# Clinical trial results:
# Clinical phase 2 trial to compare treosulfan-based conditioning therapy with busulfan-based conditioning prior to allogeneic haematopoietic stem cell transplantation (HSCT) in paediatric patients with non-malignant diseases.

## Summary

| | |
|---|---|
| EudraCT number | 2013-005508-33 |
| Trial protocol | DE AT CZ PL IT |
| Global end of trial date | |

**Results information**

| | |
|---|---|
| Result version number | v1 (current) |
| This version publication date | 03 April 2022 |
| First version publication date | 03 April 2022 |

## Trial information

**Trial identification**

| | |
|---|---|
| Sponsor protocol code | MC-FludT.16/NM |

**Additional study identifiers**

| | |
|---|---|
| ISRCTN number | - |
| ClinicalTrials.gov id (NCT number) | NCT02349906 |
| WHO universal trial number (UTN) | - |

Notes:

**Sponsors**

| | |
|---|---|
| Sponsor organisation name | medac Gesellschaft fuer klinische Spezialpraeparate mbH |
| Sponsor organisation address | Theaterstrasse 6, Wedel, Germany, 22880 |
| Public contact | Dr Jochen Kehne, medac Gesellschaft fuer klinische Spezialpräparate mbH, 0049 41038006388, j.kehne@medac.de |
| Scientific contact | Medical Expert, medac Gesellschaft fuer klinische Spezialpräparate mbH, 0049 410380060, medwiss@medac.de |

Notes:

**Paediatric regulatory details**

| | |
|---|---|
| Is trial part of an agreed paediatric investigation plan (PIP) | Yes |
| EMA paediatric investigation plan number(s) | EMEA-000088-PIP01-10 |
| Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial? | No |
| Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial? | No |

Notes:

## Results analysis stage

| | |
|---|---|
| Analysis stage | Interim |
| Date of interim/final analysis | 07 June 2021 |
| Is this the analysis of the primary completion data? | Yes |
| Primary completion date | 07 May 2020 |
| Global end of trial reached? | No |

Notes:

## General information about the trial

Main objective of the trial:

The objective of this trial was to describe the safety and efficacy of i.v. treosulfan compared to the conventional (myeloablative) dose of i.v. busulfan, each administered as part of a standardised fludarabine-containing conditioning regimen and to contribute to a PK model, which permits - in conjunction with data comparing treosulfan and busulfan in adults with malignant diseases - to extend the use of treosulfan in the paediatric population by extrapolating efficacy
The primary objective of this trial was the comparative evaluation of freedom from transplantation (treatment)-related mortality, defined as death from any transplantation (treatment)-related cause from start of conditioning treatment (visit Day -7) until day +100 after HSCT.

Protection of trial subjects:

The study was conducted in accordance with ICH GCP guidelines, applicable local laws and in compliance with the ethical principles originating in the Declaration of Helsinki.
Regulatory authorities were informed of the study and amendments in accordance with national regulations and, where necessary, the appropriate approval was obtained.
Due to the life-threatening diseases to be treated, an independent Data Monitoring Committee (DMC) was set up. All available safety and efficacy data were subjected to thorough review.

Background therapy:

This trial allowed administration of 2 different background conditioning regimens in addition to treosulfan or busulfan: one background conditioning regimen consisted of an intensified fludarabine-containing regimen with additional thiotepa (Stratum A) whereas the other consisted of the standard regimen with fludarabine only (Stratum B).
Other concomitant medication could be administered according to the hospital practice.

Evidence for comparator:

Busilvex® (i.v. busulfan) was selected as reference regimen within this trial. This reference treatment regimen was confirmed by EMA.
The drug is registered in Europe for conditioning treatment prior to conventional haematopoietic progenitor cell Transplantation.

| | |
|---|---|
| Actual start date of recruitment | 20 April 2015 |
| Long term follow-up planned | Yes |
| Long term follow-up rationale | Safety, Efficacy, Regulatory reason |
| Long term follow-up duration | 2 Years |
| Independent data monitoring committee (IDMC) involvement? | Yes |

Notes:

## Population of trial subjects

### Subjects enrolled per country

| | |
|---|---|
| Country: Number of subjects enrolled | Poland: 34 |
| Country: Number of subjects enrolled | Czechia: 6 |
| Country: Number of subjects enrolled | Germany: 44 |
| Country: Number of subjects enrolled | Italy: 22 |
| Worldwide total number of subjects | 106 |
| EEA total number of subjects | 106 |

Notes:

| Subjects enrolled per age group | |
|---|---|
| In utero | 0 |
| Preterm newborn - gestational age < 37 wk | 0 |
| Newborns (0-27 days) | 0 |
| Infants and toddlers (28 days-23 months) | 29 |
| Children (2-11 years) | 61 |
| Adolescents (12-17 years) | 16 |
| Adults (18-64 years) | 0 |
| From 65 to 84 years | 0 |
| 85 years and over | 0 |

## Subject disposition

### Recruitment

Recruitment details:

106 subjects were enrolled at 18 sites in 4 countries.
The first subject was enrolled in the study on 20 April 2015.

### Pre-assignment

Screening details:

All subjects were screened for eligibility to participate in the study to ensure that all subjects met all inclusion and exclusion criteria.
A total of 106 subjects were randomised  of which 101 subjects received Investigational medicinal product (IMP) and were included in the efficacy and safety analyses.

### Period 1

| | |
|---|---|
| Period 1 title | Randomisation |
| Is this the baseline period? | No |
| Allocation method | Randomised - controlled |
| Blinding used | Not blinded |

Blinding implementation details:

Due to the different treatment schedules of the test arm (treosulfan) and the reference arm (busulfan) with regard to the different infusion regimens, blinding of the IMP was not considered feasible within the orphan indication of paediatric subjects with a non-malignant indication for allogeneic HSCT.

### Arms

| | |
|---|---|
| Are arms mutually exclusive? | Yes |
| **Arm title** | Busulfan |

Arm description:

This arm comprises all subjects randomised to Busulfan.

| | |
|---|---|
| Arm type | Active comparator |
| Investigational medicinal product name | Busulfan |
| Investigational medicinal product code | |
| Other name | Busilvex |
| Pharmaceutical forms | Concentrate for solution for infusion |
| Routes of administration | Intravenous use |

Dosage and administration details:

Busulfan was administered i.v. on 4 consecutive days (days -7, -6, -5, and -4) prior to Hematopoietic stem-cell transplantation (HSCT) (day 0). Busulfan was to be infused in accordance with the respective hospital standard, ie, 1, 2, or 4 times daily. The required total dose was calculated on basis of the actual body weight based on the summary of product characteristics (SmPC).

| | |
|---|---|
| **Arm title** | Treosulfan |

Arm description:

This arm comprises all subjects randomised to Treosulfan.

| | |
|---|---|
| Arm type | Experimental |
| Investigational medicinal product name | Treosulfan |
| Investigational medicinal product code | |
| Other name | Trecondi |
| Pharmaceutical forms | Concentrate for solution for infusion |
| Routes of administration | Intravenous use |

Dosage and administration details:

Treosulfan was administered i.v. over 2 hours on 3 consecutive days (days -6, -5, and -4) prior to HSCT (day 0). The required total dose of treosulfan was calculated on basis of the subject’s body surface area (BSA).

| Number of subjects in period 1 | Busulfan | Treosulfan |
|---|---|---|
| Started | 54 | 52 |
| Completed | 50 | 51 |
| Not completed | 4 | 1 |
| Withdrawal prior to start of treatment with IMP | 4 | 1 |

## Period 2

| | |
|---|---|
| Period 2 title | Treatment |
| Is this the baseline period? | Yes[1] |
| Allocation method | Not applicable |
| Blinding used | Not blinded |

## Arms

| | |
|---|---|
| Are arms mutually exclusive? | Yes |
| **Arm title** | Busulfan |

Arm description:

This arm is equal to the Full Analysis Set (FAS) and includes all subjects randomised to busulfan who received at least one dose of the IMP and with at least one documented efficacy parameter.
The subjects within the FAS were analysed in their initial arm of randomisation (intention to treat principle).

| | |
|---|---|
| Arm type | Active comparator |
| Investigational medicinal product name | Busulfan |
| Investigational medicinal product code | |
| Other name | Busilvex |
| Pharmaceutical forms | Concentrate for solution for infusion |
| Routes of administration | Intravenous use |

Dosage and administration details:

Busulfan was administered i.v. on 4 consecutive days (days -7, -6, -5, and -4) prior to Hematopoietic stem-cell transplantation (HSCT) (day 0). Busulfan was to be infused in accordance with the respective hospital standard, ie, 1, 2, or 4 times daily. The required total dose was calculated on basis of the actual body weight based on the summary of product characteristics (SmPC) for Busilvex.

| | |
|---|---|
| **Arm title** | Treosulfan |

Arm description:

This arm is equal to the Full Analysis Set (FAS) and includes all subjects randomised to treosulfan who received at least one dose of the IMP and with at least one documented efficacy parameter.
The subjects within the FAS were analysed in their initial arm of randomisation (intention to treat principle).

| | |
|---|---|
| Arm type | Experimental |
| Investigational medicinal product name | Treosulfan |
| Investigational medicinal product code | |
| Other name | Trecondi |
| Pharmaceutical forms | Concentrate for solution for infusion |
| Routes of administration | Intravenous use |

Dosage and administration details:

Treosulfan was administered i.v. over 2 hours on 3 consecutive days (days -6, -5, and -4) prior to HSCT (day 0). The required total dose of treosulfan was calculated on basis of the subject's body surface area (BSA).

Notes:

[1] - Period 1 is not the baseline period. It is expected that period 1 will be the baseline period.

Justification: To be able to document the reason for "not complete" between randomization and treatment for both treatment arms separately, an additional pretreatment ("Randomization") period was created.

| **Number of subjects in period 2[2]** | Busulfan | Treosulfan |
|---|---|---|
| Started | 50 | 51 |
| Completed | 43 | 47 |
| Not completed | 7 | 4 |
| Death | 7 | 2 |
| Lost to follow-up | - | 2 |

Notes:

[2] - The number of subjects reported to be in the baseline period are not the same as the worldwide number enrolled in the trial. It is expected that these numbers will be the same.

Justification: The number of subjects in the baseline period is based on the subjects treated (Full Analysis set).

## Baseline characteristics

### Reporting groups

| Reporting group title | Busulfan |
|---|---|

Reporting group description:

This arm is equal to the Full Analysis Set (FAS) and includes all subjects randomised to busulfan who received at least one dose of the IMP and with at least one documented efficacy parameter.
The subjects within the FAS were analysed in their initial arm of randomisation (intention to treat principle).

| Reporting group title | Treosulfan |
|---|---|

Reporting group description:

This arm is equal to the Full Analysis Set (FAS) and includes all subjects randomised to treosulfan who received at least one dose of the IMP and with at least one documented efficacy parameter.
The subjects within the FAS were analysed in their initial arm of randomisation (intention to treat principle).

| Reporting group values | Busulfan | Treosulfan | Total |
|---|---|---|---|
| Number of subjects | 50 | 51 | 101 |
| Age categorical<br>Units: Subjects | | | |
| Infants and toddlers (28 days-23 months) | 14 | 14 | 28 |
| Children (2-11 years) | 26 | 31 | 57 |
| Adolescents (12-17 years) | 10 | 6 | 16 |
| Age continuous<br>Units: years | | | |
| arithmetic mean | 6.0 | 5.0 | |
| standard deviation | ± 5.3 | ± 4.4 | - |
| Gender categorical<br>Units: Subjects | | | |
| Female | 19 | 15 | 34 |
| Male | 31 | 36 | 67 |
| Summary of trial disease characteristics<br>Units: Subjects | | | |
| Primary immunodeficiencies | 28 | 23 | 51 |
| Inborn errors metabolism | 4 | 2 | 6 |
| Hemoglobinopathies | 13 | 21 | 34 |
| Bone marrow failure syndromes | 5 | 5 | 10 |
| Donor type<br>Units: Subjects | | | |
| Matched sibling donor (MSD) | 12 | 9 | 21 |
| Matched family donor (MFD) | 5 | 5 | 10 |
| Matched unrelated donor (MUD) | 32 | 36 | 68 |
| Umbilical cord blood (UCB) | 1 | 1 | 2 |
| Body surface area<br>Units: square meter | | | |
| arithmetic mean | 0.836 | 0.746 | |
| standard deviation | ± 0.396 | ± 0.297 | - |

## End points

### End points reporting groups

| Reporting group title | Busulfan |
|---|---|

Reporting group description:

This arm comprises all subjects randomised to Busulfan.

| Reporting group title | Treosulfan |
|---|---|

Reporting group description:

This arm comprises all subjects randomised to Treosulfan.

| Reporting group title | Busulfan |
|---|---|

Reporting group description:

This arm is equal to the Full Analysis Set (FAS) and includes all subjects randomised to busulfan who received at least one dose of the IMP and with at least one documented efficacy parameter.
The subjects within the FAS were analysed in their initial arm of randomisation (intention to treat principle).

| Reporting group title | Treosulfan |
|---|---|

Reporting group description:

This arm is equal to the Full Analysis Set (FAS) and includes all subjects randomised to treosulfan who received at least one dose of the IMP and with at least one documented efficacy parameter.
The subjects within the FAS were analysed in their initial arm of randomisation (intention to treat principle).

| Subject analysis set title | Treosulfan Pharmacokinetic Set |
|---|---|
| Subject analysis set type | Sub-group analysis |

Subject analysis set description:

Pooled analysis of MC FludT16/NM and MC FludT.17/M.
The PK Set included all subjects of the FAS with any treosulfan concentration measurements.

### Primary: Freedom from transplantation (treatment)-related mortality - Number

| End point title | Freedom from transplantation (treatment)-related mortality - Number[1] |
|---|---|

End point description:

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

| End point type | Primary |
|---|---|

End point timeframe:

Until day +100 after HSCT

Notes:

[1] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The data analysis is descriptive in nature. No specific confirmatory statistical hypotheses are specified.

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: Subjects | | | | |
| With event | 5 | 0 | | |
| Without event | 45 | 51 | | |

### Statistical analyses

No statistical analyses for this end point

## Primary: Freedom from transplantation (treatment)-related mortality - Incidence

| End point title | Freedom from transplantation (treatment)-related mortality - Incidence |
|---|---|

End point description:

Incidence of freedom from transplantation (treatment)-related mortality, defined as death from any transplantation (treatment)-related cause from start of conditioning treatment (visit Day -7) until day +100 after HSCT.
The associated time span of transplantation-related mortality was defined as the interval from end of HSCT to death due to transplantation-related cause whereas the time span of treatment-related mortality was defined as interval from start of conditioning treatment, ie, visit Day -7, until end of HSCT.

| End point type | Primary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | 90.0 (80.1 to 96.0) | 100.0 (94.3 to 100.0) | | |

### Statistical analyses

| Statistical analysis title | Diff. incidences |
|---|---|
| Comparison groups | Busulfan v Treosulfan |
| Number of subjects included in analysis | 101 |
| Analysis specification | Pre-specified |
| Analysis type | superiority |
| P-value | = 0.0528 |
| Method | Cochran-Mantel-Haenszel |
| Parameter estimate | Risk difference (RD) |
| Point estimate | -10 |
| Confidence interval | |
| level | 90 % |
| sides | 2-sided |
| lower limit | -19.9 |
| upper limit | -3.4 |

## Secondary: Engraftment - reconstitution of granulopoiesis

| End point title | Engraftment - reconstitution of granulopoiesis |
|---|---|

End point description:

Neutrophilic granulocytes engraftment was assessed by reconstitution of granulopoiesis.
Engraftment was defined as the first of 3 consecutive days with an absolute neutrophilic granulocytes count of more than 0.5 x10^9/L. The term "consecutive days" was defined as 3 consecutive blood samples if taken on different days.
The date of reaching engraftment was the documented "date of engraftment". Death from any cause, relapse/progression or use of rescue therapies until the date of primary graft failure or documentation of engraftment status (whatever occurred first) were competing events.
The maximum conditional cumulative incidence is reported.

| End point type | Secondary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | 100.0 (94.1 to 100.0) | 97.3 (88.7 to 100.0) | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: Engraftment - reconstitution of leukopoiesis

| End point title | Engraftment - reconstitution of leukopoiesis |
|---|---|

End point description:

Leukocyte engraftment was assessed by reconstitution of leukopoiesis.
Engraftment was defined as the first of 3 consecutive days with a total leucocyte of more than 1 x 10^9/L. The term "consecutive days" was defined as 3 consecutive blood samples if taken on different days.
The date of reaching engraftment was the documented "date of engraftment". Death from any cause, relapse/progression or use of rescue therapies until the date of primary graft failure or documentation of engraftment status (whatever occurred first) were competing events.
The maximum conditional cumulative incidence is reported.

| End point type | Secondary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | 100.0 (94.1 to 100.0) | 96.8 (87.1 to 100.0) | | |

### Statistical analyses

No statistical analyses for this end point

### Secondary: Engraftment - reconstitution of thrombopoiesis > 20 x10^9/L

| End point title | Engraftment - reconstitution of thrombopoiesis > 20 x10^9/L |
|---|---|

End point description:

Platelet (PLT) engraftment was assessed by reconstitution of thrombopoiesis.
Engraftment was defined as the first of 3 consecutive days with PLT count of at least 20 x10^9/L in the absence of PLT transfusion. The term "consecutive days" was defined as 3 consecutive blood samples if taken on different days.
The date of reaching engraftment was the documented "date of engraftment". Death from any cause, relapse/progression or use of rescue therapies until the date of primary graft failure or documentation of engraftment status (whatever occurred first) were competing events.
The maximum conditional cumulative incidence is reported.

| End point type | Secondary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | 96.8 (86.6 to 100.0) | 100.0 (93.8 to 100.0) | | |

### Statistical analyses

No statistical analyses for this end point

### Secondary: Engraftment - reconstitution of thrombopoiesis > 50 x10^9/L

| End point title | Engraftment - reconstitution of thrombopoiesis > 50 x10^9/L |
|---|---|

End point description:

Platelet (PLT) engraftment was assessed by reconstitution of thrombopoiesis.
Engraftment was defined as the first of 3 consecutive days with PLT count of at least 50 x10^9/L in the absence of PLT transfusion. The term "consecutive days" was defined as 3 consecutive blood samples if taken on different days.
The date of reaching engraftment was the documented "date of engraftment". Death from any cause, relapse/progression or use of rescue therapies until the date of primary graft failure or documentation of engraftment status (whatever occurred first) were competing events.

The maximum conditional cumulative incidence is reported.

| End point type | Secondary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | 97.1 (87.9 to 100.0) | 94.8 (86.3 to 100.0) | | |

## Statistical analyses

No statistical analyses for this end point

## Secondary: HSOS, lung toxicity, hepatic toxicity, early toxicity (until day +28) and infections of any CTCAE grade

| End point title | HSOS, lung toxicity, hepatic toxicity, early toxicity (until day +28) and infections of any CTCAE grade |
|---|---|

End point description:

Incidence of all treatment emergent significant adverse events.
Evaluation of hepatic sinusoidal obstruction syndrome ("HSOS", according to Jones et al), "Lung toxicity" (CTCAE term "Pulmonary fibrosis"), "Hepatic toxicity" (according to Bearman's criteria), Early toxicity defined as any AE occurring until day +28 and "Infections of any CTCAE grade" (non-serious and serious) until day +100.

| End point type | Secondary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| HSOS according to Jones (1987) | 10.0 (4.0 to 19.9) | 2.0 (0.1 to 9.0) | | |
| Early toxicity (any AE occurring until day +28) | 96.0 (87.9 to 99.3) | 94.1 (85.5 to 98.4) | | |
| Hepatic toxicity according to Bearman (1988) | 54.0 (41.5 to 66.2) | 51.0 (38.7 to 63.2) | | |
| Lung toxicity (CTCAE term "Pulmonary fibrosis") | 0.0 (0.0 to 5.8) | 0.0 (0.0 to 5.7) | | |
| Infections (SOC "Infections and infestations") | 70.0 (57.6 to 80.5) | 60.8 (48.3 to 72.3) | | |

## Statistical analyses

No statistical analyses for this end point

## Secondary: Incidence of complete donor type chimerism

| End point title | Incidence of complete donor type chimerism |
|---|---|

End point description:

Evaluation of donor-type chimerism on day +28, day +100, and 12 months after HSCT.
Chimerism was analysed in Peripheral blood or Bone marrow samples at visits Day +28, Day +100, Month 12.
Complete donor-type chimerism was defined if a value of >= 95% was detected. The incidences of complete donor-type chimerism were estimated as the number of subjects with complete chimerism divided by the total number of subjects at risk.

| End point type | Secondary |
|---|---|

End point timeframe:

28 days, 100 days and 12 months after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50[2] | 51[3] | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Day +28 | 82.0 (70.7 to 90.3) | 84.3 (73.5 to 91.9) | | |
| Day +100 | 84.8 (73.3 to 92.6) | 66.7 (54.3 to 77.5) | | |
| Month 12 | 76.7 (63.8 to 86.8) | 49.0 (36.5 to 61.5) | | |

Notes:

[2] - Subjects at risk:
Day +28 = 50
Day +100 = 46
Month 12 = 43

[3] - Subjects at risk:
Day +28 = 51
Day +100 = 51
Month 12 = 49

## Statistical analyses

No statistical analyses for this end point

## Secondary: Overall survival

| End point title | Overall survival |
|---|---|

End point description:

Overall survival (OS) after HSCT was defined as the probability of surviving. Survival time was defined

as the time period between end of HSCT and the date of death due to any cause.

| End point type | Secondary |
|---|---|

End point timeframe:

12, 24 and 36 months after HSCT

| **End point values** | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Month 12 | 88.0 (77.9 to 93.7) | 96.1 (88.0 to 98.8) | | |
| Month 24 | 88.0 (77.9 to 93.7) | 96.1 (88.0 to 98.8) | | |
| Month 36 | 84.0 (71.4 to 91.4) | 96.1 (88.0 to 98.8) | | |

## Statistical analyses

No statistical analyses for this end point

## Secondary: Incidence of graft failure

| End point title | Incidence of graft failure |
|---|---|

End point description:

The incidence of graft failure was defined as the probability of having a graft failure (primary or secondary) and being alive without using “stem cell infusion (re-transplant) with conditioning” rescue therapy (ie, second allogenic transplantations) between the end of HSCT and the end of the longer term follow up phase.
The associated time span was defined as the interval from day 0 to graft failure.

| End point type | Secondary |
|---|---|

End point timeframe:

12, 24 and 36 months after HSCT

| **End point values** | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Month 12 | 4.0 (0.0 to 8.6) | 15.8 (7.4 to 24.3) | | |
| Month 24 | 4.0 (0.0 to 8.6) | 21.0 (11.2 to 30.9) | | |
| Month 36 | 4.0 (0.0 to 8.6) | 24.8 (13.6 to 35.9) | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: Acute GvHD grade I-IV - number

| End point title | Acute GvHD grade I-IV - number |
|---|---|

End point description:

Acute graft versus host disease of grades I to IV - number of subjects with and without event.
Time to aGvHD was defined as the time between end of HSCT and the date of first occurrence of aGvHD. Subjects alive with no occurrence of aGvHD at 100 days after end of HSCT and no competing event were censored. Death and graft failure within 100 days after HSCT were competing events.

| End point type | Secondary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: Subjects | | | | |
| With event | 21 | 28 | | |
| Without event | 29 | 23 | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: Acute GvHD grade I-IV - cumulative incidence

| End point title | Acute GvHD grade I-IV - cumulative incidence |
|---|---|

End point description:

Acute graft versus host disease of grades I to IV - cumulative incidence.
Time to aGvHD was defined as the time between end of HSCT and the date of first occurrence of aGvHD. Subjects alive with no occurrence of aGvHD at 100 days after end of HSCT and no competing event were censored. Death and graft failure within 100 days after HSCT were competing events.

| End point type | Secondary |
|---|---|

End point timeframe:

14, 28 and 100 days after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Day 14 | 2.0 (0.0 to 5.3) | 13.7 (5.8 to 21.7) | | |
| Day 28 | 30.0 (19.3 to 40.7) | 37.3 (26.1 to 48.4) | | |
| Day 100 | 42.0 (30.5 to 53.5) | 54.9 (43.4 to 66.4) | | |

### Statistical analyses

No statistical analyses for this end point

### Secondary: Acute GvHD grade III-IV - number

| End point title | Acute GvHD grade III-IV - number |
|---|---|

End point description:

Acute graft versus host disease of grades III to IV - number of subjects with and without Event.
Time to aGvHD was defined as the time between end of HSCT and the date of first occurrence of aGvHD. Subjects alive with no occurrence of aGvHD at 100 days after end of HSCT and no competing event were censored. Death and graft failure within 100 days after HSCT were competing events.

| End point type | Secondary |
|---|---|

End point timeframe:

Until day +100 after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: subjects | | | | |
| With event | 4 | 7 | | |
| Without event | 46 | 44 | | |

### Statistical analyses

No statistical analyses for this end point

### Secondary: Acute GvHD grade III-IV - cumulative incidence

| End point title | Acute GvHD grade III-IV - cumulative incidence |
|---|---|

End point description:

Acute graft versus host disease of grades III to IV - cumulative incidence.
Time to acute GvHD (aGvHD) was defined as the time between end of HSCT and the date of first occurrence of acute GvHD. Death, relapse/progression and graft failure within 100 days after end of HSCT were competing events.

| End point type | Secondary |
|---|---|

End point timeframe:

14, 28 and 100 days after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Day 14 | 2.0 (0.0 to 5.3) | 2.0 (0.0 to 5.2) | | |
| Day 28 | 4.0 (0.0 to 8.6) | 2.0 (0.0 to 5.2) | | |
| Day 100 | 8.0 (1.7 to 14.3) | 13.7 (5.8 to 21.7) | | |

## Statistical analyses

No statistical analyses for this end point

## Secondary: Overall chronic GvHD - number

| End point title | Overall chronic GvHD - number |
|---|---|

End point description:

Overall chronic graft versus host disease - number of subjects with and without event.
Chronic GvHD was evaluated from 100 days after transplantation until the end of the longer term follow-up. Time to cGvHD was defined as the time between 100 days after end of HSCT and the first episode of cGvHD.
Subjects were at risk (evaluable) for cGvHD if they had survived graft failure-free until 100 days after end of HSCT. In addition, subjects with premature trial termination at visit Day +100 were excluded from the risk set. Subjects alive with no cGvHD at the last follow-up were censored.
Death and graft failure were competing events.

| End point type | Secondary |
|---|---|

End point timeframe:

From day +100 to last follow-up visit

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 44 | 47 | | |
| Units: subjects | | | | |
| With event | 17 | 6 | | |
| Without event | 27 | 41 | | |

## Statistical analyses

No statistical analyses for this end point

## Secondary: Overall chronic GvHD - cumulative incidence

| End point title | Overall chronic GvHD - cumulative incidence |
|---|---|

End point description:

Overall chronic graft versus host disease - cumulative incidence
Chronic GvHD was evaluated from 100 days after transplantation until the end of the longer term follow-up. Time to cGvHD was defined as the time between 100 days after end of HSCT and the first episode of cGvHD.
Subjects were at risk (evaluable) for cGvHD if they had survived graft failure-free until 100 days after end of HSCT. In addition, subjects with premature trial termination at visit Day +100 were excluded from the risk set. Subjects alive with no cGvHD at the last follow-up were censored.
Death and graft failure were competing events

| End point type | Secondary |
|---|---|

End point timeframe:

12, 24 and 36 months after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 44 | 47 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Month 12 | 38.6 (26.6 to 50.7) | 12.8 (4.8 to 20.8) | | |
| Month 24 | 38.6 (26.6 to 50.7) | 12.8 (4.8 to 20.8) | | |
| Month 36 | 38.6 (26.6 to 50.7) | 12.8 (4.8 to 20.8) | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: Moderate/severe chronic GvHD - number

| End point title | Moderate/severe chronic GvHD - number |
|---|---|

End point description:

Moderate/severe chronic graft versus host disease - number of subjects with and without event.
Chronic GvHD was evaluated from 100 days after transplantation until the end of the longer term follow-up. Time to cGvHD was defined as the time between 100 days after end of HSCT and the first episode of cGvHD.
Subjects were at risk (evaluable) for cGvHD if they had survived graft failure-free until 100 days after end of HSCT. In addition, subjects with premature trial termination at visit Day +100 were excluded from the risk set. Subjects alive with no cGvHD at the last follow-up were censored.
Death and graft failure were competing events

| End point type | Secondary |
|---|---|

End point timeframe:

From day +100 to last follow-up visit

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 44 | 47 | | |
| Units: subjects | | | | |
| With event | 10 | 5 | | |
| Without event | 34 | 42 | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: Moderate/severe chronic GvHD - cumulative incidence

| End point title | Moderate/severe chronic GvHD - cumulative incidence |
|---|---|

End point description:

Moderate or severe chronic graft versus host disease - cumulative incidence.
Chronic GvHD was evaluated from 100 days after transplantation until the end of the longer term follow-up. Time to cGvHD was defined as the time between 100 days after end of HSCT and the first episode of cGvHD.
Subjects were at risk (evaluable) for cGvHD if they had survived graft failure-free until 100 days after end of HSCT. In addition, subjects with premature trial termination at visit Day +100 were excluded from the risk set. Subjects alive with no cGvHD at the last follow-up were censored.
Death and graft failure were competing events.

| End point type | Secondary |
|---|---|

End point timeframe:

12, 24 and 36 months after HSCT

| End point values | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 44 | 47 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Month 12 | 22.7 (12.3 to 33.1) | 10.6 (3.2 to 18.0) | | |
| Month 24 | 22.7 (12.3 to 33.1) | 10.6 (3.2 to 18.0) | | |
| Month 36 | 22.7 (12.3 to 33.1) | 10.6 (3.2 to 18.0) | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: Use of rescue therapies

| End point title | Use of rescue therapies |
|---|---|

End point description:

Rescue therapies, cell therapies given in order to treat acute graft failure or relapse of the underlying disease were to be documented as rescue therapies. These included unfractionated or fractionated (eg, allo-depleted or T-cell receptor alpha-beta depleted) donor lymphocyte infusions (DLI) and stem cell infusions (SCI) with or without further conditioning regimens. Re-occurrence of transfusion dependence for red blood cells or PLT was to be documented as rescue therapy.

| End point type | Secondary |
|---|---|

End point timeframe:

Visit Day 0 (end of HSCT) until the end of the longer term follow up phase.

| **End point values** | Busulfan | Treosulfan | | |
|---|---|---|---|---|
| Subject group type | Reporting group | Reporting group | | |
| Number of subjects analysed | 50 | 51 | | |
| Units: percent | | | | |
| number (confidence interval 90%) | | | | |
| Any rescue therapy | 42.0 (30.1 to 54.6) | 41.2 (29.5 to 53.7) | | |
| DLIs | 4.0 (0.7 to 12.1) | 9.8 (3.9 to 19.5) | | |
| Stem cell boost | 2.0 (0.1 to 9.1) | 3.9 (0.7 to 11.8) | | |
| SCI (re-transplant) with conditioning | 2.0 (0.1 to 9.1) | 0.0 (0.0 to 5.7) | | |
| SCI (re-transplant) without conditioning | 0.0 (0.0 to 5.8) | 0.0 (0.0 to 5.7) | | |
| Transfusion dependence for red blood cells | 34.0 (23.0 to 46.5) | 33.3 (22.5 to 45.7) | | |
| Transfusion dependence for platelets | 28.0 (17.8 to 40.3) | 27.5 (17.4 to 39.5) | | |
| Other rescue therapies | 4.0 (0.7 to 12.1) | 7.8 (2.7 to 17.1) | | |

### Statistical analyses

No statistical analyses for this end point

## Secondary: PK result _ t1/2term

| End point title | PK result _ t1/2term |
|---|---|

End point description:

Pooled analysis of MC FludT16/NM and MC FludT.17/M.
Pharmacokinetic Parameter t1/2term = apparent terminal elimination half-life.
Analysis of PK parameters was performed in a subset of subjects treated with treosulfan. The calculation of individual treosulfan dose was adapted to the subject’s BSA group.

| End point type | Secondary |
|---|---|

End point timeframe:

PK samples were taken on day -6 (first day of treatment with treosulfan) at 5 predefined different time points between +/- 5 minutes and 7-8 hours after the end of the infusion.

| End point values | Treosulfan Pharmacokinetic Set | | | |
|---|---|---|---|---|
| Subject group type | Subject analysis set | | | |
| Number of subjects analysed | 82[4] | | | |
| Units: hour | | | | |
| geometric mean (standard deviation) | | | | |
| BSA group ≤ 0.5 m² (10 g/m² dose group) | 1.27 (± 0.178) | | | |
| BSA group > 0.5 - ≤ 1.0 m² (12 g/m² dose group) | 1.40 (± 0.173) | | | |
| BSA group > 1.0 m² (14 g/m² dose group) | 1.58 (± 0.178) | | | |

Notes:

[4] - BSA group ≤ 0.5 m²: N = 16
BSA group > 0.5 - ≤ 1.0 m²: N =37
BSA group > 1.0 m²: N = 29

## Statistical analyses

No statistical analyses for this end point

## Secondary: PK result _ AUC infinity

| End point title | PK result _ AUC infinity |
|---|---|

End point description:

Pooled analysis of MC FludT16/NM and MC FludT.17/M.
Pharmacokinetic Parameter AUC infinity = AUC from time 0 to infinite time.
Analysis of PK parameters was performed in a subset of subjects treated with treosulfan. The calculation of individual treosulfan dose was adapted to the subject's BSA group.

| End point type | Secondary |
|---|---|

End point timeframe:

PK samples were taken on day -6 (first day of treatment with treosulfan) at 5 predefined different time points between +/- 5 minutes and 7-8 hours after the end of the infusion.

| End point values | Treosulfan Pharmacokinetic Set | | | |
|---|---|---|---|---|
| Subject group type | Subject analysis set | | | |
| Number of subjects analysed | 81[5] | | | |
| Units: µg.h/mL | | | | |
| geometric mean (standard deviation) | | | | |
| BSA group ≤ 0.5 m² (10 g/m² dose group) | 1570 (± 482) | | | |
| BSA group > 0.5 - ≤ 1.0 m² (12 g/m² dose group) | 1672 (± 401) | | | |
| BSA group > 1.0 m² (14 g/m² dose group) | 1903 (± 310) | | | |

Notes:

[5] - BSA group ≤ 0.5 m²: N = 15
BSA group > 0.5 - ≤ 1.0 m²: N =37
BSA group > 1.0 m²: N = 29

## Statistical analyses

No statistical analyses for this end point

## Other pre-specified: PK result _ Cmax

| End point title | PK result _ Cmax |
|---|---|

End point description:

Pooled analysis of MC FludT16/NM and MC FludT.17/M.
Pharmacokinetic Parameter Cmax = the maximum observed plasma concentration.
Analysis of PK parameters was performed in a subset of subjects treated with treosulfan. The calculation of individual treosulfan dose was adapted to the subject's BSA group.

| End point type | Other pre-specified |
|---|---|

End point timeframe:

PK samples were taken on day -6 (first day of treatment with treosulfan) at 5 predefined different time points between +/- 5 minutes and 7-8 hours after the end of the infusion.

| **End point values** | Treosulfan Pharmacokinetic Set | | | |
|---|---|---|---|---|
| Subject group type | Subject analysis set | | | |
| Number of subjects analysed | 82[6] | | | |
| Units: µg/mL | | | | |
| geometric mean (standard deviation) | | | | |
| BSA group ≤ 0.5 m² (10 g/m² dose group) | 608 (± 209) | | | |
| BSA group > 0.5 - ≤ 1.0 m² (12 g/m² dose group) | 662 (± 286) | | | |
| BSA group > 1.0 m² (14 g/m² dose group) | 652 (± 111) | | | |

Notes:

[6] - BSA group ≤ 0.5 m²: N = 15
BSA group > 0.5 - ≤ 1.0 m²: N =38
BSA group > 1.0 m²: N = 29

## Statistical analyses

No statistical analyses for this end point

## Adverse events

### Adverse events information

Timeframe for reporting adverse events:

All AE (serious and non serious) occurring between day -7 and day +100 after HSCT were to be reported. Thereafter, only SAE with suspected relatedness (SAR) were to be reported until the end of longer-term follow-up phase.

Adverse event reporting additional description:

Adverse event reporting was based on the Safety Analysis Set. This includes all subjects enrolled in the trial who have received at least one dose of IMP.
All subjects were analysed within their arm of actual treatment.

| | |
|---|---|
| Assessment type | Systematic |

### Dictionary used

| | |
|---|---|
| Dictionary name | CTCAE |
| Dictionary version | 4.03 |

### Reporting groups

| | |
|---|---|
| Reporting group title | Busulfan - Safety Analysis Set |

Reporting group description:

This arm comprises all subjects who were randomised to Busulfan and received at least one dose of IMP.

| | |
|---|---|
| Reporting group title | Treosulfan - Safety Analysis Set |

Reporting group description:

This arm comprises all subjects randomised to treosulfan who received at least one dose of the IMP.

| Serious adverse events | Busulfan - Safety Analysis Set | Treosulfan - Safety Analysis Set | |
|---|---|---|---|
| Total subjects affected by serious adverse events | | | |
| subjects affected / exposed | 16 / 50 (32.00%) | 18 / 51 (35.29%) | |
| number of deaths (all causes) | 7 | 2 | |
| number of deaths resulting from adverse events | 4 | 0 | |
| Investigations | | | |
| Investigations - Other, specify | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Neoplasms benign, malignant and unspecified (incl cysts and polyps) | | | |
| Myelodysplastic syndrome | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 1 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Injury, poisoning and procedural complications | | | |
| Injury, poisoning and procedural complications - Other, specify | | | |

| | | | |
|---|---|---|---|
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 1 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Nervous system disorders | | | |
| Encephalopathy | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 1 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 1 | 0 / 0 | |
| General disorders and administration site conditions | | | |
| Fever | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 6 / 51 (11.76%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 6 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Multi-organ failure | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 0 / 1 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 1 | 0 / 0 | |
| Blood and lymphatic system disorders | | | |
| Blood and lymphatic system disorders - Other, specify | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 1 / 2 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Anemia | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 0 / 2 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Immune system disorders | | | |
| Immune system disorders - Other, specify | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Gastrointestinal disorders | | | |
| Vomiting | | | |

| | | | |
|---|---|---|---|
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Respiratory, thoracic and mediastinal disorders | | | |
| Bronchopulmonary hemorrhage | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 1 / 1 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Pneumonitis | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 0 / 1 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Pneumothorax | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 0 / 1 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Respiratory, thoracic and mediastinal disorders - Other, specify | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 0 / 1 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 1 | 0 / 0 | |
| Hepatobiliary disorders | | | |
| Hepatobiliary disorders - Other, specify | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 2 / 2 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Infections and infestations | | | |
| Infections and infestations - Other, specify | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 5 / 51 (9.80%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 5 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Sepsis | | | |

| | | | |
|---|---|---|---|
| subjects affected / exposed | 2 / 50 (4.00%) | 2 / 51 (3.92%) | |
| occurrences causally related to treatment / all | 0 / 2 | 0 / 2 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Lung infection | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 0 / 51 (0.00%) | |
| occurrences causally related to treatment / all | 0 / 3 | 0 / 0 | |
| deaths causally related to treatment / all | 0 / 2 | 0 / 0 | |
| Catheter related infection | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 1 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Enterocolitis infectious | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 1 | 0 / 3 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Bronchial infection | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Encephalitis infection | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |
| Upper respiratory infection | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 1 / 51 (1.96%) | |
| occurrences causally related to treatment / all | 0 / 0 | 0 / 1 | |
| deaths causally related to treatment / all | 0 / 0 | 0 / 0 | |

Frequency threshold for reporting non-serious adverse events: 5 %

| Non-serious adverse events | Busulfan - Safety Analysis Set | Treosulfan - Safety Analysis Set | |
|---|---|---|---|
| Total subjects affected by non-serious adverse events | | | |
| subjects affected / exposed | 48 / 50 (96.00%) | 49 / 51 (96.08%) | |
| Vascular disorders | | | |
| Hypertension | | | |
| subjects affected / exposed | 18 / 50 (36.00%) | 19 / 51 (37.25%) | |
| occurrences (all) | 29 | 20 | |
| Hematoma | | | |
| subjects affected / exposed | 6 / 50 (12.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 8 | 4 | |
| Hypotension | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 6 | 3 | |
| Capillary leak syndrome | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 0 | 3 | |
| General disorders and administration site conditions | | | |
| Fever | | | |
| subjects affected / exposed | 36 / 50 (72.00%) | 36 / 51 (70.59%) | |
| occurrences (all) | 71 | 58 | |
| Infusion related reaction | | | |
| subjects affected / exposed | 6 / 50 (12.00%) | 9 / 51 (17.65%) | |
| occurrences (all) | 11 | 11 | |
| Chills | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 7 / 51 (13.73%) | |
| occurrences (all) | 3 | 7 | |
| Localized edema | | | |
| subjects affected / exposed | 4 / 50 (8.00%) | 4 / 51 (7.84%) | |
| occurrences (all) | 4 | 6 | |
| Fatigue | | | |
| subjects affected / exposed | 4 / 50 (8.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 8 | 3 | |
| Pain | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 4 / 51 (7.84%) | |
| occurrences (all) | 7 | 5 | |
| Respiratory, thoracic and mediastinal disorders | | | |

| | | | |
|---|---|---|---|
| Cough | | | |
| subjects affected / exposed | 13 / 50 (26.00%) | 10 / 51 (19.61%) | |
| occurrences (all) | 19 | 11 | |
| Epistaxis | | | |
| subjects affected / exposed | 7 / 50 (14.00%) | 8 / 51 (15.69%) | |
| occurrences (all) | 11 | 9 | |
| Hypoxia | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 4 / 51 (7.84%) | |
| occurrences (all) | 3 | 5 | |
| Sore throat | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 5 / 51 (9.80%) | |
| occurrences (all) | 2 | 5 | |
| Pharyngeal mucositis | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 0 / 51 (0.00%) | |
| occurrences (all) | 3 | 0 | |
| Investigations | | | |
| Alanine aminotransferase increased | | | |
| subjects affected / exposed | 10 / 50 (20.00%) | 8 / 51 (15.69%) | |
| occurrences (all) | 13 | 9 | |
| Investigations - Other, specify | | | |
| subjects affected / exposed | 4 / 50 (8.00%) | 8 / 51 (15.69%) | |
| occurrences (all) | 5 | 11 | |
| Aspartate aminotransferase increased | | | |
| subjects affected / exposed | 7 / 50 (14.00%) | 4 / 51 (7.84%) | |
| occurrences (all) | 9 | 6 | |
| GGT increased | | | |
| subjects affected / exposed | 6 / 50 (12.00%) | 2 / 51 (3.92%) | |
| occurrences (all) | 6 | 2 | |
| Blood bilirubin increased | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 5 | 3 | |
| Cardiac disorders | | | |
| Sinus tachycardia | | | |
| subjects affected / exposed | 7 / 50 (14.00%) | 5 / 51 (9.80%) | |
| occurrences (all) | 9 | 6 | |
| Sinus bradycardia | | | |

| | | | |
|---|---|---|---|
| subjects affected / exposed | 4 / 50 (8.00%) | 1 / 51 (1.96%) | |
| occurrences (all) | 5 | 1 | |
| Nervous system disorders | | | |
| Headache | | | |
| subjects affected / exposed | 12 / 50 (24.00%) | 14 / 51 (27.45%) | |
| occurrences (all) | 18 | 20 | |
| Dizziness | | | |
| subjects affected / exposed | 6 / 50 (12.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 8 | 3 | |
| Blood and lymphatic system disorders | | | |
| Febrile neutropenia | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 4 / 51 (7.84%) | |
| occurrences (all) | 0 | 4 | |
| Hemolysis | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 1 | 3 | |
| Eye disorders | | | |
| Eye disorders - Other, specify | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 1 / 51 (1.96%) | |
| occurrences (all) | 3 | 1 | |
| Gastrointestinal disorders | | | |
| Mucositis oral | | | |
| subjects affected / exposed | 40 / 50 (80.00%) | 36 / 51 (70.59%) | |
| occurrences (all) | 43 | 36 | |
| Vomiting | | | |
| subjects affected / exposed | 32 / 50 (64.00%) | 33 / 51 (64.71%) | |
| occurrences (all) | 71 | 69 | |
| Diarrhea | | | |
| subjects affected / exposed | 23 / 50 (46.00%) | 30 / 51 (58.82%) | |
| occurrences (all) | 45 | 46 | |
| Abdominal pain | | | |
| subjects affected / exposed | 15 / 50 (30.00%) | 23 / 51 (45.10%) | |
| occurrences (all) | 25 | 39 | |
| Nausea | | | |
| subjects affected / exposed | 19 / 50 (38.00%) | 15 / 51 (29.41%) | |
| occurrences (all) | 33 | 20 | |
| Constipation | | | |

| | | | |
|---|---|---|---|
| subjects affected / exposed | 7 / 50 (14.00%) | 8 / 51 (15.69%) | |
| occurrences (all) | 9 | 13 | |
| Gastrointestinal disorders - Other, specify | | | |
| subjects affected / exposed | 4 / 50 (8.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 4 | 3 | |
| Gastritis | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 2 | 4 | |
| Stomach pain | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 2 | 3 | |
| Anal mucositis | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 1 | 3 | |
| Hepatobiliary disorders | | | |
| Hepatobiliary disorders - Other, specify | | | |
| subjects affected / exposed | 28 / 50 (56.00%) | 26 / 51 (50.98%) | |
| occurrences (all) | 41 | 36 | |
| Skin and subcutaneous tissue disorders | | | |
| Pruritus | | | |
| subjects affected / exposed | 9 / 50 (18.00%) | 14 / 51 (27.45%) | |
| occurrences (all) | 11 | 22 | |
| Skin and subcutaneous tissue disorders - Other, specify | | | |
| subjects affected / exposed | 9 / 50 (18.00%) | 13 / 51 (25.49%) | |
| occurrences (all) | 12 | 24 | |
| Rash maculo-papular | | | |
| subjects affected / exposed | 7 / 50 (14.00%) | 13 / 51 (25.49%) | |
| occurrences (all) | 8 | 20 | |
| Alopecia | | | |
| subjects affected / exposed | 6 / 50 (12.00%) | 11 / 51 (21.57%) | |
| occurrences (all) | 6 | 11 | |
| Dry skin | | | |
| subjects affected / exposed | 5 / 50 (10.00%) | 0 / 51 (0.00%) | |
| occurrences (all) | 5 | 0 | |
| Skin hyperpigmentation | | | |

| | | | |
|---|---|---|---|
| subjects affected / exposed | 3 / 50 (6.00%) | 2 / 51 (3.92%) | |
| occurrences (all) | 3 | 2 | |
| Urticaria | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 1 | 3 | |
| Hypertrichosis | | | |
| subjects affected / exposed | 0 / 50 (0.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 0 | 3 | |
| Renal and urinary disorders | | | |
| Hematuria | | | |
| subjects affected / exposed | 5 / 50 (10.00%) | 5 / 51 (9.80%) | |
| occurrences (all) | 6 | 5 | |
| Urinary frequency | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 4 / 51 (7.84%) | |
| occurrences (all) | 1 | 4 | |
| Musculoskeletal and connective tissue disorders | | | |
| Pain in extremity | | | |
| subjects affected / exposed | 6 / 50 (12.00%) | 9 / 51 (17.65%) | |
| occurrences (all) | 10 | 11 | |
| Back pain | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 2 | 3 | |
| Infections and infestations | | | |
| Infections and infestations - Other, specify | | | |
| subjects affected / exposed | 21 / 50 (42.00%) | 22 / 51 (43.14%) | |
| occurrences (all) | 40 | 31 | |
| Rhinitis infective | | | |
| subjects affected / exposed | 6 / 50 (12.00%) | 4 / 51 (7.84%) | |
| occurrences (all) | 6 | 6 | |
| Sepsis | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 2 / 51 (3.92%) | |
| occurrences (all) | 3 | 2 | |
| Lung infection | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 1 / 51 (1.96%) | |
| occurrences (all) | 3 | 1 | |
| Catheter related infection | | | |

| | | | |
|---|---|---|---|
| subjects affected / exposed | 3 / 50 (6.00%) | 1 / 51 (1.96%) | |
| occurrences (all) | 3 | 1 | |
| Skin infection | | | |
| subjects affected / exposed | 1 / 50 (2.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 1 | 4 | |
| Metabolism and nutrition disorders | | | |
| Anorexia | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 4 | 3 | |
| Iron overload | | | |
| subjects affected / exposed | 3 / 50 (6.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 4 | 3 | |
| Hyperkalemia | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 6 | 4 | |
| Hypokalemia | | | |
| subjects affected / exposed | 2 / 50 (4.00%) | 3 / 51 (5.88%) | |
| occurrences (all) | 6 | 3 | |

## More information

## Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

| Date | Amendment |
|---|---|
| 14 August 2015 | Update of the study timelines due to delayed study start<br>Clarification on withdrawal of patients who violate eligibility criteria prior to administration of IMP, i.e. withdrawals without IMP had to be replaced.<br>To ensure that the inclusion/exclusion criteria remain fulfilled until start of conditioning the most current result had to be used for assessment of eligibility in case of repeated examinations.<br>Exclusion criterion number 8 was revised.<br>Clarification of the period in which the patient registration had to be performed.<br>New safety information was added to the protocol and the patient information for parents.<br>Reference to the document "Development Summary of Product Characteristics" for Treograft was added.<br>A narrower therapeutic window range has been defined for a target AUCday0-4 in order to ensure a dose-adjustment of the full myeloablative dose in case Therapeutic drug monitoring of Busulfan has to be performed.<br>The wording for the administration time of i.v. treosulfan and i.v. fludarabine was changed from "within" 120/30 minutes to "over" 120/30 minutes.<br>In addition to prophylactic also therapeutic treatment of GvHD (eg,glucocorticoids) and anti-infective medication administered for anti-bacterial, antiviral and anti-fungal prophylactic treatment had to be documented on the CRF .<br>Exceptions from expedited SAE/SAR reporting, clarification regarding documentation of laboratory parameters as adverse events, detailed instructions on SAE reporting and Instructions on documentation of "pregnancy" as SAE were added.<br>A separate SAE report form has to be completed for each SAE.<br>A change in the number of patients subjected to blood samples has been implemented in case an interim analysis reveals that the population PK model is not sufficiently accurate, or the DMC recommend dose modifications.<br>The accepted time deviation of the first PK sample has been defined.<br>"Graft failure" has been additionally considered as a competing event for aGvHD and cGvHD. |
| 23 June 2016 | Changes in Sponsor personnel and job titles.<br>Changes in timelines.<br>Up-to-date information regarding safety was provided in the Investigator's Brochure (approved edition 9.0, released 29-Nov-2015), including the Development SmPC for treosulfan, and in the SmPC for Busilvex. Safety information was deleted from the Clinical Trial Protocol.<br>Additional blood samples were required for the validation of the POP-PK model.<br>Based on a DMC recommendation dated 15-Apr-2016, PK sampling was to be performed until adequate PK data were available for each of the age groups.<br>Editorial changes to improve readability were made and the sentences referring to the pooled statistical analysis and the interim analyses were moved.<br>Subject Information Sheets were updated according to the above mentioned changes. |

| | |
|---|---|
| 13 February 2017 | The CRO for regulatory aspects and/or monitoring outside of DE changed. In addition, the outsourcing was extended to all countries involved in the clinical trial and to project management.<br>The Sponsor's staff changed. In addition, a Medical Expert was named in order to provide a single point of contact to advise on trial related medical questions or problems. Functions of the International Coordinating Investigator and the National Coordinating Investigators were specified.<br>The definition of engraftment after HSCT was clarified in order to avoid misinterpretations. The term "consecutive days" was defined as 3 consecutive blood samples, if these were taken on different days. The second and third samples were required to confirm stable engraftment above the defined thresholds and were to be taken on the next consecutive days whenever possible; however, exceptions were accepted.<br>Update and submission of the Investigational Medicinal Product Dossier. |
| 06 June 2017 | For particular non-malignant diseases like thalassaemia major or combined immunodeficiencies the reoccurrence of disease due to loss of donor cells may require the regular administration of blood products (eg, erythrocytes or PLT) as therapeutic agents. Thus, a return to transfusion dependence associated with the reoccurrence of the underlying disease after allo-HSCT was to be documented as rescue therapy on the CRF. Further, stem cell infusions with or without further conditioning regimens could be used as rescue therapy.<br>A PIP modification was approved requesting to change the requirement to treat the vast majority of subjects (at least 85 out of 100) without additional use of thiotepa. Instead the unlimited use of the thiotepa containing therapy in qualified subjects was permitted.<br>The last regular chimerism documentation (without signs of graft failure) was to be done 12 months after transplantation. The use of rescue therapy and graft failure was to be documented until the end of the longer-term follow-up phase. A donor type chimerism analysis was only required in order to confirm secondary graft failure after sustained decline of neutrophils (≤ 0.5 x 109/L) and leucocytes (≤ 1.0 x 109/L). In some non-malignant diseases like thalassaemia major, graft failure may not manifest as marrow aplasia, but as autologous reconstitution. In these cases, conventional definitions of graft failure based on the detection of cytopaenia could not be applied, but loss of chimerism was the sign of graft failure. Loss of chimerism in the cell compartment of interest led to recurrence of disease symptoms; loss of chimerism in the red cell compartment led to haemolysis and transfusion dependence in thalassaemia. In order to get clear evidence for a late secondary graft failure, the analysis of donor type chimerism was extended to the longer term follow-up phase.<br>The definition of primary graft failure was specified as a donor-type chimerism of < 10% in BM. |

Notes:

## Interruptions (globally)

Were there any global interruptions to the trial? No

## Limitations and caveats

None reported