.

-The package leaflet included in this submission is in general, identical to the previously readability tested package leaflet. During the initial marketing authorisation application, the MAH has performed a full user testing that has successfully demonstrated the comprehensibility and usability of the PL. This conclusion can be extended to the PL submitted as part of this proposed extension of the therapeutic indication.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) to treat paediatric patients older than one month with malignant and non-malignant diseases.

Treosulfan is approved for the conditioning treatment prior to alloHSCT in malignant and non-malignant diseases in adult and in malignant diseases in paediatric patients. The MAH intends to extend the approved indication also to paediatric patients with non-malignant indications.

3.1.2. Available therapies and unmet medical need

AlloHSCT is the only potentially curative treatment option for many patients especially with AML and MDS, relapsed patients with various other haematological malignancies, as well as some non-malignant disorders (NMD) such as primary immunodeficiencies (PID), inborn errors of metabolism, haemoglobinopathies or bone marrow failure syndromes. The most common indications for an alloHSCT are AML (40.8%), followed by MDS/MPS (15.7%), and ALL (15.4%) and NMD (13.2%). Main indications for alloHSCT in children are ALL, AML and NMD [Passweg 2021].

An increasing number of children with non-malignant diseases can be cured by alloHSCT. There is a number of conditioning regimens that can be used in this treatment. Treosulfan (TREO)-based conditioning regimens is one of them [Slatter 2015; Peters 201794], although the use is not approved in the EU being the target of the application for the extension of indication under assessment.

The various conditioning regimens prior alloHSCT used in clinical practice today differ in their intensity and are currently divided into three categories: myeloablative conditioning (MAC), reduced intensity conditioning (RIC) and non-myeloablative conditioning (NMA). Myeloablative conditioning regimens cause irreversible cytopenia and Haematopoietic stem cells (HSC) support is mandatory. Non-myeloablative conditioning regimens cause minimal cytopenia and can be given also without HSC support. Reduced intensity conditioning regimens do not fit criteria for MAC or NMA regimens. They cause cytopenia of variable duration and should be given with stem cell support, although cytopenia may not be irreversible [Bacigalupo 2009]. The most frequently used medicinal products used for conditioning treatment include oral or preferably intravenous Busulfan (BU), Cyclophosphamide (CY), Melphalan (MEL), Thiotepa (TT), Fludarabine (FLU), cytarabine, amsacrine, and increasingly TREO. Total body

irradiation (TBI) has been included in some of the regimens to further reduce relapse incidence; however, it causes substantial toxicity.

In paediatric patients, various conditioning regimens are used depending on the disease (malignant or non-malignant), disease status, as well as institute-specific preferences. Reduced intensity conditioning regimens are much less frequently used than in adults because children less frequently have comorbidities which do not allow using a MAC regimen. In addition, children with non-malignant diseases like haemoglobinopathies or metabolic disorders require an intensive, myeloablative conditioning treatment to ensure a successful engraftment. In recent years, thiotepa has been increasingly incorporated into conditioning regimens for malignant as well as non-malignant diseases [Peters 2017].

Transplant-related morbidity/mortality limits the use of alloHSCT for many patients. The mortality rate at 100 days post alloHSCT ranges between 7% for patients with acute leukaemia in remission undergoing matched related donor (MRD) HSCT and 27% for patients with refractory acute leukaemia undergoing matched unrelated donor (MUD) HSCT [Pasquini 2014].

There is a high unmet medical need for new conditioning regimens with low treatment-related toxicities but no increased risk for relapse to improve the OS and ensure a good quality of life for the patients. Such a regimen should offer sufficient stem cell toxicity, immunosuppressive potential and high anti-tumour activity (in case of malignant disorders), but reduced organ toxicity (especially with respect to the liver, kidneys, lung, and the nervous system) and predictive pharmacokinetics. With the current application, treosulfan in meant to replace busulfan in the conditioning prior alloHSCT in patients with non-malignant diseases with similar clinical results and less toxicity as in adults.

3.1.3. Main clinical studies

Treosulfan-based conditioning regimens followed by alloHSCT have been investigated by the MAH in a total of 613 adult and 121 paediatric patients within seven clinical trials. According to the Paediatric Investigation Plan (PIP), two clinical transplantation studies with TREO-based conditioning were conducted in paediatric patient populations [Table 3].

Table 3 Listing of clinical studies in paediatric patients

Study ID	Study title	Objectives
MC-FludT.16/ NM	Clinical phase II trial to compare TREO-based conditioning therapy with BU-based conditioning prior to alloHSCT in paediatric patients with non-malignant diseases	To describe the safety and efficacy of TREO compared to the conventional dose BU (control), each administered as part of a standardised FLU-containing conditioning regimen and to contribute to a pharmacokinetic model
MC-FludT.17/M	Clinical phase II trial to describe the safety and efficacy of TREO-based conditioning therapy prior to alloHSCT in paediatric patients with haematological malignancies	To describe the safety and efficacy of TREO as part of a standardised FLU-containing conditioning and to contribute to a pharmacokinetic model

The final clinical study report (CSR) of **MC-FludT.17/M** (Version 2, dated 24-Mar-2020) includes longer-term follow-up results until 36 months after transplant. The CSR of study **MC-FludT.16/NM** focusses on completed 12 month follow-up data for all subjects, but also contains follow-up data available as of the data cut-off on 07-Jun-2021. Longer-term follow-up data will be collected until the last recruited subject has completed visit Month 36 and presented in an updated version of the CSR expected in 2023. Both studies are based on an approved PIP, incl. four agreed modifications.

3.2. Favourable effects

In paediatric patients, a conditioning regimen consisting of 10-14 g/m²/d × 3 TREO (Day -6/-5/-4) plus fludarabine (FT10-14) proved to be very effective and well tolerated in MC-FludT.17/M and MC-FludT.16/NM clinical trials that included a total of 121 patients with, respectively, malignant or non-malignant disorders. In the latter clinical trial, a comparison of the TREO/FLU/±TT regime with a BU/FLU/±TT regime is performed after randomisation of 1:1 of 101 patients (51:50). Most of the patients (89%) received a regimen of FT10-14 combined with thiotepa (FT10-14/TT).

Furthermore, data of TREO-based conditioning are available from the EBMT registry for a total of 1 416 patients which confirm its efficacy and safety for malignant as well as non-malignant transplant indications [Peters 2011/2017].

The main favourable effects of the use of TREO and in particularly when comparing with BU are:

- High cumulative engraftment rate, with a maximum conditional cumulative incidence of neutrophil engraftment of 97.3% in paediatric patients with non-malignant diseases (study 16/NM) and 100% in paediatric patients with malignant diseases (study MC-FludT.17/M). This is also concordant with the higher (3.9% for TREO and 4.0% for BU) primary graft failure rates observed for in patients with non-malignant diseases when compared with patients with malignant diseases (0) and higher secondary graft failure for the TREO arm of the study 16/NM (18.4%). According to the 2017 EBMT meta-analysis in paediatric patients with non-malignant diseases, neutrophil engraftment at Day 100 after alloHSCT was superior with the TREO/FLU/TT combination versus BU/FLU/TT (96.1% vs. 83.5%) [Peters 201794].

- Lower freedom from transplant (treatment) related mortality until Day +100 after HSCT: both TREO and BU give good results but the treosulfan behaves better with 100% (.16/NM; 90% CI: 94.3, 100) or 98.6% subjects (MC-FludT.17/M; 90% CI: 93.4, 99.9) without an event for treosulfan vs 90% for busulfan /90% CI: 80.1, 96.0)(unadjusted p-value 0.0267; adjusted p-value 0.0528).
- Lower transplant-related mortality: TREO-based conditioning was at 1 year very low in patients with both non-malignant (3.9%; 90% CI: 1.2, 12.0) as well as malignant diseases (1.4%; 90% CI: 0.3, 7.2%) and lower than with BU in no-malignant diseases (12.0%; 90% CI: 6.3, 22.1) (HR (TREO/BU) 0.29 (90% CI 0.0, 1.09); p value 0.1244). This was also seen in the EBMT registry analysis with no influence of age or dose of treosulfan and significantly lower TRM for TREO/FLU/TT than for BU/FLU/TT conditioning [Peters 2017]. In both treatments for non-malignant diseases the TRM was maintained over time (only a slight increase between 2 and 3 years for BU arm) however after to 2 years in malignant disease with treosulfan. TRM after TREO-based conditioning was also very low in the EBMT registry analysis. TREO dosing in the paediatric studies aimed to reach TREO plasma levels in the range observed with the 14 g/m² dose in adults. The data from both studies suggest that paediatric patients tolerate TREO-based conditioning better than adult patients and justify that there was no need to reduce the TREO dose in this patient population.

- Higher disease-free survival in patients with non-malignant diseases: Disease-free survival (DFS) at one year in paediatric patients with non-malignant diseases was highest with the TREO/FLU/TT regimen (86%). This was significantly higher than the 77.3% DFS rate observed with the BU/FLU/TT regimen (P = 0.002) [Peters 2017]. However, event-free survival was lower for TREO arm in study 16/NM than for BU at all time points but comparable between non-malignant and malignant diseases.

- Higher overall survival rate in patients with non-malignant diseases: In study 16/NM OS rate at 1 year after TREO-based conditioning was 96.1% (90% CI: 88.0, 98.8%), higher than for BU with an OS value of 88.0% (90% CI:77.9,93.7) than for malignant diseases with an OS of 91.4% (90% CI: 83.9, 95.5). This OS rate was maintained at 3 years for TREO in non-malignant diseases, decreased

to 84.0% (90% CI: 71.4, 91.4) for BU and for TREO in malignant diseases to 84.3% (90% CI: 75.5, 90.1). These data are confirmed by data from the EBMT registry.

- Low relapse rate: The conditional cumulative incidence of relapse/progression in the 70 patients treated with TREO-based conditioning in trial MC-FludT.17/M was 15.7% at 12 months and 23.0% at 24/36 months with 15.7% (n=11) of these patients requiring rescue therapy mainly of donor lymphocyte infusion (DLI) or stem cell boost. According to the EBMT registry [Peters 2017], incidence of disease relapse at 1 year after transplant is also very low in patients with non-malignant diseases and ranged from 6.1-6.8% with TREO-based conditioning to 7.2-9% with BU-based conditioning. The no difference regarding rescue therapies between TREO and BU is confirmed in the 16/NM trial. However, in study 16/NM, patients with non-malignant diseases required more rescue therapies (41.2%; especially transfusions for RBCs [33%] and platelets [27.5%]) than patients with malignant diseases in study MC-FludT.17/M.
- Lower gonadal toxicity: Results from two clinical [Faraci 2019; Leiper 2020] and one preclinical study [Levi 2018] suggest that the gonadal toxicity is lower with TREO compared to BU.

Good engraftment with lower freedom from transplant (treatment) related mortality until Day +100 after HSCT, lower transplant-related mortality, better overall survival as compared to busulfan.

Based on the engraftment and chimerism data approaching 100% and > 90%, respectively, efficacy parameters like EFS, OS, and GvHD-free and relapse-free survival confirm the effectiveness of this conditioning treatment not only in preparing for alloHSCT paediatric patients with malignant diseases but also in intended extension of the indication, i.e. non-malignant diseases as for adults.

3.3. Uncertainties and limitations about favourable effects

Long term data on efficacy will be provided as the final study report of study MC-FludT.16/NM will be submitted when available. This is of interest as allo-HSCT will be applied in the treatment for non-malignant diseases with the expectation of long-term treatment benefit.

3.4. Unfavourable effects

The most commonly reported undesirable effects of TREO are myelosuppression (leukocytopenia, thrombocytopenia, anaemia), immunosuppression, and gastrointestinal complaints (nausea, vomiting). They are usually mild and resolve after treatment. In fact, it is this toxicity that supported the development of TREO for conditioning treatment prior to HSCT, especially in the allogenic setting. Bone marrow depression and immunosuppression are therefore the desired therapeutic effects of a conditioning regimen.

The study in non-malignant diseases (MC-FludT.16/NM) also included an active-control group with 50 evaluable patients treated with the reference conditioning regimen BU/FLU ± TT.

This safety data set is supplemented by safety data derived from two registry studies of the EBMT [Peters 201193; Peters 2017] which included a total of 1 521 paediatric patients with malignant and non-malignant diseases who had been treated with TREO-based conditioning. Therefore, available safety data cover all paediatric patients which are currently treated with alloHSCT.

Two comparisons can be made based on the unfavourable effects based on the data submitted: a) a comparison between the effects with **treosulfan**- and **busulfan**-based conditioning regimens prior HSCT in paediatric patients with non-malignant diseases as gathered within the **MC-FludT.16/NM** trial; b)

and a comparison of the effects of the treosulfan-based conditioning regime prior HSCT between paediatric patients with **non-malignant diseases** (from **MC-FludT.16/NM**) and with **malignant diseases** as gathered within the **MC-FludT.17/M**.

The most common causes of death after HSCT for non-malignant diseases were infection, graft versus host disease, the original disease and transplant associated organ complications.

From the data gathered in patients with **non-malignant diseases** it is possible to observe that frequency of TEAEs, including TEAEs with at least CTCAE grade III TEAEs, and frequency of SAEs was broadly similar in the two treatment arms with the exceptions depicted below. Also, laboratory parameters were largely comparable for the 2 treatment arms throughout the trial. In trial MC-FludT.16/NM, the median level of WBCs at baseline, incidence of increased bilirubin/ALT/AST and median change levels from baseline of electrolytes (sodium, potassium) had no significant differences between both treatment groups. The same is largely verified for vital signs.

Higher rate of unfavourable effects is seen with TREO-based conditioning treatment prior allo-HSCT in non-malignant diseases when comparing with BU-based treatment: The TREO group showed higher frequency of TEAEs including drug-related TEAEs, with differences $\geqslant$ 10%, for diarrhoea, abdominal pain, maculo-papular rash and pruritus; Higher frequency, with differences $\geqslant$ 5%, was observed with TREO for infections, febrile neutropenia and vascular events but lower frequency for vomiting and respiratory disorders. The TREO group of subjects experienced more SAEs, such as infections and more general disorders (fever) and conditions that required hospitalisation or prolongation of hospitalisation than the BU group. Cumulative incidence of aGvHD of all grades as well as of grade III/IV was higher for the TREO group up to Day +100 of the HSCT. The durations of leukopenia and neutropenia were significantly longer in the TREO arm than the BU arm but the MAH clarified the potential factors associated with these events (see above Section 8.3).

Lower rate of unfavourable effects is seen with TREO-based conditioning treatment prior allo-HSCT in non-malignant diseases when comparing with BU-based treatment: The TREO group showed lower frequency of TEAEs including drug-related TEAEs, with differences $\geqslant$ 10%, for lung infection, dry skin, oral mucositis, nausea and hepatobiliary disorders. The TREO group of subjects experienced less life-threatening SAEs and SAEs resulting in death as well as drug-related SAEs than the BU group. Cumulative incidence of cGvHD was higher in the BU arm. The Kaplan Meier estimate at 12 months for subjects showing a deterioration to less than 60 points was slightly more favourable for the TREO group (8.3%) than for the BU group (12.7%).

When comparing the use of TREO prior HSCT in paediatric patients with **malignant and non-malignant diseases** similar rate of AEs of any CTCAE grade and drug-related ADRs of at least CTCAE Grade III was seen. Results were comparable in both trials when treated with TREO regarding the frequency of SAEs and the % of patients with at least one SAE. Some exceptions are listed below:

Higher rate of unfavourable effects is seen with TREO-based conditioning treatment prior alloHSCT in non-malignant diseases when comparing malignant diseases: the rate of AEs of at least CTCAE Grade III was somewhat higher (difference <5%) when comparing with patients with malignant diseases. Subjects with non-malignant disease also presented slightly higher drug-related SAE (3.9% vs 1.4%). The mean time from transplantation to death was shorter for the patients with non-malignant diseases treated with TREO than for the patients with malignant (5.75 months vs 14.97 months). Incidences of aGvHD were slightly higher in patients with non-malignant diseases (54.9%) than with malignant diseases (43.5%). In non-malignant diseases children below the age of one year experienced more respiratory grade III/IV toxicity

Lower rate of unfavourable effects is seen with TREO-based conditioning treatment prior alloHSCT in non-malignant diseases when comparing malignant diseases: the drug-related ADRS of any CTCAE grade

(difference >5%) was lower than in the case of patients with malignant diseases. Life-threatening AEs slightly more associated with the treatment of malignant diseases (difference <5%). More patients (> 5% difference) with malignant diseases experienced viremia, device-related infection, hypersensitivity reactions, hypokalaemia, psychiatric disorders, nausea, bone pain, positive viral test, and increased blood bilirubin while epistaxis, abdominal pain, hepatoxicity, puritus, alopecia, increased CRP and infusion-related reactions were found in patients transplanted for non-malignant diseases. Number of death cases were considerable higher in the trial MC-FludT.17/M (17.1%, n=12) than in the TREO arm of the MC-FludT.16/NM (3.9%, n=2) being largely due to relapse/progression in the case of the former trial. Incidences of hepatic toxicity and infections were lower (but no significant) for the patients with non-malignant diseases than for patients with malignant diseases. Cumulative incidence of cGvHD is maintained over time for patients with both malignant and non-malignant diseases but was considerably higher (difference >10%) when TREO is used in the patients with malignant diseases. The same (although with a difference >5%) was observed for the cumulative incidence of moderate/severe cGvHD. The overall Kaplan Meier estimate at 12 months for subjects showing a deterioration to less than 60 points was lower for patients with non-malignant diseases (8.3%) than with non-malignant diseases (13.6%).

Based on new literature data (Even-Or et al. 2020 [1]), the MAH proposes to amend an existing warning on section 4.4 of the SmPC and corresponding section of PL on skin toxicity following TREO-thiotepa-FLU-based conditioning regimen in non-malignant paediatric patients, when patients received sodium bicarbonate-containing hydration in the course of TREO infusion. In the response provided to the first request for supplementary information, the MAH reevaluated the issue on skin toxicity to specify the exact related ADRs and their severity. From the literature consulted no clear relation between skin toxicity and sodium bicarbonate-containing hydration could be found and only reports from clinical practice support the proposed change to the SmPC. Without being able to obtain detailed information the MAH concluded that further investigation is needed so that this issue can be detailed in the SmPC. As such, the MAH proposes to withdraw the proposed amendment to the existing warning on section 4.4 of the SmPC and corresponding PL and leave the texts has previously approved. This is acceptable, especially considering the commitment of the MAH to collect additional information within signal management to allow a proper evaluation of the issue of skin toxicity and the submission of a future variation if applicable.

3.5. Uncertainties and limitations about unfavourable effects

The MAH proposes to amend an existing warning on skin toxicity based on new literature data. Rash and dermatitis are given as examples of such skin toxicities. In the first round of the assessment the MAH was asked to specify the exact ADRs and their severity and if applicable to update the Product Information accordingly. From the literature consulted no clear relation between skin toxicity and sodium bicarbonate-containing hydration could be found and only reports from clinical practice support the proposed change. Without being able to obtain detailed information the MAH concluded that further investigation is needed so that this issue can be detailed in the SmPC.

Long term data on safety in the treatment for non-malignant diseases -with the expectation of long-term treatment benefit - will be provided as the MAH agreed to a CHMP recommendation to submit the final study report of study MC-FludT.16/NM when available.

3.6. Effects Table

Table 2. Effects Table for Trecondi (conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT) in paediatric patients older than one month with non-malignant)(data cut-off: MC-FludT.16/NM trial 07-Jun-2021)

Effect	Short description	Unit	TREO (n=51)	BU (n=50)	Uncertainties / Strength of evidence	References
Favourable Effects						
Freedom from transplantation (treatment)-related mortality	Incidence Freedom from transplantation (treatment)-related mortality until day +100 Primary endpoint	%	100.0	90.0	p[a]<0.0528 OR<0.0001	Table 11.4.1.1.A CSR (FAS)
TRM	Transplantation-related Mortality at 1 year Secondary endpoint	%	3.9	12.0	p[a]<0.1244 HR 0.29 (0.08, 1.09)	Table 11.4.1.2.A CSR (FAS)
OS	Overall survival at 1 year Secondary endpoint	%	96.1	88.0	p[a]<0.0718 HR 0.29 (0.08, 1.09)	Table 11.4.1.3.A CSR (FAS)
Graft failure	Rates of primary graft failures Secondary endpoints	%	3.9	4.0		Table 11.4.1.4.A CSR (FAS)
Graft failure	Rates of secondary graft failures Secondary endpoints	%	18.4	0.0		Table 11.4.1.4.A CSR (FAS)
Graft failure	Cumulative incidences of graft failure at 12 months Primary and secondary graft failures Secondary endpoints	%	15.8	4.0	p[a]< 0.0366 HR 5.48 (1.44, 20.91)	Table 11.4.1.4.B CSR (FAS)
Engraftment Granulopoiesis	Maximum conditional cumulative incidence reached Reconstitution of granulopoiesis Secondary endpoint	%	97.3	100.0	P[b]< 0.0521	Table 11.4.1.5.A CSR (FAS)
Neutropenia	Mean (SD) Duration of neutropenia[d]	days	19.9 (7.7)	15.9 (7.3)	P[c]< 0.0108	Table 11.4.1.5.B CSR (FAS)
Engraftment Leukopoiesis	Maximum conditional cumulative	%	96.8	100.0	P[b]< 0.2469	Table 11.4.1.5.A CSR (FAS)

Effect	Short description	Unit	TREO (n=51)	BU (n=50)	Uncertainties / Strength of evidence	References
	incidence reached Reconstitution of leukopoiesis Secondary endpoint					
Leukopenia	Mean (SD) Duration of leukopenia[d]	days	19.0 (5.7)	16.3 (7.3)	P[c]< 0.0087	Table 11.4.1.5.B CSR (FAS)
Engraftment Thrombopoiesis > 20 $x10^9$/L	Maximum conditional cumulative incidence reached Reconstitution of thrombopoiesis > 20 $x10^9$/L Secondary endpoint	%	100.0	96.8	P[b]<0.8595	Table 11.4.1.5.A CSR (FAS)
Engraftment Thrombopoiesis > 50 $x10^9$/L	Maximum conditional cumulative incidence reached Reconstitution of thrombopoiesis > 50 $x10^9$/L Secondary endpoint	%	94.8	97.1	P[b]< 0.3635	Table 11.4.1.5.A CSR (FAS)
Donor Type Chimerism	Incidence of complete donor type chimerism until Month 12 visit Subjects at risk at Day +100 visit Secondary endpoint	%	66.7	84.8	p[a]< 0.1196 OR 0.3972	Table 11.4.1.6.A CSR (FAS)
EFS	Event-free Survival at 1 year Secondary endpoint	%	80.3	86.0	p[a]<0.3343 HR 1.54 (0.74, 3.22)	Table 11.4.1.7.A CSR (FAS)
GvHD-free Survival	GvHD-free survival at 1 year Secondary endpoint	%	82.9	69.4	p[a]<0.2178 HR 0.58 (0.28, 1.20)	Table 11.4.1.8.A CSR (FAS)
Chronic GvHD-free Survival	Chronic GvHD-free Survival at 1 year Secondary endpoint	%	89.3	69.4	p[a]<0.0308 HR 0.32 (0.14, 0.76)	Table 11.4.1.9.A CSR (FAS)
Rescue Therapies	Incidence of Any Rescue Therapies Secondary endpoints	%	41.2	42.0	p[a]<0.5264 OR 0.7592	Table 11.4.1.10.A CSR (FAS)
Unfavourable Effects						
TEAE	Any Adverse event	n (%)	49 (96.1)	48 (96.0)		Table 12.2.1.A CSR (FAS)
TRAE	Any Drug-related adverse events	n (%)	41 (80.4)	37 (74.0)		Table 12.2.1.A

Effect	Short description	Unit	TREO (n=51)	BU (n=50)	Uncertainties / Strength of evidence	References
						CSR (FAS)
SAE	Any serious adverse event	n (%)	18 (35.3)	16 (32.0)		Table 12.2.1.A CSR (FAS)
SAE	Death/Life-threatening Results in death	n (%)	0 (0.0)/ 3 (5.9)	4 (8.0)/ 4 (8.0)		Table 12.2.1.A CSR (FAS)
SAE	Hospitalization or prolongation of hospitalization	n (%)	16 (31.4)	8 (16.0)		Table 12.2.1.A CSR (FAS)
STRAE	Drug-related serious adverse events	n (%)	2 (3.9)	3 (6.0)		Table 12.2.1.A CSR (FAS)
AE	Maximum CTCAE grade of adverse events Grade III	n (%)	34 (66.7)	30 (60.0)		Table 12.2.1.A CSR (FAS)
cGvHD	Overall cGvHD Cumulative incidence at 1 year	%	12.8	38.6	p[a]<0.0168 HR 0.31 (0.14, 0.69)	Table 12.2.2.2.D CSR (FAS)
Infections and infestations	Any event	n (%)	12 (23.5)	7 (14.0)		Table 12.3.1.2.A CSR (FAS)

Table 3. Effects Table for Trecondi (conditioning treatment prior to allogeneic haematopoietic stem cell transplantation (alloHSCT) in paediatric patients older than one month with malignant and)(data cut-off: MC-FludT.17/NM trial Date last subject completed - longer-term follow-up visit: 30-Sep-2019)

Effect	Short description	Unit	TREO (n=70)	Uncertainties / Strength of evidence	References
Favourable Effects					
Freedom from transplantation (treatment)-related mortality	Incidence Freedom from transplantation (treatment)-related mortality until day +100 Primary endpoint	% (90% CI)	98.6% (93.4, 99.9)		CSR (FAS)
TRM	Transplantation-related Mortality at 1 year Secondary endpoint [e]	% (90% CI)	1.4 (0.3, 7.2)		Table 11.4.1.2.A CSR (FAS)
OS	Overall survival at 1 year Secondary endpoint [e]	% (90% CI)	91.4 (83.9, 95.5)		Table 11.4.1.3.A CSR (FAS)
Relapse / Progression	Relapse / Progression Incidence after HSCT	% (90% CI)	15.7 (8.6, 22.9)		Table 11.4.1.4.A CSR (FAS)

Effect	Short description	Unit	TREO (n=70)	Uncertainties / Strength of evidence	References
RFS / PFS	Relapse-free / Progression-free Survival at 1 year [e]	% (90% CI)	82.9 90% CI (73.9, 89.0)		Table 11.4.1.5.A CSR (FAS)
Graft failure	Rates of primary graft failures Secondary endpoints	n/N (%) (90% CI)	0/70 (0.0) (0.0, 4.2)		Table 11.4.1.6.A CSR (FAS)
Graft failure	Rates of secondary graft failures Secondary endpoints	n/N (%) (90% CI)	1/69 (1.4) (0.1, 6.7)		Table 11.4.1.6.A CSR (FAS)
NRM	Non-relapse Mortality after HSCT at 1 year	% (90% CI)	1.4 (0.0, 3.8)		Table 11.4.1.7.A CSR (FAS)
Engraftment Granulopoiesis	Maximum conditional cumulative incidence reached Reconstitution of granulopoiesis Secondary endpoint	% (90% CI)	100.0 (97.7, 100.0)		Table 11.4.1.8.A CSR (FAS)
Neutropenia	Mean (SD) Duration of neutropenia[d]	days	22.3 (7.7)		Table 11.4.1.8.B CSR (FAS)
Engraftment Leukopoiesis	Maximum conditional cumulative incidence reached Reconstitution of leukopoiesis Secondary endpoint	% (90% CI)	100.0 (97.7, 100.0)		Table 11.4.1.8.C CSR (FAS)
Leukopenia	Mean (SD) Duration of leukopenia[d]	days	20.5 (6.1)		Table 11.4.1.8.D CSR (FAS)
Engraftment Thrombopoiesis > 20 $x10^9$/L	Maximum conditional cumulative incidence reached Reconstitution of thrombopoiesis > 20 $x10^9$/L Secondary endpoint	% (90% CI)	94.1 (88.4, 99.9)		Table 11.4.1.8.E CSR (FAS)
Engraftment Thrombopoiesis > 50 $x10^9$/L	Maximum conditional cumulative incidence reached Reconstitution of thrombopoiesis > 50 $x10^9$/L Secondary endpoint	% (90% CI)	91.9 (84.9, 98.8)		Table 11.4.1.8.F CSR (FAS)
Donor Type Chimerism	Incidence of complete donor	% (90% CI)	91.3 (83.6, 96.1)		Table 11.4.1.9.A

Effect	Short description	Unit	TREO (n=70)	Uncertainties / Strength of evidence	References
	type chimerism Subjects at risk at Day +100 visit Secondary endpoint				CSR (FAS)
EFS	Event-free Survival at 1 year Secondary endpoint	% (90% CI)	81.4 (72.3, 87.8)		Table 11.4.1.10.A CSR (FAS)
GvHD-free and RFS / PFS (GRFS)	GvHD-free Relapse-free / Progression-free Survival at 1 year Secondary endpoint	% (90% CI)	65.7 (55.5, 74.1)		Table 11.4.1.11.A CSR (FAS)
Chronic GvHD-free and RFS / PFS	Chronic GvHD-free and Relapse-free / Progression-free Survival at 1 yeat Secondary endpoint	% (90% CI)	67.1 (57.0, 75.4)		Table 11.4.1.12.A CSR (FAS)
Rescue Therapies	Incidence of Any Rescue Therapies Secondary endpoints	n (%)	58 (82.9)	p[a]<0.5264 OR 0.7592	Table 11.4.1.13.A CSR (FAS)
Unfavourable Effects					
TEAE	Any Adverse event	n (%)	68 (97.1)		Table 12.2.1.A CSR (FAS)
TRAE	Any Drug-related adverse events	n (%)	63 (90.0)		Table 12.2.1.A CSR (FAS)
SAE	Any serious adverse event	n (%)	23 (32.9)		Table 12.2.1.A CSR (FAS)
SAE	Death/Life-threatening Results in death	n (%)	1 (1.4)/ 6 (8.6)		Table 12.2.1.A CSR (FAS)
SAE	Hospitalization or prolongation of hospitalization	n (%)	20 (28.6)		Table 12.2.1.A CSR (FAS)
STRAE	Drug-related serious adverse events	n (%)	1 (1.4)		Table 12.2.1.A CSR (FAS)
AE	Maximum CTCAE grade of adverse events Grade III	n (%)	42 (60.0)		Table 12.2.1.A CSR (FAS)
cGvHD	Overall cGvHD Cumulative incidence at 1 year	% (90% CI)	23.9 (15.3, 32.4)		Table 12.2.2.2.B CSR (FAS)
Infections and infestations	Any event	n (%)	15 (21.4)		Table 12.3.1.2.A CSR (FAS)

Abbreviations: TREO – treosulfan; BU – busulfan; OR – Odd ratio; HR – Hazard Ratio; TEAE - treatment emergent

adverse events; TRAE – drug-related adverse events; STRAE – serious drug-related adverse events

Notes: [a]p-value is adjusted for thiotepa and disease using the stratified Cochran-Mantel-Haenszel test (freedom from TRM, Donor Type Chimerism, rescue therapies), Cox regression Model (TRM, OS, EFS, GvHD-free survival, Chronic GvHD-free Survival), Fine and Gray model (graft failure, cGvHD); [b] Based on the Pepe-Mori test; [c] Wilcoxon-Mann-Whitney test; [d] First date with neutropenia until date of engraftment (subjects at risk = subjects with neutropenia and neutrophilic granulopoiesis); [e] Based on Kaplan-Meier estimates

Hazard Ratio (Treosulfan/Busulfan) (90% CI); OS - The median duration of follow-up was 25.4 months (11.7 - 63.3 months) in the BU arm and 25.6 months (10.7 - 60.9 months) in the TREO arm.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Overall the efficacy results of the first randomised Phase II alloHSCT trial (MC-FludT.16/NM) in paediatric patients with non-malignant diseases demonstrated a benefit for the treosulfan conditioning regimen over busulfan conditioning regimen in the selected subject population regarding Freedom from transplant (treatment) related mortality until day +100 after HSCT, TRM, OS, GvHD-free and cGvHD-free survival and EFS despite a higher rate of secondary graft failures and lower complete donor-type chimerism. Therefore, in children with non-malignant diseases indicated for alloHSCT the risk of graft failure must be carefully weighed against the risk of TRM, especially when an unrelated (MUD) or partly matched alloHSCT is proposed and treosulfan is part of their preparative regimen for alloHSCT.

As per the final CTR of the MCFludT.16/NM trial, the Transplant-related mortality (TRM) after treosulfan is lower than after busulfan which translates into an OS of treosulfan-treated patients at least comparable to the one of busulfan-treated patients which is reassuring.

When comparing the two treatment arms – treosulfan and busulfan – form the MC-FludT.16/NM trial were conditioning prior HSCT was tested in patients with non-malignant diseases, it is possible to observe that the rate of TEAEs, TESAEs and significant TEAEs was similar in the 2 treatment arms with only small differences on the CTCAE SOC and Term level. No unknown risks were identified in the trial and there was no occurrence of any SUSARs. Comparable incidences of aGvHD were observed between the treatment arms, however, a significantly and clinically meaningful lower incidence of overall cGvHD was found with treosulfan. More deaths occurred in the busulfan arm than the treosulfan arm. Overall, safety results favour the treatment with treosulfan in comparison with busulfan in for conditioning prior to alloHSCT in paediatric patients with non-malignant diseases.

The second Phase II trial in paediatric patients with malignant haematological disease (MC-FludT.17/M) has shown that a BSA-adapted i.v. dose of 10 g/m^2, 12 g/m^2, or 14 g/m^2 Treosulfan given on Days -6, -5, and 4 can be successfully used as conditioning treatment before alloHSCT in the selected paediatric population. However, the selected BSA-adapted dosing required further justification/clarification. The newly proposed regime (10 g/m^2 for BSA <0.4 m^2, 12 g/m^2 for 0.4$\leqslant$BSA<0.9 m^2 and 14 g/m^2 for BSA $\geqslant$0.9 m^2) resulted in an increase in the % of values >3600 mg*h/L in all groups. Also, for the BSA levels of 0.4, 0.5, 0.9, and 1.0 m2 max AUC values of 4361, 4719, 4209 and 4147 mg*h/L were simulated. The proposed final BSA-categories for dose calculation seem adequate.

Based on the engraftment and chimerism data approaching 100% and > 90%, respectively, efficacy parameters like EFS, OS, and GvHD-free and relapse-free survival confirm the effectiveness of this conditioning treatment.

The adverse events presented were already included in section 4.8 of the SmPC related with paediatric population. Among the TESAEs, upper respiratory infection and febrile neutropenia are reported, the former is not clearly included in SmPC and the latter is included with unknown frequency. No unknown risks were identified and there was no occurrence of any SUSARs. At the end of the trial where treosulfan was included in the conditioning regime for patients with malignant diseases, 20% subjects (n=14) terminated the trial prematurely, being death from relapse/progression and transplantation-related the main cause in 17.1% of the subjects (n=12). Laboratory parameters and vital signs did not change and performance scores were as expected.

Acute GvHD Grade III-IV was reported in less than 9% of the subjects and was maintained at 100 days. Also, for chronic GvHD cumulative incidence did not change up to the end of the duration of the trial's follow-up, i.e. 36 months.

Overall, safety data gathered from the MU-FludT.17/M trial is in line with the adverse reactions listed for adults and paediatric patients already included in the approved PI and can be explained by the intensive treatment prior alloHSCT of treosulfan on top of standardized fludarabine, and in the majority of the cases also with thiotepa, as well due to the underlying malignant disease (ALL, AML, MDS, and JMML) and transplantation-procedure per se. Safety are comparable to those reported for treosulfan in other trials and comparable or even favourable to those reported for BU- or TBI-based conditioning regimens.

3.7.2. Balance of benefits and risks

Overall, the reported efficacy and safety results of these two Phase II allo-HSCT trials resulted in a positive benefit judgement for the Treosulfan-based conditioning regimen used in paediatric patients with selected malignant diseases and non-malignant diseases, with an expected and manageable safety profile confirming the approved indication in paediatric patients with malignant diseases as well as in paediatric patients with selected non-malignant diseases. The efficacy and safety results obtained from the MU-FludT.16/NM also support the benefit of using treosulfan instead of a Busulfan-based condition regimen in paediatric patients with non-malignant diseases facing a comparable safety profile and thus allow to support the request for an extension of the use of Treosulfan to this population.

3.7.3. Additional considerations on the benefit-risk balance

Furthermore, summarised results of TREO-based conditioning are available from four EBMT registry analyses [Nagler 2017; Shimoni 2015/2021; Peters 2011/2017].

A conditioning regimen consisting of 14 g/m²/d × 3 TREO Day -6/-5/-4 plus FLU (FT10 14) and preferably combined with TT resulted in a very high disease-free and overall survival rate in paediatric patients with malignant as well as non-malignant disorders. The efficacy of this regimen is supported by data from the EBMT registry that included a total of 1 416 patients with malignant or non-malignant diseases. Furthermore, a large number of published trials of other study groups support the use of TREO-based conditioning in these patients. Meanwhile, two European guidelines recommend the use of TREO-based conditioning in patients with MDS as well as primary immunodeficiencies. Paediatric patients seem to tolerate higher doses of TREO compared to adults since most safety parameters were comparable to results obtained with the FT10 regimen used in adult patients.

The data strongly suggest that TREO-based conditioning is an effective and safe treatment for paediatric patients with malignant as well as non-malignant diseases.

3.8. Conclusions

The overall B/R of Trecondi is positive.

4. Recommendations

Outcome

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

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

Extension of indication to include additional non-malignant transplant indications (non-malignant diseases in the paediatric population) for Trecondi 1 g/5 g powder for solution for infusion based on final 12-months follow-up results of study MC-FludT.16/NM; a randomised phase II interventional study aimed to compare Treosulfan-based conditioning therapy with Busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation in paediatric patients with non-malignant diseases.
Further, the MAH proposes to amend an existing warning on skin toxicity based on new literature data. Moreover, the MAH proposes to introduce a slightly modified dosing regimen according to the patient's body surface based on long-term follow-up data of paediatric study MC-FludT.17/M, a Phase II 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, as well as a final analysis of the population pharmacokinetics of treosulfan in paediatric patients. As a consequence, sections 4.1, 4.2, 4.4, 4.8 and 5.1 of the SmPC are updated. The Package Leaflet is updated in accordance. Version 1.4 of the RMP has also been submitted.

The variation leads to amendments to the Summary of Product Characteristics and Package Leaflet and to the Risk Management Plan (RMP).

Amendments to the marketing authorisation

In view of the data submitted with the variation, amendments to Annexes I and IIIB and to the Risk Management Plan are recommended.

Paediatric data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0346/2020 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

5. EPAR changes

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

Scope

Please refer to the Recommendations section above.

Summary

Please refer to Scientific Discussion 'Trecondi-H-C-004751/II/0014"