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RESEARCH**

APPLICATION NUMBER:

214759Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review

Clinical Review

Non-Clinical Review

Statistical Review

Clinical Pharmacology Review

NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	NDA 214759
Priority or Standard	Standard
Submit Date(s)	04/30/2024; 08/10/2022; 04/21/2022; 08/11/2020
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PDUFA Goal Date	01/30/2025
Division/Office	Division of Hematologic Malignancies I / Office of Oncologic Diseases
Review Completion Date	01/21/2025
Established/Proper Name	Treosulfan
(Proposed) Trade Name	Grafapex
Pharmacologic Class	Alkylating agent (Small Molecule)
Code name	
Applicant	medac Gesellschaft für klinische Spezialpräparate mbH (medac)
Dosage form	Lyophilized powder for injection
Applicant proposed Dosing Regimen	10 g/m ² intravenously given daily for three days, beginning on Day -4 prior to transplantation in combination with fludarabine
Applicant Proposed Indication(s)/Population(s)	"for use in combination with fludarabine as a preparative regimen for allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult and pediatric patients > 1 year old with acute myeloid leukemia (AML)" and "...with myelodysplastic syndrome (MDS)"
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	• Use in combination with fludarabine as preparative regimen for allo-HSCT in adult and pediatric patients 1 year and older with AML • Use in combination with fludarabine as preparative regimen for allo-HSCT in adult and pediatric patients 1 year and older with MDS
Recommended SNOMED CT Indication Disease Term for each Indication (if applicable)	
Recommended Dosing Regimen	10 g/m ² by intravenous infusion given daily for three days, beginning on Day -4 prior to transplantation in combination with fludarabine

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Reviewers of Multi-Disciplinary Review and Evaluation

Regulatory Project Manager	Brad McKenzie, PharmD
Clinical Reviewer	Joseph Wynne, MD, PhD
Clinical Team Leader	Emily Jen, MD, PhD
Statistical Reviewer	Alexei Ionan, PhD
Statistical Team Leader	Jonathon Vallejo, PhD
Associate Director for Labeling (ADL)	Elizabeth Everhart, MSN, RN, ACNP
Cross-Disciplinary Team Leader	Emily Jen, MD, PhD
Division Director (OB)	Yuan Li Shen, Dr. P.H.
Division Director (DHM1)	R. Angelo de Claro, MD
Office Director (or designated signatory authority)	Marc Theoret, MD

Additional Reviewers of Application

Nonclinical Reviewer	Moran Choe, PhD
Nonclinical Team Leader	Brenda Gehrke, PhD
Division Director (DHOT)	Haleh Saber, PhD, MS
Office of Clinical Pharmacology (OCP) Reviewer	Vicky Hsu, PhD
Office of Clinical Pharmacology (OCP) Team Leader	Ankit Shah, PhD
Division Director (OCP)	Brian Booth, PhD
OPQ/DP	Olen Stephens, PhD / Shalini Anand, PhD
OPQ/OPMA	Jin Lee, PhD / James Norman, PhD
OPQ/Micro	Julie Nemecek, PhD / Bryan Riley, PhD
OPQ/RBPM	Dahlia Walters, MS, PMP, FAC-P/PM
OPDP	Samia Alam, PharmD / Jina Kwak, PharmD
OOD-Safety	Shan Pradhan (ADS); Stacie Woods (SRPM)
OSE/DEPI	Wei Liu / Steven Bird
OSE/DMEPA	Jody Kundreskas, PharmD / Nicole Iverson, PharmD
OSE/DPV	Graca Does, MD/Katie Sullivan/Afrouz Nayernama, PharmD
OSE/DRM	Till Olickal / Naomi Boston, PharmD
OSE/SRPM	Candido Alicea, PhD / Janet Higgins

OPQ=Office of Pharmaceutical Quality
 OPDP=Office of Prescription Drug Promotion
 OSI=Office of Scientific Investigations
 OSE= Office of Surveillance and Epidemiology
 DEPI= Division of Epidemiology
 DMEPA=Division of Medication Error Prevention and Analysis
 DRISK=Division of Risk Management

Glossary

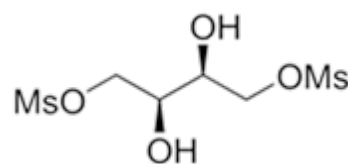
AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Conference on Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application

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NME	new molecular entity
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

1 Executive Summary

1.1. Product Introduction

Proposed Trade Name:	Grafapex	
Established Name:	Treosulfan	
Also Known As:	Treosulphan, dihydroxybusulfan, CAS # 299-75-2, Ovastat®	
Chemical Name:	1,2,3,4-butanetcol, 1,4-dimethanesulfonate; threitol 1,4-dimethanesulfonate; threitol 1,4-bismethanesulfonate; L-threitol 1,4-bis(methanesulfonate); (2S,3S)-2,3-dihydroxybutane-1,4-diyl dimethanesulfaonte; (2S,3S)-(-)-1,4,-Di(mesyloxy)-2,3-butanediol	
Molecular Formula:	C ₆ H ₁₄ O ₈ S ₂	Chemical Structure:
Molecular Weight:	278.3 g/mol	
Dosage Forms:	Injection, lyophilized (1 gm, 5 gm)	
Therapeutic Class:	Antineoplastic	
Chemical Class:	Small molecule	
Pharmacologic Class:	Alkylating agent	
Mechanism of Action:	Treosulfan is an alkylating agent. DNA alkylation is thought to be responsible for the cytotoxic activities of treosulfan. Treosulfan depleted hematopoietic stem cells and showed immuno-suppressive and antitumor activity in mouse models of leukemia.	

1.2. Conclusions on the Substantial Evidence of Effectiveness

Substantial Evidence of Effectiveness (SEE) was established with one adequate and well-controlled clinical investigation and confirmatory evidence.

MC-FludT.14/L Trial II (NCT00822393) was a multicenter, randomized, active-controlled trial comparing treosulfan to busulfan in combination with fludarabine as a preparative regimen for allogeneic HSCT. Eligible patients included adults 18 to 70 years old with AML in complete remission or MDS, Karnofsky performance status > 60%, and hematopoietic cell transplantation comorbidity index [HCT CI] score > 2 for patients < 50 years old. The patients were randomized to receive treosulfan 10 g/m² daily on days -4, -3 and -2 or to busulfan 0.8 mg/kg every 6 hours on days -4 and -3 in combination with fludarabine 30 mg/m² daily on days -6, -5, -4, -3 and -2, and hematopoietic stem cell transplantation on day 0. Antithymocyte globulin was administered as well to patients with unrelated donors.

The study was designed as a noninferiority trial with a primary endpoint of event-free survival

(EFS) with 2 years of follow-up after transplantation. Events were defined as disease relapse, graft failure, or death. The noninferiority margin on the hazard ratio scale was 1.3. If noninferiority was demonstrated, then the primary endpoint would be tested for superiority. Overall survival (OS) was a secondary endpoint, but there was no alpha allocated to testing OS.

The trial was stopped at the second interim analysis when EFS noninferiority was achieved. Since the trial continued enrolling until the results of the interim analysis were known, an additional "last analysis" was conducted using all enrolled patients (N = 570). Based on the last analysis, the Applicant concluded that the results supported noninferiority for EFS, and in sequential testing, superiority of treosulfan for EFS (based on 224 events). Superiority of treosulfan for EFS was not demonstrated at the second interim analysis, though the boundary for superiority based on the updated number of EFS events was crossed at the last analysis. However, FDA did not consider the trial to have demonstrated superiority of treosulfan for EFS at the last analysis, as this analysis was not pre-planned and had been requested by FDA.

During review of the original NDA submission, a Complete Response was issued on 30 July 2021 as the review team was unable to confirm the results of the primary endpoint analysis since the raw data needed to verify the components of the EFS endpoint were incomplete. In the subsequent resubmissions (21 April 2022, 30 April 2024), the Applicant submitted all available CBC data and some bone marrow results. In the current resubmission, the Applicant submitted the results of all bone marrow results including original reports.

For FDA's analysis of efficacy, the analysis set consisted of all randomized patients. This included 570 patients with a median age of 70 years (range 31, 70); 61% were male. Race and ethnicity data were not collected. Sixty-four percent of patients had AML. Ninety-seven percent of patients received peripheral blood stem cells.

Study MC FludT.14/L Trial II was considered a positive study based on the primary endpoint of EFS (non-inferiority p-value = 0.0000164 as compared to an efficacy boundary of 0.000149). Based on FDA-adjudication of EFS events, the hazard ratio for EFS was 0.65 (95% CI: 0.50, 0.85) in the ITT population. However, the definition of EFS used by the Applicant reflected a composite of efficacy (relapse and death) and safety (graft failure) measures. In the accepted definition of EFS for AML, graft failure is not considered an efficacy event. For patients with AML in CR, the applicable endpoint would be relapse-free survival (RFS). For patients with MDS not in CR, achievement of remission might reflect the effectiveness of treatment, but the protocol definition of EFS does not take into account failure to achieve remission and the data collection was not sufficient to reliably adjudicate achievement of CR in the MDS population. Therefore, the clinical meaningfulness of the EFS endpoint as defined in the protocol is unclear for both the AML and MDS populations. Additional analyses of EFS and related endpoints (including RFS) were conducted to explore the robustness of the result to definition and population. The results of these additional analyses consistently favored the treosulfan arm.

(b) (4)

While Study MC-FludT.14/L Trial II was not designed to test OS, this endpoint is the gold standard for establishing efficacy in oncology. Thus, FDA relied on OS, defined as the time from randomization until death from any cause, to establish the efficacy of treosulfan. The HR for OS (Cox proportional hazards model, stratified by donor type and risk group) compared to busulfan was 0.67 (95% CI: 0.51, 0.90) in the ITT population, 0.73 (95% CI: 0.51, 1.06) in patients with AML, and 0.64 (95% CI: 0.40, 1.02) in patients with MDS. Despite the limitations of the study design and available data, the results appear consistent with superiority overall. However, since there was no prespecified alpha-spending plan for OS, no p-values will be reported.

Supportive evidence for the effectiveness of treosulfan as a component of a preparative regimen was based on the clinical evidence from the dose-finding study MC-FludT.6/L which accrued a total of 55 patients, including 19 with AML and 6 with MDS. Study MC-FludT.6/L achieved its primary objective to evaluate the feasibility and tolerability of different dose levels of treosulfan prior to allogeneic HSCT. In addition, the 1-year OS outcomes for patients with hematologic malignancies who received the treosulfan-based preparative regimen at 10 g/m²/dose, 12 g/m²/dose, and 14 g/m²/dose were 70%, 78%, and 53%, respectively. Although FDA does not usually use landmark OS in a single-arm trial to support efficacy given the potential for bias, the findings in MC-FludT.6/L were reproducible in two dose cohorts (10 g/m² and 12 g/m²), the results in the 10 g/m² cohort were consistent with that of the treosulfan arm of MC-FludT.14/L Trial II (1-year OS: 79%), and the results in the 14 g/m² cohort were also consistent with the results in the treosulfan arm of MC-FludT.14/L Trial I (1-year OS: 67%). The consistency of these findings reduces concerns for bias and supports the conclusion that the 10 g/m²/dose treosulfan is effective as part of a preparative regimen.

On the basis of biology of AML and MDS and the mechanism of action of treosulfan, the efficacy of treosulfan 10 g/m²/dose can be extrapolated from the studies in adults to pediatric patients.

(b) (4)

the recommended dose for pediatric patients with AML or MDS is 10 g/m²/dose of treosulfan based on a full extrapolation of efficacy from MC-FludT.14/L Trial II and PopPK approach.

In summary, MC-FludT.14/L Trial II was a positive study based on its primary endpoint, and the overall results demonstrate the efficacy of 10 g/m²/dose treosulfan as a component of conditioning regimen prior to allogeneic HSCT. Treosulfan-based preparative regimens prior to allogeneic HSCT in patients with AML or MDS result in a survival advantage (HR 0.67 [95% CI: 0.51, 0.90]) compared to busulfan-based preparative regimens. The recommended indication for treosulfan (10 g/m²/dose) is: “for use in combination with fludarabine as a preparative regimen for allogeneic HSCT in adult and pediatric patients 1 year of age and older with AML (1.1) ... [or]... MDS (1.2).”

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

AML and MDS have high rates of morbidity and mortality, and allogeneic hematopoietic stem cell transplantation (HSCT) is required for curative therapy in patients with high-risk disease. Currently, there are no drugs approved for use as a preparative regimen prior to allogeneic HSCT for AML or MDS, and regimens are not standardized. There is a need for additional conditioning regimen options for patients with AML and MDS proceeding to allogeneic HSCT.

MC-FludT.14/L Trial II (NCT00822393) was a multicenter, randomized, active-controlled trial comparing treosulfan to busulfan in combination with fludarabine as a preparative regimen for allogeneic HSCT. Eligible patients included adults 18 to 70 years old with AML in complete remission or MDS, Karnofsky performance status > 60%, and hematopoietic cell transplantation comorbidity index [HCT CI] score > 2 for patients < 50 years of age. Patients were randomized to receive treosulfan 10 g/m² daily on days -4, -3 and -2 or to busulfan 0.8 mg/kg every 6 hours on days -4 and -3 in combination with fludarabine 30 mg/m² daily on days -6, -5, -4, -3 and -2, and hematopoietic stem cell transplantation on day 0. Antithymocyte globulin was administered as well to patients with unrelated donors.

The study was designed as a noninferiority trial with a primary endpoint of event-free survival (EFS) with 2 years of follow-up after transplantation. Events were defined as disease relapse, graft failure, or death. The noninferiority margin on the hazard ratio scale was 1.3. If noninferiority was demonstrated, then the primary endpoint would be tested for superiority. Overall survival (OS) was a secondary endpoint, but there was no alpha allocated to testing OS.

Study MC FludT.14/L Trial II was considered a positive study based on the primary endpoint of EFS (non-inferiority p-value = 0.0000164 as compared to an efficacy boundary of 0.000149). Based on FDA-adjudication, EFS showed a hazard ratio (HR) of 0.65 (95% CI: 0.50, 0.85) in the ITT population. However, EFS as defined by the Applicant reflects a composite of efficacy (relapse and death) and safety (graft failure) measures, and the clinical meaningfulness of this definition is unclear for both the AML and MDS populations. Additional analyses of EFS and related endpoints (including RFS) were conducted to explore the robustness of the result to definition and population. The results of these additional analyses consistently favored the treosulfan arm which supported the conclusion of a positive trial, (b) (4)

Although Study MC-FludT.14/L Trial II was not designed to test OS, this endpoint is the gold standard for establishing efficacy in oncology. Therefore, FDA relied on OS, defined as the time from randomization until death from any cause, to establish the efficacy of treosulfan. The HR for OS (Cox proportional hazards model, stratified by donor type and risk group) compared to busulfan was 0.67 (95% CI: 0.51, 0.90) in the ITT

population, 0.73 (95% CI: 0.51, 1.06) in patients with AML, and 0.64 (95% CI: 0.40, 1.02) in patients with MDS. Despite the limitations of the study design and available data, the results appear consistent with superiority overall. However, since there was no prespecified alpha-spending plan for OS, no p-values will be reported.

Adverse events with treosulfan were similar to those observed with busulfan-based conditioning. Common TEAEs ($\geq 20\%$) included musculoskeletal pain, stomatitis, pyrexia, nausea, edema, infection, and vomiting. Of those, musculoskeletal pain (12% higher), edema (11% higher), and infection (5% higher) occurred at a rate at least 5% higher in the treosulfan arm than the busulfan arm. The most common grade 3-5 AEs ($\geq 5\%$) in the treosulfan arm were febrile neutropenia, infection, hypertension, stomatitis, and musculoskeletal pain. Of those, infection occurred at a rate of $>5\%$ compared to the busulfan arm (6% higher in the treosulfan arm).

MC-FludT.14/L Trial I which randomized patients to treosulfan-based conditioning using $14 \text{ g/m}^2/\text{dose}$ vs busulfan-based conditioning was terminated early due to delayed hematopoietic recovery and increase early treatment-related mortality (10.2% vs 5.3% in the treosulfan and busulfan arms, respectively). FDA's analysis of the final data cut (1-year follow-up) showed no evidence of a treatment effect for this higher treosulfan dose compared to the busulfan-based regimen for the overall population (OS HR 1.15 [95% CI: 0.78, 1.69]) or when assessed individually for patients with AML or with MDS. The results of this study also confirmed that higher doses of treosulfan result in increased early morbidity and mortality in addition to higher transplant-related and non-relapse mortality. These findings warrant inclusion of a Warning and Precaution in the USPI since the 14 g/m^2 dose is published in the literature and approved for the pediatric population in some countries.

Based on the biology of AML and MDS and the mechanism of action of treosulfan, the efficacy of treosulfan $10 \text{ g/m}^2/\text{dose}$ can be extrapolated from the studies in adults to pediatric patients, and safety data for treosulfan in combination with fludarabine are available for patients ages 1 year and older. (b) (4)

the recommended dose for pediatric patients with AML or MDS is $10 \text{ g/m}^2/\text{dose}$ of treosulfan based on a full extrapolation of efficacy from MC-FludT.14/L Trial II and Population PK approach.

Given the observed OS benefit and with risk mitigation strategies in place in labeling, the clinical benefit of $10 \text{ g/m}^2/\text{dose}$ treosulfan in combination with fludarabine as a preparative regimen for allogeneic HSCT appears to outweigh the risks in adult and pediatric patients 1 year of age or older with AML or MDS.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	- Acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (MDS) have high rates of morbidity and mortality. - Allogeneic HSCT is required for curative therapy of high-risk AML and IPSS-R intermediate, high, and very high risk MDS.	AML and MDS are serious and life-threatening diseases.
Current Treatment Options	- There are no drugs approved for use as a preparative regimen prior to allogeneic HSCT for AML or MDS. - There is no consensus regarding choice of preparative regimen and there are no standardized guidelines for which patients should receive reduced-intensity or myeloablative regimens.	There is a need for additional conditioning regimen options for patients with AML and MDS proceeding to allogeneic HSCT.
Benefit	- MC-FludT.14/L Trial II (NCT00822393) was a multicenter, randomized, active-controlled trial comparing treosulfan 10 g/m²/dose to busulfan in combination with fludarabine as a preparative regimen for allogeneic HSCT in patients with AML in CR or with MDS. A total of 570 patients were randomized. - The study was positive based on its primary endpoint of EFS: HR 0.65 (95% CI: 0.50, 0.85). However, the applicability of this endpoint as defined in the protocol to the AML and MDS subpopulations was unclear. - Efficacy was established based on OS: HR 0.67 (95% CI: 0.51, 0.90) in the ITT population. Results for patients with AML and MDS were consistent with those for the overall study: OS HR 0.73 (95% CI: 0.51, 1.06) in patients with AML and 0.64 (95% CI: 0.40, 1.02) in patients with MDS. - Efficacy of 10 g/m²/dose of treosulfan in pediatric patients with AML or MDS is extrapolated based on the adult study and supported by safety data in pediatric patients aged 1 year and older.	Treosulfan 10 g/m ² /dose in combination with fludarabine is an effective preparative regimen prior to allogeneic HSCT for patients ≥ 1 year old with AML or MDS.

NDA/BLA Multi-disciplinary Review and Evaluation [NDA 214759]
 Grafapex (treosulfan)

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	• Fatal adverse reactions occurred in 1.5% of patients who received treosulfan. • The most common ($\geq 20\%$) adverse reactions were musculoskeletal pain, stomatitis, pyrexia, nausea, edema, infection, and vomiting. • Grade 3 or 4 nonhematological laboratory abnormalities included increased GGT, increased bilirubin, increased ALT, increased AST, and increased creatinine. • MC-FludT.14/L Trial I demonstrated substantial evidence of increased morbidity and mortality with 14 g/m²/day of treosulfan.	The safety profile of treosulfan 10 g/m²/dose appears similar to that of busulfan, but significant safety issues with the 14 g/m²/dose regimen including higher treatment-related mortality warrants inclusion of a Warning and Precaution in the USPI since this dose is published in the literature and approved for use in the pediatric population in some countries.

1.4. **Patient Experience Data**

Patient Experience Data Relevant to this Application (check all that apply)

☒	Patient experience data was not submitted as part of this application.
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X

Emily Jen, MD, PhD
Cross Discipline Team Leader

2 Therapeutic Context

2.1. Analysis of Condition

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for analysis of condition.

2.2. Analysis of Current Treatment Options

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for analysis of current treatment options.

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Treosulfan is not currently marketed in the United States and has no history of being marketed in the United States.

3.2. Summary of Presubmission/Submission Regulatory Activity

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for summary of presubmission/submission regulatory activity prior to July 2021.

- NDA 214759 received a Complete Response Letter on 7/30/2021- FDA noted that the Applicant had not provided substantial evidence of effectiveness of treosulfan as part of a preparative regimen for allogeneic hematopoietic stem cell transplant. FDA was unable to confirm the primary endpoint of EFS because all complete blood cell (CBC) and bone marrow results were not submitted to support adjudication of the endpoint. Additionally, because of the missing CBC and marrow results, FDA was not able to complete an assessment of whether the results of the study would support the indications for treatment of patients with AML and for treatment of patients with MDS. To address this issue, FDA asked the Applicant to submit the CBC and marrow results through the complete assessment period for EFS for MC-FLudT.14/L Trial II. FDA also noted that (b) (4) OS cannot be used as the sole basis for approval since the statistical analysis plan for Study MC-FLudT-14/L trial II neither include Type 1 error for OS nor alpha allocation for multiple OS analyses. Since no alpha was reserved for independent OS analysis and the EFS endpoint was unable to be verified due to lack of CBC and marrow data noted above, FDA concluded that without verification of the EFS analysis, any OS analysis could only be considered exploratory. To independently address the issues with OS, FDA recommended submitting data from an adequate and well-controlled trial of treosulfan vs busulfan with a prespecified analysis of OS for each indication with control of Type 1 error.
- Type A Meeting 11/23/2021- FDA and the Applicant discussed the Applicant's plan to evaluate and present additional bone marrow and CBC results gathered through the complete assessment period for EFS via the MC-FludT.14/L Trial II. FDA again asked for clarification for how missing assessments were handled. FDA also noted that there is no acceptable definition for EFS in MDS and that RFS more accurately reflects the AML patient's clinical circumstances. Discussion was also had on clinical studies to address clinical pharmacology issues include QTc assessments.
- Response to CR submitted 4/21/2022- Applicant submitted updated laboratory and chimerism data with sensitivity analyses aimed at addressing missing assessment data

through various censoring rules for EFS in response to CR comments discussed at the Type A meeting of 11/23/21.

- Acknowledgement Incomplete response letter sent 5/16/2022- Letter noted that the Applicant had not yet provided sufficient evidence of effectiveness of treosulfan as a part of a preparative regimen for allogeneic hematopoietic stem cell transplantation for adult and pediatric patients with AML or MDS. FDA asked the Applicant to update all files dependent on the newly collected laboratory data include the adtte.xpt files. However, FDA also noted continued missing data with respect to bone marrow chimerism results and assay validation.
- Second Response to CR submitted 8/10/2022- Applicant submitted updated lab files with updated dependencies as noted in the Acknowledgement Incomplete response letter sent 5/16/2022 or submitted additional files with new names to preserve the files from the initial submission. The Applicant also submitted data detailing site specific chimerism assays. Lastly, the Applicant provided an updated adtte.xpt file with the new sensitivity analyses.
- Second Acknowledgement Incomplete Response Letter sent 9/15/2022- FDA noted that detailed review of the newly submitted data continued to identify inconsistencies and a substantial extent of missing assessments. Specifically, FDA noted that blast values presented in updated lab data sets indicated that MRD assays rather than a morphological assessment of marrow blasts were erroneously being reported. Additionally, FDA identified 179 missing day +28 or Day +100 protocol required disease assessments. Therefore, FDA concluded that the Applicant had yet to provide substantial evidence of effectiveness.
- Type A meeting 12/14/2022- FDA provided additional feedback on the Applicant's proposed approach to address the deficiencies noted in FDA's Acknowledge Incomplete Response Letter issued on September 15, 2022, and all other issues noted in the CRL and in the Information Request dated August 31, 2022. FDA noted that based on the issues identified during reviews of your submissions dated April 21, and August 10, 2022, we have no confidence in the marrow data submitted in the datasets, and source documentation for marrow examinations from only 50 patients would not be sufficient to allay FDA's concerns about data integrity. Therefore, the Applicant would need to submit the source documentation for all marrow examinations that were performed through the complete assessment period for EFS for all subjects in MCFLudT.14/L Trial II.
- Third Response to CR submitted 4/30/2024- In response to the issues raised by the Agency, the Applicant performed a systematic review of all source documentation of bone marrow evaluations and provided the source documentation in original language and with English translations for all subjects from Study MC-FLudT.14/L Trial II. The results of this effort, along with specific responses from the Applicant to each of the issues raised in the Acknowledgment Incomplete Response Letter from September 15, 2022, were provided in the current submission.

NDA/BLA Multi-disciplinary Review and Evaluation [NDA 214759]
Grafapex (treosulfan)

- Major Amendment 9/12/2024- Applicant informed that additional data submission and required analysis considered a Major Amendment to the application and the PDUFA goal date has been updated to January 30, 2025

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

Excerpted from FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021):

“Two clinical sites ... were inspected for Study MC-FludT.14/L.” “Based on the inspections, OSI concluded that the conduct at the study sites was adequate and study data derived from clinical sites appeared reliable.”

4.2. Product Quality

Excerpted from FDA Integrated Quality Review of NDA 214759 (29 October 2024):

“The Applicant provided adequate information on the proposed drug product to ensure the identity, strength, purity, and quality of the proposed drug product. The overall manufacturing inspection recommendation is approval for all the facilities associated with this application. The proposed labeling and labels include adequate information to meet the regulatory requirements.”

4.3. Clinical Microbiology

See Section 4.2.

4.4. Devices and Companion Diagnostic Issues

There were no companion diagnostics or other devices in this submission.

5 Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

Excerpted from FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021):

“From a pharmacology/ toxicology perspective, there are no outstanding issues that would prevent the approval of treosulfan in patients undergoing allogeneic hematopoietic stem cell transplantation (b) (4) with AML or MDS; however, there is one nonclinical post-marketing requirement (PMR).”

“During the review of the NDA, it was determined that the nonclinical data for treosulfan submitted to the NDA in studies or literature for which the Applicant has the right to reference did not provide convincing evidence of DNA alkylation and breaks as the mechanism of action (MOA) of treosulfan. The PMR is to conduct an in vitro study to show treosulfan binds to DNA and causes DNA breakage. As the recommendation has been a complete response (see Section 1.2), the pharmacology/toxicology discipline recommends the Applicant conduct and submit the results of the study at the time of resubmission.”

Excerpted from FDA Pharmacology/Toxicology Review of NDA 214759 (11 October 2024):

“To address this issue, the Applicant conducted genetic toxicology studies, and submitted the results to demonstrate the genotoxicity of treosulfan. Genetic toxicology studies included an in vitro bacterial reverse mutation (Ames) assay and an in vitro chromosomal aberration assay.” “Overall, the study results showed that treosulfan is a genotoxic compound.”

See reviews referenced above for additional details including review conclusions for Sections 5.2 to 5.5.

5.2. Referenced NDAs, BLAs, DMFs

5.3. Pharmacology

5.4. ADME/PK

5.5. Toxicology

- 5.5.1. **General Toxicology**
- 5.5.2. **Genetic Toxicology**
- 5.5.3. **Carcinogenicity**
- 5.5.4. **Reproductive and Developmental Toxicology**
- 5.5.5. **Other Toxicology Studies**

6 Clinical Pharmacology

6.1. Executive Summary

Excerpted from NDA 214759 Clinical Pharmacology Re-submission Memo (31 October 2024):

” The Office of Clinical Pharmacology did not recommend approval then because of issues regarding the veracity of the primary study endpoint. A summary of key review issues and recommendations from the original clinical pharmacology review of NDA 214759 is provided below:

REVIEW ISSUE	RECOMMENDATIONS / COMMENTS
General dosing instructions	The recommended dosage regimen of treosulfan is 10 g/m ² /day for 3 consecutive days (Days -4, -3, and -2) in combination with fludarabine 30 mg/m ² /day for 5 consecutive days (Days -6 through -2) prior to allogeneic hematopoietic stem cell transplantation (Day 0).
Dosing in patient subgroups (intrinsic and extrinsic factors)	<i>Pediatric Patients ≥1 year of age:</i> The recommended dosage regimen of treosulfan is the same for all patients ≥1 year of age. Efficacy in pediatric patients ≥1 year of age was extrapolated from adults and dose selection was supported by PK and safety data from Studies MC-FludT.16/NM and MC-FludT.17/M. Treosulfan exposure after administration of 10 g/m² in pediatric patients with BSA <0.7 m² and with BSA 0.7 to <1.1 m² are 11% and 5% higher, respectively, compared to adults. Exposure-response analyses for safety did not indicate that an 11% increase in exposures would result in significant increase in the probability of common toxicities. <i>Renal Impairment:</i> No dosage modification is recommended for patients with mild renal impairment. An additional study will be necessary to identify a safe dose of treosulfan for patients with moderate hepatic impairment. A study in patients with severe renal impairment is not feasible and will not be required. <i>Hepatic Impairment:</i> No dosage modification is recommended for patients with mild hepatic impairment. Studies in patients with moderate or severe hepatic impairment are not needed, as these patients are typically excluded from transplantation.

“The only new data submitted in this re-submission were in vitro metabolism study results which determined that treosulfan is metabolized by CYP2D6 and its monoepoxide intermediate is metabolized by CYP2C8. The labeling has been updated to reflect this information. In vitro metabolism studies for other metabolites are currently ongoing.

This NDA is approvable from a clinical pharmacology perspective.”

Excerpted from Addendum to Clinical Pharmacology Re-submission Memo (27 November 2024):

“The previous review indicated the need for the following PMR:
Conduct an in vitro study to evaluate treosulfan and epoxide metabolites as substrates of major cytochrome P450 enzymes consistent with FDA guidance.

The in vitro assessment of treosulfan metabolism showed involvement of CYP2D6 ($t_{1/2}$ with $rCYP2D6 = 108$ min) and was noted in the previous review. Based on an updated communication with the Applicant ..., we have determined that the above PMR is no longer needed.”

“Based on a review of the submitted reports of the in vitro studies, the monoepoxide ... and diepoxide metabolites ... are not meaningfully metabolized by CYP enzymes. Of note, the $t_{1/2}$ values for positive control compounds for the respective rCYPs were very short. No labeling update is needed based on these results.”

See reviews referenced for additional details including review conclusions for Section 6.2 and 6.3.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

6.2.2. General Dosing and Therapeutic Individualization

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

6.3.2. Clinical Pharmacology Questions

7 Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 1. Listing of Clinical Trials Relevant to this NDA

Trial Identity	Trial Design Regimen/ schedule/route	Primary Endpoint(s)	Study Population	Countries
Pivotal Trial				
MC-FLudT-14/L Trial II	Phase III RCT Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 10 g/m ² , days -4, -3, -2. or <u>Busulfan</u> : 0.8 mg/kg/d, days -4, -3 <i>MUD only</i> : ATG Days -4 through -2	EFS	N = 570 Patients with AML in CR or MDS; ages 18-70 years, either aged ≥ 50 or with HCT-CI score > 2, MRD or MUD donor	US: 0 Non-US: 5
Additional Studies Addressing Efficacy				
MC-FludT.14/L Trial I	Phase III RCT Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 14 g/m ² , days -6, -5, -4. or <u>Busulfan</u> : 0.8 mg/kg/d, days -4, -3 <i>MUD only</i> : ATG Days -4 through -2	EFS	N = 320 Patients with AML in CR or MDS; ages 18-70 years, either aged ≥ 50 or with HCT-CI score > 2, MRD or MUD donor	US: 0 Non-US: 6
EBMT 2019	Registry Study <u>Treosulfan</u> : MC-FLudT.14/L Trial II <u>EBMT</u> : Flu/Mel, Bu/Cy	OS	N = 1745 AML in CR or MDS, aged 50-70 years	
CIBMTR 2019	Registry Study <u>Treosulfan</u> : MC-FludT.14/L Trial II <u>RIC</u> : Flu/Bu2 or Flu/Bu2 + ATG <u>MAC</u> : Flu/Bu4, Flu/Bu4 + ATG, or Cy/Bu4	OS	N = 1399 AML in CR or MDS, Age ≥ 50 years or HCT-CI score ≥ 2	
MC-FludT.6/L	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 10, 12, 14 g/m ² , days -6, -5, -4. <i>MUD only</i> : ATG Days -4 through -2	Feasibility and tolerability Additional endpoints: Hematopoietic recovery, OS	N = 56 Hematological malignancy, ≥18 years, MUD/MRD/misMRD donor	US: 0 Non-US: 4
Studies to Support Safety				
MC-FludT.7/AML	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 14 g/m ² , days -6, -5, -4. <i>MUD only</i> : ATG Days -4 through -2	Engraftment AESIs	N = 75 AML in CR, ages 18-60 years, MUD/MRD allo HSCT, Karnofsky ≥80%	US: 0 Non-US: 4

Table 1. Listing of Clinical Trials Relevant to this NDA

Trial Identity	Trial Design Regimen/ schedule/route	Primary Endpoint(s)	Study Population	Countries
MC- FludT.8/MDS	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 10, 12, 14 g/m ² , days -6, -5, -4. <i>MUD only</i> : ATG Days -4 through -2	Engraftment AESIs	MDS, ages 18-65, MUD/MRD allo HSCT, Karnofsky ≥80%	US: 0 Non-US: 4
MC- FludT.16/N M	RCT Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -7 to -3. and <u>Treosulfan</u> : 10, 12, or 14 g/m ² , days -6, -5, -4. or <u>Busulfan</u> : 0.8 mg/kg/d, days -7 to-4 with/without Thiotepa 5 mg/kg x 2 on day -2	Freedom from TRM	Non-malignant disease for first myeloablative allogeneic HSCT; ages 28 days to 18 years.	US: 0 Non-US: 4
MC- FludT.17/M	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -7 to -3. and <u>Treosulfan</u> : 10, 12, or 14 g/m ² , days -6, -5, -4. or <u>Busulfan</u> : 0.8 mg/kg/d, days -7 to-4 with/without Thiotepa 5 mg/kg x 2 on day -2	Freedom from TRM	28 days to 18 years, ALL, AML, MDS, JMML, requiring myeloablative alloHSCT, Karnofsky ≥70% Exclusion: - Fanconi anemia or other DNA breakage disorders	US: 0 Non-US: 30 study sites
Peters, 2011	Meta-Analysis <u>Treosulfan</u> : 12-75 mg/m ² total dosing	aGvHD, cGvHD, AEs	Nonmalignant or malignant diseases, <18 years	US: 0 Non-US: 16
Peters, 2017	Retrospective study <u>Treo/Flu</u> (Treo at 10, 12, or 14 mg/m ² /d x 3) +/- <u>Thiotepa</u> <u>Bu/Flu +/- Thiotepa</u>	OS, TRM	Pediatric patients with nonmalignant diseases	US: 0 Non-US: 37

Source: FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July

Abbreviations: RCT: Randomized controlled trial; AML: acute myeloid leukemia; CR: complete remission; MDS: myelodysplastic syndrome; MUD: matched unrelated donor; MRD: matched related donor; HCT-CI: hematopoietic cell transplant-specific comorbidity index; ATG: anti-thymocyte globulin; EFS: event-free survival; US: United State; non-US: non-United States countries; OS: overall survival; TRM: transplant-related mortality; aGVHD: acute graft versus host disease; cGVHD: chronic graft versus host disease; AE: adverse event; AESI: adverse event of special interest; Treo: treosulfan; Bu: busulfan; Flu: fludarabine; Cy: cyclophosphamide; misMRD: mismatched related donor; alloHSCT: allogeneic hematopoietic stem cell transplant.

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Section 8.1.1 and Section 8.1.2 of this review focus on review of the pivotal trial, Study MC-FludT.14/L Trial II. Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for details of additional studies in this table evaluated previously.

7.2. Review Strategy

The key materials used for the review included NDA datasets, clinical study reports, case report forms, responses to information requests, and relevant published literature.

Data sources included applicant study reports, protocols and amendments, statistical analysis plan and amendments, datasets, and literature references.

Relevant electronic datasets and information request responses:

- [\\CDSESUB1\evsprod\NDA214759](#), SDNs 0001, 0017, and 0029 Original NDA submission data sets
- [\\CDSESUB1\evsprod\NDA214759\0070](#) Resubmission, contains the response to the issues raised in Complete Response.
- [\\CDSESUB1\evsprod\NDA214759\0072](#) Information request response with analyses and datasets: overall survival analysis, subgroup analysis, time on treatment analysis.
- [\\CDSESUB1\evsprod\NDA214759\0075](#) Information request response with updated analyses and datasets based on the FDA adjudication of outcomes.
- [\\CDSESUB1\evsprod\NDA214759\0079](#) Information request response with analyses results: main efficacy endpoints of interest.

The applicant submitted information from 7 clinical trials of treosulfan, in combination with fludarabine, as preparative conditioning regimen for allogeneic HSCT. The pivotal trial MC-FludT.14/L Trial II was relevant to determining efficacy in the intended population. In addition, study MC-FludT.14/L Trial I and Studies MC-FludT.6/L were used to support safety and efficacy.

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for discussion of the review strategy for safety and information regarding the additional studies evaluated previously.

8 Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Study MC-FludT.14/L Trial II

Trial Design

Study MC-FludT.14/L Trial II was a phase III open-label, randomized clinical trial to compare treosulfan-based conditioning therapy with busulfan-based reduced-intensity conditioning (RIC) prior to allogeneic haematopoietic stem cell transplantation in patients with AML or MDS considered ineligible for myeloablative conditioning regimens. The first patient was enrolled on 13-Jun-2014 and the last patient completed 25-Jan-2018.

Per statistical analysis plan, "MC-FludT.14/L was designed as a multicentre, multinational, randomised, active-controlled, group-sequential phase III trial to show non-inferiority of Treosulfan-based conditioning therapy compared to Busulfan-based reduced-intensity conditioning (RIC) prior to allogeneic haematopoietic stem cell transplantation in patients with Acute Myeloid Leukaemia (AML) or Myelodysplastic syndromes (MDS) considered ineligible to standard conditioning regimens."

The study was designed as a non-inferiority trial with a primary endpoint of event-free survival (EFS) within 2 years. The study population consisted of adult patients with AML or MDS who were not eligible for myeloablative conditioning regimens. Patients were randomized to receive either reduced-intensity conditioning with busulfan and fludarabine, or the treosulfan and fludarabine.

Treatment Plan Overview

Patients were randomized to receive either RIC with the control (reference) arm of busulfan + fludarabine, or the test arm of treosulfan + fludarabine (Table 2).

Table 2: Treatment Overview of MC-FludT.14/L Trial II

Treatment	Day -6	-5	-4	-3	-2	-1	0	+1	+3	+6
Treosulfan (IV, 10 g/m ² within 120 minutes) – test arm			X	X	X					
Busulfan (IV, 0.8 mg/kg within 120 minutes) - control			X	X						
Fludarabine (IV, 30 mg/m ² within 30 minutes)	X	X	X	X	X					
ATG-S-Fresenius / Grafalon (IV, 10 mg/kg in MUD) – Germany, Hungary, Italy, Poland			X	X	X					
ATG-Thymoglobulin (IV, 2.5 mg/kg in MUD) – France					X	X				
Allogeneic stem cell transplantation							X			
Cyclosporine-A (IV, PO, dose level adjusted)						X	X	X...	X...	X...
Methotrexate (IV, mg/m ² /day)								15	10	10
Source Data: Clinical Protocol, adapted from Appendix 16-16.1.1										

All patients received fludarabine (30 mg/m²/d) on days -6 to -2. Patients on the control arm received busulfan (0.8 mg/kg) on days -4 and -3. Patients receiving busulfan received anti-seizure prophylaxis. Patients on the treatment arm received treosulfan (10 mg/m²/d) on days -4 to -2. Additionally, patients who received a MUD received ATG. The type of ATG and dosing varied by country.

GVHD prophylaxis was identical in both arms. All patients received level-adapted cyclosporine from Day -1 through Day +100 and methotrexate on days +1, +3, and +6, with folic acid rescue.

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for further details regarding the study design.

Study Endpoints

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for the full list of endpoints and results. This review focuses on the assessment of the event-free survival (primary endpoint) and the overall survival.

Selected Protocol-Specified Endpoints

Primary Endpoint: Event-Free Survival (within 24 months post-transplantation)

Per the statistical analysis plan, the primary efficacy endpoint was “event-free survival (EFS) within 2 years after transplantation measured from time of end of HSCT (= day 0) to time of event. The associated time span is defined as the interval from day 0 to relapse, graft failure or death (whatever occurs first). The date of the event is either the documented date of relapse/progression or the date of death or the date of primary (date of examination of neutrophilic granulocytes) or secondary graft failure (date of examination of neutrophilic granulocytes).”

Statistical Reviewer’s Comment

The following key issues were identified with event-free survival, as specified in the statistical analysis plan:

1. **Day Zero:** Transplantation (HSCT). The period of regulatory interest starts from randomization.
2. **Timeframe:** Within 24 months. The period of regulatory interest is not restricted to 24 months.
3. **Events:** Sufficient data to confirm the outcomes were missing in the initial submissions. FDA adjudicated the events with the additional submitted data. See the Additional Endpoints per FDA Assessment section below for further details.
4. **Variables used in the Cox-proportional hazards model:** risk group, donor type, center. Including center is not appropriate (see the Statistical Issues section).

5. **Interpretation:** *Interpretation of the treatment effect based on event-free survival is challenging. The Applicant utilized a definition for EFS in this trial that was not standard and had not been discussed with FDA prior to the study. For AML studies, FDA has accepted an EFS endpoint that represent the time from randomization until the time of an event. Events are considered induction treatment failure, relapse for those who have induction treatment success, or death. The eligibility criteria for this study required patients with AML to be in remission prior to transplant. Therefore, for the AML patients a Relapse-Free Survival (RFS) endpoint would have more accurately reflected the events within this clinical context. This analysis was requested as discussed below. Additional issues with the Applicant's AML EFS event definition were the inclusion of primary or secondary graft failure as events and the inclusion of cytogenetic and molecular relapse events without evidence of morphological relapse.*

EFS is not an established clinical benefit endpoint for new drugs for MDS and has not been used for regulatory purposes in MDS. As with the AML patients, the Applicant's MDS EFS event definition included the use of primary or secondary graft failure as events, which is a nonstandard EFS event. Moreover, the MDS EFS definition did not include treatment failure as an event even though most MDS patients were not in remission (and were not required to be) at that time of transplant. Additionally, patients were not formally assessed for response after transplant with post-transplant assessments focusing on engraftment instead. Therefore, the interpretation of relapse events for the MDS patient was unclear since the patients were not formally assessed for remission.

Issues 1-4 were addressed by FDA by defining a different endpoint: event-free survival (from randomization). See the Additional Endpoints per FDA Assessment section below for further details.

Overall Survival (within 24 months post-transplantation)

Per statistical analysis plan, overall survival was defined as "the time length between day 0 and the date of death due to any cause within the 24-month study period (i.e. excluding post-surveillance evaluation)."

Day Zero: Transplantation (HSCT)

Timeframe: within 24 months

Statistical Reviewer's Comment

The following key issues were identified with the overall survival, as specified in the statistical analysis plan:

1. **Day Zero:** *Transplantation (HSCT). The period of regulatory interest starts from randomization.*

2. **Timeframe:** *Within 24 months. The period of regulatory interest is not restricted to 24 months.*

These issues were addressed by FDA by defining a different endpoint: overall survival (post-randomization). See the Additional Endpoints per FDA Assessment section below for further details.

Additional Endpoints per FDA Assessment

This section lists additional endpoints used for regulatory consideration, as defined and adjudicated by FDA.

Event-Free Survival (post-transplantation)

We refer to the EFS (post-transplantation) herein as the endpoint with the same definition as the primary endpoint with several distinctions:

Day Zero: Transplantation

Timeframe: Unrestricted

Events: Adjudicated by the FDA

Event-Free Survival (post-randomization)

We refer to the EFS (post-randomization) herein as the endpoint with the same definition as the primary endpoint with several distinctions:

Day Zero: Randomization

Timeframe: Unrestricted

Events: Adjudicated by the FDA

This was considered as the primary endpoint for review purposes. The FDA EFS adjudication process is summarized below.

- All EFS events were adjudicated as defined in the protocol including use of the protocol definitions of relapse for AML, “relapse” of MDS (including relapse and progression), and graft failure (including primary and secondary GF).
- Patients without a prior event were censored at last follow up.
- The timing of events from PARAMCD=EFSSBITT in the updated adtte.xpt data file was compared to the source documentation.
- Relapse was confirmed based on the laboratory, bone marrow, and source documentation data resulting in updates to either the time of relapse or the occurrence of relapse for some patients.
- Review of the updated bone marrow data identified subjects whose baseline bone marrow results indicated bone marrow blast counts above the inclusion criterion for either AML or MDS. These patients were censored at randomization.

Relapse-Free Survival (AML)

RFS was not an original trial endpoint. However, RFS is an acceptable regulatory endpoint in studies for treatments of patients with AML in remission. FDA defines RFS, for the purposes of this review, as the time from randomization to the date of morphologic relapse or death from any cause, whichever occurs first. In the study population with AML:

- FDA adjudicated baseline remission status based on laboratory and bone marrow results as described above for EFS.
- The start date was revised to be date of randomization rather than end of HSCT.
- Censoring was applied at the time of events not included in FDA's definition of RFS (i.e., graft failure, cytogenetic relapse, molecular relapse) due to lack of consistent follow-up and documentation of morphologic relapse after these events had been called.

Overall Survival (post-randomization)

For the regulatory consideration purposes, the overall survival is defined as the time from randomization until death due to any cause (not limited by a 24-month period). We refer to this FDA-defined endpoint as overall survival (post-randomization) herein.

Day Zero: Randomization

Timeframe: Unrestricted

This is the main endpoint of interest from the regulatory perspective. No alpha was allocated, and no testing boundary was set for overall survival hypothesis testing. Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for further details.

Analysis Populations

The protocol-specified analysis population for the primary analysis of EFS was the full analysis set (FAS), defined as all randomized patients who were treated at least one time with the study medication and with at least one documented efficacy parameter. Herein we will refer to all randomized patients as the intention-to-treat (ITT) analysis set.

Statistical Analysis Plan

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021). Per protocol, the primary analysis of EFS utilized a Cox model "(stratified by centre) with donor type (MUD vs. MRD), prognostic risk group (I vs. II) and treatment as factors". The primary analysis was intended to test non-inferiority based on EFS, with a non-inferiority margin of HR=1.3. Study MC-FLudT1.4 Trial II was designed with three interim analyses for efficacy and futility, planned to occur at 9.4%, 28.5%, and 49.7% information fraction (described in further detail in Section 8.1.2. The associated boundaries were based on the primary analysis of EFS, such that the trial would be stopped if efficacy or futility was met for the NI test of EFS.

If the test of non-inferiority for EFS was found to be significant, a test of superiority would be conducted. Per protocol, “In case of statistical significance, superiority with respect to the primary study endpoint will be tested based on the points to consider on switching between superiority and noninferiority (CPMP/EWP/482/99).” This corresponds to a test of superiority at the alpha level utilized for the NI test.

As noted above, the statistical analysis plan included center as one of the stratification factors. For the review purposes, this factor was excluded from the models used to assess additional endpoints per FDA assessment due to the excessive number of levels (See the Statistical Issues section for details).

Statistical Reviewer’s Comment: *The 30 July 2021 FDA Multidisciplinary Review noted issues with the report submitted to justify the NI margin (inconsistency of EFS definition across studies, inconsistency of trial populations, lack of identification of a trial with a population exchangeable with Study MC-FludT1.4 Trial II). Furthermore, issues concerning the relevance of EFS in the enrolled population are detailed above.*

(b) (4)

Protocol Amendments

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

8.1.2. Study Results

Preamble:

The following is a brief summary of the Study MC-FludT.14/L (Trial II) background, as described in the Clinical Study Report (v1.0, 5 March 2020):

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

When reviewing the results of the second interim analysis, the DMC recommended to stop recruitment for this trial since the primary objective – the proof of non-inferiority of treosulfan compared to busulfan – had already been achieved. The previous CTR (V 1.0 dated 15-Sep-2017) described the results from the 476 patients available at that time and constitutes the confirmatory analysis of this trial. This updated report describes the results of the last analysis

of all 570 patients enrolled in the trial including post surveillance data. Of these, 551 patients qualified for the FAS. In total, 234 events were observed representing an information fraction of 48.6% based on the 481 events planned for in the final analysis foreseen in the Clinical Trial Protocol if the trial had not been stopped prematurely.”

FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) identified the following issues with the submitted data, among others, which resulted in a Complete Response:

- “The primary endpoint of Study MC-FludT-14/L Trial II was event-free survival (EFS). Because all complete blood cell (CBC) and marrow results were not submitted, we were unable to independently confirm the results of the analysis of the primary endpoint of EFS for Study MC-FludT-14/L Trial II.
- Additionally, because of the missing CBC and marrow results, we were not able complete our assessment of whether the results of the study would support the indications for treatment of patients with AML and for treatment of patients with MDS.”

This review focuses on the evaluation of efficacy in Study MC-FludT-14/L Trial II. The evaluation of efficacy relied on the additional data submitted with the application to address the deficiencies identified in the Complete Response. The FDA adjudication of the submitted data is summarized in Section 8: Statistical and Clinical and Evaluation.

Study MC-FludT-14/L Trial II was stopped at the second interim analysis with 168 EFS events observed in the FAS population. A total of n=476 patients were randomized at this time; n=460 were included in the FAS. In a Type C meeting with FDA convened on 17 Sep 2019, FDA conveyed concerns regarding the low information fraction associated with the second interim analysis. FDA recommended the interim and final analyses be submitted for review. Following this meeting, the Applicant submitted a final CSR, dated 05 March 2020. The date of the last patient completed was 25 Jan 2018 in this report.

Table 3 describes the two analysis populations (FAS and ITT) across the two reports.

Table 3. Description of Analysis Populations Across Reports

Document (database lock date)	Analysis Population	N
Interim CSR (31 May 2017)	FAS	460
	ITT	476
Final CSR (16 March 2018)	FAS	551
	ITT	570

This review focuses on the last analysis of all 570 patients enrolled in the trial, unless stated otherwise.

The key questions that this review of Study MC-FludT/14/Trial II is aiming to address are the following:

1. Has Study MC-FludT.14/L Trial II reached its primary objective based on the event-free survival outcome, as adjudicated by the FDA?
2. Has Study MC-FludT.14/L Trial II demonstrated an advantage on overall survival and other efficacy outcomes for the treosulfan study group over the busulfan study group?
3. Has Study MC-FludT.14/L Trial II demonstrated favorable benefit-risk for the proposed indications for patients with AML and for patients with MDS?

Compliance with Good Clinical Practices

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Financial Disclosure

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Data Quality and Integrity

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for discussion of the data quality and integrity of the initial submission. To address the issues noted in the Complete Response Letter (July 30, 2021), the Applicant provided all available CBC data during their first resubmission (April 21, 2022 – SDN 58 and SDN 60) and some additional bone marrow results. In this current submission (April 30, 2024-SDN 70), the Applicant has submitted the results from an additional 486 bone marrow reports for a total 2,452 bone marrow reports. The Applicant has also provided copies of the original bone marrow reports and English language translations of these reports per Division request. These reports include 99.6% of screening marrows, 94.5% of possible day +28 marrows, 77.1% of possible day +100 marrows, and 1246 additional marrows from later time points which allowed for FDA adjudication of the primary endpoint per the Applicant's EFS event criteria.

Patient Disposition

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Protocol Violations/Deviations

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Table of Demographic Characteristics

Table 4. Baseline Characteristics in MC FludT.14/L Trial II (ITT Population)

Baseline Characteristics	Treosulfan N = 280	Busulfan N = 290
Age, median (range), years	60.0 (37, 70)	60.5 (31, 70)
Age, n (%) 18-64 years 65-70 years	204 (73%) 76 (27%)	218 (75%) 72 (25%)
Sex, n (%) - Male - Female	171 (61%) 109 (39%)	176 (61%) 114 (39%)
Weight, median (range), kg	80.0 (48.0, 144.0)	78.1 (46.0, 141.9)
Disease, n (%) - AML - MDS	192 (69%) 88 (31%)	173 (60%) 117 (40%)
AML remission status, n (%) - CR1 >CR1	164 (85%) 28 (15%)	148 (86%) 25 (14%)
MDS risk group, n (%) - Lower risk - Higher risk	20 (23%) 68 (77%)	20 (17%) 97 (83%)
HCT-CI score ≤2 >2	116 (41%) 164 (59%)	118 (41%) 172 (59%)
Donor - Matched related - Matched unrelated	66 (24%) 214 (76%)	68 (23%) 222 (77%)

Source: Adapted from FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021)

Abbreviations: AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; CR1: complete remission 1; HCT-CI: hematopoietic cell transplant-specific comorbidity index

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for additional analyses of other demographic and baseline characteristics of the study population.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Efficacy Results – Primary Endpoint

EFS Adjudication

As noted above, the Applicant defined EFS as the time from transplant until an event of disease relapse, graft failure, or death, whichever occurs first. Disease relapse for AML was considered morphologic relapse, cytogenetic relapse, or molecular relapse. Disease relapse/progression for MDS was considered recurrence of marrow or peripheral blasts >5% if they were >5% at study entry and the patient had a marrow CR after transplant, reappearance of cytogenetic abnormalities with a $\geq 5\%$ decline in donor chimerism, or reduction in peripheral counts (decrease of $\geq 50\%$ from maximum response level (after engraftment) in granulocytes or platelets or reduction in HgB concentration by ≥ 1.5 g/dl from maximum response level (after engraftment) or transfusion dependency) accompanied by absolute decline in donor chimerism by $\geq 5\%$. The Applicant included either primary graft failure or secondary graft failure as an EFS event. During review of the initial submission, FDA attempted to verify if Study MC-FludT.14/L Trial II met its primary endpoint as planned, completed, and reported by the Applicant. The clinical team noted that they required more comprehensive laboratory data and bone marrow data to complete an adjudication of the primary EFS endpoint as defined by the Applicant. Since this information was not initially collected as part of the study, the Applicant had to request the missing information from the individual study sites.

The Applicant conducted an additional database lock on 16 Mar 2018 and characterized all EFS events observed (regardless of timing). Once collected, the Applicant utilized an external hematologist to adjudicate the EFS events based on the newly collected data (Table 5 below). FDA review noted two subjects with missed events. One patient had a missed secondary graft failure event that went on to receive a second transplant who had been previously censored at last follow up without an event. The other patient had a missed late relapse that had been previously censored at last follow up. The remaining six adjustments noted during the Applicant's review of the new data were to update the timing of relapse events based on the first evidence of relapse in the source documentation.

Table 5. Applicant's Adjudication of EFS Events

Subject Identifier	Planned Treatment	EFS day	EFS day Applicant's Adjudication	Censor EFS	Censor EFS Applicant Adjudication	Event or Censoring EFS	Event or Censoring EFS Applicant's Adjudication	Applicant's explanation
(b) (6)	Busulfan	282	93	1	1	RELAPSE/ PROGRESSION	RELAPSE/ PROGRESSIO N	relapse date adjusted
	Treosulfan	1050	902	0	1	NO EVENT (RELAPSE/ PROGRESSION, DEATH OR GRAFT FAILURE)	RELAPSE/ PROGRESSIO N	relapse
	Treosulfan	108	83	1	1	RELAPSE/ PROGRESSION	RELAPSE/ PROGRESSIO N	relapse date adjusted
	Busulfan	99	78	1	1	RELAPSE/ PROGRESSION	RELAPSE/ PROGRESSIO N	relapse date adjusted
	Treosulfan	1081	1027	1	1	RELAPSE/ PROGRESSION	RELAPSE/ PROGRESSIO N	relapse date adjusted

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Grafapex (treosulfan)

Subject Identifier	Planned Treatment	EFS day	EFS day Applicant's Adjudication	Censor EFS	Censor EFS Applicant Adjudication	Event or Censoring EFS	Event or Censoring EFS Applicant's Adjudication	Applicant's explanation
(b) (6)	Busulfan	1286	89	0	1	NO EVENT (RELAPSE/ PROGRESSION, DEATH OR GRAFT FAILURE)	SECONDARY GRAFT FAILURE	Secondary graft failure
	Treosulfan	111	97	1	1	RELAPSE/ PROGRESSION	RELAPSE/ PROGRESSION	relapse date adjusted
	Busulfan	266	229	1	1	RELAPSE/ PROGRESSION	RELAPSE/ PROGRESSION	relapse date adjusted

Source: Applicant's adjudication analysis

With this current submission, FDA was able to review the source documentation for labs and bone marrow reports assembled by the Applicant and adjudicate EFS per the Applicant's definition. This included screening laboratory data for evidence of relapse or graft failure events and verifying that the data provided substantiates the occurrence and timing of EFS events. FDA agreed with the Applicant's EFS event updates in Table 5 above but noted 8 additional subjects that required adjudication (Table 6 below). Five patients required censoring from the analysis as source documentation (including translated bone marrow biopsy reports) revealed that their pretransplant disease assessment was not in range for inclusion. This meant that patients had bone marrow blasts $\geq 5\%$ for AML patients or $\geq 20\%$ for MDS patients. For two patients, FDA adjusted the timing of their relapse date based on review of the source data. Lastly, for one patient, FDA adjudicated their relapse event as not supported by source documentation with follow-up showing the patient was well and without relapse almost a year after the previously reported date. This patient was changed to censored at last follow up. These adjudications were submitted to the Applicant who agreed with FDA's justifications for the updates.

Table 6. FDA Adjudication of EFS events

USUBJID	ADT_APL	CNSR_APL	DESC_APL	ADT_FDA	CNSR_FDA	DESC_FDA	Rationale
MC-FludT.14/L_A-(b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	sensor initial blast	Censor, baseline blasts out of range per inclusion criteria. Analysis date is 1 day after start date (1 day after randomization date for ITT analysis)
MC-FludT.14/L_A-(b) (6)		1	RELAPSE/PROGRESSION		1	RELAPSE/PROGRESSION	changed date of event based on review of patient data, biopsy on this day showed morphologic relapse
MC-FludT.14/L_A-(b) (6)		1	RELAPSE/PROGRESSION		0	sensor initial blast	Censor, baseline blasts out of range per inclusion criteria
MC-FludT.14/L_A-(b) (6)		1	RELAPSE/PROGRESSION		0	sensor last follow up	no relapsed documented per updated bone marrow reports, changed analysis date and changed to censor at last follow up

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Grafapex (treosulfan)

USUBJID	ADT_APL	CNSR_APL	DESC_APL	ADT_FDA	CNSR_FDA	DESC_FDA	Rationale
MC-FludT.14/L_A-(b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	sensor initial blast	Censor, baseline blasts out of range per inclusion criteria
MC-FludT.14/L_A-(b) (6)		1	DEATH		0	sensor initial blast	Censor, baseline blasts out of range per inclusion criteria
MC-FludT.14/L_A-(b) (6)		0	NO EVENT (RELAPSE/PROGRESSION, DEATH OR GRAFT FAILURE)		0	sensor initial blast	Censor, baseline blasts out of range per inclusion criteria
MC-FludT.14/L_A-(b) (6)		1	RELAPSE/PROGRESSION		1	RELAPSE/PROGRESSION	changed date of event based on review of patient data

Source: FDA analysis of source documentation.

During the adjudication process, FDA also made note of which relapse criteria were used to identify an event. AML relapse events were categorized as either morphologic relapse, molecular relapse, or cytogenetic relapse. MDS relapse events were categorized as morphologic relapse, cytogenetic relapse, or decreased counts/reduced chimerism. These are indicated in the EFS events shown in Table 7 below.

Table 7. FDA-Adjudicated Post-Randomization EFS Events Based on the Applicant's Event Criteria

Event or Censoring Description	Planned Treatment		570
	Busulfan	Treosulfan	total
DEATH	55	35	90
AML Death	24	17	41
MDS Death	31	18	49
NO EVENT (RELAPSE/PROGRESSION, DEATH OR GRAFT FAILURE)	154	185	339
AML no event	95	126	221
MDS no event	59	59	118
PRIMARY GRAFT FAILURE	1	1	2
AML Primary graft failure	0	0	0
MDS Primary graft failure	1	1	2
SECONDARY GRAFT FAILURE	9	0	9
AML Secondary graft failure	5	0	5
MDS Secondary graft failure	4	0	4
RELAPSE/PROGRESSION	71	59	130
AML Morphologic relapse	44	46	90
AML Molecular relapse	4	2	6
AML Cytogenetic relapse	1	3	4
MDS Morphologic relapse	18	8	26
MDS Cytogenetic relapse	3	2	5
MDS decreased counts/reduced chimerism	1	1	2

Source: FDA analysis of source documentation.

EFS Definitions

Multiple definitions of EFS were explored during the review. Table 8 presents the various definitions, time points of assessment, and the number of events observed.

Table 8: EFS Definitions Utilized in FDA Review

Endpoint Definition	Time frame	FDA Adjudication	Database Lock Date	Events Observed
EFS (within 24 months post-transplantation)	24 months post-HSCT	No	31 May 2017	168
		No	16 Mar 2018	224
EFS (post-transplantation)	Any time post-HSCT	No	16 Mar 2018	234
		Yes	16 Mar 2018	229*
EFS (post-randomization)	Any time post-randomization	Yes	16 Mar 2018	231

*Upon FDA review, 2 additional events were noted to not have been included in the Applicant's final version of EFS (as submitted in adtte.xpt dated 09 Sep 2024). Thus, this endpoint should have included 231 events per FDA adjudication.

Event-Free Survival (within 24 months post-transplantation)

The hazard ratio for EFS, as performed at the *interim* analysis in the FAS population with 168 events was 0.65 (95% CI: 0.47, 0.90). The p-value for non-inferiority testing was 0.0000164, which was significant as compared to the efficacy boundary of 0.000149. The p-value for superiority testing was 0.0051268, which did not cross the efficacy boundary. This endpoint had 224 events at the *last* analysis, which would correspond to a 46.6% information fraction. The efficacy boundary based on this information fraction would have been 0.00097. The hazard ratio for EFS, as defined in the protocol and performed at the *last* analysis, within 2 years post-transplantation, using Cox proportional hazards model with risk group and donor type, was 0.64 (95% CI: 0.49, 0.83), p-value (one-sided) for superiority was 0.0004 (efficacy stopping boundary was 0.00097).

Statistical Reviewer's Comment: *The 224 events corresponding to the "last" analysis are based on additional events observed for the primary endpoint (EFS within 24 months post-transplantation). While this endpoint met the superiority boundary at this time, this does not account for the previous analysis, though the alpha spent at the prior analyses was small. This boundary is hypothetical – it corresponds to a trial which would have conducted an interim analysis at this time. This analysis was not planned but requested by FDA. While the boundary is useful for providing context for the p-value, it does not represent the traditional boundary utilized for demonstrating efficacy.*

Event-Free Survival (post-transplantation)

FDA conducted additional analyses on EFS to assess its robustness to 1) adjudication of events and 2) the 2-year analysis window. These analyses use data from the final CSR, which includes events occurring after the 2-year analysis window. The analyses that follow do not use site as a stratification factor, as this resulted in a high number of strata (site/center factor has 22 levels).

In the final CSR date 16 Mar 2018, the Applicant reported the hazard ratio for EFS (post-transplantation), unrestricted to any period, as 0.65 (95% CI: 0.49, 0.84). With FDA adjudication, the hazard ratio for EFS (post-transplantation), unrestricted to any period, was 0.65 (95% CI: 0.50, 0.85), p-value (one-sided) was 0.0007 (Table 9).

Table 9 shows the summaries. These EFS events include post-surveillance events, i.e., those which occur beyond 2 years post-HSCT. Per the final CSR, this represents “an information fraction of 48.6% based on the 481 events planned for in the final analysis foreseen in the Clinical Trial Protocol if the trial had not been stopped prematurely. The information fraction of 48.6% corresponds to an event-driven alpha level of 0.001262 within this last analysis according to the O’Brien-Fleming α -spending function.” The database lock for the last analysis occurred on 16 March 2018.

Table 9. Study MC-FludT.14/L Trial II FDA-Adjudicated Event-Free Survival (post-transplantation) Summary

	Busulfan N=283	Treosulfan N=268
Events, n (%)	135 (47.7%)	94 (35.1%)
Death, n (%)	55 (19.4%)	35 (13.1%)
Relapse/Progression, n (%)	71 (25.1%)	58 (21.6%)
Primary Graft Failure, n (%)	1 (0.4%)	1 (0.4%)
Secondary Graft Failure, n (%)	8 (2.8%)	0 (0.0%)
Median (Range)	36.5 (0, 40)	NE
Hazard Ratio (95% Confidence Interval) ¹	0.65 (0.50, 0.85)	
p-value (one-sided) ²	0.0007	

¹ Based on a Cox proportional hazards model stratified by donor type and risk group

² Log-rank test for superiority stratified by donor type and risk group

NE = Not estimable

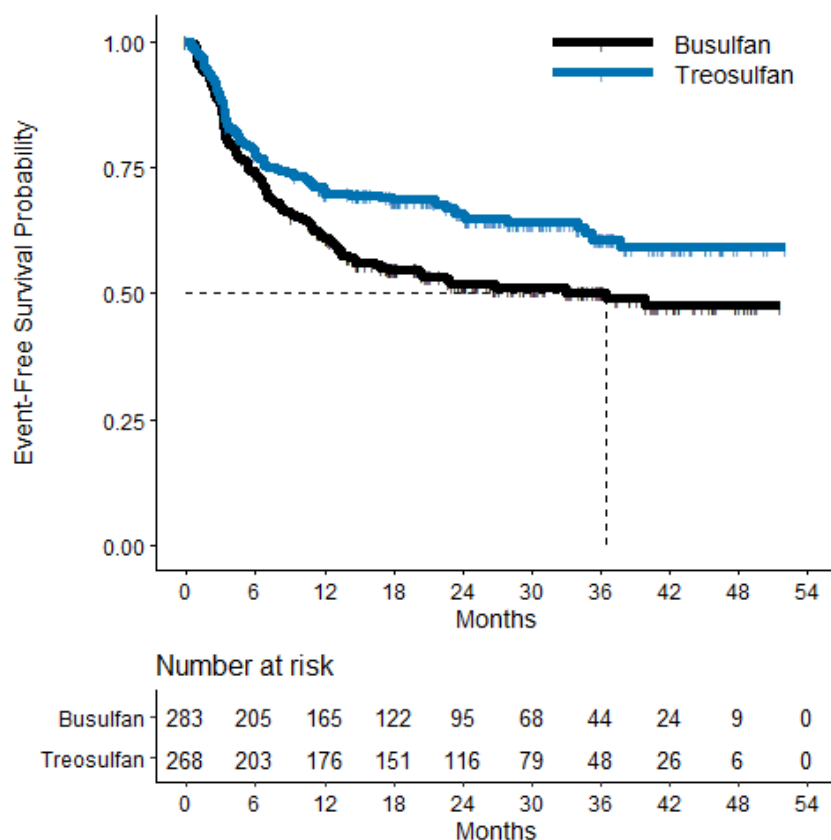
Database lock date: 16 March 2018

Source: FDA Analysis

Statistical Reviewer’s Comment: The 234 EFS events referenced above include events beyond 2 years post-HSCT, which is inconsistent with the primary endpoint of Study MC-FludT-14/L Trial II. The reported information fraction of 48.6% is not representative of how this quantity is typically reported; therefore, the associated boundary has no clear meaning. This percentage might be used to contextualize how much additional information was encoded in the additional events included in the final CSR, although this may be misleading. For instance, had the trial been designed for EFS at any time, the targeted final number of events may have been different. For these reasons, the p-value and boundary above should not be interpreted as demonstrating

efficacy based on this endpoint. This trial was not designed for this definition of EFS, nor was the associated boundary.

Figure 1. Study MC-FludT.14/L Trial II FDA-Adjudicated Event-Free Survival (post-transplantation) Kaplan-Meier Plot



Database lock date: 16 March 2018
Source: FDA Analysis

Efficacy Results – Secondary and other relevant endpoints

Overall Survival (within 24 months post-transplantation)

The HR for overall survival (within 24 months post-transplantation) in the FAS population reported at the *interim* analysis was 0.61 (95% CI: 0.42, 0.88) with 82/240 (34%) of events in the busulfan group and 52/220 (24%) of events in the treosulfan group (adjusted for donor type as factor, and risk group and center as strata using Cox regression model). This analysis was included in the interim CSR.

The HR for overall survival (within 24 months post-transplantation) in the FAS population at the *last* analysis was 0.63 (95% CI: 0.47, 0.86) with 107/283 (38%) of events in the busulfan group and 72/268 (27%) events in the treosulfan group (adjusted for donor type and risk group as stratification factors). Seventy-three percent (95% CI: 67%, 78%) of patients in the treosulfan

group and 60% (95% CI: 54%, 66%) of patients in the busulfan group were alive at 24 months. The results suggest an OS advantage in the treosulfan group as compared to the busulfan group. This endpoint was not specified in the analysis plan to be formally tested.

Dose/Dose Response

There was no exploration of dose/dose response in this study. See discussion of dose/response in Section 8.1.3 and in the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Durability of Response

See discussion of time-to-event endpoints.

Persistence of Effect

See discussion of time-to-event endpoints.

Efficacy Results – Secondary or exploratory COA (PRO) endpoints

No PRO endpoints were included in this study.

Additional Analyses Conducted on the Individual Trial

Event-Free Survival (post-randomization)

The hazard ratio for EFS (post-randomization) in the ITT population was 0.65 (95% CI: 0.50, 0.85), p-value (one-sided) is 0.0006. Table 10 and Figure 2 show the summaries. The one-sided significance level resulting from an event-driven O'Brien-Fleming type group-sequential efficacy stopping boundary was 0.001262.

Statistical Reviewer's Comment: *The p-value and boundary above should not be interpreted as demonstrating efficacy based on this endpoint. As noted above, this trial was not designed for this definition of EFS, nor was the associated boundary. However, this boundary may provide useful context for interpreting the results of this endpoint.*

Table 10. Study MC-FludT.14/L Trial II FDA-Adjudicated Event-Free Survival (post-randomization) Summary

	Busulfan N=290	Treosulfan N=280
Events, n (%)	136 (46.9%)	95 (33.9%)
Death, n (%)	55 (19.0%)	35 (12.5%)
Relapse/Progression, n (%)	71 (24.5%)	59 (21.1%)
Primary Graft Failure, n (%)	1 (0.3%)	1 (0.4%)
Secondary Graft Failure, n (%)	9 (3.1%)	0 (0.0%)
Median (Range)	33.4 (1, 40)	NE
Hazard Ratio (95% Confidence Interval) ¹	0.65 (0.50, 0.85)	
p-value (one-sided) ²	0.0006	

¹ Based on a Cox proportional hazards model stratified by donor type and risk group

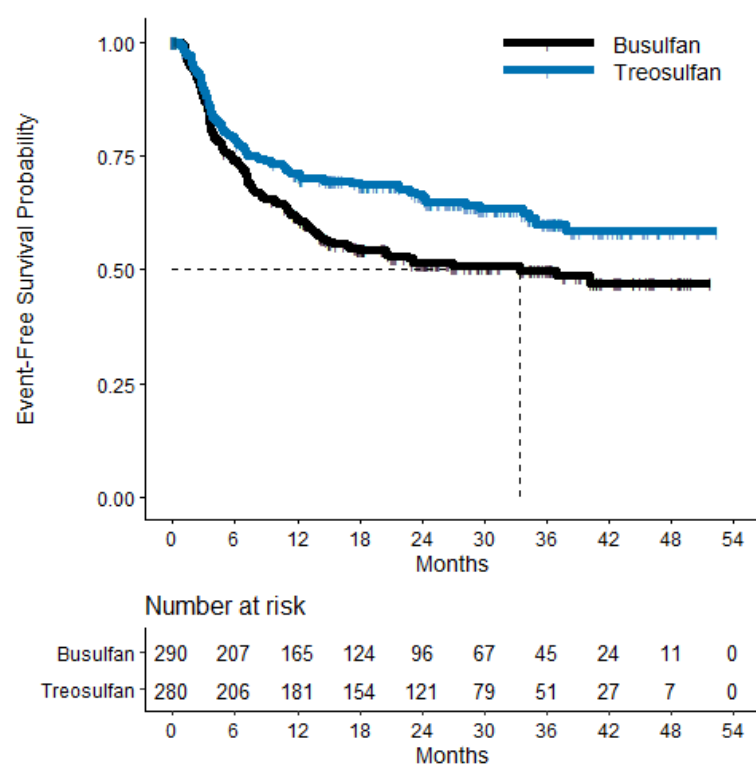
² Log-rank test stratified by donor type and risk group

NE = Not estimable

Database lock date: 16 March 2018

Source: FDA Analysis

Figure 2. Study MC-FludT.14/L Trial II FDA-Adjudicated Event-Free Survival (post-randomization) Kaplan-Meier Plot



Database lock date: 16 March 2018

Source: FDA Analysis

Clinical Reviewer's Comment: As shown above, the adjudicated EFS results analyzed by FDA either within 24 months of transplant or with an unrestricted timeframe resulted in results which were consistent with the per protocol definition and analysis of EFS. While Study MC FludT.14/L Trial II met its primary analysis of EFS, the use of an EFS endpoint that comprised nonstandard EFS events of unclear clinical benefit made these results unsuitable (b) (4). It is important to note that a properly defined EFS endpoint could support findings of efficacy for AML with active disease (U.S. Food and Drug Administration, 2022). However, since the patients with AML enrolled on Study MC-FludT.14/L Trial II were in remission, an RFS endpoint would better capture the clinical benefit in this context (because failing to achieve remission was not a risk for these patients and is a key event for an EFS endpoint). FDA analyzed RFS for patients with AML in an exploratory analysis detailed below. For patients with MDS, while recent criteria propose a definition for EFS in MDS, the clinical meaningfulness of these definitions have not been established. Available data indicate that CR remains the response endpoint associated with the greatest long-term benefit, and a definition of EFS that captures failure to achieve remission might be an acceptable endpoint. However, the protocol EFS definition did not include failure to achieve remission as a component of treatment failure and the frequency of CBC and bone marrow data collection did not allow for reliable assessment of remission status. Without this remission assessment, it is less clear how to interpret subsequent disease relapse/progression events. Ultimately, the OS best captured clinical benefit of treosulfan as discussed below.

Overall Survival (post-randomization)

The hazard ratio for OS (post-randomization) was 0.67 (95% CI: 0.51, 0.90) in the ITT population. Table 11 and Figure 3 summarize these results. The results show an OS advantage of treosulfan compared to busulfan.

Table 11. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) Summary

	Busulfan N=290	Treosulfan N=280
Events, n (%)	112 (38.6%)	81 (28.9%)
Survival at 24 Months, % (95% CI)	60 (54, 66)	73 (67, 78)
Hazard Ratio (95% CI) ¹	0.67 (0.51, 0.90)	

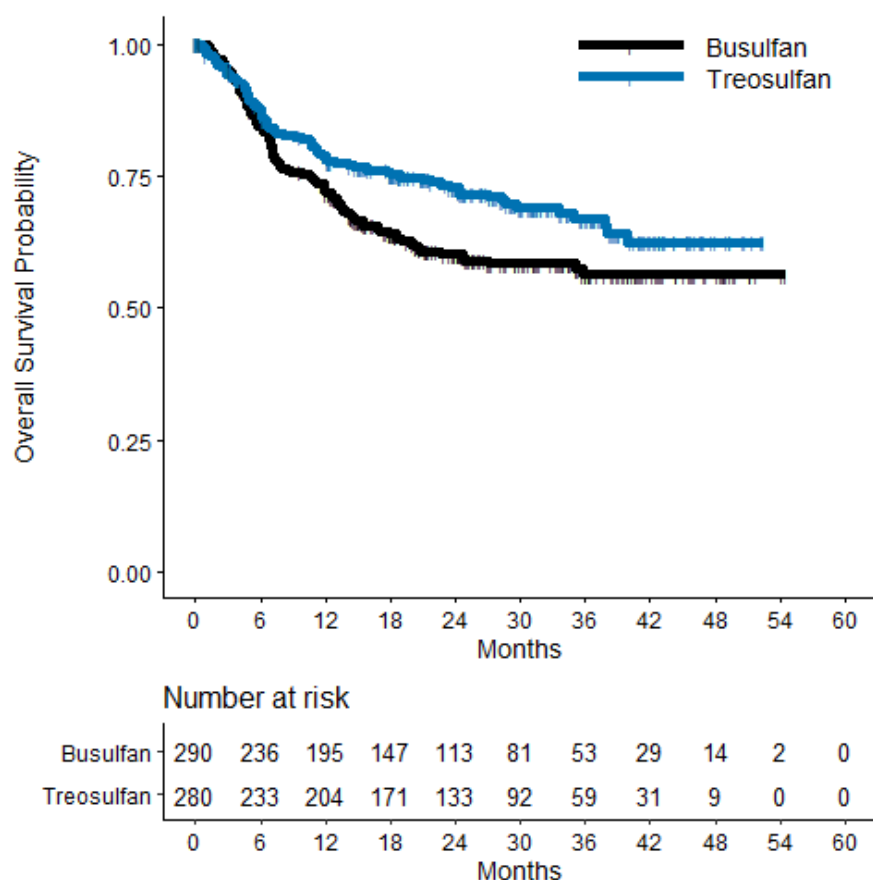
¹ Based on a Cox proportional hazards model stratified by donor type and risk group

CI = Confidence Interval

Database lock date: 16 March 2018

Source: FDA Analysis

Figure 3. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) Kaplan-Meier Plot



Database lock date: 16 March 2018
 Source: FDA Analysis

Subgroup Analyses: Overall Survival (post-randomization)

Overall Survival (post-randomization) in AML

The hazard ratio for OS (post-randomization) was 0.73 (95% CI: 0.51, 1.06) in the AML population. Table 12 and Figure 4 show the summaries. The results suggest an OS advantage of treosulfan compared to busulfan in the AML subgroup.

Table 12. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) in AML Summary

	Busulfan N=173	Treosulfan N=192
Events, n (%)	61 (35.3%)	54 (28.1%)
Survival at 24 Months, % (95% CI)	65 (57, 72)	73 (66, 79)
Hazard Ratio (95% CI) ¹	0.73 (0.51, 1.06)	

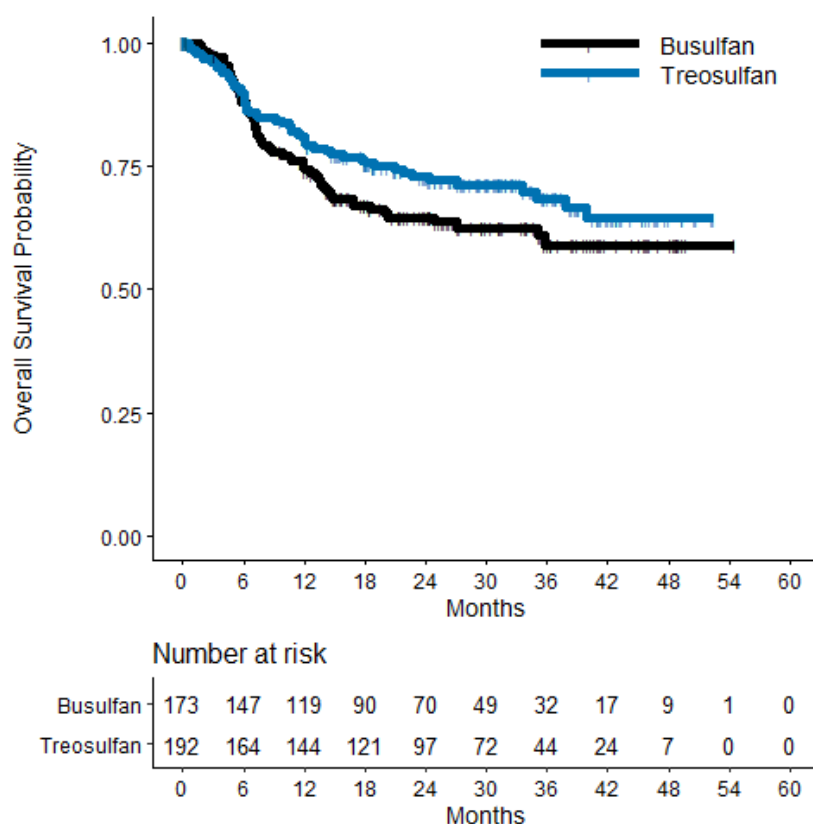
¹ Based on a Cox proportional hazards model stratified by donor type and risk group

CI = Confidence Interval

Database lock date: 16 March 2018

Source: FDA Analysis

Figure 4. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) in AML Kaplan-Meier Plot



Database lock date: 16 March 2018

Source: FDA Analysis

Overall Survival (post-randomization) in MDS

The hazard ratio for OS (post-randomization) was 0.64 (95% CI: 0.40, 1.02) in the MDS population. Table 13 and Figure 5 show the summaries. The results suggest an OS advantage of treosulfan compared to busulfan for the MDS subgroup.

Table 13. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) in MDS Summary

	Busulfan N=117	Treosulfan N=88
Events, n (%)	51 (43.6%)	27 (30.7%)
Survival at 24 Months, % (95% CI)	54 (44, 63)	73 (62, 81)
Hazard Ratio (95% CI) ¹	0.64 (0.40, 1.02)	

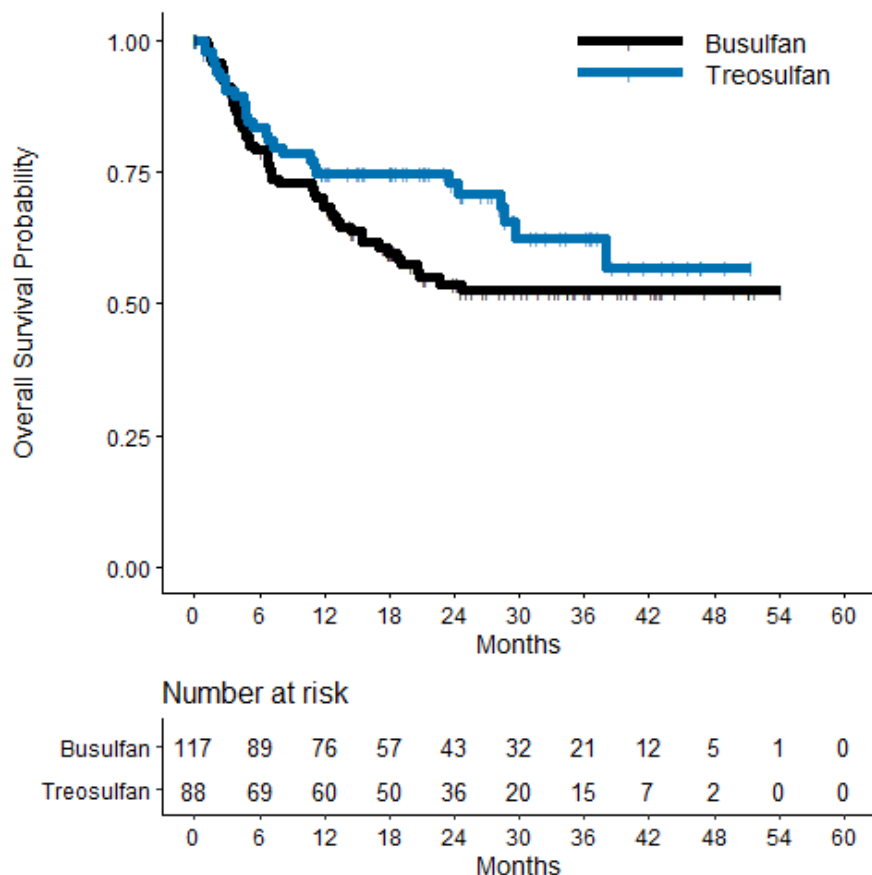
¹ Based on a Cox proportional hazards model stratified by donor type and risk group

CI = Confidence Interval

Database lock date: 16 March 2018

Source: FDA Analysis

Figure 5. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) in MDS Kaplan-Meier Plot



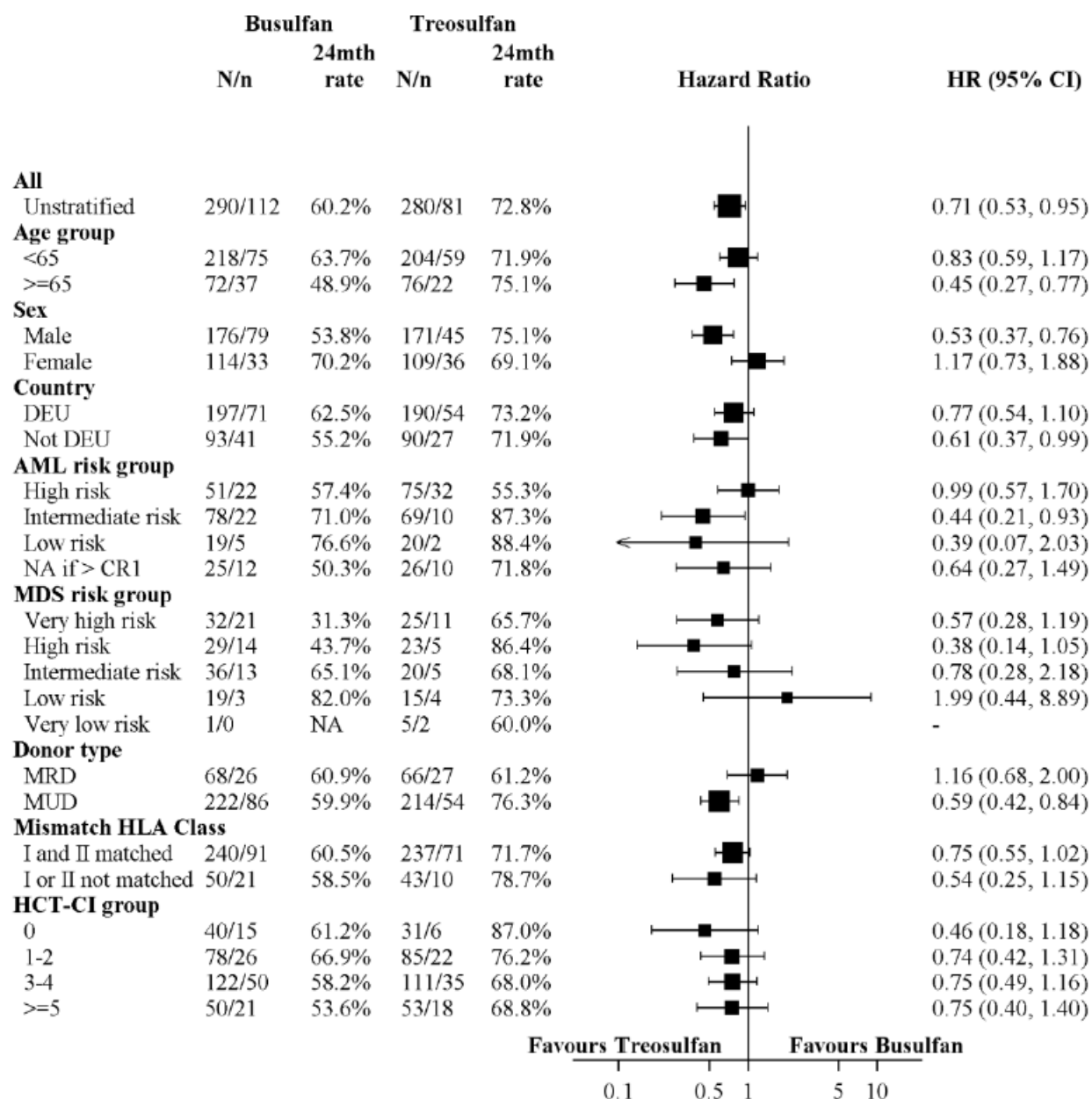
Database lock date: 16 March 2018

Source: FDA Analysis

Subgroup Analyses: OS (post-randomization)

Figure 6 shows subgroup analyses for OS (post-randomization) in the AML and MDS populations. Most point estimates of hazard ratios are below one, suggesting favorable outcomes for the treosulfan group compared to the busulfan group.

Figure 6. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) Subgroup Analyses (AML and MDS)



N = Number of patients, n = number of events

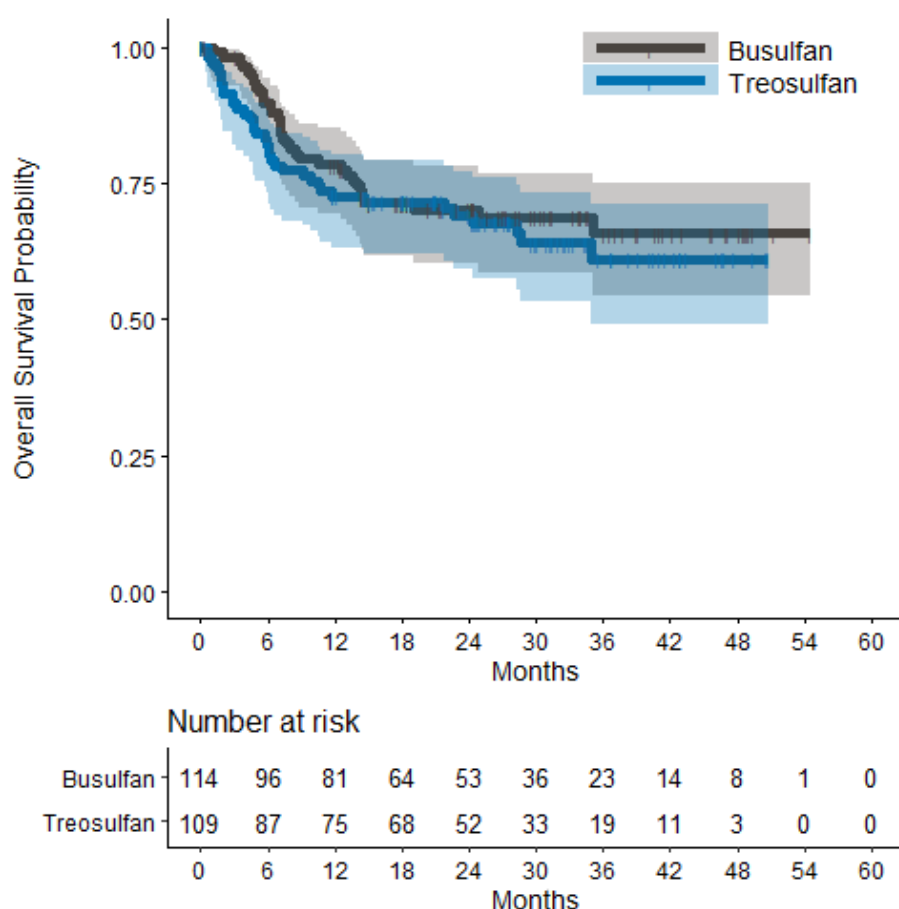
Note: Hazard ratios are based on an unstratified Cox proportional hazards model

Database lock date: 16 March 2018

Source: Adapted from Applicant's information request response received on 30 July 2024

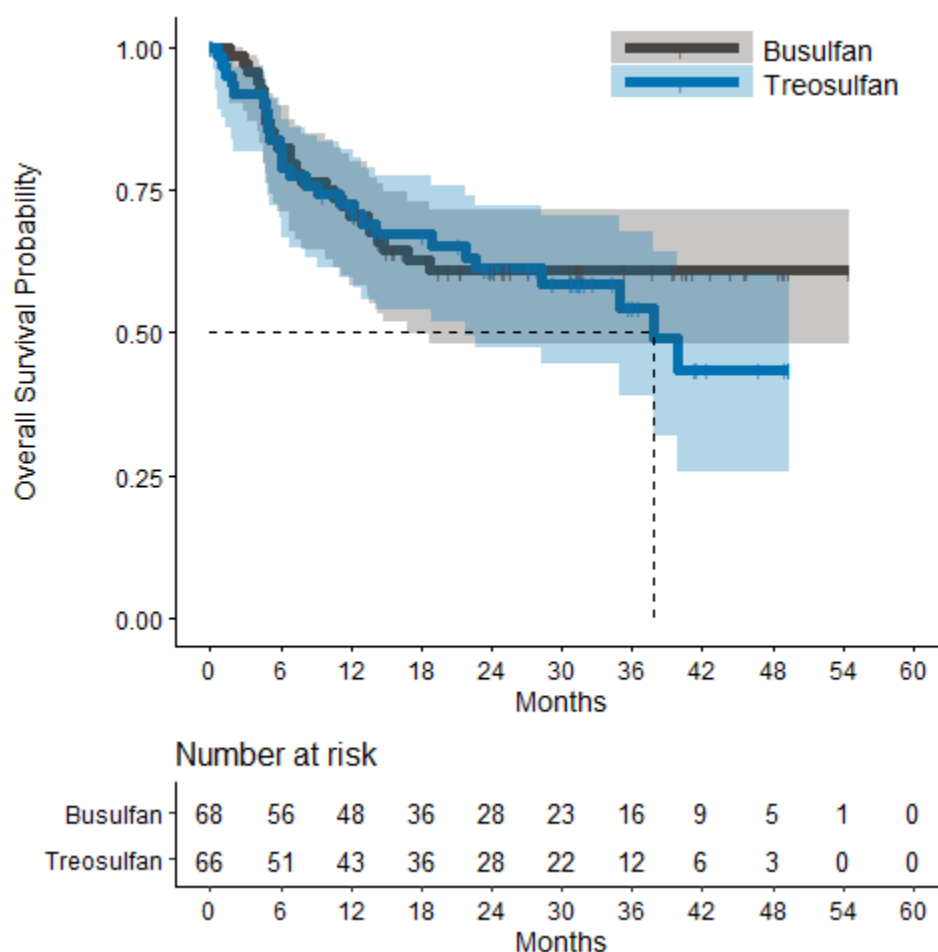
Some point estimates of hazard ratios are above one: female group, low risk MDS group, and MRD group. Low risk MDS group results are challenging to interpret due to low number of events: three events in the busulfan group and four events in the treosulfan group. For the female and MRD subgroups, Kaplan-Meier plots are shown below in Figure 7 and Figure 8. Survival curves with 95% pointwise confidence intervals overlap – there appears to be no substantial differences between treatment groups in the female subgroup. In the MRD subgroup, the result is likely due to a few influential events occurring later in the follow-up period.

Figure 7. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) in Female Subgroup Kaplan-Meier Plot



Database lock date: 16 March 2018
 Source: FDA Analysis

Figure 8. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) in MRD Subgroup Kaplan-Meier Plot



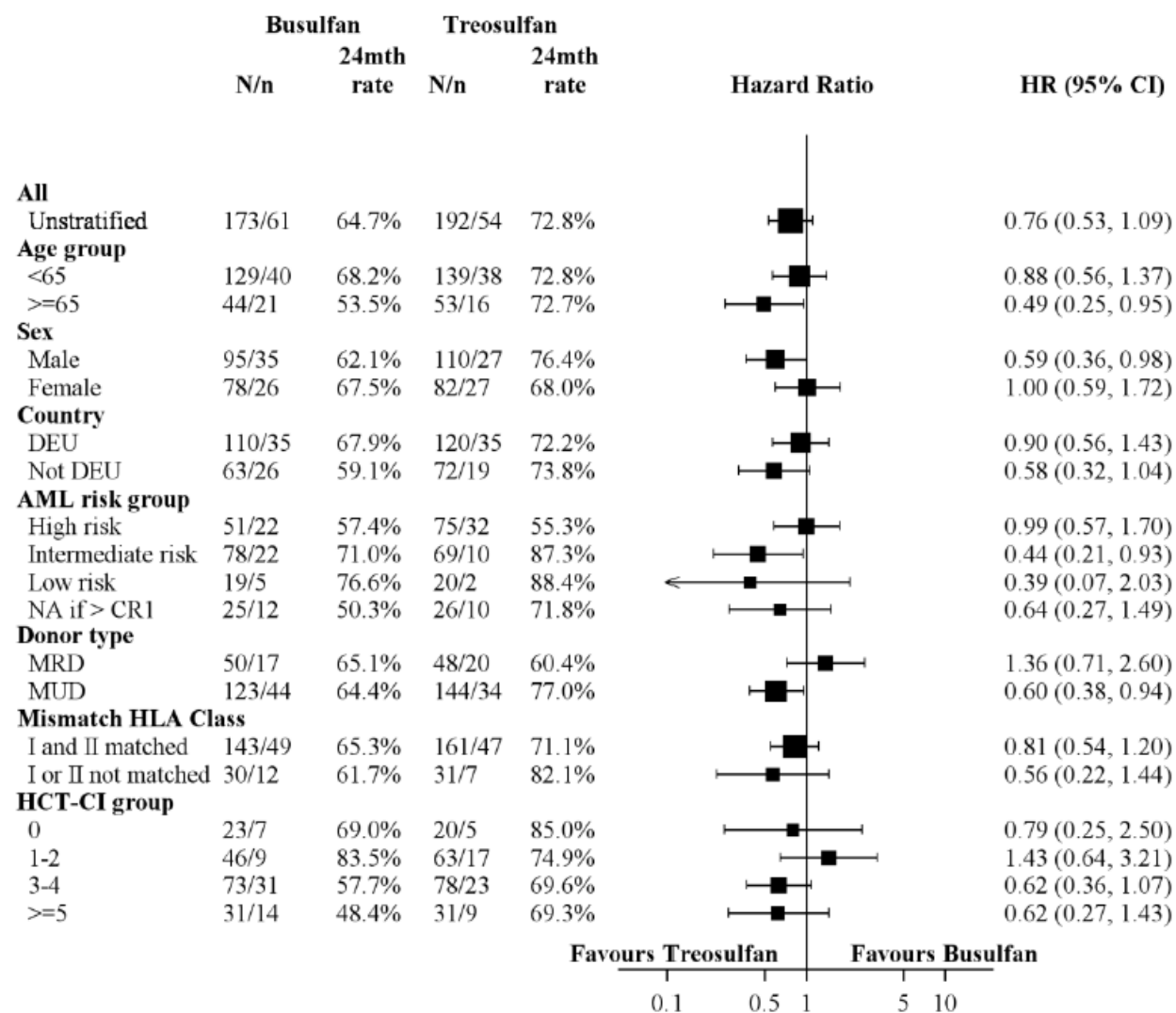
Database lock date: 16 March 2018
Source: FDA Analysis

Clinical Reviewer's Comment on MRD vs MUD: As noted in the analysis above and during the initial review of the data, the benefit demonstrated in the OS endpoint appears to be driven by the patients utilizing a MUD for transplant. It is not clear why this might have been observed, but it is most likely that this apparent difference is a chance finding since the imbalance in the number of events was small. Clinically, patients receiving a transplant from a MUD versus MRD have differing competing risks post-transplant of GVHD, infection, and relapse that are all affected by the different donor type. There will be a higher risk of GVHD with MUD but greater chance of a graft versus leukemia effect (lower relapse chance). Moreover, the need for anti-thymocyte globulin (ATG) to be added to the transplant conditioning regimen of MUD transplants to reduce the higher risk of GVHD is at the expense of increased infectious risk and the potential for a decreased graft versus leukemia effect. Within these competing and varying risks, it is unclear how the use of treosulfan versus busulfan would have further altered these risks to only benefit the patients receiving a MUD transplant. However, it is important to

consider that the trial was not designed to test treosulfan against a placebo control; rather it was designed to show the efficacy of treosulfan as an alkylating agent used in conditioning compared to an active control arm of busulfan conditioning.

Subgroup Analyses: OS (post-randomization) in AML

Figure 9. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) Subgroup Analyses (AML)



N = Number of patients, n = number of events

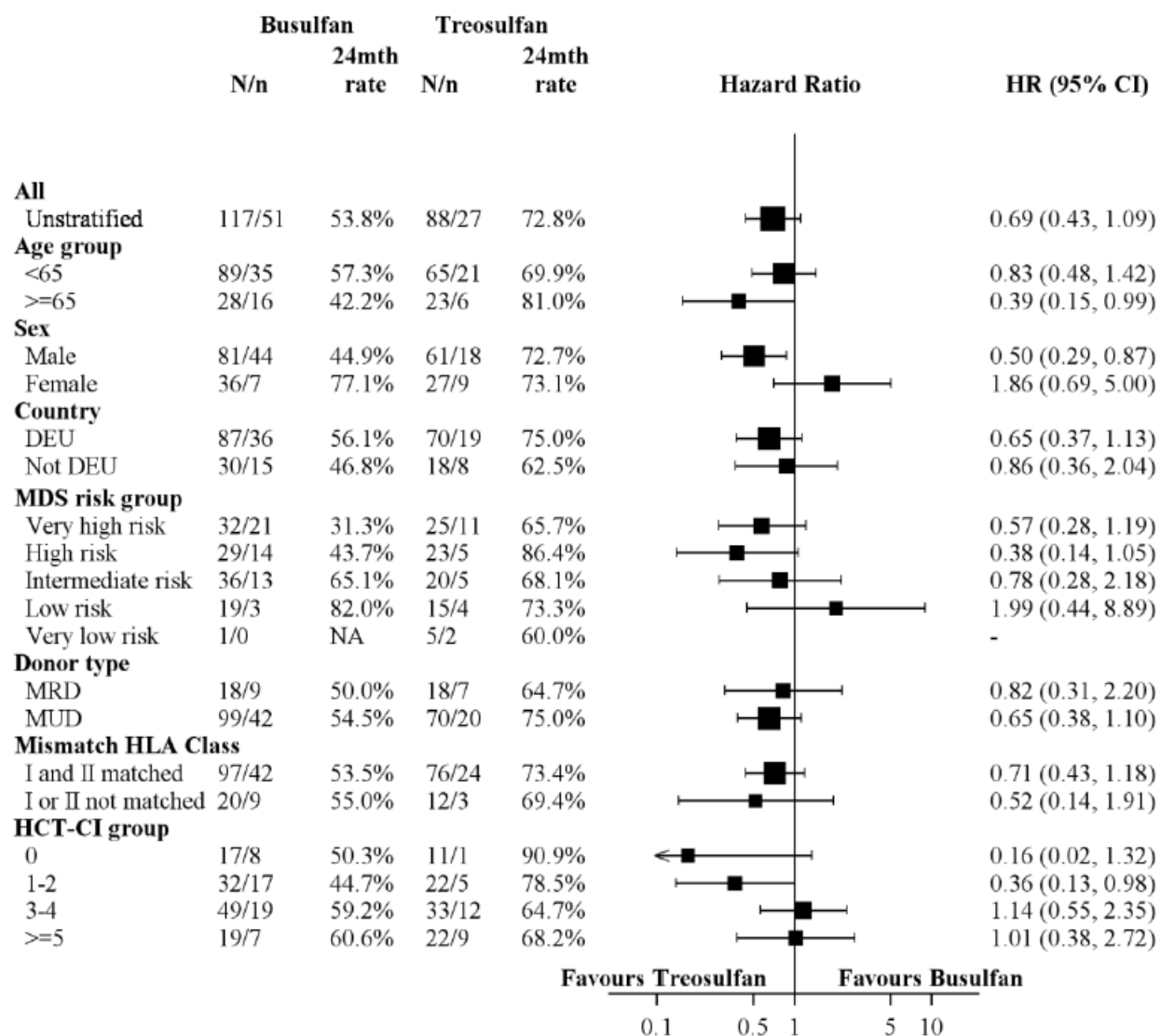
Note: Hazard ratios are based on an unstratified Cox proportional hazards model

Database lock date: 16 March 2018

Source: Adapted from Applicant's information request response received on 30 July 2024

Subgroup Analyses: OS (post-randomization) in MDS

Figure 10. Study MC-FludT.14/L Trial II Overall Survival (post-randomization) Subgroup Analyses (MDS)



N = Number of patients, n = number of events

Note: Hazard ratios are based on an unstratified Cox proportional hazards model

Database lock date: 16 March 2018

Source: Adapted from Applicant's information request response received on 30 July 2024

Subgroup Analyses: EFS (within 24 months post-transplantation)

Table 14 summarizes subgroup results for EFS (within 24 months post-transplantation) in the FAS population reported at interim analysis.

Table 14. Study MC-FludT.14/L Trial II Subgroup Analyses: EFS (within 24 months post-transplantation)

Outcome	Busulfan	Treosulfan	HR (95% CI)
EFS, Events/N (%)	100/240 (42%)	68/220 (31%)	0.65 (0.47, 0.90)
AML, Events/N (%)	53/138 (55%)	48/155 (64%)	0.75 (0.51, 1.12)
MDS, Events/N (%)	47/102 (44%)	20/65 (65%)	0.66 (0.39, 1.11)

Database lock date: 31 May 2017
Source: FDA Analysis

Statistical Reviewer's Comment: *These results suggest that the positive result observed at the interim analysis was not driven by either the AML or MDS populations.*

Subgroup Analyses: Relapse-Free Survival (AML Population)

FDA conducted an ad hoc analysis of RFS in patients with AML. As noted above, a properly defined EFS endpoint could support findings of efficacy for either AML or MDS. However, since patients with AML enrolled on Study MC-FludT.14/L Trial II were in remission, an RFS endpoint better captures the potential benefit in this clinical context (because failing to achieve remission, which is a key event for an EFS endpoint, was not a risk for patients already in remission). Additionally, the Applicant's inclusion of graft failure as an EFS event complicates interpretation of the EFS endpoint. Graft failure is assessed in all trials concerning HSCT; however, graft failure is considered a safety issue since this is a complication of the transplant procedure.

RFS events for AML were adjudicated by FDA based on the EFS adjudications noted above. RFS was defined as the time from randomization until morphologic relapse or death from any cause, whichever comes first. The Applicant included three criteria for AML relapse in their EFS definition: hematologic, cytogenetic, and molecular. However, only hematologic relapse criteria are fit for regulatory considerations. Moreover, investigation of the data showed that little follow up data was available for patients once an event of molecular or cytogenetic relapse was called. Similar issues with lack of follow up data were noted for patients with primary or secondary graft failure. Therefore, to achieve the EFS to RFS adjudication in the AML population, FDA censored patients at the time of molecular or cytogenetic relapse and primary or secondary graft failure since follow up data for morphological relapse was not routinely collected after these events were called. This is shown conceptually in Table 15 and with detailed patient adjudications in Table 16 below. The Applicant agreed with FDA adjudications for the AML RFS analysis.

Table 15. FDA's EFS to RFS Event Censoring Procedure

Event Description	EFS Event (n)	RFS Event/ censor (n)
Death	Event (41)	Event
Relapse		
Morphologic	Event (90)	Event
Cytogenetic	Event	Censor (4)
Molecular	Event	Censor (6)
Graft failure		
Primary	Event	Censor (0)
Secondary	Event	Censor (5)

Source: FDA analysis of source data

Table 16. FDA-Adjudicated RFS Events for Patients with AML Based on Morphologic Relapse

USUBJID	ADT_APL	CNSR_APL	DESC_APL	ADT_FDA	CNSR_FDA	DESC_FDA	Rationale
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	censor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	censor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	SECONDARY GRAFT FAILURE	(b) (6)	0	censor at graft failure	Censor at time of graft failure
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	censor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	censor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	censor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	censor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	censor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	SECONDARY GRAFT FAILURE	(b) (6)	0	censor at graft failure	Censor at time of graft failure
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	SECONDARY GRAFT FAILURE	(b) (6)	0	censor at graft failure	Censor at time of graft failure
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	SECONDARY GRAFT FAILURE	(b) (6)	0	censor at graft failure	Censor at time of graft failure

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USUBJID	ADT_APL	CNSR_APL	DESC_APL	ADT_FDA	CNSR_FDA	DESC_FDA	Rationale
MC-FludT.14/L_A- (b) (6)	(b) (6)	1	RELAPSE/PROGRESSION	(b) (6)	0	sensor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)		1	RELAPSE/PROGRESSION		0	sensor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse
MC-FludT.14/L_A- (b) (6)		1	SECONDARY GRAFT FAILURE		0	sensor at graft failure	Censor at time of graft failure
MC-FludT.14/L_A- (b) (6)		1	RELAPSE/PROGRESSION		0	sensor at mol/cyto relapse	Relapse event changed to censor due to lack of documentation of morphologic relapse

Source: FDA analysis of source data

The hazard ratio for RFS in the AML population was 0.72 (0.51, 1.03). Table 17 and Figure 11 show the summaries. The results suggest an RFS advantage of treosulfan compared to busulfan.

Table 17. Study MC-FludT.14/L Trial II Relapse-Free Survival in AML Summary

	Busulfan N=173	Treosulfan N=192
Events, n (%)	68 (39.3%)	61 (31.8%)
Death, n (%)	24 (13.9%)	17 (8.9%)
Relapse/Progression, n (%)	44 (25.4%)	44 (22.9%)
Relapse-Free Survival at 24 Months, % (95% CI)	59% (50%, 66%)	69% (61%, 75%)
Hazard Ratio (95% CI) ¹	0.72 (0.51, 1.03)	

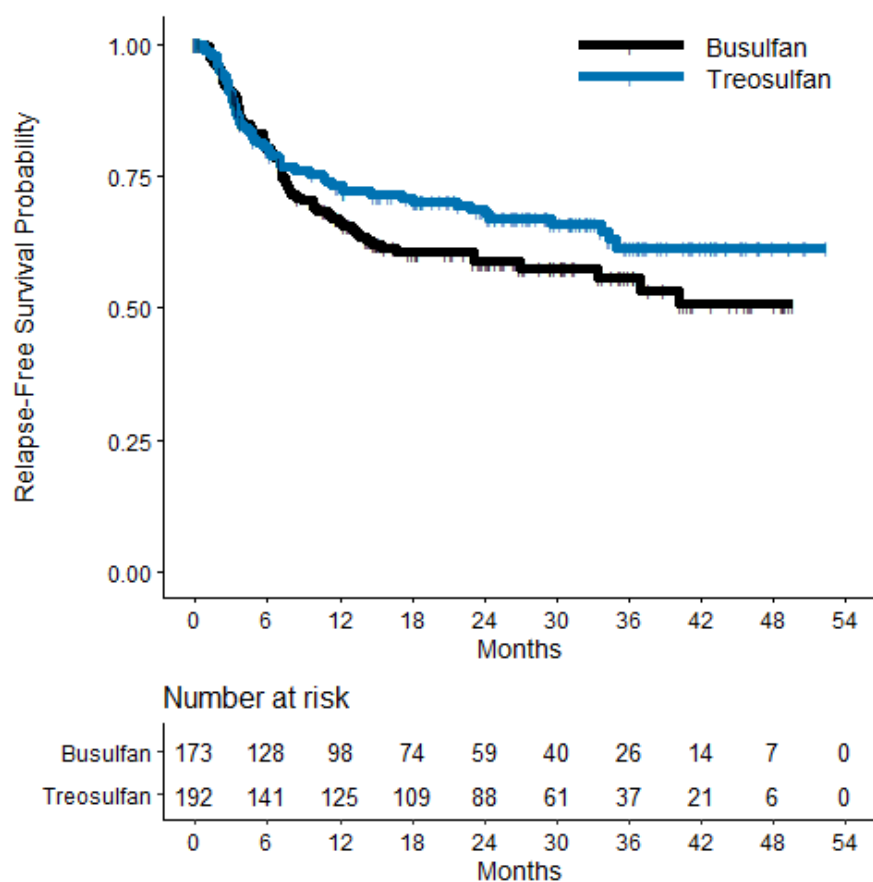
¹ Based on a Cox proportional hazards model stratified by donor type and risk group

CI = Confidence Interval

Database lock date: 16 March 2018

Source: FDA Analysis

Figure 11. Study MC-FludT.14/L Trial II Relapse-Free Survival in AML Kaplan-Meier Plot



Database lock date: 16 March 2018
 Source: FDA Analysis

8.1.3 Integrated Review of Effectiveness

Methods

The Applicant proposed an indication for treosulfan "for use in combination with fludarabine as a preparative regimen for allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult and pediatric patients > 1 year old with acute myeloid leukemia (AML)" and "...with myelodysplastic syndrome (MDS)." The proposed dose of treosulfan was of 10 g/m²/dose for use in the combination described. (b) (4)

The clinical development program in AML and MDS included two randomized control trials, three single-arm trials, one dose-escalation study, and two registry studies (See Table 35 in FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021)). All clinical trials were conducted outside the US. The pivotal trial MC-FludT.14/L Trial II was relevant to determining efficacy in the intended population. In addition, MC-FludT.14/L Trial I and MC-FludT.6/L were used to support the proposed 10 g/m² treosulfan dose. Other studies included in the submission were reviewed but were not considered to have contributed to assessment of efficacy for the proposed indications. See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for full list of studies and limitations of those studies.

MC-FludT.14/L Trial II

Overview

The Applicant proposed to use Study MC-FludT.14/L Trial II (Trial 14 - II; treosulfan 10 g/m²/dose) to support the proposed indication. Trial 14 - II was a multicenter, randomized, active-controlled trial comparing treosulfan to busulfan in combination with fludarabine as a preparative regimen for allogeneic HSCT. Eligible patients included adults 18 to 70 years old with AML or MDS, Karnofsky performance status > 60%, and age ≥ 50 years or hematopoietic cell transplantation comorbidity index [HCT CI] score > 2. The patients were randomized to receive treosulfan 10 g/m² daily on days -4, -3 and -2 or to busulfan 0.8 mg/kg every 6 hours on days -4 and -3 in combination with fludarabine 30 mg/m² daily on days -6, -5, -4, -3 and -2, and hematopoietic stem cell transplantation on day 0. Antithymocyte globulin was administered as well to patients with unrelated donors.

In general, a randomized controlled trial with a survival-based endpoint is appropriate to support an indication for a drug in a preparative regimen for allogeneic HSCT. FDA frequently requires two trials to determine efficacy, but one trial might be sufficient if the trial is adequately designed and well-conducted, and the results are internally consistent, clinically meaningful, and statistically persuasive.

Overview of Issues with Data Collection

The following issues regarding data collection were noted in the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021):

- “The primary endpoint of Study MC-FLudT-14/L Trial II was event-free survival (EFS). Because all complete blood cell (CBC) and marrow results were not submitted, we were unable to independently confirm the results of the analysis of the primary endpoint of EFS for Study MC-FLudT-14/L Trial II.
- Additionally, because of the missing CBC and marrow results, we were not able complete our assessment of whether the results of the study would support the indications for treatment of patients with AML and for treatment of patients with MDS.”

Additional data were submitted by the Applicant in the current resubmission. FDA adjudicated time-to-event outcomes based on these data. Relapse was confirmed based on the laboratory, bone marrow, and source documentation data resulting in updates to either the time of relapse or the occurrence of relapse for some patients.

The extent of the submitted data is limited. Per Complete Response to the FDA Complete Response Letter 30 July 2021, page 10: "... the collection of some of the marrow data was considered not mandatory under the study protocol MC-FludT.14/L Trial II, particularly beyond Day +100 and in case of lack of an event. However, in some instances, bone marrow was collected beyond Day +100 and details of these assessments are provided." Given the lack of reliable data collection beyond Day 100 (and "in case of lack of an event"), outcomes relying on such data beyond Day 100 might be challenging to interpret.

Overview of Issues with Trial Design

FDA noted several issues with the design of Study MC-FludT.14/L Trial II. These include:

- Inclusion of two distinct clinical populations, MDS and AML, without a pre-specified plan to formally test each subpopulation.
- The definition of the primary endpoint EFS, which included non-efficacy events such as graft failure and whose applicability to the AML and MDS subpopulations is unclear.
- The primary objective of non-inferiority for EFS, with a NI margin lacking sufficient justification.
- Unusual choices for the primary analysis, including use of an analysis population of FAS rather than the ITT and inclusion of a high number of stratification levels (due to inclusion of site as a stratification factor).
- Lack of a pre-specified testing plan for OS.
- Multiple early interim analyses for efficacy which limited the overall available information on secondary endpoints.

- Study conduct based solely on the NI test of EFS, which precluded further enrollment and follow-up for other key analyses and endpoints (e.g., the superiority test of EFS and analyses related to OS).
- Per the unireview dated 30 Jul 2021 regarding assessment of response in patients with MDS: “results of marrow examinations which would allow for assessment of response were not collected systematically, so response cannot be assessed.”

Overview of Endpoint Definitions

Table 18 shows differences in definitions for the main outcomes of interest. FDA relied primarily on the outcomes listed in the *Definitions per FDA* column for the benefit-risk assessment. Furthermore, while Study MC-FludT.14/L Trial II utilized the FAS for efficacy analyses, FDA relied on the ITT population and subsets thereof (namely, the AML and MDS subpopulations of the ITT).

Table 18. Study MC-FludT.14/L Trial II Outcome Definition Differences

Outcome	Definition per SAP	Definition per FDA
EFS		
Designation	EFS (within 24 months post-transplantation)	EFS (post-randomization)
Day zero	HSCT	Randomization
Events	As submitted by the Applicant	Adjudicated by FDA
Variables	Risk group, donor type, center	Risk group, donor type
OS		
Designation	OS (within 24 months post-transplantation)	OS (post-randomization)
Day zero	HSCT	Randomization
Variables	Risk group, donor type, center	Risk group, donor type
RFS		
Designation	NA	RFS
Day zero	NA	Randomization
Events	NA	Adjudicated by FDA
Variables	NA	Risk group, donor type

Source: FDA Analysis

FDA used a modified statistical analysis plan for assessing time-to-event outcomes. The results reported herein use a Cox proportional hazards model stratified by donor type and risk group only. See Section 8.3: Statistical Issues for further details.

Overview of Efficacy Outcomes per FDA Definition

Table 19 shows hazard ratios and 95% confidence intervals for selected outcomes as defined by FDA in Table 18 across key populations of interest.

Table 19. Study MC-FludT.14/L Trial II Efficacy Summary Across FDA-Defined Outcomes and Populations

Outcome	ITT (AML and MDS) HR (95% CI)	AML HR (95% CI)	MDS HR (95% CI)
EFS (post-randomization)	0.65 (0.50, 0.85)	0.69 (0.49, 0.95)	0.60 (0.38, 0.94)
OS (post-randomization)	0.67 (0.51, 0.90)	0.73 (0.51, 1.06)	0.64 (0.40, 1.02)
RFS	NA	0.72 (0.51, 1.03)	NA

Note: Hazard ratios and 95% Confidence Intervals, based on Cox proportional hazards model stratified by risk group and donor type.

Database lock date: 16 Mar 2018

Source: FDA Analysis

The results show favorable outcomes for treosulfan compared to busulfan in the ITT population across multiple efficacy endpoints as discussed below.

Primary Endpoint

Event-Free Survival

Study MC-FludT.14/L Trial II was stopped early for efficacy at 35% (168/481 planned events in the FAS population) information fraction of EFS. Early stopping for efficacy might result in an overestimation of a treatment effect. The hazard ratio for EFS, as performed at the *interim* analysis was 0.65 (95% CI: 0.47, 0.90). The p-value for non-inferiority testing was 0.0000164, which was significant as compared to the efficacy boundary of 0.000149. The p-value for superiority testing was 0.0051268, which did not cross the efficacy boundary. Results for EFS at the *last* analysis suggested the p-value would have crossed the corresponding superiority boundary, though this analysis was ad-hoc and the corresponding boundary hypothetical in nature.

Per the statistical analysis plan, the primary efficacy endpoint was “event-free survival (EFS) within 2 years after transplantation measured from time of end of HSCT (= day 0) to time of event. The associated time span is defined as the interval from day 0 to relapse, graft failure or death (whatever occurs first). The date of the event is either the documented date of relapse/progression or the date of death or the date of primary (date of examination of neutrophilic granulocytes) or secondary graft failure (date of examination of neutrophilic granulocytes).” This is not a standard definition used for regulatory considerations in AML or MDS populations. Per FDA's Acute Myeloid Leukemia: Developing Drugs and Biological Products for Treatment Guidance for Industry, EFS is defined as the "time from randomization to the date of: induction treatment failure, relapse for those who have induction treatment success (e.g., complete remission), or death from any cause, whichever comes first". Interpretation of the results based on the protocol-defined EFS outcome may be challenging.

Has Study MC-FludT.14/L Trial II reached its primary objective based on the event-free survival outcome, as adjudicated by the FDA?

Grafapex (treosulfan)

Study MC-FludT.14/L Trial II reached its primary objective of demonstrating that EFS (post-transplantation, within 2 years) outcomes were non-inferior on the treosulfan arm compared to the busulfan arm. Data were submitted in support of adjudication of this endpoint for this submission. After adjudication, FDA found that the results for EFS as originally reported were largely consistent with those that were adjudicated.

Results were similar for other definitions of EFS. The hazard ratio for EFS (post-randomization, without time restriction) was 0.65 (95% CI: 0.50, 0.85), indicative of a positive treatment effect of treosulfan compared to busulfan.

As noted above, EFS as defined per protocol includes both safety and efficacy events and is not aligned with FDA guidance. Furthermore, FDA guidance for the treatment of AML states that EFS may be considered as an efficacy endpoint in populations not in remission, but that RFS is the appropriate for populations that are in remission at baseline. As the AML subpopulation was in remission at baseline and the MDS subpopulation was not, any conceptual “event-free” survival endpoint would likely differ for these two subpopulations. Therefore, EFS results in the enrolled population has no clear interpretation. (b) (4)

Secondary and Other Endpoints

Study MC-FludT.14/L Trial II was stopped at the second interim analysis based on a significant test of EFS non-inferiority. While superiority of EFS was an alpha-controlled, secondary objective, the associated statistical test did not meet significance at the second interim analysis. Furthermore, OS was not specified as an endpoint to be tested. For trials with important secondary objectives, a common design is one which continues to follow for the secondary objective even if the primary objective is met. This approach was not utilized in Study MC-FludT.14/L Trial II. Thus, the results of secondary endpoints must be considered in light of these limitations.

Overall Survival

For Study MC-FludT.14/L Trial II, the analysis of OS was considered exploratory. FDA assessed OS in the ITT population with using the full follow-up period. No alpha-controlled hypothesis testing was prespecified for the overall survival or for testing AML and MDS subgroups. Not controlling for multiplicity might result in erroneous conclusions.

Per statistical analysis plan, OS is defined as “the time length between day 0 and the date of death due to any cause within the 24-month study period (i.e. excluding post-surveillance evaluation).” Day zero is the time of allogeneic hematopoietic stem cell transplantation. This is not a standard OS definition used for regulatory purposes, which typically uses 1) randomization as the start of the period of interest 2) has no restriction on follow-up (i.e., no limit set to 24 months). Since the OS is both safety and efficacy endpoint, the regulatory interest is in the full unrestricted survival period starting from randomization, referred to OS (post-randomization) herein.

Has Study MC-FludT.14/L Trial II demonstrated an advantage on overall survival and other efficacy outcomes for the treosulfan study group over the busulfan study group?

Table 19 shows hazard ratios and 95% confidence intervals for the OS in the ITT population as well as in AML and MDS populations separately. These results are based on the final CSR with database lock 16 Mar 2018. Based on the reverse Kaplan-Meier estimate, median follow-up time for OS (post-randomization) was 29.6 months (range: 0, 54.5) in the busulfan group and 29.5 months (range: 0, 52.5) in the treosulfan group. The hazard ratio for OS (post-randomization) was 0.67 (95% CI: 0.51, 0.90) in the ITT population, demonstrating an OS advantage in the treosulfan group compared to the busulfan group. The upper bounds of the 95% confidence intervals slightly exceed one in the AML and in the MDS subgroups, suggesting that some residual uncertainty regarding efficacy in these subgroups remains. Nonetheless, the results for patients with AML and MDS are consistent with the overall results of the study.

In Study MC-FludT.14/L Trial II, treatment with treosulfan-based conditioning resulted in improved OS compared to busulfan-based conditioning in the ITT population. Since FDA verified that Study MC-FludT.14/L Trial II was a positive study based on its EFS primary endpoint, FDA could consider the OS endpoint as an acceptable endpoint for assessment of the efficacy of treosulfan for use in transplant conditioning. Unlike EFS, OS was not subject to the same interpretation issues based on the events included in the EFS definition and incomplete capture of follow up monitoring. Moreover, since monitoring for OS is simpler, there are fewer concerns for missed events compared to an EFS endpoint. The timing of OS events is unambiguous compared to EFS events and because patients can be reliably censored at the time of last follow up/known well without concern for a missed event occurring prior to that last follow up encounter.

Overall survival is both safety and efficacy endpoint and remains the gold standard in clinical trials. The results show a substantial treatment effect on the overall survival in the full study population. However, alpha-controlled hypothesis testing was not prespecified. Therefore, p-values should not be included in the USPI. To communicate the full survival functions in treosulfan and busulfan groups in patients with AML and, separately, in patients with MDS, including Kaplan-Meier plots in the USPI is recommended, along with the treatment effect point estimates and confidence intervals.

Additional Efficacy Endpoints

The Applicant prespecified several secondary endpoints discussed in the original review, but these largely consisted of safety endpoints or endpoints that could not be used in support of the OS results to demonstrate the effectiveness of treosulfan for treatment of AML or MDS.

For treatment of patients with AML transplanted in remission, RFS would be the usual endpoint to support the OS findings. To investigate RFS in Study MC-FludT.14/L Trial II, FDA reviewed the available EFS data and determined how to appropriately convert the Applicant's EFS analysis into a traditional RFS analysis. This showed that the hazard ratio for RFS in the AML patients

was 0.72 (95% CI: 0.51, 1.03). This is also well-captured by the RFS at 24 months which showed estimates of 59% (95% CI: 50%, 55%) versus 69% (95% CI: 61%, 75%) for busulfan and treosulfan, respectively. This suggests that when focused on the RFS events of hematologic relapse or death, that treatment with treosulfan showed a lower risk of these events compared to treatment with busulfan further supporting the findings of efficacy in the AML population demonstrated in the OS analysis.

For treatment of MDS not in remission, EFS (defined appropriately) or CR that is durable might be suitable to assess activity. However, because CBC and marrow examination data were not collected systematically to assess achievement of remission in this protocol, achievement of CR and/or treatment failure as an event for EFS cannot be assessed.

Subpopulations

Has Study MC-FludT.14/L Trial II demonstrated favorable benefit-risk for the proposed indications for patients with AML and for patients with MDS?

Disease

FDA considers treatment of AML and MDS to be two distinct indications which would typically warrant separate sources of substantial evidence of effectiveness to support approval. Study MC-FludT.14/L Trial II enrolled both populations with no plan to formally assess them separately. In addition, the sample size for each subpopulation was not based on power considerations or to ensure a certain number of patients in each subgroup. These features limited the ability Study MC-FludT.14/L Trial II to establish substantial evidence for either disease alone.

However, FDA considered it may be reasonable to assess efficacy in a combined ITT population of AML and MDS provided the endpoint used had equivalent interpretation for both diseases, which the OS analysis provided. For trials with such mixed populations, it is critical that a sufficient number of patients in each subpopulation be enrolled so that differential treatment effects can be identified and described. This appears to have been the case for Study MC-FludT.14/L Trial II, which enrolled 365 patients with AML and 205 patients with MDS.

As noted above, the definition and relevance of EFS to each setting is unclear. While the treatment effect on EFS appears to be similar in the two subpopulations, FDA does not consider this as direct evidence of effectiveness overall or for either subpopulation. FDA found the OS results to be similar across the two populations, with a hazard ratio of 0.73 (95% CI: 0.51, 1.06) for the AML population and 0.64 (95% CI: 0.40, 1.02) for the MDS population (Table 19). Taken together, these observations suggest 1) that the positive result observed in Study MC-FludT.14/L Trial II was not driven by either the AML or MDS subgroup and 2) that the observed treatment effect on OS was not driven by either the AML or MDS subgroup.

Given these considerations, FDA considers Study MC-FludT.14/L Trial II to have demonstrated

favorable benefit-risk for the proposed indications for patients with AML and for patients with MDS.

Race and Ethnicity

Data on race and ethnicity were not collected for any of the studies included in this NDA. This deficiency leaves some uncertainty about the generalizability of the efficacy outcomes with respect to the US population. While the results from the European region of multiregional trials have generally been similar to North American results (Wilson, J NCCN, 2022) and, based on treosulfan's mechanism of action as an alkylating agent, there is no reason to believe that the benefit-risk profile demonstrated in Study MC-FludT.14/L Trial II would be expected to differ by racial or ethnic background, the lack of racial or ethnic reporting in this application did not allow for assessment of efficacy or safety results by race and ethnicity. Additional information is needed to ensure there are no unexpected differences in the benefit-risk profile in US racial and ethnic groups. To address this, the Applicant has agreed to assess the PK and exposure profile and registry-based outcomes by race and ethnicity in PMC #1 and PMC #2, respectively.

Donor Type

In MC-FludT.14/L Trial II, 76% of patients had unrelated donors and 24% had related donors. Table 20 shows OS (post-randomization) by donor type.

Table 20. Study MC-FludT.14/L Trial II OS (post-randomization) by Donor Type

Subgroup	ITT (AML + MDS) HR (95% CI)	AML HR (95% CI)	MDS HR (95% CI)
Related Donor	1.16 (95% CI: 0.68, 2.0)	1.36 (95% CI: 0.71, 2.6)	0.82 (95% CI: 0.31, 2.2)
Unrelated Donor	0.59 (95% CI: 0.42, 0.84)	0.6 (95% CI: 0.38, 0.94)	0.65 (95% CI: 0.38, 1.1)

Note: Results are based on an unstratified Cox proportional hazards model

Database lock date: 16 March 2018

Source: FDA Analysis

As noted during the original review, the treatment effect appears to be driven by the subpopulation of patients with unrelated donors, and the treatment effect for this subset appears consistent whether they have AML or MDS. For patients with related donors, the HR is greater than one, and the results are inconsistent across disease subgroups. The biological basis for this observation is unclear. Higher HR estimates for the MRD group in the AML subpopulation are likely due to a few influential events observed after two years of follow-up (e.g., see Figure 8). Regarding the MRD group in the MDS subpopulation, since less than 10 events in each treatment group were observed in this subgroup, the results are likely unreliable due to few observed events. There is insufficient information to assess the potential lack of effectiveness. The results should be interpreted with caution.

Dose/Dose Response

Study MC-FludT.6/L

Excerpted from FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021):

Study MC-FludT.6/L was a multicenter single-arm dose-escalation trial to test the tolerability of treosulfan and fludarabine as a preparative regimen for allogeneic HSCT from an HLA-matched sibling, 1 antigen-mismatched sibling, or HLA-matched unrelated donor. Eligible patients were ≥ 18 years old with a "chemosensitive" hematological malignancy indicated for HSCT but who were considered at high risk for toxicities with standard MAC regimens. Patients were treated in sequential cohorts (n=15 each) at treosulfan dose levels of 10 gm/m², 12 gm/m², or 14 gm/m². The aim of the protocol was to determine a dose and schedule for later trials. The primary efficacy endpoint was hematopoietic recovery. Overall survival was an additional endpoint.

Table 21. Study MC-FludT.6/L: Selected Outcomes

	Percentage of Patients* with Outcome by Treosulfan Dose Used in Combination with Fludarabine		
Outcome	10 gm/m ² (N=20)	12 gm/m ² (N=18)	14 gm/m ² (N=17)
Day-28 ANC > 0.5 Gi/L	100%	100%	94%
Day-28 platelets > 50 Gi/L	70%	94%	65%
No secondary graft failure	100%	100%	100%
Day-100 complete chimerism	95%	94%	93%
1-year overall survival	70%	78%	59%

Source: Study MC-FludT.6/L Clinical Study Report Tables 11.4.1.1C, 11.4.1.1E, 11.4.1.1A, 11.4.1.2A and Figures 11.4.1.4A and 11.4.1.6A.

*Percentage of patients with available data.

A total of 55 patients were treated on this study, including 19 with AML and 6 with MDS. As discussed in Section 8.1.4 of the original NDA review, hematopoietic recovery and high-grade donor chimerism were achieved at all dose levels, and the 1-year OS was worst in the 14 g/m²/day x 3 cohort. Despite these findings, the Applicant chose the 14 g/m²/day x 3 regimen for the initial pivotal trial, MC-FludT.14/L Trial I.

Study MC-FludT.14/L Trial I

Adapted from FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) and updated based on current review of MC-FludT.14/L Trial II

Study MC-FludT.14/L Trial I was the initial pivotal trial which randomized patients to treosulfan-based conditioning therapy with a dose of 14 g/m²/day x 3 to busulfan-based reduced-intensity conditioning therapy in adults with AML or MDS. As discussed in FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) Sections 8.1.2 and 8.3.5, this study was terminated early due to delayed hematopoietic recovery and increase early treatment-related mortality. FDA's analysis of the final data cut (1-year follow-up) showed no evidence of a treatment effect for the overall population or when assessed individually for patients with AML

or with MDS. The second pivotal trial, MC-FludT.14/L Trial II, commenced using the lower treosulfan dose of 10 g/m²/day x 3. Table 22 shows the results of selected efficacy outcomes in ITT population of MC-FludT.14/L Trial I and Trial II.

Table 22. Analyses of OS vs Busulfan by Treosulfan Dose

Outcome	Population	Treosulfan Dose	
		MC-FludT.14/L Trial I 14 g/m ² /day x 3 days	MC-FludT.14/L Trial II 10 g/m ² /day x 3 days
Hazard ratio for OS (Treosulfan vs busulfan)	ITT	1.15 (0.78, 1.69)	0.67 (0.51, 0.90)
	AML	1.27 (0.81, 2.01)	0.73 (0.51, 1.06)
	MDS	0.99 (0.40, 2.45)	0.64 (0.40, 1.02)
OS at 1 year (Treosulfan arm vs busulfan arm)	ITT	67% vs 75%	79% vs 72%
	AML	66% vs 76%	81% vs 74%
	MDS	68% vs 70%	75% vs 68%

Source: Adapted from FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) and updated based on FDA analysis of MC-FludT.14/L Trial II in this review

The results suggest that the effectiveness for the treosulfan dose of 10 g/m²/day x 3 days in comparison to busulfan x 2 days is better than that for the treosulfan dose of 14 g/m²/day x 3 days for the ITT population and for patients with either AML or MDS.

Clinical TL Comment: Clinical evidence from Study MC-FludT.6/L support the benefit of treosulfan for use as part of a preparative regimen. The 1-year OS outcomes for patients with hematologic malignancies who received the treosulfan-based preparative regimen at 10 g/m²/dose were consistent with those in the treosulfan arm of the pivotal trial. Although FDA does not usually use landmark OS in a single-arm trial to support efficacy due to concerns regarding the potential for bias, the findings in MC-FludT.6/L were reproducible in the two lower dose cohorts in the study (see Table 21). Additionally, the results of MC-FludT.6/L were reproducible across studies (see Table 21 and Table 22): 1-year OS in the 10 g/m² cohort of MCFludT.6/L was consistent with that of the treosulfan arm of MC-FludT.14/L Trial II and similarly, 1-year OS in the 14 g/m² cohort was consistent with the results in the treosulfan arm of MC-FludT.14/L Trial I. Together, the consistency of these findings reduces concerns of bias and increase the confidence that the observed OS outcomes in MC-FludT.6/L reflect the clinical benefit of 10 g/m²/dose of treosulfan as part of a preparative regimen. Furthermore, the results of these studies also support a dose-dependent effect of treosulfan on OS, with worse OS seen with the treosulfan 14 g/m²/dose compared to lower doses of treosulfan in MC-FludT.6/L and compared to the busulfan regimen in MC-FludT.14/L Trial I.

Additional Efficacy Considerations

Extrapolation to Pediatric Patients

As discussed in the original unireview, [REDACTED]

(b) (4)

Grafapex (treosulfan)

(b) (4) MC-FludT.16/NM was a phase 2 study for nonmalignant diseases that randomized patients to a 14 g/m²/dose equivalent of treosulfan or standard busulfan. Because this was not performed in a disease that is being considered for an indication for treosulfan, it was not evaluated for efficacy. The second study, MC-FludT.17/M, was a nonrandomized study for pediatric patients with malignant diseases; all patients received a 14 g/m²/dose equivalent of treosulfan. (b) (4)

8.1.4 Integrated Assessment of Effectiveness

The results of MC-FludT.14/L Trial II demonstrate the efficacy of 10 g/m²/dose treosulfan as a component of a preparative regimen prior to allogeneic HSCT. Patients not eligible for myeloablative conditioning were randomized to either treosulfan-based or busulfan-based reduced intensity conditioning. Patients who received treosulfan-based conditioning had longer OS compared to patients who received busulfan-based conditioning (HR 0.67 [95% CI: 0.51, 0.90]). These findings are supported by clinical evidence from Study MC-FludT.6/L where the 1-year OS outcome in patients who received the 10 g/m²/dose treosulfan-based preparative regimen was similar to that in the treosulfan arm of MC-FludT.14/L Trial II. Although FDA does not usually use landmark OS in a single-arm trial to support efficacy given the potential for bias, the OS findings in MC-FludT.6/L were reproducible in the two lower dose cohorts and were consistent with the OS results for patients treated with treosulfan across two other trials. The consistency of these findings reduces concerns for bias and supports the conclusion that the 10 g/m²/dose treosulfan is effective as part of a preparative regimen.

Extrapolation of efficacy to the pediatric population is reasonable based on the biology of AML and MDS and the mechanism of action of treosulfan as an alkylating agent. (b) (4)

(b) (4) the recommended dose for pediatric patients with AML or MDS is 10 g/m²/dose of treosulfan based on a full extrapolation of efficacy from MC-FludT.14/L Trial II and Population PK approach. (b) (4)

Treosulfan-based preparative regimens prior to allogeneic HSCT in patients with AML or MDS result in a survival advantage compared to busulfan-based preparative regimens; however, it is not clear where treosulfan will be placed in clinical practice. The recommended indication for treosulfan (10 g/m²/dose) is: “for use in combination with fludarabine as a preparative regimen for allogeneic HSCT in adult and pediatric patients 1 year of age and older with AML (1.1) [or] MDS (1.2).”

Review of Safety

Please refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for the original review of safety. No new safety information has been submitted in response to the Complete Response by the Applicant.

Selected analyses supporting safety information included in labeling are excerpted from FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) and presented below.

8.2.1 Safety Review Approach

No new safety data were included in this resubmission. See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for detailed safety review approach.

Studies MC-FludT.6/L, MC-FludT.7/AML, MC-FludT.8/MDS, MC-FludT.14/L I, MC-FludT.14/L II, MC-FludT.16/NM, and MC-FludT.17/M were included in the integrated summary of safety submitted by the Applicant. We performed the safety analysis on MC-FludT.6L, MC-FludT.7/AML, MC-FludT.8/MDS, MC-FludT.14/L I, and MC-FludT.14/L II for the adult population, and on MC-FludT.16/NM and MC-FludT.17/M for the pediatric safety analysis.

Summaries of data and safety analyses by the clinical reviewer were performed using JMP version 15.0 (SAS Institute, Inc., Cary, NC) and MedDRA Adverse Events Diagnostic (MAED, FDA, Silver Spring, MD), versions 2.5 (through 3/21/2021) and 3.0 (after 3/21/2021). Summaries of laboratory data were performed using JMP version 15.0 and Palantir (Palantir Technologies, Inc., Denver, CO, ©2017-2021). Postmarket analysis was conducted using Empirica Signal (Oracle, Santa Clara, CA, ©2014).

8.2.2 Review of the Safety Database

Overall Exposure

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for details on overall exposure.

Adequacy of the safety database

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for review of adequacy of the safety database.

8.1.5 Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for review.

Categorization of Adverse Events

Adverse events were reported down to the verbatim term. The adverse events were coded using MedDRA version 20.0 for all studies and for the pooled safety population. Where indicated in the tables or text, some adverse events are presented as grouped terms. Regimen-related toxicity (RRT) is defined as directly caused by the conditioning regimen. Bearman's RRT criteria are used unless otherwise specified. Treatment-emergent adverse events (TEAEs) included events that started from the start of study drug administration through HSCT day +30, unless otherwise indicated.

Table 23. Grouped Terms used in Safety Analyses

Grouped Term	Basis of Grouping
Bacterial infection	HLGT Bacterial infectious disorders
Fungal infection	HLGT Fungal infectious disorders
Infection	HLGT Infections - pathogen unspecified
Viral infection	HLGT Viral infectious disorders
Fatigue	HLT Asthenic conditions
Dyspnoea	HLT Breathing abnormalities
Diarrhoea	HLT Colitis (excl infective)
Diarrhoea	HLT Diarrhoea (excl infective)
Rash	HLT Erythemas
Abdominal pain	HLT Gastrointestinal and abdominal pains (excl oral and throat)
Oedema	HLT Oedema NEC
Rash	HLT Rashes, eruptions and exanthems NEC
Renal impairment	HLT Renal failure and impairment
Renal impairment	HLT Renal function analyses
Stomatitis	HLT Stomatitis and ulceration
Cystitis noninfective	PT Custom Cystitis noninfective - Cystitis haemorrhagic, Cystitis noninfective, Haematuria
Encephalopathy	PT Custom Encephalopathy - Agitation, Aphasia, Ataxia, Cognitive disorder, Confusional state, Delirium, Depressed level of consciousness, Disturbance in attention, Encephalopathy, Epilepsy, Hallucination, Lethargy, Memory impairment, Posterior reversible encephalopathy syndrome, Restlessness, Seizure, Somnolence
Hepatotoxicity	PT Custom Hepatotoxicity - Blood bilirubin increased, Hepatic failure, Hepatic pain, Hepatocellular injury, Hepatomegaly, Hepatorenal syndrome, Hepatosplenomegaly, Hepatotoxicity, Jaundice, Ocular icterus, Veno-occlusive liver disease
Hypersensitivity	PT Custom Hypersensitivity - Anaphylactic reaction, Hypersensitivity
Musculoskeletal pain	PT Custom Musculoskeletal pain - Arthralgia, Back pain, Bone pain, Flank pain, Limb discomfort, Musculoskeletal chest pain, Myalgia, Neck pain, Non-cardiac chest pain, Pain in extremity, Pain in jaw, Spinal pain
Tachycardia	PT Custom Tachycardia - Atrial fibrillation, Atrial flutter, Atrial tachycardia, Sinus tachycardia, Supraventricular tachycardia, Tachycardia
Cardiac failure	SMQB Cardiac failure excluding oedema
Haemorrhage	SMQB Haemorrhage terms (excl laboratory terms)

Embolism	SMQN Embolic and thrombotic events excluding VOD
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Source: Standard Grouped Terms, Reviewer's Grouped Terms

Routine Clinical Tests

8.1.6 Safety Results

Deaths

In the full safety database, there were 14 deaths within 30 days of the last dose of treosulfan and 57 deaths (43 additional deaths) within 100 days of the last dose of treosulfan.

In MC-FludT.14/L Trial II, there were 4 deaths (1.5%) from fatal adverse reactions due to treosulfan by Day +30; there were two additional deaths that were adjudicated as transplant-related mortality. In the busulfan arm, there were two deaths prior to Day +30, both of which were adjudicated as adverse reactions due to busulfan. In the treosulfan arm, there were 17 deaths (6%) by transplant day +100, compared to 19 deaths (7%) by transplant day + 100 in the busulfan arm. However, we note that for six patients, we did not have sufficient information to adjudicate the cause of death.

Serious Adverse Events

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Dropouts and/or Discontinuations Due to Adverse Effects

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Significant Adverse Events

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Treatment Emergent Adverse Events and Adverse Reactions

When evaluating TEAEs by Preferred Term (PT), the most common TEAEs were those expected after allo-HSCT. The most common adverse reactions ($\geq 10\%$) on the treosulfan arm of MC-FludT.14/L Trial II included musculoskeletal pain, stomatitis, pyrexia, nausea, edema, infection, vomiting, rash, diarrhea, headache, febrile neutropenia, abdominal pain, hypertension, hemorrhage, fatigue, constipation, tachycardia, and hepatotoxicity. The most common grade 3-5 ARs ($\geq 10\%$) on the treosulfan arm were febrile neutropenia and infection. Rare but potentially serious events that occurred on the treosulfan arm of MC-FludT.14/L Trial II included ventricular arrhythmias (including tachycardia and extrasystoles; N = 5, 1.852%), erythema multiforme (N = 8, 2.963%), palmar-plantar erythrodysaesthesia syndrome (N = 5, 1.852%), and cardiac failure (N = 8, 2.963%).

Table 24. Treatment Emergent Adverse Events by Preferred Term* in >5% of Treosulfan-Treated Patients through Day +30, MC-FludT.14/L Trial II

Preferred Term*	All Grades				Grades 3-5			
	Treosulfan N = 270		Busulfan N = 283		Treosulfan N = 270		Busulfan N = 283	
	N	%	N	%	N	%	N	%
Musculoskeletal pain	105	38.889	75	26.502	14	5.185	7	2.473
Stomatitis	102	37.778	135	47.703	16	5.926	21	7.42
Pyrexia	93	34.444	101	35.689	2	0.741	9	3.18
Nausea	89	32.963	116	40.989	8	2.963	17	6.007
Oedema	77	28.519	51	18.021	2	0.741	4	1.413
Infection	63	23.333	51	18.021	35	12.963	20	7.067
Vomiting	59	21.852	55	19.435	2	0.741	4	1.413
Rash	46	17.037	37	13.074	3	1.11	4	1.413
Diarrhoea	45	16.667	52	18.375	4	1.481	4	1.413
Headache	44	16.296	52	18.375	3	1.11	2	0.707
Febrile neutropenia	40	14.815	31	10.954	40	14.815	31	10.954
Abdominal pain	40	14.815	38	13.428	7	2.592	5	1.767
Hypertension	38	14.074	60	21.201	21	7.778	27	9.541
Haemorrhage	37	13.704	40	14.134	4	1.481	3	1.06
Fatigue	34	12.593	42	14.841	3	1.11	1	0.353
Constipation	33	12.222	33	11.661	1	0.37	0	0
Tachycardia	27	10	15	5.3	4	1.481	6	2.12
Hepatotoxicity	27	10	22	7.774	10	3.704	9	3.18
Renal impairment	24	8.889	24	8.481	5	1.852	1	0.353
Decreased appetite	24	8.889	26	9.187	5	1.852	4	1.413
Chills	20	7.407	16	5.654	1	0.37	0	0
Hypotension	19	7.037	12	4.24	5	1.852	5	1.767
Weight increased	19	7.037	18	6.36	0	0	0	0
Pruritus	17	6.296	12	4.24	1	0.37	0	0
Dizziness	17	6.296	14	4.947	0	0	1	0.353
Pain	16	5.926	8	2.827	1	0.37	0	0
Encephalopathy	16	5.926	17	6.007	5	1.852	3	1.06
Viral infection	16	5.926	20	7.067	4	1.481	5	1.767
Hypersensitivity	16	5.926	22	7.774	2	0.741	1	0.353
Dyspnoea	15	5.556	23	8.127	2	0.741	4	1.413

Source: Reviewer's analysis, MAED, ISS population (ADSL, ADAEGT2 datasets)

*Includes grouped terms

Laboratory Findings

As treosulfan is used as part of a conditioning regimen for allogeneic HSCT, cytopenias occur in nearly 100% of patients. Other laboratory abnormalities are listed below. There were no significant differences in the maximum grades of laboratory abnormalities between the treosulfan and busulfan arms of MC-FludT.14/L Trial II.

Table 25. Selected Grades 3-4 Laboratory Abnormalities through Transplant Day +30 in Study MC-FludT.14/L Trial II

Laboratory Abnormality	Treosulfan N = 270 %	Busulfan N = 283 %
Gamma Glutamyl Transferase Increased	16	28
Bilirubin Increased	6	5
Alanine Aminotransferase Increased	6	4
Aspartate Aminotransferase Increased	4	1
Creatinine Increased	3	0.7

Source: FDA Review, ISS population

Vital Signs, Electrocardiograms (ECGs), QT, and Immunogenicity

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

8.2.5 Analysis of Submission-Specific Safety Issues

Treosulfan Dose (14 g/m²)

Trial MC-FludT.14/L Trial I used a treosulfan dose of 14 g/m² for patients randomized to receive treosulfan. This study was stopped early due to increased transplant-related morbidity and mortality observed in the treosulfan arm. Treatment-emergent adverse events through HSCT day +30 are shown in Table 26. There were increased TEAEs of hepatotoxicity, stomatitis, musculoskeletal pain, infection, and febrile neutropenia in the treosulfan cohort, including grades 3-5 TEAEs.

Table 26. Treatment Emergent Adverse Events by Preferred Term* in >5% of Treosulfan-Treated Patients through Day +30, MC-FludT.14/L Trial I

Preferred Term*	All Grades				Grades 3-5			
	Treosulfan N = 168		Busulfan N = 152		Treosulfan N = 168		Busulfan N = 152	
	N	%	N	%	N	%	N	%
Hepatotoxicity	108	64.29	72	47.37	22	13.1	12	7.89
Nausea	108	64.29	74	48.68	10	5.95	6	3.95
Stomatitis	105	62.5	105	69.08	18	10.71	25	16.45
Musculoskeletal pain	101	60.12	56	36.84	26	15.48	11	7.24
Fatigue	83	49.4	66	43.42	10	5.95	5	3.29
Diarrhoea	71	42.26	44	28.95	12	7.14	3	1.97
Oedema	68	40.48	50	32.89	3	1.79	2	1.32
Vomiting	66	39.29	39	25.66	3	1.79	0	0

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Grafapex (treosulfan)

Febrile neutropenia	64	38.1	44	28.95	63	37.5	42	27.63
Haemorrhage	58	34.52	49	32.24	5	2.98	3	1.97
Infection	57	33.93	40	26.32	38	22.62	17	11.18
Rash	51	30.36	34	22.37	4	2.38	2	1.32
Abdominal pain	47	27.98	30	19.74	10	5.95	6	3.95
Hypertension	42	25	38	25	13	7.74	5	3.29
Headache	39	23.21	36	23.68	4	2.38	7	4.61
Hypotension	34	20.24	22	14.47	5	2.98	5	3.29
Dizziness	34	20.24	28	18.42	1	0.6	0	0
Decreased appetite	31	18.45	24	15.79	7	4.17	8	5.26
Hypersensitivity	30	17.86	26	17.11	4	2.38	2	1.32
Constipation	29	17.26	13	8.55	0	0	1	0.66
Non-cardiac chest pain	26	15.48	11	7.24	6	3.57	2	1.32
Encephalopathy	26	15.48	17	11.18	4	2.38	6	3.95
Tachycardia	25	14.88	11	7.24	7	4.17	3	1.97
Weight increased	25	14.88	12	7.89	0	0	0	0
Dyspnoea	25	14.88	21	13.82	5	2.98	7	4.61
Chills	21	12.5	19	12.5	2	1.19	2	1.32
Pyrexia	18	10.71	22	14.47	1	0.6	0	0
Insomnia	18	10.71	12	7.89	0	0	2	1.32
Hyperhidrosis	17	10.12	12	7.89	1	0.6	1	0.66
Pruritus	13	7.74	15	9.87	0	0	1	0.66
Depression	13	7.74	9	5.92	1	0.6	3	1.97
Dyspepsia	12	7.14	7	4.61	0	0	0	0
Myalgia	11	6.55	2	1.32	2	1.19	0	0
Bacterial infection	11	6.55	3	1.97	6	3.57	2	1.32
Anxiety	11	6.55	5	3.29	2	1.19	0	0
Tremor	9	5.36	2	1.32	1	0.6	0	0

Source: Reviewer's analysis, MAED, ISS population (ADSL, ADAEGT2 datasets)

*Includes grouped terms

8.2.6 Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

No COAs were included in this application.

8.2.7 Safety Analyses by Subgroups

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for additional subgroup analyses.

Drug-Donor Interactions

Treosulfan was used as part of the conditioning regimen for patients undergoing both matched related donor and matched unrelated donor allogeneic HSCT. TEAEs were assessed in the treosulfan arm of MC-FludT.14/L Trial II in the related and unrelated donor populations. There

were more TEAEs observed in patients with matched unrelated donors, though this may be due to the difference in patient numbers between these two groups. When evaluating Grade 3-5 TEAEs, infection was more common in patients with a matched related donor (N = 11, 17%) than in those receiving a matched unrelated donor (N = 24, 12%).

A comparison between the treosulfan and busulfan groups was performed. All-grade musculoskeletal pain, edema, infection, and hemorrhage were more common in patients with matched related donors receiving treosulfan-based conditioning. Of note, grade 3-5 infection was higher in the treosulfan group than the busulfan group (17.46% vs 4.412%, p-value = 0.022).

8.2.8 Specific Safety Studies/Clinical Trials

Dose Dependency for Adverse Events

As MC-FludT.14/L Trial I, in which patients randomized to treosulfan received a dose of 14 g/m², was stopped early because of increased transplant-related morbidity and mortality, a comparison was performed between all adult patients who received 10 g/m² and those who received 14 g/m² of treosulfan. We observed higher rates of hepatotoxicity, GI toxicity, infection (including febrile neutropenia), and musculoskeletal AEs in patients receiving the 14 g/m² dose of treosulfan, as seen in Table 27.

Table 27. Treosulfan Dose Comparison of 10 g/m² and 14 g/m² in Cohort 3 (MC-FludT.6, MC-FludT.7/AML, MC-FludT.8/MDS, MC-FludT.14/L Trial I, and MC-FludT.14/L Trial II)

Preferred Term*	Treosulfan 10 g/m ² N = 290		Treosulfan 14 g/m ² N = 305		RD
	N	%	N	%	
Hepatotoxicity	28	9.66	116	38.03	28.38
Nausea	100	34.48	189	61.97	27.48
Febrile neutropenia	47	16.21	126	41.31	25.1
Fatigue	38	13.1	111	36.39	23.29
Diarrhoea	55	18.97	117	38.36	19.4
Haemorrhage	46	15.86	95	31.15	15.29
Infection	69	23.79	118	38.69	14.9
Vomiting	66	22.76	113	37.05	14.29
Stomatitis	108	37.24	155	50.82	13.58
Headache	49	16.9	90	29.51	12.61
Hypertension	41	14.14	81	26.56	12.42
Abdominal pain	43	14.83	83	27.21	12.39
Hypersensitivity	18	6.21	52	17.05	10.84
Insomnia	9	3.1	42	13.77	10.67
Musculoskeletal pain	69	23.79	103	33.77	9.98
Bone pain	46	15.86	77	25.25	9.38

Grafapex (treosulfan)

Non-cardiac chest pain	8	2.76	36	11.8	9.04
Dyspepsia	6	2.07	33	10.82	8.75
Weight increased	20	6.9	47	15.41	8.51
Rash	52	17.93	76	24.92	6.99
Constipation	37	12.76	60	19.67	6.91
Encephalopathy	19	6.55	40	13.11	6.56
Hypotension	21	7.24	42	13.77	6.53
Hyperhidrosis	1	0.34	20	6.56	6.21
Decreased appetite	27	9.31	46	15.08	5.77
Dizziness	18	6.21	36	11.8	5.6
Renal impairment	30	10.34	16	5.25	-5.1
CRP increased	18	6.21	3	0.98	-5.22
GGT increased	27	9.31	7	2.3	-7.02
AST increased	28	9.66	5	1.64	-8.02
ALT increased	32	11.03	7	2.3	-8.74
Pyrexia	98	33.79	41	13.44	-20.35

Source: Reviewer's analysis, ISS population (ADSL, ADAEGT2 datasets)

*Includes Grouped Terms

Clinical TL Comment: As noted in the original review, across the safety population, patients who received 14 g/m²/dose of treosulfan experienced substantially more toxicity, including infectious complications, GI toxicity, and hepatotoxicity, compared to those who received the 10 g/m²/dose of treosulfan. In MC-FludT.14/L Trial I, the higher rates of early fatal adverse reactions confirm an increased risk of morbidity and mortality with the 14 g/m² dose of treosulfan. These findings warrant inclusion of a warning and precaution in the USPI.

8.2.9 Additional Safety Explorations

Human Carcinogenicity or Tumor Development

An analysis of the SOC "Neoplasms benign, malignant, and unspecified (including cysts and polyps)" in MC-FludT.14/L Trial II revealed one patient in the treosulfan arm and one patient in the busulfan arm with these events.

Human Reproduction and Pregnancy

Because of the proposed mechanism of action of treosulfan, pregnancy and reproduction are discouraged. There have been no studies or literature reports of subjects who became pregnant while receiving treosulfan, or of children who were born of parents who were receiving treosulfan.

Pediatrics and Assessment of Effects on Growth

Two pediatric studies were submitted as part of this application. All pediatric patients received doses of treosulfan that were equivalent to 14 g/m² dosing. Safety analysis was performed comparing the pediatric patients to the recommended dose of treosulfan (10 g/m²). The

majority of pediatric patients received thiotepa in addition to fludarabine and treosulfan for their conditioning. Analyses comparing pediatric and adult studies (Cohorts 4 and 1) excluded patients who received thiotepa.

An analysis between the treosulfan arm and the busulfan arm in the Phase 2 randomized study MC-FludT.16/NM was conducted. Nearly all of the patients in this analysis received a preparative regimen of either treosulfan or busulfan, fludarabine, and thiotepa. The majority of TEAEs were similar between the two arms. Notable exceptions included higher rates of abdominal pain, rash, pruritis, hemorrhage, and infusion-related reactions in the treosulfan arm of this study.

Pediatric patients from both MC-FludT.16/NM and MC-FludT.17/M who received a preparative regimen of treosulfan + fludarabine (Cohort 4) were then compared to adult patients who received the recommended treosulfan-based conditioning regimen (Cohort 3, 10 g/m²). The pediatric patient numbers are small, as the majority of patients received a preparative regimen that included thiotepa and are excluded from this analysis. The analysis revealed higher rates of hepatotoxicity and gastrointestinal toxicities in the pediatric patients who received a dose equivalent to 14 g/m² compared with adult patients who received the 10 g/m² dose.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

8.2.10 Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

Expectations on Safety in the Postmarket Setting

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021).

8.2.11 Integrated Assessment of Safety

The primary data in support of safety for the proposed indication of treosulfan came from study MC-FludT.14/L Trial II, in which 270 patients with either AML or MDS were exposed to treosulfan as part of a conditioning regimen prior to allogeneic HSCT. All patients received two doses of treosulfan on days -4 and -3 prior to HSCT, at a dose of 10 g/m²/dose.

The study population was monitored for death, adverse events, serious adverse events, and laboratory abnormalities. By Day +30 following HSCT, there were 4 deaths (1.5%) on the treosulfan arm, all due to fatal pulmonary adverse reactions, as compared to 2 deaths (0.7%) on the busulfan (control) arm.

Grafax (treosulfan)

Adverse events with treosulfan were mainly similar to those observed with busulfan-based conditioning. Common TEAEs ($\geq 20\%$) included musculoskeletal pain, stomatitis, pyrexia, nausea, edema, infection, and vomiting. Of those, musculoskeletal pain (12% higher), edema (11% higher), and infection (5% higher) occurred at a rate at least 5% higher in the treosulfan arm than the busulfan arm. The most common grade 3-5 AEs ($\geq 5\%$) in the treosulfan arm were febrile neutropenia, infection, hypertension, stomatitis, and musculoskeletal pain. Of those, infection occurred at a rate of $>5\%$ compared to the busulfan arm (6% higher).

Table 28. Summary of Major Safety Events from MC-FludT.14/L Trial II through Day +30

	Treosulfan (N = 270)	Busulfan (N = 283)
Any TEAE	250 (92.6%)	272 (96.1%)
Grade 3-5 TEAE	148 (54.8%)	151 (53.4%)
SAE	23 (8.5%)	20 (7.1%)
Fatal Adverse Reactions	4 (1.5%)	2 (0.7%)

Source: FDA analysis, ISS population (ADSL, ADAEGT2 datasets), clinical study report

Safety data supporting the dose of treosulfan ($10 \text{ g/m}^2/\text{dose}$) were from studies MC-FludT.6/L, MC-FludT.7/AML, MC-FludT.8/MDS, and MC-FludT.14/L Trials I and II. In this comparison, 290 patients were dosed at $10 \text{ g/m}^2/\text{dose}$, and 305 patients were dosed at $14 \text{ g/m}^2/\text{dose}$.

Higher rates of hepatotoxicity, GI toxicity, infection (including febrile neutropenia), and musculoskeletal AEs were reported in patients receiving the 14 g/m^2 dose of treosulfan compared to the $10 \text{ g/m}^2/\text{dose}$.

In the pediatric studies, very few patients under the age of 1 year received a regimen without thiotepe. This limits the conclusions regarding the safety of the proposed treosulfan treatment regimen to patients 1 year and older. Higher rates of hepatotoxicity and GI toxicity were reported in pediatric patients who received $14 \text{ g/m}^2/\text{dose}$ -equivalent of treosulfan compared to adults who received the 10 g/m^2 dose. (b) (4)

8.3 Statistical Issues

This NDA has received two separate Complete Responses, dated 30 July 2021 and 15 September 2022. These Complete Responses were issued mainly for inadequate data collection and study design. Key issues:

- The Study MC-FludT.14/L Trial II was stopped early for efficacy at 35% information fraction of EFS. Early stopping for efficacy might result in an overestimation of a treatment effect.

- No alpha-controlled hypothesis testing was prespecified for the overall survival. Not controlling for multiplicity might result in erroneous conclusions.
- The EFS definition per protocol is different from the definition typically used for regulatory consideration. Clinical and regulatory relevance of the EFS endpoint, as defined in the protocol, is questionable.
- Adequate data were not submitted to confirm the EFS results in the previous submissions. Additional data were submitted, but the reliability of data is likely limited beyond Day 100.
- The full study population consists of two heterogeneous subpopulations that usually have separate indications: AML and MDS. As there were no prespecified hypotheses testing and alpha allocation for these subpopulations, residual uncertainty remains regarding the benefit observed in these subpopulations.

Refer to the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021) for further details. The relevant statistical issues are detailed below.

Data Collection

The following issues regarding data collection were noted in the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021):

- “The primary endpoint of Study MC-FLudT-14/L Trial II was event-free survival (EFS). Because all complete blood cell (CBC) and marrow results were not submitted, we were unable to independently confirm the results of the analysis of the primary endpoint of EFS for Study MC-FLudT-14/L Trial II.
- Additionally, because of the missing CBC and marrow results, we were not able complete our assessment of whether the results of the study would support the indications for treatment of patients with AML and for treatment of patients with MDS.”

The extent of the submitted data is limited. Per Complete Response to the FDA Complete Response Letter 30 July 2021, page 10: “The applicant would like to respectfully submit that the collection of some of the marrow data was considered not mandatory under the study protocol MC-FludT.14/L Trial II, particularly beyond Day +100 and in case of lack of an event. However, in some instances, bone marrow was collected beyond Day +100 and details of these assessments are provided.” Given the lack of reliable data collection beyond Day 100 (and “in case of lack of an event”), outcomes relying on such data beyond Day 100 might be challenging to interpret.

Event-Free Survival Definition

Per statistical analysis plan, the primary efficacy endpoint is “event-free survival (EFS) within 2 years after transplantation measured from time of end of HSCT (= day 0) to time of event. The associated time span is defined as the interval from day 0 to relapse, graft failure or death (whatever occurs first). The date of the event is either the documented date of relapse/progression or the date of death or the date of primary (date of examination of neutrophilic granulocytes) or secondary graft failure (date of examination of neutrophilic

granulocytes).” This is not a standard definition used for regulatory considerations in AML or MDS populations. Per FDA's *Acute Myeloid Leukemia: Developing Drugs and Biological Products for Treatment Guidance for Industry*, EFS is defined as the "time from randomization to the date of: induction treatment failure, relapse for those who have induction treatment success (e.g., complete remission), or death from any cause, whichever comes first". Interpretation of the results based on the protocol-defined EFS outcome may be challenging.

Overall Survival Definition

Per statistical analysis plan, OS is defined as “the time length between day 0 and the date of death due to any cause within the 24-month study period (i.e. excluding post-surveillance evaluation).” Day zero is the time of allogeneic hematopoietic stem cell transplantation. This is not a standard OS definition used for regulatory purposes, which typically uses 1) randomization as the start of the period of interest 2) has no restriction on follow-up (i.e., no limit set to 24 months). Since the OS is both safety and efficacy endpoint, the regulatory interest is in the full unrestricted survival period starting from randomization, referred to OS (post-randomization) herein.

No Alpha Control and No Prespecified Boundary for Overall Survival Testing

Overall survival was one of the secondary outcomes. The OS results show a relatively high magnitude of a treatment effect with the hazard ratio equal to 0.67 (95% CI: 0.51, 0.90; 193 events) in the AML and MDS population. No alpha was allocated for testing overall survival and no boundary for the interim analysis was prespecified. However, these results were considered to reflect a meaningful improvement on the gold-standard endpoint in oncology.

Subgroups

The full study population consists of two distinct populations: patients with AML and patients with MDS. While the OS (post-randomization) results appear to be supportive of the benefit in AML and MDS subgroups, there was no prespecified alpha-controlled hypothesis testing for these subgroups and the upper bounds of the 95% confidence intervals exceed one, suggesting that some residual uncertainty about the treatment effect in each of the subgroups remains.

Additionally, further subgroup of interest included donor type: MUD vs MRD. As noted in the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021), “Due to differences in minor and other unmeasured major antigens, HLA-matched unrelated donors have a higher degree of histoincompatibility with the recipient than HLA-identical sibling donors, so the minimum intensity of the preparative regimen needed for successful HSCT may differ by donor type.” Higher HR estimates for the MRD group in the AML subpopulation are likely due to a few influential events observed after two years of follow-up. Regarding the MDS subgroup, since less than 10 events in each treatment group were observed in this subgroup, the results are likely unreliable due to few observed events. Harm to patients with MRD cannot be ruled out. The subgroup results should be interpreted with caution.

Differences in Treatment Regimen

As noted in the FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021),

there were differences in treatment regimens between study groups:

- “Trial 14 - II compared fludarabine in combination with 3 days of treatment with treosulfan to a combination with 2 days of busulfan. Any conclusions regarding superiority would need to take into account the difference between arms in the number of days of treatment with treosulfan vs the number of days of treatment with busulfan.
- Additionally, in Trial 14 - II, patients with unrelated donors also received ATG in the preparative regimen. ATG dosing varied by geographic region. Any conclusions related to the subgroup analysis by donor would need to take into consideration the differences in treatment regimens by donor type”

The differences in the treatment regimen might have an impact on the treatment effect interpretation.

Stratification Factors

Per statistical analysis plan for the overall survival, “Cox proportional hazards regression model stratified by centre with donor type (MUD vs. MRD), risk group (I vs. II) and treatment as factors will be fitted.” The site factor has 22 levels, the donor type factor has two levels, and the risk group factor has two levels. Including the site in a Cox proportional hazards model would result in small or zero number of events in some strata, making the interpretation of the results challenging (e.g., see Silcocks (2012)). Furthermore, this practice is not typically recommended for trials with few patients per center. Per ICH E9 (page 17), “In some trials, for example, some large mortality trials with very few subjects per center, there may be no reason to expect the centers to have any influence on the primary or secondary variables because they are unlikely to represent influences of clinical importance. In other trials, it may be recognized from the start that the limited numbers of subjects per center will make it impracticable to include the center effects in the statistical model. In these cases, it is not considered appropriate to include a term for center in the model, and it is not necessary to stratify the randomization by center in this situation”. For this reason, the results reported herein and in the USPI use a Cox proportional hazards model stratified by donor type and risk group.

Proportional Hazards Assumptions

The treatment effects in Study MC-FludT.14/L Trial II were quantified primarily with the hazard ratios. The hazard ratios are estimated with a Cox proportional hazards model, which relies on proportional hazards assumptions. A plateauing effect or crossing curves can be seen on most Kaplan-Meier plots for the EFS and the OS outcomes. A plateauing effect or crossing curves might indicate that the proportional hazards assumption does not hold. Additional analyses were conducted to quantify the treatment effects with various alternative statistics that do not rely on the proportional hazards assumptions. The results of these analyses do not change the conclusions based on the hazard ratios, suggesting favorable outcomes for the treosulfan compared to the busulfan.

8.4 Conclusions and Recommendations

The review team was able to verify or clarify the efficacy and safety endpoints as provided by the Applicant. The efficacy of 10 g/m²/dose treosulfan as a component of a conditioning regimen prior to allogeneic HSCT was established based on improvement in overall survival with HR 0.67 (95% CI: 0.51, 0.90) demonstrated in Study MC-FludT.14/L Trial II and on supportive evidence provided by Study MC-FludT.6/L.

The safety of treosulfan 10 g/m²/dose was generally similar to that of busulfan in Study MC-FludT.14/L Trial II with a nominally higher incidence of musculoskeletal pain and infection in the treosulfan arm. One major safety concern is the high morbidity and mortality with the 14 g/m²/dose treosulfan regimen established in MC-FludT.14/L Trial I and supported by Study MC-FludT.6/L. These findings warrant inclusion of a Warning and Precaution in the USPI since this dose is published in the literature and approved for the pediatric population in some countries.

The efficacy of treosulfan at 10 g/m²/dose was extrapolated to pediatric patients undergoing HSCT for an AML or MDS based on the efficacy demonstrated in the adult pivotal study and safety was established based on data from pediatric studies in patients 1 year and older. (b) (4)



8.4.1 Approach to Substantial Evidence of Effectiveness

Select from the options below to indicate how substantial evidence of effectiveness (SEE) was established (if applicable). If there are multiple indications, repeat items 1–3 for each indication.

1. Verbatim indication (*enter approved indication if the application was approved and the Applicant's proposed indication if the application received a complete response*):

Use in combination with fludarabine as preparative regimen for allo-HSCT in adult and pediatric patients 1 year and older with AML

2. SEE was established with (*check **one** of the options for traditional or accelerated approval pathways and complete response not due to lack of demonstrating SEE*)

- a. Adequate and well-controlled clinical investigation(s):

- i. ☐ Two or more adequate and well-controlled clinical investigations, **OR**
- ii. ☐ One adequate and well-controlled clinical investigation with highly persuasive results that is considered to be the scientific equivalent of two clinical investigations

OR

- b. ☒ One adequate and well-controlled clinical investigation and confirmatory evidence^{1,2,3}

OR

- c. ☐ Evidence that supported SEE from a prior approval (*e.g., 505(b)(2) application relying only on a previous determination of effectiveness; extrapolation; over-the-counter switch*)²

3. Complete response, if applicable

- a. ☒ SEE was established
b. ☐ SEE was not established (*if checked, omit item 2*)

1. Verbatim indication (*enter approved indication if the application was approved and the Applicant's proposed indication if the application received a complete response*):

Use in combination with fludarabine as preparative regimen for allo-HSCT in adult and pediatric patients 1 year and older with MDS

2. SEE was established with (*check **one** of the options for traditional or accelerated approval pathways and complete response not due to lack of demonstrating SEE*)

d. Adequate and well-controlled clinical investigation(s):

- i. ☐ Two or more adequate and well-controlled clinical investigations, **OR**
ii. ☐ One adequate and well-controlled clinical investigation with highly persuasive results that is considered to be the scientific equivalent of two clinical investigations

OR

- e. ☒ One adequate and well-controlled clinical investigation and confirmatory evidence^{1,2,3}

OR

- f. ☐ Evidence that supported SEE from a prior approval (*e.g., 505(b)(2) application relying only on a previous determination of effectiveness; extrapolation; over-the-counter switch*)²

3. Complete response, if applicable

- a. ☒ SEE was established
b. ☐ SEE was not established (*if checked, omit item 2*)

¹ FDA draft guidance for industry *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products* (2019)

² FDA guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products* (1998)

³ Demonstrating Substantial Evidence of Effectiveness Based on One Adequate and Well-Controlled Clinical Investigation and Confirmatory Evidence (2023)]

NDA/BLA Multi-disciplinary Review and Evaluation [NDA 214759]
Grafapex (treosulfan)

X

Alexei Ionan, PhD
Primary Statistical Reviewer

X

Jonathon Vallejo, PhD
Statistical Team Leader

X

Joseph Wynne, MD
Primary Clinical Reviewer

X

Emily Jen, MD, PhD
Clinical Team Leader

9 Advisory Committee Meeting and Other External Consultations

This Application was not discussed by an advisory committee.

10 Pediatrics

Because treosulfan has orphan drug designation, PREA does not apply. The original NDA application was submitted on August 11, 2020, therefore FDARA does not apply.

11 Labeling Recommendations

11.1 Prescription Drug Labeling

The table below describes substantive changes to the USPI for NDA 214759 Grafapex (treosulfan). See the approval letter for final, approved labeling.

Summary of Significant Labeling Changes (High level changes and not direct quotations)		
Section	Proposed Labeling	Approved Labeling
1 Indications and Usage	Proposed indications for adult and pediatric patients 1 year of age and older with AML and with MDS.	The approved indication is for AML and MDS in adult and pediatric patients 1 year of age and older.
2 Dosage and Administration	Proposed subsections 2.1 Recommended Dosage and 2.2 Preparation and Administration Instructions	FDA generally agreed with the applicant's proposed text in subsection 2.1 but modified subsection 2.2 to reorganize the presentation of the data.
5 Warnings and Precautions (W&P)	Proposed the following W&P: 5.1 Myelosuppression 5.2 Seizures 5.3 Skin Disorders 5.4 Injection Site Reactions and Tissue Necrosis 5.5 Secondary Malignancies 5.6 Increased Early Morbidity and Mortality at Dosages Higher than Recommended 5.7 Embryo-Fetal Toxicity	FDA agreed with the proposed warnings but made the following substantive edits - 5.1: Added that the time to neutrophil counts >0.5 Gi/L after allogeneic hematopoietic stem cell transplantation occurred at a median of 18 days and added range. Added instructions that the preparative regimen should not begin if stem cell donor is not available and that blood cell counts should be monitored daily until hematopoietic recovery. 5.2: Removed "(b) (4)" from the reports of seizures as seizures have occurred in both adult and pediatric patients.
6 Adverse Reactions	Included data from MC-fludT.14/L Trial II	FDA modified the fatal adverse reactions to include

		those that were regimen-related within 30 days of transplantation and noted that all fatalities were due to pulmonary adverse reactions. FDA split the most common adverse reactions and laboratory abnormalities into two sentences; for laboratory abnormalities, FDA reported selected grade 3 or 4 nonhematological abnormalities and added text that all patients on Grafapex and fludarabine experienced anemia, neutropenia, and thrombocytopenia. The incidence cutoff for the adverse reactions table was changed (b) (4) to 10%. In the listing of adverse reactions that occurred in (b) (4) 10% of patients, FDA (b) (4) any terms that were included in grouped terms in the adverse reactions table.
8 Use in Specific Populations	Included 8.4 Pediatric Use	FDA modified subsection 8.4 to include that the basis of approval in pediatric patients 1 year of age and older with AML and MDS was based on data in adults with additional safety and pharmacokinetic data in pediatric patients. FDA noted that the incidence of hepatic and GI adverse reactions was increased in pediatric patients compared to adults.

		A Juvenile Animal Toxicity Data section with included with in subsection 8.4 to note that delayed physical development was noted in rats who received high doses of treosulfan. FDA removed (b) (4) as there are no actionable recommendations.
12 Clinical Pharmacology	12.2 Pharmacodynamics – noted that treosulfan exposure was associated with increased incidence of infection-related death. 12.3 Pharmacokinetics – included pharmacokinetic properties and data.	In subsection 12.2 Pharmacodynamics, FDA agreed with the PD statement concerning infection-related death and added that the treosulfan time course of pharmacodynamic response is unknown as required by 21 CFR 201.57(c)(13)(i)(B). In subsection 12.3 Pharmacokinetics, FDA modified the data to align with best practices and guidance recommendations throughout. Within the Specific Populations heading, FDA added that the effect of moderate or severe renal or hepatic impairment, and age over 65 years on treosulfan pharmacokinetics is unknown. Other edits made throughout this subsection for better clarity. Included pharmacokinetic data in pediatric patients 1 year of age and older
14 Clinical Studies	Proposed inclusion of results in AML and MDS (b) (4).	FDA agreed with the inclusion of results for patients with AML and MDS.

		FDA reported that efficacy was established on the basis of overall survival (OS). FDA included the hazard ratios for OS in the randomized population (stratified by donor type and risk group) and separately in patients with AML and patients with MDS in text and removed (b) (4) . FDA added 3 K-M plot figures for OS – 1 for all randomized patients, 1 for patients with MDS, and 1 for patients with AML. FDA removed (b) (4)
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11.2 Patient Labeling

There is no patient labeling.

Other Prescription Drug Labeling
Not applicable

12 Risk Evaluation and Mitigation Strategies (REMS)

Not applicable.

13 Postmarketing Requirements and Commitment

Recommended Postmarketing Requirements and Commitments:

PMR #1

Conduct a clinical pharmacokinetic trial to evaluate a serious potential risk of increased drug toxicity and determine an appropriate, safe dose of treosulfan in patients with moderate renal impairment (estimated glomerular filtration rate or creatinine clearance 30-59 mL/min). This trial should be designed and conducted in accordance with the FDA Guidance for Industry titled "Pharmacokinetics in Patients with Impaired Renal Function: Study Design, Data Analysis, and Impact on Dosing and Labeling."

PMR #2

Conduct a clinical trial to characterize the serious potential risk of QTc prolongation (i.e., exclude large mean increases of >20 msec in the QTc interval) with treosulfan and its active metabolite. Design and conduct the study in accordance with the ICH E14 Guidances for Industry titled, E14 Clinical Evaluation of QT/QTc and E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs Questions and Answers (R3) Guidance for Industry.

PMR #3

Conduct a clinical pharmacokinetic trial to characterize the serious potential risk of increased drug toxicity and further characterize the safety of treosulfan by evaluating the pharmacokinetics of the active monoepoxide intermediate [(2S,3S)-1,2-epoxybutane-3,4-diol-4-methanesulfonate] in adult patients after administration of treosulfan.

PMC #1

Conduct a clinical trial to assess the pharmacokinetics of treosulfan among U.S. racial and ethnic groups. This study should characterize the exposure (including pharmacokinetics data) of treosulfan.

PMC #2

Conduct a registry-based observational study to assess outcomes by race and ethnicity for patients undergoing allogeneic hematopoietic stem cell transplantation using treosulfan-fludarabine as the preparative regimen. Include subgroup analyses by race and ethnicity for overall survival, nonrelapse mortality and relapse-free survival.

14 Division Director (OB) Comments

X

Yuan Li Shen, Dr. P.H.
Office of Biostatistics
Division of Biometrics IX

15 Division Director (Clinical) Comments

X

R. Angelo de Claro, MD
Office of Oncologic Diseases
Division of Hematologic Malignancies I (DHMI)

16 Office Director (or designated signatory authority) Comments

This application was reviewed by the Oncology Center of Excellence (OCE) per the OCE Intercenter Agreement. My signature below represents an approval recommendation for the clinical portion of this application under the OCE.

X

Marc R. Theoret, MD
Deputy Director
Oncology Center of Excellence/FDA

17 Appendices

17.1 References

E9 Statistical Principles for Clinical Trials <https://www.fda.gov/media/71336/download>

Silcocks P. (2012). How many strata in an RCT? A flexible approach. *British journal of cancer*, 106(7), 1259–1261. <https://doi.org/10.1038/bjc.2012.84>

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Wilson BE, Pearson SA, Barton MB, Amir E. Regional Variations in Clinical Trial Outcomes in Oncology. *J Natl Compr Canc Netw*. 2022 Aug;20(8):879-886.e2. doi: 10.6004/jnccn.2022.7029. PMID: 35948036.

17.2 Financial Disclosure

See FDA Multidisciplinary Review and Evaluation of NDA 214759 (30 July 2021)

Covered Clinical Study (Name and/or Number):

Was a list of clinical investigators provided:	Yes ☐	No ☐ (Request list from Applicant)
Total number of investigators identified: _____		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): _____		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): _____		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____		

Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) _____		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

17.3 Nonclinical Pharmacology/Toxicology

N/A

17.4 OCP Appendices (Technical documents supporting OCP recommendations)

N/A

17.5 Additional Clinical Outcome Assessment Analyses

N/A

NDA 214759 Grafapex (treosulfan) for Injection, for Intravenous Use

Multidisciplinary Review Signatures

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Clinical Reviewer	Joseph Wynne, MD, PhD	OOD/DHM1	Sections: 2, 3, 7, 8, 9, 11	Select one:
				☒ Authored
				☐ Approved
Signature:				
Clinical Team Leader	Emily Jen, MD, PhD	OOD/DHM1	Sections: 2, 3, 7, 8, 9, 11	Select one:
				☐ Authored
				☒ Approved
Signature:				
Statistics Reviewer	Alexei Ionan, PhD	OB/DBIX	Sections: 7, 8	Select one:
				☒ Authored
				☐ Approved
Signature:				
Statistics Team Leader	Jonathon Vallejo, PhD	OB/DBIX	Sections: 7, 8	Select one:
				☐ Authored
				☒ Approved
Signature:				
Statistics Division Director	Yuan Li Shen Dr. P.H.	OB/DBIX	Sections: 7, 8	Select one:
				☐ Authored
				☒ Approved
Signature:				
Associate Director for Labeling (ADL)	Elizabeth Everhart, MSN, RN, ACNP	OOD	Sections: 11	Select one:
				☒ Authored
				☐ Approved
Signature:				
Cross-Disciplinary Team Leader (CDTL)	Emily Jen, MD, PhD	OOD/DHM1	Sections: 1, 4, 5, 6, 8, 11	Select one:
				☒ Authored
				☐ Approved
Signature: See Appended Electronic Signature Page				
Division Director	Angelo de Claro, MD	OOD/DHM1	Sections: All	Select one:
				☐ Authored
				☒ Approved
Signature: See Appended Electronic Signature Page				

Supervisory Associate Director (Acting) / Signatory	Marc R. Theoret, MD	OOD	Sections: All	Select one:	
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				☒	Approved
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MARC R THEORET
01/21/2025 02:31:07 PM

ADDENDUM TO CLINICAL PHARMACOLOGY RE-SUBMISSION MEMO

Application Number	NDA 214759, SDN 70
Link to EDR	\\CDSESUB1\evsprod\NDA214759\0070
Submission Date	4-30-24
Submission Type	NDA Re-Submission (3 rd re-submission)
Brand Name	GRAFAPEX [®]
Generic Name	Treosulfan
Proposed Dosage	10 g/m ² on Days -4, -3, -2 in combination with fludarabine before hematopoietic stem cell infusion on Day 0
Dosage Form and Strength	1 g/vial and 5 g/vial treosulfan as a lyophilized powder in a single-dose vial
Route of Administration	Intravenous (IV)
Proposed Indication(s)	▪ Use in combination with fludarabine as a preparative regimen for allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult and pediatric patients ≥ 1 year old with acute myeloid leukemia (AML) ▪ Use in combination with fludarabine as a preparative regimen for alloHSCT in adult and pediatric patients ≥ 1 year old with myelodysplastic syndrome (MDS)
Applicant	medac GmbH
Associated INDs	IND 054552
OCP Review Team	Vicky Hsu, Ph.D. Ankit Shah, Ph.D.

This is an Addendum to the clinical pharmacology review that was checked into DARRTS on **10-31-24**. The purpose of this Addendum is to correct a PMR from the previous review.

The previous review indicated the need for the following PMR:

Conduct an in vitro study to evaluate treosulfan and epoxide metabolites as substrates of major cytochrome P450 enzymes consistent with FDA guidance.

The in vitro assessment of treosulfan metabolism showed involvement of CYP2D6 (t_{1/2} with rCYP2D6 = 108 min) and was noted in the previous review.

Based on an updated communication with the Applicant (see below), we have determined that the above PMR is no longer needed.

Clinical Pharmacology Information Request

Reference is made to your Complete Response to the CRL and Acknowledgement of Incomplete Response (dated 4-26-24), where you stated that “CYP450 reaction phenotyping with the monoepoxide and the diepoxide metabolites began later and has not yet been completed” and that the reports will be submitted when available (page 33 of 37).

Reference is also made to your response to our PMR-PMC template milestone dates, where you indicated that the final report submission for these metabolites studies occurred in Apr 2024. We note the submission of Reports “CYP1269 R14A” and CYP1269 R14B” to the NDA.

Clarify the discrepancy and whether these aforementioned reports are the formal final study reports.

medac GmbH Response

The applicant confirms that the reports CYP1269 R14A und CYP1269 R14B, as filed under CTD Section 5.3.2.2, are the formal final study reports of the CYP450 reaction phenotyping with the treosulfan monoepoxide and the diepoxide metabolites. We apologize for the confusion created.

Based on a review of the submitted reports of the in vitro studies, the monoepoxide (**Table 1**) and diepoxide metabolites (**Table 2**) are not meaningfully metabolized by CYP enzymes. Of note, the $t_{1/2}$ values for positive control compounds for the respective rCYPs were very short. No labeling update is needed based on these results.

Table 1: Reaction phenotyping (recombinant enzymes) – data for the treosulfan monoepoxide EBDM

CYP isoform	$t_{1/2}$ (min)	SE $t_{1/2}$ (min)	n
1A2	>135	NA	5
2B6	>135	NA	4 a
2C8	108	34.4	5
2C9	>135	NA	5
2C19	>135	NA	4 a
2D6	>135	NA	5
3A4	>135	NA	4 a

SE = Standard error of the curve fitting; n = number of time points used to fit curve; NA = Not applicable a 0 minute time point excluded. Source: Applicant's final report for Study cyp1269 r14a

Table 2: Reaction phenotyping (recombinant enzymes) – data for the treosulfan diepoxide DEB

CYP isoform	$t_{1/2}$ (min)	SE $t_{1/2}$ (min)	n
1A2	>135	NA	5
2B6	>135	NA	5
2C8	>135	NA	5
2C9	>135	NA	5
2C19	>135	NA	5
2D6	>135	NA	5
3A4	>135	NA	5

SE = Standard error of the curve fitting; n = number of time points used to fit curve; NA = Not applicable. Source: Applicant's final report for Study cyp1269 r14b

Based on these findings, the review team has determined that the above PMR that was noted in the clinical pharmacology review (dated **10-31-24**) is no longer needed and will NOT be issued as part of the approval of this NDA.

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CLINICAL PHARMACOLOGY RE-SUBMISSION MEMO

Application Number	NDA 214759, SDN 70
Link to EDR	\\CDSESUB1\evsprod\NDA214759\0070
Submission Date	4-30-24
Submission Type	NDA Re-Submission (3 rd re-submission)
Brand Name	GRAFAPEX®
Generic Name	Treosulfan
Proposed Dosage	10 g/m ² on Days -4, -3, -2 in combination with fludarabine before hematopoietic stem cell infusion on Day 0
Dosage Form and Strength	1 g/vial and 5 g/vial treosulfan as a lyophilized powder in a single-dose vial
Route of Administration	Intravenous (IV)
Proposed Indication(s)	▪ Use in combination with fludarabine as a preparative regimen for allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult and pediatric patients ≥ 1 year old with acute myeloid leukemia (AML) ▪ Use in combination with fludarabine as a preparative regimen for alloHSCT in adult and pediatric patients ≥ 1 year old with myelodysplastic syndrome (MDS)
Applicant	medac GmbH
Associated INDs	IND 054552
OCP Review Team	Vicky Hsu, Ph.D. Ankit Shah, Ph.D.

The clinical pharmacology of treosulfan was extensively reviewed in the original submission (Unireview [link](#), DARRTS 7-30-21). Briefly, the Applicant is seeking approval of treosulfan as part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). The proposed dosing regimen is treosulfan 10 g/m²/day for 3 consecutive days (Days -4, -3, and -2) in combination with fludarabine 30 mg/m²/day for 5 consecutive days (Days -6 through -2) prior to allogeneic hematopoietic stem cell transplantation (alloHSCT; Day 0).

Pivotal evidence to support efficacy in the proposed population is based on the results of Study MC-FLudT.14/L Trial II. Key review issues include the adequacy of the proposed treosulfan dosing regimen in adult patients, pediatric extrapolation to identify a treosulfan dosing regimen appropriate for pediatric patients ≥1 year of age, and evaluation of the Applicant's proposals for patients with organ impairment. The Applicant provided pharmacokinetic studies in adults and pediatric patients, a population PK model of treosulfan, and a dose-response evaluation in adults to support the dose selection in the general population and specific populations. The

Applicant did not provide adequate data to resolve other review questions including characterization of treosulfan metabolism and effects of treosulfan on QTc interval.

The Office of Clinical Pharmacology did not recommend approval then because of issues regarding the veracity of the primary study endpoint.

A summary of key review issues and recommendations from the original clinical pharmacology review of NDA 214759 is provided below:

REVIEW ISSUE	RECOMMENDATIONS / COMMENTS
General dosing instructions	The recommended dosage regimen of treosulfan is 10 g/m ² /day for 3 consecutive days (Days -4, -3, and -2) in combination with fludarabine 30 mg/m ² /day for 5 consecutive days (Days -6 through -2) prior to allogeneic hematopoietic stem cell transplantation (Day 0).
Dosing in patient subgroups (intrinsic and extrinsic factors)	<i>Pediatric Patients ≥1 year of age:</i> The recommended dosage regimen of treosulfan is the same for all patients ≥1 year of age. Efficacy in pediatric patients ≥1 year of age was extrapolated from adults and dose selection was supported by PK and safety data from Studies MC-FludT.16/NM and MC-FludT.17/M. Treosulfan exposure after administration of 10 g/m² in pediatric patients with BSA <0.7 m² and with BSA 0.7 to <1.1 m² are 11% and 5% higher, respectively, compared to adults. Exposure-response analyses for safety did not indicate that an 11% increase in exposures would result in significant increase in the probability of common toxicities. <i>Renal Impairment:</i> No dosage modification is recommended for patients with mild renal impairment. An additional study will be necessary to identify a safe dose of treosulfan for patients with moderate hepatic impairment. A study in patients with severe renal impairment is not feasible and will not be required. <i>Hepatic Impairment:</i> No dosage modification is recommended for patients with mild hepatic impairment. Studies in patients with moderate or severe hepatic impairment are not needed, as these patients are typically excluded from transplantation.

The previous clinical pharmacology review identified 5 potential post-marketing requirements / commitments (PMR / PMCs), which are still applicable and should be issued for this NDA (see Table below).

Key Issue(s) to be Addressed	Rationale	Key Considerations for Design Features
Determination of an appropriate safe treosulfan dose for use in patients with moderate renal	There is evidence of renal elimination of treosulfan. Renal impairment may increase treosulfan systemic exposures and require dose adjustment.	(PMR 1) Conduct a clinical trial to evaluate the pharmacokinetics and safety of treosulfan in patients with normal renal function and patients with moderate renal impairment.

Key Issue(s) to be Addressed	Rationale	Key Considerations for Design Features
Evaluation of treosulfan and epoxide metabolites as substrates of metabolizing enzymes.	The Applicant did not evaluate treosulfan or its epoxide metabolites as substrates of major metabolizing enzymes. This evaluation is necessary to describe risk of drug-drug interactions and effects of hepatic impairment on treosulfan safety and PK.	(PMR 2) Conduct an <i>in vitro</i> study to evaluate treosulfan and epoxide metabolites as substrates of major cytochrome P450 enzymes consistent with FDA guidance.
Evaluation of the effects of treosulfan on the QT interval.	The effects of treosulfan on the QT interval have not been evaluated. A QT study should be performed to evaluate the cardiac safety of treosulfan.	(PMR 3) Conduct a clinical trial to evaluate the effects of treosulfan on the QT interval in patients receiving the recommended dosage of treosulfan.
Evaluation of treosulfan active metabolite PK in adult patients.	The PK of treosulfan parent drug was evaluated in 24 adult patients. The PK of active epoxide metabolites was not evaluated in adult patients.	(PMR 4) Evaluate the PK of treosulfan active epoxide metabolites in adult patients.
Determination of the effects of race and ethnicity on the PK of treosulfan.	Race and ethnicity data were not collected in the pivotal study or adult PK sub-study of treosulfan. The effects of race and ethnicity on the PK of treosulfan is therefore unknown.	(PMC 1) Conduct a clinical trial to evaluate the effects of race and ethnicity on the pharmacokinetics of treosulfan.

The only new data submitted in this re-submission were in vitro metabolism study results which determined that treosulfan is metabolized by CYP2D6 and its monoepoxide intermediate is metabolized by CYP2C8. The labeling has been updated to reflect this information. In vitro metabolism studies for other metabolites are currently ongoing (relates to PMR 2, above).

This NDA is approvable from a clinical pharmacology perspective. The PMRs and PMC listed above should be issued to the applicant.

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10/31/2024 09:39:31 AM

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 214759
Supporting document/s: 70
Applicant's letter date: April 30, 2024
CDER stamp date: April 30, 2024
Product: GRAFAPEX (treosulfan) for Injection
Indication: Use in combination with fludarabine as a preparative regimen for allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult and pediatric patients 1 year of age and older with acute myeloid leukemia (AML)
Applicant: Medac Gesellschaft für klinische Spezialpräparate mbH (GmbH)
Review Division: DHM I/DHOT
Reviewer: Moran Choe, Ph.D (DHOT)
Supervisor/Team Leader: Brenda Gehrke, Ph.D (DHOT)
Clinical Division Director: Angelo De Claro, MD (DHM I)
Project Manager: Bradley McKenzie, PharmD

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1 Executive Summary

1.1 Introduction

GRAFAPEX (treosulfan) is an alkylating drug. The Applicant, Medac GmbH, submitted a Class 2 Resubmission of the original NDA 214759. The Applicant is seeking marketing approval for GRAFAPEX (treosulfan) for the use in combination with fludarabine as a preparative regimen for alloHSCT in adult and pediatric patients 1 year of age and older with AML. The current submission is a resubmission of the original NDA that was submitted under section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act on August 11, 2020.

1.2 Brief Discussion of Nonclinical Findings

The relevant nonclinical pharmacology, pharmacokinetics, and toxicology studies for treosulfan to support NDA 214759 were previously reviewed in the Multidisciplinary Review and Evaluation (Unireview; DARRTS date: July 30, 2021) for the initial submission of NDA 214759. During the review of the NDA, it was determined that the nonclinical data for treosulfan submitted to the NDA in studies or literature for which the Applicant has the right to reference did not provide convincing evidence of DNA alkylation and breaks as the mechanism of action (MOA) of treosulfan or to address genotoxicity potential of the drug. The FDA issued a Complete Response Letter due to Clinical deficiencies. In the letter, FDA recommended the Applicant conduct and submit the results of nonclinical studies to address the comment from Pharmacology/Toxicology discipline.

To address this issue, the Applicant conducted genetic toxicology studies, and submitted the results to demonstrate the genotoxicity of treosulfan. Genetic toxicology studies included an in vitro bacterial reverse mutation (Ames) assay and an in vitro chromosomal aberration assay.

In the in vitro bacterial reverse mutation assay, treosulfan tested at up to 3160 µg per plate, with or without metabolic activation, caused a concentration-dependent mutagenic effect in the *Salmonella typhimurium* strains. In the in vitro chromosomal aberration assay using human lymphocyte cultures, treosulfan tested at up to 50 µg/mL showed clastogenic properties with respect to chromosomal or chromatid damage with or without metabolic activation. Overall, the study results showed that treosulfan is a genotoxic compound.

1.3 Recommendations

1.3.1 Approvability

There are no Pharmacology/Toxicology approvability issues with the proposed GRAFAPEX (treosulfan) for Injection drug product for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Appropriate labeling changes have been made to the United States Prescribing Information (USPI) to be consistent with the Pregnancy and Lactation Labeling Rule (PLLR) format and current labeling practices. A sentence regarding the genetic toxicology studies has been added under Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility.

2.2 Previous Reviews Referenced

Refer to the Pharmacology/Toxicology review in the original NDA Multidisciplinary Review and Evaluation for NDA 214759 submitted to DARRTS on July 30, 2021.

3 Genetic Toxicology

3.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Mutagenicity study of treosulfan in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay (in vitro)

Study no.:	38665
Study report location:	4.2.3.3.1
Date of study initiation:	September 17, 2021
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Treosulfan Lyophilisate 1000 mg, F190859A, 98.9%

Key Study Findings

- Treosulfan was positive for mutagenicity in the reverse mutation assay.
- Treosulfan induced a concentration-dependent mutagenic effect and at least 2-fold increase in the number of revertant colonies for test strains TA100 and TA1535 with or without metabolic activation. No mutagenic effect was observed for the test strains TA98, TA1537 and WP2 *uvrA*.
- Cytotoxicity was observed in all tester strains at 3160 µg/plate, resulting in a reduction in the number of spontaneous revertant colonies. No test item precipitation was observed.

Methods

Strains:	4 strains of <i>Salmonella typhimurium</i> (TA98, TA100, TA1535, TA1537) and 1 strain of <i>Escherichia coli</i> (WP2 <i>uvrA</i>)
Concentrations in definitive study:	10, 31.6, 100, 316, 1000 and 3160 µg/plate
Basis of concentration selection:	cytotoxicity at ≥3160 µg/plate in tester strain TA100 with and without S9 mix
Negative control:	sterile deionized water
Positive control:	2-aminoanthracene, 2-nitrofluorene, sodium azide, 4-nitroquinoline-N-oxide, benzo[a]pyrene
Formulation/Vehicle:	sterile deionized water
Incubation & sampling time:	50 hours at 37°C

Study Validity

The mean number of revertant colonies observed for the negative/solvent control was comparable to historical control data obtained from the previous studies. All positive controls used induced the expected increase in the mean revertant colonies relative to the negative/solvent control, thereby confirming the responsiveness of the strains and the sensitivity of the test system.

Results

Summary of mutagenic effects of treosulfan

Concentration in µg test item/plate	TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
Plate incorporation test without metabolic activation					
10.0	-	-	-	-	-
31.6	-	-	-	-	-
100	-	-	4.9	-	-
316	-	-	9.8	-	-
1000	-	2.7	12.3	-	-
3160	c	c	c	c	c
Plate incorporation test with metabolic activation					
10.0	-	-	-	-	-
31.6	-	-	-	-	-
100	-	-	-	-	-
316	-	2.3	10.4	-	-
1000	-	2.2	12.4	-	-
3160	c	c	c	c	c
Pre-incubation test without metabolic activation					
10.0	-	-	-	-	-
31.6	-	-	2.7	-	-
100	-	-	5.0	-	-
316	-	2.1	9.3	-	-
1000	-	c	9.2	-	-
3160	c	c	c	c	c
Pre-incubation test with metabolic activation					
10.0	-	-	-	-	-
31.6	-	-	2.1	-	-
100	-	-	3.3	-	-
316	-	2.6	7.5	-	-
1000	-	c	6.1	-	-
3160	c	c	c	c	c

Numbers shown are fold change values compared to the control, -: fold change values <2,
c: cytotoxic concentration

(Excerpted from Applicant's report)

3.2 *In Vitro* Assays in Mammalian Cells

In vitro assessment of the clastogenic activity of treosulfan in cultured human peripheral lymphocytes

Study no.:	38666
Study report location:	4.2.3.3.1
Date of study initiation:	September 27, 2021
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Treosulfan Lyophilisate 1000 mg, F190859A, 98.9%

Key Study Findings

- Treosulfan was positive for clastogenicity in the in vitro chromosome aberrations assay in human peripheral lymphocytes.
- Treosulfan induced 1.0 - 9.0% of chromosomal aberrations (excluding gaps) of the cells at concentrations of 3.125 - 50 µg/mL medium (4-hour exposure) and 1.3 - 8.4 % of chromosomal aberrations at concentrations of 3.125 to 25 µg/mL medium (24-hour exposure) in the absence of metabolic activation.
- Treosulfan induced chromosomal aberrations (excluding gaps) of the cells at concentrations of 3.125 - 50 µg/mL medium (4-hour exposure) in the presence of metabolic activation.

Methods

Cell line:	human peripheral lymphocytes
Concentrations in definitive study:	3.125, 6.25, 12.5, 25, and 50 µg/mL +S9 activation (4 hours) and -S9 activation (4 or 24 hours)
Basis of concentration selection:	cytotoxicity at ≥31.6 µg/mL with or without S9 activation
Negative control:	sterile deionized water
Positive control:	cyclophosphamide monohydrate (+S9), mitomycin C (-S9)
Formulation/Vehicle:	sterile deionized water
Incubation & sampling time:	4 and 24 hours in the absence of activation system and 4 hours in the presence of activation system.

Study Validity

The positive controls induced a significant increase in the percentage of cells with structural aberrations. The percentage of cells with structural aberrations in the negative and positive controls was comparable to the historical control data.

Results

Summary of the clastogenic activity of treosulfan - without metabolic activation (-S9 mix)

4-h exposure				24-h exposure			
Treatment (µg/mL medium)	Mitotic index ^{#1}	Number of metaphases scored	% of cells with aberrations excluding gaps	Treatment (µg/mL medium)	Mitotic index ^{#1}	Number of metaphases scored	% of cells with aberrations excluding gaps
Aqua ad iniectionabilia				Aqua ad iniectionabilia			
0	1.00	300	0.3	0	1.00	300	0.3
Treosulfan Lyophilisate				Treosulfan Lyophilisate			
3.125	1.03	300	1.0	3.125	0.70	300	1.3
6.25	1.27	300	2.0	6.25	0.49	300	2.0
12.5	0.86	300	2.0	12.5	0.46	300	4.3 *
25	1.02	300	9.0 *	25	0.28	143 ^{#2}	8.4 *
50	0.37	300	9.0 *	50	0.17	^{#3}	
Mitomycin C				Mitomycin C			
0.2	0.72	300	8.3 *	0.2	0.50	300	7.7 *

* = significantly different from negative control ($p \leq 0.05$)
 #1 = mitotic index: number of metaphases/1000 cells; negative control = 1.00
 #2 = no more metaphases of sufficient quality for evaluation due to cytotoxicity
 #3 = no further evaluation was carried out

(Excerpted from Applicant's report)

Summary of the clastogenic activity of treosulfan – with metabolic activation (+S9 mix)

4-h exposure			
Treatment (µg/mL medium)	Mitotic index ^{#1}	Number of metaphases scored	% of cells with aberrations excluding gaps
Aqua ad iniectionabilia			
0	1.00	300	1.3
Treosulfan Lyophilisate			
3.125	1.22	300	0.7
6.25	1.15	300	1.3
12.5	0.69	300	1.3
25	0.84	210 ^{#2}	8.6 *
50	0.32	160 ^{#2}	10.0 *
Cyclophosphamide			
20	0.94	300	8.0 *

* = significantly different from negative control ($p \leq 0.05$)
 #1 = mitotic index: number of metaphases/1000 cells; negative control = 1.00
 #2 = no more metaphases of sufficient quality for evaluation due to cytotoxicity

(Excerpted from Applicant's report)

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HALEH SABER
10/11/2024 02:42:49 PM

**CLINICAL TEAM LEADER REVIEW
DIVISION OF HEMATOLOGIC MALIGNANCIES 1**

Application Number	NDA 214759.65
Submission Type	Resubmission
Submission Date	8/10/2022
Applicant	medac
Proposed Trade Name	(b) (4)
Nonproprietary Name	Treosulfan
Dosage Form and Strength	Injection, lyophilized (1000 mg, 5000 mg)
Route of Administration	Intravenous
Proposed Dosing Regimen	10 g/m ² body surface area (BSA) per day as a two-hour intravenous infusion, given on three consecutive days (day -4, -3, -2) before hematopoietic stem cell infusion (day 0)
Proposed Indication	As part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).
Team Leader	Donna Przepiorka, MD, PhD

Regulatory Background: NDA 214759 was submitted 8/11/2020, and a CRL was issued on 7/30/2021. A resubmission with response to the CRL was received 4/21/2022, and an Acknowledge Incomplete Response Letter was issued on 5/16/2022 due to deficiencies in the datasets. SDN 65 includes a response to the CRL and responses to the issues in the 5/16/2022 letter.

Background: During review of the datasets in preparation for endpoint adjudication, a number of implausible laboratory results were identified. An Information Request was sent to the Applicant on 9/1/2022. The Applicant clarified several typographical errors that needed to be corrected. Two issues remained unresolved:

1. In lb.xpt, there were 86 instances where the marrow blast percentage (BLAST) was < 1 % and in some cases displayed as low as 0.01 %, numbers too low to be plausible for a blast count by morphology. In their 9/6/2022 response, the Applicant indicated that "Documented values <1%, however, are obviously not related to classical microscopic morphological assessments of bone marrow smears or histological BM specimens. *It is reasonable to assume* that the study sites performed, e.g., multiparameter flow cytometric (MFC) detection of minimal residual disease (MRD) of bone marrow aspirates to detect leukemic blasts and documented these results to the best of their abilities."

Review: The Applicant's response supports the uncertainty regarding whether the data for the parameter BLAST is a true marrow blast count. As such, this data file is unreviewable for the purposes of endpoint adjudication.

2. In adchim.xpt, there are 245 cases in which there is a result for a marrow chimerism study, but there are no marrow results in adlb.xpt based on the data element for parameter BLAST.

In their 9/6/2022 response, the Applicant indicated that "The questioned marrow results (leukemic blasts in bone marrow) are not available." They further asserted that marrow results were not required if there were no signs of relapse or progression and that additional marrow results were in adcefa.xpt.

Marrows on Day +28 and Day +100 were required by protocol independent of whether there were signs of relapse. Assessment of the discordant cases indicated that 179 of the missing were from Day +28 or Day +100, and that the data available in adcefa.xpt accounted for only 24 of the missing Day +28 or Day +100 cases.

Review: From the presence of marrow chimerism data without concordant marrow blast counts, one can conclude that there are still data that exist but are not submitted in accordance with the protocol.

3. In the table on page 65 of the Complete Response to the CRL received in SDN 65, the Applicant indicated that presence or absence of dysplasia was not a data element collected, and that the Cheson 2006 criteria were used.

Review: The Cheson criteria require analysis of outcomes with and without incorporating the presence of dysplasia. Consequently, it may not be possible to assess the overall response rate, the endpoint used for regulatory decision-making, for the subgroup of patients with MDS.

Summary Statement: Comment #1 of the CRL advised that submission of the CBC and marrow results through the assessment period for EFS would be needed to confirm that the results of Study MC-FLudT-14/L Trial II. The current version of the dataset is incomplete, and the parameter for the marrow blast count contains misleading information.

Recommendation: Acknowledge Incomplete Response Letter

Letter-Ready Comments

1. You have not provided substantial evidence of effectiveness of treosulfan as a part of a preparative regimen for allogeneic hematopoietic stem cell transplantation for adult and pediatric patients with AML or MDS for the following reasons:
 - a. A major element of the response criteria for both AML and MDS is the percentage of blasts on morphological examination of the marrow. In response to our 9/1/2022 query regarding implausible results in lb.LBSTRESN for LBTESTCD==BLAST, you indicated that this variable may include results of MRD assays rather than the percentage of marrow blasts. As such, there is insufficient information to confirm the primary endpoint of Study MC-FLudT-14/L Trial II.
 - b. In response to our 9/1/2022 query identifying cases where there were results for marrow chimerism studies in adchim.xpt but no results for blasts in lb.xpt on the same date, you indicated that marrow results were not required in the absence of signs of relapse, and that additional blast percentages were submitted in adcefa.xpt. According to the Flow

Chart / Clinical and Laboratory Tests in the protocol for Study MC-FLudT-14/L Trial II, disease status by marrow examination was required for at least Day +28 and Day +100. Assessment of the discordant cases indicated that 179 of the missing cases were from Day +28 or Day +100, which would be required by protocol independent of whether there were signs of relapse. Additionally, the data available in adcefa.xpt accounted for only 24 of the missing Day +28 or Day +100 cases. Therefore, it appears that there is still substantial missing data.

To address this issue, submit a pdf of the source documentation for all marrow results (i.e., copies of the pathology reports) through the complete assessment period for EFS for MC-FLudT.14/L Trial II, submit a revision of lb.xpt (and all dependent data files) to separate out results of the MRD assays from the percentage of marrow blasts, and submit a revision of adnewrel.xpt to include the results for percentage of marrow blasts from both lb.xpt and adcefa.xpt. Whether the results will be adequate to support approval of both indications will be a review issue.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

DONNA PRZEPIORKA
09/15/2022 02:56:35 PM

NDA/BLA Multidisciplinary Review and Evaluation

Application Number	NDA 214759
Application Type	Type 1: New Molecular Entity
Priority or Standard	Standard
Submit Date	8/11/2020
Received Date	8/11/2020
PDUFA Goal Date	8/11/2021
Office/Division	OOD/DHM1
Review Completion Date	7/30/2021
Applicant	Medac GmbH
Established Name	Treosulfan
(Proposed) Trade Name	Grafapex®
Pharmacologic Class	Alkylating agent
Formulations	Injection, lyophilized (1 gm, 5 gm)
Applicant Proposed Indication/Population	As part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).
Recommendation on Regulatory Action	Complete Response

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Grafapex (treosulfan)

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Reviewers of the Multidisciplinary Review and Evaluation

Nonclinical Reviewer	Amy Skinner, PhD
Nonclinical Team Leader	Brenda Gehrke, PhD
Office of Clinical Pharmacology Reviewer(s)	Lauren Price, PharmD; Ruojing Li, PhD; Jianghong Fan, PhD
Office of Clinical Pharmacology Team Leader(s)	Olanrewaju Okusanya, PharmD, MS; Lian Ma, PhD; Yuching Yang, PhD
Clinical Reviewer	Cara Rabik, MD, PhD
Clinical Team Leader	Donna Przepiorka, MD, PhD
Statistical Reviewer	Wenjuan Gu, PhD
Statistical Team Leader	Lisa Rodriguez, PhD
Cross-Discipline Team Leader	Donna Przepiorka, MD, PhD
Associate Director for Labeling (OOD)	Elizabeth Everhart, MSN, ACNP
Division Director (DHOT)	Haleh Saber, PhD
Division Director (OCP)	Brian Booth, PhD
Division Director (OB)	Thomas E. Gwise, PhD
Division Director (DHM1)	Angelo De Claro, MD
Office Director (or designated signatory authority)	Marc Theoret, MD

Additional Reviewers of Application

Regulatory Project Manager (DHM1)	Kristopher Kolibab, PhD
Associate Director for Safety (OCE)	Abhilasha Nair, MD
OPDP	Rachael Conklin, MS, RN
OPQ	Kabir Shahjahan, PhD; Paresma Patel, PhD; Olen Stephens, PhD; Anamitro Banerjee, PhD; Jin Lee, PhD; Ying Zhang, PhD; Elizabeth Bearr, PhD; Gouri Chattopadhyay, PhD; Sherita McLamore, PhD
OSE/DMEPA	Stephanie DeGraw, PharmD; Nicole Iverson, PharmD; Hina Mehta, PharmD
OSE/DRM	Till Olickal, PhD, PharmD; Naomi Boston, PharmD
OSI	Anthony Orencia, MD; Min Lu, MD, MPH

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Grafapex (treosulfan)

Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Conference on Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Event
NDA	new drug application

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NME	new molecular entity
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report PD pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy SAE serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

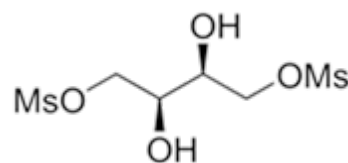
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Grafapex (treosulfan)

1 Executive Summary

1.1 Product Introduction

Proposed Trade Name:	Grafapex	
Established Name:	Treosulfan	
Also Known As:	Treosulphan, dihydroxybusulfan, CAS # 299-75-2, Ovastat®	
Chemical Name:	1,2,3,4-butanetcol, 1,4-dimethanesulfonate; threitol 1,4-dimethanesulfonate; threitol 1,4-bismethanesulfonate; L-threitol 1,4-bis(methanesulfonate); (2S,3S)-2,3-dihydroxybutane-1,4-diyl dimethanesulfaonte; (2S,3S)-(-)-1,4,-Di(mesyloxy)-2,3-butanediol	
Molecular Formula:	C ₆ H ₁₄ O ₈ S ₂	Chemical Structure:
Molecular Weight:	278.3 g/mol	
Dosage Forms:	Injection, lyophilized (1 gm, 5 gm)	
Therapeutic Class:	Antineoplastic	
Chemical Class:	Small molecule	
Pharmacologic Class:	Alkylating agent	
Mechanism of Action:	Treosulfan is an alkylating agent. DNA alkylation is thought to be responsible for the cytotoxic activities of treosulfan. Treosulfan depleted hematopoietic stem cells and showed immuno-suppressive and antitumor activity in mouse models of leukemia.	

NDA 214759 for Grafapex was submitted under the 505(b)(1) pathway for the indication "as part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS)" using a treosulfan dose of 10 g/m² per day combination with fludarabine based on the results of Study MC-FLudT-14/L Trial II. The Applicant also proposed to include in the USPI Section 14 a description of [REDACTED] (b) (4)

1.2 Conclusions on the Substantial Evidence of Effectiveness

A complete response is recommended under 21 CFR 314.125(b)(5) due to lack of substantial evidence of effectiveness.

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Grafapex (treosulfan)

MC-FLudT-14/L Trial II (NCT00822393) was a multicenter, randomized, active-controlled trial comparing treosulfan to busulfan in combination with fludarabine as a preparative regimen for allogeneic HSCT. Eligible patients included adults 18 to 70 years old with AML or MDS, Karnofsky performance status > 60%, and age ≥ 50 years or hematopoietic cell transplantation comorbidity index [HCT CI] score > 2. The patients were randomized to receive treosulfan 10 g/m² daily on days -4, -3 and -2 or to busulfan 0.8 mg/kg every 6 hours on days -4 and -3 in combination with fludarabine 30 mg/m² daily on days -6, -5, -4, -3 and -2, and hematopoietic stem cell transplantation on day 0. Antithymocyte globulin was administered as well to patients with unrelated donors.

The study was designed as a noninferiority trial with a primary endpoint of event-free survival (EFS) with 2 years of follow-up after transplantation. Events were defined as disease relapse, graft failure, or death. The noninferiority margin on the hazard ratio scale was 1.3. If noninferiority was demonstrated, then the primary endpoint would be tested for superiority. Overall survival (OS) was a secondary endpoint, but there was no alpha allocated to testing OS.

The trial was stopped at the second interim analysis when EFS noninferiority was achieved. Since the trial continued enrolling until the results of the interim analysis were known, an additional "last analysis" was conducted using all enrolled patients. On the basis of the last analysis, the Applicant concluded that the results supported noninferiority for EFS, and in sequential testing, superiority of treosulfan for EFS (48.6% information level). Superiority of treosulfan for OS was not demonstrated at the second interim analysis but was at the last analysis.

The review team was not able to confirm the results of the primary endpoint analysis, since the raw data needed to verify the components the EFS endpoint were incomplete. Additionally, in the absence of a prespecified plan for testing OS for superiority with control of Type 1 error, the results of the OS analysis were not considered substantial evidence of effectiveness.

1.3 Benefit-Risk Assessment

In the absence of evidence to conclude effectiveness, a benefit-risk assessment cannot be formulated.

1.4 Patient Experience Data

Patient Experience Data Relevant to this Application

X	Patient experience data were not submitted as part of this application.
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NDA Multidisciplinary Review and Evaluation

NDA 214759

Grafapex (treosulfan)

1.5 Comments to Applicant

1. You have not provided substantial evidence of effectiveness of treosulfan as a part of a preparative regimen for allogeneic hematopoietic stem cell transplantation for adult and pediatric patients with AML or MDS for the following reasons:
 - The primary endpoint of Study MC-FLudT-14/L Trial II was event-free survival (EFS). Because all complete blood cell (CBC) and marrow results were not submitted, we were unable to independently confirm the results of the analysis of the primary endpoint of EFS for Study MC-FLudT-14/L Trial II.
 - Additionally, because of the missing CBC and marrow results, we were not able complete our assessment of whether the results of the study would support the indications for treatment of patients with AML and for treatment of patients with MDS.

To address this issue, submit the CBC and marrow results through the complete assessment period for EFS for MC-FLudT.14/L Trial II. Whether the results would be adequate to support approval of both indications will be a review issue.

2. (b) (4) OS cannot be used as the sole basis for approval for the following reasons:

- The statistical plan for Study MC-FLudT-14/L Trial II did not include control of Type 1 error for overall survival (OS).
- There was no prespecified boundary for OS interim analyses and no alpha allocation for multiple OS analyses. Therefore, p-values are nominal, and analysis of OS is considered to be exploratory.

To address this issue, submit data from an adequate and well-controlled trial of treosulfan vs busulfan with a prespecified analysis of OS for each indication with control of Type 1 error.

Additional Comments

Clinical/Statistical

1. Treatment of AML and treatment of MDS are considered different indications. In order to obtain approval for both indications, you would need to demonstrate substantial evidence of effectiveness for each individually.

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Grafapex (treosulfan)

2. Your subgroup analyses of EFS and OS suggest a substantial difference in treatment effect by donor type. Clarify how the results support your claim of noninferiority of treosulfan for HSCT from both related and unrelated donors (with the latter using ATG).
3. You conducted your efficacy analyses in the Full Analysis Set (FAS). For a randomized trial with EFS and/or OS endpoints, the analyses should be conducted on the intent-to-treat (ITT) population.
4. For Study MC-FludT.14/L II, the variable RACE in ADSL has no data element for any of the study subjects. Consequently, the submission did not provide adequate information to ensure that the study results are applicable to the US population. Consider conducting a study to assess outcomes by race and ethnicity for patients undergoing allogeneic HSCT using treosulfan-fludarabine as the preparative regimen. Include subgroup analyses by race and ethnicity for overall survival, nonrelapse mortality and relapse-free survival with at least 3 years of follow-up.
5.  (b) (4)

Clinical Pharmacology

6. Address the following in the NDA resubmission:
 - a. Provide data to determine an appropriate safe dose of treosulfan for patients with moderate renal impairment (CL_{cr} 30-59 mL/min). This should be conducted in accordance with the FDA Guidance for Industry titled “Pharmacokinetics in Patients with Impaired Renal Function: Study Design, Data Analysis, and Impact on Dosing and Labeling.”
 - b. Provide data to support the evaluation of the effects of treosulfan on the QTc interval. This should be done in accordance with ICH E14.
 - c. Provide data evaluating treosulfan and its epoxide metabolites as substrates of metabolizing enzymes *in vitro*.
 - d. Provide data evaluating the pharmacokinetics (PK) of treosulfan active metabolites in adult patients.
 - e. Provide data evaluating the effects of race and ethnicity on treosulfan PK.

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Nonclinical

7. We recommend that prior to the resubmission of the NDA, you conduct an in vitro study demonstrating DNA alkylation and breakage with treosulfan to adequately characterize the alkylating and genotoxicity properties and determine the potential serious risk of reproductive and developmental toxicity and carcinogenicity with treosulfan. This study should be submitted to the NDA with the resubmission.

2 Therapeutic Context

2.1 Analysis of Condition

Acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (MDS) have high rates of morbidity and mortality.

2.1.1 Acute Myeloid Leukemia

AML is the most common type of acute leukemia in adult patients, with an incidence of 21,450 patients in the US in 2019. Fifty percent of patients with AML die of their disease. The incidence of AML increases with age. As the population of the United States ages, the incidence and median age of AML continues to increase. The median age of diagnosis is approximately 68 years [1]. Older patients often do not tolerate intensive chemotherapy, underscoring the need for new therapies.

AML is a life-threatening condition. Allogeneic hematopoietic stem cell transplantation (HSCT) is required for curative therapy of high risk AML and IPSS-R intermediate, high, and very high risk MDS [2, 3]. A meta-analysis of 24 trials evaluating allogeneic HSCT, autologous HSCT, and chemotherapy alone for AML identified a significant reduction in hazard of death if allogeneic HSCT occurs in CR1 as compared to autologous HSCT or chemotherapy alone (hazard ratio 0.9, 95% CI 0.82-0.97) [4]. Further analysis revealed that the benefit of allogeneic HSCT was significant only in intermediate-risk (HR 0.76) and poor-risk (HR 0.69) AML, but not in favorable-risk (HR 1.06) AML. Additionally, the combined HR for relapse-free survival was 0.8 (0.74-0.86) across all risk groups and 0.78 (0.73-0.84) for intermediate and poor-risk patients.

2.1.2 Myelodysplastic Syndrome

MDS encompasses a group of hematopoietic stem cell disorders, which have dysplasia in myeloid, erythroid, or megakaryocytic lineages and are associated with cytopenias [5]. The median age of diagnosis is 70 years [5]. The most recent SEER 5-year age-adjusted incidence

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rate (2014-2018) is 4.2 per 100,000, with a higher incidence in men (5.8 per 100,000 compared to 3.1 per 100,000 in women) [6].

Risk classification of MDS is performed using the Revised International Prognostic Scoring System (IPSS-R). Patients are classified as having very low, low, intermediate, high, or very high risk MDS based on bone marrow cytogenetics, bone marrow blast percentage, and cytopenias [7]. Patients with intermediate, high-risk, or very high-risk MDS based on IPSS-R require allogeneic stem cell transplantation for curative therapy [8].

2.2 Analysis of Current Treatment Options

Currently, no drugs are approved for use as a preparative regimen prior to allogeneic HSCT for AML or MDS.

Drugs used in the standard of care for preparative regimen for allogeneic HSCT in AML and MDS include, but are not limited to, cyclophosphamide, busulfan, fludarabine, melphalan, ATG, and radiation therapy. Table 1 provides comparisons of various HSCT preparative regimens from 1996-2020.

Table 1. Comparisons of Selected HSCT Conditioning Regimens, 1996-2020

Study	Comparison					Conclusion
	Regimen A	Regimen B	Outcome	A (%)	B (%)	
Ringden, et al. [12] – AML in CR1	MAC Cy + TBI	MAC Cy + Busulfan (PO)	LFS (2 y)	66	64	Intermediate risk defined as 2 nd or later remission or 1 st relapse. No statistical difference between Cy/TBI and Cy/Bu in either risk group.
Ringden, et al. [12] – intermediate risk AML			RR (2 y)	19	23	
			TRM (2 y)	19	16	
			LFS (2 y)	47	32	
			RR (2 y)	44	55	
			TRM (2 y)	15	28	
Uberti, et al. [13]	MAC Cy + TBI (std)	MAC Cy + Busulfan (PO)	OS (5 y)	32	35	No statistical difference between Cy/TBI and Cy/Bu.
RR (1 y)			9	10		
TRM (1 y)			43	48		
Bornhauser, et al; Fasslrinner, et al. [15, 16]	MAC Cy/TBI	RIC Flu/TBI	DFS 3 y	56	58	RIC has similar NRM and decreased toxicity than MAC, with no evidence of increased risk of late relapse.
			DFS 10 y	43	55	
			OS 3 y	58	61	
			OS 10 y	47	60	
			RI 3 y	26	28	
			RI 9 y	30	30	
Ringden, et al. [19]	MAC Bu/Cy	RIC Flu/Bu	NRM 3 y	18	13	RIC is associated with decreased TRM; there was no
			NRM 10 y	26	16	
			LFS (5 y)	58%	83%	
			OS (5 y)	62	76	
			RR	35	12	

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			TRM (5 y)	10.5	11.5	significant difference between RIC and MAC for OS, LFS, or RR.
Nagler, et al. [11]	MAC Cy + TBI	MAC Cy + Busulfan (IV)	LFS (2 y)	64	61	Bu/Cy not significantly different from Cy/TBI
			OS (2 y)	69	68	
			RI (2 y)	21	26	
			NRM (2 y)	15	12	
Scott, et al (2017, 2020) [14, 21]	MAC Flu/Bu4 Bu/Cy TBI	RIC Flu/Bu2 Flu/Mel	RFS 18 mo	67.8	47.3	MAC should be standard of care in fit patients with AML or MDS despite higher rates of TRM with MAC.
			RFS 4 y	58	34	
			OS 18 mo	77.5	67.7	
			OS 4 y	65	49	
			RR (18 mo)	13.5	48.3	
			TRM (18 mo)	15.8	4.4	
Kröger, et al. (2017, 2018) [17, 18]	MAC Bu/Cy	RIC Bu/Flu	RFS 2y	58	62	In MDS or sAML with <20% blasts, RIC is “at least equivalent” to MAC
			RFS 5y	51	57	
			OS 2 y	63	76	
			OS 5 y	53	69	
			RR 2 y	15	17	
			RR 5 y	18	22	
			NRM 1 y	25	17	
			NRM 5 y	30	22	
Zhou, et al. [1]	RIC Flu/Bu	RIC Flu/Mel	LFS (5 y)	25	28	RIC regimen remains an individual decision based on perceived risk and benefit
			OS (5 y)	31	35	
			RR (5 y)	55	36	
			NRM (5 y)	21	36	
Source: Reviewer’s analysis of literature search						
Abbreviations: HSCT: hematopoietic stem cell transplant; AML: acute myeloid leukemia; Cy: cyclophosphamide; TBI: total body irradiation; Bu: busulfan; Flu: fludarabine; Mel: melphalan; LFS: leukemia-free survival; OS: overall survival; RR: relapse rate; TRM: transplant-related mortality; NRM: non-relapse mortality RI: relapse incidence; DFS: disease-free survival; RFS: relapse-free survival; MDS: myelodysplastic syndrome; sAML: secondary AML.						

2.2.1 Myeloablative conditioning

Preparative regimens for allogeneic HSCT are not standardized and range greatly from institution to institution and country to country. Myeloablative conditioning (MAC) results in complete eradication of host hematopoiesis and severe immunosuppression. Severe myelosuppression may directly kill leukemia stem cells, and AML is further attacked by donor cells, causing a graft versus leukemia (GVL) effect. The most common myeloablative preparative regimens are cyclophosphamide (120 mg/m²) and total body irradiation and busulfan (14-16 mg/kg) and cyclophosphamide (120-200 mg/m²). However, MAC is associated with high rates of transplant-related morbidity and mortality, particularly in older patients with pre-existing comorbidities [9].

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2.2.2 Reduced intensity conditioning

Reduced intensity conditioning (RIC) regimens have lower rates of transplant-related morbidity and mortality, but they range in their myelosuppressive and immunosuppressive characteristics. In general, the CIBMTR defines RIC regimens as TBI ≤ 5 Gy (single dose) or ≤ 8 Gy (fractionated), busulfan doses ≤ 9 mg/kg, or melphalan doses ≤ 150 mg/m². The most frequently used RIC regimens in a 2015 publication included fludarabine with either low-dose TBI or an alkylating agent [10]. A T-cell depleting agent such as ATG or Campath is used often, especially for matched unrelated donors.

2.2.3 MAC versus RIC

Table 1 shows a comparison of HSCT conditioning regimens over the last 25 years. For patients with AML, MAC regimens are generally not significantly different from each other [11-13].

Comparison of MAC vs RIC for patients with AML has not led to consensus. A 2017 publication of a study conducted by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) showed decreased transplant-related mortality with RIC HSCT. The study population included patients with MDS or AML aged 18-65 with HCT-CI ≤ 4 . While overall survival (OS) was similar between the groups, MAC HSCT resulted in a significantly decreased risk of relapse-free survival (RFS) compared to RIC HSCT [14]. Interestingly, patients with MDS did not have a difference in RFS. However, multiple regimens were available for both RIC and MAC HSCT, with no one standard of care. Sixty-four percent of MAC HSCTs were Flu/Bu4, 30% were Bu/Cy, and the remainder were Cy/TBI. RIC HSCT regimens included Flu/Bu2 (81%) and Flu/Mel (19%) [14]. Other studies have not shown differences in OS or event-free survival, however [15-19]. For AML patients with detectable minimal residual disease, MAC may result in improved survival [20].

Within RIC regimens, two of the most commonly used conditioning regimens are fludarabine + busulfan and fludarabine + melphalan. The CIBMTR published an analysis of their transplant registry, comparing 622 AML patients who received a fludarabine + melphalan RIC HSCT and 791 AML patients who received a fludarabine + busulfan RIC HSCT. Fludarabine + melphalan was associated with higher transplant-related mortality but lower relapse risk; overall survival and leukemia-free survival were similar between the two regimens [1].

2.2.4 Patient Selection for RIC

While RIC HSCTs are safe and have efficacy, particularly in an older, less fit population, MAC regimens continue to confer a benefit in survival and relapse. However, as the age of AML diagnosis increases, more AML patients are undergoing RIC HSCT. While there are no standard guidelines for which patients should receive RIC vs MAC, one trial made the following recommendations for RIC [14]:

1. Patients >65 years of age OR
2. Patients with HCT comorbidity index (HCT-CI) scores ≥ 3 .

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HCT-CI scoring includes categories for age and a number of potential comorbidities in different organ-system classes. A multi-institutional retrospective analysis of approximately 3000 patients validated the use of the HCT-CI score for TRM. Age >20 was associated with increasing risk of TRM, with statistical significance for all patients 40 years of age and older. An HCT-CI of 0, 1, or 2 resulted in no statistical significance in the hazard ratio for survival; survival rates were >60% at two years for both RIC and MAC. An HCT-CI score of 3 or 4 resulted in two-year survival of 50% for RIC and 45% for MAC; an HCT-CI ≥ 5 had two-year survival rates of 35 and 29%, for RIC and MAC, respectively [9].

Based on these data, for patients with AML and MDS who are either ≥ 65 years of age, or who have an HCT-CI score ≥ 3 should be considered for RIC allogeneic HSCT.

3 Regulatory Background

3.1 U.S. Regulatory Actions and Marketing History

Treosulfan is not currently marketed in the United States and has no history of being marketed in the United States.

3.2 Summary of Presubmission/Submission Regulatory Activity

Interactions between the Applicant and the Agency were conducted under IND 054552. The clinical trials supporting this application were conducted outside the US and not under the IND. The key presubmission regulatory activities for this agent are as follows:

- IND 054552 was received in October 1997. It was inactivated in March 2003 and terminated in July 2010 due to lack of activity for more than 5 years.
- IND 054552 was reinstated and reactivated in 2013 to provide a source for cross references.
- Type B EOP2 Meeting 9/4/2014 - Key advice included that US Busulfex would be expected as the comparator, data was needed to show that EFS is a valid surrogate of OS in the population of patients transplanted for AML in CR and with MDS, data would be needed to serve as the basis for the noninferiority margin, the design does not provide any assurance that there would be sufficient power to test long-term outcomes within disparate groups by disease, donor type and MHC compatibility, (b) (4)

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- Orphan drug designation was granted 4/8/2015 for treosulfan as a “conditioning treatment prior to HSCT in malignant and nonmalignant diseases in adults and pediatric patients.”
- Type C Meeting responses 12/3/2015 - Key advice included the need to use US Busulfex as the comparator in the US, the need for justification for use of EFS rather than OS as the basis for determining noninferiority, the need to demonstrate that the constancy assumption was met in the determination of the noninferiority margin, the need to provide data showing the magnitude of the treatment effect of Bu-Flu over a comparator (such as chemotherapy alone) for the intended population, the need to justify why a loss of nearly 10 percentage points of OS is not clinically meaningful relative to the treatment effect of Bu-Flu over a comparator, the need to justify the design not addressing the heterogeneity in the eligible population with regard to disease, disease status, donor type and MHC compatibility, (b) (4), that the ITT population (all randomized) should be used for the primary efficacy analysis, and that OS and EFS should be conducted based on event-driven analyses.
- Type C Meeting 9/17/2019 - Key advice included the need to provide data showing the magnitude of the treatment effect of Bu-Flu over a comparator for the intended population, the need to justify why a loss of nearly 10 percentage points of EFS is not clinically meaningful relative to the treatment effect of Bu-Flu over that comparator, and that the analyses should be event-driven and that the confidence intervals and significance levels be adjusted accordingly. Additionally, since EFS at 2nd interim analysis used only a 28% information level, the data did not appear sufficiently mature as a basis for a marketing application.
- Type C Written Response Only 2/24/2020 - FDA advised the Applicant to evaluate the potential for treosulfan to prolong the QT/QTc prolongation interval, and to identify the pathways by which treosulfan and its active metabolites, if any, are eliminated to determine the need to assess the effect of organ impairment on their systemic exposures.
- Type B Pre-NDA Meeting 4/2/2020 - FDA noted that in the proposed “Justification of NI Margin”, the assertion that BuFlu has an equivalent treatment effect vs. chemotherapy to other conditioning regimens was not supported by data, that the noninferiority margin was based on a point estimate rather than incorporating the variability of the historical estimate, and that the proposed post-hoc justification of the noninferiority margin should document all studies considered for such developing such a margin, and the reasons for including or excluding studies in the subsequent analyses.
- Type C Written Responses Only 7/6/2020 - FDA provided advice on the design of Study MC-FludT.18/QT to assess for effects on QTc, indicated that the NDA should include a complete

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assessment of the safety of the drug, including effects on QTc, and requested that any delay in conduct of the QT study should be justified in the NDA submission.

- NDA 214759 was received on 8/11/2020 and filed on 10/7/2020 with a standard review classification. The following filing and review issues were noted:
 - For MC-FludT.14/L II, the post-HSCT bone marrow studies and the CBC results post Day +100 were not submitted from the clinical sites as part of the CRF, we are not able to independently verify the primary endpoint of EFS at 24 months. Without these data, our ability to perform a substantive review will be limited.
 - For Study MC-FludT.14/L II, the variable RACE in ADSL has no data element for any of the study subjects. The submitted package lacks adequate information to ensure that the study results are applicable to the US population.
- Midcycle Communication was held 1/10/2021. The FDA reiterated the above review issues.
- Late Cycle Communication was held 5/5/2021. In addition to the above, the FDA identified the following issue:
 - Study MC-FludT.14/L Trial II included patients with matched related donors (MRD) and matched unrelated donors (MUD). Survival (OS) was significantly different between the treosulfan and busulfan arms for the ITT population with a hazard ratio (HR) of 0.65 (95% CI: 0.48-0.88), but on subgroup analysis, there was no apparent treatment effect in the patients with MRDs (HR 1.002 (0.55, 1.84)). (b) (4)

3.3 Foreign Regulatory Actions and Marketing History

Treosulfan was developed in 1964 and has a foreign regulatory and marketing history.

- Approved for ovarian cancer in eight European countries in 1973 as an oral formulation and in 1996 as an IV formulation.
- June 2019: Approved in the European Union / EEA for conditioning therapy prior to allogeneic HSCT in adult patients with malignant and non-malignant diseases, and in pediatric patients >1 month of age with malignant diseases.
- August 2020: Approved in Switzerland for the conditioning therapy prior to allogeneic HSCT in adult patients with malignant and non-malignant diseases, and in pediatric patients >1 month of age with malignant diseases.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1 Office of Scientific Investigations (OSI)

Two clinical sites(Site 01 Essen and Site 27 Mainz) were inspected for Study Mc-FludT.14/L. Site 01 Essen has the largest number of patients enrolled (~20% of the randomized population) and a disproportionate number of protocol violations. Site 27 Mainz has a disproportionate hazard ratio for the primary endpoint (0.21 vs 0.69 for the entire study population) and a disproportionate number of protocol violations. Based on the inspections, OSI concluded that the conduct at the study sites was adequate and study data derived from clinical sites appeared reliable.

4.2. Product Quality

Not applicable

4.3 Devices and Companion Diagnostic Issues

There were no companion diagnostics or other devices in this submission.

5 Nonclinical Pharmacology/Toxicology

ALL Acute lymphoblastic leukemia
cALL common acute lymphoblastic leukemia
.....(b) (4)
FACS fluorescence activated cell sorting
IC₅₀ inhibitory concentration yielding 50% response
IL-2 interleukin 2
IFN-γ interferon-γ
.....(b) (4)
NOD/SCID Non-obese diabetic/ severe combined immunodeficient (mice)
PMR postmarketing requirement
PND postnatal development
TNF-α tumor necrosis factor-α

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5.1 Executive Summary

Treosulfan (L-Threitol 1,4-bismethanesulfonate) is an alkylating drug and is closely related to busulfan with the addition of 2 hydroxyl groups to facilitate solubility in water. Since treosulfan was synthesized 60 years ago and has been widely used as a cytotoxic drug to treat ovarian cancer for many decades, it is well-characterized in peer-reviewed scientific literature. The Applicant (Medac GmbH) provided references to published studies in support of the marketing application. Treosulfan is indicated in combination with fludarabine as a preparative regimen for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). Except as specifically identified, all nonclinical data and information discussed below and necessary for approval of this NDA (#214759) are owned by Medac GmbH or are data for which Medac GmbH has a right of reference. Any information or data that Medac GmbH does not own or have a written right to reference is for descriptive purposes only and is not relied upon for approval of this NDA.

In cellular assays employing primary pediatric leukemic cells and leukemic cell lines, 72-hour treosulfan exposure had a cytotoxic effect with inhibitory concentration yielding 50% response (IC_{50}) values ranging from 0.7 to 490 μ M. Cells from patients with common acute lymphoblastic leukemia (cALL) were most sensitive, while cells from a patient with mature B-ALL were most resistant to treosulfan. In most cases, under the conditions tested, cells were more sensitive to treosulfan than to busulfan. Immune cells were also sensitive to treosulfan cytotoxicity, as T cells, NK cells, and stem cells isolated from healthy donors yielded IC_{50} s from <0.4 to 49.7 μ M, with stem cells exhibiting the most sensitive effect.

Treosulfan showed in vivo myeloablative and immunosuppressive activity in a murine allogeneic bone marrow transplant model. BALB/c mice received 1.5 g/kg/day of treosulfan intraperitoneally (i.p.) x 3 days. Stem cells (assessed via colony forming units, CFUs) were rapidly depleted by post-treatment Day 1 and were negligible for the remaining 12 days of the experiment. Fluorescence activated cell sorting (FACS) analysis also showed decreases in T and B cells. The number of immune cells producing tumor necrosis factor (TNF)- α or interferon (IFN)- γ remained constant or decreased slightly, while the percentage of interleukin 2 (IL-2)-producing T cells decreased to 50% of control values on Days 6-12. A mixed lymphocyte reaction test demonstrated that treosulfan-induced T-cell depletion suppressed alloreactivity in host splenic cells (BALB/c) mixed with C57/black stimulator lymphocytes.

The Applicant also included several references describing treosulfan's antileukemic and antineoplastic activity in vivo. Antitumor activity was demonstrated in mice bearing sarcoma or carcinoma tumors.

In cardiovascular in vitro safety pharmacology studies, treosulfan did not exhibit the potential to induce QT prolongation. There were also no changes in cardiovascular parameters when

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fasting dogs received 5-200 mg/kg of treosulfan intravenously (i.v.) and were monitored via ECG for 4 hours. Central nervous system (CNS) functions were not altered in mice that received 100 mg/kg of treosulfan i.p. or in a fasted dog that received 200 mg/kg of treosulfan via oral administration (p.o.).

To assess the safety of treosulfan, the Applicant conducted a GLP-compliant toxicology study up to 4 weeks in adult Sprague-Dawley rats. Rats received treosulfan at doses of 10, 50, or 150 mg/kg via i.v. once per day x 28 days, followed by a 2-week recovery period. The Applicant assessed 2 different formulations of treosulfan, a powdered form and a lyophilisate form. Toxicokinetic (TK) analysis showed that exposures were dose-proportional and were comparable between males and females. Exposures and toxicities were also generally comparable between formulations. There was no apparent accumulation over time. Drug-related adverse findings included bone marrow suppression, lymphoid atrophy in spleen, thymic hemorrhage and atrophy, GI tract hyperplasia, muscle necrosis, multi-organ inflammation, and injection site reactions. Inflammation was observed in the kidney, colon, urinary bladder, and prostate gland. The male and female reproductive organs (epididymis, testes, prostate, and ovary) were also targets of toxicity. Other findings included optic nerve hemorrhage, and Peyer's patch hyperplasia. There were 2 unscheduled deaths, both animals were in the high dose group and received the lyophilisate formulation. One female died in the 3rd week of dosing and was found to have edematous lungs and dilated intestines filled with fluid. The second animal was a male in the recovery cohort that had to be euthanized 1 day prior to the scheduled sacrifice (Day 42). The male had swelling in several areas including the nose/snout, paw, and testes that was discovered, upon histology analysis, to be a malignant leukemia. Males treated with either formulation also had enlarged epididymis and testes, which was not reversed during the recovery phase. Females treated with the powdered formulation had dose-dependent shrunken ovaries. Animals receiving either formulation had a dose-dependent increase in brain weight.

Treosulfan is genotoxic and induced a concentration-dependent increase in percent of anaphases with aberrations in onion root tips. The percentage of cells with aberrant anaphases increased from 3% to 21% when cells were treated with 50 mM of treosulfan or 0.05 mM of diepoxybutane (DEB), respectively. Dedicated carcinogenicity or fertility studies were not conducted with treosulfan, as they are not needed to support the use of a drug intended to treat patients with advanced cancer. Cytotoxic drugs that demonstrate genotoxicity are generally recognized as being carcinogenic, and in the general toxicology study, one animal treated with treosulfan presented with leukemia. A 7-month chronic-dose study conducted in Wistar rats revealed dose-dependent fertility impairment in males and females (i.e. arrested spermatogenesis and absent corpora lutea and follicles). To assess the potential for developmental toxicity, the Applicant conducted a juvenile toxicity study in which juvenile rats received treosulfan dosed at 10, 50, or 100 mg/kg/day i.v. in main and recovery groups during postnatal development days (PND) 10 to 35. Since the hematopoietic organs are targets of treosulfan, the findings in juvenile animals were typically comparable to the findings observed

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in adult animals. Juvenile rats had delayed development, observed as reduced body weight and a dose-dependent reduction in the size of male reproductive organs (testes and prostate). Moreover, females at 100 mg/kg experienced a significant delay in sexual maturation (vaginal opening delayed by 1.8 days). A pharmacokinetic assessment of the blood-brain barrier penetration of treosulfan showed that following single intravenous administration of 500 mg/kg to juvenile or young adult rats, plasma exposures (C_{max}) were comparable between groups but juvenile rats had exposures 2-fold higher than those observed in young adult rats. A local tolerance test conducted in New Zealand White rabbits was negative for drug-related findings.

There is no information on the presence of treosulfan in milk. As many drugs are secreted in breastmilk and because treosulfan has the potential to cause serious toxicities, the label includes a warning not to breastfeed during treatment with treosulfan and for 1 week after the final dose. The 1-week recommendation is based on the short half-life of the drug ($5 \times T_{1/2} < 1$ week).

No embryofetal developmental toxicity was conducted. Drugs that are genotoxic and target rapidly dividing cells are expected to cause embryofetal lethality or malformations (see ICH S9). The product label will include this information and will provide a duration of contraception consistent with the FDA guidance *Oncology Pharmaceuticals: Reproductive Toxicity Testing and Labeling Recommendations* for genotoxic drugs.

From a pharmacology/ toxicology perspective, there are no outstanding issues that would prevent the approval of treosulfan in patients undergoing allogeneic hematopoietic stem cell transplantation (b) (4) with AML or MDS; however, there is one nonclinical post-marketing requirement (PMR). During the review of the NDA, it was determined that the nonclinical data for treosulfan submitted to the NDA in studies or literature for which the Applicant has the right to reference did not provide convincing evidence of DNA alkylation and breaks as the mechanism of action (MOA) of treosulfan. The PMR is to conduct an in vitro study to show treosulfan binds to DNA and causes DNA breakage.

As the recommendation has been a complete response (see Section 1.2), the pharmacology/ toxicology discipline recommends the Applicant conduct and submit the results of the study at the time of resubmission.

5.2 Referenced NDAs, BLAs, DMFs

None

5.3 Pharmacology

Primary Pharmacology

In vitro pharmacology

Treosulfan exhibited antileukemic activity in pediatric tumor cells and cell lines. Munkelt et al. (2008) employed flow cytometry to quantify the cytotoxic effect of 72-hour exposure to treosulfan on primary pediatric leukemic cell specimens (bone marrow aspirates, pleural effusions, and/ or peripheral blood) obtained from 20 children at the time of diagnosis, during therapy, or at relapse. Under the conditions tested, treosulfan showed greater activity than busulfan in all specimens except for 1 cALL relapse specimen (Table 2). Treosulfan demonstrated the greatest activity toward stem cells (CD34+) isolated from a healthy donor ($IC_{50} = <0.4 \mu M$).

Table 2. Representative IC_{50} s for Treosulfan in Primary Pediatric Leukemic Cells, Cell Lines, and Immune Cells from Healthy Donors

Diagnosis	Disease Status	Treosulfan IC_{50} (μM)	Busulfan IC_{50} (μM)
cALL	initial	0.7	8.1
cALL	initial	52.5	243.6
cALL	initial	13.1	81.2
cALL	progression	61.3	243.6
cALL	relapse	183.8	162.4
cALL	relapse	122.5	406.0
Mature B-ALL	initial	490.0	538.0
Pre T-ALL	initial	5.3	126.0
Mature T-ALL	initial	1.8	12.2
Mature T-ALL	relapse	1.8	52.8
Mature T-ALL	progression during relapse therapy	14.0	89.1

Cell Line	Treosulfan IC_{50} (μM)	Busulfan IC_{50} (μM)
H9 (T-ALL)	55.0	122.0
Molt4/8 (T-ALL)	9.8	15.7
K562 (CML)	147.0	540.0

Healthy Donor	Treosulfan IC_{50} (μM)	Busulfan IC_{50} (μM)
T-cells Donor 1	12.3	122.0
T-cells Donor 2	45.5	89.8
NK cells Donor 4	49.7	144.6
NK cells Donor 5	1.8	9.0
Stem cells (CD34+)	<0.4	7.3

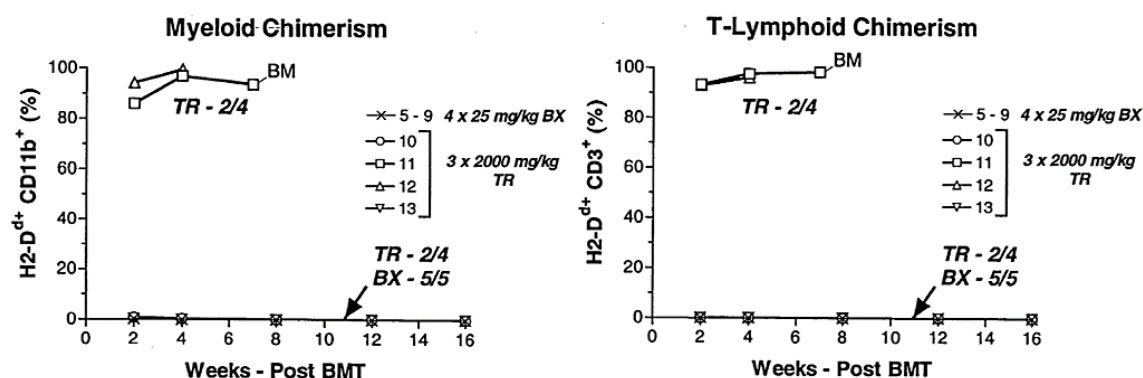
cALL = common acute lymphoblastic leukemia; CML = chronic myeloid leukemia; NK = natural killer

(Adapted from publication)

In vivo pharmacology

Treosulfan had myeloablative properties in vivo. The Applicant assessed the ability of treosulfan to condition mice for allogeneic bone marrow transplant (Expt. (b) (4) 008). Investigators treated C57BL/6J adult male mice (5/group) with a 4-day conditioning regimen of either 4 daily doses of 25 mg/kg Busulfex or 3 daily doses of 2000 mg/kg treosulfan administered i.p., followed by transplantation with 10×10^6 bone marrow cells from B10.A donors. All Busulfex-treated mice survived the 16-week post-transplant period with little demonstration of toxicity. In contrast, treosulfan-treated mice experienced weight loss and diarrhea, and 1 mouse died. Donor chimerism (anti-H-2D^d staining) on myeloid and lymphoid cells showed no donor cells in Busulfex-treated mice. Two treosulfan-treated mice exhibited 85-100% donor chimerism; mice had grossly inflamed small intestines upon necropsy and may have developed GVHD (Figure 1).

Figure 1. Donor Chimerism in Peripheral Mouse Blood Following Busulfex or Treosulfan Conditioning and Allogeneic BMT



Bx = Busulfex; TR = treosulfan; BMT = bone marrow transplant (Excerpted from the Applicant's submission)

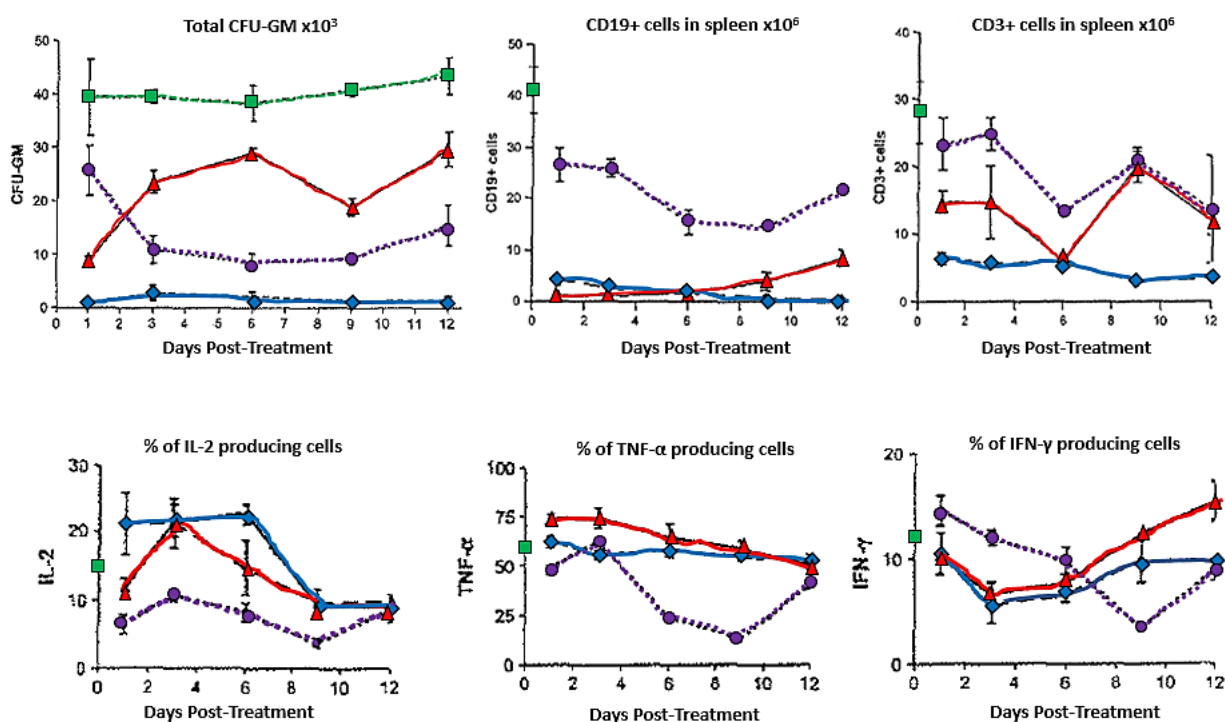
Treosulfan had myeloablative and immunosuppressive properties in studies conducted in mice. Sjöö et al. (2006) exposed BALB/c mice to treosulfan at 1.5 g/kg/day via i.p. injection for 3 consecutive days, then conducted stem cell and immune cell assays to characterize the effects. Stem cell quantity was rapidly depleted by post-treatment Day 1 and remained negligible through the remainder of the experiment (post-treatment Day 12), as assessed via colony forming units-granulocyte macrophage (CFU-GM; **Error! Reference source not found.**, top row, left panel). FACS analysis revealed decreases in splenic B and T cells by post-treatment Day 1, reaching nadirs of 12.5% and 25% of controls, respectively, that remained suppressed through post-treatment Day 12 (**Error! Reference source not found.**, top row, middle and right panels). Immunophenotyping showed comparable decreases in CD4+ T helper and CD8+ T killer cells, rather than elimination of specific subpopulations (not shown). Treosulfan treatment resulted in an increase in the percentage of IL-2-producing cells on post-treatment Days 1-3 following 6-hour in vitro activation with PMA/ Ionomycin. This IL-2-producing CD3+ T-cell population decreased to 50% of controls from post-treatment Days 6-12 (Figure 2, bottom row, left panel). The number of splenic cells producing TNF- α remained constant (Figure 2, bottom row, middle panel). The percentage of T cells producing IFN- γ only slightly decreased over the 12 post-treatment days (Figure 2, bottom row, right panel). Finally, a mixed lymphocyte reaction experiment demonstrated that treosulfan-induced T-cell depletion suppressed alloreactivity in host splenic cells (BALB/c) mixed with C57/black stimulator lymphocytes (data not shown).

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Figure 2. Treosulfan Effects on Bone Marrow Stem Cells and Immune Cells



Control = green squares/ dotted line; treosulfan = blue diamonds/ solid line; busulfan = purple circles/ dotted line; cyclophosphamide = red triangles/ solid line
(Adapted from publication)

Treosulfan also had antineoplastic and antileukemic activity in several xenotransplantation models. In one published study the Applicant referenced, NOD/SCID mice bearing subcutaneously implanted human pediatric pre-B acute lymphoblastic leukemic (ALL) cells grew palpable tumors (about 5 mm) in 18 days, and then received a single intraperitoneal (i.p.) bolus injection of treosulfan at 1000, 2000, or 3000 mg/kg (Fichtner, 2003). Mice in the 1000 mg/kg group had delayed tumor growth (~7 days) compared to the saline-treated controls. Mice that received 2000 mg/kg had no evidence of tumors by Day 25, but tumors recurred in all mice by Day 55. Mice that received 3000 mg/kg also did not show any presence of tumor, but 1/8 had recurrent tumor by Day 55 (data not shown). In another Applicant-referenced study, NOD/SCID mice bearing Ewing's sarcoma (ES) cell line-derived tumors (CHP-100 cells) received an i.p. bolus injection of 3000 mg/kg of treosulfan. While control mice had a mean tumor volume of ~3500 mm³ on Day 13, treosulfan-treated mice had a mean tumor volume ~100 mm³, demonstrating activity against ES tumors in vivo (not shown).

Secondary Pharmacology

None

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Safety Pharmacology

Cardiovascular function

Study # CYP1269 R11-R12: Test of Treosulfan on hERG, hNav1.5, hCav1.2, hKir2.1 and hKCNQ1/minK Cardiac Ion Channels Using Automated Patch Approach

Investigators incubated HEK293 cells stably expressing human hERG potassium channel with treosulfan lyophilisate (200, 1000, or 5000 μM) or 0.3 μM of the reference article, E-4031, and measured potassium current using the patch-clamp technique in a non-GLP study. For comparison, the C_{max} in adults administered a clinical dose of 10 g/m^2 i.v. treosulfan is approximately 300 $\mu\text{g}/\text{mL}$ (~ 1000 μM). Treosulfan had no effect on hERG tail current ($\text{IC}_{50} > 5000$ μM). The positive and negative controls behaved as expected. Investigators further evaluated the effects of 200, 1000, and 5000 μM treosulfan against additional cardiac ion channels using the patch clamp assay: hNav1.5 (peak and late), hCav1.2, hKir2.1, and hKCNQ1/minK. The % inhibition of each cardiac ion channel that was recorded when cells were exposed to 5000 μM is shown in Table 3.

Table 3. Summary of % Current Inhibitions in Cardiac Channel Assays

currents:	hERG tail	hNav1.5 Na ⁺ (peak ¹)	hNav1.5 Na ⁺ (peak ²)	hNav1.5 Na ⁺ (late)	hCav1.2 L-type Ca	hKir2.1 rapid delay K ⁺	hKCNQ1/minK slow delay K ⁺
% inhibition at 5000 μM	1.5%	16.8%	34.5%	18.3%	18.3%	3.7%	6.1%

1 = tonic block 2=Use-dependent block

Treosulfan (10 $\mu\text{g}/\text{mL}$) had no effect on contractions of isolated guinea pig ileum produced by acetylcholine or histamine (Leo, 1978b). It also had no effect on isoprenaline-induced dilation of isolated cat tracheal muscle following exposure to concentrations ranging from 3 to 20 $\mu\text{g}/\text{mL}$.

In vivo evaluation of cardiovascular effects of treosulfan in female fasting adult mongrel dogs showed that i.v. dosing of 5-200 mg/kg of treosulfan had no effect on arterial or venous blood pressure, or the carotid occlusion reflex (Leo, 1978b). Moreover, 4-hour ECG evaluations in these treosulfan-exposed dogs were unremarkable. Female adult rats exposed to i.v. doses of treosulfan at 20 mg/kg , then 40 mg/kg 30 minutes later exhibited no changes in blood pressure.

Respiratory and CNS Function

Mice that received 100 mg/kg of treosulfan i.p. were not protected from convulsions induced by i.v. injection of pentetrazole or from the convulsant effects induced by electroshock (Leo, 1978b). Treosulfan (50 mg/kg p.o.) offered no analgesic effect to mice following i.p. injection of 50 mg/kg of acetic acid. It also offered no conduction anesthesia to electric stimulation in tail nerves following i.v. injection of 2% treosulfan. Ham/ICR mice administered 150 or 300 mg/kg of treosulfan i.p. showed no signs of abnormal behavior. A fasted female dog administered 200 mg/kg of treosulfan p.o. exhibited no change in respiration, heart rate, or pupil size for 4 hours post-dose.

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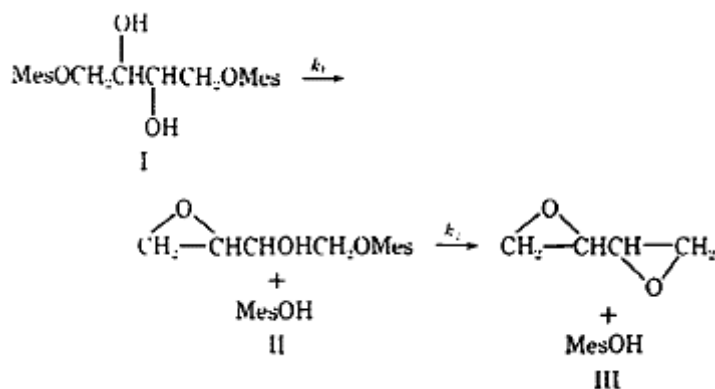
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Grafapex (treosulfan)

5.4 ADME/PK

The conversion of treosulfan to the epoxide compound is pH-dependent, and negligible <pH 6. As such, transformation from treosulfan to the mono- or diepoxide species, and ratios of all 3 species, will differ based on the pH in the various in vivo compartments.

Treosulfan transformation into mono- and diepoxide species



I = Treosulfan; II = (2S,3S)-1,2-epoxy-3,4-butanediol + 4-methanesulfonate (MSA);

III = (2S,3S)-diepoxybutane (DEB) + MSA

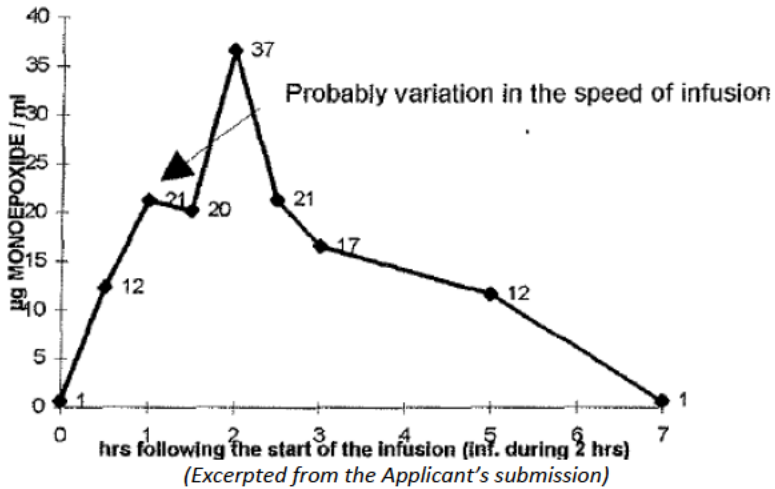
(Excerpted from Feit, 1970)

Type of Study	Major Findings
Protein Binding	Treosulfan did not bind to plasma proteins
Biotransformation	
Study # FEIT 1997: Analysis of (2S,3S)-Diepoxybutane (L-Diepoxybutane) and (2S,3S)-1,2-Epoxy-3,4-Butanediol 4-Methansulfonate (Monoepoxide) in Human Plasma	Investigators prepared plasma aliquots from a male patient following 2-hour i.v. administration of 43.68 g (20.6 g/m ²) treosulfan. - L-diepoxytubane was not detected in the plasma up to 7 hours after IV infusion of treosulfan - Plasma concentration of monoepoxide (in figure) peaked 2 hours after administration:

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Grafapex (treosulfan)

LEO 1978a: Considerations on the Pharmacokinetics of Treosulfan	 (b) (4)																																																																							
Distribution																																																																								
Study # MDC/01: [³H]-Treosulfan: Distribution and Excretion studies in Rats	Highest concentrations (tissue: blood ratios) of [³H]-treosulfan within 30 min following single i.v. administration of 100 mg/kg to male albino rats <table><tr><th>Tissue</th><th>5 min</th><th>30 min</th></tr><tr><td>Urinary bladder</td><td>283.35</td><td>18.21</td></tr><tr><td>Kidney</td><td>3.13</td><td>1.79</td></tr><tr><td>Tooth pulp</td><td>1.22</td><td>1.22</td></tr><tr><td>Ex-orbital lachrymal gland</td><td>0.94</td><td>1.19</td></tr><tr><td>Small intestine wall</td><td>0.90</td><td>1.17</td></tr><tr><td>Bulbo-urethral gland</td><td>1.08</td><td>1.02</td></tr><tr><td>Lung</td><td>1.07</td><td>1.06</td></tr><tr><td>Bone marrow</td><td>0.65</td><td>0.89</td></tr></table> Listed in order from highest (5 or 30 min) to lowest concentrations - Although bone marrow does not have the highest concentration, its values are shown for comparison as it is the intended target organ - Radioactivity was also detected in the brain, spinal cord, myocardium, eye, epididymis, prostate, testes, Harderian gland, preputial gland Plasma and brain concentrations of treosulfan after single i.v. bolus injection of 500 mg/kg of treosulfan <table><tr><th>Age</th><th>Tissue</th><th>Sex</th><th>T_{1/2} Treosulfan (h)</th><th>C_{max} Treosulfan (µM)</th><th>AUC Treosulfan (µM x h)</th></tr><tr><td rowspan="4">Juvenile (PND10)</td><td rowspan="2">Plasma</td><td>M</td><td>1.04</td><td>3108</td><td>4846</td></tr><tr><td>F</td><td>1.15</td><td>2015</td><td>4273</td></tr><tr><td rowspan="2">Brain</td><td>M</td><td>2.40</td><td>133</td><td>580</td></tr><tr><td>F</td><td>3.07</td><td>130</td><td>533</td></tr><tr><td rowspan="4">Adult (34-35 days)</td><td rowspan="2">Plasma</td><td>M</td><td>0.68</td><td>2938</td><td>2483</td></tr><tr><td>F</td><td>0.68</td><td>3471</td><td>2699</td></tr><tr><td rowspan="2">Brain</td><td>M</td><td>1.99</td><td>88</td><td>227</td></tr><tr><td>F</td><td>2.21</td><td>70</td><td>197</td></tr></table>	Tissue	5 min	30 min	Urinary bladder	283.35	18.21	Kidney	3.13	1.79	Tooth pulp	1.22	1.22	Ex-orbital lachrymal gland	0.94	1.19	Small intestine wall	0.90	1.17	Bulbo-urethral gland	1.08	1.02	Lung	1.07	1.06	Bone marrow	0.65	0.89	Age	Tissue	Sex	T _{1/2} Treosulfan (h)	C _{max} Treosulfan (µM)	AUC Treosulfan (µM x h)	Juvenile (PND10)	Plasma	M	1.04	3108	4846	F	1.15	2015	4273	Brain	M	2.40	133	580	F	3.07	130	533	Adult (34-35 days)	Plasma	M	0.68	2938	2483	F	0.68	3471	2699	Brain	M	1.99	88	227	F	2.21	70	197
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Study # (b) (4) 27700: Pharmacokinetic Study and Assessment of Blood-Brain Barrier Penetration of Treosulfan and its Transformation Products After Single Intravenous Administration to Juvenile and Young Adult CD® Rats -Non-GLP-																																																																								

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Grafapex (treosulfan)

Excretion																																																			
Study # MDC/01: [³ H]-Treosulfan: Distribution and Excretion studies in Rats	Recovery of radioactivity in a male albino rats after i.v. administration of a single dose of [³H]-treosulfan at 100 mg/kg <table><tr><th>Sample</th><th>Time (h)</th><th>Mean %AR* (n = 3)</th></tr><tr><td rowspan="8">Urine</td><td>0-6</td><td>43.9</td></tr><tr><td>6-24</td><td>13.9</td></tr><tr><td>24-48</td><td>1.9</td></tr><tr><td>48-72</td><td>0.8</td></tr><tr><td>72-96</td><td>0.7</td></tr><tr><td>96-120</td><td>1.1</td></tr><tr><td>120-144</td><td>0.5</td></tr><tr><td>144-168</td><td>0.6</td></tr><tr><td>Total urine</td><td>0-168</td><td>63.2</td></tr><tr><td rowspan="7">Feces</td><td>0-24</td><td>10.6</td></tr><tr><td>24-48</td><td>0.8</td></tr><tr><td>48-72</td><td>0.30</td></tr><tr><td>72-96</td><td>0.4</td></tr><tr><td>96-120</td><td>0.5</td></tr><tr><td>120-144</td><td>0.3</td></tr><tr><td>144-168</td><td>0.2</td></tr><tr><td>Total Feces</td><td>0-168</td><td>13.1</td></tr><tr><td>Carcass</td><td>168</td><td>0.9</td></tr><tr><td>Cage Wash</td><td>168</td><td>1.7</td></tr><tr><td>Total Recovery</td><td>0-168</td><td>78.9</td></tr></table> *%AR = % administered radioactivity Loss of significant amount of radioactive material is likely due to loss of volatile components	Sample	Time (h)	Mean %AR* (n = 3)	Urine	0-6	43.9	6-24	13.9	24-48	1.9	48-72	0.8	72-96	0.7	96-120	1.1	120-144	0.5	144-168	0.6	Total urine	0-168	63.2	Feces	0-24	10.6	24-48	0.8	48-72	0.30	72-96	0.4	96-120	0.5	120-144	0.3	144-168	0.2	Total Feces	0-168	13.1	Carcass	168	0.9	Cage Wash	168	1.7	Total Recovery	0-168	78.9
	Sample	Time (h)	Mean %AR* (n = 3)																																																
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Study # MDC/01: [³ H]-Treosulfan: Distribution and Excretion studies in Rats	PK Parameters in a male albino rat after i.v. administration of a single dose of [³H]-treosulfan at 100 mg/kg <table><tr><th>Parameter</th><th>Value</th></tr><tr><td>C_{max} (µg equivalent/mL)</td><td>83.3</td></tr><tr><td>T_{max} (hours)</td><td>0.08</td></tr><tr><td>AUC₀₋₂₄ (µg equivalent/mL*hr)</td><td>253.1</td></tr><tr><td>AUC_{inf} (µg equivalent/mL*hr)</td><td>471.3</td></tr><tr><td>initial t½ (hours)</td><td>0.48</td></tr><tr><td>terminal t½ (hours)</td><td>25.8</td></tr></table> (Excerpted from the Applicant's submission)	Parameter	Value	C _{max} (µg equivalent/mL)	83.3	T _{max} (hours)	0.08	AUC ₀₋₂₄ (µg equivalent/mL*hr)	253.1	AUC _{inf} (µg equivalent/mL*hr)	471.3	initial t½ (hours)	0.48	terminal t½ (hours)	25.8																																				
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5.5 Toxicology

5.5.1 General Toxicology

Study title/ number: 4-Week Subchronic Toxicity Study of Treosulfan by Repeated Intravenous Bolus Injection to CD® Rats/ Study # (b) (4) 37259

Key Study Findings

- There were 2 unscheduled deaths in the 150 mg/kg (high dose) group that received the lyophilizate formulation; one of these was a male in the 150 mg/kg treosulfan lyophilizate recovery group sacrificed on Day 42 due to malignant leukemia in the bone marrow, head, paws, and tail
- The target organs included the bone marrow (myelosuppression, decreased bone marrow cells), spleen (lymphoid atrophy), thymus (hemorrhage, atrophy), GI tract (hyperplasia), muscle (necrosis), injection site, and reproductive organs (infiltrates/ inflammation in prostate, changes in epididymis and testicular weights).
- Additional toxicities included hemorrhage of the optic nerve and multi-organ inflammation

GLP compliance: Yes

Methods

Dose and frequency of dosing:	Treosulfan: 10, 50, or 150 mg/kg once daily (QD) x 28 days, or Treosulfan Lyophilisate: 10, 50, or 150 mg/kg QD x 28 days
Route of administration:	IV bolus
Formulation/Vehicle:	Sterile 0.45% NaCl; sterile 0.9% NaCl
Species/Strain:	Rat/ CD®/Sprague-Dawley
Number/Sex/Group:	Main study: 10/sex/group Recovery: 3/sex/control; 9/sex/group
Age:	53-63 days
Satellite groups/ unique design:	None (TK study performed with the Recovery animals)
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: changes from control

Parameters	Major findings
Mortality	1 female in the 150 mg/kg treosulfan lyophilisate group died on Day 17. Histopathology showed enlarged

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Grafapex (treosulfan)

	adrenals, edematous lungs, small spleen and thymus, and dilated intestines filled with liquid. Moreover, there was congestion and atrophy in bone marrow, congestion in kidneys, liver, lungs, spleen, and thymus, a granuloma of foreign body in the lungs, lymphoid atrophy in spleen, erosion of glandular mucosa in stomach, and atrophy and acute hemorrhage in thymus. 1 male in the 150 mg/kg treosulfan lyophilisate recovery group was sacrificed on Day 42 (1 day before scheduled recovery sacrifice) due to swelling in several areas: tail, nose/snout, paw, and testes. Necropsy revealed enlarged kidneys and a hardened, brown-discolored liver. Histopathology showed the animal suffered from malignant leukemia in the bone marrow, head, paws, and tail, which caused the swelling in these areas. There were lymphocytic infiltrations and granulomatous inflammation in the epididymis; epidermal hyperplasia, exudative inflammation, and/or epidermal ulceration in the head, paws, and tail; bacterial infection in the paws and tail; an eosinophilic infiltration in the pelvis of the right kidney; hematopoiesis and interstitial fibrosis in the liver; increased hematopoiesis in the spleen; granuloma of foreign body in the tongue; and ulceration, chronic inflammation, and hypertrophy with atypical cells in the urinary bladder.
Clinical Signs	Unremarkable
Body Weights	Males exposed to 150 mg/kg of treosulfan gained less weight than males in all other groups. Females exposed to 150 mg/kg of treosulfan or treosulfan lyophilisate gained less weight than females in other groups.
Change in Body Weight Male Body Weight Red arrow marks end of treatment phase and beginning of recovery T. Lyophilisate = treosulfan lyophilisate formulation Female Body Weight	
Ophthalmoscopy	Unremarkable

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Grafapex (treosulfan)

Hematology							
% Difference from Control in Hematologic Parameters							
Treosulfan		Male			Female		
Test	Day	10 mg/kg	50 mg/kg	150 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg
Eosinophils	29	12%	-38%*	-83%**	-1%	-35%	-91%**
	43	83%	85%	-9%	99%	97%	89%
Leukocytes	29	-17%	-39%	-68%**	-15%	-36%	-65%**
	43	-19%	-20%	-41%**	5%	-14%	-31%
Lymphocytes	29	-20%	-41%**	-73%**	-16%	-39%**	-68%**
	43	-24%	-29%	-46%**	-1%	-20%	-37%
Treosulfan Lyophilisate Formulation							
Eosinophils	29	-51%**	-60%**	-95%**	-6%	-49%*	-90%**
	43	89%	61%	6%	86%	122%	36%
Leukocytes	29	-11%	-36%	-64%**	8%	-23%	-65%
	43	0%	-22%	-27%	15%	-13%	-17%
Lymphocytes	29	-9%	-37%**	-71%**	8%	-30%*	-70%**
	43	-7%	-29%	-32%*	11%	-21%	-21%
Values >100% are shown in bold; *p < 0.05; **p < 0.01							
Clinical Chemistry				Unremarkable			
Urinalysis							
Urinalysis Parameters							
Treosulfan		Male			Female		
Test	Unit	10 mg/kg	50 mg/kg	150 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg
Ery	++ or +++	0, 0R	2, 2R	0, 2R	0, 0R	0, 1R	0, 0R
Hgb	250 ery/ μ L	0, 0R	1, 2R	0, 1R	0, 0R	0, 1R	0, 0R
Treosulfan Lyophilisate							
Ery	++ or +++	0, 2R	4, 1R	1, 2R	0, 0R	0, 0R	0, CE
Hgb	250 ery/ μ L	0, 3R	4, 1R	1, 3R	0, 0R	0, 0R	0, CE
Male and female control groups were unremarkable (n = 10) per control and treated groups during treatment phase (n = 3) for control and (n = 9) for treated groups during recovery (R) phase Ery = erythrocytes; ++ = few in all fields examined; +++ = many in all fields examined CE = collection error in recovery phase; sample missed container							
Gross Pathology				Aside from the male and female animals in the 150 mg/kg treosulfan lyophilisate group that were sacrificed/ died prematurely, all other animals had unremarkable necropsies			

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Grafapex (treosulfan)

Organ Weights												
% Difference from Control in Organ to Body Weight Ratio												
Terminal	Male						Female					
Organ/ Tissue	Treosulfan (mg/kg)			T. Lyophilisate mg/kg			Treosulfan (mg/kg)			T. Lyophilisate mg/kg		
	10	50	150	10	50	150	10	50	150	10	50	150
BRAIN	3%	4%	11%	-3%	-1%	7%	0%	3%	9%	0%	2%	9%
EPIDIDYMIS (L)	3%	14%	16%	3%	-1%	8%						
EPIDIDYMIS (R)	6%	7%	9%	-5%	-7%	4%						
GLAND, ADRENAL (L)	-5%	-1%	5%	-6%	2%	0%	-6%	1%	-10%	-2%	-7%	-6%
GLAND, ADRENAL (R)	-8%	10%	-2%	-5%	2%	7%	0%	8%	8%	4%	4%	8%
HEART	5%	2%	6%	-1%	-3%	4%	4%	5%	8%	9%	8%	9%
KIDNEY (L)	8%	5%	1%	2%	3%	9%	-1%	-3%	3%	-2%	2%	8%
KIDNEY (R)	7%	5%	1%	3%	3%	10%	2%	0%	4%	-2%	2%	6%
LUNG	0%	-1%	13%	-4%	-2%	9%	0%	5%	14%	-6%	-4%	15%
OVARY (L)							21%	4%	11%	15%	3%	16%
OVARY (R)							12%	13%	4%	13%	11%	11%
SPLEEN	-4%	-7%	-28%	-4%	-11%	-32%	2%	-9%	-24%	-7%	-12%	-31%
TESTIS (L)	7%	9%	11%	1%	1%	13%						
TESTIS (R)	6%	7%	10%	-1%	-2%	12%						
THYMUS	2%	2%	-12%	16%	0%	-11%	-3%	-8%	-11%	7%	0%	-25%
Recovery	Treosulfan (mg/kg)			T. Lyophilisate mg/kg			Treosulfan (mg/kg)			T. Lyophilisate mg/kg		
Organ/ Tissue	10	50	150	10	50	150	10	50	150	10	50	150
BRAIN	-6%	-2%	3%	-9%	-10%	-4%	-8%	-9%	-8%	-7%	-4%	1%
EPIDIDYMIS (L)	5%	10%	16%	7%	4%	21%						
EPIDIDYMIS (R)	-1%	5%	12%	6%	2%	16%						
GLAND, ADRENAL (L)	-6%	-10%	-6%	3%	-17%	-10%	-16%	-12%	-7%	-13%	-23%	-6%
GLAND, ADRENAL (R)	-1%	-5%	6%	-7%	-17%	-7%	-7%	-2%	-1%	-3%	-14%	2%
HEART	-5%	-5%	-4%	-10%	-9%	-7%	-1%	2%	6%	-2%	2%	-1%
KIDNEY (L)	-1%	4%	5%	1%	-1%	8%	-4%	-1%	5%	-3%	-5%	0%
KIDNEY (R)	-2%	4%	1%	-2%	-2%	5%	-1%	-1%	5%	-3%	-4%	2%
LUNG	-9%	-13%	-3%	1%	-9%	1%	-5%	-9%	4%	-4%	-8%	12%
OVARY (L)							-4%	4%	-7%	15%	10%	-2%
OVARY (R)							-9%	-10%	1%	3%	8%	-9%
SPLEEN	-3%	-3%	-8%	-1%	-11%	-4%	-8%	-12%	-11%	-9%	-14%	-16%
TESTIS (L)	0%	3%	10%	-3%	-4%	0%						
TESTIS (R)	0%	2%	10%	-4%	-4%	-1%						
THYMUS	-7%	-9%	-8%	-3%	-10%	-12%	9%	0%	4%	4%	7%	7%
Histopathology												
Adequate battery: Yes												

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Grafapex (treosulfan)

Histopathology Observations in Rats										
Formulation: Treosulfan			Male				Female			
Organ/ Tissue	Finding		0 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg	0 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg
Kidney – left	basophilia, tubular, focal/ multifocal	mild	2			3, 2R	1			1R
	infiltrate, focal/ multifocal	min	2			3, 7R	2			1, 3R
Kidney – right	basophilia, tubular, focal/ multifocal	min	1			1, 2R				1R
	infiltrate, focal; multifocal; pelvis	min				2, 1R				1, 3R
Liver	congestion	min					2			5, 2R
		mild	1			2, 3R	3			1, 7R
	fatty change, diffuse; multifocal; periportal	mild	1			3, 2R	1			2R
LN, cervical	atrophy, lymphoid	mild				3, 1R				
		mark				2				
	erythro- phagocytosis	min				4			1, 2R	
Gland, harderian	infiltrate, focal; multifocal	min								1R
	inflammation, chronic, focal/ multifocal	mild								2R
LN mesenteric	granuloma	min	1	1	1	4	2			1
	hyperplasia, lymphoid	min			1, 1R	6, 3R	1	1R	2, 2R	3, 6R
Urinary bladder	inflammation	mild			1, 1R	3, 2R			1	1
	hyperplasia, urothelial				1R	3R				
	hypertrophy, urothelial; focal/ multifocal	min			3, 3R	1R			1	6R
		mild			4, 1R	10, 7R				1, 1R
	ulcer, urothelial; focal, multifocal	mild				1R				
Nerve, optic	hemorrhage, focal; multifocal	min				3				1
	Pigment, brown; macrophage, focal	min				2R				2R
Site, injection	posttraumatic change, perivascular, focal; vascular, focal/ multifocal	min				2	1			1R
		mild	1			1				4
Bone marrow – femur	fatty bone marrow, increased	min	1			1, 1R				
		mild				7, 2R				2, 1R
	red bone marrow, decreased	min				1				
		mild				7				2
Spleen	atrophy,	min				1R				1

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Grafapex (treosulfan)

	lymphoid	mild				10, 1R				9
	congestion	min	3	3, 3R	2, 6R	6, 4R		3, 2R	2, 2R	4R
		mild	1	1R	3, 3R	2, 2R			1R	2, 1R
Muscle, skeletal	infiltrate, lymphohistiocytic, focal, multifocal	min		2R	1, 1R	2, 5R		1	3, 1R	3R
	necrosis, myofiber, focal, multifocal	min				1R				2R
Thymus	atrophy	min				2				
	hemorrhage, acute, focal/ multifocal	min	4			8, 3R	4			3, 4R
		mild				1R				
Gland, prostate	infiltrate, focal, multifocal		1			2, 4R				
	inflammation, chronic, focal/ multifocal	min				1R				
		mild				1, 2R				
Gland, Seminal vesicle	infiltrate, lymphohistio-granulocytic, focal	min				2				
Colon	hyperplasia, mucosa-associated lymphoid tissue	min				1				1R
Peyer's patch	hyperplasia	min				3, 1R				1

Formulation: Treosulfan Lyophilisate			Male				Female			
Organ/ Tissue	Finding		0 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg	0 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg
Kidney – left	basophilia, tubular, focal/ multifocal	mild	2			1, 4R	1			2R
	infiltrate, focal; multifocal	min	2			2, 5R	2			3, 4R
Kidney – right	basophilia, tubular, focal/ multifocal	min	1			3, 3R				
	Infiltrate, focal; multifocal; pelvis	min				1, 3R				1, 1R
Liver	congestion					1R	2			3, 2R
		mild	1			4, 4R	3			2, 5R
	fatty change, diffuse; multifocal; periportal	mild	1			1, 1R	1			3, 3R
LN, cervical	atrophy, lymphoid	mild								2

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Grafapex (treosulfan)

	erythro-phagocytosis	min			1R	1R		2	1R	3R
Gland, harderian	infiltrate, focal; multifocal	min				2R				1R
	inflammation, chronic, focal, multifocal	min				2R				
		mild				2R				3R
LN mesenteric	granuloma	min	1	1R	1, 4R	5, 1R	2	2		2, 1R
	hyperplasia, lymphoid	min		1R	2, 1R	8, 3R	1	2R	3	2, 5R
Urinary bladder	inflammation	mild		1R	2	3			1	
	hyperplasia, urothelial	min			1R	1R				
	hypertrophy, urothelial; focal, multifocal	min			2, 4R	4R			1R	4, 5R
		mild			5, 1R	8				1
	ulcer, urothelial; focal, multifocal	mild			1	1, 2R				
Nerve, optic	hemorrhage, focal; multifocal	min								1
Site, injection	posttraumatic change, perivascular, focal; vascular, focal/ multifocal	min				1	1			2R
		mild	1			1, 1R				1
Bone marrow – femur	fatty bone marrow, increased	min	1			2, 3R				1, 1R
		mild				5, 2R				3, 2R
	red bone marrow decreased	min				3				
		mild				4, 1R				4
Spleen	atrophy, lymphoid	min				3				6
		mild				7				4, 1R
	congestion	min	3	6, 4R	8, 5R	3, 3R		1, 6R	5, 4R	2, 5R
		mild	1	1, 2R	2R	2, 4R			1R	2, 1R
Muscle, skeletal	Infiltrate, lymphohistiocytic, focal, multifocal	min		3, 2R	2, 1R	1, 1R		1, 6R	2, 7R	5, 5R
	necrosis, myofiber, focal, multifocal	min			1R	1R		1R	3R	
Thymus	atrophy, acute, focal/ multifocal	min				2				1
	hemorrhage, acute, focal/ multifocal	min	4			4, 3R	4			4, 4R
		mild				3R				1R
Prostate gland	infiltrate, focal, multifocal		1			3, 4R				
Colon	hyperplasia, mucosa-associated lymphoid tissue	min				2				3
Peyer's patch	atrophy, lymphoid	min								2
	hyperplasia, lymphoid	min				1				3

R = Recovery phase

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Grafapex (treosulfan)

Toxicokinetics						
Toxicokinetic Parameters for Treosulfan in Adult Rats						
Treosulfan mg/kg/day	Male			Female		
	10	50	150	10	50	150
Day 1						
$T_{1/2}$ (hr)	0.35	0.64	0.74	0.36	0.62	0.71
C_{max} (μ M)	8.02	40.97	115.00	6.97	39.83	124.00
$AUC_{(0-last)}$ (μ M*hr)	3.93	25.72	81.85	3.41	23.04	80.89
Day 28						
$T_{1/2}$ (hr)	0.45	0.70	0.80	0.38	0.71	0.75
C_{max} (μ M)	6.60	42.67	123.00	7.46	43.00	122.33
$AUC_{(0-last)}$ (μ M*hr)	3.55	26.02	86.93	3.65	25.92	85.47
$AUC_{(0-last)}$ AR	0.93	1.01	1.06	1.07	1.125	1.06
T. Lyophilisate	Male			Female		
	10	50	150	10	50	150
Day 1						
$T_{1/2}$ (hr)	0.38	0.75	0.78	0.36	0.78	0.82
C_{max} (μ M)	7.95	38.63	116.33	7.27	35.90	94.27
$AUC_{(0-last)}$ (μ M*hr)	3.87	23.49	80.36	3.56	23.16	72.04
Day 28						
$T_{1/2}$ (hr)	0.39	0.65	1.36	0.40	0.78	0.81
C_{max} (μ M)	7.74	36.80	125.33	7.71	43.00	127.33
$AUC_{(0-last)}$ (μ M*hr)	3.80	23.34	90.95	3.73	25.40	85.69
$AUC_{(0-last)}$ AR	0.98	0.99	1.13	1.05	1.10	1.19

AR = accumulation ratio = AUC Day 28 / AUC Day 1

Effects on Reproductive Organs

Study Title/ Study Number: Submission to the Licensing Authority in Respect to Marketing a New Drug Treosulfan Leo, Vol. 2, Experimental and Biological Studies/ Leo-1978b

Key Study Findings

- Treosulfan demonstrated dose-dependent effects on male and female reproductive organs. Effects on spermatogenesis and ovary function indicate that treosulfan can impair fertility in both males and females.

GLP compliance:

Unknown

Methods

Dose and frequency of dosing:

5 or 50 mg/kg/day x 6 days per week x 7 months

Route of administration:

Stomach tube

Formulation/Vehicle:

Dissolved in saccharose solution (2 mL/kg)

Species/Strain:

Rat/ Wistar

Number/Sex/Group:

10/sex/group

Study design:

1 male and 1 female rat from each group were killed after dosing for 30, 60, 90, 120, 150, and 180 days. Major organs and reproductive

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Grafapex (treosulfan)

organs were removed for histological examination

Observations and Results

Histopathology Findings

Observations	5 mg/kg	50 mg/kg
Male	Spermatogenesis was reduced or arrested	Spermatogenesis was markedly reduced or arrested
Female	Normal corpora lutea and follicles Normal uterus	Absent corpora lutea and follicles in a few animals Uterus showed some degree of atrophy

5.5.2 Genetic Toxicology

Other Genetic Toxicity Studies

Study title/ number: Studies on Epoxide Formation from (2S,3S)-Threitol 1,4-Bimethanesulfonate. The Preparation and Biological Activity of (2S,3S)-1,2-Epoxy-3,4-butanediol 4-Methanesulfonate/ Feit 1970

Key Study Findings:

- Treosulfan induced a concentration-dependent increase in % of anaphases with aberrations in onion root tips
- Monoepoxide induced 8-fold higher % of anaphase cells with aberrations vs. treosulfan
- Diepoxide induced 15-fold higher % of anaphase cells with aberrations vs. treosulfan

GLP compliance: Not stated

Test system: Comparison of cytological effects induced in onion root tips following 1 hour exposure to treosulfan (20, 50 mM), monoepoxide (0.5, 2 mM), or diepoxide (0.05, 0.1 mM).
Study is valid: Yes (peer-reviewed)

5.5.3 Carcinogenicity

No study or assessment conducted.

5.5.4 Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Fertility Studies

No dedicated fertility studies were conducted. Refer to the general toxicology section for treosulfan effects on reproductive organs.

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Grafapex (treosulfan)

Juvenile Development

Study title/ number: Subchronic Toxicity Study of Treosulfan by Intravenous Administration to Juvenile CD® Rats with a 5-Week Recovery Period/ Study # 26054

Key Study Findings

- The original high dose was set at 250 mg/kg/day; however, several animals died prematurely at that dose and the dose was reduced to 100 mg/kg/day
- Adverse findings in males and females included reduced neutrophils
- Males in the 100 mg/kg group gained less weight over time than males in other groups and had minimal interstitial lymphocytic infiltrates
- Females in the 100 mg/kg group had a 1.8-day delay in the time point of vaginal opening and minimal reduction in splenic cellularity with concomitant reduction in spleen weight upon necropsy

GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 10, 50, or 100 mg/kg in main study and recovery groups on postnatal day (PND) 10 to 35

0, 10, 50, or 250 mg/kg/day in satellite groups on PND 10 to 31

Route of administration: IV bolus injection

Formulation/Vehicle: 0.9% NaCl solution

Species/Strain: Rat/ CD®/Sprague-Dawley

Number/Sex/Group: Main study: 10/sex/group

Recovery: 4/sex/group

Satellite groups: 30/sex/group

Unique study design:

Deviation from study protocol No

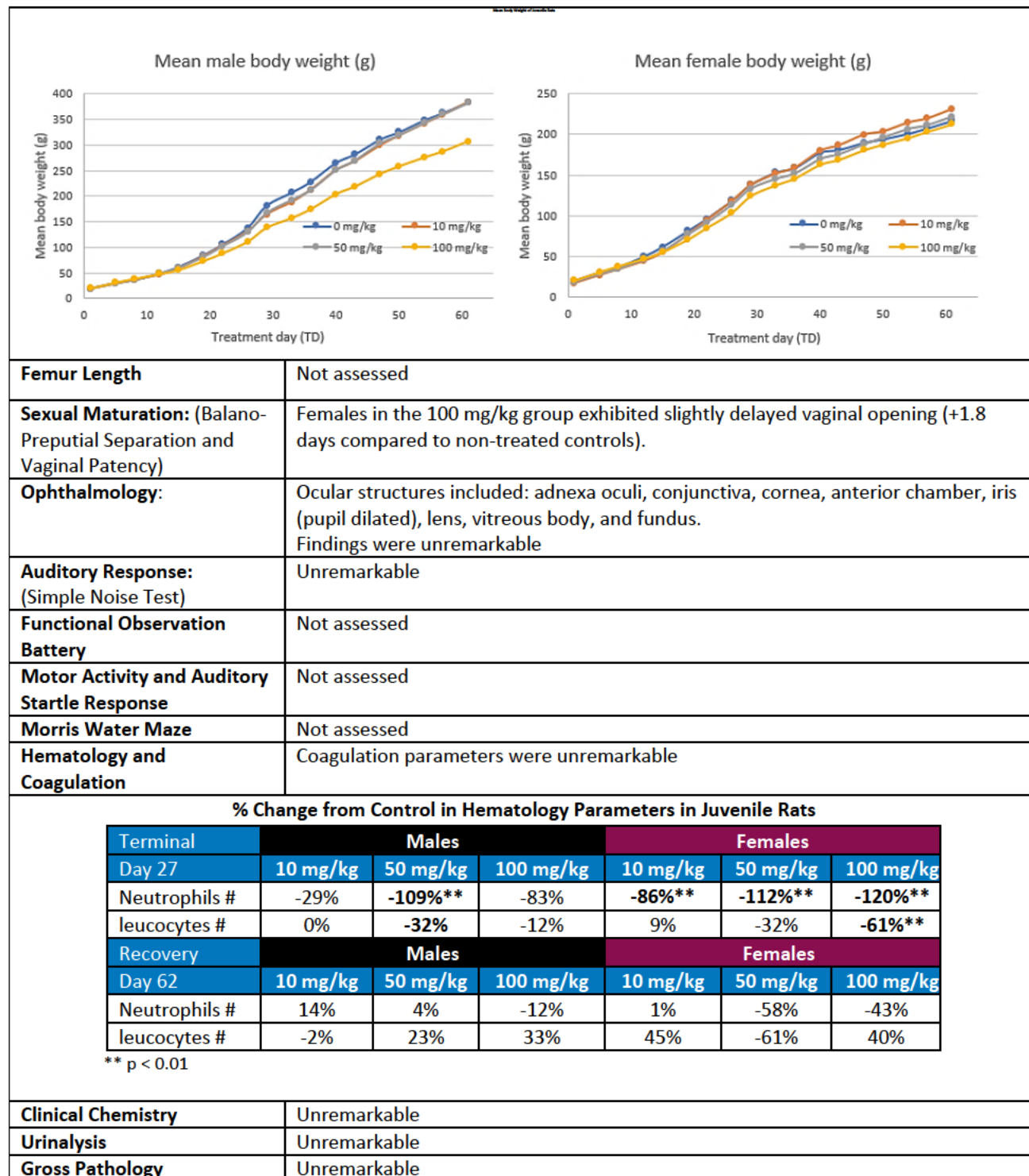
affecting interpretation of results:

Parameters	Major findings
Mortality	10 satellite animals at 250 mg/kg died prematurely between PND 19 and PND 30, resulting in a dose reduction to 100 mg/kg in the main study and recovery animals.
Clinical Signs	There were no drug-related changes at the injection site or appearance of clinical signs in treated animals.
Body Weights and Feed Consumption	Although males in the 100 mg/kg group gained significantly less weight than other groups, they consumed a comparable quantity of food. In contrast, females in the 100 mg/kg group gained a similar amount of weight as other groups but consumed significantly more food (+16%) from PND 52 to PND 66

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Grafapex (treosulfan)



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Grafapex (treosulfan)

Organ Weights

% Difference from Control in Relative Organ Weight

	Male			Female		
	10 mg/kg	50 mg/kg	100 mg/kg	10 mg/kg	50 mg/kg	100 mg/kg
Brain (R)	4% -5%	6% 1%	14%** 0%	1% -8%	3% -2%	11% 3%
Gonads* (L/R) (R)	-3%/ -4% -9%/ -5%	-9%/ -11% -13%/ -7%	-26%**/ -26%** 0%/ 4%	11%/ 24% -8%/ -24%	10%/ 14% -34%/ -26%	17%/ -3% -15%/ -4%
Lungs (R)	4% 2%	9% 13%	12%** 22%	-1% -10%	1% -17%	2% 3%
Spleen (R)	-1% 8%	4% -4%	-13% 22%	-5% 6%	-10% 8%	-26%** 12%
Thymus (R)	-21% 15%	-16% -8%	-27%** 3%	2% 2%	-15% 0%	-6% 13%

Relative body weight = g organ weight/ kg of body weight

*Male gonads = testes/ female gonads = ovaries

(L/R) = Left/ right; (R) = Recovery Phase; **p < 0.01

Histopathology (Adequate Battery: Yes)

Histopathology Observations in Juvenile Rats

Organ/ Tissue	Finding	severity	Male		Female	
			0 mg/kg	100 mg/kg	0 mg/kg	100 mg/kg
Epididymis	interstitial lymphocytic infiltrates	min	3	7		
Eye	Rosette formation	min	0	1	1	2
Injection site	perivascular hemorrhage	min	5	10	7	9
Larynx	lympho-histiocytic infiltrates	min	1	2		
Liver (Oil-Red-O)	Fatty infiltrates, single hepatocyte	min	4	0	4	8
Prostate	Juvenile condition	min	8			
		mild		10		
Spleen	reduced cellularity	min				5
Testis	atrophy of germinal epithelium	min	0	1		
	hyperplasia, leydig cells	min	0	1		
Trachea	gland dilatation	min	1	3	2	5, 3R
Urinary bladder	Proteinaceous content		1, 1R	2, 2R		
	lympho-histiocytic infiltrates	min	0	1		
	transitional cell hyperplasia	min	0	2		

min(imal) < mild

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Grafapex (treosulfan)

Toxicokinetics		Toxicokinetic Parameters for Treosulfan (Juvenile Study; Rats)						
	Males				Females			
	10 mg/kg	50 mg/kg	100* mg/kg	250 mg/kg	10 mg/kg	50 mg/kg	100* mg/kg	250 mg/kg
Postnatal Day 10 (Treatment Day 1)								
T _{max} (hr)		1.36		1.36		1.13		1.39
C _{max} (μM)	19.884	96.783	209.714	524.284	17.050	73.693	145.862	364.656
AUC(0-last) (μM*hr)		90.091	266.103	665.257		77.327	223.626	559.066
Postnatal Day 31 (Treatment Day 22)								
T _{max} (hr)								
C _{max} (μM)	13.895	76.505		408.946	12.120	61.908		284.392
AUC(0-last) (μM*hr)								
Accumulation Ratio	0.70	0.79		0.78	0.71	0.84		0.78

NA: Not applicable; Accumulation ratio = C_{max} Day 31/C_{max} Day 1
T_{max} was only assessed at 2 timepoints
Values for the 100 mg/kg dose were extrapolated based on linear dose-related exposure

Embryo-Fetal Development

No embryo-fetal development studies were conducted.

5.5.5 Other Toxicology Studies

Local Tolerance

The right ear of each New Zealand White adult rabbit (n = 5) received 50 mg of treosulfan injected intra-arterially and 10 mg of treosulfan injected peri-venously parallel to the marginal vein of the same ear (Leo 1985). Left ears served as controls. There were no drug-related effects throughout a 5-day observation period.

Impurity Qualification

Chemistry and Manufacturing Controls (CMC) consulted with Pharmacology/Toxicology and Clinical Pharmacology regarding the potential safety risk of (b) (4). The Applicant reported a level of no more than (NMT) (b) (4) % of (b) (4) in any treosulfan batch. Clinical Pharmacology stated that from their perspective, there was no safety concern with the (b) (4) % limit for (b) (4). Pharmacology/Toxicology agrees that from a Pharmacology/Toxicology perspective there are no safety concerns since (b) (4) and as treosulfan has been in clinical use in Europe for many years, any adverse events caused by elevated (b) (4) levels would have been reported.

OPMA consulted with Pharmacology/Toxicology regarding the potential risk of (b) (4) -derived extractables per vial of treosulfan drug product, as patients exposed to the maximum daily dose of 16.2 g/day of treosulfan would be exposed to a total of

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Grafapex (treosulfan)

(b) (4) mcg/day of extractables. The individual extractable substances are made up of hetero- versus homogeneous unknowns; each below (b) (4) mcg/day. Following a discussion with OPMA and CMC reviewers, Pharmacology/Toxicology concluded that this level is acceptable from a Pharmacology/Toxicology perspective because 1) treosulfan is indicated in combination with fludarabine for patients with advanced cancer, 2) patients will only receive treosulfan for 3 consecutive days, 3) treosulfan is already known to be genotoxic and carcinogenic, 4) fludarabine is also genotoxic and 5) (b) (4) used in Europe (where treosulfan has been used in patients with ovarian cancer for nearly 50 years).

6 Clinical Pharmacology

6.1 Executive Summary

The Applicant is seeking approval of treosulfan as part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). The proposed dosing regimen is treosulfan 10 g/m²/day for 3 consecutive days (Days -4, -3, and -2) in combination with fludarabine 30 mg/m²/day for 5 consecutive days (Days -6 through -2) prior to allogeneic hematopoietic stem cell transplantation (alloHSCT; Day 0).

Pivotal evidence to support efficacy in the proposed population is based on the results of Study MC-FLudT.14/L Trial II. Key review issues include the adequacy of the proposed treosulfan dosing regimen in adult patients, pediatric extrapolation to identify a treosulfan dosing regimen appropriate for pediatric patients ≥ 1 year of age, and evaluation of the Applicant's proposals for patients with organ impairment. The Applicant provided pharmacokinetic studies in adults and pediatric patients, a population PK model of treosulfan, and a dose-response evaluation in adults to support the dose selection in the general population and specific populations. The Applicant did not provide adequate data to resolve other review questions including characterization of treosulfan metabolism and effects of treosulfan on QTc interval.

Recommendations

The Office of Clinical Pharmacology has reviewed the information contained in NDA 214759. This NDA is not approvable from a Clinical Pharmacology perspective given that the Agency was not able to independently verify the primary endpoint supporting evidence of effectiveness (Refer to **Section 8**). The key review issues with specific recommendations and comments are summarized below.

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Grafapex (treosulfan)

REVIEW ISSUE	RECOMMENDATIONS/COMMENTS
Pivotal or supportive evidence of effectiveness	In Study MC-FludT.14/L Trial II, treatment with the proposed dosing regimen of treosulfan + fludarabine improved overall survival (OS) with a hazard ratio (HR) and 95% confidence interval (CI) of 0.65 (0.48, 0.88) compared to treatment with busulfan + fludarabine. However, the Agency was not able to independently verify the primary endpoint of event-free survival (EFS) for Study MC-FludT.14/L Trial II (Refer to Section 8).
General dosing instructions	The recommended dosage regimen of treosulfan is 10 g/m ² /day for 3 consecutive days (Days -4, -3, and -2) in combination with fludarabine 30 mg/m ² /day for 5 consecutive days (Days -6 through -2) prior to allogeneic hematopoietic stem cell transplantation (Day 0).
Dosing in patient subgroups (intrinsic and extrinsic factors)	<i>Pediatric Patients ≥1 year of age:</i> The recommended dosage regimen of treosulfan is the same for all patients ≥1 year of age. Efficacy in pediatric patients ≥1 year of age was extrapolated from adults and dose selection was supported by PK and safety data from Studies MC-FludT.16/NM and MC-FludT.17/M. Treosulfan exposure after administration of 10 g/m² in pediatric patients with BSA <0.7 m² and with BSA 0.7 to <1.1 m² are 11% and 5% higher, respectively, compared to adults. <i>Renal Impairment:</i> No dosage modification is recommended for patients with mild renal impairment. An additional study will be necessary to identify a safe dose of treosulfan for patients with moderate hepatic impairment. A study in patients with severe renal impairment is not feasible and will not be required. <i>Hepatic Impairment:</i> No dosage modification is recommended for patients with mild hepatic impairment.

Clinical Pharmacology Outstanding Issues

Key Issue(s) to be Addressed	Rationale	Key Considerations for Design Features
Determination of an appropriate safe treosulfan dose for use in patients with moderate renal impairment.	There is evidence of renal elimination of treosulfan. Renal impairment may increase treosulfan systemic exposures and require dose adjustment.	Conduct a clinical trial to evaluate the pharmacokinetics and safety of treosulfan in patients with normal renal function and patients with moderate renal impairment.
Evaluation of treosulfan and epoxide metabolites as substrates of metabolizing enzymes.	The Applicant did not evaluate treosulfan or its epoxide metabolites as substrates of major metabolizing enzymes. This evaluation is necessary to describe risk of drug-drug interactions and effects of hepatic impairment on treosulfan safety and PK.	Conduct an <i>in vitro</i> study to evaluate treosulfan and epoxide metabolites as substrates of major cytochrome P450 enzymes consistent with FDA guidance.

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Key Issue(s) to be Addressed	Rationale	Key Considerations for Design Features
Evaluation of the effects of treosulfan on the QT interval.	The effects of treosulfan on the QT interval have not been evaluated. A QT study should be performed to evaluate the cardiac safety of treosulfan.	Conduct a clinical trial to evaluate the effects of treosulfan on the QT interval in patients receiving the recommended dosage of treosulfan.
Evaluation of treosulfan active metabolite PK in adult patients.	The PK of treosulfan parent drug was evaluated in 24 adult patients. The PK of active epoxide metabolites was not evaluated in adult patients.	Evaluate the PK of treosulfan active epoxide metabolites in adult patients.
Determination of the effects of race and ethnicity on the PK of treosulfan.	Race and ethnicity data were not collected in the pivotal study or adult PK sub-study of treosulfan. The effects of race and ethnicity on the PK of treosulfan is therefore unknown.	Conduct a clinical trial to evaluate the effects of race and ethnicity on the pharmacokinetics of treosulfan.

6.2 Summary of Clinical Pharmacology Assessment

6.2.1 Pharmacology and Clinical Pharmacokinetics

Treosulfan is an alkylating agent with cytotoxic activity. The PK of treosulfan was described using a population PK model. The final population PK (PPK) model for treosulfan is a two-compartment model with linear elimination and intercompartmental clearance. BSA is included as a significant covariate on CL, V1, V2 and Q in the final model, supporting a BSA-based dosing regimen.

The area under the curve (AUC; mean $\pm$ SD) in adult patients was $1,157 \pm 193$ hr·mcg/mL after administration of 10 g/m². There was no dose accumulation.

Distribution: The volume of distribution in adult patients is about 41.1 liters (CV%: 19.2%). In animal studies, treosulfan penetration through the blood brain-barrier is limited [see Section 5.4]. Treosulfan does not bind to plasma albumin.

Elimination: The median terminal half-life of treosulfan is 2.2 hours.

Metabolism: Treosulfan is converted into an active monoepoxide intermediate [(2S,3S) 1,2 epoxybutane 3,4 diol 4 methanesulfonate] and finally to L diepoxibutane [(2S,3S) 1,2:3,4 diepoxybutane].

Excretion: A median of 42% of the treosulfan dose is excreted unchanged with the urine within 24 hours, and 89% of this unchanged fraction is excreted within the first 8 hours after administration.

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6.2.2 General Dosing and Therapeutic Individualization

General Dosing

The recommended regimen of treosulfan in adults and pediatric patients ≥ 1 year of age is treosulfan 10 g/m²/day for 3 consecutive days (Days -4, -3, and -2) in combination with fludarabine 30 mg/m²/day for 5 consecutive days (Days -6 through -2) prior to allogeneic hematopoietic stem cell transplantation (Day 0).

Therapeutic Individualization

No dosage adjustment is recommended for patients with mild renal impairment (CLcr 60-89 mL/min) or mild hepatic impairment [total bilirubin less than or equal to upper limit of normal (ULN) with AST greater than ULN or total bilirubin greater than 1 to 1.5 times ULN with any AST]. No information is provided or requested for patients with moderate or severe hepatic impairment as they are typically excluded from transplantation. Additional data will be necessary to inform the dosing of treosulfan in patients with moderate renal impairment (CLcr 30 – 59 mL/min).

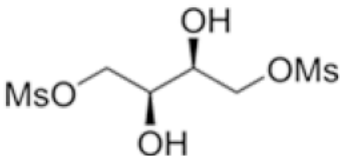
Outstanding Issues

The following issues were not adequately addressed by the available data:

- Determination of an appropriate safe dose of treosulfan for patients with moderate renal impairment (CLcr 30-59 mL/min)
- Evaluation of the effects of treosulfan on the QTc interval
- Evaluation of treosulfan and its epoxide metabolites as substrates of metabolizing enzymes *in vitro*
- Evaluation of the PK of treosulfan active metabolites in adult patients
- Determination of the effects of race and ethnicity on treosulfan PK

6.3 Comprehensive Clinical Pharmacology Review

6.3.1 General Pharmacology and Pharmacokinetic Characteristics

General Information	
Description	 Chemical structure: Treosulfan has the molecular formula C₆H₁₄O₈S₂ and a molecular weight of 278.3 g/mole.

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Bioanalysis	In Study MCFludT.14/L Trial I PK Sub-study, treosulfan in human plasma and urine was measured using an HPLC assay. Accuracy (%RE) and precision (%CV) of the quality controls (QCs) for the runs used in measuring treosulfan plasma and urine concentrations were ≤15%. In Studies MC-FludT.16/NM and MC-FludT.17/M, treosulfan and monoepoxide in human plasma were measured using an LC-MS/MS assay. Accuracy (%RE) and precision (%CV) of the QCs for the runs used in measuring treosulfan and monoepoxide plasma concentrations were ≤15%.
Drug Exposure at Steady State	The area under the curve (AUC; mean ± SD) in adult patients was 1,157 ± 193 hr·mcg/mL. Treosulfan exposure after administration of 10 g/m ² in pediatric patients with BSA <0.7 m ² and with BSA 0.7 to <1.1 m ² is 11% and 5% higher, respectively, compared to adults.
Range of Effective Dose or Exposure	Patients receiving a higher dose of treosulfan (14 g/m ² /day for 3 consecutive days; 1.4 times the recommended dose) in Study MC-FludT.14/L Trial I had a higher incidence of early fatal and/or serious adverse reactions including infections, febrile neutropenia, musculoskeletal pain, and hepatotoxicity.
Accumulation	There was no dose accumulation with once daily administration of treosulfan over 3 consecutive days.
Active Moieties	Treosulfan is converted into an active monoepoxide intermediate [(2S,3S)-1,2-epoxybutane-3,4-diol-4-methanesulfonate] and finally to L-diepoxybutane [(2S,3S)-1,2:3,4-diepoxybutane].
QT Prolongation	The effects of treosulfan on the QT interval in humans have not been evaluated. <i>In vitro</i> , treosulfan does not significantly inhibit hERG at a concentration of 1,000 μM.
Distribution	
Volume of Distribution	The volume of distribution in adult patients is 41.1 liters (CV%: 19.2%). The treosulfan volume of distribution increases with BSA. In animal studies, treosulfan penetration through the blood brain-barrier is limited [see Section 5.4].
Plasma Protein Binding	Treosulfan does not bind to plasma albumin.
Elimination	
Terminal Elimination Half-Life (SD)	The median terminal half-life of treosulfan is 2.2 hours in adults and 1.5 hours in pediatric patients. In pediatric patients, the median terminal half-life of the active monoepoxide intermediate is 1.6 hrs. No data are available on the PK of the monoepoxide intermediate in adults.
Metabolism	
Primary Metabolic Pathway(s)	Treosulfan converts to mono- and di-epoxide metabolites in a temperature- and pH-dependent manner in (b) (4) human plasma. No studies have been conducted to evaluate treosulfan or its active metabolites as substrates of major metabolizing enzymes. In pediatric patients (age range 4 months to 17 years), there was no relationship between age and the monoepoxide:parent AUC_{inf} ratio, suggesting no significant age-related differences in metabolism of treosulfan among pediatric patients. No monoepoxide PK data were available from adult patients; additional data will be needed to evaluate treosulfan and metabolite PK in adults.

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Excretion	
Primary Excretion Pathways, % dose (SD)	A median of 42% of the treosulfan dose is excreted unchanged in the urine within 24 hours, and 89% of this unchanged fraction is excreted within the first 8 hours after administration.
Drug Interactions	
Inhibition/Induction of Metabolism	<i>In vitro</i> , a combined sample of treosulfan and its metabolites inhibits CYP2C19 and CYP3A at clinically relevant concentrations. A combined sample of treosulfan and its metabolites does not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, or CYP2D6 at clinically relevant concentrations. Evaluation of treosulfan as an inducer of CYP1A2, CYP2B6, and CYP3A was inconclusive due to cytotoxicity caused by incubation of treosulfan.
Inhibition of Transporter Systems	<i>In vitro</i> , a combined sample of treosulfan and its metabolites inhibits P-gp. A combined sample of treosulfan and its metabolites does not inhibit BCRP, BSEP, MATE1, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, or OCT2 at clinically relevant concentrations.

6.3.2 Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

No. The Agency was not able to independently verify the primary endpoint of EFS for Study MC-FludT.14/L Trial II (Refer to **Section 8**). Therefore, the efficacy evaluation for the current submission focused on overall survival (OS) results in Study MC-FludT.14/L Trial II. In Study MC-FludT.14/L Trial II, 570 adult patients planned to undergo allogeneic HSCT for AML or MDS were randomized 1:1 to receive treosulfan 10 g/m²/day x 3 days + fludarabine 30 mg/m²/day x 5 days or busulfan 0.8 mg/kg Q6H x 8 doses + fludarabine 30 mg/m²/day x 5 days. Treatment with the proposed dosing regimen of treosulfan + fludarabine improved OS with a HR (95% CI) of 0.65 (0.48, 0.88) compared to treatment with busulfan + fludarabine in the overall population. In adult patients with AML, treosulfan + fludarabine improved OS with a HR (95% CI) of 0.61 (0.41, 0.92) compared to treatment with busulfan + fludarabine while in adult patients with MDS treosulfan + fludarabine improved OS with a HR (95% CI) of 0.62 (0.34, 1.10) compared to treatment with busulfan + fludarabine. Refer to **Section 8.1.1** for a detailed assessment of treosulfan efficacy in Trial II.

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. Treatment with the proposed dosing regimen resulted in improved overall survival compared to treatment with busulfan + fludarabine in adult patients with AML or MDS (Refer to **Section 8.1.1**).

Use of a higher dose of treosulfan (14 g/m²/day x 3 days) was evaluated in Study MC-FludT.14/L Trial I. Study MC-FludT.14/L Trial I was identical in design to Trial II with the exception of the

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treosulfan dose (14 g/m^2) and treosulfan schedule (Days -6, -5, and -4). A planned interim analysis of Trial I showed that the treosulfan 14 g/m^2 + fludarabine arm had poorer survival and higher infectious complications than the busulfan + fludarabine arm and the trial was stopped early (Refer to **Section 8.1.2**). The differing outcomes in Trial I vs Trial II are attributed to the difference in treosulfan dose which lead to higher incidence of early fatal and/or serious adverse reactions.

The relationship between exposure and time to neutrophil engraftment [defined as time from Day 0 to the first of 3 consecutive days with absolute neutrophil count (ANC) $>0.5 \times 10^9/\text{L}$] was assessed in adult patients. No significant E-R relationship between treosulfan AUC versus time to engraftment was identified; however, the adult E-R analysis had significant limitations including a small number of patients (24), a single dose level (14 g/m^2) with narrow exposure range, and non-daily blood counts to assess ANC.

Separate exposure-response analyses for safety were conducted using data from 82 pediatric patients treated with treosulfan (10, 12, or 14 g/m^2) + fludarabine in Studies MC-FludT.16/NM and MC-FludT.17/M. There was no significant relationship identified between treosulfan exposure (AUC or C_{max}) and common adverse events (infection and gastrointestinal disorders). In addition, no significant relationship was identified between treosulfan exposure and duration of neutropenia (days with ANC $<0.5 \times 10^9/\text{L}$) in pediatric patients. Limitations of the pediatric E-R analysis include administration of thiotepea in some (but not all) patients and non-daily blood counts to assess ANC.

In summary, treosulfan doses higher than 10 g/m^2 are not appropriate for this population due to the risk of increased early morbidity and mortality with doses higher than recommended; do not exceed the recommended dosage of treosulfan.

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

Limited pharmacokinetic data were available from adult patients treated with treosulfan. Study MC-FludT.14/L Trial I included a PK Sub-study which collected PK samples from 24 adult patients after administration of treosulfan 14 g/m^2 . PK samples were not collected in Study MC-FludT.14/L Trial II. A population PK (PPK) model was developed using data from the 24 adult patients in the Study MC-FludT.14/L Trial I PK Sub-study and 83 pediatric patients treated with treosulfan 10, 12, or 14 g/m^2 in two pediatric studies (MC-FludT.16/NM and MC-FludT.17/M). The final PPK model was described by a two-compartment model with linear elimination and intercompartmental clearance. BSA is included as a significant covariate on CL, V1, V2 and Q in the final model, supporting a BSA-based dosing regimen. Given the variability of treosulfan PK parameters (CV% 16.7% for $\text{AUC}_{24\text{h}}$ and 19.2% for Vd), the proposed treosulfan dosing regimen of 10 g/m^2 is acceptable. Refer to the Pharmacometrics review in **Section 16.4.1** for detailed review of the final PPK model.

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There were no clinically significant differences in the pharmacokinetics of treosulfan based on sex, mild renal impairment (CL_{cr} 60-89 mL/min), or mild hepatic impairment (total bilirubin less than or equal to ULN with AST greater than ULN or total bilirubin greater than 1 to 1.5 times ULN with any AST).

Pediatric Patients: Given the need for alternatives in pediatric patients, information regarding an appropriate dose of treosulfan for pediatric patients was evaluated. The Applicant provided safety and PK data from 115 pediatric patients administered treosulfan prior to alloHSCt in the following two clinical studies:

- MC-FludT.16/NM: open-label, randomized (1:1) study of treosulfan + fludarabine (n=46) vs busulfan + fludarabine (n=45) prior to alloHSCt in 91 pediatric patients (age range 4 months to 17 years) with non-malignant diseases. 82.5% (75/88) of treated patients received an intensified regimen with the addition of thiotepea.
- MC-FludT.17/M: open-label, single-arm study of treosulfan + fludarabine prior to alloHSCt in 70 pediatric patients (age range 4 months to 17 years) with hematological malignancies. 92.8% (65/70) of patients received an intensified regimen with the addition of thiotepea.

In both pediatric studies, the Applicant selected the dose of treosulfan by matching the treosulfan AUC_{24h} in adults following the 14 g/m² dosing regimen. (b) (4)

. Refer to

Section 7.1 for additional description of the two pediatric studies and **Section 8.3.6** for a comparison of safety outcomes for pediatric patients vs adults enrolled in Trial II.

Extrapolation of the proposed indication in adults to include pediatric patients ≥ 1 year was evaluated during the review of this application as an approach to support a pediatric indication. To support a pediatric full extrapolation approach, FDA requested that the Applicant identify pediatric doses that would match treosulfan AUC_{24h} in adults after administration of treosulfan 10 g/m². Given the increased morbidity and mortality observed in adults treated with treosulfan 14 g/m²/day x 3 days, (b) (4) this higher exposure is not acceptable for a pediatric extrapolation approach. The Applicant utilized their population PK model to conduct simulations of treosulfan AUC_{24h} after administration of different doses (8 to 11 g/m²) to pediatric patients with BSA ranging from 0.25 to 2.25 m² with patients categorized into three groups based on BSA: ≤ 0.7 m², 0.7 to 1.1 m², and > 1.1 m². Based on the Applicant's simulation results (Error! Reference source not found.), the Applicant proposed the following dose levels in pediatric patients to obtain comparable treosulfan AUC_{24h} to an average adult after administration of treosulfan 10 g/m²:

- 9.0 g/m² if BSA ≤ 0.7 m² or in the age range 1 to 4 years
- 9.5 g/m² if $0.7 \text{ m}^2 < \text{BSA} \leq 1.1 \text{ m}^2$ or in the age range 5 to 9 years
- 10.0 g/m² if BSA $> 1.1 \text{ m}^2$ or in the age range 10 to 18 years

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Table 4. Simulated AUC_{24h} for different age and BSA values by dose regimen. Highlighted dosing regimens are equal or higher than target AUC_{24h} of average American adult

Age [yr]	BSA [m ²]	8 g/m ²	8.5 g/m ²	9 g/m ²	9.5 g/m ²	10 g/m ²	10.5 g/m ²	11 g/m ²
1	0.462	1026	1090	1154	1218	1283	1347	1411
2	0.552	1006	1069	1132	1195	1258	1321	1383
3	0.63	992	1054	1116	1177	1239	1301	1363
4	0.707	979	1040	1101	1163	1224	1285	1346
5	0.779	969	1029	1090	1150	1211	1271	1332
6	0.864	958	1017	1077	1137	1197	1257	1317
7	0.954	947	1006	1066	1125	1184	1243	1303
8	1.05	937	996	1054	1113	1172	1230	1289
9	1.139	929	987	1045	1103	1161	1219	1277
10	1.24	920	978	1035	1093	1150	1208	1265
11	1.366	911	967	1024	1081	1138	1195	1252
12	1.474	903	959	1016	1072	1129	1185	1242
13	1.576	896	952	1008	1064	1120	1176	1232
14	1.671	891	946	1002	1057	1113	1169	1224
15	1.755	886	941	996	1052	1107	1162	1218
16	1.792	884	939	994	1049	1105	1160	1215
17	1.817	882	937	993	1048	1103	1158	1213
18	1.853	880	935	990	1045	1100	1155	1211

Source: Applicant population PK study report 2021

Independent simulations conducted by FDA compared the treosulfan AUC_{24h} among different BSA groups and dosing regimens to evaluate the Applicant's proposed pediatric dosing regimens. The simulation results, shown in Figure 3 indicate that the Applicant's proposed pediatric dosing regimens are expected to provide similar treosulfan AUC_{24h} compared to the median treosulfan AUC_{24h} in adult patients after administration of treosulfan 10 g/m² (target exposure).

The administration of treosulfan 10 g/m² in pediatric patients with BSA ≤ 0.7 m², 0.7 to 1.1 m², and >1.1 m² is predicted to result in treosulfan AUC_{24h} 11.2% higher, 4.7% higher, and 2.8% lower, respectively, compared to the target exposure in adults (

Figure 3). As such, a simplified dosing regimen of 10 g/m² for the entire pediatric population with BSA range of 0.25 to 2.25 m² is not expected to provide a clinically significant difference in exposure compared to the target exposure in adults.

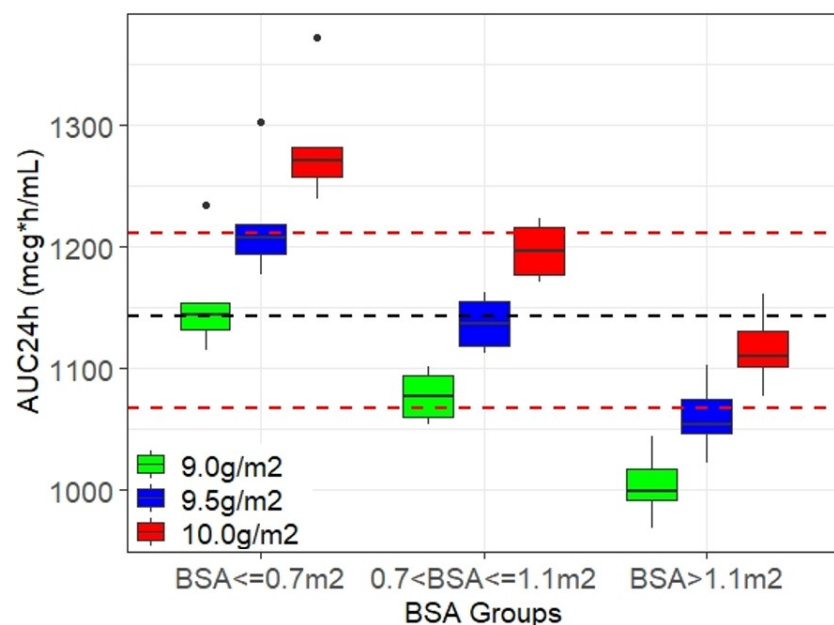
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Figure 3: Comparison of AUC_{24h} in pediatric population among different dosing regimens and BSA groups



Red dash lines: 25th and 75th percentiles of adult AUC_{24h} following 10g/m²; black dash line: median of adult AUC_{24h} following 10g/m² (target exposure);

Source: reviewer's analysis

Exposure-response analyses for safety based on the two pediatric studies did not indicate that an 11.2% increase in exposure would result significant increase in the probability of common toxicities (infections and gastrointestinal disorders) given the variability of the drug exposure. Therefore, for the convenience of dosing and to avoid potential dosing errors with the Applicant's proposed dosing regimens, FDA recommends a treosulfan dose of 10 g/m² for all pediatric patients ≥1 year.

Geriatric Patients: Pharmacokinetic differences between patients <65 years of age compared to patients ≥65 years of age were evaluated using data from the Trial I PK Sub-study. The geometric mean AUC_{24h} (1,126 μg*h/mL for <65 years vs 1,225 μg*h/mL for ≥65 years) and C_{max} (554 μg/mL for <65 years vs 556 μg/mL for ≥65 years) were similar between age groups. Given the small number of patients ≥65 years of age with pharmacokinetic data (n=4), the effects of geriatric age on treosulfan PK were inconclusive. In addition, in MC-FludT.14/L Trial II there were no significant differences in treosulfan safety or effectiveness observed between patients 65 years of age or older (n=73) and younger adult patients (n=197) on the treosulfan + fludarabine arm. Refer to **Section 8.3.6** for additional information.

Renal Impairment: Treosulfan is excreted in the urine (median 42% recovery unchanged). The effects of renal impairment on treosulfan PK were evaluated using PK data from the Trial I PK Sub-study. A trend towards lower treosulfan clearance and higher treosulfan AUC was observed

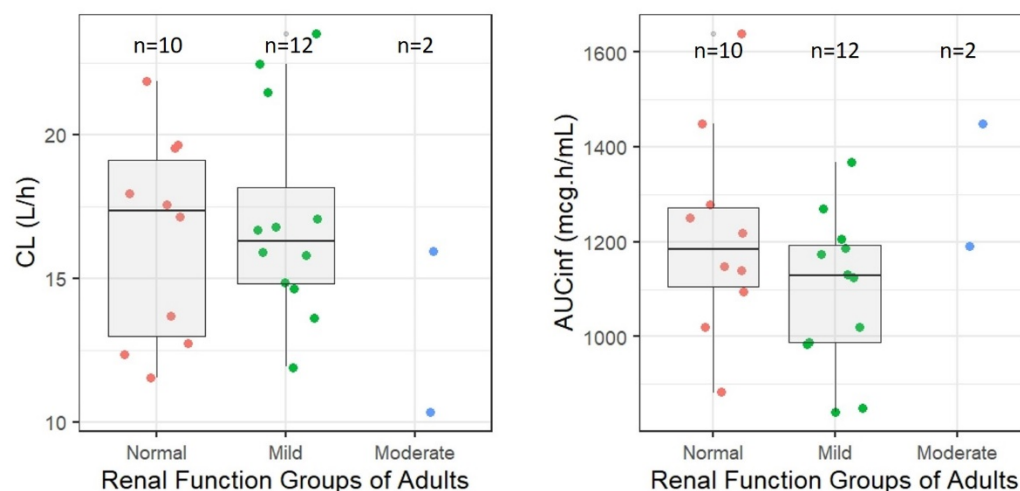
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in patients with moderate renal impairment (CLcr 30 to <60 mL/min; **Figure 4**). However, the observation was limited by the small number of patients with moderate renal impairment (n=2). No patients with severe renal impairment (CLcr <30 mL/min) were enrolled in the Trial I PK Sub-study.

Figure 4: Estimates of Treosulfan Clearance (Left) and AUC_{inf} (Right) by Renal Function



Note: Parameters assuming administration of 10 g/m².

Source: Reviewer's Analysis

The effects of renal impairment on treosulfan safety were evaluated using data from Trial II. No PK samples were collected in Trial II; therefore, creatinine clearance was utilized as a surrogate for treosulfan exposure in subgroup safety analyses. CLcr was a statistically significant predictor of non-relapse mortality (NRM) at 24 months. In addition, a trend towards increased safety events [including infection-related mortality (IRM) at 24 months, Grade 3+ infection, and Grade 3+ GI toxicity] was observed for patients with renal impairment (**Table 5**). The evaluation of safety was limited by the small number of patients with moderate renal impairment (n=3). No patients with severe renal impairment were enrolled in Trial II.

Table 5. Safety Subgroup Analysis by Renal Function in Trial II – Treosulfan Arm

	Normal (n=137)	Mild (n=115)	Moderate (n=3)
NRM at 24 months	10.1%	14.0%	66.7%
IRM at 24 months	5.4%	14.0%	33.3%
Grade 3+ infection	11.7%	19.1%	33.3%
Grade 3+ GI toxicity	9.5%	14.8%	66.7%

Source: Response to IR received 4 Jan 2021

Given the urinary excretion and observed trends in both PK and safety in patients with renal impairment, the Applicant should conduct a renal impairment study to identify a safe dose of treosulfan in patients with moderate renal impairment. Given the rarity of performing alloHSCT

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in patients with severe renal impairment, a study in this population would not be feasible and will therefore not be required.

Hepatic Impairment: The effects of hepatic impairment on treosulfan safety were evaluated using data from Trial II. There was no apparent trend in safety events for patients with hepatic impairment categorized per NCI-ODWG (**Table 6**). However, the evaluation of safety was limited by the small number of patients with moderate hepatic impairment (n=4). No patients with severe hepatic impairment were enrolled in Trial II as these patients are generally not considered appropriate candidates for allogeneic HSCT. No dose modification is required for patients with mild hepatic impairment.

Table 6. Safety Subgroup Analysis by Hepatic Function in Trial II – Treosulfan Arm

	Normal (n=186)	Mild (n=78)	Moderate (n=4)
NRM at 24 months	13.0%	10.3%	0
IRM at 24 months	10.0%	7.7%	0
Grade 3+ infection	12.9%	20.5%	25%
Grade 3+ GI toxicity	14.0%	9.0%	0

Source: Response to IR received 4 Jan 2021

Race and Ethnicity: The Applicant did not collect information on race or ethnicity from Study MC-FludT14.L Trial I, including the PK sub-study. Race was recorded for pediatric patients in Studies MC-FludT.16/NM and MC-FludT.17/M; however, of the 83 pediatric patients with PK data, 80 were White, 2 were Asian, and 1 was Black. Therefore, the effects of race and ethnicity on treosulfan PK are unknown given that there were too few patients with differing races to assess the effects of race on treosulfan PK. Additional information will be necessary to evaluate these effects.

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

Treosulfan was evaluated *in vitro* as an inhibitor or inducer of major CYP enzymes and as an inhibitor of major transporters. Separate *in vitro* stability studies were conducted to evaluate the relative concentration of treosulfan and its active metabolites under conditions used in the *in vitro* CYP and transporter studies. Based on the stability studies, both treosulfan and its active metabolites are present *in vitro* and the relative concentrations change over time (reduction in parent treosulfan with corresponding increase in metabolites). Therefore, it is not possible to determine the contribution of each moiety to the observed results.

CYP Enzymes:

In vitro, a combined sample of treosulfan and its metabolites inhibits CYP2C19 and CYP3A at clinically relevant concentrations. For CYP2C19, the treosulfan reversible IC₅₀ was 972 µM and for CYP3A, the treosulfan reversible IC₅₀ was 1,870 µM (calculated based on initial treosulfan

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concentration). A combined sample of treosulfan and its metabolites does not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, or CYP2D6 at clinically relevant concentrations ($IC_{50} > 10,000 \mu M$ based on initial treosulfan concentration).

Physiologically based pharmacokinetic (PBPK) modelling was used to predict the effects of treosulfan administration on CYP3A, CYP2C19, and P-gp substrates *in vivo*. The PBPK model and mechanistic static model were used to predict the magnitude of enzyme mediated DDI caused by treosulfan and its metabolite, EBDM, respectively. Results of the analysis showed that the potential DDI of treosulfan as a CYP3A4 inhibitor and a CYP2C19 inhibitor cannot be excluded. Refer to **Section 16.4.2** for detailed review of the Applicant's PBPK model.

Given the short half-life of treosulfan (2.2 hrs) and context of use (3-day course), no *in vivo* DDI study is required. For certain CYP3A or CYP2C19 substrates where minimal concentration changes may lead to serious or life-threatening toxicities providers should monitor for adverse reactions and reduce the dosage, as needed, if recommended in the prescribing information of these substrates.

Evaluation of treosulfan as an inducer of CYP1A2, CYP2B6, and CYP3A in human hepatocytes was inconclusive due to cytotoxicity caused by incubation of treosulfan.

Transporter Systems:

In vitro, a combined sample of treosulfan and its metabolites inhibits P-gp. At an initial treosulfan concentration of $3,000 \mu M$, treosulfan incubation inhibited P-gp mediated transport of loperamide by 51.1% ($IC_{50} \sim 3,000 \mu M$). Based on PBPK analysis, the potential DDI of treosulfan as a P-gp inhibitor cannot be excluded; however, the risk of clinically significant DDIs is low.

In vitro, a combined sample of treosulfan and its metabolites does not inhibit BCRP, BSEP, MATE1, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, or OCT2 at clinically relevant concentrations (no concentration-dependent inhibition up to at least $3,000 \mu M$ initial treosulfan concentration).

Has the cardiac safety of treosulfan, including effects on the QT interval, been adequately characterized?

In vitro, treosulfan does not significantly inhibit hERG at a concentration of $1,000 \mu M$. The Applicant has not systematically evaluated the effects of treosulfan on the QT interval in patients after administration of treosulfan. A total of 613 patients have received treosulfan in trials of treosulfan as part of a preparative regimen prior to transplantation. Post-baseline ECGs were available from only one study (MC-FludT.6/L). Two of 35 evaluable patients had a change to abnormal ECG post-baseline (one asymptomatic T-wave negativity and one sick sinus/atrial fibrillation).

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Given the mechanism of action (alkylating agent) and context of use (preparative regimen prior to alloHSCT) for treosulfan, a thorough QT study in healthy volunteers is not feasible. A QT study designed to exclude large mean increases (>20 msec) in the QT/QTc interval should be conducted to evaluate the effects of treosulfan on the QT interval in patients receiving the recommended dosage of treosulfan.

7 Sources of Clinical Data and Review Strategy

7.1 Table of Clinical Studies

Table 7. Listing of Clinical Trials Relevant to this NDA

Trial Identity	Trial Design Regimen/ schedule/route	Primary Endpoint(s)	Study Population	Countries
Pivotal Trial				
MC-FLudT-14/L Trial II	Phase III RCT Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 10 g/m ² , days -4, -3, -2. or <u>Busulfan</u> : 0.8 mg/kg/d, days -4, -3 <i>MUD only</i> : ATG Days -4 through -2	EFS	N = 570 Patients with AML in CR or MDS; ages 18-70 years, either aged ≥ 50 or with HCT-CI score > 2, MRD or MUD donor	US: 0 Non-US: 5
Additional Studies Addressing Efficacy				
MC-FludT.14/L Trial I	Phase III RCT Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 14 g/m ² , days -6, -5, -4. or <u>Busulfan</u> : 0.8 mg/kg/d, days -4, -3 <i>MUD only</i> : ATG Days -4 through -2	EFS	N = 320 Patients with AML in CR or MDS; ages 18-70 years, either aged ≥ 50 or with HCT-CI score > 2, MRD or MUD donor	US: 0 Non-US: 6
EBMT 2019	Registry Study <u>Treosulfan</u> : MC-FLudT.14/L Trial II <u>EBMT</u> : Flu/Mel, Bu/Cy	OS	N = 1745 AML in CR or MDS, aged 50-70 years	
CIBMTR 2019	Registry Study <u>Treosulfan</u> : MC-FludT.14/L Trial II <u>RIC</u> : Flu/Bu2 or Flu/Bu2 + ATG <u>MAC</u> : Flu/Bu4, Flu/Bu4 + ATG, or Cy/Bu4	OS	N = 1399 AML in CR or MDS, Age ≥ 50 years or HCT-CI score ≥ 2	

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Trial Identity	Trial Design Regimen/ schedule/route	Primary Endpoint(s)	Study Population	Countries
Studies to Support Safety				
MC- FludT.6/L	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 10, 12, 14 g/m ² , days -6, -5, -4. <i>MUD only</i> : ATG Days -4 through -2	Feasibility and tolerability	N = 56 Hematological malignancy, ≥18 years, MUD/MRD/misMRD donor	US: 0 Non-US: 4
MC- FludT.7/AML	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 14 g/m ² , days -6, -5, -4. <i>MUD only</i> : ATG Days -4 through -2	Engraftment AESIs	N = 75 AML in CR, ages 18-60 years, MUD/MRD allo HSCT, Karnofsky ≥80%	US: 0 Non-US: 4
MC- FludT.8/MDS	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -6 to -2. and <u>Treosulfan</u> : 10, 12, 14 g/m ² , days -6, -5, -4. <i>MUD only</i> : ATG Days -4 through -2	Engraftment AESIs	MDS, ages 18-65, MUD/MRD allo HSCT, Karnofsky ≥80%	US: 0 Non-US: 4
MC- FludT.16/NM	RCT Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -7 to -3. and <u>Treosulfan</u> : 10, 12, or 14 g/m ² , days -6, -5, -4. or <u>Busulfan</u> : 0.8 mg/kg/d, days -7 to -4 with/without Thiotepa 5 mg/kg x 2 on day -2	Freedom from TRM	Non-malignant disease for first myeloablative allogeneic HSCT; ages 28 days to 18 years.	US: 0 Non-US: 4
MC- FludT.17/M	Phase II Transplant conditioning: <u>Fludarabine</u> : (30 mg/m ²), days -7 to -3. and <u>Treosulfan</u> : 10, 12, or 14 g/m ² , days -6, -5, -4. or <u>Busulfan</u> : 0.8 mg/kg/d, days -7 to -4 with/without Thiotepa 5 mg/kg x 2 on day -2	Freedom from TRM	28 days to 18 years, ALL, AML, MDS, JMML, requiring myeloablative alloHSCT, Karnofsky ≥70% Exclusion: - Fanconi anemia or other DNA breakage disorders	US: 0 Non-US: 30 study sites
Peters, 2011	Meta-Analysis <u>Treosulfan</u> : 12-75 mg/m ² total dosing	aGvHD, cGvHD, AEs	Nonmalignant or malignant diseases, <18 years	US: 0 Non-US: 16
Peters, 2017	Retrospective study <u>Treo/Flu</u> (Treo at 10, 12, or 14 mg/m ² /d x 3) +/- <u>Thiotepa</u> <u>Bu/Flu +/- Thiotepa</u>	OS, TRM	Pediatric patients with nonmalignant diseases	US: 0 Non-US: 37

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Source: Reviewer's analysis, Clinical Study Reports

Abbreviations: RCT: Randomized controlled trial; AML: acute myeloid leukemia; CR: complete remission; MDS: myelodysplastic syndrome; MUD: matched unrelated donor; MRD: matched related donor; HCT-CI: hematopoietic cell transplant-specific comorbidity index; ATG: anti-thymocyte globulin; EFS: event-free survival; US: United State; non-US: non-United States countries; OS: overall survival; TRM: transplant-related mortality; aGVHD: acute graft versus host disease; cGVHD: chronic graft versus host disease; AE: adverse event; AESI: adverse event of special interest; Treo: treosulfan; Bu: busulfan; Flu: fludarabine; Cy: cyclophosphamide; misMRD: mismatched related donor; alloHSCT: allogeneic hematopoietic stem cell transplant.

During the course of the review, FDA identified other transplant protocols that appeared to have been sponsored by the Applicant, and additional information was requested. In their response received 6/21/2021, the Applicant identified the additional protocols listed below:

Study Identifier

Study Design



(b) (4)

Because no data from these trials were included in this submission, FDA could not include the results in the analyses for this review.

7.2 Review Strategy

The key materials used for the review of efficacy and safety included:

- NDA datasets, clinical study reports, case report forms, responses to information requests
- Relevant published literature
- Relevant information in the public domain

Data sources included applicant study reports, protocols and amendments, SAP and amendments, data sets, and literature references.

Electronic data sets in the ADaM format and SAS programs were located at <\\CDESUB1\evsprod\NDA214759>, SDNs 0001, 0017, and 0029.

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The applicant submitted information from 7 clinical trials of treosulfan, in combination with fludarabine, as preparative conditioning regimen for allogeneic HSCT. The pivotal trial MC-FludT.14/L II was relevant to estimating efficacy in the intended population. In addition, study MC-FludT.14/L I was used to support efficacy. The registry studies from EBMT and CIBMTR were evaluated for supporting the biological activity.

Studies MC-FludT.6.L, MC-FludT.7/AML, MC-FludT.8/MDS, MC-FludT.14/L I, MC-FludT.14/L II, MC-FludT.16/NM, and MC-FludT.17/M were included in the integrated summary of safety submitted by the Applicant. We performed the safety analysis on MC-FludT.6L, MC-FludT.7/AML, MC-FludT.8/MDS, MC-FludT.14/L I, and MC-FludT.14/L II for the adult population, and on MC-FludT.16/NM and MC-FludT.17/M for the pediatric safety analysis.

Summaries of data and safety analyses by the clinical reviewer were performed using JMP version 15.0 (SAS Institute, Inc., Cary, NC) and MedDRA Adverse Events Diagnostic (MAED, FDA, Silver Spring, MD), versions 2.5 (through 3/21/2021) and 3.0 (after 3/21/2021). Summaries of laboratory data were performed using JMP version 15.0 and Palantir (Palantir Technologies, Inc., Denver, CO, ©2017-2021). Postmarket analysis was conducted using Empirica Signal (Oracle, Santa Clara, CA, ©2014).

8 Statistical and Clinical Evaluation

8.1 Review of Relevant Individual Trials Used to Support Efficacy

8.1.1 Study MC-FLudT-14/L Trial II

"Clinical Phase III Trial to Compare Treosulfan-Based Conditioning Therapy With Busulfan-Based Reduced-Intensity Conditioning (RIC) Prior to Allogeneic Haematopoietic Stem Cell Transplantation in Patients with AML Or MDS Considered Ineligible To Standard Conditioning Regimens - Trial II"

INVESTIGATIONAL PLAN

Trial Design

Design and Objectives

The study was designed as a non-inferiority Phase III trial to compare treosulfan-based conditioning therapy to busulfan-based reduced-intensity conditioning therapy in adult patients with high-risk hematologic malignancies. Patients were randomized between the two arms of the study.

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The primary endpoint of the study was event-free survival at 2 years after transplantation. Events were defined as disease relapse, graft failure, or death.

Secondary endpoints included overall survival (OS), incidence of relapse (RI), non-relapse mortality (NRM), and transplant-related mortality (TRM) at 2 years after transplantation. Additional secondary objectives included CTCAE grade III+ mucositis between days -6 and +28, engraftment at day +28, donor chimerism at days +28 and +100, acute and chronic GvHD within 2 years of transplantation, and other CTCAE grade III/IV events between days -6 and +28.

Key Eligibility Criteria

The study population consisted of adult patients with AML or high risk MDS who were not eligible for myeloablative conditioning regimens. The study defined this group of patients as patients who were at least 50 years of age at the time of HSCT or with an HCT-CI score > 2. Karnofsky performance score was at least 60%.

Treatment Plan Overview

Patients were randomized to receive either RIC with the control (reference) arm of busulfan + fludarabine, or the test arm of treosulfan + fludarabine (Table 8).

Table 8. Treatment Overview of MC-FludT.14/L Trial II

Treatment	Day -6	-5	-4	-3	-2	-1	0	+1	+3	+6
Treosulfan (IV, 10 g/m ² within 120 minutes) – test arm			X	X	X					
Busulfan (IV, 0.8 mg/kg within 120 minutes) - control			X	X						
Fludarabine (IV, 30 mg/m ² within 30 minutes)	X	X	X	X	X					
ATG-S-Fresenius / Grafalon (IV, 10 mg/kg in MUD) – Germany, Hungary, Italy, Poland			X	X	X					
ATG-Thymoglobulin (IV, 2.5 mg/kg in MUD) – France					X	X				
Allogeneic stem cell transplantation							X			
Cyclosporine-A (IV, PO, dose level adjusted)						X	X	X...	X...	X...
Methotrexate (IV, mg/m ² /day)								15	10	10
Source Data: Clinical Protocol, adapted from Appendix 16-16.1.1										

All patients received fludarabine (30 mg/m²/d) on days -6 to -2. Patients on the control arm received busulfan (0.8 mg/kg) on days -4 and -3. Patients receiving busulfan received anti-seizure prophylaxis. Patients on the treatment arm received treosulfan (10 mg/m²/d) on days -4 to -2. Additionally, patients who received a MUD received ATG. The type of ATG and dosing varied by country.

GVHD prophylaxis was identical in both arms. All patients received level-adapted cyclosporine from Day -1 through Day +100 and methotrexate on days +1, +3, and +6, with folic acid rescue.

Monitoring Schedule

Study subjects underwent screening within three weeks prior to the start of conditioning

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treatment, including routine blood tests, tests of end organ function, disease evaluation, performance status, and viral serologies. Monitoring for efficacy and safety is shown in Table 9. Of note, for patients who did not have signs of relapse, bone marrow studies following day +100 were not required.

Table 9. Monitoring Overview of MC-FludT.14/L Trial II

	Day -6 to +27	Day +28	Day +100	Month 6, 9, 12	Month 15, 18, 21	Month 24
Efficacy						
CBC	-6, -3, -1, +2, +6, +10, +14, +16, +20, +24 (until engraftment)	X	X			
Chimerism		BM	BM or PB			
Disease status		BM, PB	BM, PB	PB*	PB*	PB*
Graft failure, relapse, death		X	X	X	X	X
Safety						
Physical Exam	Day 0	X	X	X	X	X
Concomitant medication	X	X				
HSOS/VOD	X	X				
CTCAE Grade I-V	Continuous documentation					
SAE	Continuous documentation		If related to conditioning			
Serum chemistry	-6, -3, -1, +2, +6, +10, +14, +16, +20, +24	X				
aGvHD	Continuous documentation					
cGvHD				Continuous documentation		
GvHd prophylaxis or treatment	Continuous documentation (starting from day -1)					
<i>*Bone marrow if suspected relapse</i>						
<i>Source: Adapted from clinical protocol for MC-FludT.14/L Trial II</i>						

Statistical Analysis Plan

The submitted SAP was version 7.0, dated Dec. 03, 2019.

Analysis Set

Full analysis set (FAS): all randomized patients who were treated with at least one dose of study medication and had at least one efficacy parameter documented after baseline. The patients within the FAS were to be analyzed with respect to the groups into which they were randomized (intention to treat principle). Sponsor's analyses were based on FAS.

Start point for Time-to-event Endpoints

Allogeneic hematopoietic stem cell transplantation.

Stat Reviewer's Comment: This FAS population used by Sponsor is not an ITT population. FDA re-did all analyses based on the ITT population and started from time of randomization.

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Sample Size Determination

The noninferiority margin is HR=1.3. No hazard rate of the control arm and accrual rate was specified in the SAP. According to Page 61 of the DMC Charter, at 1-sided $\alpha=2.5\%$, at most 481 events or 930 patients would be enrolled. The trial would have a power of 80%. Assuming that roughly 3% of patients have to be excluded from FAS, at most 960 patients would be enrolled.

Clinical TL Comment: In the document "Internal Report. Estimating the treatment effect of busulfan-based conditioning to justify the non-inferiority margin of the phase III study MC-FludT.14/L Trial II" (February 27, 2020) in Module 5.3.5.3, the Applicant provide several cases of published comparative analyses or randomized studies of HSCT vs chemotherapy for EFS, RFS, and OS (using various definitions of each) for multiple different populations of patients with AML or MDS. No particular publication was identified as having a population exchangeable with that in Study MC-FludT.14/L Trial II. This document does not provide justification for the NI margin prespecified in this trial.

Stat Reviewer's Comment: Under IND 054552, in the Meeting Minutes of Type B Pre-NDA Meeting held on Apr 02 2020, FDA requested that the report justifying the noninferiority margin of MC-FludT.14/L (Trial II) should be placed in the Module 5.3.5.4. The Sponsor clarified that the noninferiority margin was prespecified and cannot be changed. The Agency acknowledged the Sponsor's clarifications on the noninferiority margin and reiterated that the noninferiority margin use and interpretation in efficacy assessment would be a review issue.

In the NDA submission, no report justifying the noninferiority margin was submitted. However, because FDA could not independently verify the primary endpoint of EFS for Study MC-FludT.14/L II, review was focused on the OS analysis. Therefore, no IR was issued for EFS noninferiority related issues.

Analysis of Efficacy Endpoints

Primary:

Kaplan-Meier methods was used to calculate the 12-and 24-month EFS rate and the associated 95% CIs. Noninferiority was tested using a Cox proportional hazards regression model stratified by center with donor type (MUD vs. MRD), risk group (I vs. II) and treatment as factors. The three factors in the Cox model were stratification factors used in the randomization procedure.

Stat Reviewer's Comment: FDA recommends stratified log-rank test for testing superiority and Cox model stratified by stratification factors at randomization to estimate HR and 95% CI.

Secondary:

The Kaplan-Meier method was used to estimate survival probabilities and 95% CIs. The unstratified log-rank test was used to test for a difference between the two treatment arms.

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Cox models similar to the model used for primary endpoint analysis would be used to estimate the hazard ratio (HR) and 95% CIs.

Interim Analyses for Efficacy

Three interim analyses were pre-specified in the study protocol, which allowed to stop the trial as soon as non-inferiority has been demonstrated. The interim analyses were scheduled based on a combination of number of patients and number of events. O'Brien-Fleming boundary was used.

If significant non-inferiority could be shown at interim or final analysis, the study would stop enrollment and superiority with respect to the primary study endpoint would be sequentially tested.

Table 10. Planned stopping boundaries for efficacy and futility monitoring

Interim Look	Information			Stopping Boundaries Efficacy*		Stopping Boundaries Futility**	
	Sample Size	Number of events	IF[%]	HR-scale	p-value scale	HR-scale	p-value scale
1	220	45	9.4%	0.1497	0.0000	3.1962	0.9987
2	460	137	28.5%	0.6518	0.0000	1.4426	0.7287
3	700	239	49.7%	0.8847	0.0015	1.2207	0.3133
Final analysis	930	481	100%	1.0864	0.0245	1.08464	0.0245

IF) Information fraction is defined as the ratio of the information at the current time-point to the maximum information committed to the study

*) Stop for efficacy, if HR or p-value fall below the associated stopping boundaries

**) Stop for futility, if HR or p-value exceed the associated stopping boundaries or if CP_H1 or

CP_observed fall below the documented thresholds (see study protocol, version 05 dated 10-Jul-2015, Table 10.2.7B)

Source: Table 7.3.A, Page 40 of SAP Version 7.0

Protocol Amendments

The first two protocol amendments relate to MC-FludT.14/L Trial I (see 8.1.2). Protocol versions 4-6 (Amendments 3-5) were submitted for MC-FludT.14/L Trial II, with protocol version 4 as the initial protocol for MC-FludT.14/L Trial II (Table 11).

Table 11. Protocol Amendments for MC-FludT.14/L Trial II

Amendment	Summary of changes to protocol.
3 (Protocol v 4.0)	Summary of changes (1 January 2013): • Primary and secondary objectives changed to "... within 2 years after transplantation." • Dose of treosulfan changed to 10 g/m²/day on days -4 to -2. • Non-inferiority margin specified at 1.3. • Revised sample size was submitted, along with updated interim analysis plan. • Addition of a DMC for supervision of the study.

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4 (Protocol v 5.0)	Summary of changes (10 July 2015): • The updated language of the primary objective is: “To show at least non-inferiority of treosulfan-based conditioning to RIC therapy based on IV busulfan and to compare the associated safety profiles. The aim of the study is to compare EFS within 2 years after transplantation between treosulfan-based conditioning and busulfan-based conditioning. Events are defined as relapse, graft failure, or death.” • The statistical plan was updated to state that patients in Trial II would be evaluated separately from those in Trial I.
5 (Protocol v 6.0)	Summary of changes (2 December 2016) : • The range of acceptable IMP dose deviation was aligned between sections 9.2 and 10.2.2 • Mucositis removed as an AESI and plan updated for exploratory analysis only.
Source: MC-FludT.14/L Trial II CSR (Appendix 16)	

STUDY RESULTS

First subject enrolled: June 13, 2013

Final subject enrolled: January 25, 2018

Planned enrollment: at most 930 patients (481 events)

Actual enrollment of randomized subjects: 570 patients

Compliance with Good Clinical Practices

As this study was performed only in Europe, the FDA checklist for foreign clinical trial data was performed. The study met all qualifications (Table 12).

Table 12. Checklist for Foreign Clinical Trial Data

1. Investigator's qualifications	<i>CTR Section 6 CTR Appendix 16.1.4</i>
2. Description of research facilities	<i>CTR Section 6</i>
3. Detailed summary of the protocol, study results, and if requested, copies of the study records and primary source documents	<i>5.3.5</i>
4. Description of the drug substance and drug product batches used	<i>3.2.S, 3.2.P</i>
5. Documentation that the study is adequate and well-controlled (21 CFR 314.126)	<i>X</i>
6. Name/address of IEC, list and qualifications of IEC members	<i>Appendix 16.1, CTR 5.1</i>
7. Summary of the IEC's decisions	<i>CTR 5.1</i>
8. Description of the consent process	<i>CTR 5.3</i>
9. Description of incentive to subjects	<i>N/A</i>
10. Description of monitoring process and controls for compliance	<i>CTR</i>
11. Description of investigator training and documentation of commitment to GCP	<i>2.5.1.6, CTR 9.6</i>

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Financial Disclosure

The financial disclosure form for MC-FludT.14/L Trial II was submitted with the application. No investigators with disclosable financial interests were identified.

Data Quality and Integrity

The quality and integrity of the submitted data were sufficient to review the application for the secondary endpoint of overall survival. Insufficient data were provided to independently verify the primary endpoint of event free survival at 2 years.

Patient Disposition

Five hundred seventy patients were randomized to busulfan or treosulfan arms (Figure 5). Following randomization, subjects in each arm did not receive study drug, primarily due to screening failures. In addition, two patients in the treatment arm were not evaluable for efficacy endpoints as they did not receive HSCT. The full analysis set is comprised of 283 patients in the busulfan arm and 268 patients in the treosulfan arm.

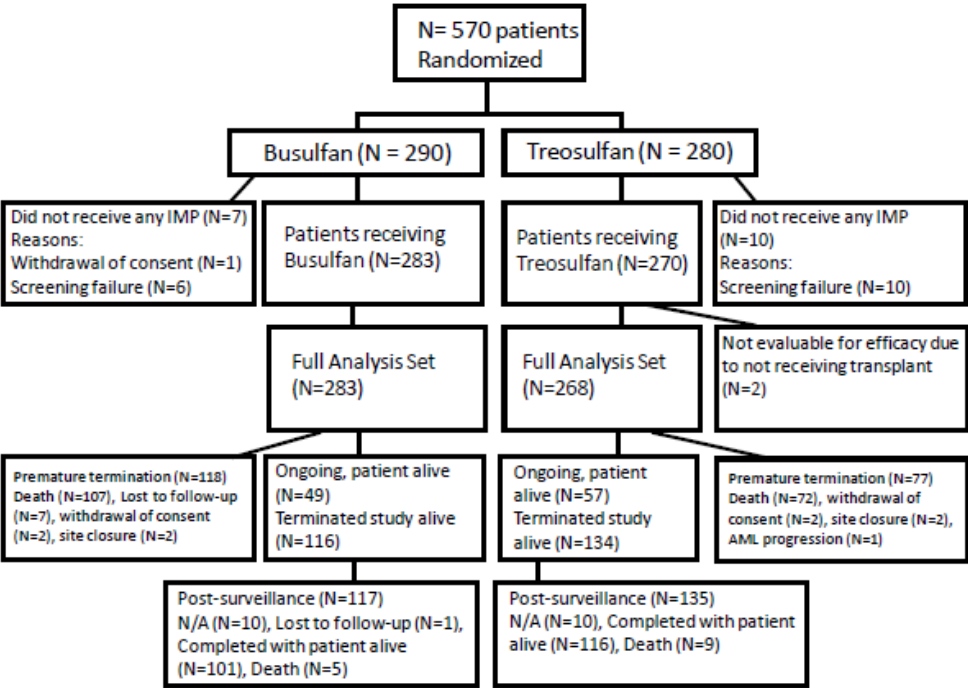


Figure 5. Distribution of Patients in MC-FludT.L/14 Trial II

Source: Reviewer’s Analysis, Adapted from MC-FludT.14/L II Clinical Trial Report

Protocol Violations/Deviations

The clinical trial report contained reports of 490 of 570 patients with reported deviations (86% of total patients). Of these, 201 of 570 patients (35.3%) had a major protocol deviation. Slightly greater than 17% of patients had a procedural deviation, with missing evaluations at different timepoints. There were 32 patients (5.6%) with treatment deviations. Table 13 further characterizes the categories of major protocol deviations.

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Table 13. Major Protocol Deviations by Category in MC-FludT.14/L Trial II

Category	Treosulfan (N = 280)	Busulfan (N = 290)	All Patients (N = 570)
Patients with any deviation (N, %)	91 (32.5%)	110 (37.9%)	201 (35.3%)
<i>Procedure deviation</i>			
Any deviation	45 (16.1%)	54 (18.6%)	99 (17.4%)
Missing baseline evaluations	23 (8.2%)	29 (10%)	52 (9.1%)
Missing evaluations from day -6 and day +28	6 (2.1%)	0 (0%)	6 (1.1%)
Missing evaluations from day +29 to day +100	9 (3.2%)	6 (2.1%)	15 (2.6%)
Missing evaluations from day +100 and month +24	17 (6.1%)	23 (7.9%)	40 (7%)
<i>Adherence to other relevant specifications of the protocol</i>			
Any deviation	28 (10%)	24 (8.3%)	52 (9.1%)
Informed consent	16 (5.7%)	17 (5.9%)	33 (5.8%)
Non-compliance	12 (4.3%)	7 (2.4%)	19 (3.3%)
Participation in another study	0 (0%)	1 (0.3%)	1 (0.2)
<i>Randomization error</i>			
Incorrect stratification	15 (5.4%)	24 (8.3%)	39 (6.8%)
<i>Treatment deviation</i>			
Any deviation	12 (4.3%)	20 (6.9%)	32 (5.6%)
Treosulfan or Busulfan	8 (2.9%)	15 (5.2%)	23 (4%)
Methotrexate	4 (1.4%)	3 (1%)	7 (1.2%)
ATG	1 (0.4%)	3 (1%)	4 (0.7%)
<i>Time schedule deviation</i>			
Incorrect timing of examinations at baseline	7 (2.5%)	4 (1.4%)	11 (1.9%)
<i>Inclusion/exclusion error at study entry</i>			
Inclusion/exclusion criteria violated	1 (0.4%)	1 (0.3%)	2 (0.4%)
<i>Excluded concomitant medication</i>			
Concomitant disease therapy in the absence of relapse/progression	1 (0.4%)	0 (0%)	1 (0.2 %)

Source: MC-FludT.14/L Trial II Clinical Study Report

Demographic Characteristics

The demographic characteristics of the intent-to-treat (ITT) population are shown in Table 14. There were no significant differences between the treosulfan and busulfan groups.

Table 14. MC-FludT.14/L Trial II Demographics (ITT)

Demographic Parameter	Category/ Statistic	Treatment Group		
		Treosulfan N=280	Busulfan N=290	All Patients N=570
Age at Randomization	N	280	290	570
	Mean (SD)	59.4 (6.51)	60 (6.04)	59.7 (6.28)
	Median (Min, Max)	60 (37, 70)	60 (31, 70)	70 (31, 70)
Age Category at Randomization (N, %)	≤ 18 years	0 (0%)	0 (0%)	0 (0%)
	18 - 64 years	204 (73%)	218 (75%)	422 (74%)
	65 - 74 years	76 (27%)	72 (25%)	148 (26%)
	≥ 75 years	0 (0%)	0 (0%)	0 (0%)

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Demographic Parameter	Category/ Statistic	Treatment Group		
		Treosulfan N=280	Busulfan N=290	All Patients N=570
Sex, N (%)	Female	109 (39%)	114 (39%)	223 (39%)
	Male	171 (61%)	176 (61%)	347 (61%)
Country, N (%)	Germany	190 (68%)	197 (68%)	387 (68%)
	France	6 (2%)	8 (3%)	14 (2%)
	Hungary	17 (6%)	18 (6%)	35 (6%)
	Italy	27 (10%)	30 (10%)	57 (10%)
	Poland	40 (14%)	37 (13%)	77 (14%)
	USA	0 (0%)	0 (0%)	0 (0%)
Source: Reviewer's analysis, FludT.L/14.II ADSL Dataset. Note: no racial or ethnic data was collected because of local regulations.				

Other Baseline Characteristics

Table 15 shows the disease characteristics of patients enrolled in MC-FludT.14/L Trial II. Based on disease risk status, patients received a designated risk score, in order to evaluate both MDS and AML patients in the same analysis.

Table 15. MC-FludT.14 Trial II Disease Characteristics (ITT)

Disease Characteristics	Category/ Statistic	Treatment Group		
		Treosulfan N=280	Busulfan N=290	All N=570
Diagnosis (N, %)	AML	192 (69%)	173 (60%)	365 (64%)
	MDS	88 (31%)	117 (40%)	205 (36%)
AML Risk Classification (N, %)	High risk	75 (39%)	51 (29%)	126 (35%)
	Intermediate risk	69 (36%)	78 (45%)	147 (41%)
	Low risk	20 (11%)	19 (11%)	39 (11%)
	NA if > CR1	26 (14%)	25 (14%)	51 (14%)
Remission Status, AML only (N, %)	CR1	164 (85%)	148 (86%)	312 (85%)
	>CR1	28 (15%)	25 (14%)	53 (15%)
IPSS-R Classification (MDS Only) (N, %)	Very high risk	25 (28%)	32 (27%)	57 (28%)
	High risk	23 (26%)	29 (25%)	52 (25%)
	Intermediate risk	20 (23%)	36 (31%)	56 (27%)
	Low risk	15 (17%)	19 (16%)	34 (17%)
	Very low risk	5 (6%)	1 (1%)	6 (3%)
Designated Risk Group (All) (N, %)	Risk group I	129 (46%)	153 (53%)	282 (50%)
	Risk group II	149 (54%)	137 (47%)	286 (50%)
HCT-CI Score (N, %)	≤ 2	116 (41%)	118 (41%)	234 (41%)
	> 2	164 (59%)	172 (59%)	336 (59%)
Donor Type (N, %)	MRD	66 (24%)	68 (23%)	134 (24%)
	MUD	214 (76%)	222 (77%)	436 (76%)
Donor Age (years)-Median (range)	MRD	55 (33 - 74)	57 (34 - 70)	56 (33 - 74)
	MUD	31 (18 - 59)	30 (18 - 58)	31 (18 - 59)
Source of Stem Cells (N, %)	Bone Marrow	8 (3%)	7 (2%)	15 (3%)
	Peripheral Blood	260 (97%)	276 (98%)	536 (97%)

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Grafapex (treosulfan)

Disease Characteristics	Category/ Statistic	Treatment Group		
		Treosulfan N=280	Busulfan N=290	All N=570
Blast % at Randomization	Mean (SD)	3.26 (3.5)	3.76 (3.8)	3.51% (3.7)
	Median (Min, Max)	2.5 (0, 20)	2.5 (0, 19)	2.5 (0, 20)

Source: Reviewer's Analysis, FludT.L/14.II ADSL Dataset.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Conditioning preparative regimens were administered at the research sites by qualified study personnel. Patients did not receive any experimental treatments within 4 weeks prior to the study. Concomitant medications for GvHD prophylaxis were prescribed in the trial; all other prophylactic and concomitant medications were recorded in the CRF.

Efficacy Results – Primary Endpoint

The study was designed as an NI study with the option to test superiority at the final analysis. Enrollment was stopped at the second interim analysis. The final analysis was based on 48.6% information. Superiority of Treosulfan was not achieved at the second interim analysis but was at the last analysis, which included 570 patients and 234 events. While the trial showed treosulfan was superior to the control with respect to the primary endpoint at the final analysis, EFS [HR was 0.64, (0.49, 0.84)], there are reporting issues that cause FDA to question the usefulness of the result. The lack of 'Day +100 bone marrow' reports calls the EFS evaluation into question. FDA was not able to independently verify the primary endpoint of EFS. Therefore, efficacy review and analyses were focused on the overall survival, which was found to be supportive.

EFS

The first interim analysis happened at 40 EFS events, and DMC recommended to continue enrollment. One hundred sixty-four events happened at the second interim analysis. Non-inferiority was achieved with 476 patients, and enrollment was stopped. The final analysis included 168 events. Superiority of Treosulfan was not achieved at the second interim analysis but was at the last analysis, which included 570 patients and 234 events.

Table 16. Actual Stopping boundaries for efficacy and futility monitoring shows the actual stopping boundaries for efficacy and futility monitoring. Because noninferiority margin was achieved, the trial stopped at 48.6% information. As well studied in literature, results of early interim analyses tend to overestimate treatment efficacy. This early stop may result in insufficient follow-up.

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Table 16. Actual Stopping boundaries for efficacy and futility monitoring

Interim Look	Information			Stopping Boundaries Efficacy*		Stopping Boundaries Futility**	
	Sample Size	Number of events	IF[%]	HR-scale	p-value scale	HR-scale	p-value scale
1	221	40	8.3%	0.1144	7.693E-15	3.4251	0.9989
2	460	164	34.1%	0.73174	0.000124	1.398117	0.679246
Final confirmatory Analysis after early stopping	460	168	34.9%	0.743677	0.000149	1.392550	0.671973
Last analysis incl. post surveillance	551	234	48.6%	0.875859	0.001262	1.220195	0.313994

IF) Information fraction is defined as the ratio of the information at the current time-point to the maximum information committed to the study

*) Stop for efficacy, if HR or p-value fall below the associated stopping boundaries

**) Stop for futility, if HR or p-value exceed the associated stopping boundaries or if CP_H1 or CP_observed fall below the documented thresholds (see study protocol, version 05 dated 10-Jul-2015, Table 10.2.7B)

Source: Table 7.3.B, Page 41 of SAP Version 7.0

Because the post-HSCT bone marrow studies and the CBC results after Day +100 were not submitted, the Agency was not able to independently verify the primary endpoint of EFS for Study MC-FludT.14/L II.

Efficacy review and analyses will focus on the overall survival.

OS

FDA uses a Cox model stratified by the stratification factors of randomization to calculate the hazard ratio (HR) and 95% confidence interval (CI). All analyses are conducted on the ITT population, which includes all randomized patients according to their assigned treatment at randomization. The starting point of OS is time of randomization, not time of transplant.

The median (range) of follow-up time was 29.5 months (0-54.8) months as determined by reverse Kaplan-Meier method. The HR is 0.65 (0.48, 0.88). Figure 6 shows the Kaplan-Meier Curve for OS in ITT population.

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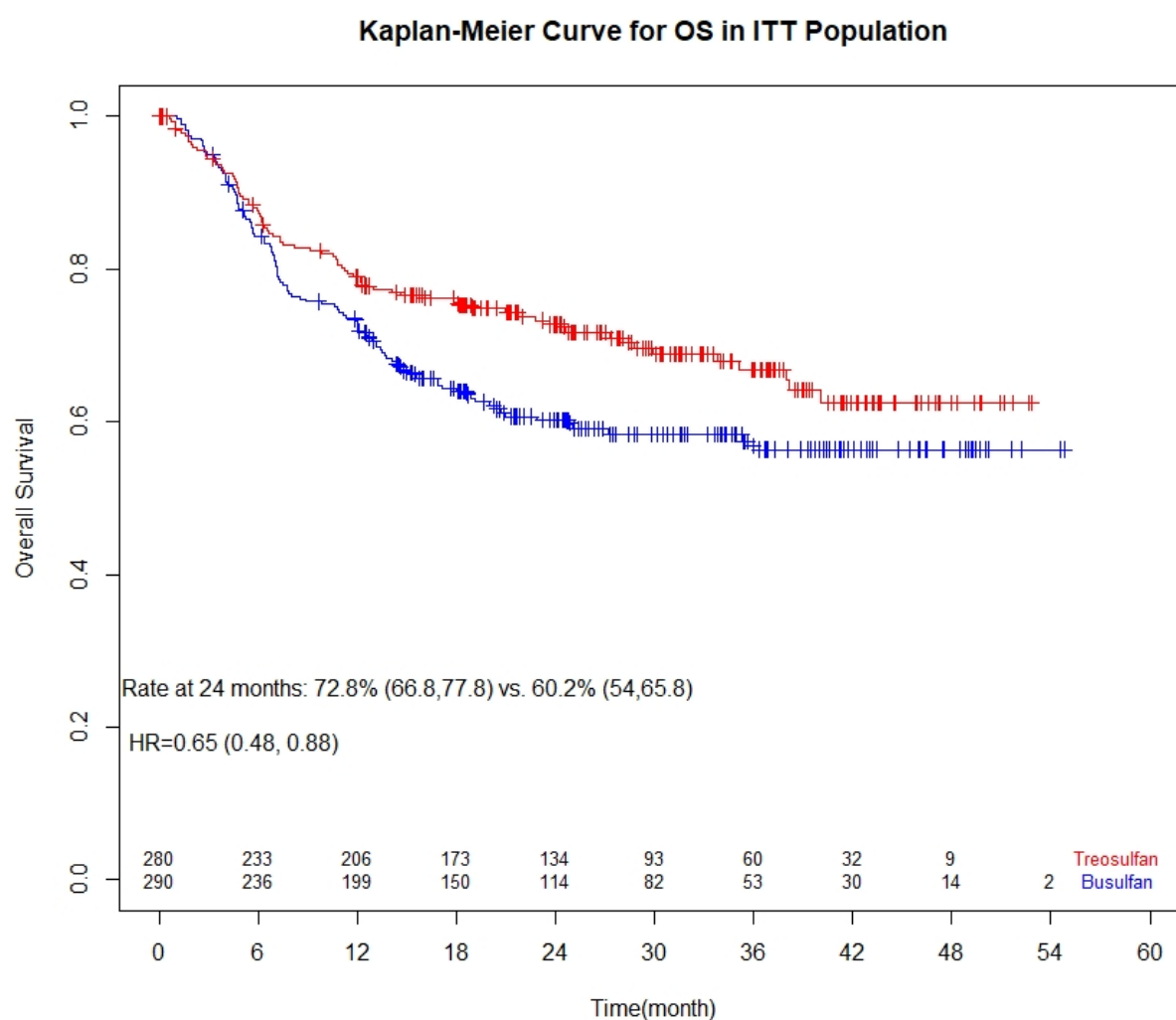
Grafapex (treosulfan)

Table 17. Number of Events and Censoring in ITT Population

	Busulfan	Treosulfan
Number of Patients	290	280
Events (%)	112 (38.6%)	81 (28.9%)
Censoring (%)	178 (61.4%)	199 (71.1%)

Source: FDA Analysis

Figure 6. Kaplan-Meier Curve for OS in ITT Population



Source: FDA Analysis

Subpopulations

There were 365 patients diagnosed as AML and 205 as MDS. Table 18 shows the number of

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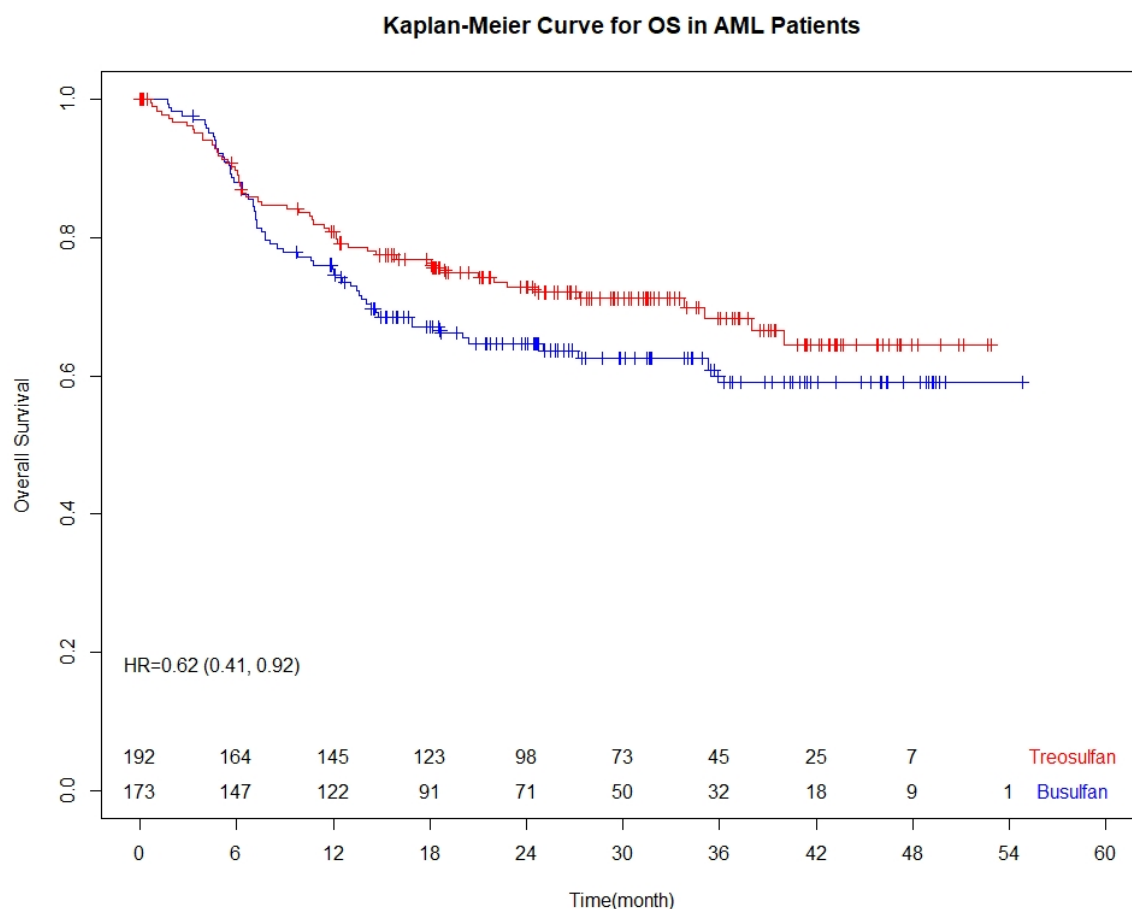
deaths and censorings within each subpopulation. The HR is 0.61 (0.41, 0.92) for the AML subpopulation and 0.62 (0.34, 1.10) for the MDS subpopulation.

Table 18. Number of Events and Censoring in AML and MDS subpopulations

	AML		MDS	
	Busulfan	Treosulfan	Busulfan	Treosulfan
Number of Patients	173	192	117	88
Events (%)	61 (35.3%)	54 (28.1%)	51 (43.6%)	27 (30.7%)
Censoring (%)	112 (64.7%)	138 (71.9%)	66 (56.4%)	61 (69.3%)

Source: FDA Analysis

Figure 7. Kaplan-Meier Curve for OS in AML Patients



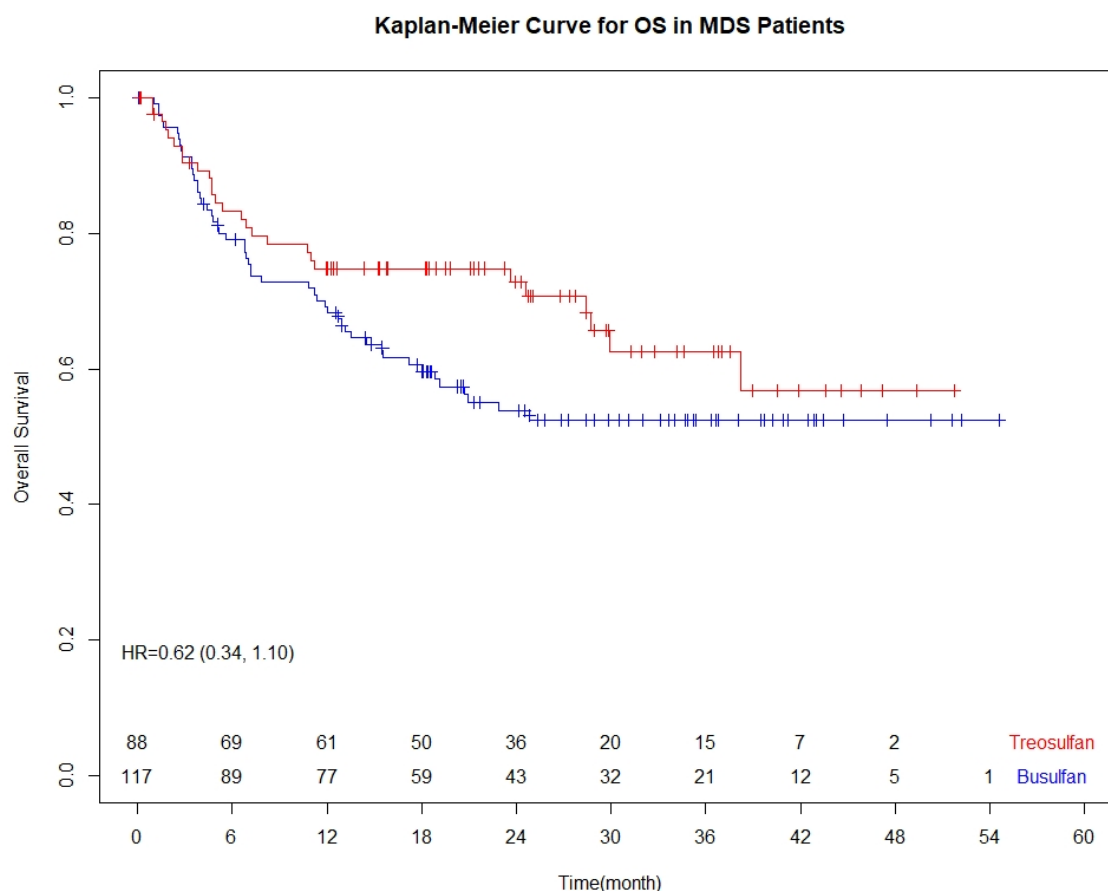
Source: FDA Analysis

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Figure 8. Kaplan-Meier Curve for OS in MDS Patients



Source: FDA Analysis

There were 115 deaths (31.5%) in the set of AML patients and 78 deaths (38.1%) in the set MDS patients. In AML patients, 15.6% died of relapse or progression and 8.3% died of transplantation related cause. In MDS patients, 9.3% died of relapse or progression and 24.9% died of transplantation related cause.

Table 19. Cause of death by disease

	AML (N=365)			MDS (N=205)		
	Treosulfan	Busulfan	Total	Treosulfan	Busulfan	Total
Death, n (%)	54 (28.1%)	61 (35.3%)	115 (31.5%)	27 (30.7%)	51 (43.6%)	78 (38.1%)
Cause of Death n (%)						
Relapse/progression	30 (15.6%)	32 (18.5%)	62 (17.0%)	4 (4.6%)	15 (12.8%)	19 (9.3%)
Transplantat related	16 (8.3%)	24 (13.9%)	40 (11.0%)	17 (19.3%)	34 (29.1%)	51 (24.9%)
Secondary malignancy				1 (1.1%)	1 (0.9%)	2 (1.0%)
Unknown	6 (3.1%)	5 (2.9%)	11 (3.0%)	4 (4.6%)	1 (0.9%)	5 (2.4%)
Other	2 (1.0%)		2 (0.6%)	1 (1.1%)		1 (0.5%)

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Efficacy Results – Exploratory and Other COA (PRO) Endpoints

The additional exploratory endpoints as reported by the Applicant are shown in Table 20. Except where indicated, the Applicant's analyses utilized the FAS.

Table 20. Study MC-FludT.14/L Trial II: Applicant's Analyses of Secondary Endpoints

Outcome	Set	Treosulfan Arm (n=268)	Busulfan Arm (n=283)	Source Table
Primary graft failure	FAS	0.4%	0.4%	14.2.7A
Secondary graft failure	FAS	0	2.9%	14.2.7A
Day-28 complete chimerism	EVAL ¹	93%	83%	11.4.1.8.A
Day-100 complete chimerism	EVAL	86%	80%	11.4.1.8.A
CIF ANC > 0.5 Gi/L	FAS	SHR 1.06 (0.91, 1.24)		11.4.1.7.A
CIF Platelets > 50 Gi/L	FAS	SHR 0.79 (0.67, 0.92)		11.4.1.7.F
CIF relapse/progression	FAS	SHR 0.82 (0.59, 1.16)		11.4.1.3.A
CIF nonrelapse mortality	FAS	SHR 0.63 (0.41, 0.97)		11.4.1.5.A
GRFS ²	FAS	HR 0.73 (0.57, 0.92)		11.4.1.9.A
CRFS ³	FAS	HR 0.70 (0.55, 0.88)		11.4.1.10.A

Source: Study MC-FludT.14/L Clinical Study Report dated 5/3/2020

Abbreviations: ANC, absolute neutrophil count; CIF, cumulative incidence function; CRFS, chronic GVHD- and relapse-free survival, EVAL, evaluable set; FAS, full analysis set; GRFS, GVHD- and relapse-free survival; HR, hazard ratio; SHR, subhazard ratio

¹ Evaluable subset of the FAS

² Survival free of Grade 3-4 acute GVHD, extensive chronic GVHD and relapse

³ Survival free of extensive chronic GVHD and relapse

Clinical TL Review Comment: *The endpoints based on hematopoietic recovery, chimerism and nonrelapse mortality are safety issues rather than efficacy issues. GRFS and CRFS represent a mixture of safety and efficacy data. The relapse/progression endpoint might be considered as supportive of efficacy, but the results of analyses using cumulative incidence with competing risks are difficult to interpret when the incidence of competing risks is so high. As such, none of the prespecified secondary endpoint provide information to address a determination of efficacy. Since the patients with MDS were transplanted when not in remission, achievement of remission might reflect effectiveness, but this was not a prespecified endpoint; however, results of marrow examinations which would allow for assessment of response were not collected systematically, so response cannot be assessed.*

There were no efficacy-related patient-reported outcomes submitted for review.

Additional Analyses Conducted on the Individual Trial

All FDA analyses were based on the ITT population unless specifically annotated.

Boundary Analysis for OS

There was no alpha-allocation plan for OS or any other secondary endpoints. Therefore, the

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significance of OS is hard to interpret. All p-values are nominal and cannot be included in labeling since there was no pre-specified plan to control multiplicity.

To facilitate decision making, we calculated O'Brien-Fleming (OBF) boundaries and Pocock boundaries using the information fraction of EFS. OBF assigns more weight toward later analysis, while Pocock boundary assigns alpha to each analysis equally. Therefore, OBF is more conservative compared to Pocock and recommended in analyses for primary and key secondary endpoints.

For comparison, in a hypothetical well-controlled trial with EFS as primary endpoint and OS as key secondary endpoint, the fixed sequence testing procedure at each prespecified interim analysis would be to test EFS first, if EFS achieved statistical significance according to the OBF boundary, then test OS at the same significance level. Because this trial was designed as a noninferiority test and used the margin of HR=1.3, which was more liberal than what was usually recommended by FDA, it stopped early at 48.6% information of EFS. Although not prespecified, information fraction of OS at the stopping point would be even smaller. We then applied the less strict Pocock boundary to compare with significance level from OBF.

From this boundary analysis, we wanted to see how significant the OS results would be assuming the same information fraction as EFS at the time points of interim analyses. Please note these boundaries are exploratory only and based on a hypothetical exercise.

We conducted a stratified log-rank test for OS at the last analysis. The p-value was 0.0049. At this point, the OBF boundary is 0.0025 and the Pocock boundary is 0.014. The OS results crossed the Pocock boundary, but not the OBF boundary. Therefore, there would have been different conclusions regarding statistical significance based on the different methods. As previously specified, the OBF boundary is more conservative and assigns more weight toward later analysis, while the Pocock boundary assigns weights equally to early and late analyses. These mixed results suggest that it is not clear if OS would have been considered to be significant; however, no definitive conclusions can be made based on the OS results because the multiplicity approach was not prespecified.

Table 21. O'Brien Fleming and Pocock Boundaries for OS using Information Fraction of EFS

Interim Look	Sample Size	# of Events	IF(%)	Pocock		OBF	
				Upper	Lower	Upper	Lower
1	221	40	8.3	0.003	0.003	<.0001	<.0001
2	460	164	34.1	0.009	0.009	0.0001	0.0001
Confirmatory	460	168	34.9	0.006	0.006	0.0001	0.0001
Last	551	234	48.6	0.007	0.007	0.0013	0.0013

Source: FDA Analysis

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Subgroup Analysis for OS

We conducted an exploratory subgroup OS analysis to investigate if there is any detrimental trend in the subgroups that may raise safety concerns. Table 22 summarizes the HR and 95% CI of OS in these interested subgroups by treatment arms. Figure 9 is a forest plot that demonstrating OS results in both the subgroups and the overall population. The same Cox model for OS analysis in the overall population was applied here. The overall HR is 0.65 (0.48, 0.88). In the subgroups, most HRs are less than 1 except for the low or very low risk MDS and the MRD donor type group. However, the sample size of these two groups are relatively small and the 95% CI of HRs cover 1. There was no CI that could indicate a clearly detrimental effect in any subgroup because all confidence intervals were fairly wide. This demonstrates the uncertainty in the hazard ratio estimates presented.

Table 22. Trial 14-II: HR and 95% CI in subgroups by treatment arms.

Subgroup	Busulfan(N/d)	Treosulfan(N/d)	HR(95%CI)
Age			
<65	218/75	204/59	0.71(0.49,1.02)
>=65	72/37	76/22	0.49(0.23,1.02)
Sex			
Female	114/33	109/36	0.97(0.57,1.65)
Male	176/79	171/45	0.48(0.32,0.72)
Country			
DEU	197/71	190/54	0.71(0.49,1.02)
Not DEU	93/41	90/27	0.56(0.33,0.94)
Diagnosis			
AML	173/61	192/54	0.61(0.41,0.92)
MDS	117/51	88/27	0.62(0.35,1.11)
AML Risk Group			
High risk	51/22	75/32	0.84(0.46,1.52)
Intermediate risk	78/22	69/10	0.41(0.17,1)
Low risk	19/5	20/2	0.17(0.02,1.7)
NA if gt CR1	25/12	26/10	0.6(0.2,1.75)
MDS Risk Group			
Very high risk	32/21	25/11	0.46(0.16,1.31)
High risk	29/14	23/5	0.33(0.06,2.01)
Intermediate risk	36/13	20/5	0.42(0.06,2.86)
Low or very low risk	20/3	20/6	1.48(0.33,6.57)
Donor Type			
MRD	68/26	66/27	1(0.55,1.83)
MUD	222/86	214/54	0.56(0.4,0.8)

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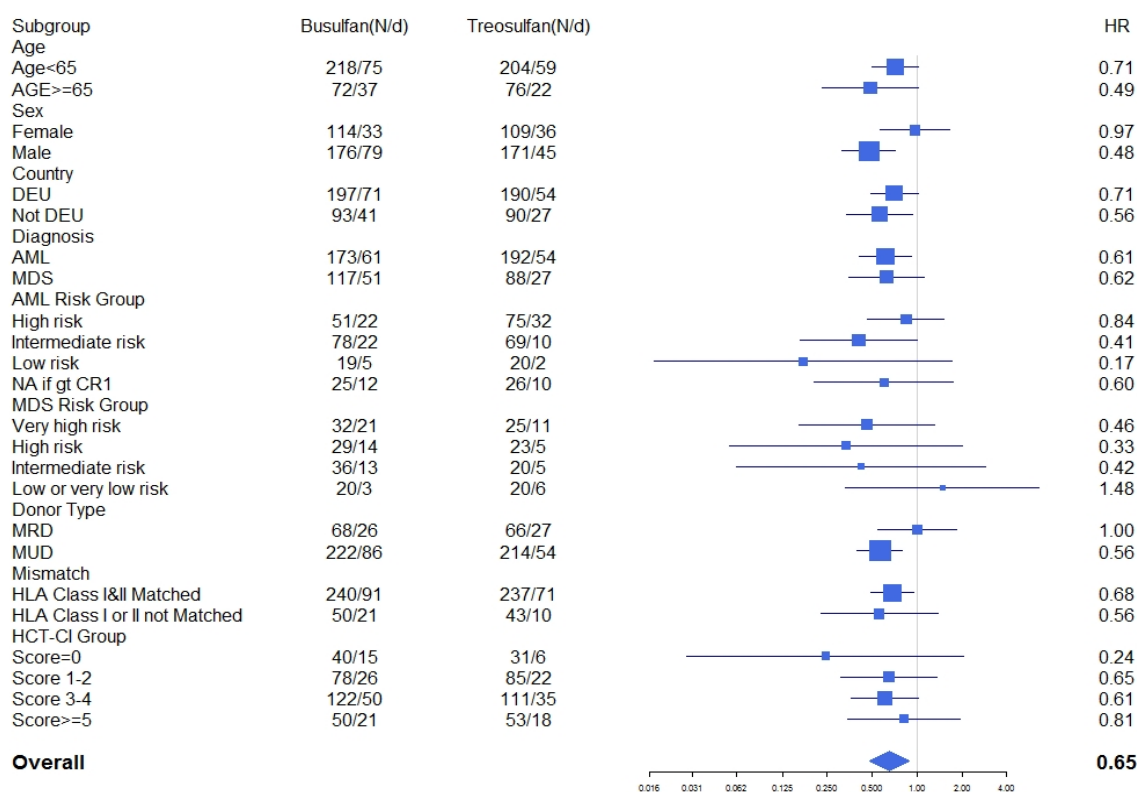
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Grafapex (treosulfan)

Subgroup	Busulfan(N/d)	Treosulfan(N/d)	HR(95%CI)
Mismatch			
HLA Class I&II Matched	240/91	237/71	0.68(0.49,0.96)
HLA Class I or II not Matched	50/21	43/10	0.56(0.23,1.37)
HCT-CI Group			
Score=0	40/15	31/6	0.24(0.03,2.06)
Score 1-2	78/26	85/22	0.65(0.31,1.36)
Score 3-4	122/50	111/35	0.61(0.36,1.03)
Score>=5	50/21	53/18	0.81(0.34,1.93)
Overall			0.65(0.48,0.88)

Source: FDA Analysis

Figure 9. Trial 14-II Forest plot to show OS results in subgroups



Source: FDA Analysis

Comparison of Analysis Sets

Sponsor's results were based on FAS and time started from transplant. However, FDA applied the Intent-to-treat (ITT) principle and started at randomization when calculate time. To investigate the impact of different analysis sets, we compared number of patients with missing

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information between the two treatment arms. As shown in Table 23, the number of subjects who did not receive any study treatment or had no efficacy data were comparable between the two treatment arms.

Table 23. Patients with missing information in two treatment arms

	Busulfan	Treosulfan	Total
ITT	290	280	570
FAS	283	268	551
No IMP given	7 (2.4%)	10 (3.6%)	17 (3.0%)
No efficacy endpoint available	0	2 (0.7%)	2 (0.4%)

Source: FDA Analysis

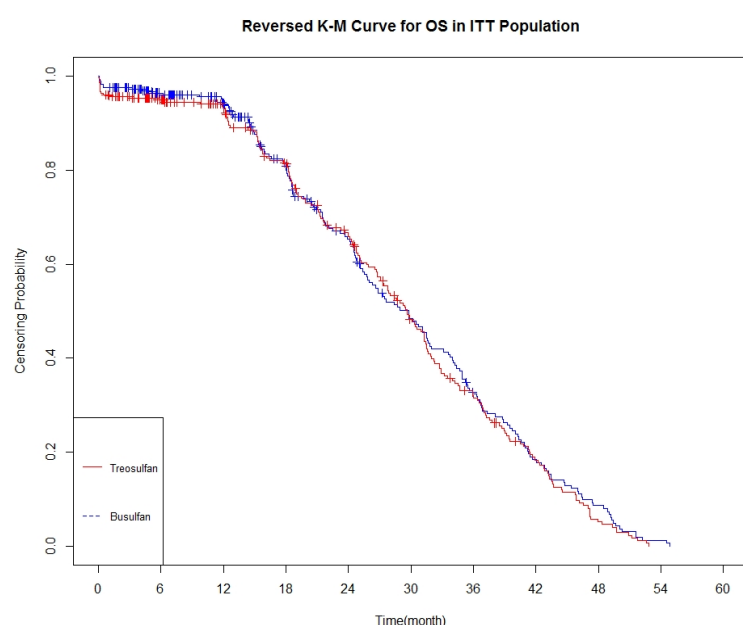
Analysis of Trial Conduct

To further understand the quality of OS data, we performed the following analyses:

- **Informative Censoring for OS**

In this analysis we reversed the censoring indicator for death, which means censoring is now counted as event and death as censoring. We then used Kaplan-Meier method to make a reversed censoring survival curve for the two treatment arms. If there is any informative censoring in one of the treatment arms, the survival curves will separate from each other. Figure 10 shows the reversed K-M curve for OS in the ITT population, which does not indicate that informative censoring is present.

Figure 10. Reversed censoring curve for OS in ITT population



Source: FDA Analysis

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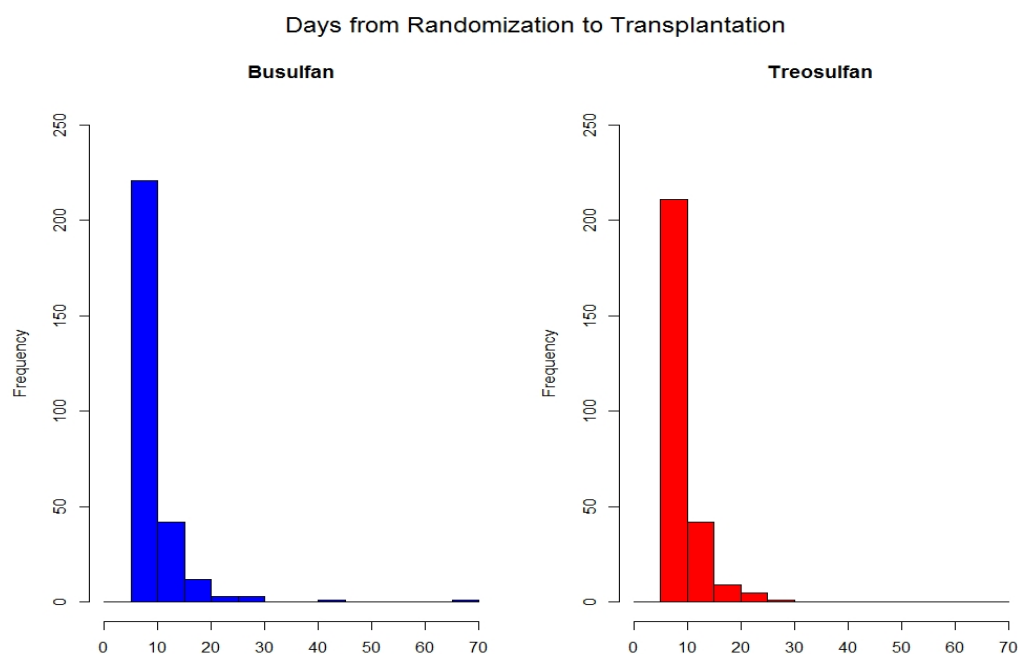
- **Time from randomization to transplantation**

Since the indication is conditioning treatment before transplantation, we wanted to assess if there was any difference in time from randomization to transplantation in different treatment arms. Table 24 summarizes days between randomization and transplantation by treatment arms. Figure 11 shows the histogram of days from randomization to transplantation by treatment arms. To further compare difference in the two treatment arms, we made a Kaplan-Meier curve for transplantation. In this curve, we treated transplantation as event, and calculated time from randomization to transplantation. Patients who did not receive transplant were censored at the last visit. Figure 12 shows the results, which does not indicate any imbalance in the distribution of time from randomization to transplantation between the two arms.

Table 24. Days between randomization and transplantation by treatment arms

Days between randomization and HSCT	Busulfan (N=290)	Treosulfan (N=280)	Total (N=570)
n	283	268	551
Mean (SD)	9.52 (5.503)	9.06 (3.494)	9.30 (4.637)
Median (Q1, Q3)	8 (7, 10)	8 (7, 9)	8 (7, 10)
Min, Max	6, 70	6, 26	6, 70
Missing	7	12	19

Figure 11. Histogram for days between randomization and transplantation



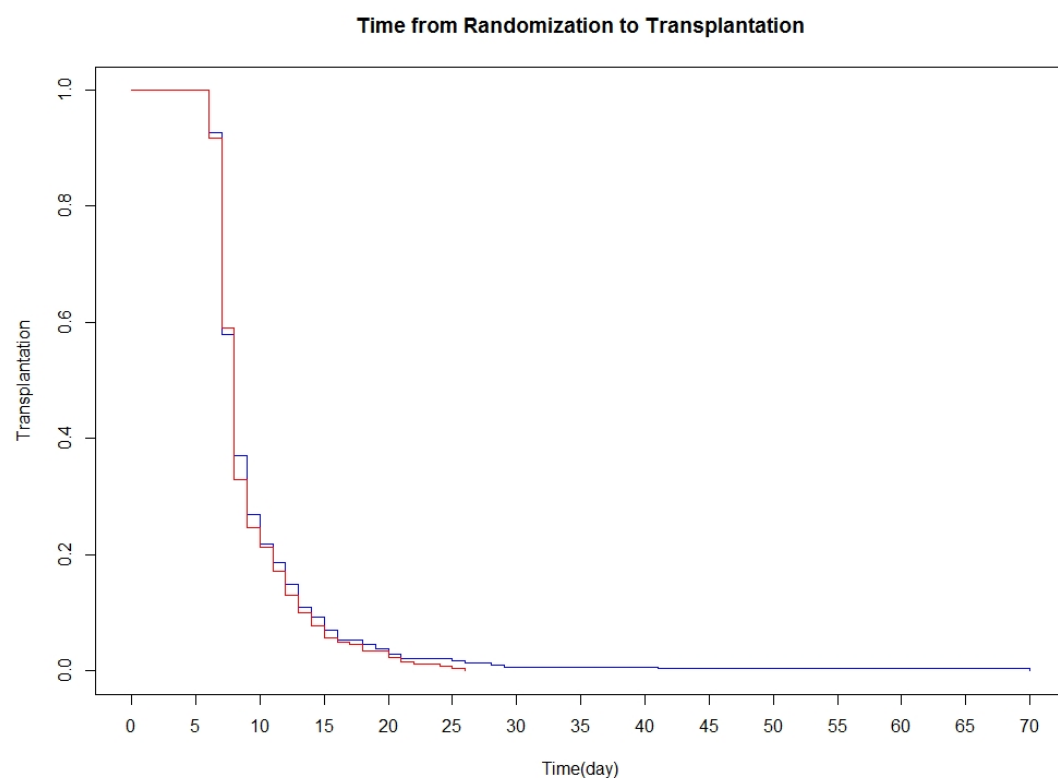
Source: FDA Analysis

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Figure 12. Kaplan-Meier curve for time between randomization and transplantation



Source: FDA Analysis

Table 25 shows time from diagnosis to randomization by treatment arms. It is shown that the distribution of days between diagnosis and randomization are comparable between two arms. No different randomization time from diagnosis caused by treatment was found.

Table 25. Time from diagnosis to randomization by treatment arms

Days between diagnosis and randomization	Busulfan (N=290)	Treosulfan (N=280)	Total (N=570)
n	288	279	567
Mean (SD)	298.54 (398.337)	296.68 (432.646)	297.63 (415.206)
Median (Q1, Q3)	164 (106.5, 345)	161 (108, 297)	164 (107, 321)
Min, Max	0, 3897	7, 4127	0, 4127
Missing	2	1	3

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Grafapex (treosulfan)

8.1.2 Study MC-FludT.14/L Trial I

"Clinical Phase III Trial To Compare Treosulfan-Based Conditioning Therapy With Busulfan-Based Reduced-Intensity Conditioning (RIC) Prior To Allogeneic Haematopoietic Stem Cell Transplantation In Patients With AML Or MDS Considered Ineligible To Standard Conditioning Regimens - Trial I"

INVESTIGATIONAL PLAN

Trial Design

Design and Objectives

The study was designed as a non-inferiority Phase III trial to compare treosulfan-based conditioning therapy to busulfan-based reduced-intensity conditioning therapy in adult patients with high-risk hematologic malignancies. Patients were randomized between the two arms of the study.

The primary endpoint of the study was event-free survival at 2 years after transplantation. Events were defined as disease relapse, graft failure, or death.

Secondary endpoints included overall survival (OS), incidence of relapse (RI), non-relapse mortality (NRM), and transplant-related mortality (TRM) at 2 years after transplantation. Additional secondary objectives included CTCAE grade III+ mucositis between days -6 and +28, engraftment at day +28, donor chimerism at days +28 and +100, acute and chronic GvHD within 2 years of transplantation, and other CTCAE grade III/IV events between days -6 and +28.

Key Eligibility Criteria

The study population consisted of adult patients with AML or high risk MDS who were not eligible for myeloablative conditioning regimens. The study defined this group of patients as patients who were at least 50 years of age at the time of HSCT or with an HCT-CI score > 2. Karnofsky performance score was at least 60%.

Treatment Plan Overview

Patients were randomized to receive either RIC with the control (reference) arm of busulfan + fludarabine, or the test arm of treosulfan + fludarabine (Table 26). All patients received fludarabine (30 mg/m²/d) on days -6 to -2. Patients on the control arm received busulfan (0.8 mg/kg) on days -4 and -3. Patients receiving busulfan received anti-seizure prophylaxis. Patients on the treatment arm received treosulfan (10 mg/m²/d) on days -6 to -4. Additionally, patients who received a MUD received ATG. The type of ATG and dosing varied by country.

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Table 26. Treatment Overview of MC-FludT.14/L Trial I

Treatment	Day -6	-5	-4	-3	-2	-1	0	+1	+3	+6
Treosulfan (IV, 14 g/m ² within 120 minutes) – test arm	X	X	X							
Busulfan (IV, 0.8 mg/kg within 120 minutes) – control			X	X						
Fludarabine (IV, 30 mg/m ² within 30 minutes)	X	X	X	X	X					
ATG-S-Fresenius / Grafalon (IV, 10 mg/kg in MUD) – Germany, Hungary, Italy, Poland			X	X	X					
ATG-Thymoglobulin (IV, 2.5 mg/kg in MUD) – France					X	X				
Allogeneic stem cell transplantation							X			
Cyclosporine-A (IV, PO, dose level adjusted)						X	X	X...	X...	X...
Methotrexate (IV, mg/m ² /day)								15	10	10
<i>Source: Reviewer's analysis.</i>										

GVHD prophylaxis was identical in both arms. All patients received level-adapted cyclosporine from Day -1 through Day +100 and methotrexate on days +1, +3, and +6, with folic acid rescue.

Monitoring Schedule

Table 27. Monitoring Overview of MC-FludT.14/L Trial I

	Day -6 to +27	Day +28	Day +100	Month 6, 9, 12
Efficacy				
CBC	-6, -3, -1, +2, +6, +10, +14, +16, +20, +24 (until engraftment)	X	X	
Chimerism		BM	BM or PB	
Disease status		BM, PB	BM, PB	PB*
Graft failure, relapse, death		X	X	X
Safety				
Physical Exam		X	X	X
Concomitant medication	X	X		
HSOS/VOD	X	X		
CTCAE Grade I-V	Continuous documentation			
SAE	Continuous documentation		If related to conditioning	
Serum chemistry	-6, -3, -1, +2, +6, +10, +14, +16, +20, +24	X		
aGvHD	Continuous documentation			
cGvHD				Continuous
GvHd prophylaxis / treatment	Continuous documentation (starting from day -1)			
*Bone marrow if suspected relapse				
Source: Adapted from clinical protocol for MC-FludT.14/L Trial I (Appendix 16.1 of CSR)				

Study subjects underwent screening within three weeks prior to the start of conditioning

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treatment, including routine blood tests, tests of end organ function, disease evaluation, performance status, and viral serologies. Monitoring for efficacy and safety is shown in Table 27. Of note, for patients who did not have signs of relapse, bone marrow studies following day +100 were not required.

Statistical Analysis Plan

This study used the same statistical analysis plan as Trial 14-II. Please refer to 8.1.1.

Protocol Amendments

Two protocol amendments were submitted prior to amendment 3, which closed Part I of the study (Table 28). Amendment 3 resulted in the initial protocol (version 4.0) for MC-FludT.14/L Trial II (see 8.1.1 for details).

Table 28. Protocol Amendments for MC-FludT.14/L Trial I

Amendment	Summary of Changes
1 (Protocol v 2.0)	Summary of changes (23 September 2009) Updated safety and efficacy data from previous clinical trials of treosulfan + fludarabine prior to HSCT.
2 (Protocol v 3.0)	Summary of changes (13 November 2009) Addition of PK testing.
Source: MC-FludT.14/L CSR, Appendix 16	

STUDY RESULTS

Compliance with Good Clinical Practices

As this study was performed only in Europe, the FDA checklist for foreign clinical trial data was performed. The study met all qualifications (Table 29).

Table 29. Checklist for Foreign Clinical Trial Data

1. Investigator's qualifications	CSR Section 6, CSR Appendix 16.1.4
2. Description of research facilities	CSR Section 6
3. Detailed summary of the protocol, study results, and if requested, copies of the study records and primary source documents	5.3.5.1
4. Description of the drug substance and drug product batches used	3.2.S, 3.2.P
5. Documentation that the study is adequate and well-controlled (21 CFR 314.126)	X
6. Name/address of IEC, list and qualifications of IEC members	CSR Appendix 16.1.3, CSR 5.1

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7. Summary of the IEC's decisions	CSR 5.1
8. Description of the consent process	CSR 5.3
9. Description of incentive to subjects	N/A
10. Description of monitoring process and controls for compliance	CSR
11. Description of investigator training and documentation of commitment to GCP	2.5.1.6, CSR 9.6
Source: MC-FludT.14/L Trial I CSR	

Financial Disclosure

The financial disclosure form for MC-FludT.14/L Trial II was submitted with the application. No investigators with disclosable financial interests were identified.

Data Quality and Integrity

The quality and integrity of the submitted data were sufficient to review the application for the secondary endpoint of overall survival and safety.

Patient Disposition

Three hundred thirty patients were randomized to busulfan or treosulfan arms (Figure 13). Following randomization, subjects in each arm did not receive study drug. Following treatment administration 1 patient in the control arm and two patients in the treatment arm were not evaluable for efficacy endpoints. The evaluable patient set is comprised of 112 patients in the busulfan arm and 112 patients in the treosulfan arm.

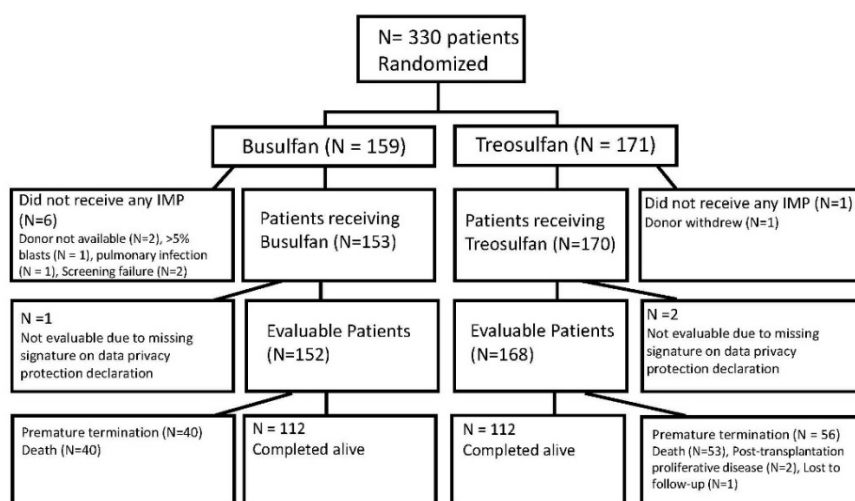


Figure 13. Distribution of Patients in MC-FludT.14/L Trial I

Source: Adapted from MC-FludT.14/L Trial I CSR.

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Protocol Violations/Deviations

The clinical study report contained reports of 236 of 330 patients with reported protocol deviations (72% of total patients) (Table 30). Of these, 120 (75.5%) were treated with busulfan and 116 (67.8%) were treated with treosulfan. Of note, there were more deviations regarding administration of the investigational drug product in patients receiving busulfan (12.6%) than in patients receiving treosulfan (3.5%). Six patients did not receive any busulfan, and one patient did not receive any treosulfan.

Table 30. Major Protocol Deviations by Category in MC-FludT.14/L Trial I

Category	Treosulfan (N = 171)	Busulfan (N = 159)	All Patients (N = 330)
Patients with any deviation (N, %)	116 (67.8%)	120 (75.5%)	236 (71.5%)
<i>Procedure deviation</i>			
Any deviation	31 (18.1%)	36 (22.6%)	67 (20.3%)
Missing baseline evaluations	0 (0%)	3 (0.6%)	3 (1.6%)
Missing evaluations from day -6 and day +28	4 (2.3%)	9 (5.7%)	13 (3.9%)
Missing evaluations from day +29 to day +100	13 (7.6%)	5 (3.1%)	18 (5.5%)
<i>Adherence to other relevant specifications of the protocol</i>			
Any deviation	13 (7.6%)	4 (2.5%)	17 (5.2%)
Deviations in relapse evaluation	9 (5.3%)	2 (1.3%)	11 (3.3%)
Non-compliance	2 (1.2%)	1 (0.6%)	3 (0.9%)
Deviations in graft failure evaluation	2 (1.2%)	1 (0.6%)	3 (0.9%)
<i>Randomization error</i>			
Incorrect stratification	10 (5.8%)	18 (11.3%)	28 (8.8%)
<i>Treatment deviation</i>			
Any deviation	58 (33.9%)	64 (40.3%)	122 (37%)
Treosulfan or Busulfan	6 (3.5%)	20 (12.6%)	26 (7.9%)
Fludarabine	6 (3.5%)	6 (3.8%)	12 (3.6%)
Methotrexate	19 (11.1%)	10 (6.3%)	20 (8.8%)
ATG	22 (12.9%)	16 (10.1%)	38 (11.5%)
Ca-Folate	32 (18.7%)	24 (15.1%)	56 (17%)
<i>Time schedule deviation</i>			
Any deviation	67 (39.2%)	64 (40.3%)	131 (39.7%)
<i>Inclusion/exclusion error at study entry</i>			
Inclusion/exclusion criteria violated	3 (1.8%)	2 (1.3%)	5 (1.5%)
<i>Excluded concomitant medication</i>			
Concomitant disease therapy in the absence of relapse/progression	0 (0%)	2 (1.3%)	2 (0.6%)
<i>Source: Adapted from MC-FludT.14/L Trial I Clinical Study Report</i>			

Demographic Characteristics

Table 31 shows the demographic characteristics of the ITT population enrolled on MC-FludT.14/L Trial I. There were no significant differences between the two randomized groups.

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Table 31. Demographic Characteristics of MC-FludT.14/L Trial I

Demographic Parameter	Category/ Statistic	Treatment Group		
		Treosulfan N=169	Busulfan N=158	All Patients N=327
Age at Randomization	N	169	158	327
	Mean (SD)	57.3 (8.21)	57.7 (7.78)	57.5 (7.99)
	Median (Min, Max)	59 (21-70)	58 (24-70)	58 (21-70)
Age Category at Randomization (N, %)	≤ 18 years	0 (0%)	0 (0%)	0 (0%)
	18 – 64 years	140 (83%)	124 (78%)	264 (81%)
	65-74 years	29 (17%)	34 (22%)	63 (19%)
	≥ 75 years	0 (0%)	0 (0%)	0 (0%)
Sex (N, %)	Female	92 (54%)	69 (44)	161 (49%)
	Male	77 (46%)	89 (56%)	166 (51%)
Country (N, %)	Finland	3 (2%)	2 (1%)	5 (2%)
	France	7 (4%)	4 (3%)	11 (3%)
	Germany	127 (74%)	119 (75%)	246 (75%)
	Hungary	4 (2%)	4 (3%)	8 (2%)
	Italy	14 (8%)	15 (9%)	29 (9%)
	Poland	16 (9%)	15 (9%)	31 (9%)
Source: Reviewer's analysis. FludT.L/14.I DM legacy Dataset. Note: no racial or ethnic data was collected because of local regulations				

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Table 32 shows baseline disease characteristics of the ITT population. There were more patients enrolled on the study with AML than MDS, though the breakdown between the two treatment arms was similar.

Table 32. MC-FludT.14/L Trial I Subject Disease Characteristics

Disease Characteristics	Category/Statistic	Treatment Group		
		Treosulfan N = 169	Busulfan N = 158	All N = 327
Diagnosis (N, %)	AML	131 (78%)	112 (71%)	243 (74%)
	MDS	38 (22%)	46 (29%)	84 (26%)
Risk Classification (N, %)	High risk (AML, MDS)	52 (31%)	46 (29%)	98 (30%)
	Intermediate risk (AML)	43 (25%)	35 (22%)	78 (24%)
	Intermediate risk 1 (MDS)	20 (12%)	20 (13%)	40 (12%)
	Intermediate risk 2 (MDS)	14 (9%)	19 (12%)	33 (10%)
	Low risk (AML, MDS)	9 (5%)	8 (5%)	17 (5.2%)
	NA if > CR1 (AML)	31 (18%)	30 (19%)	61 (19%)
Designated Risk Group (N, %)	Risk group I (low + intermediate)	86 (51%)	82 (52%)	168 (51%)
	Risk group II (high + >CR1)	83 (49%)	76 (48%)	159 (49%)
HCT-CI Score (N, %)	HCT-CI ≤ 2	72 (43%)	56 (35%)	128 (39%)
	HCT-CI > 2	97 (57%)	102 (65%)	199 (61%)

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Donor Type (N, %)	MRD	45 (27%)	34 (22%)	79 (24%)
	MUD	123 (73%)	124 (78%)	247 (76%)
Source of Stem Cells (N, %)	Bone marrow	11 (7%)	17 (11%)	28 (9%)
	Peripheral blood	157 (93%)	135 (89%)	292 (91%)
Blast % at Randomization	Mean (SD)	2.84 (2.60)	2.93 (2.93)	2.89 (2.76)
	Median (Min, Max)	2.5 (0, 15)	2.5 (0, 17)	2.5 (0, 17)
<i>Source: Reviewer's analysis. FludT.L/14.I DM, TR, CL legacy datasets.</i>				

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Conditioning preparative regimens were administered at the research sites by qualified study personnel. Patients did not receive any experimental treatments within 4 weeks prior to the study. Concomitant medications for GvHD prophylaxis were prescribed in the trial; all other prophylactic and concomitant medications were recorded in the CRF.

Efficacy Results – Primary Endpoint

The trial discontinued after planned interim analysis. It was determined that dose should be lower in this elderly population because neutropenic phase was much longer than control. A higher mortality was also found in the Treosulfan group. As early as 28 days after transplantation, 7 patients (4.2%) of the Treosulfan treatment group, and none of the patients of the Busulfan treatment group had died.

As stated in the clinical report: “Consistently with the longer duration of neutropenia in patients receiving Treosulfan, a higher frequency of patients with CTCAE Grade III/IV infections was observed in the Treosulfan treatment group (54.8% vs. 36.2%). This observation is also reflected in the higher frequency of SAEs reported for the Treosulfan treatment group (11.3% vs. 5.9%), most notably infections (6.5% vs. 2.0%). Likewise, the cumulative incidence of infection related TRM on Day +100 was higher in the Treosulfan treatment group (7.9%) compared to the Busulfan treatment group (4.0%, one-sided $p=0.0630$, adjusted for donor type, risk group and centre using Cox regression model).

The Treosulfan group had a higher cumulative incidence of NRM and a significantly higher cumulative incidence of TRM on Day +100 for patients receiving Treosulfan (10.2%) compared to patients receiving Busulfan (5.3%, $p=0.077$)."

Efficacy Results – Secondary and Other Relevant Endpoints

Not applicable.

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Subpopulations

Because this trial was stopped due to safety concern, subpopulation analysis on OS was conducted to further investigate any detrimental OS effect. Table 33 and Figure 14 show the HR (95%) of OS by selected subgroups. In this trial, treosulfan had detrimental effect on OS. This effect was consistent within analyzed subgroups and no significantly larger detrimental effect was found in the subgroup analysis.

Table 33: Trial 14-I HR and 95% CI in subgroups by treatment arms.

Subgroup	Busulfan(N/d)	Treosulfan(N/d)	HR(95%CI)
Age			
<65	124/47	140/50	1.05(0.68,1.63)
>=65	34/10	29/15	1.58(0.59,4.2)
Sex			
Female	69/23	92/35	1.54(0.83,2.86)
Male	90/34	79/30	1.09(0.62,1.91)
Country			
DEU	119/43	127/48	1.1(0.71,1.7)
Not DEU	40/14	44/17	1.36(0.61,3.04)
Diagnosis			
AML	112/39	131/50	1.27(0.81,2.01)
MDS	46/18	38/15	0.99(0.4,2.45)
AML Risk Group			
High risk	41/16	49/19	0.94(0.44,2.03)
Intermediate risk	35/11	43/15	1.24(0.53,2.89)
Low risk	6/1	8/1	N/A
NA if gt CR1	30/11	31/15	2.12(0.83,5.44)
MDS Risk Group			
Very high risk	5/2	5/1	N/A
High risk	20/6	20/7	2.4(0.43,13.42)
Intermediate risk	19/9	14/7	0.98(0.22,4.43)
Low or very low risk	2/1	1/0	N/A
Donor Type			
MRD	34/14	45/16	0.7(0.3,1.64)
MUD	124/43	123/49	1.31(0.85,2.02)
Mismatch			
HLA Class I&II Matched	114/39	127/46	1.07(0.67,1.72)
HLA Class I or II not Matched	45/18	43/19	1.05(0.49,2.27)

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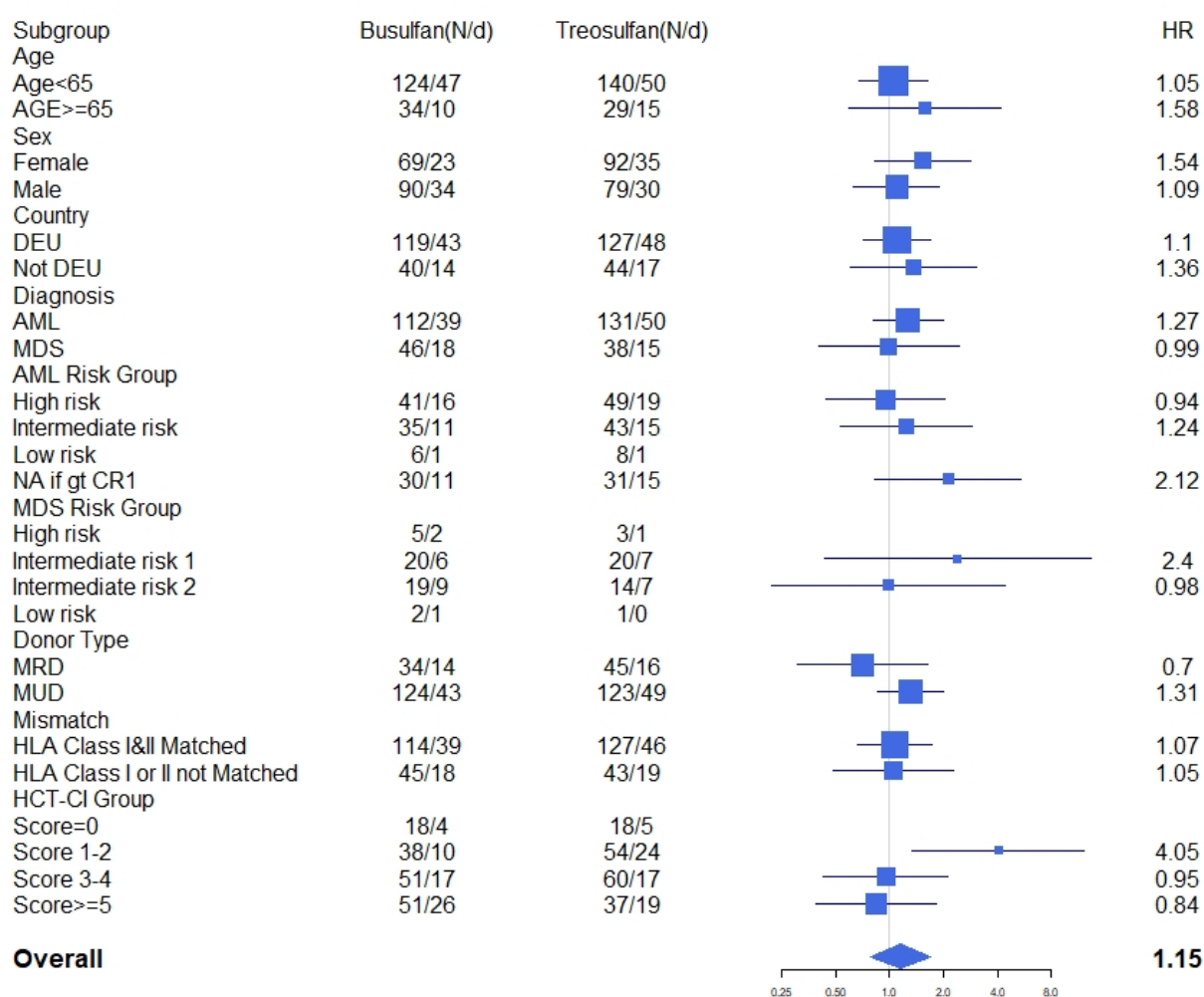
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HCT-CI Group			
Score=0	18/4	18/5	NA
Score 1-2	38/10	54/24	4.05(1.33,12.29)
Score 3-4	51/17	60/17	0.95(0.43,2.13)
Score>=5	51/26	37/19	0.84(0.39,1.83)
Overall			1.15(0.78,1.69)

Source: FDA Analysis

Figure 14. Trial 14-I Forest plot to show OS results in subgroups



Source: FDA Analysis

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8.1.3 Registry Studies

8.1.3.1 Retrospective EBMT-PD/IE-WP analysis:

Re-analysis of EBMT-registry data on Fludarabine/Melphalan and Busulfan/Cyclophosphamide based conditioning treatment compared to Fludarabine/Treosulfan based conditioning of adult AML and MDS patients treated in MC-FludT.14/L Trial II phase III by matched pairs.

Key study objectives were comparison of OS, relapse (RI), and NRM, all at 2 years after allo-HSCT. Comparison patients (N = 1493) received conditioning with either Flu-Mel or Bu/Cy without TBI. The MC-FludT.14/L Trial II cohort consisted of 252 patients, with patients < 50 years of age excluded from the analysis. The EBMT patient cohort had a lower HCT-CI score, higher Karnofsky score, lower recipient age, and higher donor age than the MC-FludT.14/L Trial II cohort. Four matched comparisons were performed as follows:

1. MDS: Flu/Mel vs Flu/Treo
2. MDS: Bu/Cy vs Flu/Treo
3. AML: Flu/Mel vs Flu/Treo
4. AML: Bu/Cy vs Flu/Treo

In MDS, Flu/Treo was statistically superior to Bu/Cy in OS. In MDS, no other comparisons were statistically significant. In AML, Flu/Treo was statistically superior to Flu/Mel and Bu/Cy in OS and NRM.

Multivariable sensitivity analyses were also performed. In MDS, Flu/Treo again was statistically superior to Bu/Cy, with no other analyses reaching significance. In AML, Flu/Treo was statistically superior to Flu/Mel and Bu/Cy in NRM and OS. Relapse incidence was comparable among groups.

Stat Reviewer's Comment: Patient level data were not submitted. Therefore, FDA could not independently verify the results.

8.1.3.2 CIBMTR SC17-04b

Comparison of allogeneic transplantation outcomes for acute myeloid leukemia and myelodysplastic syndrome with full or less intensity busulfan-fludarabine regimens and busulfan-cyclophosphamide regimen in the United States to a treosulfan-containing regimen in a medac-sponsored European clinical trial MC-FludT.14/L (Trial II).

The treosulfan “trial” population is identical to the population above in MC-FludT.14/L Trial II (N = 268). The comparison population included patients from 96 transplant centers. Conditioning regimens for this analysis included the following:

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RIC:

- Busulfan + fludarabine (Flu/Bu2) (N = 318)
- Flu/Bu + ATG (N = 199)

MAC

- Busulfan + fludarabine (Flu/Bu4) (N = 327)
- Flu/B4 + ATG (N = 169)
- Busulfan + cyclophosphamide (Cy/Bu4) (N = 317)

The primary endpoint for the analysis was 2-year OS. Secondary endpoints included 2-year DFS, 2-year EFS, relapse, TRM/NRM, and incidence of aGVHD and cGVHD. There were no differences in HCT-CI scores across the groups. Cy/Bu4 was more likely to be given to younger patients than the other regimens. OS, DFS, and EFS were higher for patients in MC-FludT.14/L Trial II than those in the CIBMTR database. This is likely due to higher relapse rates with the RIC and Flu/B4 + ATG regimens and higher TRM with the other MAC regimens. aGVHD did not differ between the groups. cGVHD was higher in the trial regimen when compared to the RIC and ATG-containing regimens but was comparable to the rate observed in the Cy/Bu4 population.

Stat Reviewer's Comment: Patient level data were not submitted. Therefore, FDA could not independently verify the results.

8.1.4 Additional Studies in AML or MDS

MC-FludT.6/L

Allogeneic hematopoietic stem cell transplantation in patients with advanced hematological malignancies after treosulfan-based conditioning therapy - A clinical phase II study

Study MC-FludT.6/L was a multicenter single-arm dose-escalation trial to test the tolerability of treosulfan and fludarabine as a preparative regimen for allogeneic HSCT from an HLA-matched sibling, 1 antigen-mismatched sibling, or HLA-matched unrelated donor. Eligible patients were ≥ 18 years old with a "chemosensitive" hematological malignancy indicated for HSCT but who were considered at high risk for toxicities with standard MAC regimens. Patients were treated in sequential cohorts (n=15 each) at treosulfan dose levels of 10 gm/m², 12 gm/m², or 14 gm/m² (see Table 12 for details of the regimen). The aim of the protocol was to determine a dose and schedule for later trials. The primary efficacy endpoint was hematopoietic recovery. Overall survival was an additional endpoint.

There were 55 patients accrued, including 19 with AML and 6 with MDS among the various dose cohorts. The median age was 50 years (range, 18 - 66 years); 60% were male and 40% were female. Table 34 shows the selected efficacy outcomes by dose as reported by the Applicant.

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Table 34. Study MC-FludT.6/L: Selected Efficacy Outcomes

Outcome	Percentage of Patients* with Outcome by Treosulfan Dose Used in Combination with Fludarabine		
	10 gm/m ² (N=20)	12 gm/m ² (N=18)	14 gm/m ² (N=17)
Day-28 ANC > 0.5 Gi/L	100%	100%	94%
Day-28 platelets > 50 Gi/L	70%	94%	65%
No secondary graft failure	100%	100%	100%
Day-100 complete chimerism	95%	94%	93%
1-year overall survival	70%	78%	59%

Source: Study MC-FludT.6/L Clinical Study Report Tables 11.4.1.1C, 11.4.1.1E, 11.4.1.1A, 11.4.1.2A and Figures 11.4.1.4A and 11.4.1.6A.

*Percentage of patients with available data.

The Applicant concluded that efficacy and safety of the combination of treosulfan and fludarabine were comparable at all 3 treosulfan dose levels.

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

Study MC-FludT.7/AML was a multicenter single-arm trial to test treosulfan and fludarabine as a preparative regimen for allogeneic HSCT from an HLA-matched sibling or HLA-matched unrelated donor. Eligible patients were 18-60 years old with AML indicated for HSCT. The preparative regimen consisted of treosulfan at a dose level of 14 gm/m² in combination with fludarabine (see Table 7 for details of the regimen). The primary objective was "evaluation of engraftment". Overall survival was an additional endpoint. The target accrual was 75 patients. The sample size was calculated to allow for at least 65 patients eligible to assess the safety outcomes in comparison to historical data using busulfan. Seventy-five patients were enrolled. The median age was 45 years (range, 19 - 59 years); 51% were male and 49% were female. At study entry, 80% were in first CR and the remainder in later CR. The Applicant reported that neutrophil recovery at Day 28 was achieved by 93%, complete chimerism at Day 100 was achieved by 92%, Day-100 nonrelapse mortality was 3%, and overall survival at 1 year was 77% (Study MC-FludT.7/AML Clinical Study Report Tables 11.4.1.1A, 11.4.1.2A, 14.2.4A, and 14.2.6A). The Applicant concluded that the regimen "has myeloablative potential as demonstrated by the fast and durable eradication of the physiologic recipient haematopoiesis. Long-term outcome data are in good accordance with data from the literature on AML transplantation after standard or myeloablative conditioning. " (Study MC-FludT.7/AML Clinical Study Report Section 13).

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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)

Study MC-FludT.8/MDS was a multicenter single-arm trial to test treosulfan and fludarabine as a preparative regimen for allogeneic HSCT from an HLA-matched sibling or HLA-matched unrelated donor. Eligible patients were 18-60 years old with MDS indicated for HSCT. The preparative regimen consisted of treosulfan at a dose level of 14 gm/m² in combination with fludarabine (see Table 7 for details of the regimen). The primary objective was "evaluation of engraftment". Overall survival was an additional endpoint. The target accrual was 45 patients. The sample size was calculated to allow for assessment of safety outcomes in comparison to historical data using busulfan. Forty-six patients were enrolled; one patient was subsequently diagnosed as AML and excluded from the outcome analyses. The median age was 50 years (range, 22 - 63 years); 47% were male and 53% were female. At study entry, 47% had excess blasts. The Applicant reported that neutrophil recovery at Day 28 was achieved by 96%, complete chimerism at Day 100 was achieved by 93%, Day-100 nonrelapse mortality was 9%, and overall survival at 1 year was 82% (Study MC-FludT.8/MDS Clinical Study Report Tables 11.4.1.1A, 11.4.1.2A, 14.2.4A, and 14.2.6A). A response assessment using the standard response criteria was not provided. The Applicant concluded that the regimen "has myeloablative potential as demonstrated by the fast and durable eradication of the physiologic recipient haematopoiesis. Long-term outcome data seemed to be in favour of this new regimen when compared with data from the literature." (Study MC-FludT.8/MDS Clinical Study Report Section 13).

MC-FludT.17/M

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 MC-FludT.17/M was a multicenter single-arm trial to test treosulfan and fludarabine as a preparative regimen for allogeneic HSCT from an HLA-matched or 1-allele mismatched related or unrelated donors. Eligible patients were 28 days to < 18 years old with ALL, AML, MDS or JMML indicated for HSCT. The preparative regimen consisted of BSA-adapted treosulfan dosing equivalent to exposure from 14 gm/m² in adults in combination with fludarabine and thiopeta (see Table 7 for details of the regimen). The primary objective was to estimate freedom from transplant-related mortality at Day 100. Overall survival was an additional endpoint. The target accrual was 70 patients. The sample size was calculated to allow a descriptive analysis of hematopoietic recovery, toxicity and PK parameters as part of an agreed PIP. Seventy patients were included in the analyses. The median age was 9.5 years (range, < 1 - 17 years); 63% were

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male and 37% were female. There were 29 patients with AML and 10 with MDS. (b) (4)

(Study MC-FludT.17/M Clinical Study Report Tables 14.2.1A and 14.2.3A). The Applicant concluded that this regimen "can successfully be used as conditioning treatment before allo-HSCT in the selected paediatric population with malignant haematological diseases." (Study MC-FludT.17/M Clinical Study Report Section 13).

8.2 Integrated Review of Effectiveness

8.2.1 Assessment of Efficacy Across Trials

Methods

The Applicant proposed an indication for treosulfan "as part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS)." The proposed dose of treosulfan was of 10 g/m²/dose for use in the combination described. (b) (4)

The clinical development program included two randomized control trials, three single-arm trials and two registry studies (Table 35). All clinical trials were conducted outside the US.

Table 35. Clinical Studies Submitted to Demonstrate the Effectiveness of Treosulfan in Combination with Fludarabine as a Preparative Regimen for Patients with AML or MDS

Trial	Design	Population	Treosulfan Dose	Contribution
MC-FLudT-14/L Trial II	Randomized Phase 3	Adults*- AML, MDS	10 g/m ² /dose	Pivotal Trial
MC-FLudT-14/L Trial I	Randomized Phase 3	Adults*- AML, MDS	14 g/m ² /dose	Pivotal Trial
MC-FludT.7/AML	Single-arm Phase 2	Adults- AML	14 g/m ² /dose	Dose selection
MC-FludT.8/MDS	Single-arm Phase 2	Adults- MDS	14 g/m ² /dose	Dose selection
MC-FludT.17/M	Single-arm Phase 2	Children - Heme cancers	14 g/m ² /dose	Dose selection
MC-FludT.6/L	Dose-escalation	Adults- Heme cancers	10-14 g/m ² /dose	Dose selection
EBMT 2019	Registry Study	Age 50-70 yrs; AML, MDS	10 g/m ² /dose	Supporting
CIBMTR 2019	Registry Study	Adults*- AML, MDS	10 g/m ² /dose	Supporting

Source: FDA analysis

*Specifically age ≥ 50 years or with an HCT-CI > 2

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There were several issues identified with the elements of the clinical development program:

- Patient level data were not submitted for the registry studies. Therefore, FDA cannot independently verify the results. As such, the registry studies do not contribute to the determination of the effectiveness of treosulfan.
- EFS and OS are the acceptable endpoints for approval of a drug for use in a preparative regimen for allogeneic HSCT for patients with AML or MDS. Since Studies MC-FludT.7/AML and MC-FludT.8/MDS are single-arm trials, they were not designed adequately to assess the time-to-event endpoints of EFS and OS. As such, Studies MC-FludT.7/AML and MC-FludT.8/MDS do not contribute to the determination of the effectiveness of treosulfan.
- Similarly, the single-arm trial Study MC-FludT.17/M is not designed adequately to assess EFS or OS and would not support an indication in the pediatric patients with AML or MDS.
- Studies MC-FludT.14/L Trial I (Trial 14 - I; treosulfan 14 g/m²/dose) was failed trial. The study was stopped early at the first interim analysis which reported an EFS HR=1.23 (95% CI 0.84, 1.80) and an OS HR=1.30 (0.85, 2.00) in the Full Analysis Set. The trial was stopped early because of a safety concern.

The Applicant proposed to use Study MC-FludT.14/L Trial II (Trial 14 - II; treosulfan 10 g/m²/dose) to support the proposed indication. Trial 14 - II was a multicenter, randomized, active-controlled trial comparing treosulfan to busulfan in combination with fludarabine as a preparative regimen for allogeneic HSCT. Eligible patients included adults 18 to 70 years old with AML or MDS, Karnofsky performance status > 60%, and age ≥ 50 years or hematopoietic cell transplantation comorbidity index [HCT CI] score > 2. The patients were randomized to receive treosulfan 10 g/m² daily on days -4, -3 and -2 or to busulfan 0.8 mg/kg every 6 hours on days -4 and -3 in combination with fludarabine 30 mg/m² daily on days -6, -5, -4, -3 and -2, and hematopoietic stem cell transplantation on day 0. Antithymocyte globulin was administered as well to patients with unrelated donors.

In general, a randomized controlled trial with a survival-based endpoint is appropriate to support an indication for a drug in a preparative regimen for allogeneic HSCT. FDA frequently requires two trials to determine efficacy, but one trial might be sufficient if the trial is adequately-designed and well-conducted, and the results are internally consistent, clinically meaningful, and statistically very persuasive. Potential issues with the design of Trial 14 - II in particular included:

- Trial 14 - II was designed as a noninferiority trial with a primary endpoint of EFS within 2 years of follow-up. FDA was not involved in the review of this protocol prior to conduct of the study, and there was no pre-existing agreement on an acceptable noninferiority margin in this setting.

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- For Trial 14 - II, EFS was defined as the interval from Transplant Day 0 to relapse, graft failure or death (whatever occurs first); patients alive without an event were censored at 2 years from HSCT.
 - For patients with AML transplanted in remission, relapse-free survival (RFS) would be the usual endpoint to assess efficacy.
 - For patients with MDS transplanted not in remission, EFS might be an acceptable endpoint if defined to reflect the need to achieve remission.
- OS was a secondary endpoint of Trial 14 - II. The analysis of OS was planned as exploratory; the timing of the OS analysis was not event-driven, and there was no prespecified alpha-spending plan to correct for multiplicity.
- In Trial 14 - II, the efficacy analyses were planned to be conducted in the subgroup of patients who were randomized, received at least one dose of study drug, and had at least one efficacy assessment postbaseline (Full Analysis Set; FAS) and in a subgroup of the FAS without selected protocol deviations (Per Protocol Set; PPS). However, for a randomized trial, the ITT population is the preferred analysis set.
- Trial 14 - II compared fludarabine in combination with 3 days of treatment with treosulfan to a combination with 2 days of busulfan. Any conclusions regarding superiority would need to take into account the difference between arms in the number of days of treatment with treosulfan vs the number of days of treatment with busulfan.
- AML and MDS are different diseases, so any trial used to support both indications would need to test for efficacy in each individually. Subgroup analyses of efficacy by diagnosis were prespecified in Trial 14 - II.
- Due to differences in minor and other unmeasured major antigens, HLA-matched unrelated donors have a higher degree of histoincompatibility with the recipient than HLA-identical sibling donors, so the minimum intensity of the preparative regimen needed for successful HSCT may differ by donor type. Any trial used to support an indication using both related and unrelated donors would need to test for efficacy by each individually. Subgroup analyses of efficacy by donor type were prespecified in Trial 14 - II.
- Additionally, in Trial 14 - II, patients with unrelated donors also received ATG in the preparative regimen. ATG dosing varied by geographic region. Any conclusions related to the subgroup analysis by donor would need to take into consideration the differences in treatment regimens by donor type.

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Primary Endpoint

Event-Free Survival

For Trial 14 - II, the Applicant's last analysis of EFS for submission was the last analysis at 234 events (total 48.6% information level). At the prespecified second interim analysis (164 events, 34.1% information level), the Data Monitoring Committee (DMC) recommended to stop recruitment because non-inferiority was achieved. Recruitment continued until interim analysis results became available for the DMC. Therefore, the last analysis had more events than the second interim analysis. The Applicant provided results for the full follow-up period with median follow-up of 29.7 months in the treosulfan arm and 29.4 months in the busulfan arm. The HR for EFS was 0.64 (0.48, 0.87) in the PPS and 0.64 (0.48, 0.87) in the FAS. The Applicant concluded that the results supported noninferiority, and in sequential testing, superiority of treosulfan.

However, the Applicant did not submit all CBC and bone marrow results, so FDA was unable to verify the primary endpoint independently and could not confirm that this was a positive trial. Although, in general, FDA does not accept clinical trials for which the primary endpoint cannot be confirmed, for this submission the Division Director indicated that the OS endpoint would be an acceptable alternative for the assessment of efficacy.¹

Secondary and Other Endpoints

Overall Survival

For Trial 14 - II, the analysis of OS was considered exploratory. FDA assessed OS in the ITT population with using the full follow-up period. Table 36 shows the results of FDA's analyses of OS with a median follow-up of 29.5 months (range, 0 - 54.8 months). No p-value is shown, as this analysis was exploratory.

Table 36. Trial 14 - II: FDA Analyses of OS

Population	Treosulfan Arm (3 days of dosing) (N)	Busulfan Arm (2 days of dosing) (N)	HR* (95% CI)
ITT	280	290	0.65 (0.48, 0.88)
AML	192	173	0.62 (0.41, 0.92)
MDS	88	117	0.62 (0.34, 1.10)

Source: FDA analysis

* Stratified by risk group, donor type and study center

The HR and upper bound of the 95% CI for the patients with AML, without any multiplicity

¹ NDA 214759 Division Director Filing Review dated 10/7/2020.

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adjustment, reflect a better estimated survival for the treosulfan regimen over the busulfan regimen for this indication.

The OS HR and 95% CI for the patients with MDS failed to demonstrate superiority for treosulfan, and the lack of superiority is supported by the Applicant's analysis in the FAS (HR 0.63; 0.37; 1.10).

Additional Efficacy Endpoints

The Applicant prespecified several secondary endpoints for Trial 14 - II, described in 8.1.1, but most of these were safety endpoints and none was suitable to use in support of the OS demonstrating effectiveness for treatment of AML or MDS.

For treatment of **AML in remission**, RFS would be a suitable endpoint to support a finding of superiority in OS. Because CBC and marrow examination data were not collected systematically in Trial 14 - II, RFS cannot be assessed, and there are no secondary endpoints to corroborate the OS outcome for the patients with AML.

For treatment of **MDS not in remission**, EFS (defined suitably) or CR that is durable might be suitable to assess activity. Because CBC and marrow examination data were not collected systematically in this protocol, EFS and CR cannot be assessed, and there are no secondary endpoints to corroborate an efficacy outcome for the patients with MDS.

Subpopulations

Exploratory subpopulations prespecified for analysis of OS in Trial 14 - II included donor type, risk group, combination of donor type and risk group, disease (AML and MDS), age group (< 50 years vs. ≥ 50 years), HCT-CI Score (≤ 2 vs. > 2), remission status in AML (CR1 vs. > CR1), disease status at study entry in MDS (untreated vs. treated), risk group within AML patients, and risk group within MDS patients. Race and ethnicity were not included in the subpopulation analyses as data on race and ethnicity were not collected. This deficiency leaves some uncertainty about the generalizability of the efficacy outcomes with respect to the US population.

Table 22 in Section 8.1.1 shows the results of the FDA subpopulation analysis of OS for Trial 14 - II. In general, where the subgroups were large enough for credible analyses, the results demonstrated consistency in the subpopulations with the result in the ITT population overall. The exception was the subpopulation by donor type. Table 37 shows the results of FDA's analysis of OS by donor for the ITT population and in patients with AML or with MDS separately.

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Table 37. Trial 14 - II: FDA Analyses of OS by Donor Type and Disease

Population/Disease	Treosulfan vs Busulfan HR* (95% CI) for OS by Donor Type	
	Related Donor	Unrelated Donor
ITT	HR 1.002 (0.55, 1.84)	HR 0.56 (0.48, 0.88)
AML	HR 0.97 (0.46, 2.03)	HR 0.51 (0.31, 0.82)
MDS	HR 0.48 (0.07, 2.37)	HR 0.65 (0.34, 1.18)

Source: FDA analysis

*Stratified by risk group and study center

The results in Table 37 show that the treatment effect appears to be driven by the subpopulation of patients with unrelated donors (HR 0.56), and the treatment effect appears to be consistent with patients with unrelated donors whether they have AML (HR 0.51) or MDS (HR 0.65). For the patients with related donors, the treatment effect is much smaller or negative than for those with related donors, and the HR is inconsistent across the disease subgroups, although the number of patients with MDS and related donors is small. Of note, as shown in Appendix Figure 16.5.1, within a disease population, the busulfan arms appear to have similar OS (e.g., 16.5.1 A vs B red lines for patients with AML), but for either disease, the OS appears to be worse with treosulfan for patients with related donors in comparison to those with unrelated donors. The biological basis for this observed difference is unclear; it may be related to the use of ATG that was limited to those with unrelated donors, or to some unmeasured genetic element in the older related donors. Additional information is needed to assess this potential lack of effectiveness.

Dose/Dose Response

The Applicant reported that a) for autologous HSCT, the single-dose MTD of treosulfan was 47 gm/m², and b) in an investigator-initiated pilot study of treosulfan 10 g/m²/day x 3 in combination with fludarabine was tolerable as a preparative regimen for allogeneic HSCT and produced a high rate of complete donor chimerism (Study MC-FludT.6/L Clinical Study Report Section 9.4.4). For Study MC-FludT.6/L, the dose-escalation trial for development of the proposed preparative regimen, the Applicant studied treosulfan doses 10, 12 and 14 g/m²/day x 3 in combination with fludarabine. As discussed in Section 8.1.4, hematopoietic recovery and high-grade donor chimerism were achieved at all dose levels, and the 1-year OS was worst in the 14 g/m²/day x 3 cohort. Nonetheless, the Applicant chose the 14 g/m²/day x 3 regimen for the initial pivotal trial, MC-FludT.14/L Trial I (Trial 14 - I).

As discussed in Sections 8.1.2 and 8.3.5, Trial 14 - I was terminated early due to delayed hematopoietic recovery and increase early treatment-related mortality. FDA's analysis of the final data cut (1-year follow-up) showed no evidence of a treatment effect for the overall population or when assessed individually for patients with AML or with MDS. The second pivotal trial, Trial 14 - II, commenced using the lower treosulfan dose of 10 g/m²/day x 3.

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Table 38 shows the results of selected efficacy outcomes in ITT population of Trial 14 - I and Trial 14 - II.

Table 38. Analyses of OS vs Busulfan by Treosulfan Dose

Outcome	Population	Treosulfan Dose	
		Trial 14 - I 14 g/m ² /day x 3 days	Trial 14 - II 10 g/m ² /day x 3 days
Hazard ratio for OS (Treosulfan vs busulfan)	ITT	1.15 (0.78, 1.69)	0.65 (0.48, 0.88)
	AML	1.27 (0.81, 2.01)	0.62 (0.41, 0.92)
	MDS	0.99 (0.40, 2.45)	0.62 (0.34, 1.10)
OS at 1 year (Treosulfan arm vs busulfan arm)	ITT	67% vs 75%	79% vs 72%
	AML	66% vs 76%	81% vs 74%
	MDS	68% vs 70%	75% vs 74%

Source: FDA analysis

The results suggest that the effectiveness for the treosulfan dose of 10 g/m²/day x 3 days in comparison to busulfan x 2 days is better than that for the treosulfan dose of 14 g/m²/day x 3 days for the ITT population and for patients with either AML or MDS.

Additional Efficacy Considerations

Extrapolation to Pediatric Patients

(b) (4)

MC-FludT.16/NM was a phase 2 study for nonmalignant diseases that randomized patients to a 14 g/m²/dose equivalent of treosulfan or standard busulfan. Because this was not performed in a disease that is being considered for an indication for treosulfan, it was not evaluated for efficacy. The second study, MC-FludT.17/M, was a nonrandomized study for pediatric patients with malignant diseases; all patients received a 14 g/m²/dose equivalent of treosulfan. (b) (4)

8.2.2 Integrated Assessment of Effectiveness

In the absence of verification of the primary endpoint, and without prespecification of Type-1 error for multiple comparisons, Study 14-II does not provide considerable evidence of effectiveness.

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8.3 Review of Safety

8.3.1 Safety Review Approach

The FDA reviewed the safety data for 1,206 patients in clinical trials (Table 39). These included 728 patients treated with treosulfan and 478 treated with busulfan (active control). FDA utilized the following cohorts for this safety review:

- Cohort 1: The 270 patients treated with treosulfan in MC-FludT.14/L Trial II were used to assess the safety profile of treosulfan in the intended population, as compared to the standard RIC of busulfan + fludarabine.
- Cohort 2: The 168 patients treated with treosulfan in MC-FludT.14/L Trial I were used to assess potential safety signals at the treosulfan dose of 14 g/m², as compared to the standard RIC of busulfan + fludarabine.
- Cohort 3: The 290 patients treated with treosulfan at 10 g/m² (MC-FludT.6/L and MC-FludT.14/L Trial II) and the 305 patients treated with treosulfan at 14 g/m² (MC-FludT.6/L, MC-FludT.7/AML, MC-FludT.8/MDS, and MC-FludT.14/L Trial I) were compared to assess differences in dose-related toxicity.
- Cohort 4: The 158 pediatric patients (treosulfan, N = 115; busulfan, N = 43) enrolled in MC-FludT.16/NM and MC-FludT.17/M were assessed for safety signals within the pediatric population. The 12 pediatric patients who received treosulfan + fludarabine (without thiotepea) were compared to the 10 g/m² population from Cohort 3.

All analyses were performed using the ISS datasets (12/11/2020, SDN 0017).

Table 39. Safety Database and Dosing Schema

			Treosulfan (dose)			Busulfan
Study	Treosulfan-Fludarabine	Thiotepa	10 g/m²	12 g/m²	14 g/m²	N/A
MC-FludT.6/L	Yes	Without	20	18	17	0
MC-FludT.7/AML	Yes	Without	0	0	75	0
MC-FludT.8/MDS	Yes	Without	0	0	45	0
MC-FludT.14/L I	Yes	Without	0	0	168	152
MC-FludT.14/L II	Yes	Without	270	0	0	283
Total			290	18	305	435
MC-FludT.16/NM	Yes	With Thiotepa	4	25	9	37
		Without Thiotepa	5	1	1	6
MC-FludT.17/M	Yes	With Thiotepa	5	23	37	0
		Without Thiotepa	1	3	1	0
Total			15	52	48	43
Source: Reviewer's Analysis, ISS ADSL dataset						

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Treosulfan is an alkylating agent, and it is related to busulfan. The common toxicities for busulfan in the setting of allo-HSCT are myelosuppression, hepatitis veno-occlusive disease (VOD), nausea, vomiting, stomatitis, diarrhea, fever, abdominal pain, anxiety, anorexia, insomnia, and electrolyte abnormalities. Of note, busulfan has an increased risk for seizures, which has not been identified for treosulfan.

As treosulfan is closely related to busulfan, and is thought to act as an alkylating agent, the known safety profile for busulfan was vital for this analysis.

Animal toxicity studies revealed that the target organ of treosulfan is the bone marrow, with leukopenia, neutropenia, reticulocytopenia, thrombocytopenia, and lymphopenia observed in the repeat-dose toxicity studies in rats, dogs, and monkeys. There was no evidence of dose-limiting functional or structural changes to the liver, kidneys, heart, lungs, or central nervous system.

The main safety signal that arose during the development program occurred during MC-FludT.14/L Trial I (treosulfan dose 14 g/m²). Because of higher than anticipated early morbidity and mortality in the treosulfan arm of the study, the study was paused. An amendment was submitted, and the protocol reopened with a lower dose of treosulfan (MC-FludT.14/L Trial II, treosulfan dose 10 g/m²).

8.3.2 Review of the Safety Database

Overall Exposure

Overall exposure of all patients who were treated in the development plan is demonstrated in Table 40.

Table 40. Treosulfan or Busulfan Regimens in the Safety Population

Treatment	Day -7	-6	-5	-4	-3	-2	-1	0
Treosulfan (IV, 10 g/m ²). Total dose = 30 g/m ² . (N = 270 in MC-FludT.14/L II (X); N = 35 in MC-FludT.6/L, MC-FludT.16/NM, and MC-FludT.17/NM (X*))		X*	X*	X*				
Treosulfan (IV 12 g/m ²). Total dose = 36 g/m ² . (N = 70 in MC-FludT.6/L, MC-FludT.16/NM, and MC-FludT.17/M)		X	X	X				
Treosulfan (IV, 14 g/m ²). Total dose = 42 g/m ² . (N = 353 in MC-FludT.6/L, MC-FludT.7/AML, MC-FludT.8/MDS, MC-FludT.14/L I, MC-FludT.16/NM, and MC-FludT.17/M)		X	X	X				
Busulfan (IV, 0.8 mg/kg) . Total dose = 1.6 mg/kg – 3.2 mg/kg. (N = 435 in MC-FludT.14/L Trials I and II (X). N = 43 in MC-FludT.16/NM and MC-FludT.17/M (X*)).	X*	X*	X*	X*				
Source: Clinical Study Reports, Clinical Trial Protocols								

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Relevant characteristics of the safety population

The demographics of the safety population for the cohorts 1, 2, and 3 are shown in Table 41. In general, more male patients than female patients were enrolled on these. Nearly all of the patients in these cohorts were in the 18-64 year age group, with 20% of patients in the 65-74 year age group, and no patients age 75 or greater.

Table 41. Key Characteristics of Safety Cohorts 1, 2, and 3

Study ID	MC-FludT.6/L	MC-FludT.7/AML	MC-FludT.8/MDS	MC-FludT.14/L Trial I	MC-FludT.14/L Trial II	All
	N = 55	N = 75	N = 45	N = 320	N = 553	N = 1048
Sex	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Female	22 (40%)	37 (49%)	24 (53%)	159 (50%)	217 (39%)	459 (44%)
Male	33 (60%)	38 (51%)	21 (47%)	161 (50%)	336 (61%)	589 (56%)
Age Group						
18-64 years	53 (96%)	75 (100%)	45 (100%)	258 (81%)	412 (75%)	843 (80%)
65-74 years	2 (4%)	0 (0%)	0 (0%)	62 (19%)	141 (25%)	205 (20%)
>75 years	0 (%)	0 (%)	0 (%)	0 (%)	0 (%)	0 (%)
Treatment Arm						
Busulfan	0 (%)	0 (%)	0 (%)	152 (48%)	283 (51%)	435 (42%)
Treosulfan	55 (100%)	75 (100%)	45 (100%)	168 (52%)	270 (49%)	613 (58%)
Dose of Treosulfan (g/m²)						
10	20 (36%)	0 (%)	0 (%)	0 (%)	270 (100%)	290 (47%)
12	18 (33%)	0 (%)	0 (%)	0 (%)	0 (%)	18 (3%)
14	17 (31%)	75 (100%)	45 (100%)	168 (100%)	0 (%)	305 (50%)

Source: ISS ADSL dataset. Reviewer's analysis.

The key characteristics of cohort 4 (pediatric population) is shown in Table 42. More male patients than female patients were enrolled on MC-FludT.16/NM and MC-FludT.17/M. Most patients were in either the 2-11 year or 12-16 year age groups. Five patients were 17 or older and are analyzed separately, but they are not included with the adult patients above because of differences in the preparative regimen. Data on race were collected with the pediatric studies, with over 90% of the patients identifying as "White." Most pediatric patients received thiotepla, in addition to fludarabine and either busulfan or treosulfan as part of their preparative regimen and are excluded from comparison with cohort 1.

Table 42. Key Characteristics of Safety Cohort 4

	MC-FludT.16/NM	MC-FludT.17/M	All
	N = 88	N = 70	N = 158
Sex	N (%)	N (%)	N (%)
Female	28 (32%)	26 (37%)	54 (34%)
Male	60 (68%)	44 (63%)	104 (66%)
Age Group			
28 days to 23 months	26 (30%)	9 (13%)	35 (22%)
2 to 11 years	46 (52%)	28 (40%)	74 (47%)
12 to 16 years	14 (16%)	30 (43%)	44 (28%)

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≥ 17 years	2 (2%)	3 (4%)	5 (3%)
Race			
Asian	6 (7%)	0 (0%)	6 (4%)
Black or African-American	3 (3%)	0 (0%)	3 (2%)
Other	4 (5%)	0 (0%)	4 (3%)
White	75 (85%)	70 (100%)	145 (92%)
Treatment Arm			
Busulfan	43 (49%)	0 (0%)	43 (27%)
Treosulfan	45 (51%)	70 (100%)	115 (73%)
Dose of Treosulfan (g/m²)			
10	9 (20%)	6 (9%)	15 (13%)
12	26 (58%)	26 (37%)	52 (45%)
14	10 (22%)	38 (54%)	48 (42%)
Thiotepa			
With Thiotepa	75 (85%)	65 (93%)	140 (89%)
Without Thiotepa	13 (15%)	5 (7%)	18 (11%)
<i>Source: ISS ADSL dataset; Reviewer's Analysis</i>			

Adequacy of the Safety Database

The safety database was assessed for adequacy with the following conclusions:

- Because data on race and ethnicity were not collected, the safety database was not adequate to extrapolate to the demographics of the United States population.
- There were sufficient patient numbers to assess for rare adverse reactions.
- There were not sufficient patients in the “≥75 years of age” age group to assess safety signals in that population.
- There were sufficient patients to evaluate differences by sex.
- There were sufficient patients treated at the 10 and 14 g/m² dose levels to assess for a dose-toxicity relationship.

8.3.3 Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

The doses of concomitant medications were not provided in cm.xpt or adcm.xpt. Table 43 shows the duration of follow-up for mortality, SAE reporting, TEAE reporting, and laboratory testing for each of the studies included in the ISS. MC-FludT.6/L, which was the dose finding Phase 2 study, had the longest follow-up for SAE, TEAE, and safety labs. Additionally, Cohort 4 had longer SAE and SAR follow-up than the other studies included in Cohorts 1, 2, and 3.

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Table 43. ISS Population: Planned Duration of Follow-up for Safety Data by Protocol

Protocol	Duration of follow-up for mortality	Duration of follow-up for SAE reporting	Duration of follow-up for TEAE	Duration of follow-up for safety lab testing
MC-FludT.6/L	24 months	24 months	6 months	6 months
MC-FludT.7/AML	1 year after last patient included: Median 715 days	SAEs – 28 days; SARs – 1 year after last patient included	28 days	100 days
MC-FludT.8/MDS	1 year after last patient included: Median 780 days	SAEs – 28 days; SARs – 1 year after last patient included	28 days	100 days
MC-FludT.14/L Trial I	1 year after last patient included: Median 22-24 months	SAEs – 28 days; SARs – 1 year after last patient included	28 days	28 days (except CBC: 100 days)
MC-FludT.14/L Trial II	1 year after last patient included: Median 29 months	SAEs – 28 days; SARs – 1 year after last patient included	28 days	28 days (except CBC: 100 days)
MC-FludT.16/NM	Until the last subject completed Visit Month 36; Current Median FU: 12 months	SAEs – 100 days SARs – 3 years after last patient recruited.	100 days	28 days (except CBC: 100 days)
MC-FludT.17/M	Until the last subject completed Visit Month 36; Median Follow-up: 42 months	SAEs – 100 days SARs – 3 years after last patient recruited.	100 days	28 days (except CBC: 100 days)

Source: Adapted from Applicant's 4/28/2021 response to Information Request

Categorization of Adverse Events

Adverse events were reported down to the verbatim term. The adverse events were coded using MedDRA version 20.0 for all studies and for the pooled safety population. Where indicated in the tables or text, some adverse events are presented as grouped terms (Table 44). Regimen-related toxicity (RRT) is defined as directly caused by the conditioning regimen. Bearman's RRT criteria are used unless otherwise specified. Treatment-emergent adverse events (TEAEs) included events that started from the start of study drug administration through HSCT day +30, unless otherwise indicated.

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Table 44. Grouped Terms used in Safety Analyses

Grouped Term	Basis of Grouping
Bacterial infection	HLGT Bacterial infectious disorders
Fungal infection	HLGT Fungal infectious disorders
Infection	HLGT Infections - pathogen unspecified
Viral infection	HLGT Viral infectious disorders
Fatigue	HLT Asthenic conditions
Dyspnoea	HLT Breathing abnormalities
Diarrhoea	HLT Colitis (excl infective)
Diarrhoea	HLT Diarrhoea (excl infective)
Rash	HLT Erythemas
Abdominal pain	HLT Gastrointestinal and abdominal pains (excl oral and throat)
Oedema	HLT Oedema NEC
Rash	HLT Rashes, eruptions and exanthems NEC
Renal impairment	HLT Renal failure and impairment
Renal impairment	HLT Renal function analyses
Stomatitis	HLT Stomatitis and ulceration
Cystitis noninfective	PT Custom Cystitis noninfective - Cystitis haemorrhagic, Cystitis noninfective, Haematuria
Encephalopathy	PT Custom Encephalopathy - Agitation, Aphasia, Ataxia, Cognitive disorder, Confusional state, Delirium, Depressed level of consciousness, Disturbance in attention, Encephalopathy, Epilepsy, Hallucination, Lethargy, Memory impairment, Posterior reversible encephalopathy syndrome, Restlessness, Seizure, Somnolence
Hepatotoxicity	PT Custom Hepatotoxicity - Blood bilirubin increased, Hepatic failure, Hepatic pain, Hepatocellular injury, Hepatomegaly, Hepatorenal syndrome, Hepatosplenomegaly, Hepatotoxicity, Jaundice, Ocular icterus, Veno-occlusive liver disease
Hypersensitivity	PT Custom Hypersensitivity - Anaphylactic reaction, Hypersensitivity
Musculoskeletal pain	PT Custom Musculoskeletal pain - Arthralgia, Back pain, Bone pain, Flank pain, Limb discomfort, Musculoskeletal chest pain, Myalgia, Neck pain, Non-cardiac chest pain, Pain in extremity, Pain in jaw, Spinal pain
Tachycardia	PT Custom Tachycardia - Atrial fibrillation, Atrial flutter, Atrial tachycardia, Sinus tachycardia, Supraventricular tachycardia, Tachycardia
Cardiac failure	SMQB Cardiac failure excluding oedema
Haemorrhage	SMQB Haemorrhage terms (excl laboratory terms)
Embolism	SMQN Embolic and thrombotic events excluding VOD
<i>Source: Standard Grouped Terms, Reviewer's Grouped Terms</i>	

Routine Clinical Tests

See 8.1.1 and 8.1.2 for descriptions of the frequency of clinical assessments for studies MC-FludT.14/L Trials I and II. The frequency of clinical assessments is adequate to assess the risks of serious safety signals identified by HSCT day +30. There are late RRTs associated with HSCT that occur rarely but can be serious; other than graft versus host disease (GVHD), these were not captured reliably in the pivotal study MC-FludT.14/L Trial II (Cohort 1).

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8.3.4 Safety Results

Deaths

In the full safety database, there were 14 deaths within 30 days of the last dose of treosulfan and 57 deaths (43 additional deaths) within 100 days of the last dose of treosulfan (Table 45).

Table 45. Deaths in the ISS through HSCT Day +30 and Day +100

Study	Population	Treatment	Number (N) Treated	Death within 30 days of HSCT		Death within 100 days of HSCT	
				N	%	N	%
FludT.6/L	Hematologic malignancies (adults)	Treosulfan: 10 g/m ²	20	0	0%	2	10%
		Treosulfan: 12 g/m ²	18	1	6%	2	11%
		Treosulfan: 14 g/m ²	17	0	0%	4	24%
FludT.7/AML	AML (adults)	Treosulfan: 14 g/m ²	75	0	0%	5	7%
FludT.8/MDS	MDS (adults)	Treosulfan: 14 g/m ²	45	0	0%	4	9%
FludT.14/L I	AML or MDS (adults)	Treosulfan: 14 g/m ²	168	7	4%	18	15%
		Busulfan: 0.8 mg/kg	152	0	0%	13	9%
FludT.14/L II	AML or MDS (adults)	Treosulfan: 10 g/m ²	270	5	2%	17	6%
		Busulfan: 0.8 mg/kg	283	2	1%	19	7%
FludT.16/NM	Nonmalignant disorders (children)	Treosulfan: 10-14 g/m ²	45	0	0%	0	0%
		Busulfan: 0.8 mg/kg	43	0	0%	4	9%
FludT.17/M	Hematologic malignancies (children)	Treosulfan: 10-14 g/m ²	70	1	1%	1	1%

Source: Reviewer's Analysis, ISS ADSL dataset.

Deaths from Cohort 1 were assessed and causes of death were adjudicated (Table 46). In MC-FludT.14/L Trial II, there were 4 deaths (1.5%) from fatal adverse reactions due to treosulfan by Day +30; there were two additional deaths that were adjudicated as transplant-related mortality. In the busulfan arm, there were two deaths prior to Day +30, both of which were adjudicated as adverse reactions due to busulfan. In the treosulfan arm, there were 17 deaths (6%) by transplant day +100, compared to 19 deaths (7%) by transplant day + 100 in the busulfan arm. However, we note that for six patients, we did not have sufficient information to adjudicate the cause of death.

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Grafapex (treosulfan)

Table 46. Causes of Death, MC-FludT.14/L Trial II through HSCT Day+100

Subject	Transplant day of death	Treatment Arm	Applicant Cause of Death	FDA Cause of Death
(b) (6)	+13	Treosulfan	TRM – Hemorrhage, Renal Failure, Infection (bacterial)	AR RRT – Pulm
	+13	Busulfan	TRM – Infection (bacterial), MOF	AR RRT – Liver
	+14	Treosulfan	TRM – MOF, Infection (bacterial, viral, fungal)	TRM – Infection
	+14	Treosulfan	TRM – MOF, Infection	AR RRT – Pulm
	+21	Busulfan	TRM – VOD/HSOS, Infection (unknown)	AR RRT – Liver
	+22	Treosulfan	Other – Sepsis	AR RRT – Pulm
	+22	Treosulfan	TRM – Infection (bacterial)	AR RRT – Pulm
	+31	Treosulfan	TRM – Hemorrhage	TRM – Hemorrhage
	+33	Busulfan	TRM – Infection (unknown)	AR RRT – Cardiac
	+33	Busulfan	TRM – Pulmonary toxicity, interstitial pneumonitis, skin toxicity, renal failure, cardiac toxicity, infection (unknown)	AR RRT – Pulm
	+36	Busulfan	TRM – Hemorrhage	AR RRT – Pulm
	+40	Treosulfan	TRM – Infection (fungal)	Unrelated
	+40	Busulfan	Relapse/Progression	Primary disease – relapse
	+41	Busulfan	TRM – Infection (bacterial)	AR RRT – Pulm
	+44	Treosulfan	TRM – Infection (bacterial)	TRM – Infection
	+44	Busulfan	Relapse/Progression	Primary disease – relapse
	+46	Treosulfan	TRM – Infection (bacterial)	Insufficient information
	+51	Treosulfan	TRM – GVHD, infection (viral)	Insufficient information
	+52	Treosulfan	TRM – Infection (bacterial, viral)	Insufficient information
	+63	Treosulfan	TRM – Infection (viral)	TRM – Infection
	+69	Busulfan	TRM – Infection (bacterial)	TRM – Infection
	+70	Busulfan	Relapse/Progression	Primary disease – relapse
	+70	Busulfan	TRM – Infection (bacterial), MOF	TRM – Acute GVHD
	+73	Busulfan	TRM – GVHD, MOF	TRM – Acute GVHD
	+74	Busulfan	TRM – Infection (bacterial), GVHD	Insufficient information
	+77	Treosulfan	TRM – GVHD, Infection (bacterial)	TRM – Acute GVHD
	+77	Busulfan	Secondary malignancy	Insufficient information
	+79	Treosulfan	TRM – Infection (bacterial)	Insufficient information
	+81	Treosulfan	TRM – GVHD	TRM – Acute GVHD
	+85	Busulfan	Relapse/Progression	Primary disease – relapse
	+89	Treosulfan	Relapse/Progression	Primary disease – relapse
	+91	Busulfan	TRM – Infection (bacterial), GVHD	TRM – Acute GVHD
	+93	Treosulfan	Relapse/Progression	Primary disease – relapse
	+97	Busulfan	Relapse/Progression	Primary disease – relapse
	+97	Busulfan	TRM – GVHD	TRM – Acute GVHD
	+99	Busulfan	Relapse/Progression	Primary disease – relapse

Source: Reviewer's Analysis, MC-FludT.14/L Trial II Study Report, ISS ADSL database, Clinical Report Forms
Abbreviations: TRM: transplantation-related mortality; AR: adverse reaction; GVHD: graft versus host disease; MOF: multisystem organ failure.

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Grafapex (treosulfan)

Deaths in the treosulfan arm from Table 46 that are due to regimen-related toxicity that began prior to Day +30 after HSCT are detailed below.

Subject (b) (6) was a 63-year-old woman with AML in CR1 (2% bone marrow blasts), with a past medical history significant for acute renal impairment (resolved), hypertension, and diabetes. The subject received treosulfan + fludarabine as conditioning for allo-HSCT and received a PBSC as prescribed by the protocol with a matched related donor. On Day + 11, the subject is reported to have developed a lung infection, based on physical symptoms of pneumonia. On Day +12, the subject developed hemolysis and bronchopulmonary hemorrhage. The subject died on Day +13. The lung infection and bronchopulmonary hemorrhage were grade 5. The Applicant considered lung infection and bronchopulmonary hemorrhage as possibly related to treosulfan.

Reviewer's Comment: The FDA adjudicated the cause of death to be AR RRT – pulmonary based on the time to event analysis and the narrative provided by the Applicant, which did not provide a microbial etiology.

Subject (b) (6) was a 56-year-old man with MDS who received a conditioning regimen with treosulfan + fludarabine and a PBSC allo-HSCT from a matched unrelated donor. On Day +1, the subject developed grade 3 lung infection, with symptoms of dyspnea, fever, CRP elevation, and respiratory insufficiency. This was further specified as pneumonia and progressed to grade 4. On Day +7, the subject developed grade 2 febrile neutropenia. On Day +9, the patient developed grade 5 sepsis, with symptoms of hypotension, acidosis, kidney failure, and respiratory insufficiency. The subject died on Day +14. The Applicant assessed the cause of death as sepsis with lung infection as life-threatening. Both were considered by the Applicant as possibly related to treosulfan.

Reviewer's Comment: The FDA adjudicated the cause of death to be AR RRT – pulmonary based on the narrative provided by the Applicant. No microbial etiology was provided.

Subject (b) (6) was a 66-year-old man with AML who received a conditioning regimen with treosulfan + fludarabine and a PBSC allo-HSCT from a matched related donor. On Day +11, the patient experienced grade 4 lung infection, which worsened on Day +19 with progression to life-threatening respiratory fatigue. On Day +22, the patient experienced grade 5 sepsis and grade 5 kidney failure. The Applicant considered lung infection and sepsis as unlikely to be related to treosulfan.

Reviewer's Comment: The FDA adjudicated the cause of death to be AR RRT – pulmonary. This is based on the time to event and the narrative provided by the Applicant, which did not provide a microbial etiology.

Subject (b) (6) was a 66-year-old woman with MDS who received a conditioning regimen with

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Grafapex (treosulfan)

treosulfan + fludarabine and a PBSC allo-HSCT from a matched unrelated donor. On Day +12, the patient developed a grade 5 lung infection which led to death on Day +22.

Reviewer's Comment: *The FDA adjudicated the cause of death to be AR RRT – pulmonary. This is based on the time to event and the narrative provided by the Applicant. No microbial etiology was provided.*

Serious Adverse Events

In MC-FludT.14/L Trial II, 23 patients (8.5%) who received treosulfan had at least one serious adverse event (SAE), compared to 20 patients (7.1%) who received busulfan. The most common SAE was infection, with thirteen patients on the treosulfan arm (4.8%) and eight patients on the busulfan arm (2.8%) experiencing this SAE by D+30 (Table 47).

Table 47. Serious Adverse Events (SAEs) by Preferred Term* in MC-FludT.14/L Trial II by HSCT Day +30

Preferred Term*	Treosulfan (N = 270)		Busulfan (N = 283)	
	N	%	N	%
Infection	13	4.815	8	2.827
Haemorrhage	4	1.481	0	0
Renal impairment	3	1.111	1	0.353
Aspiration	1	0.37	0	0
C-reactive protein increased	1	0.37	0	0
Cardiac failure	1	0.37	0	0
Cytokine release syndrome	1	0.37	0	0
Embolism	1	0.37	0	0
Hypertension	1	0.37	0	0
Syncope	1	0.37	0	0
Tachycardia	1	0.37	0	0
Capillary leak syndrome	1	0.37	1	0.353
Ileus	1	0.37	1	0.353
Viral infection	1	0.37	1	0.353
Respiratory failure	1	0.37	2	0.707
Acute respiratory distress syndrome	0	0	1	0.353
Diarrhoea	0	0	1	0.353
Dyspnoea	0	0	1	0.353
Ileal perforation	0	0	1	0.353
Pyrexia	0	0	1	0.353
Toxicity to various agents	0	0	1	0.353
Vomiting	0	0	1	0.353
Hepatotoxicity	0	0	3	1.06
Source: Reviewer's Analysis, MAED; ISS ADSL, ADAEGT2 Datasets				
*Includes grouped terms (see Table 42)				

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Grafapex (treosulfan)

Similarly, the most common grade 3-5 SAE was infection in both arms, followed by hemorrhage and renal impairment (Table 48).

Table 48. Grade 3-5 SAEs by Preferred Term* in MC-FludT.14/L Trial II by HSCT Day +30

Preferred Term*	Treo sulfan (N = 270)		Busulfan (N = 283)	
	N	%	N	%
Infection	13	4.815	8	2.827
Haemorrhage	4	1.481	0	0
Renal impairment	2	0.741	0	0
Aspiration	1	0.37	0	0
C-reactive protein increased	1	0.37	0	0
Cardiac failure	1	0.37	0	0
Hypertension	1	0.37	0	0
Syncope	1	0.37	0	0
Tachycardia	1	0.37	0	0
Capillary leak syndrome	1	0.37	1	0.353
Ileus	1	0.37	1	0.353
Viral infection	1	0.37	1	0.353
Respiratory failure	1	0.37	2	0.707
Acute respiratory distress syndrome	0	0	1	0.353
Dyspnoea	0	0	1	0.353
Ileal perforation	0	0	1	0.353
Toxicity to various agents	0	0	1	0.353
Hepatotoxicity	0	0	2	0.707
<i>Source: Reviewer's Analysis, MAED; ISS ADSL, ADAEGT2 datasets</i>				
<i>*Includes grouped terms (see Table 42)</i>				

Dropouts and/or Discontinuations Due to Adverse Effects

There were no dropouts or discontinuations due to adverse effects.

Significant Adverse Events

See Section 8.3.5 for a discussion of acute and chronic graft versus host disease (GVHD).

Treatment Emergent Adverse Events and Adverse Reactions

Treatment emergent adverse events (TEAEs) through HSCT Day +30 in MC-FludT.14/L Trial II by system organ class (SOC) are shown in Table 49. Most TEAEs by SOC occur at similar rates between the treosulfan arm and the busulfan arm. Of note, cardiac disorders were more common in among patients who received treosulfan.

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Grafapex (treosulfan)

Table 49. Treatment Emergent Adverse Events by System Organ Class (SOC) through Day +30, MC-FludT.14/L Trial II

SOC	Treosulfan (N = 270)		Busulfan (N = 283)	
	N	%	N	%
Gastrointestinal disorders	184	68.148	208	73.498
General disorders and administration site conditions	151	55.926	150	53.004
Musculoskeletal and connective tissue disorders	102	37.778	77	27.208
Skin and subcutaneous tissue disorders	79	29.259	73	25.795
Investigations	77	28.519	76	26.855
Nervous system disorders	76	28.148	86	30.389
Infections and infestations	73	27.037	69	24.382
Vascular disorders	67	24.815	84	29.682
Metabolism and nutrition disorders	60	22.222	55	19.435
Respiratory, thoracic and mediastinal disorders	52	19.259	61	21.555
Cardiac disorders	41	15.185	25	8.834
Blood and lymphatic system disorders	40	14.815	31	10.954
Renal and urinary disorders	31	11.481	26	9.187
Psychiatric disorders	21	7.778	25	8.834
Ear and labyrinth disorders	17	6.296	24	8.481
Immune system disorders	17	6.296	22	7.774
Eye disorders	12	4.444	27	9.541
Injury, poisoning and procedural complications	8	2.963	9	3.18
Hepatobiliary disorders	4	1.481	6	2.12
Reproductive system and breast disorders	4	1.481	7	2.473
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1	0.37	1	0.353
Surgical and medical procedures	1	0.37	0	0
<i>Source: Reviewer's analysis, MAED; ADSL and ADAEGT2 datasets.</i>				

When evaluating TEAEs by Preferred Term (PT), the most common TEAEs were those expected after allo-HSCT (Table 50). The most common adverse reactions ($\geq 10\%$) on the treosulfan arm of MC-FludT.14/L Trial II included musculoskeletal pain, stomatitis, pyrexia, nausea, edema, infection, vomiting, rash, diarrhea, headache, febrile neutropenia, abdominal pain, hypertension, hemorrhage, fatigue, constipation, tachycardia, and hepatotoxicity. The most common grade 3-5 ARs ($\geq 10\%$) on the treosulfan arm were febrile neutropenia and infection. Rare but potentially serious events that occurred on the treosulfan arm of MC-FludT.14/L Trial II included ventricular arrhythmias (including tachycardia, see Grouped Term in Table 44 above, and extrasystoles; N = 5, 1.852%), erythema multiforme (N = 8, 2.963%), palmar-plantar erythrodysesthesia syndrome (N = 5, 1.852%), and cardiac failure (N = 8, 2.963%).

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Grafapex (treosulfan)

Table 50. Treatment Emergent Adverse Events by Preferred Term* in >5% of Treosulfan-Treated Patients through Day +30, MC-FludT.14/L Trial II

Preferred Term*	All Grades				Grades 3-5			
	Treosulfan (N = 270)		Busulfan (N = 283)		Treosulfan (N = 270)		Busulfan (N = 283)	
	N	%	N	%	N	%	N	%
Musculoskeletal pain	105	38.889	75	26.502	14	5.185	7	2.473
Stomatitis	102	37.778	135	47.703	16	5.926	21	7.42
Pyrexia	93	34.444	101	35.689	2	0.741	9	3.18
Nausea	89	32.963	116	40.989	8	2.963	17	6.007
Oedema	77	28.519	51	18.021	2	0.741	4	1.413
Infection	63	23.333	51	18.021	35	12.963	20	7.067
Vomiting	59	21.852	55	19.435	2	0.741	4	1.413
Rash	46	17.037	37	13.074	3	1.11	4	1.413
Diarrhoea	45	16.667	52	18.375	4	1.481	4	1.413
Headache	44	16.296	52	18.375	3	1.11	2	0.707
Febrile neutropenia	40	14.815	31	10.954	40	14.815	31	10.954
Abdominal pain	40	14.815	38	13.428	7	2.592	5	1.767
Hypertension	38	14.074	60	21.201	21	7.778	27	9.541
Haemorrhage	37	13.704	40	14.134	4	1.481	3	1.06
Fatigue	34	12.593	42	14.841	3	1.11	1	0.353
Constipation	33	12.222	33	11.661	1	0.37	0	0
Tachycardia	27	10	15	5.3	4	1.481	6	2.12
Hepatotoxicity	27	10	22	7.774	10	3.704	9	3.18
Renal impairment	24	8.889	24	8.481	5	1.852	1	0.353
Decreased appetite	24	8.889	26	9.187	5	1.852	4	1.413
Aspartate aminotransferase increased	23	8.519	14	4.947	12	4.444	7	2.473
Alanine aminotransferase increased	23	8.519	18	6.36	14	5.185	9	3.18
Chills	20	7.407	16	5.654	1	0.37	0	0
Gamma-glutamyltransferase increased	20	7.407	34	12.014	12	4.444	25	8.834
Hypotension	19	7.037	12	4.24	5	1.852	5	1.767
Weight increased	19	7.037	18	6.36	0	0	0	0
Pruritus	17	6.296	12	4.24	1	0.37	0	0
Dizziness	17	6.296	14	4.947	0	0	1	0.353
Pain	16	5.926	8	2.827	1	0.37	0	0
C-reactive protein increased	16	5.926	15	5.3	8	2.963	10	3.534
Encephalopathy	16	5.926	17	6.007	5	1.852	3	1.06
Viral infection	16	5.926	20	7.067	4	1.481	5	1.767
Hypersensitivity	16	5.926	22	7.774	2	0.741	1	0.353
Dyspnoea	15	5.556	23	8.127	2	0.741	4	1.413
Hypomagnesaemia	14	5.185	8	2.827	0	0	0	0

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Grafapex (treosulfan)

Source: Reviewer's analysis, MAED, ISS population (ADSL, ADAEGT2 datasets)

*Includes grouped terms (see Table 42)

Table 51 shows the analysis for TEAEs deemed related or possibly related to the conditioning regimen by the Applicant.

Table 51. Related Treatment Emergent Adverse Events by Preferred Term* in >5% of Treosulfan-Treated Patients through Day +30, MC-FludT.14/L Trial II

Preferred Term*	Treosulfan (N = 270)		Busulfan (N = 283)	
	N	%	N	%
Stomatitis	87	32.222	108	38.163
Nausea	58	21.481	82	28.975
Vomiting	40	14.815	34	12.014
Fatigue	22	8.148	24	8.481
Alanine aminotransferase increased	21	7.778	14	4.947
Infection	21	7.778	14	4.947
Aspartate aminotransferase increased	20	7.407	9	3.18
Hepatotoxicity	19	7.037	17	6.007
Abdominal pain	18	6.667	16	5.654
Decreased appetite	17	6.296	15	5.3
Diarrhoea	16	5.926	31	10.954
Rash	15	5.556	11	3.887
Gamma-glutamyltransferase increased	14	5.185	29	10.247
Headache	14	5.185	18	6.36

Source: Reviewer's analysis, MAED, ISS population (ADSL, ADAEGT2 datasets)
*Includes grouped terms (see Table 42)

Reviewer's Comment: The FDA notes that the Grouped Term of musculoskeletal pain was not considered to be a related AE by the Applicant. However, the timing of this GT indicates that it is an adverse reaction following treosulfan conditioning. We note higher rates of infectious complications in patients following treosulfan-based preparative regimens compared to busulfan-based preparative regimens. We also note a higher rate of fatal ARs through Day +30 after HSCT in patients who received treosulfan-based preparative regimens.

Laboratory Findings

As treosulfan is used as part of a conditioning regimen for allogeneic HSCT, cytopenias occur in nearly 100% of patients. Other laboratory abnormalities are listed below (Table 52). There were no significant differences in the maximum grades of laboratory abnormalities between the treosulfan and busulfan arms of MC-FludT.14/L Trial II.

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Grafapex (treosulfan)

Table 52. Selected Laboratory Abnormalities (Nonhematologic) by Maximum Grade in MC-FludT.14/L Trial II

Laboratory Analyte	Treo sulfan N = 270		Busulfan N = 283	
	Grades 1-4 (n/N evaluab le (%))	Grades 3-4 (n/N evaluab le (%))	Grades 1-4 (n/N evaluab le (%))	Grades 3-4 (n/N evaluab le (%))
Creatinine (umol/L) Increased	212/268 (79)	7/268 (2.6)	199/283 (70)	2/283 (0.7)
Bilirubin, total (umol/L) Increased	165/268 (62)	16/268 (6)	130/283 (46)	14/283 (4.9)
Glucose (mmol/L) Increased	157/264 (59)	6/264 (2.3)	146/280 (52)	7/280 (2.5)
Gamma Glutamyl Transferase (U/L) Increased	185/268 (69)	38/268 (14)	230/283 (81)	76/283 (27)
Alanine Aminotransferase (U/L) Increased	133/268 (50)	16/268 (6)	123/283 (43)	11/283 (3.9)
Aspartate Aminotransferase (U/L) Increased	108/267 (40)	10/267 (3.7)	84/283 (30)	4/283 (1.4)
Alkaline Phosphatase (U/L) Increased	63/265 (24)	1/265 (0.4)	50/283 (18)	0/0 (0.0)
Sodium (mmol/L) Decreased	93/268 (35)	16/268 (6)	102/283 (36)	14/283 (4.9)
Potassium (mmol/L) Decreased	68/268 (25)	13/268 (4.9)	56/283 (20)	4/283 (1.4)
Potassium (mmol/L) Increased	49/268 (18)	0/0 (0.0)	63/283 (22)	2/283 (0.7)
Source: FDA Analysis, ISS (ADLB)				

Vital Signs

Vital signs were provided for the following timepoints: within 3 weeks of HSCT, day 0, day 28, day +100, month 6, month 9, month 12, month 15, month 18, and month 24. There were no vital signs submitted from the time periods around treosulfan or busulfan infusions to assess for evidence of infusion reactions. There were not sufficient vital signs collected to assess for outliers within the 30 days following HSCT.

QT/Electrocardiograms (ECGs)

Formal QT/QTc testing has not been completed. Under “Investigations,” “electrocardiogram abnormality” was identified in one patient on the busulfan arm of MC-FludT.14/L Trial II and zero patients on the treosulfan arm. Ventricular arrhythmias were identified as rare but potentially serious ARs and are discussed in in the TEAE and AR section above.

Table 53 shows other electrocardiographic abnormalities in MC-FludT.14/L Trial II through HSCT Day +30.

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Table 53. Electrocardiographic abnormalities identified in MC-FludT.14/L Trial II through Day +30

SOC	PT	Treosulfan (N = 270)		Busulfan (N = 283)	
		N	%	N	%
Cardiac disorders		35	12.963	22	7.774
Cardiac disorders	Bundle branch block left	1	0.37	0	0
Cardiac disorders	Conduction disorder	1	0.37	0	0
Cardiac disorders	Atrial fibrillation	11	4.074	4	1.413
Cardiac disorders	Atrial flutter	0	0	1	0.353
Cardiac disorders	Atrial tachycardia	2	0.741	0	0
Cardiac disorders	Sinus bradycardia	4	1.481	3	1.06
Cardiac disorders	Sinus node dysfunction	0	0	1	0.353
Cardiac disorders	Sinus tachycardia	8	2.963	5	1.767
Cardiac disorders	Supraventricular tachycardia	8	2.963	5	1.767
Cardiac disorders	Ventricular arrhythmia	2	0.741	2	0.707
Cardiac disorders	Ventricular extrasystoles	1	0.37	0	0
Cardiac disorders	Ventricular tachycardia	3	1.111	2	0.707
Cardiac disorders	Cyanosis	0	0	1	0.353
Cardiac disorders	Palpitations	1	0.37	1	0.353

Source: Reviewer's analysis, MAED, ISS population (ADSL, ADAEGT2 datasets)

Immunogenicity

No immunogenicity data were submitted.

8.3.5 Analysis of Submission-Specific Safety Issues

Treosulfan Dose (14 g/m²)

Trial MC-FludT.14/L Trial I used a treosulfan dose of 14 g/m² for patients randomized to receive treosulfan. This study was stopped early due to increased transplant-related morbidity and mortality observed in the treosulfan arm. Treatment-emergent adverse events through HSCT day +30 are shown in Table 54. There were increased TEAEs of hepatotoxicity, stomatitis, musculoskeletal pain, infection, and febrile neutropenia in the treosulfan cohort, including grades 3-5 TEAEs.

Table 54. Treatment Emergent Adverse Events by Preferred Term* in >5% of Treosulfan-Treated Patients through Day +30, MC-FludT.14/L Trial I

Preferred Term*	All Grades				Grades 3-5			
	Treosulfan (N = 168)		Busulfan (N = 152)		Treosulfan (N = 168)		Busulfan (N = 152)	
	N	%	N	%	N	%	N	%
Hepatotoxicity	108	64.29	72	47.37	22	13.1	12	7.89
Nausea	108	64.29	74	48.68	10	5.95	6	3.95
Stomatitis	105	62.5	105	69.08	18	10.71	25	16.45
Musculoskeletal pain	101	60.12	56	36.84	26	15.48	11	7.24

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Fatigue	83	49.4	66	43.42	10	5.95	5	3.29
Diarrhoea	71	42.26	44	28.95	12	7.14	3	1.97
Oedema	68	40.48	50	32.89	3	1.79	2	1.32
Vomiting	66	39.29	39	25.66	3	1.79	0	0
Febrile neutropenia	64	38.1	44	28.95	63	37.5	42	27.63
Haemorrhage	58	34.52	49	32.24	5	2.98	3	1.97
Infection	57	33.93	40	26.32	38	22.62	17	11.18
Rash	51	30.36	34	22.37	4	2.38	2	1.32
Abdominal pain	47	27.98	30	19.74	10	5.95	6	3.95
Hypertension	42	25	38	25	13	7.74	5	3.29
Headache	39	23.21	36	23.68	4	2.38	7	4.61
Hypotension	34	20.24	22	14.47	5	2.98	5	3.29
Dizziness	34	20.24	28	18.42	1	0.6	0	0
Decreased appetite	31	18.45	24	15.79	7	4.17	8	5.26
Hypersensitivity	30	17.86	26	17.11	4	2.38	2	1.32
Constipation	29	17.26	13	8.55	0	0	1	0.66
Non-cardiac chest pain	26	15.48	11	7.24	6	3.57	2	1.32
Encephalopathy	26	15.48	17	11.18	4	2.38	6	3.95
Tachycardia	25	14.88	11	7.24	7	4.17	3	1.97
Weight increased	25	14.88	12	7.89	0	0	0	0
Dyspnoea	25	14.88	21	13.82	5	2.98	7	4.61
Chills	21	12.5	19	12.5	2	1.19	2	1.32
Pyrexia	18	10.71	22	14.47	1	0.6	0	0
Insomnia	18	10.71	12	7.89	0	0	2	1.32
Hyperhidrosis	17	10.12	12	7.89	1	0.6	1	0.66
Pruritus	13	7.74	15	9.87	0	0	1	0.66
Depression	13	7.74	9	5.92	1	0.6	3	1.97
Dyspepsia	12	7.14	7	4.61	0	0	0	0
Myalgia	11	6.55	2	1.32	2	1.19	0	0
Bacterial infection	11	6.55	3	1.97	6	3.57	2	1.32
Anxiety	11	6.55	5	3.29	2	1.19	0	0
Tremor	9	5.36	2	1.32	1	0.6	0	0

Source: Reviewer's analysis, MAED, ISS population (ADSL, ADAEGT2 datasets)

*Includes grouped terms (see Table 42)

Graft versus Host Disease

MC-FludT-14.L Trial II was further analyzed for differences between occurrence of acute (aGVHD) and chronic (cGVHD) graft versus host disease between the treosulfan and busulfan arms (Table 55). For analysis of both aGVHD and cGVHD, there was no censoring of the population for death or other events. The rate of aGVHD is defined as the proportion of the safety population diagnosed with aGVHD by HSCT Day +100. There was no risk difference of >10% for any grade of aGVHD. The rate of cGVHD is defined as the proportion of the safety population diagnosed with cGVHD through month +24. There was no risk difference of ≥ 10 for either limited or extensive cGVHD between the treosulfan and busulfan arms.

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Grafapex (treosulfan)

Table 55. Comparison of GVHD rates between Treosulfan and Busulfan in MC-FludT.14/L Trial II

	Treosulfan (N = 270)		Busulfan (N = 283)	
	N	%	N	%
Acute GVHD				
Total (Grades 1-4)	141	52%	163	58%
Grades 2-4	52	19%	68	24%
Grades 3-4	17	6%	24	8.5%
Grade 4	5	2%	9	2%
Chronic GVHD				
Total	138	51%	138	49%
Limited	96	36%	75	27%
Extensive	42	16%	62	22%
<i>Source: Reviewer's Analysis, ADGVHD data file (MC-FludT.14/L Trial II)</i>				

Other late onset regimen-related toxicities were not able to be evaluated as discussed in Section 8.3.2.

8.3.6 Safety Analyses by Subgroups

Drug-Demographic Interactions

TEAEs by Sex

TEAEs with an RD of >5% between female and male patients are shown in Table 56. Women were more likely to experience GI-related toxicities, including nausea, vomiting, diarrhea, and constipation than men in Trial MC-FludT.14/L Trial II. Men receiving treosulfan were more likely to have hepatotoxicity and renal impairment. Vomiting was significantly different between women and men ($p = 0.00082$). Vomiting was not more likely in women receiving treosulfan (33%) compared to women receiving busulfan (28%, RD 4.5).

Table 56. TEAEs by Preferred Term* through Day +30 with a Risk Difference of >5%: Patients by Sex in MC-FludT.14/L Trial II (Treosulfan Cohort)

Preferred Term*	Women (N = 107)		Men (N = 163)		RD
	N	%	N	%	
Vomiting	35	32.71	24	14.72	17.99
Nausea	41	38.32	48	29.45	8.87
Stomatitis	45	42.06	57	34.97	7.09
Oedema	35	32.71	42	25.77	6.94
Diarrhoea	22	20.56	23	14.11	6.45
Constipation	17	15.89	16	9.82	6.07
Headache	21	19.63	23	14.11	5.52
Bone pain	18	16.82	19	11.66	5.17
Pruritus	10	9.35	7	4.29	5.05
Renal impairment	6	5.61	12	7.36	-5.44

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<i>Hypomagnesaemia</i>	2	1.87	28	17.18	-5.49
<i>Abdominal pain</i>	12	11.21	21	12.88	-5.96
<i>Hepatotoxicity</i>	6	5.61	18	11.04	-7.28
<i>Source: Reviewer's analysis, ISS population (ADSL, ADAEGT2 datasets)</i>					
<i>*Includes Grouped Terms (See Table 42)</i>					

TEAEs by Age

TEAEs with an RD of >5% between adult (ages 17-64) and elderly (ages 65-74) patients are shown in Table 57. When comparing TEAEs by age group, only infection was more likely to occur in elderly patients (those ages 65-74) compared to younger adults with an RD of >10%. In comparing elderly patients who received treosulfan to those who received busulfan-based conditioning, infection occurred more frequently in the treosulfan arm (32% vs 22%, RD 9.45).

Table 57. TEAEs by Preferred Term* through Day +30 with a Risk Difference of >5%: Patients by Age Group in MC-FludT.14/L Trial II (TreoSulfan Cohort)

Preferred Term*	Adults (Ages 17-64, N = 197)		Elderly (Age 65-74, N = 73)		Risk Difference
	N	%	N	%	
Stomatitis	78	39.59	24	32.88	6.72
Bone pain	30	15.23	7	9.59	5.64
Oedema	59	29.95	18	24.66	5.29
<i>Viral infection</i>	9	4.57	7	9.59	-5.02
<i>Arthralgia</i>	17	8.63	10	13.7	-5.07
<i>Fatigue</i>	22	11.17	12	16.44	-5.27
<i>Diarrhoea</i>	30	15.23	15	20.55	-5.32
<i>Hypotension</i>	11	5.58	8	10.96	-5.38
<i>Vomiting</i>	40	20.3	19	26.03	-5.72
<i>Decreased appetite</i>	14	7.11	10	13.7	-6.59
<i>Infection</i>	40	20.3	23	31.51	-11.2
<i>Source: Reviewer's Analysis, MAED, ADSL and ADAEGT2 datafiles.</i>					
<i>*Includes Grouped Terms (See Table 42)</i>					

TEAEs by Race

All studies were performed in Europe. No data on race or ethnicity were collected as part of any study. Therefore, no comparisons can be made.

Drug-Disease Interactions

TreoSulfan was used as part of the conditioning regimen for patients with the diagnoses of AML and MDS. As these are separate diseases, TEAEs were assessed in the treosulfan arm of MC-FludT.14/L Trial II in the AML and MDS populations separately (Table 58). No TEAEs were more common in patients with AML. Patients with an underlying diagnosis of MDS were statistically ($p < 0.05$) more likely to have TEAEs of hemorrhage, hyperglycemia, cough, edema, hyponatremia, and encephalopathy.

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Table 58. TEAEs by Preferred Term* Through Day +30 with a Risk Difference of >5%: Patients by Disease (AML or MDS) in Trial MC-FludT.14/L Trial II (Treo sulfan Cohort)

Preferred Term*	AML (N = 185)		MDS (N = 85)		RD
	N	%	N	%	
Hypersensitivity	8	4.324	8	9.412	-5.087
Hyponatraemia	3	1.622	6	7.059	-5.437
Abdominal pain	24	12.973	16	18.824	-5.851
Hyperglycaemia	3	1.622	7	8.235	-6.614
Stomatitis	66	35.676	36	42.353	-6.677
Cough	5	2.703	8	9.412	-6.709
Musculoskeletal pain	68	36.757	37	43.529	-6.773
Encephalopathy	7	3.784	9	10.588	-6.804
Headache	26	14.054	18	21.176	-7.122
Diarrhoea	26	14.054	19	22.353	-8.299
Febrile neutropenia	22	11.892	18	21.176	-9.285
Haemorrhage	18	9.73	19	22.353	-12.623
Oedema	45	24.324	32	37.647	-13.323

Source: Reviewer's Analysis, MAED, ADSL and ADAEGT2 datafiles.
 *Includes Grouped Terms (See Table 42)

Drug-Donor Interactions

Treo sulfan was used as part of the conditioning regimen for patients undergoing both matched related donor and matched unrelated donor allogeneic HSCT. TEAEs were assessed in the treo sulfan arm of MC-FludT.14/L Trial II in the related and unrelated donor populations (Table 59). There were more TEAEs observed in patients with matched unrelated donors, though this may be due to the difference in patient numbers between these two groups. When evaluating Grade 3-5 TEAEs, infection was more common in patients with a matched related donor (N = 11, 17%) than in those receiving a matched unrelated donor (N = 24, 12%).

Table 59. TEAEs by Preferred Term* Through Day +30 with a Risk Difference of >5%: Patients by Donor Type (Matched Related or Matched Unrelated Donor) in Trial MC-FludT.14/L Trial II (Treo sulfan Cohort)

Preferred Term*	Matched Related Donor (N = 63)		Matched Unrelated Donor (N = 207)		RD
	N	%	N	%	
Decreased appetite	9	14.286	15	7.246	7.039
Hypotension	2	3.175	17	8.213	-5.038
Weight increased	2	3.175	17	8.213	-5.038
Chills	2	3.175	18	8.696	-5.521
Rash	8	12.698	38	18.357	-5.659
Nausea	18	28.571	71	34.3	-5.728
Hypertension	6	9.524	32	15.459	-5.935
Dizziness	1	1.587	16	7.729	-6.142

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Pruritus	1	1.587	16	7.729	-6.142
Abdominal pain	6	9.524	34	16.425	-6.901
Febrile neutropenia	6	9.524	34	16.425	-6.901
Viral infection	0	0	16	7.729	-7.729
Stomatitis	20	31.746	82	39.614	-7.867
Oedema	14	22.222	63	30.435	-8.213
Headache	6	9.524	38	18.357	-8.834
Hepatotoxicity	2	3.175	25	12.077	-8.903
Tachycardia	2	3.175	25	12.077	-8.903
Gamma-glutamyltransferase increased	0	0	20	9.662	-9.662
Musculoskeletal pain	18	28.571	87	42.029	-13.458
Diarrhoea	4	6.349	41	19.807	-13.458
Pyrexia	10	15.873	83	40.097	-24.224
<i>Source: Reviewer's Analysis, MAED, ADSL and ADAEGT2 datafiles.</i>					
<i>*Includes Grouped Terms (See Table 42)</i>					

In addition, given the potential signal for decreased efficacy of treosulfan in the matched related donor group, a comparison between the treosulfan and busulfan groups was performed, as shown in Table 60. All-grade musculoskeletal pain, edema, infection, and hemorrhage were more common in patients with matched related donors receiving treosulfan-based conditioning. Of note, grade 3-5 infection was higher in the treosulfan group than the busulfan group (17.46% vs 4.412%, p-value = 0.022).

Table 60. Treatment Emergent Adverse Events by Preferred Term* in >10% of Treosulfan-Treated Patients through Day +30, Matched Related Donors, MC-FludT.14/L Trial II

Preferred Term*	All Grades				Grades 3-5			
	Treosulfan (N = 63)		Busulfan (N = 68)		Treosulfan (N = 63)		Busulfan (N = 68)	
	N	%	N	%	N	%	N	%
Stomatitis	20	31.746	33	48.529	4	6.349	8	11.765
Musculoskeletal pain	18	28.571	14	20.588	1	1.587	1	1.471
Nausea	18	28.571	27	39.706	1	1.587	3	4.412
Oedema	14	22.222	9	13.235	0	0	0	0
Infection	13	20.635	8	11.765	11	17.46	3	4.412
Vomiting	13	20.635	11	16.176	0	0	1	1.471
Pyrexia	10	15.873	10	14.706	0	0	0	0
Decreased appetite	9	14.286	3	4.412	2	3.175	1	1.471
Haemorrhage	8	12.698	3	4.412	2	3.175	0	0
Rash	8	12.698	4	5.882	0	0	0	0

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Constipation	8	12.698	7	10.294	0	0	0	0
Fatigue	7	11.111	11	16.176	1	1.587	0	0
Source: Reviewer's Analysis, MAED, ADSL and ADAEGT2 datafiles. *Includes Grouped Terms (See Table 42)								

Drug-Drug Interactions

There were no additional drug-drug interaction analyses performed.

8.3.7 Clinical Outcomes Assessments Informing Tolerability/Safety

No Clinical Outcomes Assessments were performed in these studies.

8.3.8 Specific Safety Studies/Clinical Trials

Dose Dependency for Adverse Events

As MC-FludT.14/L Trial I, in which patients randomized to treosulfan received a dose of 14 g/m², was stopped early because of increased transplant-related morbidity and mortality, a comparison was performed between all adult patients who received 10 g/m² and those who received 14 g/m² of treosulfan. We observed higher rates of hepatotoxicity, GI toxicity, infection (including febrile neutropenia), and musculoskeletal AEs in patients receiving the 14 g/m² dose of treosulfan, as seen in Table 61.

Table 61. Treosulfan Dose Comparison of 10 g/m² and 14 g/m² in Cohort 3 (MC-FludT.6, MC-FludT.7/AML, MC-FludT.8/MDS, MC-FludT.14/L Trial I, and MC-FludT.14/L Trial II)

Preferred Term*	Treosulfan 10 g/m ² (N = 290)		Treosulfan 14 g/m ² (N = 305)		RD
	N	%	N	%	
Hepatotoxicity	28	9.66	116	38.03	28.38
Nausea	100	34.48	189	61.97	27.48
Febrile neutropenia	47	16.21	126	41.31	25.1
Fatigue	38	13.1	111	36.39	23.29
Diarrhoea	55	18.97	117	38.36	19.4
Haemorrhage	46	15.86	95	31.15	15.29
Infection	69	23.79	118	38.69	14.9
Vomiting	66	22.76	113	37.05	14.29
Stomatitis	108	37.24	155	50.82	13.58
Headache	49	16.9	90	29.51	12.61
Hypertension	41	14.14	81	26.56	12.42
Abdominal pain	43	14.83	83	27.21	12.39
Hypersensitivity	18	6.21	52	17.05	10.84
Insomnia	9	3.1	42	13.77	10.67
Musculoskeletal pain	69	23.79	103	33.77	9.98
Bone pain	46	15.86	77	25.25	9.38

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Non-cardiac chest pain	8	2.76	36	11.8	9.04
Dyspepsia	6	2.07	33	10.82	8.75
Weight increased	20	6.9	47	15.41	8.51
Rash	52	17.93	76	24.92	6.99
Constipation	37	12.76	60	19.67	6.91
Encephalopathy	19	6.55	40	13.11	6.56
Hypotension	21	7.24	42	13.77	6.53
Hyperhidrosis	1	0.34	20	6.56	6.21
Decreased appetite	27	9.31	46	15.08	5.77
Dizziness	18	6.21	36	11.8	5.6
Renal impairment	30	10.34	16	5.25	-5.1
CRP increased	18	6.21	3	0.98	-5.22
GGT increased	27	9.31	7	2.3	-7.02
AST increased	28	9.66	5	1.64	-8.02
ALT increased	32	11.03	7	2.3	-8.74
Pyrexia	98	33.79	41	13.44	-20.35

Source: Reviewer's analysis, ISS population (ADSL, ADAEGT2 datasets)

*Includes Grouped Terms (See Table 42)

Reviewer's Comment: The FDA notes that there is notably more toxicity, including infectious complications, GI toxicity, and hepatotoxicity, in patients who received 14 g/m²/dose of treosulfan compared to 10 g/m²/dose of treosulfan. In addition, the higher rates of early fatal adverse reactions in MC-FludT.14/L Trial I underscore the increased risk of morbidity and mortality with the higher dose of treosulfan.

Time Dependency for Adverse Events

There was insufficient follow-up with detailed safety results to allow an assessment of late toxicities.

8.3.9 Additional Safety Explorations

Human Carcinogenicity or Tumor Development

An analysis of the SOC "Neoplasms benign, malignant, and unspecified (including cysts and polyps)" in MC-FludT.14/L Trial II revealed one patient in the treosulfan arm and one patient in the busulfan arm with these events.

Human Reproduction and Pregnancy

Because of the proposed mechanism of action of treosulfan, pregnancy and reproduction are discouraged. There have been no studies or literature reports of subjects who became pregnant while receiving treosulfan, or of children who were born of parents who were receiving treosulfan.

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Grafapex (treosulfan)

Pediatrics and Assessment of Effects on Growth

Two pediatric studies were submitted as part of this application. All pediatric patients received doses of treosulfan that were equivalent to 14 g/m² dosing. Safety analysis was performed comparing the pediatric patients to the recommended dose of treosulfan (10 g/m²). The majority of pediatric patients received thiotepea in addition to fludarabine and treosulfan for their conditioning. Analyses comparing pediatric and adult studies (Cohorts 4 and 1) excluded patients who received thiotepea.

An analysis between the treosulfan arm and the busulfan arm in the Phase 2 randomized study MC-FludT.16/NM is seen in Table 62. Nearly all of the patients in this analysis received a preparative regimen of either treosulfan or busulfan, fludarabine, and thiotepea. The majority of TEAEs were similar between the two arms. Notable exceptions included higher rates of abdominal pain, rash, pruritis, hemorrhage, and infusion-related reactions in the treosulfan arm of this study.

Table 62. TEAEs by Preferred Term* through HSCT Day +30 with a rate of >10%: MC-FludT.16/NM

Preferred Term*	Treo sulfan (N = 45)		Busulfan (N = 43)	
	N	%	N	%
Stomatitis	32	71.11	33	76.74
Pyrexia	28	62.22	28	65.12
Diarrhoea	25	55.56	19	44.19
Hepatotoxicity	25	55.56	24	55.81
Vomiting	25	55.56	25	58.14
Abdominal pain	21	46.67	14	32.56
Rash	18	40	6	13.95
Hypertension	15	33.33	16	37.21
Nausea	14	31.11	16	37.21
Pruritus	12	26.67	6	13.95
Headache	12	26.67	8	18.6
Alopecia	11	24.44	5	11.63
Haemorrhage	11	24.44	9	20.93
Infusion related reaction	9	20	4	9.3
Musculoskeletal pain	9	20	8	18.6
Viral infection	9	20	9	20.93
Constipation	7	15.56	4	9.3
Alanine aminotransferase increased	7	15.56	5	11.63
Infection	7	15.56	12	27.91
Chills	6	13.33	2	4.65
Oedema	5	11.11	2	4.65
Cystitis noninfective	5	11.11	5	11.63
Source: ISS ADSL and ADAEGT2 datasets. Reviewer's analysis.				
*Includes Grouped Terms (See Table 42)				

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Pediatric patients from both MC-FludT.16/NM and MC-FludT.17/M who received a preparative regimen of treosulfan + fludarabine (Cohort 4) were then compared to adult patients who received the recommended treosulfan-based conditioning regimen (Cohort 3, 10 g/m²) (Table 63). The pediatric patient numbers are small, as the majority of patients received a preparative regimen that included thiotepea and are excluded from this analysis. The analysis revealed higher rates of hepatotoxicity and gastrointestinal toxicities in the pediatric patients compared with adult patients.

Table 63. TEAEs by Preferred Term* Through Day +30 with a Risk Difference of >5%: Pediatric No Thiotepea - Pediatric Patients (Cohort 4) vs Adult patients (Cohort 3, 10 g/m²)

Preferred Term	Pediatric (N = 12)		Adult (N = 290)		RD
	N	%	N	%	
Hepatotoxicity	6	50	28	9.66	40.34
Stomatitis	7	58.33	108	37.24	21.09
Vomiting	5	41.67	66	22.76	18.91
Abdominal pain	4	33.33	43	14.83	18.51
Pyrexia	6	50	98	33.79	16.21
Infusion related reaction	2	16.67	2	0.69	15.98
Rash	4	33.33	52	17.93	15.4
Alopecia	2	16.67	5	1.72	14.94
Pollakiuria	2	16.67	6	2.07	14.6
Diarrhoea	4	33.33	55	18.97	14.37
Anal inflammation	1	8.33	0	0	8.33
Engraftment syndrome	1	8.33	0	0	8.33
Haemolysis	1	8.33	0	0	8.33
Pneumonitis	1	8.33	1	0.34	7.99
Urticaria	1	8.33	1	0.34	7.99
Dysphagia	1	8.33	2	0.69	7.64
Influenza like illness	1	8.33	2	0.69	7.64
Ear pain	1	8.33	3	1.03	7.3
Hyperkalaemia	1	8.33	3	1.03	7.3
Hypoxia	1	8.33	3	1.03	7.3
ALT increased	2	16.67	32	11.03	5.63
Hypokalaemia	1	8.33	9	3.1	5.23
CRP increased	0	0	18	6.21	-6.21
Dizziness	0	0	18	6.21	-6.21
Dyspnoea	0	0	18	6.21	-6.21
Pruritus	0	0	18	6.21	-6.21
Encephalopathy	0	0	19	6.55	-6.55
Weight increased	0	0	20	6.9	-6.9
Hypotension	0	0	21	7.24	-7.24
Headache	1	8.33	49	16.9	-8.56
Decreased appetite	0	0	27	9.31	-9.31
GGT increased	0	0	27	9.31	-9.31
AST increased	0	0	28	9.66	-9.66
Tachycardia	0	0	32	11.03	-11.03
Constipation	0	0	37	12.76	-12.76

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<i>Fatigue</i>	0	0	38	13.1	-13.1
<i>Hypertension</i>	0	0	41	14.14	-14.14
<i>Infection</i>	1	8.33	69	23.79	-15.46
<i>Haemorrhage</i>	0	0	46	15.86	-15.86
<i>Febrile neutropenia</i>	0	0	47	16.21	-16.21
<i>Oedema</i>	1	8.33	80	27.59	-19.25
<i>Musculoskeletal pain</i>	0	0	118	40.69	-40.69
<i>Source: ISS ADSL and ADAEGT2 datasets. Reviewer's analysis.</i>					
<i>*Includes Grouped Terms (See Table 42)</i>					

Of Grade 3-5 TEAEs in these cohorts, stomatitis had a risk difference >10% in pediatric patients compared to adults (Table 64).

Table 64. Grade 3-5 TEAEs by Preferred Term* through Day +30 with a Risk Difference of >5%: - Pediatric Patients (No Thiotepea, Cohort 4) vs Adult patients (Cohort 3, 10 g/m²)

Preferred Term	Pediatric (N = 12)		Adult (N = 290)		RD
	N	%	N	%	
Stomatitis	3	25	17	5.86	19.14
Dysphagia	1	8.33	1	0.34	7.99
Hyperkalaemia	1	8.33	1	0.34	7.99
Hypoxia	1	8.33	1	0.34	7.99
Pyrexia	1	8.33	2	0.69	7.64
Hypokalaemia	1	8.33	5	1.72	6.61
Nausea	1	8.33	8	2.76	5.57
<i>AST increased</i>	0	0	15	5.17	-5.17
<i>GGT increased</i>	0	0	19	6.55	-6.55
<i>Hypertension</i>	0	0	22	7.59	-7.59
<i>Infection</i>	0	0	39	13.45	-13.45
<i>Febrile neutropenia</i>	0	0	47	16.21	-16.21
<i>Source: ISS ADSL and ADAEGT2 datasets. Reviewer's analysis.</i>					
<i>*Includes Grouped Terms (See Table 42)</i>					

Reviewer's Note: Treosulfan in pediatric studies was dosed to achieve similar exposure to 14 g/m² treosulfan dosing in adults. An equivalent dose of 14 g/m² in children is not supported by the data provided. (b) (4)

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Not applicable.

8.3.10 Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

Empirica Signal was used to evaluate the postmarket experience of treosulfan (Figure 15). The

Grafapex (treosulfan)

Drug=TREOSULFAN (Subset: Thru 2021)



Source: Reviewer's Analysis, Empirica Signal

Safety in the postmarket setting is anticipated to be similar to that observed in the pivotal trial submitted and reviewed in this application. It is recognized that treosulfan may be used in populations other than those included in the indication statement. Additionally, patients with lower performance status, higher HCT-CI score, and inadequate organ function may receive treosulfan outside of a clinical trial. For these reasons, the predictability of treosulfan in the postmarket setting is not completely predictable.

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8.3.11 Integrated Assessment of Safety

The primary data in support of safety for the proposed indication of treosulfan came from study MC-FludT.14/L Trial II, in which 270 patients with either AML or MDS were exposed to treosulfan as part of a conditioning regimen prior to allogeneic HSCT. All patients received two doses of treosulfan on days -4 and -3 prior to HSCT, at a dose of 10 g/m²/dose.

The study population was monitored for death, adverse events, serious adverse events, and laboratory abnormalities (Table 65). By Day +30 following HSCT, there were 4 deaths (1.5%) on the treosulfan arm, all due to fatal pulmonary adverse reactions, as compared to 2 deaths (0.7%) on the busulfan (control) arm.

Adverse events with treosulfan were mainly similar to those observed with busulfan-based conditioning. Common TEAEs (≥20%) included musculoskeletal pain, stomatitis, pyrexia, nausea, edema, infection, and vomiting. Of those, musculoskeletal pain (12% higher), edema (11% higher), and infection (5% higher) occurred at a rate at least 5% higher in the treosulfan arm than the busulfan arm. The most common grade 3-5 AEs (≥5%) in the treosulfan arm were febrile neutropenia, infection, hypertension, stomatitis, and musculoskeletal pain. Of those, infection occurred at a rate of >5% compared to the busulfan arm (6% higher in the treosulfan arm).

Safety data supporting the dose of treosulfan (10 g/m²/dose) were from studies MC-FludT.6/L, MC-FludT.7/AML, MC-FludT.8/MDS, and MC-FludT.14/L Trials I and II. In this comparison, 290 patients were dosed at 10 g/m²/dose, and 305 patients were dosed at 14 g/m²/dose.

Summary of Major Safety Events

Table 65. Summary of Major Safety Events from MC-FludT.14/L Trial II through Day +30

	Treosulfan (N = 270)	Busulfan (N = 283)
Any TEAE	250 (92.6%)	272 (96.1%)
Grade 3-5 TEAE	148 (54.8%)	151 (53.4%)
SAE	23 (8.5%)	20 (7.1%)
Fatal Adverse Reactions	4 (1.5%)	2 (0.7%)
<i>Source: Reviewer's analysis, ISS population (ADSL, ADAEGT2 datasets), clinical study report</i>		

SUMMARY AND CONCLUSIONS

8.4 Statistical Issues

Pivotal Trial 14-II

Table 66 summarizes statistical issues for Study 14-II and our assessment for them. Because the primary endpoint EFS could not be independently verified by FDA, the review and analysis were focused on OS.

Table 66. Summary of Statistical Issues of Pivotal Trial 14-II

Issues	Sponsor	FDA	Outcome
Primary Endpoint	EFS	Cannot be verified and NI margin not justified	Prespecified primary endpoint and analysis cannot be used
Analysis Set	FAS	ITT	Results are consistent
Analysis Method for OS	Unstratified Log-rank test Adjusted Cox Model	Stratified Log-rank test Stratified Cox Model	When not adjusting for multiplicity and early stopping, OS results are supportive based on HR and 95% CI in the ITT population and the AML and MDS subpopulation
	No alpha boundary was prespecified for OS	Exploratory analysis under hypothetical prespecifications	Mixed results were obtained in exploratory analysis using different methods for multiplicity

- **Analysis set:** ITT and FAS population are similar outcome
- **Interpretation of p-values for OS:** Since there was no pre-specified plan to control multiplicity, p-values are nominal and cannot be included in labeling. To facilitate decision making, we calculated OBF boundaries and Pocock boundaries using information fraction of EFS as an exploratory analysis. As stated in section 8.1.1, the OS result (nominal p-value=0.0049) did not cross the OBF boundary (alpha=0.0025) but crossed the Pocock boundary (alpha=0.014).
- **Trial conduct:** We investigated the informative censoring for OS, time from randomization to transplant and time from diagnosis to randomization between treatment arms. We did not find any trial conduct issue that may impact the data quality or potentially introduce bias.

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Trial 14-I

This trial discontinued after planned interim analysis. It was determined that dose should be lower in this elderly population because neutropenic phase was much longer than control.

This trial preceded Trial 14-II and used the same SAP, therefore all analyses reported by sponsor was based on the FAS population. We did subgroup analysis on OS to investigate any significantly larger detrimental effect on OS comparing to the overall effect. FDA used the ITT population in all analyses. No significantly larger detrimental effect was found in subgroups we analyzed.

8.5 Conclusions and Recommendations

Study 14-II is the sole pivotal trial submitted to support efficacy. Because the primary endpoint EFS could not be independently verified by FDA for Study 14-II, no conclusions can be made based on the primary endpoint. Therefore, the statistical review and analysis were focused on OS for Study 14-II.

There was no prespecified multiplicity approach to test OS after EFS, but there would have been different conclusions regarding statistical significance for OS based on Pocock or O'Brien-Fleming boundaries. These mixed results suggest that it is not clear if OS would have been considered to be significant if there was a prespecified approach; however, no definitive conclusions can be made based on the OS results because a multiplicity approach was not prespecified. The hazard ratio was 0.62 with 95% CI (0.41, 0.92) in AML patients and 0.62 with 95% CI (0.34, 1.10) in MDS patients.

Regarding other statistical issues, the ITT and FAS population were similar and no trial conduct issues were found. From the statistical perspective, the effect of treosulfan as observed in the OS results appears to be supportive; however, interpreting this data is challenging because there was no prespecified plan to control multiplicity.

In the absence of verification of the primary endpoint, and without prespecification of Type-1 error for multiple comparisons, Study 14-II does not provide considerable evidence of effectiveness. There is insufficient evidence of effectiveness to outweigh any risks.

9 Advisory Committee Meeting and Other External Consultations

This application was not discussed by an advisory committee.

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10 Pediatrics

Because treosulfan has orphan drug designation and is not a molecularly targeted agent, PREA did not apply.

11 Labeling Recommendations

In light of the regulatory recommendation, labeling review was not completed.

12 Risk Evaluation and Mitigation Strategies (REMS)

Not applicable

13 Postmarketing Requirements and Commitments

Not applicable

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14 Division Director (DHM1)

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15 Office Director (or designee)

The clinical portion of this application was reviewed by the Oncology Center of Excellence (OCE) per the OCE Intercenter Agreement.

16 Appendices

16.1 References

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16.2 Financial Disclosure

Covered Clinical Study (Name and/or Number):

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: ____		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): ____		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455):		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: ____ Significant payments of other sorts: ____ Proprietary interest in the product tested held by investigator: ____ Significant equity interest held by investigator in S Sponsor of covered study: ____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes	No (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes	No (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) ____		
Is an attachment provided with the reason:	Yes	No (Request explanation from Applicant)

16.3 Nonclinical Pharmacology/Toxicology

No additional data.

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16.4 OCP Appendices

16.4.1 Pharmacometrics Review

A population pharmacokinetics (PPK) model was developed by the Applicant to characterize the PK of treosulfan in adults and pediatric patients received allogeneic transplant. Patients intrinsic and extrinsic factors which influence the PK and PK variability of treosulfan were identified. The exposure-response (E-R) relationships for efficacy and safety were explored using multivariate analyses. In this review, FDA Pharmacometrics Reviewer verified the Applicant's analyses, as well as conducted independent analyses to evaluate the Applicant's dose selection.

1. Population PK Analyses

Originally, the Applicant developed PPK model based on seven historical studies (from mostly adults and only a limited number of children,

Table 67) and 2 new pediatric studies (**Table 68**). However, due to the invalidated bioanalytical methods for the quantification of treosulfan, all PK data of the non-medac-sponsored studies were removed from the PPK analysis upon the FDA request. The updated dataset for PPK analysis included 1 historical medac sponsored trial (MC-FludT.14/L Trial I with 24 adult subjects) and 2 new pediatric medac trials (MC-FludT.16/NM and MC-FLudT.17/M with 83 subjects). The summary of the continuous covariates for the subjects included in the updated final PPK model are shown in

Table 69. In addition, the dataset included 63 male and 44 female subjects.

Table 67. Historical trials included in the final PPK analysis

Study	Description	No. of Subjects	Dose level (g/m ²); Single or multiple dose	Planned Infusion duration (hours)
FHCRC 1931	Adults + Children; Allogeneic Transplant	20	12-14; MD	2
GLOWKA	Children; Allogeneic Transplant	5	10-12; SD	2
MC_TC2_L	Adults; Allogeneic Transplant	20	12-14; MD	2
SOLCA3	Adults; Conventional Therapy	18	8-10; MD	0.5, 2
SYKORA	Children; Allogeneic Transplant	14	12-14; MD	2
MC_FLUDT14_L	Adults; Allogeneic Transplant	24	14; MD	2
SOLCA5	Adults; Autologous Transplant	15	20-47; MD	2

Source: Applicant population PK study report 2020

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Table 68. Study overview with planned number of subjects and dose schedule for the two new pediatric studies

Study	Description	Planned number of study subjects	Planned number of subjects for PK analysis	Route and planned dose schedule
MC-FludT.16/NM	Compare Treosulfan-based conditioning therapy with Busulfan-based conditioning prior to Allo-HSCT in children with non-malignant diseases	At least 100 children from 28 days to less than 18 years including at least: - 40 subjects from 28 days to less than 4 years; - 40 subjects from 4 to < 12 years.	- in all subjects <2 years of age - in a minimum of 12 subjects in the age group 2-<5 years of age - in a minimum of eight subjects in the age group 5-<10 years of age	One infusion of 2 hours on three consecutive days
MC-FludT.17/M	Describe safety and efficacy of Treosulfan-based conditioning therapy prior to Allo-HSCT in children with haematological malignancies	At least 70 children from 28 days to less than 18 years including at least: - 30 subjects from 28 days to less than 10 years; - 30 subjects from 10 years to less than 18 years; - 50 subjects receiving a first HSCT.	- in a minimum of eight subjects in the age group 10-17 years of age	One infusion of 2 hours on three consecutive days

Source: Applicant population PK study report 2020

Table 69. Summary of continuous covariates for the subjects included in studies MC-FludT.14/L Trial I, MC-FludT.16/NM and MC-FludT.17/M in the dataset for the updated PPK analysis

	BSA [m ²]	AGE [yr]	WGT [kg]	HGT [cm]	CCG [ml/min/1.73m ²]
n	107	107	107	107	107
Mean	1.123	17.80	37.49	128.2	153.5
SD	0.57	21.55	27.32	37.73	60.17
Min-Max	0.30-2.25	0.33-70	5.60-114	57.0-189	47.0-403.7
Median	1.00	8.00	28.10	131.0	152.4
5% perc	0.40	0.61	7.65	68.0	69.30
95% perc	2.00	63.00	84.45	174.0	256.18

Source: Applicant population PK study report 2021

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The final PPK model for treosulfan is a two-compartment model with linear elimination and intercompartmental clearance (Figure 16). To account for the bioassay differences between historical (MC-FludT.14/L Trial I) and new data (MC-FludT.16/NM and MC-FludT.17/M), a SHIFT model parameter was included to describe this difference ($C_{p,new} = \text{SHIFT} \times C_p$ and $C_{p,hist} = C_p$ where C_p represents the predicted concentration). BSA effect was included as a significant covariate on CL, V1, V2 and Q in the final model.

Figure 16. Model structure of the final PPK model of treosulfan

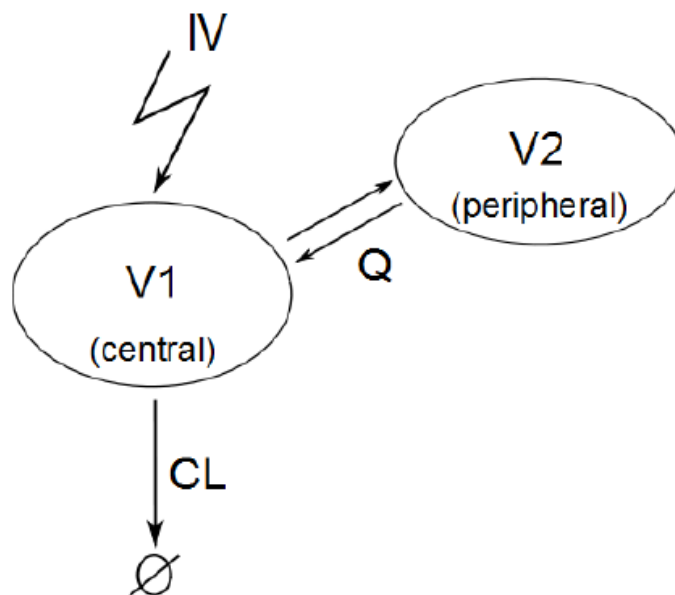


Figure 1: Two compartmental model describing disposition of Treosulfan. After IV administration into the central compartment V1, Treosulfan is distributed into the peripheral compartment V2 and eliminated. Both processes have linear kinetics determined by inter-compartmental clearance Q and clearance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

Source: Applicant population PK study report 2020

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Parameter estimates of the updated final model are listed in Table 70.

Table 70. Parameter estimates of the final PPK model

Parameter	Value	RSE
V1 [L]	29.45	6.34
CL [L/h]	15.83	3.63
V2 [L]	7.64	16.01
Q [L/h]	5.46	30.51
BSA on V1	1.72	8.38
BSA on CL	1.11	4.49
BSA on V2	0.82	8.32
SHIFT bioassay	1.30	5.47
IIV V1	0.236	35.7
IIV CL	0.174	10.76
IIV V2	0.195	34.99
IIV Q	0.263	45.35
$\Omega(V1,CL)$	0.693	17.04
σ CCV	0.185	11.06
OFV	6298.941	

IIV=variance of between-subject variability (on sd scale)

Ω =covariance between between-subject variability

σ =variance of residual error ϵ

Note: the relative standard error (RSE) on the between-subject variability and residual error are reported on the approximate standard deviation scale (SE/variance estimate)/2.

Source: Applicant population PK study report 2021

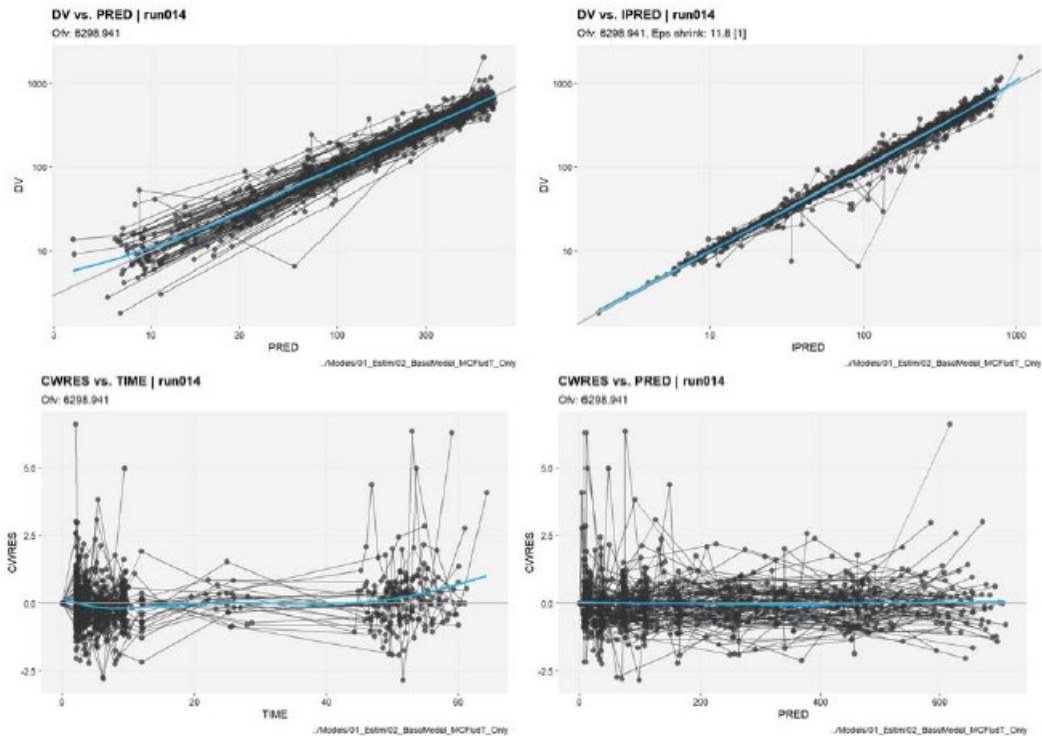
The goodness-of-fit (GOF) plots for pooled data are shown in **Figure 17**. The VPC plots stratified by BSA groups and studies are shown in **Figure 18**.

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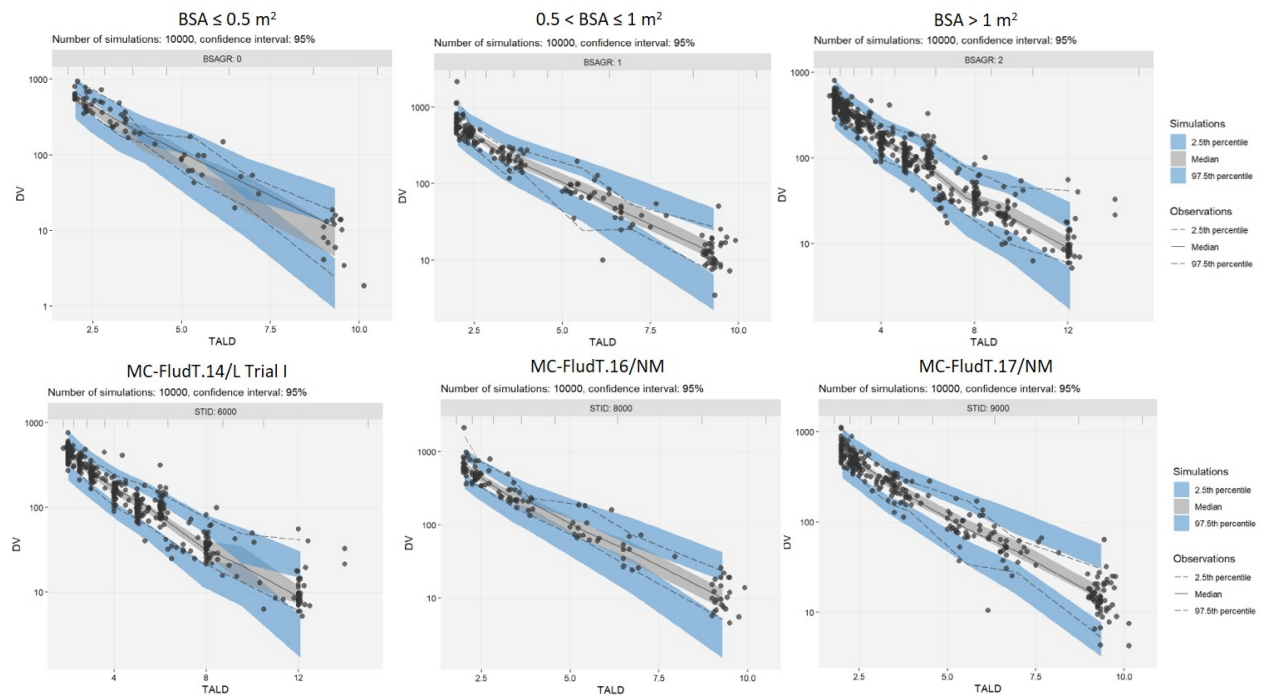
Grafapex (treosulfan)

Figure 17. Goodness-of-fit plots for pooled data



Source: Applicant population PK study report 2021

Figure 18. VPCs of the final model displayed by BSA groups and study



Source: Applicant population PK study report 2021

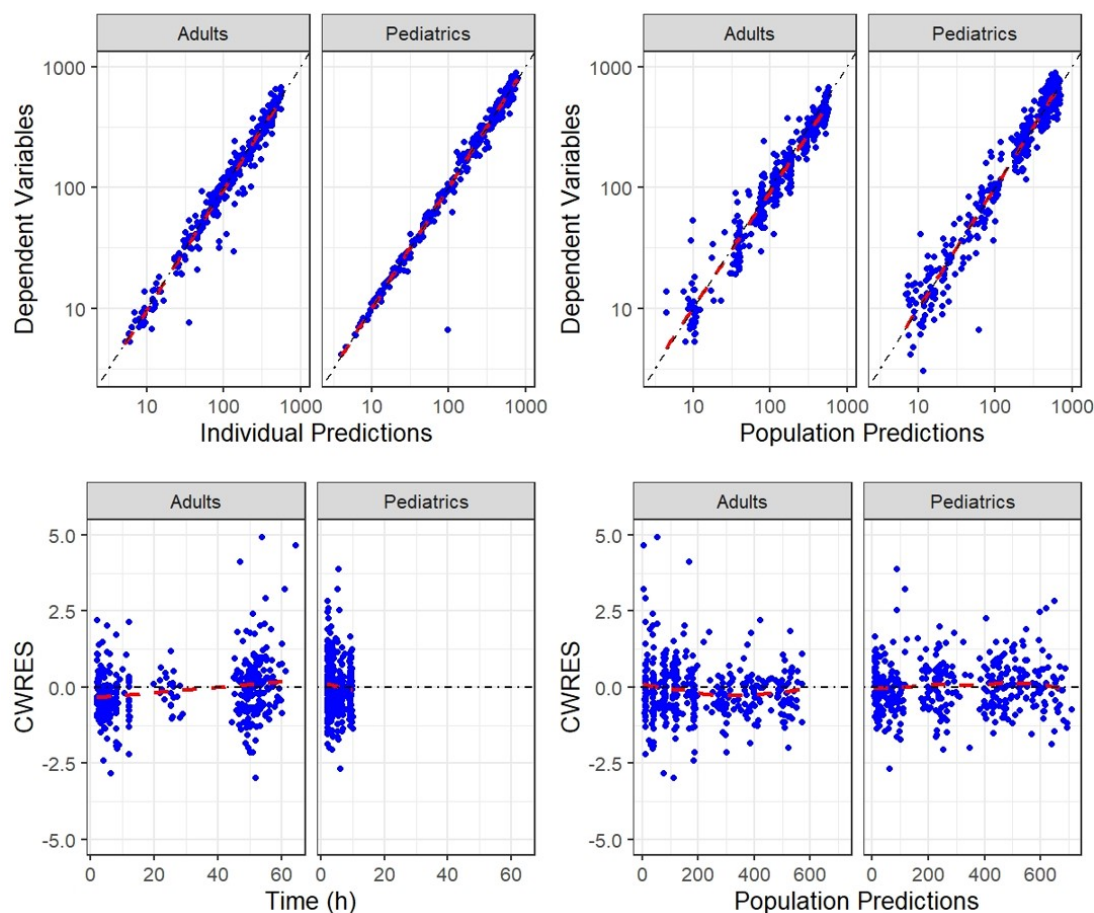
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The Reviewer also evaluated the GOF for adults and pediatrics, respectively. As shown in **Figure 19**, in both adult and pediatric population, the updated PPK model adequately described the observed treosulfan concentrations.

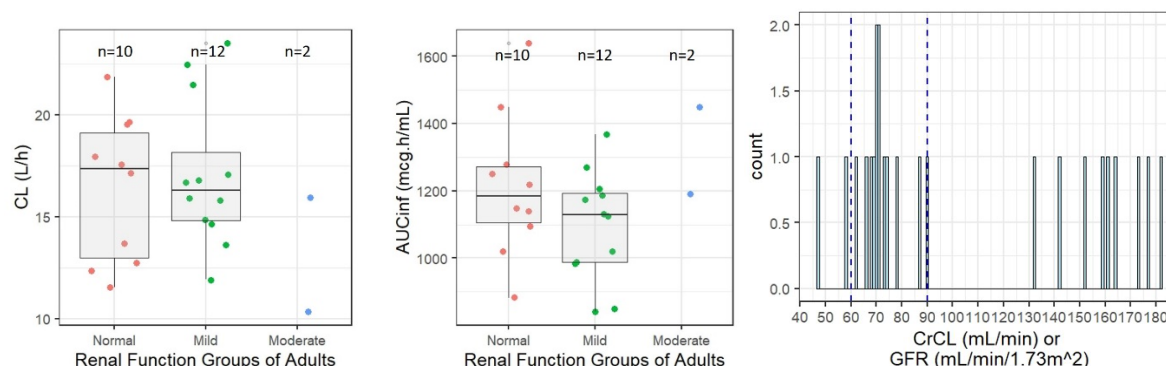
Figure 19. Goodness-of-fit plots stratified by population



Source: Reviewer's analysis

Based on the GOF and VPC plots, the Applicant's updated final PPK analysis is acceptable for describing the PK of treosulfan in adult and pediatric patients. In the Applicant's PPK model development, multiple parameters could not be precisely estimated, and the effect of renal impairment on CL was removed. Thus, the Reviewer conducted independent analysis to evaluate the impact of renal function on treosulfan clearance and exposure in adults. As shown in **Figure 20**, a trend of lower BSA-adjusted CL and higher AUCinf (but not significant) in moderate renal impairment subjects (n=2) was observed compared to subjects with normal renal function (n=10). Mild renal impairment (n=12) appears to have no impact on CL and AUCinf of treosulfan. However, it is important to note that the number of subjects in the moderate renal impairment category was very limited. In addition, the lowest CrCL included in the analysis was 47 mL/min, which is still much higher than the lower cutoff of the moderate renal impairment (30 mL/min).

Figure 20. Apparent CL (BSA-adjusted) and AUC_{inf} of treosulfan in different renal function groups



Source: Reviewer's analysis

2. Exposure-response (E-R) analysis for efficacy

2.1 Adult patients

The Applicant evaluated the potential relationship between exposure and time to engraftment (defined as time from Day 0 to neutrophil engraftment (first of 3 consecutive days with ANC >0.5 x10⁹/L)) in adult patients based on linear regression model, Kaplan-Meier curves and multivariate analyses. The original analyses were based on both historical trials (SOLCA3 and SOLCA5 were excluded due to lack of information on baseline demographic and disease characteristics) and medac-sponsored trials with the dose range from 10 g/m² single dose to 14 g/m² multiple dose. No significant E-R relationship between AUC versus time to engraftment was identified. The updated E-R analyses based on updated PPK model only included 24 adult patients following the dosing regimen of 14 g/m² in the historical medac-sponsored trial MC-FludT.14/L Trial I. No PK data were collected from Trial II at the recommended dosing regimen of 10 g/m². Thus, with a limited number of subjects and narrow exposure range at a higher than recommended dosing regimen, the updated E-R analyses in adult patients are not considered informative.

2.2 Pediatric patients

Upon FDA's request, the Applicant conducted E-R analyses for time to engraftment based on 2 pediatric trials using Cox proportional hazards models with AUC and C_{max} estimated by the updated PPK analyses. No significant relationship between treosulfan exposure and time to engraftment was demonstrated. In addition, no evidence was observed with regard to a potential effect of baseline demographic and disease characteristic variables on time to engraftment. However, because some of the pediatric patients in the studies FludT.16/NM and MC-FludT.17/M received thiotepa, the E-R analyses results should be interpreted with caution.

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3. Exposure-response analyses for safety

Applicant original E-R analyses for safety were based on pooled data from adult and pediatric patients included in the original PPK analysis. After the update by removing the invalid PK data, only 24 adult patients in trial MC-FludT.14/L Trial I were retained, and all of them were dosed with 14 g/m² treosulfan. In addition, the safety in adult patients were compared between MC-FludT.14/L Trial I (14 g/m²/day × 3 days of treosulfan on -6 to -4 day) and Trial II (10 g/m²/day × 3 days of treosulfan on -4 to -2 day). Compared to the proposed dosing regimen (10 g/m²/day × 3 days on -4 to -2), the higher dose regimen (14 g/m²/day × 3 days of treosulfan on -6 to -4 day) resulted in longer duration of neutropenia (17.5 vs. 14 days), higher infection rate (11.9% vs 8.1%) and higher infection-related death (16.1% vs 9.3%). Thus, given the available dose-response relationship for safety as well as the narrow exposure range within the 24 adult patients with valid PK data, no additional exposure-safety analyses in adults were conducted based on updated PK parameters. For details on dose-response relationship for safety, refer to sections 6.3.2 and 8.3.5 in the unireview.

For pediatric patients, the Applicant conducted multivariate E-R analyses for safety endpoints, including duration of neutropenia, infections, infection-related death and non-relapse mortality based on the updated PK parameters in patients (n=82) from trials MC-FludT.17/M and MC-FludT.16/NM, upon FDA's request. Both AUC and C_{max} were assessed. The potential effects of age, [REDACTED], disease type, donor type and Karnofsky/Lansky performance score were investigated. Treosulfan dose in pediatric PK patients (10, 12, or 14 g/m²) was BSA-adapted according to trial protocols MC-FludT.16/NM and MC-FludT.17/M. Again, because some of the pediatric patients in the studies FludT.16/NM and MC-FLudT.17/M received thiotepea, the E-R analyses results should be interpreted with caution.

Infection-related mortality

Out of 82 children from both malignant and non-malignant diseases trials, only 1 pediatric patient (1.2%) with the event of infection-related death was observed. This patient had a significant increased AUC and C_{max}. Because of the very limited number of subject (n=1), the statistically significant impact of AUC and C_{max} are not considered meaningful.

Infection

Out of 82 pediatric patients, 33 (40.2%) subjects with infections with at least CTCAE grade III were observed, and they were equally distributed between malignant and non-malignant diseases. No significant effect of treosulfan AUC or C_{max}, baseline demographic and disease characteristic variables could be detected by logistic regression models.

Gastrointestinal (GI) disorders

Out of 82 patients, 41 (50.0%) subjects with GI disorder with at least CTCAE grade III were observed. No significant effect of treosulfan AUC or C_{max} was detected by logistic regression models. Only the age of patients had a significant effect on risk for GI disorder.

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Duration of neutropenia

In the 80 pediatric patients, no significant effect of treosulfan AUC or C_{max} , baseline demographic and disease characteristic variables could be detected by linear regression models. In addition, no evidence was observed with regard to a potential effect of baseline demographic and disease characteristic variables.

4. PK Simulation to Support Pediatric dose selection

The Applicant proposed the following dose levels in pediatric patients to obtain comparable treosulfan AUC24h to an average adult after administration of treosulfan 10 g/m² (refer to section 6.3.2):

- 9.0 g/m² if BSA ≤ 0.7 m² or in the age range 1 to 4 years
- 9.5 g/m² if 0.7 m² < BSA ≤ 1.1 m² or in the age range 5 to 9 years
- 10.0 g/m² if BSA > 1.1 m² or in the age range 10 to 18 years

The Applicant's pediatric dosing regimen was identified to provide similar exposure in pediatrics to adult patients administrated with 10 g/m² of treosulfan (Table 71).

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Table 71. Simulated AUC24h for different BSA and age groups by dose regimen. Highlighted dosing regimens are equal or higher than target AUC24h of average American adult

Age [yr]	BSA [m ²]	8 g/m ²	8.5 g/m ²	9 g/m ²	9.5 g/m ²	10 g/m ²	10.5 g/m ²	11 g/m ²
1	0.462	1026	1090	1154	1218	1283	1347	1411
2	0.552	1006	1069	1132	1195	1258	1321	1383
3	0.63	992	1054	1116	1177	1239	1301	1363
4	0.707	979	1040	1101	1163	1224	1285	1346
5	0.779	969	1029	1090	1150	1211	1271	1332
6	0.864	958	1017	1077	1137	1197	1257	1317
7	0.954	947	1006	1066	1125	1184	1243	1303
8	1.05	937	996	1054	1113	1172	1230	1289
9	1.139	929	987	1045	1103	1161	1219	1277
10	1.24	920	978	1035	1093	1150	1208	1265
11	1.366	911	967	1024	1081	1138	1195	1252
12	1.474	903	959	1016	1072	1129	1185	1242
13	1.576	896	952	1008	1064	1120	1176	1232
14	1.671	891	946	1002	1057	1113	1169	1224
15	1.755	886	941	996	1052	1107	1162	1218
16	1.792	884	939	994	1049	1105	1160	1215
17	1.817	882	937	993	1048	1103	1158	1213
18	1.853	880	935	990	1045	1100	1155	1211

Source: Applicant's population PK report 2021.

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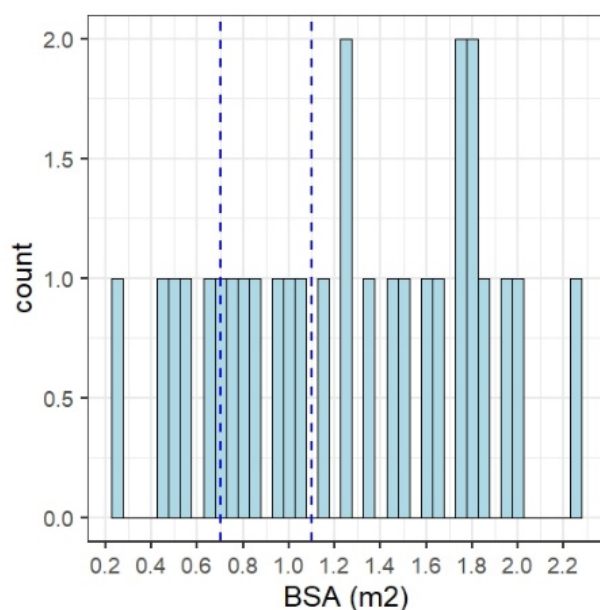
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To evaluate whether the Applicant proposed pediatric dosing regimen is reasonable, the Reviewer conducted independent simulation and compared the predicted AUC_{24h} among different BSA groups and dosing regimens. Average BSA per age group was calculated by the Applicant from average population body weight and height surveyed in the United States. The simulation was conducted for 1000 replicates to generate a reasonable number of virtual subjects (n=28000) for each dosing regimen. The BSA distribution included for each simulation is shown in **Figure 21**.

Simulation was performed using the Applicant developed final PPK model. The simulations used 1000 replicates of the Applicant generated virtual population as discussed above with 28 subjects per replicate. The software used to perform the simulation was NONMEM 7.4. The simulation result shows that the Applicant proposed pediatric dosing regimen is expected to produce similar AUC_{24h} compared to the median AUC_{24h} in adult following 10 g/m² dosing (target exposure). However, a simplified dosing regimen of 10 g/m² for the entire pediatric population with BSA range of 0.25 to 2.25 m² is also predicted to provide comparable AUC_{24h} without clinically significant difference compared to the target exposure in adults. For pediatric patients with BSA ≤ 0.7 m², AUC_{24h} is expected to be 11.2% higher than the target exposure; for pediatric subjects with BSA within 0.7 to 1.1 m², AUC_{24h} is expected to be 4.7% higher than the target exposure; for pediatric subjects with BSA > 1.1 m², AUC_{24h} is expected to be 2.8% lower than the target exposure in adults, as shown in **Figure 22**. Thus, for the convenience of dosing and to avoid potential dosing error, we recommend the 10 g/m² dosing regimen for all pediatric patients with the age ≥ 1 year.

Figure 21. BSA distribution in the simulation for pediatric dosing selection



Blue dash lines: BSA of 0.7 m² and 1.1 m²

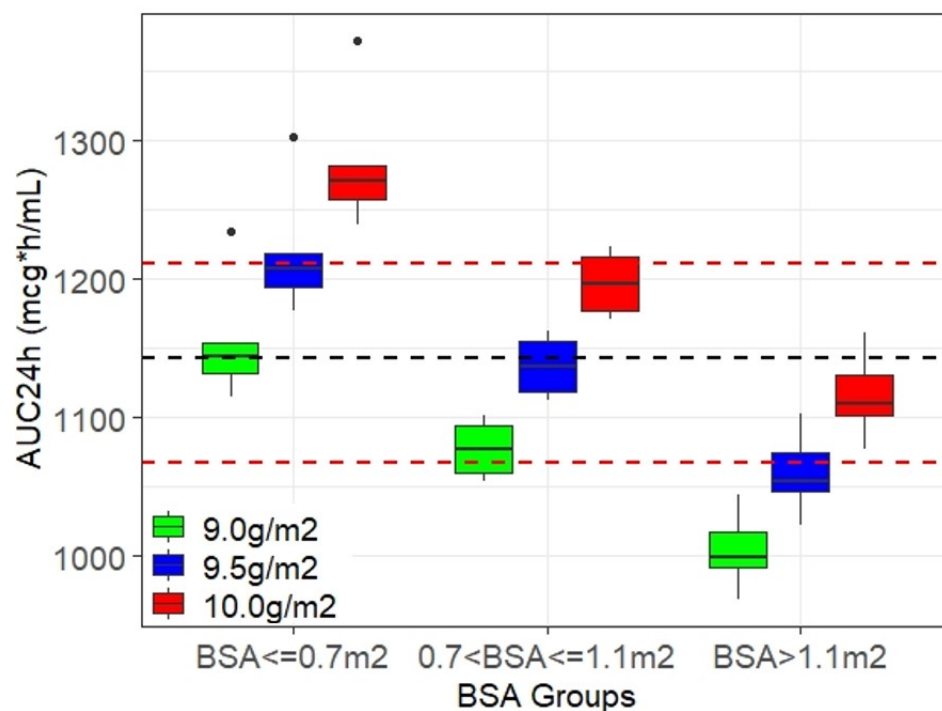
Source: Reviewer's analysis

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Figure 22. Comparison of AUC_{24h} in pediatric population among different dosing regimens and BSA groups



Red dash lines: 25th and 75th percentiles of adult AUC_{24h} following 10g/m²; black dash line: median of adult AUC_{24h} following 10g/m² (target exposure);

Source: reviewer's analysis

16.4.2 PBPK Review

Executive Summary

The objective of this review is to evaluate the adequacy of the Applicant's PBPK report, entitled "Treosulfan DDI" to predict the effects of treosulfan on the PK of 1) midazolam (a CYP3A4 substrate), omeprazole (a CYP2C19 substrate) and digoxin (a P-gp substrate).

The Division of Pharmacometrics has reviewed the PBPK report, supporting modeling files, and the Applicant's response to the FDA's information requests (IRs) submitted on December 21, 2020, March 3, 2021 and April 27, 2021 and concluded the followings.

- The treosulfan PBPK model is adequate to predict the treosulfan PK profiles following 2 hours infusion of 14 g/m² for three consecutive days.
- There is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited an inhibitory effect on CYP enzymes and P-gp in in vitro incubation systems.
- The PBPK model and mechanistic static model were used to predict the magnitude of enzyme mediated DDI caused by treosulfan and its metabolite, EBDM, respectively. Results

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of analysis showed that the potential DDI of treosulfan as a CYP3A4 inhibitor and a CYP2C19 inhibitor cannot be excluded.

- The PBPK model was used to predict the magnitude of P-gp mediated DDI caused by treosulfan. Results of analysis showed that the potential DDI of treosulfan as a P-gp inhibitor cannot be excluded. This could be important for P-gp substrates with a narrow therapeutic index.

Applicant's PBPK Modeling Effort

PBPK software

PK-Sim V8.0 (Open Systems Pharmacology) was used by the Applicant to develop the PBPK models and for DDI predictions. The reviewer used the PK-Sim V 9.0 for analyses.

Model development

Treosulfan

The treosulfan PBPK model was developed for intravenous dosing in healthy subjects. The plasma protein binding of treosulfan is considered to be negligible. The population pharmacokinetic model estimated volume of distribution is about 40 L, indicating that treosulfan may distribute into both extracellular and intracellular fluid. The standard PK-Sim 3-compartment model (intracellular, interstitial and vascular space) with a single permeation barrier between the interstitial and intracellular space in each organ was used to characterize the distribution of treosulfan.

Treosulfan is a water-soluble prodrug that is converted non-enzymatically at pH 7.4 and 37 °C to two active metabolites (EBDM and DEB) along with the release of methanesulfonic acid. Treosulfan's nonenzymatic activation is pH- and temperature- dependent and is stopped at pH <5.0². This activation process occurs in both blood and tissue compartments. Treosulfan is excreted in the kidney via glomerular filtration, and the fraction of unchanged treosulfan excreted in urine is about 15-40%. The fraction excreted via glomerular filtration was estimated based on the observed accumulative amount of unchanged treosulfan excreted in the urine. The observed plasma PK of treosulfan following the intravenous infusion was used to estimate the systemic transformation rate of treosulfan.

In vitro studies indicated that treosulfan was a reversible inhibitor for CYP2C19 with an IC₅₀ value of 972 µM. Treosulfan was a reversible inhibitor for CYP3A4 using midazolam as a substrate with an IC₅₀ value of 1870 mM but did not show inhibitory effect on CYP3A4 when using testosterone as a substrate. In vitro transport studies showed that treosulfan inhibited P-gp-mediated transport of loperamide with an IC₅₀ value of ~3000 mM.

² Michał Romański, Jacek Wachowiak, Franciszek K Główka Treosulfan Pharmacokinetics and its Variability in Pediatric and Adult Patients Undergoing Conditioning Prior to Hematopoietic Stem Cell Transplantation: Current State of the Art, In-Depth Analysis, and Perspectives. Clin Pharmacokinet. 2018 Oct;57(10):1255-1265.

Victim drug models

Midazolam

The midazolam PBPK model was used to predict the DDI potential of treosulfan as a CYP3A4 inhibitor. The Applicant's midazolam PBPK model was based on Hanke et al.³ The performance of midazolam PBPK model as a CYP3A substrate was validated with 32 clinical studies. Representative model validation and DDI evaluation results were shown in Figure 23.

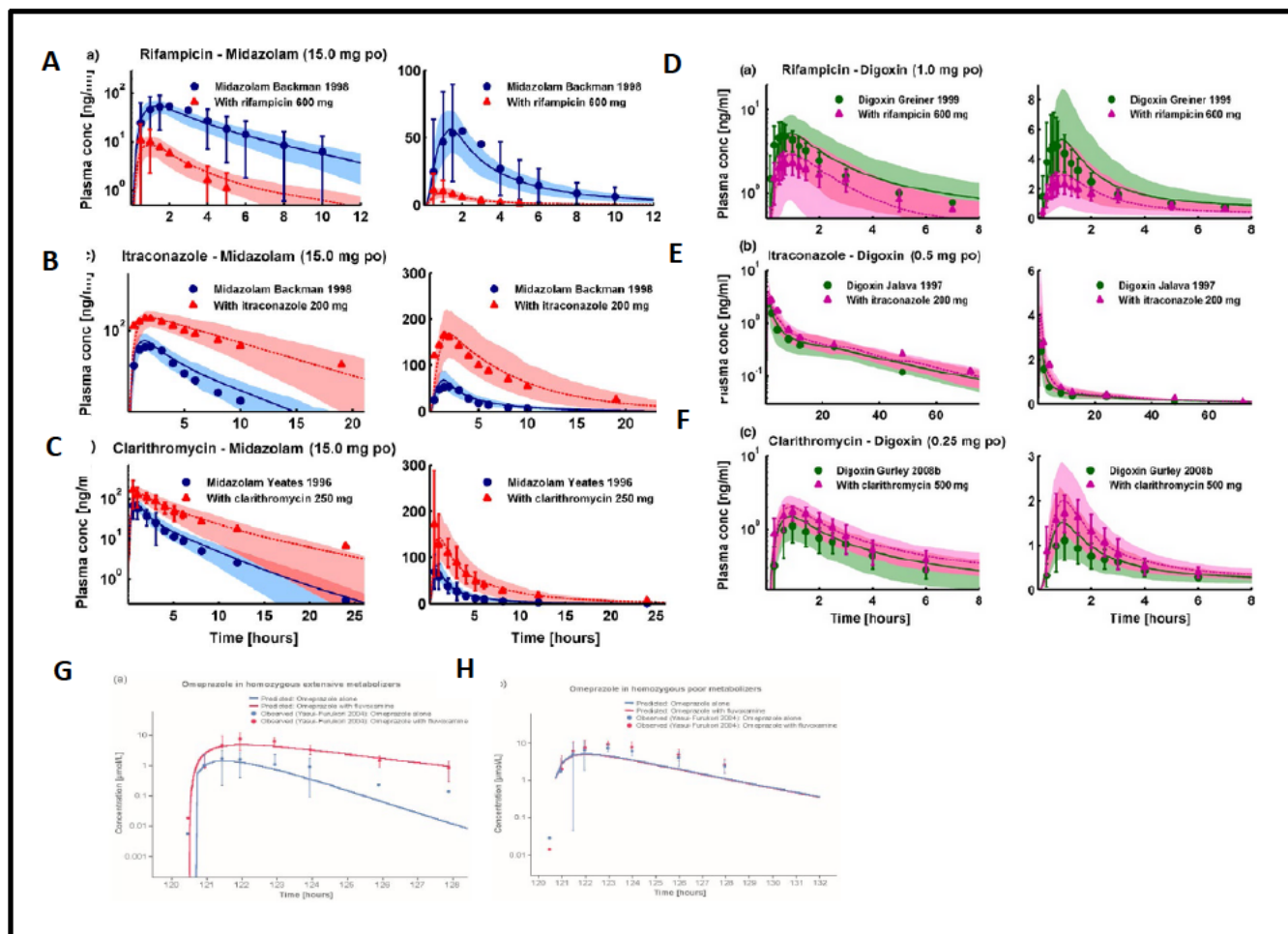


Figure 23. Midazolam, digoxin and omeprazole PBPK model validation.

Representative observed and model simulated midazolam PK profiles with and without rifampicin (A), itraconazole (B), or clarithromycin (C) coadministration⁴, digoxin PK profiles with

³Nina Hanke, Sebastian Frechen, Daniel Moj, Hannah Britz, Thomas Eissing, Thomas Wendl, Thorsten Lehr. PBPK Models for CYP3A4 and P-gp DDI Prediction: A Modeling Network of Rifampicin, Itraconazole, Clarithromycin, Midazolam, Alfentanil, and Digoxin. CPT Pharmacometrics Syst Pharmacol. 2018 Oct;7(10):647-659.

⁴Nina Hanke, Sebastian Frechen, Daniel Moj, Hannah Britz, Thomas Eissing, Thomas Wendl, Thorsten Lehr. PBPK Models for CYP3A4 and P-gp DDI Prediction: A Modeling Network of Rifampicin, Itraconazole, Clarithromycin, Midazolam, Alfentanil, and Digoxin. CPT Pharmacometrics Syst Pharmacol. 2018 Oct;7(10):647-659.

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and without rifampicin (D), itraconazole (E), or clarithromycin (F) coadministration⁵, and omeprazole PK profiles with and without fluvoxamine coadministration in homozygous extensive metabolizers (G), or with and without fluvoxamine coadministration in homozygous poor metabolizers (H)⁶.

Esomeprazole and Romeprazole

Omeprazole is a proton pump inhibitor. It is administered as a racemic mixture of its two enantiomers, esomeprazole and R-omeprazole. Omeprazole is metabolized mainly by CYP2C19 and CYP3A4. Both enantiomers are competitive and time-dependent inhibitors of CYP2C19. The omeprazole PBPK models were used to predict the DDI potential of treosulfan as a CYP2C19 inhibitor. The Applicant's esomeprazole and R-omeprazole PBPK models were based on Kanacher et al.⁷. The DDI between omeprazole and CYP2C19 inhibitors was validated with 2 clinical studies. Representative model validation and DDI evaluation results were shown in Figure 23.

Digoxin

Digoxin is a P-gp substrate. The digoxin PBPK model was used to predict the DDI potential of treosulfan as a P-gp inhibitor. The Applicant's digoxin PBPK model was based on Hanke et al.⁸. The DDI between digoxin and P-gp inhibitors was validated with 12 clinical studies. Representative model validation and DDI evaluation results were shown in Figure 23.

FDA's assessment

The Applicant conducted in vitro studies to evaluate the inhibitory effect of treosulfan toward CYPs and transporters and the in-vitro Ki values for CYP3A4, CYP2C19 and P-gp were incorporated into the PBPK model to evaluate the DDI potential of treosulfan as a perpetrator with CYP3A4, CYP2C19 and P-gp substrates. (b) (4)

information requests were issued

⁵ Nina Hanke, Sebastian Frechen, Daniel Moj, Hannah Britz, Thomas Eissing, Thomas Wendl, Thorsten Lehr. PBPK Models for CYP3A4 and P-gp DDI Prediction: A Modeling Network of Rifampicin, Itraconazole, Clarithromycin, Midazolam, Alfentanil, and Digoxin. CPT Pharmacometrics Syst Pharmacol. 2018 Oct;7(10):647-659.

⁶ Tobias Kanacher, Andreas Lindauer, Enrica Mezzalana, Ingrid Michon, Celine Veau, Jose David Gómez Mantilla, Valerie Nock, Angèle Fleury. A Physiologically-Based Pharmacokinetic (PBPK) Model Network for the Prediction of CYP1A2 and CYP2C19 Drug-Drug-Gene Interactions with Fluvoxamine, Omeprazole, S-mephenytoin, Moclobemide, Tizanidine, Mexiletine, Ethinylestradiol, and Caffeine. Pharmaceutics. 2020 Dec 8;12(12):1191.

⁷ Tobias Kanacher, Andreas Lindauer, Enrica Mezzalana, Ingrid Michon, Celine Veau, Jose David Gómez Mantilla, Valerie Nock, Angèle Fleury. A Physiologically-Based Pharmacokinetic (PBPK) Model Network for the Prediction of CYP1A2 and CYP2C19 Drug-Drug-Gene Interactions with Fluvoxamine, Omeprazole, S-mephenytoin, Moclobemide, Tizanidine, Mexiletine, Ethinylestradiol, and Caffeine. Pharmaceutics. 2020 Dec 8;12(12):1191.

⁸ Nina Hanke, Sebastian Frechen, Daniel Moj, Hannah Britz, Thomas Eissing, Thomas Wendl, Thorsten Lehr. PBPK Models for CYP3A4 and P-gp DDI Prediction: A Modeling Network of Rifampicin, Itraconazole, Clarithromycin, Midazolam, Alfentanil, and Digoxin. CPT Pharmacometrics Syst Pharmacol. 2018 Oct;7(10):647-659.

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requesting the Applicant to evaluate the time dependent treosulfan stability in incubation media to better understand the in vitro inhibition study results.

Applicant's response to FDA's IR



FDA's assessment

1. CYP inhibition study

The in vitro treosulfan buffer stability study indicated that both treosulfan and EBDM were present in the in vitro incubation system, even at the beginning of the incubation. The concentration of EBDM at time 0 was 2121 mM in 10,000 mM treosulfan solution in phosphate buffer in the in vitro buffer stability study, where the samples were prepared and analyzed directly. In the in vitro CYP inhibition study, a time interval would be expected

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between the solution preparation and initiation of pre-incubation, thus the concentration of EBDM at time 0 may be higher than 2121 mM in 10,000 mM treosulfan solution in phosphate buffer. In addition, the EBDM concentrations in the phosphate buffer appeared to reach a plateau at around 2 hours post-incubation. Therefore, the fact that observed inhibitory effect was not altered after pre-incubation for 30 min would not be adequate to justify that the inhibitory effect was not caused by EBDM. There is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on CYP enzymes in vitro. For further discussion regarding the DDI potential of treosulfan as a CYP inhibitor, please refer to the result section.

2. P-gp inhibition study

Both treosulfan and its two metabolites, EBDM and DEB were present in the in vitro transport system after 60 min incubation. In addition, P-gp preferentially transports more lipophilic substrates. Given the ranked lipophilicity is DEB>EBDM>treosulfan, there is also an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on P-gp in in vitro system. For further discussion regarding the DDI potential of treosulfan as a P-gp inhibitor, please refer to the result section.

PBPK model application

The developed PBPK model was used to simulate the DDI for treosulfan in the following scenarios.

- To predict the effect of treosulfan on the PK of midazolam (a CYP3A4 substrate) following multiple intravenous infusion of treosulfan (14 g/m², QD for 3 days) and a single oral dose administration of midazolam (2 mg) on day 2 in healthy subjects.
- To predict the effect of treosulfan on the PK of omeprazole (a CYP2C19 substrate) following multiple intravenous infusion of treosulfan (14 g/m², QD for 3 days) and multiple oral dose administration of omeprazole (10 mg, QD for 3 days) in healthy subjects.
- To predict the effect of treosulfan on the PK of digoxin (a P-gp substrate) following multiple intravenous infusion of treosulfan (14 g/m², QD for 3 days) and multiple oral dose administration of digoxin (1 mg, QD for 3 days) in healthy subjects.

Results

1. Can treosulfan PBPK model describe treosulfan PK in healthy subjects?

Yes. The treosulfan model was able to capture the observed treosulfan PK profiles and accumulative amount of unchanged treosulfan in the urine following 2 hr intravenous infusion of 14 g/m² on three consecutive days ([Figure 24 and Table 72](#)).

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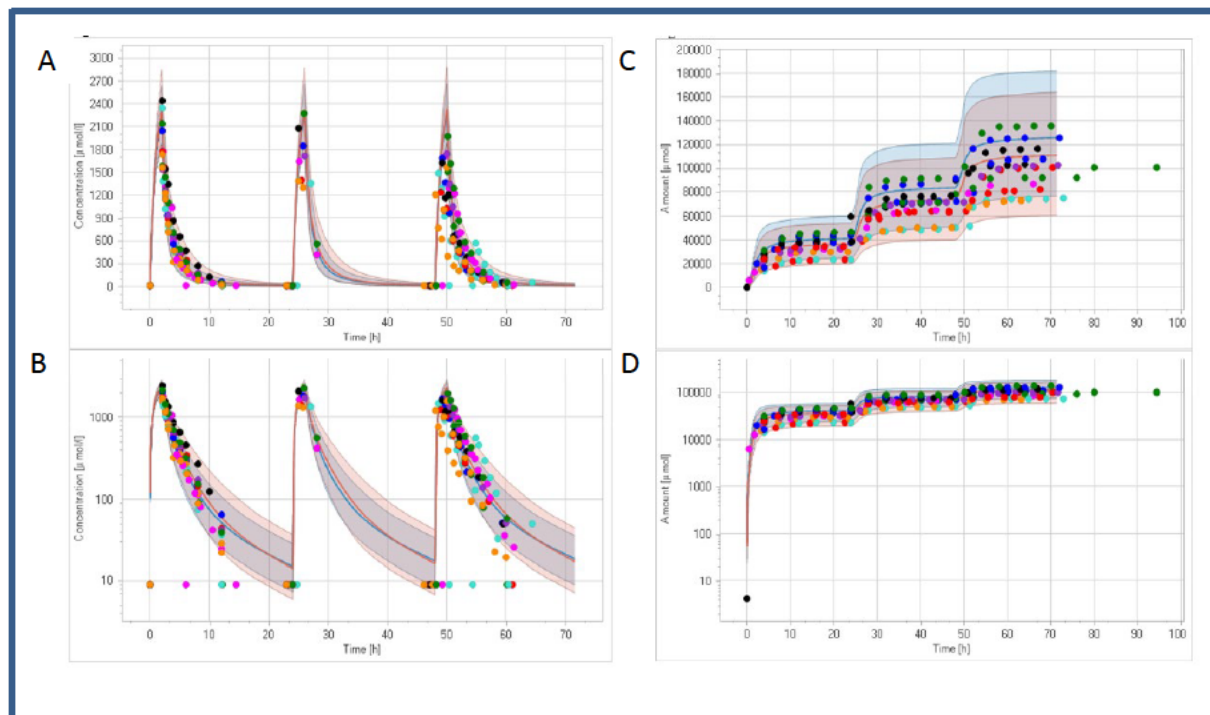


Figure 24. Observed and simulated treosulfan concentration-time profiles

Observed and simulated treosulfan concentration-time profiles following 2 hr intravenous infusion of 14 g/m² on three consecutive days (A and B). Observed and simulated accumulative amount of unchanged treosulfan excreted in the urine following 2 hr intravenous infusion of 14 g/m² on three consecutive days (C and D). A and C: linear scale and B and D: logarithmic scale. Source: Observed data were obtained from patients with AML or MDS in Study MC-FludT.14L, while simulated data were generated in healthy subjects.

Table 72. Observed and simulated treosulfan geometric mean PK parameters and predicted-to-observed ratios following 2 hr intravenous infusion of 14 g/m² on three consecutive days.

		Observed	Simulated	Predicted-to-observed ratio
AUC (h*ug/mL)	Day 1	1461	1943	1.33
Cmax (ug/mL)		470	639	1.36
AUC (h*ug/mL)	Day 3	1503	1984	1.32
Cmax (ug/mL)		434	643	1.48
Amount of treosulfan excreted in the urine (mg)	Day 1	10339	9560	0.93
	Day 2	20573	19344	0.94
	Day 3	30088	29176	0.97

Source: Observed data were obtained from patients with AML or MDS in Study MC-FludT.14L, while simulated data were generated in healthy subjects.

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Grafapex (treosulfan)

2. Can treosulfan PBPK model predict the effect of treosulfan on the PK of midazolam (a CYP3A4 substrate)?

As aforementioned, there is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on CYP3A4 in in vitro incubation system. Two potential scenarios are discussed in more detail as follows.

Scenario 1: Treosulfan is a more potent inhibitor of CYP3A4.

The Applicant's treosulfan model was used to predict the effect of treosulfan on the PK of midazolam after incorporating the in vitro obtained K_i value of 1213 mM (calculated based on in vitro measured IC_{50} value of 1870 mM) against CYP3A4. About 2-fold increase in midazolam exposure was predicted when coadministered with treosulfan. The effect of a time difference in dosing of midazolam at 1 and 2 hours before treosulfan treatment or 0, 1, 2, 3 and 4 hours after treosulfan treatment was also evaluated. Applicant's analysis showed that an about 2-fold increase in midazolam exposure would be expected if midazolam is given within 2 hours after the treosulfan administration, otherwise, the midazolam exposure change would be expected to be less than 1.5-fold. The reviewer further conducted a sensitivity analysis to assess the effect of CYP3A4 K_i values on the predicted midazolam exposure. The model predicted a 6-fold increase in midazolam exposure when a 10-fold lower K_i value was applied.

Scenario 2: The metabolite, EBDM, is a more potent inhibitor of CYP3A4.

The Applicant's treosulfan model did not include the metabolite, EBDM. Thus, the inhibition potential of EBDM on a CYP3A substrate (such as midazolam) cannot be evaluated using a PBPK modeling approach. Using a mechanistic static model described in the FDA guidance on in vitro drug interaction studies⁹, the reviewer's analysis showed that midazolam AUC ratios were less than 1.2 and about 3 when the K_i value of EBDM on CYP3A pathway was set as the same value as the in vitro K_i value and a tenth of in vitro value, respectively. The assumptions for these calculations are 1) f_m CYP3A4 for midazolam was assumed to be 1.0; 2) the EBDM K_i value against CYP3A4 was assumed to be the same as the EBDM concentration that was formed from treosulfan at a concentration of 1870 μ M (IC_{50}) in the phosphate buffer at time 0 in the in vitro buffer stability study.

In conclusion, there is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on CYP3A4 in in vitro system. The potential DDI of treosulfan as a CYP3A4 inhibitor cannot be excluded.

⁹ <https://www.fda.gov/media/134582/download>

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3. Can treosulfan PBPK model predict the effect of treosulfan on the PK of omeprazole (a CYP2C19 substrate)?

As aforementioned, there is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on CYP2C19 in in vitro incubation system. Two potential scenarios are discussed in more detail as follows.

Scenario 1: Treosulfan is a more potent inhibitor of CYP2C19.

The Applicant's treosulfan model was used to predict the effect of treosulfan on the PK of omeprazole after incorporating the in vitro derived K_i value of 778 mM (calculated based on in vitro measured IC_{50} value of 972 mM) against CYP2C19. About 1.6-fold increase in omeprazole exposure was predicted when coadministered with treosulfan. The effect of a time difference in dosing of omeprazole at 1 and 2 hours before treosulfan treatment or 0, 1, 2, 3 and 4 hours after treosulfan treatment was also evaluated. The simulated results showed that an about 1.6-fold increase in omeprazole exposure would be expected if omeprazole is given within 1 hour before or after the treosulfan administration, otherwise, the omeprazole exposure change would be expected to be less than 1.3-fold. The reviewer further conducted a sensitivity analysis to assess the effect of CYP2C19 K_i values on the predicted omeprazole exposure. The simulated omeprazole AUC ratios were about 3.0 and 4.0 using a 10-fold lower K_i value for both CYP2C19 and CYP3A4 and a 40-fold¹⁰ lower CYP2C19 and a 10-fold lower CYP3A4 K_i value than the in vitro determined, respectively, when coadministered with treosulfan.

Scenario 2: The metabolite, EBDM, is a more potent inhibitor of CYP2C19.

The Applicant's treosulfan model did not include the metabolite, EBDM. Thus, the inhibition potential of EBDM on a CYP2C19 substrate (such as omeprazole) cannot be evaluated using a PBPK modeling approach. Using a mechanistic static model¹¹, the reviewer's analysis showed that omeprazole AUC ratios were about 1.3 and 3 when the K_i value of EBDM on CYP2C19 pathway was set as the same value as the in vitro value and a tenth of in vitro value, respectively. The assumptions for these calculations are 1) f_m CYP2C19 for omeprazole was assumed to be 0.86¹²; 2) the EBDM in vitro K_i value against CYP2C19 was assumed to be the same as the EBDM concentration that was formed from treosulfan at a concentration of 972 mM (IC_{50}) in the phosphate buffer at time 0 in the in vitro buffer stability study.

¹⁰ Caiping Yao, Kent L Kunze, William F Trager, Evan D Kharasch, René H Levy. Comparison of in vitro and in vivo inhibition potencies of fluvoxamine toward CYP2C19. Drug Metab Dispos. 2003 May;31(5):565-71.

¹¹ <https://www.fda.gov/media/134582/download>

¹² Koji Chiba, Keiko Shimizu, Motohiro Kato, Takaaki Nishibayashi, Kazuki Terada, Nobuo Izumo, Yuichi Sugiyama. Prediction of inter-individual variability in the pharmacokinetics of CYP2C19 substrates in humans. Drug Metab Pharmacokinet. 2014;29(5):379-86.

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In conclusion, there is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on CYP2C19 in in vitro system. The potential DDI of treosulfan as a CYP2C19 inhibitor cannot be excluded.

4. Can treosulfan PBPK model predict the effect of treosulfan on the PK of digoxin (a P-gp substrate)?

As aforementioned, there is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on P-gp in in vitro incubation system. Two potential scenarios are discussed in more detail as follows.

Scenario 1: Treosulfan is a more potent inhibitor of P-gp.

The Applicant's treosulfan model was used to predict the effect of treosulfan on the PK of digoxin after incorporating the in vitro obtained K_i value of 1774 mM (calculated based on in vitro measured IC_{50} value of 3000 mM) against P-gp. About 1.1-fold increase in digoxin exposure was predicted when coadministered with treosulfan. The reviewer further conducted a sensitivity analysis to assess the effect of P-gp K_i values on the predicted digoxin exposure. The simulated digoxin AUC ratios were about 1.35 and 1.56 using a 20-fold and 94-fold lower P-gp K_i values¹³ than the in vitro determined, respectively, when coadministered with treosulfan.

Scenario 2: The metabolite, EBDM, is a more potent inhibitor of P-gp.

The Applicant's treosulfan model did not include the metabolite, EBDM. Thus, the inhibition potential of EBDM on a P-gp substrate cannot be evaluated using a PBPK modeling approach. The P-gp mediated interaction by EBDM would be expected to be lower than that caused by treosulfan, given the ratio of EBDM to treosulfan concentration in the in vitro systems (~ 0.2) was significantly higher than those observed in vivo (~ 0.01 in study MC-FludT.16/NM and MC-FludT.17/M).

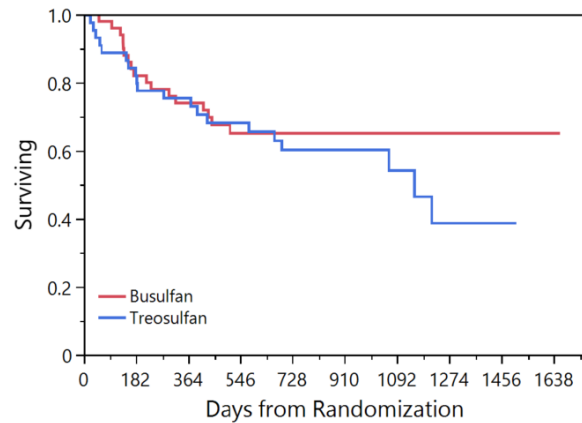
In conclusion, there is an uncertainty in regard to which moiety (treosulfan or its metabolites) exhibited inhibitory effect on P-gp in in vitro system. The potential DDI of treosulfan as a P-gp inhibitor cannot be excluded. This could be important for P-gp substrates with a narrow therapeutic index.

¹³ https://www.certara.com/app/uploads/2017/06/Howgate_2017_PAGE_P-glycoprotein.pdf

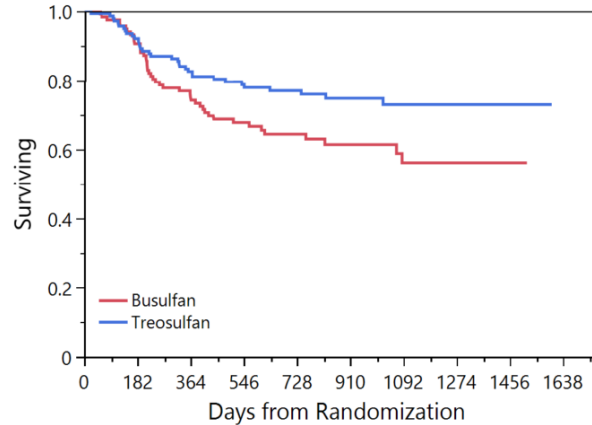
16.5 Additional Clinical Analyses

16.5.1 Kaplan Meier Estimated of OS by Disease and Donor Type

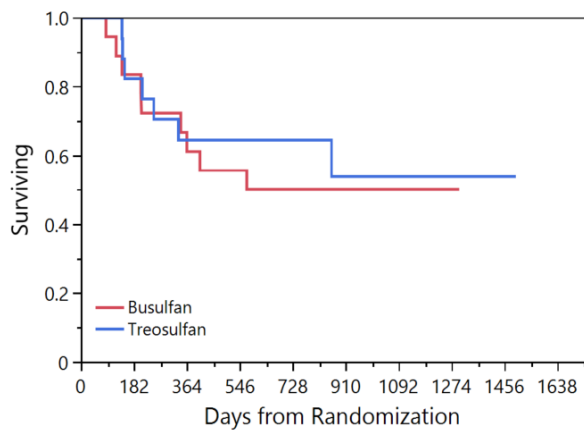
A. AML with Related Donor



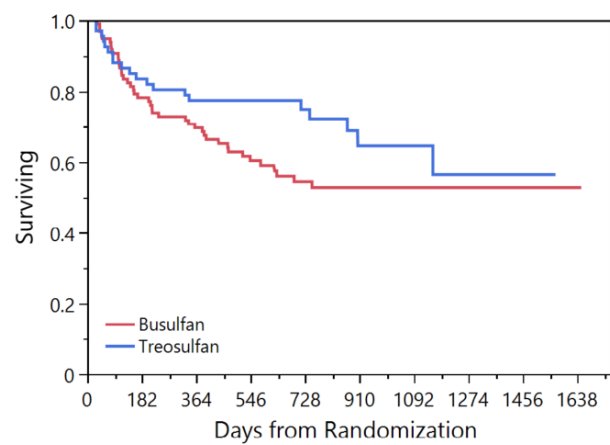
B. AML with Unrelated Donor



C. MDS with Related Donor



D. MDS with Unrelated Donor



Source: FDA Analysis

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Grafapex (Tresulfan)

DISCIPLINE	REVIEWER	OFFICE/ DIVISION	SECTIONS	AUTHORED/ APPROVED
Nonclinical Reviewer	Amy Skinner, PhD	OOD/DHOT	Sections: 5, 16.3	X Authored Approved
	Signature: Amy M. Skinner -S Digitally signed by Amy M. Skinner -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Amy M. Skinner -S, 0.9.2342.19200300.100.1.1=2002675349 Date: 2021.05.10 13:27:41 -04'00'			
Nonclinical Team Leader	Brenda Gehrke, PhD	OOD/DHOT	Sections: 5, 16.3	Authored X Approved
	Signature: Brenda Gehrke -S Digitally signed by Brenda Gehrke -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Brenda Gehrke -S, 0.9.2342.19200300.100.1.1=0012062023 Date: 2021.05.10 12:18:17 -04'00'			
Nonclinical Deputy Director	Haleh Saber, PhD	OOD/DHOT	Sections: 5, 16.3	Authored X Approved
	Signature: Haleh Saber -S Digitally signed by Haleh Saber -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Haleh Saber -S, 0.9.2342.19200300.100.1.1=1300212858 Date: 2021.07.27 10:06:20 -04'00'			
Clinical Pharmacology Reviewer	Lauren Price, PharmD	OCP/DCPI	Sections: 6, 16.4	X Authored Approved
	Signature: Lauren Price -S Digitally signed by Lauren Price -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Lauren Price -S, 0.9.2342.19200300.100.1.1=2001978474 Date: 2021.05.10 16:38:55 -04'00'			
Clinical Pharmacology Team Leader	Olanrewaju Okusanya, PharmD, MS	OCP/DCPI	Sections: 6, 16.4	Authored X Approved
	Signature: Olanrewaju Okusanya -S Digitally signed by Olanrewaju Okusanya -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001410838, cn=Olanrewaju Okusanya -S Date: 2021.05.11 15:59:30 -04'00'			
Clinical Pharmacology Division Director	Brian Booth, PhD	OCP/DCPI	Sections: 6, 16.4	Authored X Approved
	Signature: Brian P. Booth -S Digitally signed by Brian P. Booth -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Brian P. Booth -S, 0.9.2342.19200300.100.1.1=1300137436 Date: 2021.07.29 15:40:37 -04'00'			
Pharmacometrics Reviewer	Ruojing Li, PhD	OCP/DPM	Sections: 6, 16.4	X Authored Approved
	Signature: Ruojing Li -S (Affiliate) Digitally signed by Ruojing Li -S (Affiliate) DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Ruojing Li -S (Affiliate), 0.9.2342.19200300.100.1.1=2002080834, cn=Ruojing Li -S (Affiliate) Date: 2021.05.11 15:54:58 -04'00'			
Pharmacometrics Reviewer	Jianghong Fan, PhD	OCP/DPM	Sections: 6, 16.4	X Authored Approved
	Signature: Jianghong Fan -S Digitally signed by Jianghong Fan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Jianghong Fan -S, 0.9.2342.19200300.100.1.1=2001454698 Date: 2021.05.09 14:13:07 -04'00'			

NDA Multidisciplinary Review and Evaluation

NDA 214759

Grafapex (Treosulfan)

DISCIPLINE	REVIEWER	OFFICE/ DIVISION	SECTIONS	AUTHORED/ APPROVED
Pharmacometrics Team Leader	Lian Ma, PhD	OCP/DPM	Sections: 6, 16.4	Authored X Approved
	Signature: Lian Ma -S Digitally signed by Lian Ma -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Lian Ma -S, 0.9.2342.19200300.100.1.1=2000625336 Date: 2021.05.11 12:06:21 -04'00'			
Pharmacometrics Team Leader	Yuching Yang, PhD	OCP/DPM	Sections: 6, 16.4	Authored X Approved
	Signature: Yuching Yang -S Digitally signed by Yuching Yang -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yuching Yang -S, 0.9.2342.19200300.100.1.1=2000846164 Date: 2021.05.06 13:55:28 -04'00'			
Statistical Reviewer	Wenjuan Gu, PhD	OOD/DBIX	Sections: 8.1, 8.2, 8.4, 8.5	X Authored Approved
	Signature: Wenjuan Gu -S Digitally signed by Wenjuan Gu -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Wenjuan Gu -S, 0.9.2342.19200300.100.1.1=0012027801 Date: 2021.05.11 15:17:42 -04'00'			
Statistical Team Leader	Lisa Rodriguez, PhD	OB/DBIX	Sections: 8.1, 8.2, 8.4, 8.5	Authored X Approved
	Signature: Lisa R. Rodriguez -S Digitally signed by Lisa R. Rodriguez -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001011155, cn=Lisa R. Rodriguez -S Date: 2021.05.11 12:35:56 -04'00'			
Division Director (OB)	Thomas Gwise, PhD	OB/DBIX	Sections: 8.1, 8.2, 8.4, 8.5	Authored X Approved
	Signature: Thomas E. Gwise -S Digitally signed by Thomas E. Gwise -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300369224, cn=Thomas E. Gwise -S Date: 2021.07.28 08:01:26 -04'00'			
Clinical Reviewer	Cara Rabik, MD, PhD	OOD/DHMI	Sections: 2, 3, 7, 8, 9, 10, 16.5	X Authored Approved
	Signature: Cara A. Rabik -S Digitally signed by Cara A. Rabik -S Date: 2021.05.07 14:03:18 -04'00'			
Clinical Team Leader	Donna Przepiorka, MD, PhD	OOD/DHMI	Sections: 2, 3, 7, 8, 9, 10, 16.5	Authored X Approved
	Signature: Donna Przepiorka -S 2021.05.06 10:35:02 -04'00'			
Associate Director for Labeling	Elizabeth Everhart, MSN, ACNP	OOD	Sections: 11	X Authored Approved
	Signature: Elizabeth E. Everhart -S Digitally signed by Elizabeth E. Everhart -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000363858, cn=Elizabeth E. Everhart -S Date: 2021.05.06 10:51:26 -04'00'			

NDA Multidisciplinary Review and Evaluation

NDA 214759

Grafapex (Treosulfan)

DISCIPLINE	REVIEWER	OFFICE/ DIVISION	SECTIONS	AUTHORED/ APPROVED
Cross-Discipline Team Leader	Donna Przepiorka, MD, PhD	OOD/DHMI	Sections: 1, 4, 12, 13	X Authored Approved
	Signature: <i>{See appended electronic signature page}</i>			
Division Director (Clinical)	R. Angelo de Claro, MD	OOD/DHMI	Sections: All	Authored X Approved
	Signature: <i>{See appended electronic signature page}</i>			
Office Director or designee	Marc Theoret, MD	OOD	Sections: All	Authored X Approved
	Signature: <i>{See appended electronic signature page}</i>			

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

DONNA PRZEPIORKA
07/30/2021 11:38:18 AM

ROMEO A DE CLARO
07/30/2021 11:47:17 AM

MARC R THEORET
07/30/2021 01:30:35 PM

My signature indicates that I have considered the assessments and recommendations included in this Multidisciplinary review in determining the regulatory action.