19 June 2025
EMA/232925/2025
Committee for Advanced Therapies (CAT)
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zemcelpro

International non-proprietary name: dorocubicel / allogeneic umbilical cord-derived CD34- cells, non-expanded

Procedure No. EMEA/H/C/005772/0000

Note

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

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List of abbreviations

Abbreviation	Definition
ADR	adverse drug reaction
AE	adverse event
AESI	adverse event of special interest
ALL	acute lymphoblastic leukaemia
ALT	Alanine transaminase
AML	acute myelogenous leukaemia
ANC	Absolute neutrophil counts
ASBMT	American Society for Blood and Marrow Transplantation
AS	Active Substance
AST	aspartate aminotransferase
ASTCT	American Society for Transplantation and Cellular Therapy
ATG	anti-thymocyte globulin
ATMP	Advanced therapy medicinal products
BM	bone marrow
BMT CTN	Blood and Marrow Transplant Clinical Trials Network
BUN	blood urea nitrogen
CB	cord blood
CBU	cord blood unit
CCRM	Centre for Commercialization of Regenerative Medicine
CD34-	CD34-negative
CD34+	CD34-positive
CETC	Centre d'Excellence en Thérapie Cellulaire
CI	confidence interval
CIBMTR	Centre for International Blood and Marrow Transplant Research
CIR	cumulative incidence of relapse
CML	chronic myeloid leukaemia
COP	Cryptogenic organising pneumonia
CRFS	Chronic graft versus host disease-free and relapse-free survival
CRL3	CULLIN3-E3 ubiquitin ligase
CSR	Clinical Study Report

Abbreviation	Definition
CRF	Control rate freezer
CTCAE	Common Toxicity Criteria for Adverse Events
EBMT	European Society for Bone Marrow Transplantation
ECG	electrocardiogram
ECT-001	Excellthera-001 technology
ECT-001-CB	UM171-expanded umbilical cord blood cells in an optimized culture system
ELN	European LeukemiaNet
EMA	European medicines agency
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration (US)
G-CSF	granulocyte-colony stimulating factor
GRFS	graft versus host disease-free and relapse free survival
GVHD	graft versus host disease
haplo	Haploidentical familial donor
HCT-CI	hematopoietic cell transplantation specific comorbidity index
HL	Hodgkin's lymphoma
HLA	human leukocyte antigen
HMR	Hôpital Maisonneuve-Rosemont
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplant
ICU	intensive care unit
IgG	Immunoglobulin G
IPS	Idiopathic pneumonia syndrome
ISSE	Integrative Study of Safety and Efficacy
ITT	Intent-to-treat
IV	intravenous
KPS	Karnofsky performance score
MAA	Marketing authorisation application
MDS	myelodysplastic syndromes
mFAS	modified Full Analysis Set

Abbreviation	Definition
MMF	Mycophenolate mofetil
MMUD	Mismatched unrelated donor
MSD	Matched sibling donor
MUD	Matched unrelated donor
NAS	New active substance
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NHL	Non-Hodgkin lymphoma
NIH	National Institutes of Health
NK	natural killer cells
NMDP	National Marrow Donor Program
NR	not reported
NRM	Non relapse mortality
NSG	Nod SCID gamma
OS	overall survival
PAH	pulmonary alveolar haemorrhage
PB	peripheral blood
PBSC	Peripheral blood stem cells
PD	Pharmacodynamic
PFS	progression-free survival
pINN	proposed International Nonproprietary Names
PIP	paediatric investigational plan
PK	pharmacokinetic
PO	*Per os*
PP	Per protocol
PRIME	Priority medicines
PTCy	post-transplant cyclophosphamide
PTLD	post-transplant lymphoproliferative disease
qPCR	quantitative polymerase chain reaction
RFS	Relapse Free-Survival
RMAT	regenerative medicine advanced therapy
SAE	serious adverse event

Abbreviation	Definition
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
sFAS	Supportive full analysis set
smFAS	Supportive modified full analysis set
SOC	system organ class
TBI	total body irradiation
TCR	T cell receptor
TNC	total nucleated cells
TRM	Transplant related mortality
UCB	umbilical cord blood
US	United States
USA	United States

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Cordex Biologics International Limited submitted on 23 November 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Zemcelpro, through the centralised procedure under Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

Zemcelpro was designated as an orphan medicinal product EU/3/20/2271 on 22 April 2020 in the following condition: Treatment in haematopoietic stem cell transplantation.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Zemcelpro as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/zemcelpro

The applicant applied for the following indication: treatment of adult patients with haematological malignancies requiring an allogeneic haematopoietic stem cell transplantation following myeloablative conditioning for whom no other type of suitable donor cells is available

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8(3) of Directive 2001/83/EC - complete and independent application. The applicant indicated that dorocubicel was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on the applicant's own tests and studies and bibliographic literature substituting/supporting certain tests or studies.

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0426/2023 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0426/2023 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

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

1.5. Applicant's requests for consideration

1.5.1. Conditional marketing authorisation

The applicant requested consideration of its application for a conditional marketing authorisation in accordance with Article 14-a of the above-mentioned Regulation.

1.5.2. Accelerated assessment

The applicant requested accelerated assessment in accordance with Article 14 (9) of Regulation (EC) No 726/2004.

1.5.3. New active substance status

The applicant requested the active substance dorocubicel contained in the above medicinal product to be considered as a New Active Substance (NAS), as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.5.4. Scientific recommendation on classification

The applicant Cordex Biologics International Limited submitted on 23 November 2023 an application for a scientific recommendation on classification to the EMA for Zemcelpro, which was designated as an Advanced Therapy Medicinal Product (ATMP) on 25 September 2020.

1.6. PRIME

Zemcelpro was granted eligibility to PRIME on 10 December 2020 in the following indication: Urgent allogeneic haematopoietic stem cell transplantations.

Eligibility to PRIME was granted at the time in view of the following:

- There is an unmet medical need for patients who do not have access to any other type of transplant (due to absence of HLA-matched siblings, HLA-matched unrelated donors or haploidentical donors) yet require urgent transplantation. Based on literature reviews, 15% of patients do not have access to HLA-matched unrelated donors or haploidentical donors.
- Clinical data available for 49 patients, mostly enrolled phase I/II open label trials (Trial ECT-001-CB.001, Trial ECT-001-CB.002 Trial ECT-001-CB.003), support the potential to address the unmet medical need. The outcome of transplant procedure as measured by time to neutrophil engraftment (500/µL) was achieved at D+18 (median) and was independent of CD34+ cell dose. Time-to-transplant related mortality clinical data at 2 years is also promising.
- The above results are considered sufficiently robust in comparison to standard Umbilical Cord Blood (UCB) transplants to support PRIME eligibility.

Upon granting of eligibility to PRIME, the Rapporteur was appointed by the CAT.

A kick-off meeting was held on 23 April 2021. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

Regarding quality development: the proposed approach to demonstrate comparability of fresh and cryopreserved product; release specifications; stability program; the manufacturing process to inform

the possibility of process performance qualification.

Regarding non-clinical development: overall adequacy of the non-clinical data package to support an MAA.

Regarding clinical development: the overall clinical data package to support an MAA; choice of endpoints, methodology and proposed efficacy analyses; contextualisation of single-arm data via data to support conditional MA and the comprehensive data to confirm the clinical efficacy and long-term safety in the target population.

1.7. Protocol assistance

The applicant received the following protocol assistance on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
16 December 2021	EMA/SA/0000069181	Johannes Hendrikus Ovelgonne, Ole Weis Bjerrum, Romaldas Mačiulaitis
13 October 2022	EMA/SA/0000099914	Mette Linnert Jensen, Silvijus Abramavicius

The protocol assistance pertained to the following quality, non-clinical, and clinical aspects:

EMA/SA/0000069181 – Quality, Non-clinical and Clinical development

- Whether both fresh and cryopreserved ECT-001-CB-DP1 present similar quality attributes that lead to comparable clinical outcomes; the proposed drug product release specifications for ECT-001-CB-DP1 and ECT-001-CB-DP2 with respect to the chosen test parameters or acceptance criteria; the proposed stability programme to establish the shelf-life of ECT-001-CB-DP1 and ECT-001-CB-DP2; whether process performance qualification runs could be performed as part of clinical trial manufacturing and some process validation activities may be deferred to the post-authorisation phase; the proposed format and content of Module 3 for the forthcoming marketing authorisation application (MAA).
- Adequacy of the non-clinical development package to support the filing of an MAA.
- The proposed clinical data package for an MAA in patients requiring an urgent allogeneic haematopoietic stem cell transplant without access to a readily available suitable donor; appropriateness of the primary efficacy endpoint, statistical methodology, and efficacy analyses to support an MAA; whether the retrospective analysis performed to determine a benchmark of historical data to evaluate neutrophil engraftment in cord blood transplantation is an acceptable surrogate control in lieu of performing a randomised controlled study; the proposed safety and efficacy data package for conditional marketing authorisation; adequacy of the proposed post-approval commitments to confirm robustness of the clinical efficacy and long-term safety; whether the design and methodology of a draft proposal of a retrospective matched-controlled analysis, in collaboration with registries, and in particular the matching procedure constitute a valid surrogate for a randomised trial; the design, endpoints, and analysis methods for primary and secondary endpoints for study ECT-001-CB.008 which is proposed as a post-approval commitment to confirm clinical benefit and long-term efficacy.

EMA/SA/0000099914 - Clinical development

- The proposed retrospective matched-controlled analysis in order to contextualise the results from the single-arm trial ECT-001-CB.001.

1.8. Steps taken for the assessment of the product

The CAT Rapporteur and Co-Rapporteur were:

CAT Rapporteur: Emmely de Vries CAT Co-Rapporteur: Jan Mueller-Berghaus

The application was received by the EMA on	23 November 2023
Accelerated Assessment procedure was agreed-upon by CAT and CHMP on	14 December 2023
The procedure started on	20 June 2024
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	9 September 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	23 September 2024
The CAT Co-Rapporteur's first assessment (critique) was circulated to all CAT and CHMP members on	24 September 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT during the meeting on	3 October 2024
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on The procedure was reverted from accelerated assessment to standard assessment on	11 October 2024
The applicant submitted the responses to the CAT consolidated List of Questions on	20 January 2025
The following GMP and GCP inspections were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection at 2 sites (sponsor ad clinical investigator site) in Canada between 18/11/2024 and 29/11/2024. The outcome of the inspection carried out was issued on	10 January 2025 and 13 January 2025
– A GMP inspection at one manufacturing site in Canada on 25 October 2024. The outcome of the inspection carried out was issued on	20 Jan 2025
The CAT Rapporteur circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	25 February 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 March 2025
The CAT agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	21 March 2025

The applicant submitted the responses to the CAT List of Outstanding Issues on	14 May 2025
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	28 May 2025
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive draft opinion for granting a marketing authorisation to Zemcelpro as well as a report on New Active Substance (NAS) status of the active substance contained in the medicinal product on	13 June 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Zemcelpro as well as a report on New Active Substance (NAS) status of the active substance contained in the medicinal product on	19 June 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Haematological malignancies are a heterogeneous group of blood cell cancers, which include acute leukaemias, myelodysplastic syndromes (MDS), myeloproliferative disorders, lymphomas and myelomas (Arber et al., 2016[1]; Swerdlow et al, 2016[2]). Globally, the management of haematological malignancies is based on autologous or allogeneic HSCT, radiation therapy, and treatments such as chemotherapies, immunotherapies, targeted therapies, and other innovative therapies such as CAR-T.

The initially applied indication for Zemcelpro was:

Treatment of adult patients with haematological malignancies requiring an allogeneic hematopoietic stem cell transplantation who lack a readily available suitable donor.

2.1.2. Epidemiology

In 2022, a total of 19,011 allogeneic transplantations from related or unrelated donors were reported by 689 European centres. Most of those allogeneic transplantations were performed for myeloid malignancies (n= 10,433; 58.4%) or lymphoid malignancies (n=4,674; 26.2%) (Passweg et al, 2024[3]).

Generally, the preferred source of stem cells is human leukocyte antigen (HLA)-matched sibling donor

[1] Arber, D. A., Orazi, A., Hasserjian, R., et al. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, The Journal of the American Society of Hematology, 127(20), 2391-2405.
[2] Swerdlow, S. H., Campo, E., Pileri, S. A., et al. (2016). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, The Journal of the American Society of Hematology, 127(20), 2375-2390.
[3] Passweg JR, Baldomero H, Ciceri F, et al. (2024) Hematopoietic cell transplantation and cellular therapies in Europe 2022. CAR-T activity continues to grow; transplant activity has slowed: a report from the EBMT. Bone Marrow Transplant. 2024 Jun;59(6):803-812.

(MSD), as they are easy to test and collect, and have been associated with favourable prognosis in several indications. However, MSD are often not available to patients. Indeed, in the 2022 EBMT survey data, only 27% (n=5,068) of allogeneic transplants came from MSD (Passweg et al, 2024[4]). The next preferred source would be a HLA-matched unrelated donor (MUD). The likelihood of a patient finding a MUD in public registries is variable, reaching 50% for patients of European ancestry, but dropping significantly below 30% for minority ethnicities (Asian or Hispanic descent) and being as low as 5-10% for some minorities (African descent), due to their greater genetic diversity (Barker et al. 2010[5]; Barker et al. 2019a[6]; Dumont-Lagacé et al. 2022[7]). When a MSD is not available, the average delay between the initiation of the search for an unrelated donor and transplantation is 3 to 4 months (Cheuk, 2013[8]). Unfortunately, almost one third of patients will not have either an MSD or MUD (Barker et al. 2019a[9]), and will therefore need to find an alternative source of stem cells (Zhu et al. 2021[10]).

2.1.3. Biologic features

In the treatment of haematological malignancies, hematopoietic stem cell transplantation (HSCT) is used to allow cancer patients to receive doses of chemotherapy that are higher than the bone marrow can usually tolerate; bone marrow function is then restored by replacing the marrow with donor HSCs.

2.1.4. Clinical presentation

Allogeneic HSCT is the only potential curative treatment option for several haematologic malignancies such as certain types of acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), MDS, lymphoma and other diseases.

2.1.5. Management

Choosing the best option between standard MSD or MUD and alternative donors (cord blood [CB], haploidentical donor [haplo], or mismatched unrelated donor [MMUD]) for allogeneic HSCT is decided on a case-by-case manner by physicians and/or transplant committees as certain patient disease conditions would favour one type of transplant versus other conditions. In addition to the clinical disease being treated, physicians assess variables that positively or negatively affect the outcome of HSCT. These include: source of HSC (bone marrow versus peripheral blood, the latter being typically linked to greater T cell dose and thus associated with more graft versus host disease [GVHD]), HLA-mismatch (associated with GVHD, could be good for disease control), donor age (an older donor has been shown to result in inferior overall survival [OS], increased risk of non-relapse mortality (NRM) and acute GVHD, Mehta et al. 2023[11]), anti-HLA antibodies (associated with graft rejection), etc.

[4] Passweg JR, Baldomero H, Ciceri F, et al. (2024) Hematopoietic cell transplantation and cellular therapies in Europe 2022. CAR-T activity continues to grow; transplant activity has slowed: a report from the EBMT. Bone Marrow Transplant. 2024 Jun;59(6):803-812.
[5] Barker, J. N., Byam, C. E., Kernan, N. A., et al. (2010). Availability of cord blood extends allogeneic hematopoietic stem cell transplant access to racial and ethnic minorities. Biology of Blood and Marrow Transplantation, 16(11), 1541-1548.
[6] Barker, J. N., Boughan, K., Dahi, P. B., et al. (2019a). Racial disparities in access to HLAmatched unrelated donor transplants: a prospective 1312-patient analysis. Blood advances, 3(7), 939-944.
[7] Dumont-Lagacé, M., Feghaly, A., Meunier, M. C., et al. (2022). UM171 expansion of cord blood improves donor availability and HLA matching for all patients, including minorities. Transplantation and Cellular Therapy, 28(7), 410-e1.
[8] Cheuk, D. K. (2013). Optimal stem cell source for allogeneic stem cell transplantation for hematological malignancies. World journal of transplantation, 3(4), 99.
[9] Barker, J. N., Boughan, K., Dahi, P. B., et al. (2019a). Racial disparities in access to HLAmatched unrelated donor transplants: a prospective 1312-patient analysis. Blood advances, 3(7), 939-944.
[10] Zhu X, Tang B, Sun Z (2021) Umbilical cord blood transplantation: Still growing and improving. Stem Cells Transl Med, 10 (Suppl 2):S62-S74.
[11] Mehta RS, Ramdial J, Marin D, et al. (2023) Impact of Donor Age in Haploidentical-Post-Transplantation Cyclophosphamide versus Matched Unrelated Donor Post-Transplantation Cyclophosphamide Hematopoietic Stem Cell Transplantation in Patients with Acute Myeloid Leukaemia. Transplant Cell Ther, 29(6):377.e1-377.e7.

MSD is the preferred donor type for a HSCT, with the best overall results (Nagler et al. 2023[12], Al Hamed et al. 2023[13]). The next preferred source would be a MUD. Unfortunately, almost one third of patients will not have a MSD or a MUD (Barker et al. 2019[14];), and will therefore need an alternative transplant (Zhu et al. 2021[15]).

Three alternative transplant options are available for patients without matched donor: 1) a haplo familial donor 2) a MMUD and 3) CB; each option with their respective advantages and disadvantages.

Historically, transplants from mismatched donors (i.e., haplo and MMUD) were associated with increased risk of transplant related mortality (TRM) because of the higher frequency of graft-versus-host disease (GVHD) and infectious complications, leading to decreased survival (Lee et al. 2007[16]). The recent use of post-transplant cyclophosphamide (PTCy) in haplo and MMUD transplants has significantly reduced the incidence of severe GVHD previously seen with these mismatched transplants (Leick and Chen 2022[17]; Al Hamed et al. 2023[18]; Brissot et al. 2019[19]).

CB grafts, the third alternative transplant option, are more permissive to HLA mismatch than other unrelated adult donors. In addition, cord blood units (CBU) are readily available (in 2-4 weeks) as several hundred thousands of units have been collected, quality controlled, and cryopreserved across the world. Furthermore, CB transplants have been associated with a potent graft-vs-leukaemia effect, as several studies have demonstrated lower relapse rate with CB transplants compared to HLA matched donors in the setting of high-risk acute leukaemia (Milano et al. 2016[20], Borrill et al. 2023[21]). Other key attributes of CB transplants include: absence of risk to the donor (Gragert er al. 2014[22]), reduced incidence of chronic GVHD (Pidala et al. 2011[23]), and near absence of viral transmission from the donor.
Whereas an HLA-compatible CB transplant appears available for most, it is the problem of cell dose that limits their utilisation. Physicians could select larger CB units that have a suboptimal HLA-match to the recipient. While allowing for safe reconstitution, each HLA mismatch increases transplant related mortality (TRM) by 5-10% (Yokoyama et al. 2020[24]; Eapen et al. 2014[25]).

Double CB unit transplants were introduced as a possible solution for slow engraftment in CB transplantation. Compared to suitably sized single CB unit transplantation, little overall advantage of

[12] Nagler A, Labopin M, Mielke S, et al. (2023) Matched related versus unrelated versus haploidentical donors for allogeneic transplantation in AML patients achieving first complete remission after two induction courses: a study from the ALWP/EBMT. Bone Marrow Transplant, 58(7):791-800.
[13] Al Hamed R, Ngoya M, Galimard JE, et al. (2023) Unrelated or haploidentical allogeneic hematopoietic cell transplantation in second complete remission for acute myeloid leukaemia- Improved outcomes over time: A European Society for Blood and Marrow Transplantation Acute Leukaemia Working Party study. Cancer, 129(17):2645-2654.
[14] Barker, J. N., Boughan, K., Dahi, P. B., et al. (2019a). Racial disparities in access to HLAmatched unrelated donor transplants: a prospective 1312-patient analysis. Blood advances, 3(7), 939-944.
[15] Zhu X, Tang B, Sun Z (2021) Umbilical cord blood transplantation: Still growing and improving. Stem Cells Transl Med, 10 (Suppl 2):S62-S74.
[16] Lee S, Klein J, Haagenson M, et al. (2007) High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood, 110(13):4576-4583.
[17] Leick M, Chen YB (2022) A glimpse into what happens after PTCy. Blood, 139(4):479-481.
[18] Al Hamed R, Ngoya M, Galimard JE, et al. (2023) Unrelated or haploidentical allogeneic hematopoietic cell transplantation in second complete remission for acute myeloid leukaemia- Improved outcomes over time: A European Society for Blood and Marrow Transplantation Acute Leukaemia Working Party study. Cancer, 129(17):2645-2654.
[19] Brissot E, Labopin M, Ehninger G, et al. (2019) Haploidentical versus unrelated allogeneic stem cell transplantation for relapsed/refractory acute myeloid leukaemia: a report on 1578 patients from the Acute Leukaemia Working Party of the EBMT. Haematologica, 104(3):524-532.
[20] Milano, F., Gooley, T., Wood, B., et al. (2016). Cord-blood transplantation in patients with minimal residual disease. N Engl J Med, 375, 944-953.
[21] Borrill R, Poulton K, Wynn R. (2023) Immunology of cord blood T-cells favors augmented disease response during clinical pediatric stem cell transplantation for acute leukemia. Frontiers in Pediatrics;11
[22] Gragert, L., Eapen, M., Williams, E., et al. (2014). HLA match likelihoods for hematopoietic stem-cell grafts in the US registry. New England Journal of Medicine, 371(4), 339-348.
[23] Pidala, J. (2011). Graft-vs-host disease following allogeneic hematopoietic cell transplantation. Cancer Control, 18(4), 268-276
[24] Yokoyama H, Morishima Y, Fuji S, et al. (2020) HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation. Impact of HLA Allele Mismatch at HLA-A, -B, -C, and -DRB1 in Single Cord Blood Transplantation. Biol Blood Marrow Transplant, 26(3):519-528.
[25] Eapen M, Klein JP, Ruggeri A, et al. (2014) Centre for International Blood and Marrow Transplant Research, Netcord, Eurocord, and the European Group for Blood and Marrow Transplantation. Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. Blood, 2;123(1):133-40.

double CB has been reported. Double CB transplant did not accelerate engraftment, but it did lower the risk of graft failure (Barker et al, 2005[26], Scaradavou et al, 2013[27]). Although some studies suggest potential benefit of infusing two CB units for prevention of relapse (Wang et al, 2019[28], Labopin et al, 2014[29]), other studies show possible increased toxicity with infusion of two CB units, with a higher risk of TRM and severe acute and chronic GVHD (Michel et al, 2016[30], Zheng et al, 2018[31], Wang et al, 2019[32], Wagner et al, 2014[33]) with no advantages in terms of PFS (Ruggeri et al, 2014[34], Zheng et al, 2018[35], Wang et al, 2019[36]). For these reasons, recommendations are to prioritise single CB transplantation, unless no suitably sized unit can be found (Ruggeri et al, 2014[37], Michel et al, 2016[38], Zheng et al, 2018[39], Wang et al, 2019[40], Wagner et al, 2014[41]).

The percentage of patients receiving an allogeneic HCT derived from a umbilical cord blood (UCB) in the US decreased from 11% in 2012 to 4% in 2022 (annual report CIBMTR). In Europe, 19.011 allogeneic HCTs were reported in 2022 of which 14.881 were in adults. In total 250 allogeneic HCTs were derived from UCB; 136 patients were <18 years and 112 patients were adults. The relative proportion of allogeneic HCTs derived from UCBs in adults was 0.8% (Passweg et al, 2024).

The likelihood of identifying a CBU with a ≥4/6 HLA match and a size suitable for single CBU transplantation differs for racial and ethnic groups. For White Europeans, the likelihood is 96% while for African Americans the likelihood is 81% (Gragert et al, 2014). When cellularity in a single CBU is not sufficient, a double umbilical cord blood transplantation can be considered. The percentage of patients that lack access to any type of suitable donor (including double-cord) is unclear, but expected to be very limited.

Other cell therapy approaches

Another cell therapy approach includes treatment with CAR-T cells, although superiority over HSCT has not been demonstrated yet.

Recently, Omisirge (omidubicel), a CB expansion technology based on a 21-day expansion of CB-derived HSCs using nicotinamide was approved in the US by the FDA for use in adults and paediatric patients 12 years and older with hematologic malignancies who are planned for umbilical CB transplantation following myeloablative conditioning to reduce the time to neutrophil recovery and the

[26] Barker J N, Weisdorf D J, DeFor T E, et al. (2005) Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood;105(3):1343-1347.
[27] Scaradavou A, Brunstein CG, Eapen M, et al. (2013) Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukaemia. Blood;121(5):752-758.
[28] Wang L, Gu, ZY, Liu SF, et al. (2019) Single-versus double-unit umbilical cord blood transplantation for hematologic diseases: a systematic review. Transfusion Medicine Reviews; 33(1):51-60.
[29] Labopin M, Ruggeri A, Gorin NC, et al. (2014) Cost-effectiveness and clinical outcomes of double versus single cord blood transplantation in adults with acute leukaemia in France. Haematologica;99(3):535-40.
[30] Michel G, Galambrun C, Sirvent A, et al. (2016) Single-vs double-unit cord blood transplantation for children and young adults with acute leukaemia or myelodysplastic syndrome. Blood;127(26):3450-3457.
[31] Zheng CC, Zhu XY, Tang BL, et al. (2018) Double vs. single cord blood transplantation in adolescent and adult hematological malignancies with heavier body weight (≥ 50 kg). Hematology;23(2):96-104.
[32] Wang L, Gu, ZY, Liu SF, et al. (2019) Single-versus double-unit umbilical cord blood transplantation for hematologic diseases: a systematic review. Transfusion Medicine Reviews; 33(1):51-60.
[33] Wagner Jr JE, Eapen M, Carter S, et al. (2014) One-unit versus two-unit cord-blood transplantation for hematologic cancers. N Engl J Med.;371(18):1685-1694.
[34] Ruggeri, A., Labopin, M., Sormani, MP, et al. (2014) Engraftment kinetics and graft failure after single umbilical cord blood transplantation using a myeloablative conditioning regimen. Haematologica, 99(9):1509.
[35] Zheng CC, Zhu XY, Tang BL, et al. (2018) Double vs. single cord blood transplantation in adolescent and adult hematological malignancies with heavier body weight (≥ 50 kg). Hematology;23(2):96-104.
[36] Wang L, Gu, ZY, Liu SF, et al. (2019) Single-versus double-unit umbilical cord blood transplantation for hematologic diseases: a systematic review. Transfusion Medicine Reviews; 33(1):51-60.
[37] Ruggeri, A., Labopin, M., Sormani, MP, et al. (2014) Engraftment kinetics and graft failure after single umbilical cord blood transplantation using a myeloablative conditioning regimen. Haematologica, 99(9):1509.
[38] Michel G, Galambrun C, Sirvent A, et al. (2016) Single-vs double-unit cord blood transplantation for children and young adults with acute leukaemia or myelodysplastic syndrome. Blood;127(26):3450-3457.
[39] Zheng CC, Zhu XY, Tang BL, et al. (2018) Double vs. single cord blood transplantation in adolescent and adult hematological malignancies with heavier body weight (≥ 50 kg). Hematology;23(2):96-104.
[40] Wang L, Gu, ZY, Liu SF, et al. (2019) Single-versus double-unit umbilical cord blood transplantation for hematologic diseases: a systematic review. Transfusion Medicine Reviews; 33(1):51-60.
[41] Wagner Jr JE, Eapen M, Carter S, et al. (2014) One-unit versus two-unit cord-blood transplantation for hematologic cancers. N Engl J Med.;371(18):1685-1694.

incidence of infection. It has not been approved in the EU.

The treatment with curative intent of patients with haematological malignancies who lack a readily available suitable donor (due to absence of HLA-matched siblings, HLA-matched unrelated donors, haploidentical donors or CB of sufficient cell quantity) represents a clinical challenge and novel treatment options are needed.

2.2. About the product

Zemcelpro is a cell therapy manufactured by expanding allogeneic cord blood (CB)- derived HSCs ex vivo. Zemcelpro is a combination package consisting of two drug products.

The active ingredients are the cells contained in the two fractions of Zemcelpro, both derived from the same patient-specific umbilical cord blood unit (CBU):

- the fraction of 7-day expanded CD34+ cells (CD34+ cell fraction; INN dorocubicel)
- the fraction of remaining unexpanded CD34- (CD3+) cells from the CB, which contains immune cells from the donor, including T lymphocytes (unexpanded CD34- cells, sometimes referred to as ECT-001-CB-DP2)

The expansion technology combines the use of the proprietary small molecule UM171, a pyrimido-indole derivative which preserves hematopoietic stem cells functions and prevents their differentiation in vitro (Fares et al. 2014[42] and Fares et al. 2017[43]), and a fed-batch culture system, which leads to the reduction of endogenously produced negative regulators of stem cell function. By expanding HSC, the use of UM171 allows the expansion of small cord units that hold the best HLA matched CB to increase accessibility to CB transplant, minimize complications, and ensure long-term in vivo engraftment for positive clinical outcomes.

Therefore, dorocubicel is a cryopreserved UM171 ((1R, 4R)-N1-(2-benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine dihydrobromide) expanded allogeneic hematopoietic progenitor cell therapy derived from a single cord blood unit and used as an allogeneic stem cell donor source.

The primary mechanism of action of dorocubicel lies in promoting hematopoietic recovery and immune reconstitution through the activity of expanded CD34+ hematopoietic stem cells.

The unexpanded CD34- cells, consisting primarily of CD3+ T cells, play a complementary role by supporting immune reconstitution and providing graft-versus-leukemia (GVL) effects post-transplantation.

Hematopoietic stem/progenitor cells from Zemcelpro migrate to the bone marrow where they divide, mature, and differentiate in all haematological cell lineages. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin.

The initially claimed therapeutic indication is: "Zemcelpro is indicated for the treatment of adult patients with haematological malignancies requiring an allogeneic hematopoietic stem cell transplantation who lack a readily available suitable donor".

And the final approved indication is: "*Zemcelpro is indicated for the treatment of adult patients with*

[42] Fares I, Chagraoui J, Gareau Y, et al. (2014) Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. Science;345(6203):1509-1512.
[43] Fares I, Chagraoui J, Lehnertz B, et al. (2017) EPCR expression marks UM171-expanded CD34+ cord blood stem cells. Blood. 22;129(25):3344-3351.

haematological malignancies requiring an allogeneic hematopoietic stem cell transplantation following myeloablative conditioning for whom no other type of suitable donor cells is available."

Zemcelpro is provided as a single infusion consisting of ≥ 0.23 x 10^6 CD34+ cells/mL and ≥0.53 x 10^6 CD3+ cells/mL dispersion for infusion in up to 4 cryopreserved infusion bags each.

2.3. Type of application and aspects on development

The CHMP and CAT agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on:

- It was agreed that a high unmet medical need exists in the EU for patients with haematological malignancies who do not have access to any other type of transplant (due to absence of HLA-matched siblings, HLA-matched unrelated donors or haploidentical donors or CB of sufficient cell quantity) and novel treatment options with curative intent are therefore needed for this patient population.
- To justify that their product can fulfil the unmet need, the applicant presented data from 5 clinical studies over multiple years of development program (launched in 2016). The total pivotal population at the time of agreement of the accelerated assessment was 36 patients with a median follow-up of 17 months from a pooled analysis of 3 studies (001, 002, and 004) representing a population with high risk leukemia/MDS.
- The presented pooled clinical efficacy data as well as the matched controlled analysis provided preliminary evidence of efficacy and safety of Zemcelpro.
- At the time of the request, the safety profile of Zemcelpro had been assessed across the 5 clinical studies (n=101) encompassing a heterogenous group of patients. The adverse event profile is comparable to standard transplant patients and no concerning safety signals have been identified.
- Based on the presented results it was considered likely that Zemcelpro would fulfil the unmet medical need for patients with haematological malignancies who do not have access to any other type of transplant.
- The limitations to the presented data (e.g. short follow up, small sample size, heterogenous population, interpretation of the matched controlled analysis due to lack of detail) were noted.

In conclusion, despite it was perceived that the dossier could raise challenges in the interpretation of data to substantiate the benefit/risk balance, given the unmet need it was considered that the available data could support a conditional marketing authorisation and an accelerated assessment timetable was recommended.

However, during assessment the CAT and CHMP concluded that it was no longer appropriate to pursue accelerated assessment, as there were major objections regarding the manufacturing and dosing of the product not resolvable under accelerated assessment timelines.

In addition, the applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14-a of Regulation (EC) No 726/2004, based on the following criteria:

- The benefit-risk balance is positive.

  The clinical development program for ECT-001-CB (UM171-expanded umbilical cord blood cells in an optimized culture system) currently comprises 5 studies. As of 15 March 2024, 123 patients had been enrolled in ECT-001-CB clinical trials, of whom 116 had been transplanted.

The applicant considers the extent of the data provided with the data cut-off of 15 March 2024 sufficient to indicate a promising safety and efficacy profile of ECT-001-CB as a stem cell source that can address the unmet medical need of adult patients with haematological malignancies without suitable readily available donors.

- It is likely that the applicant will be able to provide comprehensive data.

  In order to confirm long-term clinical benefit of ECT-001-CB in a similar patient population, the applicant proposed to commit to provide final data from the on-going ECT-001-CB studies as well conduct three additional studies.
  - Study ECT-001-CB.011: In contrast to the sought indication of the MAA for which a controlled randomised trial is not possible or ethical, ECT-001-CB.011, a multi-centre, randomised controlled study will compare ECT-001-CB to best available conventional alternative HSC source (haplo, MMUD) in patients with high-risk leukaemia/myelodysplasia in 208 patients with the primary objective to determine whether ECT-001-CB transplantation improves EFS over allogeneic transplantation with the best available HSC source.
  - Additional data from the young adult sub-population from study ECT-001-CB.010 (included in the PIP).
  - Finally, a prospective, observational, registry-based study (ECT-001-CB.012) with the objective to collect real-world safety and efficacy data on patients that have received commercially available ECT-001-CB will help to further define the risk-benefit profile of ECT-001-CB specifically in patients without access to a readily available suitable donor.

- Unmet medical needs will be addressed.

  There is an unmet medical need for patients who do not have access to any other type of transplant (due to absence of HLA-matched siblings, HLA-matched unrelated donors or haploidentical donors) yet require urgent transplantation. Based on literature reviews, 15% of patients do not have access to HLA-matched unrelated donors or haploidentical donors.

  Clinical data available for 87 patients, enrolled in phase I/II and phase II open label trials (Trial ECT-001-CB.001, Trial ECT-001-CB.002, and Trial ECT-001-CB.004), support the potential to address the unmet medical need. The outcome of transplant procedure as measured by time to neutrophil engraftment (500/µL) was achieved at Day 20 (median in Pivotal population) and was independent of CD34+ cell dose. Time-to- transplant related mortality clinical data at 2 years is also promising.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

  Patients with haematological diseases that require an allogeneic HSCT but cannot access a readily suitable donor have a high unmet need. In such instances, the patient would then be directed to either palliative or experimental treatments.
  According to the applicant, the presented data supports the request for a conditional marketing authorisation for ECT-001-CB for the treatment of patients with haematological malignancies requiring an allogeneic HSCT who lack of readily available suitable donor and demonstrates that ECT-001-CB is of major interest from the point of view of public health.

2.4. Quality aspects

2.4.1. Introduction

Zemcelpro is a cryopreserved allogeneic hematopoietic stem and progenitor cell therapy containing two cell components, namely the expanded CD34+ cells and unexpanded CD3+ components, both derived from the same patient-specific umbilical cord blood unit (CBU).

Zemcelpro (ECT-001-CB) is a combination package containing two finished products (FP):

- FP1 (ECT-001-CB-DP1): dorocubicel (INN), the UM171-expanded CD34+ cells and
- FP2 (ECT-001-CB-DP2): unexpanded CD34- cell fraction, from which the CD3+ cells are the active fraction (important for immune recovery in CBU (Cord Blood Unit) transplantation).

The medicinal product is packaged in:

- up to four infusion bags containing a dispersion for infusion of at least 0.23×10^6 viable CD34+ cells/mL (FP1) and
- four infusion bags containing a dispersion for infusion of at least 0.53×10^6 viable CD3+ cells/mL (FP2)

Each infusion EVA bag contains 20 mL dispersion for infusion suspended in a dimethyl sulfoxide (DMSO) solution.

2.4.2. Active substance

2.4.2.1. General information

- **Dorocubicel (ECT-001-CB-DP1)**

The active substance (AS) is described as a cell dispersion. Dorocubicel is a mixture of CD34+ cells, which includes the more primitive stem cells subset called hematopoietic stem and progenitor cells (HSPC), long-term hematopoietic stem cells (HSC), dendritic cells and mast cells.

The primary mechanism of action of dorocubicel lies in promoting hematopoietic recovery and immune reconstitution through the activity of UM171-expanded CD34+ hematopoietic stem cells. The expansion of these CD34+ cells in dorocubicel enhances the therapeutic potential of the graft by increasing the quantity and quality of cells that support rapid and sustained haematopoiesis.

The expansion technology combines the use of the proprietary small molecule UM171, a pyrimido-indole derivative which preserves hematopoietic stem cells functions and prevents their differentiation *in vitro*, and a fed-batch culture system, which leads to the reduction of endogenously produced negative regulators of stem cell function. By expanding HSC, the use of UM171 allows the expansion of small cord units that hold the best human leukocyte antigen (HLA) matched CB to increase accessibility to CB transplant, minimize complications, and ensure long-term *in vivo* engraftment for positive clinical outcomes.

- **Unexpanded CD34- cells (umbilical cord derived cells from which the CD3+ cells are the active fraction (ECT-001-CB-DP2)**

ECT-001-CB-DP2 contains mature immune cells that play a role in the prevention of graft rejection, including the donor CD3+ T cells which provide immune protection early post-transplant and contribute to the graft-vs-leukemia (GVL) effect. Within the lymphoid cell population, ECT-001-CB-DP2 contains a large proportion of CD4+ T cells, CD8+ T cells, CD19+ B cells and 21.0% CD16/CD56+ NK cells. As the CD34- component is minimally manipulated in the manufacturing process of ECT-001-CB, the cell

composition, and the viability of CD3 cells in ECT-001-CB-DP2 are highly correlated with those of the starting material, the CBU.

Mechanistically, the CD34- unexpanded fraction in Zemcelpro has the same role as the CD34- cells contained in standard of care allogeneic hematopoietic stem cell transplant. The CD34- cells, consisting primarily of CD3+ T cells, play a complementary role by supporting immune reconstitution and providing GVL effects post-transplantation. These unexpanded CD3+ cells are required for, and contribute to, the therapeutic benefits of allogeneic hematopoietic stem cell transplants, by providing immunologic support, enhancing anti-tumor activity and reducing the risk of graft failure.

2.4.2.2. Manufacture, characterisation and process controls [Dorocubicel (ECT-001-CB-DP1) and Unexpanded CD34- cells (ECT-001-CB-DP2)]

The ECT-001-CB manufacturing process is continuous and there is no formal separation between active substance and finished product hence there is FP information in the AS part.

The active substances are manufactured by Centre C3i Inc., 5415 De L'Assomption Boulevard, Montreal, Qc, H1T 2M4, Canada.

This site underwent an EU GMP inspection and a valid GMP certificate has been submitted.

A request for exemption from performing finished product release testing in Europe (Annex 5.9 GMP) and maintaining reference samples and retention samples in their fully packaged unit on the basis of limited material availability was accepted after additional justification. The applicant clarified that the batch size cannot be upscaled and any re-testing would deprive patients of necessary doses. After importation into Europe the finished product is released by the Qualified Person (QP).

The names and addresses of all the facilities responsible for the manufacture, testing and physical site of importation of dorocubicel and ECT-001-CB-DP2 have been provided. All manufacturing sites are GMP compliant.

At the start of the manufacturing process, a single CBU from a single donor is processed to isolate the CD34+ cells from the remaining CD34- cells. The manufacturing process is continuous: from CBU to cryopreservation of the final product. No reprocessing is permitted.

Dorocubicel

The CD34+ cell fraction consists of cord blood-derived CD34+ cells that are expanded *in vitro* in the presence of the small molecule UM171 and hematopoietic growth factors.

The active substance manufacturing consists of: I) thawing of cryopreserved CBU, II) antibody labelling for CD34+ cells and cell selection, III) expansion of CD34+ cells in the presence of UM171, IV) harvest, formulation and V) cryopreservation.

The process flow diagram for downstream processing of the CD34+ cell fraction has also been provided.

The batch definition was changed from two cryobags with dose capping to four cryobags without dose capping. The viable CD34+ cell dose will be adjusted by selecting the appropriate number of 20 mL bags to provide the maximal dose. The applicant also provided information about shipping and preparation (including thawing) for infusion at the bedside.

ECT-001-CB-DP2

The CD34- fraction, which contains cells that were not selected during step II), is collected at the end of that step and is processed independently. Also, the CD34- manufacturing process is continuous:

from CBU Unit to cryopreservation of the final product. Manufacture of ECT-001-CB-DP2 consists of concentration, formulation and cryopreservation of the CD34- cell fraction.

The cells are formulated and cryopreserved until shipment. The dispersion for infusion is thawed just prior to infusion. Final formulation occurs with HSA and DMSO in Plasma-Lyte A Injection. The applicant has clarified that the CD34+ and CD34- cells are processed in parallel and a hold time for CD34- cells has been included in the manufacturing process description.

- **Control of materials *[Dorocubicel (ECT-001-CB-DP1) and Unexpanded CD34- cells (ECT-001-CB-DP2)]***

The starting material is an erythro-depleted donor-screened CBU commercially acquired from a qualified CBU bank. The qualified CBU banks are listed in the dossier and information was provided according to which standards and principles the harvest of CBU comply. The procurement of CBU's is in accordance with the relevant EU legislation. CBUs are selected from authorised cells banks (i.e. in accordance with relevant EU legislation i.e. Directive 2004/23/EC or 2002/98/EC and implementing Directives).

The CBU is selected within 2 months prior to transplant according to a selection algorithm that respects the minimum requirement for the HLA matching and cell dose. The maternal donors of all CBUs are screened and tested, and an overview of the virus tests performed was included upon request. It was clarified that in case of a positive test (only for the pathogens that do not belong to the panel required by the Directive) the use of the CBU will be evaluated case by case jointly with the treating transplant center. It was confirmed that tests for infectious diseases are carried out by a qualified laboratory using CE-marked test kits.

It was demonstrated that the CBU size within the acceptance criteria is not predictive of batch rejection and that the acceptance criterion is appropriate. Further information was provided on the selection and acceptance criteria (e.g. HLA matching and size) of the CBU. Evidence was provided to show that human serum albumin (HSA) and Human Immune Globulin Intravenous (IVIG) used in the manufacture originates from EU registered products. The applicant has presented an overview of the raw materials of non-animal or non-human origin used during AS manufacturing. The qualitative composition of cell culture medium was provided. Upon request, a confirmation was provided that an agreement is in place with the supplier to notify the MAH in case of changes to cell culture medium. For the reagent, a description of the manufacturing process, materials used, controls and justification of specifications as well as shelf-life and stability data were provided upon request.

UM171

UM171 ((1R, 4R)-N1-(2-benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine dihydrobromide dihydrate, $C_{25}H_{27}N_9 \bullet 2\ HBr$) is provided as a non-sterile, low bioburden solution in DMSO.

Sufficient information about UM171 has been provided, including manufacturers, chemical structure, details about the presentation and a discussion about the impurities and microbiological quality.

Sufficient information has been provided on the synthesis flow scheme and on characterisation of UM171.

Three steps are necessary to produce UM171 bulk powder, which includes several in-process checks (IPCs), in-process verifications (IPVs) and defined acceptance criteria which were sufficiently detailed.

The bulk UM171 powder is then formulated in DMSO, filtered and filled into single-use vials.

The filled vials are stoppered and crimped. The filling and stoppering operation are performed in a laminar flow hood. Samples of the finished product are taken for final release testing. A summary of

the critical manufacturing steps and the associated process controls of UM171 solution has been adequately provided.

Control of Materials [UM171]

Starting materials, raw material, and consumables are inspected, tested when applicable and released for use in the manufacturing process of UM171 solution. The information provided is sufficient.

Characterisation [UM171]

The structure of UM171 has been confirmed. Sufficient information has been provided.

Specifications [UM171]

The specifications of UM171 include tests for appearance, identity, assay, concentration, impurities, fill volume, bioburden and endotoxins.

Reference standards or materials [UM171]

There is no commercial reference standard available for UM171. In-house samples for UM171, intermediates, and potential impurities were prepared. The reference standards were analyzed and certified.

UM171 Primary Reference Standard (PRS) has been fully characterised and certified. Results of full characterisation have been provided.

Container closure system [UM171]

UM171 Solution is filled into 2 mL clear Type I clear glass vials. The vials are stoppered with a sterilized rubber stopper and crimped sealed with an aluminium seal. The technical drawings for the vial, stopper, and seal have been provided.

In conclusion, the information included in the dossier about the UM171 solution is considered sufficiently detailed.

Manufacturing process development and validation *[Dorocubicel (ECT-001-CB-DP1) and Unexpanded CD34- cells (ECT-001-CB-DP2)]*

Several changes were made in the manufacturing process in the pre-clinical phase as well as in the clinical phase. All changes were assessed as having no adverse impact on cell expansion or product safety.

The process evaluation and validation activities entailed a risk assessment to identify Critical Process Parameters (CPPs) and critical material attributes. Tabulated CPP, IPC and hold times with acceptance criteria were provided upon request.

Process Performance Qualification (PPQ) included several consecutively manufactured commercial-scale lots of dorocubicel (CD34+) and ECT-001-CB-DP2. The applicant has identified critical quality attributes (CQAs), potential CPPs, CPPs, and potential critical material attributes. In general, the build-up of the process validation exercise is acceptable, an appropriate risk assessment was performed. Additional information was provided on temperature control, medial fills, shipping temperature and the sterilising capacity of the sterilising filter for UM171.

Characterisation

ECT-001-CB consists of two fractions, dorocubicel dispersion for infusion (ECT-001-CB-DP1/formulated cryopreserved expanded CD34+ cells) and ECT-001-CB-DP2 (formulated cryopreserved unexpanded CD34- cells).

Dorocubicel

UM171 expanded cord blood derived CD34+ cells (Dorocubicel) have been characterised in terms of identity, purity, quantity immunophenotype, product and process related impurities and safety (sterility, endotoxins, mycoplasma) by analysing several clinical batches. The impact of cryopreservation was further characterized (see finished product section). The expansion of CD34 cells in the presence of UM171 is characterized by a high percentage of CD34+ cells after the 7-day culture.

Additional characterisation was performed to demonstrate that CD34+ cells maintain the ability to differentiate into different hematopoietic cell lineages. Phenotypic characterisation is performed on samples of fresh cells used in the first clinical study and upon request study results of the impact of cryopreservation on the phenotype distribution were provided. Potency entails a combination of three parameters: Fold expansion of CD34+ cells, CD34+ viability and the Colony-forming unit assay (CFU). The long-term engraftment, which is considered crucial for the efficacy of dorocubicel, was further characterised upon request. Limited characterisation of CD34- subpopulations was performed because this cell fraction is not further purified or otherwise treated.

Process-related impurities include raw materials and reagents not intended to be present in the final product. A risk assessment associated with the raw materials used in the production of ECT-001-CB was provided. Theoretical levels of process-related impurities in dorocubicel dispersion for infusion were calculated using dilution factors. Two clearance studies were performed, one for UM171 and another one for cytokines. Sufficient clearance was shown for all process-related impurities. The residual UM171 per dose found is acceptable from a toxicological point of view. The applicant provided an updated risk assessment, covering all possible sources of nitrosamines and the risk of the presence of nitrosamines is very low and no testing for nitrosamines is required. This is acceptable. ECT-001-CB being a cellular product is outside the scope of ICH Q3D for Elemental Impurities.

ECT-001-CB-DP2

ECT-001-CB-DP2 is obtained following separation of the CD34+ and CD34- cells of the CBU and contains the CD34- cells. The CD34- fraction is minimally manipulated. The CD34- cells are washed for removal of selection reagents, formulated and cryopreserved. The composition of the ECT-001-CB-DP2 has been characterised in terms of identity, purity, quantity, immunophenotype, safety. Characterisation of the minimally manipulated CD34- cells (including immunophenotypes) showed that the CD3+ cell content, is similar in native cord blood and FP2. Additional characterisation of cord blood and FP2 was performed during product development phases and clinical studies. Cryopreservation of FP2 was introduced at the beginning of the medicinal product development. The CD3+ cells after thawing retained the ability to proliferate under stimulation.

2.4.2.1. Specification [Dorocubicel (ECT-001-CB-DP1) and Unexpanded CD34- cells (ECT-001-CB-DP2)]

Due to the continuous manufacturing process, there is no active substance release testing for both FP1 and FP2. The transition from active substance to finished product does not include any hold steps, hence, specification, analytical procedures, validation of analytical procedures, batch analysis and justification of specifications, respectively are provided in the final product section. Considering the nature of the product, the applicant’s approach is considered acceptable.

2.4.2.2. Reference Standards or Materials [Dorocubicel (ECT-001-CB-DP1) and Unexpanded CD34- cells (ECT-001-CB-DP2)]

Due to the continuous manufacturing process, there is no release testing of the AS for both FP1 and FP2. Refer to the finished product section for information on reference standards or materials used in testing of the finished product.

2.4.2.3. Container Closure System [Dorocubicel (ECT-001-CB-DP1) and Unexpanded CD34- cells (ECT-001-CB-DP2)]

Due to the continuous manufacturing process, the AS are not stored before processing into the finished product. Refer to the finished product section for information on the container closure system.

2.4.2.1. Stability [Dorocubicel (ECT-001-CB-DP1) and Unexpanded CD34- cells (ECT-001-CB-DP2)]

Due to the continuous manufacturing process, and as no hold step is foreseen at active substance level before manufacturing of finished product, no stability studies have been performed at that level. Therefore, there are no stability data for AS for both FP1 and FP2. This is acceptable.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Dorocubicel dispersion for infusion consists of formulated cryopreserved expanded CD34+ cells. Dorocubicel FP is composed of 80 mL of cryopreservation medium (consisting of a mixture of DMSO, HSA, and Plasma-Lyte A) containing per ml ≥0.23 x 10^6 CD34+ cells as active substance.

The patient-specific product is presented cryopreserved in four cryobags, each containing 20 mL. It was clarified that all four (4) bags are shipped to the treatment site. Generally, all four bags will be infused into the patient, but less can be used to get to the intended dose (i.e. up to 4 bags). After thawing, the product is administered by intravenous infusion without further dilution.

Dorocubicel must be infused before ECT-001-CB-DP2 i.e. Dorocubicel dispersion for infusion is meant to be sequentially administered to the patient followed by the unexpanded CD34- cell fraction (ECT-001-CB-DP2 dispersion for infusion) derived from the same unit of cord blood.

ECT-001-CB-DP2 is composed of 80 ml of cryopreserving medium (consisting of a mixture of DMSO, HSA, and Plasma-Lyte A) containing per ml ≥0.53 x10^6 CD3+ cells as active substance. The product is presented cryopreserved in four cryobags, each containing 20 ml of product. After thawing the product is administered by intravenous infusion without further dilution. It was clarified that all four (4) bags of FP2 will be administered to the patient.

Pharmaceutical development [Dorocubicel and ECT-001-CB-DP2 dispersion for infusion]

The overall objective of ECT-001-CB pharmaceutical development was to create a product that meets all aspects of the quality target product profile (QTPP) of dorocubicel dispersion for infusion and ECT-001-CB-DP2 dispersion for infusion.

The overall quality attributes of ECT-001-CB were identified from the QTPP for subsequent use during development. The quality attributes along with justification for what attributes are deemed CQAs and those attributes that are deemed non-critical have been adequately discussed and provided.

Development of the release specification for ECT-001-CB first required identification of CQAs and establishment of acceptance criteria appropriate to control the CQAs proposed.

The changes to the manufacturing process from proof of concept to early clinical phase have been presented as part of process development per each process step. The applicant provided, upon request, an overview of major process changes introduced during development and linked these to defined process names, batch numbers, and the relevant clinical studies. Additional comparability information was provided for relevant process changes. The introduction of cryopreservation was considered most impactful, because it was introduced after the first clinical study.

The comparability data between fresh and cryopreserved product shows that cryopreservation process leads to a decrease in percent and concentration of viable CD34 cells. Nevertheless, post-cryopreservation samples still largely meet the defined acceptance criteria. Further details regarding the comparability between fresh and cryopreserved dorocubicel are presented in Section 2.6.2 Clinical pharmacology. The control strategy was modified to use the post-cryopreservation viable CD34+ cells dose as the release criterion. This was accepted. In addition, the applicant agreed to perform a post-approval evaluation on the batches used in the planned confirmatory trial, to further evaluate the dose parameters (Part of Specific Obligation – see clinical part).

Based on the provided data the impact of cryopreservation on long-term engraftment is considered sufficiently characterised also supported by the fact that clinical engraftment was similar to the fresh product.

2.4.3.1. Manufacture of the product and process controls [Dorocubicel and Unexpanded CD34- cells (*ECT-001-CB-DP2) dispersion for infusion]*

Due to the continuous manufacturing process for dorocubicel and ECT-001-CB-DP2 dispersion for infusion, information on the facilities responsible for the manufacture and testing of dorocubicel dispersion for injection is provided in the active substance section.

Manufacture of ECT-001-CB-DP2 dispersion for infusion beginning with starting material thaw is a continuous process with no reprocessing steps permitted.

The CD34+ cells are expanded *in vitro*, in the presence of UM171 and an optimised culture system with medium containing a mixture of growth factors and UM171. It is then formulated in HSA and DMSO in Plasma-Lyte A Injection, and finally cryopreserved (ECT-001-CB-DP1 dispersion for infusion).

The unexpanded CD34- fraction is formulated in HSA and DMSO in Plasma-Lyte A Injection, then cryopreserved (ECT-001-CB-DP2 dispersion for infusion).

Each lot of ECT-001-CB is patient specific, having been selected by an HLA-matching process. The ECT-001-CB manufacturing process is continuous and there is no formal separation between active substance and finished product. For further details on the manufacturing process please refer to the active substance section.

Dorocubicel dispersion and FP2 (ECT-001-CB-DP2) for infusion are distributed in CE-marked cryopreservation bags (working volume 10 – 30 mL) indicated for freezing blood and blood component. After filling, the bags are heat sealed, wrapped in an overwrap bag and put in an aluminium cassette and finally cryopreserved. Container closure integrity testing has been performed. Upon request the applicant justified that adequate controls are in place to ensure integrity of the finished product containers prior to use and specifications used for releasing the bags and information on their sterilisation was provided.

Extractables/leachables have been evaluated and the risk to patient safety was found to be negligible. Sufficient information is provided on compatibility of the container closure system with the transfusion kits that may be used for infusion of the dorocubicel and FP2.

A transfusion kit for infusion equipped with a 170-260 nm filter must be used to infuse dorocubicel and FP2 (SmPC). Upon request, characterisation of dorocubicel-FP1 and ECT-001-CB-DP2 on the potential impact of cell aggregation and visible cellular aggregates following thaw was performed. Additional controls and mitigation of the level of cellular aggregates before cryopreservation and after thaw has been described. As cellular aggregates may be present in the cryobags after thaw, also the impact of a filter on the infused dose was evaluated and justified.

It was clarified that all four bags of each dorocubicel and FP2 will be shipped to the clinical centre. The description of this procedure and the associated controls was adequately provided.

2.4.3.2. Product specification

Dorocubicel

The release specification for Dorocubicel includes: appearance, identity (viable CD34 cell number), purity (% viable CD34 cell and total nucleated cells viability), quantity (viable CD34 cell dose, viable CD34 cell concentration and fill volume), potency (viable CD34 fold expansion, CD34 cell viability and CFU), safety (mycoplasma, endotoxin concentration, endotoxin dose and sterility) and quality (pH and osmolality) tests.

For Dorocubicel, relevant acceptance criteria for release and stability were tightened based on clinical experience and post-cryopreservation acceptance criteria were introduced upon request. The dose is reflected as viable CD34+ cells/kg. The quantification of CD34+ cells is performed using a modified ISHAGE (International Society of Hematotherapy and Graft Engineering) flow cytometry method discussed in Analytical Methods section. For Dorocubicel potency, CD34+ cell viability and fold expansion as well as CFU (colony forming units assay) are tested for release.

For dorocubicel-FP1, the release and stability acceptance criteria were tightened upon request. The applicant agreed to perform a post-approval evaluation on the batches used in the planned confirmatory trial, to further evaluate the dose parameters (part of the Specific Obligation – see clinical part).

ECT-001-CB-DP2

The release specification for ECT-001-CB-DP2 (FP2) includes: appearance, identity (viable CD3 cell number), purity (% viable CD3 cells and total nucleated cells viability), quantity (viable CD3 cell dose and fill volume), potency (CD3 cell viability), safety (endotoxin concentration, endotoxin dose and sterility) and quality (pH and osmolality) tests.

For FP2, relevant acceptance criteria were tightened based on clinical experience and post-cryopreservation acceptance criteria were introduced upon request. The dose is reflected as viable CD3+ cells/kg using modified ISHAGE flow cytometry method.

Analytical methods [Dorocubicel and Unexpanded CD34- cells (ECT-001-CB-DP2) dispersion for infusion]

Summaries of the analytical procedures used for release and stability testing of dorocubicel dispersion for infusion and ECT-001-CB-DP2 dispersion for infusion have been provided. Analytical procedures were developed and validated following ICH Q2(R1).

An overview and short description of the methods used has been provided and also a detailed description of the methods in Standard Operating Procedure (SOPs). Non-compendial methods are adequately validated.

For the sterility method the panel of microorganisms tested was updated in line with Ph. Eur. 2.6.27 and the method was validated in line with Ph. Eur. 5.1.6 and Ph. Eur. 2.6.27. The provided data demonstrate the results for the validation parameters of the assay are acceptable and the method is considered validated.

The visual inspection validation reports were provided upon request. The range of the ISHAGE method was documented. The descriptions of the non-compendial methods were updated with more detailed information on the critical reagents/components and equipment used. The information provided is adequate.

Colony Forming Unit (CFU) Assay (Dorocubicel dispersion for infusion)

A hematopoietic colony forming unit (CFU) assay is performed on the finished product. The CFU assay is a well-established functional assay used to quantify the progenitor cells from a cell sample. This assay is not a measure of long-term HSC potency but instead measures the ability of clonal progenitor cells to proliferate and differentiate into various hematopoietic lineages.

The CFU method which complies with Ph. Eur. 2.7.28 (Colony-forming cell assay for human haematopoietic progenitor cells), was optimised.

ISHAGE (Dorocubicel and ECT-001-CB-DP2 dispersion for infusion)

A flow cytometric assay based on the International Society of Hematotherapy and Graft Engineering (ISHAGE), named ISHAGE All-in-One (AIO) antibody panel, is performed on the finished products. The ISHAGE method which complies with Ph. Eur. 2.7.23 (Numeration of CD34/CD45+ cells in haematopoietic products), was optimised by the applicant.

Batch Analysis [Dorocubicel and Unexpanded CD34- cells (ECT-001-CB-DP2) dispersion for infusion]

Dorocubicel dispersion for infusion and ECT-001-CB-DP2 dispersion for infusion have been used in 5 clinical trials since January 2016. Three consecutively manufactured lots of ECT-001-CB were manufactured by the proposed process for the purpose of PPQ. Results for the three dorocubicel dispersion for infusion PPQ lots have been provided. All PPQ lots complied with the commercial release specification.

Container closure system [Dorocubicel and Unexpanded CD34- cells (ECT-001-CB-DP2) dispersion for infusion]

Dorocubicel dispersion for infusion and ECT-001-CB-DP2 dispersion for infusion are distributed in cryopreservation bags, which have a working volume of 10 – 30 mL. The bags are made from Ethyl Vinyl Acetate (EVA) and certified for use according to USP 23, Class VI and ISO 10993 and produced under US and European cGMP conditions. The cryopreservation bags are purchased pre-sterilized by gamma radiation and the fluid path is sterile and non-pyrogenic. The bag comes with one male luer fitting with cap, two female luers with caps and one needle-free injection port. It is a CE marked medical device.

Compatibility of dorocubicel dispersion for infusion and ECT-001-CB-DP2 dispersion for infusion with the cryopreservation bags has been established through the long-term and in-use stability studies. An extractables and leachables risk assessment was conducted on the bags and has been adequately

discussed and presented. Additionally, a risk assessment of extractables and leachables from infusion equipment (e.g., primary infusion tubing, secondary infusion tubing and tubing with a 170 – 260 micron filter) was performed. A summary of the risk assessment has been provided.

The suitability of the primary container closure system has been shown based on results from extractable and leachable testing, container closure integrity testing, and long-term and in-use stability studies.

The cryopreserved bags of ECT-001-CB are placed in individual clear overwrap and aluminum cassettes and stored at ≤ -150 °C in the vapor phase of liquid nitrogen until ready for shipment. The cassettes are then placed inside a dewar which stores the product in the vapor phase of liquid nitrogen during shipment.

2.4.3.1. Stability of the product [Dorocubicel and Unexpanded CD34- cells (ECT-001-CB-DP2) dispersion for infusion]

The proposed shelf life for Dorocubicel and FP2 (ECT-001-CB-DP2 dispersion for infusion) stored in the cryopreservation bags is 12 months when stored at ≤ -150° C. After thawing ECT-001-CB-DP2 dispersion for infusion may be held for a maximum of 1 hour at room temperature (RT) at 15 °C – 30 °C prior to administration.

To support the shelf life claims for Dorocubicel and FP2, the applicant has presented preliminary stability studies long term and in-use after thawing at RT.

Data is available for 3 lots of dorocubicel dispersion for infusion and ECT-001-CD-DP2 dispersion for infusion placed in a primary long term stability study at ≤ -150 °C with lots manufactured using the commercial process and tested against the proposed commercial stability specification and stored in the final product container. Supportive data was also presented.

Based on the stability data provided, the claimed shelf life for Dorocubicel and FP2 of 12 months when stored at ≤ -150 °C is acceptable. After thawing Dorocubicel and FP2 may be held for a maximum of 1 hour at RT prior to administration.

2.4.3.1. Adventitious agents [Dorocubicel and Unexpanded CD34- cells (ECT-001-CB-DP2) dispersion for infusion]

ECT-001-CB is a cellular therapy, and therefore, the manufacturing process of the finished product does not include viral inactivation and/or reduction steps. The safety of the finished product with respect to adventitious agents is ensured through control of the source CBUs, raw materials and excipients of biological origin, the manufacturing process, and the inclusion of appropriate in-process controls and finished product testing.

The two fractions of ECT-001-CB (dorocubicel dispersion for infusion and ECT-001-CB-DP2 dispersion for infusion) which comprise a single patient dose are both derived from the same CBU. The sourcing of these CBUs is carefully controlled. The raw materials and excipients of biological origin used in the production of the finished products are also selected and controlled to minimize the risk of introduction of adventitious agents. The manufacturing process is performed in a closed manner as much as possible, and aseptic process validation has been successfully designed for the entire manufacturing and fill process. Lastly, the products are tested and released for non-viral adventitious agents, including microbes, mycoplasma, yeast and molds.

Three materials of human origin are used in the manufacturing process of ECT-001-CB. All three materials are medicinal approved products in the EU and linked to a certified PMF.

The applicant has adequately discussed the virological safety of CBU. The procurement of CBU's should be in accordance with the relevant EU legislation, i.e. Directive 2004/23/EC or 2002/98/EC.

The risk of potential presence of adventitious agents in the final product due to the use of materials of biological origin has been sufficiently discussed. Adventitious agents safety including TSE have been sufficiently assured.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Several quality Major objections were raised and have been addressed satisfactorily by the applicant. A valid GMP certificate had been issued for the manufacturing site (Centre C3i Inc, Canada) and was provided. The criterion for size of patient-matched small CBUs has been appropriately justified. The release and stability acceptance criteria for both dorocubicel-DP1 and ECT-001-CB-DP2 have been tightened upon request and further adequate justification was provided.

The applicant has also satisfactorily addressed the multidisciplinary Major objections. More evidence has been provided that a sufficient dose will be given of Dorocubicel and FP2, each will be released on both pre- and post-cryopreservation dose. For FP2 it was clarified that all four bags will be given, making it similar to the dose given in the clinical studies. The applicant agreed to perform a post-approval evaluation on the batches used in the planned confirmatory trial, to further evaluate the dose parameters (part of the Specific Obligation – see clinical part). Based on the provided data the impact of cryopreservation on long-term engraftment is considered sufficiently characterised also supported by the fact that clinical engraftment was similar to the fresh product.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall quality of Zemcelpro is considered acceptable. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines.

The manufacturing process of the active substance is adequately described, controlled and validated. The active substance is well characterised and appropriate specifications are set. The manufacturing process of the finished product has been satisfactorily described and validated. The quality of the finished product is controlled by adequate test methods and specifications. Adventitious agents safety including TSE have been sufficiently assured.

The applicant agreed to perform a post-approval evaluation on the batches used in the planned confirmatory trial, to further evaluate the dose parameters (part of the Specific Obligation – see clinical part).

The CHMP endorses the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

2.4.6. Recommendation for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommended a point for further investigation.

The CHMP endorses the CAT assessment regarding the recommendation for future quality development as mentioned above.

2.5. Non-clinical aspects

2.5.1. Introduction

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Zemcelpro (or ECT-001-CB) contains two drug products (DP), both derived from the same patient-specific umbilical cord blood unit (CBU):

- Dorocubicel (ECT-001-CB-DP1 or DP1): containing the CD34+ hematopoietic stem and progenitor cell fraction that has been *ex vivo* expanded with UM171, but also containing CD34- cells, such as dendritic cells and mast cells, and,

- ECT-001-CB-DP2 (or DP2): containing the unexpanded CD34- cell fraction of the cord blood, primarily containing mature immune cells like CD3+ T cells.

The combination of both is to be infused in patients (although not at the same time), and together these products will translate into a therapeutic effect. In the non-clinical dossier, the applicant has only focused on the evaluation of the DP1 fraction (dorocubicel).

Dorocubicel

For the production of dorocubicel, human CD34+ cord blood cells are expanded with UM171 and cultured with several growth factors to obtain the drug product ECT-001-CB-DP1. In a fed-batch system, various culture conditions were tested, and the corresponding cells were transplanted into sub-lethally irradiated female NSG mice to following engraftment and reconstitution. The human cell product has been used in these immunocompromised animals, because the applicant explained that UM171 had no activity on mouse HSCs (i.e. homologous murine product not possible).

The applicant has adapted their production process of DP1 several times during the product development. The cell product is cryopreserved and subsequently thawed before use in the patient.

The applicant has conducted non-clinical *in vitro* studies with human CD34+ cord blood (or peripheral blood) cells to select the optimal UM171 dose for maintenance of primitive stem cell characteristics, to characterize the cellular phenotype after UM171-related expansion and to determine the reversibility of the UM171 effect when UM171 was withdrawn from the cell culture. In addition, the impact of culture conditions changes (growth factors, culture duration, formulation) on the phenotype and function of the UM171-cultured cells was evaluated. Moreover, *in vitro* studies were performed to unravel the intracellular and molecular mechanisms of UM171 in CD34+ cells. *In vivo* engraftment studies were conducted to show primary and secondary engraftment of UM171-cultured human CD34+ cord blood cells, thereby also evaluating the impact of changes in culture conditions. With their non-clinical program, the applicant aimed to show the proof-of-concept of dorocubicel and to substantiate process adaptations in the manufacturing of the DP1 fraction.

The mechanism-of-action of UM171 has been evaluated in 2 studies. In the first study (IRIC/2022-01-001), the mechanism on molecular/signaling pathway level was evaluated. Transcriptomic analysis of CD34+ cells cultured with UM171 indicated that this chemical agent induces pro-inflammatory signalling via NF-κB, important for HSC self-renewal/expansion. In addition, UM171 induces an endothelial protein C receptor (EPCR)-dependent anti-inflammatory response to prevent exacerbated NFκB signaling and oxidative stress, as such preventing HSC cytotoxicity.

In the second study (IRIC/2022-01-002), the UM171-related mechanism on a more epigenetic level was investigated. Therefore, a genome-wide CRISPR-Cas9 knockout screen was performed with two hematopoietic cell lines and additional genetic experiments were conducted. According to the applicant, UM171 acts on KBTBD4 (in a ubiquitin-ligase complex, $CRL3^{KBTBD4}$) to mediate enhanced degradation of the LSD1/CoREST epigenetic complex, leading to epigenetic changes to retain the expansive capacity of HSCs.

T cell reconstitution was evaluated in adult patients following transplantation with UM171- expanded or unmanipulated cord blood cells (ECT-001-CB.001 Phase I/II trial). UM171 treatment led to an increase in the number and proliferative capacity of common lymphoid progenitor cells *in vitro*. Whether this UM171 effect on T cell reconstitution was also observed *in vivo* in patients has been assessed clinically (see Clinical section).

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were submitted.

The applicant did not provide any (literature) information on potential secondary PD data related to UM171.

It is not anticipated that HSCs will have pharmacological activity other than engraftment and blood cell lineage reconstitution. Therefore, the absence of secondary PD data for the cell product can be endorsed.

2.5.2.3. Safety pharmacology programme

The applicant has conducted an *in vitro* safety PD study to evaluate the potential impact of (residual) UM171 in the product on hERG channel activity. UM171 (0.1 to 10 µM) did decrease the current through the channel, but this was not statistically significant compared to baseline. Taken together, UM171 appeared not to have a relevant blocking effect on the channel activity. It is agreed that no risk from residual UM171 on hERG channels is to be expected *in vivo*.

According to ICH S7A and B, safety PD studies such as the hERG assay should be performed in compliance with GLP. However, the study was not GLP compliant. However, since the anticipated residual UM171 level in dorocubicel (8 ng/kg for a 70 kg adult) is negligible, the lack of GLP compliance is considered acceptable.

2.5.2.4. Pharmacodynamic drug interactions

No dedicated non-clinical pharmacodynamic drug interaction studies have been submitted.

From a non-clinical perspective, the absence of a dedicated PD interaction study can be endorsed.

2.5.3. Pharmacokinetics

The applicant did not study the distribution of ECT-001-CB-DP1 / dorocubicel or ECT-001-CB-DP2, but instead studied pharmacokinetics (absorption and metabolism) of UM171, the compound used in 7-day culture of donor umbilical cord blood (UCB) to generate dorocubicel.

2.5.3.1 Analytical methods

No validation reports for analytical methods were provided.

The UM171 pharmacokinetic studies in mice, rat and cynomolgus are non-GLP and are considered of limited human relevance as only traces of UM171 (8 ng/kg) will be IV injected in human with the DP1 fraction, dorocubicel. Upon clinical administration of Zemcelpro, human pharmacokinetic parameters of UM171 are not determined as levels are below limits of detection. Absence of validated methods to analyse UM171 in animal plasma can be agreed.

2.5.3.2 Absorption

The applicant conducted three non-GLP studies to evaluate the pharmacokinetic characteristics of UM171. Irradiated female NSG mice (n=3) and male Sprague Dawley Rats (n=3) were dosed with a single IV dose of 2 mg/kg and 1 mg/kg UM171 respectively. In addition to that, 3 male cynomolgus monkey were dosed with three consecutive IV doses (1 week apart) of 2.3, 23 and 230 µg/kg. At the lowest dose (2.3 µg/kg) UM171 levels were below quantification (0.5 nM).

In mice dosed 2 mg/kg UM171, half-life of UM171 (T½) was 3.1 hours, the volume of distribution/concentration (Vd/Vss) 21.0 L/kg and the clearance (CL) 151.9 mL/min/kg. The dose corresponds to a Human Equivalent Dose (HED) of ~0.163 mg/kg (allometric scaling based on body surface area), which is 20,000 times higher than the highest expected potential exposure for a 70 kg patient (8 ng/kg).

In rats dosed 1 mg/kg UM171, T½ was 4.4 hours, Vd/Vss 74.6 L/kg and CL 190.2 mL/min/kg. The dose corresponds to a HED of approximately 0.161 mg/kg (allometric scaling based on body surface area), exceeding highest expected potential exposure for a 70 kg patient (8 ng/kg) 20,000 times.

In monkeys dosed IV with 23 or 230 µg/kg UM171, T½ was 4.15 and 5.86 hours respectively, Vd/Vss 8.56 and 6.92 L/kg respectively and CL 34.9 and 24.7 mL/min/kg respectively. The highest dose (230 mg/kg) corresponds to a HED of approximately 0.065 mg/kg (allometric scaling based on body surface area), exceeding the highest potential exposure for a 70 kg patient (8 ng/kg) 8,000 times. An HPLC method was used to determine the UM171 concentration in cynomolgus monkey plasma. No method validation report was found, however, the measured concentration falls within the lower and upper limits of the calibration curve. Therefore, the method is considered fit for purpose.

The fast half-life of ~4 or 5 hours, the limited Volume of distribution (at least in the monkey) and the fast clearance suggest that UM171 is rapidly eliminated from the body. The margin between the doses given to the animals and the human dose (allometrically scaled using body surface area) is high, suggesting that humans will be exposed to UM171 far below measurable levels.

2.5.3.3 Distribution

No distribution studies were submitted.

Absence of distribution studies for UM171 can be agreed as it is considered that UM171 is a process-related impurity that will not be detectable in human due to the anticipated low level. The distribution of the UM171-treated cells is not evaluated. This is agreed as it is considered that these cells will not distribute differently from a normal allogenic stem cell transplantation.

2.5.3.4 Metabolism

The measured metabolism of UM171 in liver microsomes over time was used to calculate the half-life and clearance. The calculated half-life of UM171 in liver microsomes was 3.6 and 1.7 hours in human and rat liver microsomes, respectively, and the clearance was 5.4 mL/min/kg and 12.5 mL/min/kg in human and rat liver microsomes, respectively.

Metabolic stability of UM171 was evaluated in rat, mouse, and human liver microsomes. Three metabolites were observed in mice and rats. M1 corresponded to a double hydroxylation (M+32), and M2/M3 corresponded to a hydroxylation (M+16). None of these metabolites were formed in incubations without NADPH. These or other metabolites were not observed in human liver microsomes.

2.5.3.5 Excretion

No excretion studies were submitted, which was considered acceptable.

2.5.3.6 Pharmacokinetic drug interaction studies

No pharmacokinetic drug interaction or other pharmacokinetic studies were submitted. This was considered acceptable.

2.5.4. Toxicology

Single dose toxicity and mutagenicity/ genotoxicity was tested separately for UM171, the compound used in 7-days culture of donor umbilical cord blood (UCB) to generate dorocubicel and of UM171 treated cells/ dorocubicel. It should be noted that administration of single doses of 2.3, 23, and 230 µg/kg each one week apart, to cynomolgus monkey is considered a single dose study as the clearance is rather quick and will be nearly complete after 24 hours.

2.5.4.1. Single dose toxicity

UM171

Safety data were collected as part of the non-GLP PK study in rats (**ECT/2018-11-001**). A dose of 1 mg/kg of UM171 formulated in 5% dextrose was administered intravenously to Sprague-Dawley rats at the dosing volume of 2 mL/kg. The PK study included one control rat and three UM171-treated animals, and all animals were observed daily for 8 days post-injection. On Day 8 blood was collected, and the animals were sacrificed. After complete necropsy liver, spleen, kidneys and bone marrow were evaluated by histopathology analyses. Only some mild extra-medullary hematopoiesis was observed in the spleen. There is no indication of toxicity upon single IV dose of 1 mg/kg UM171 (HED of 0.161 mg/kg), a dose 20,000 times higher than the residual levels of UM171 measured in dorocubicel for a 70 kg patient.

A study was conducted to evaluate PK parameters of IV dosed UM171 to cynomolgus monkey (study **30827**, see pharmacokinetics section). This study is considered a single dose study, as the doses are given 7 days apart and the dose is mainly cleared from the animal within the first 24 hours. Three male cynomolgus monkey were dosed with three consecutive IV doses (1 week apart) of 2.3, 23 and 230 µg/kg. Blood was collected for clinical pathology (haematology, coagulation, and clinical chemistry) for all animals prior to the start of each treatment (cycle) and 24 hours post each dose (i.e. 6 total assessments per animal). Red blood cells (RBC) and Haemoglobin levels decreased after dosing and decreased also over the course of the 3 weeks treatment period. Reticulocytes did decrease after each dose whereas lymphocytes and neutrophils did increase post-dose. It is not clear how these changes occur, whether this might be related to the pharmacodynamic mode of action of UM171 or whether it is related to its toxicity. Clinical chemistry evaluation revealed post-dose increases, compared to pre-treatment values, in alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), and alkaline phosphatase (ALP). The applicant reports that changes might indicate liver metabolism of UM171 at the doses tested. However, no information of metabolism in

monkeys is provided in the pharmacokinetics part. Therefore, liver changes could also be indicative for the toxicity of the compound. However, the study report indicates that most values were within the testing facility's historical reference ranges for the parameters, and the changes were not dose dependent. Safety data from this study demonstrates that three single intravenous doses of UM171 spaced 7 days apart, increasing from 2.3 to 230 µg/kg, are not associated with any adverse toxicity in cynomolgus monkeys. In addition to that, the highest dose (230 mg/kg) corresponds to a HED of approximately 0.065 mg/kg (allometric scaling based on body surface area), exceeding the highest expected potential exposure for a 70 kg patient (8 ng/kg)8,000 times. Therefore, the findings will not be of relevance for human.

UM171 treated cells

Study **UofT_2013_001** was submitted as part of the pharmacology studies and as such summarized and discussed in the pharmacology section. The applicant also collected some safety data in this study which is summarized and discussed below.

Sub-lethally irradiated female NSG mice received a transplantation with human CD34+ umbilical cord cells, unmanipulated or expanded for 12-16 days (3F, with or without 35 nM UM171). Unmanipulated cell doses ranged from $1x10^3$ to $2.5x10^4$ cells, while expanded cell doses ranged from $5x10^4$ to $5x10^5$ cells, the latter representing a human equivalent of $2.5x10^7$ cells/kg and producing an average human engraftment above 10%. For secondary transplantation, 5 animals/group received bone marrow from animals transplanted with unmanipulated CB cells or CB cells cultured 12 days with or without UM171. All 15 animals were submitted for histopathology 16 weeks post-transplantation.

In all groups of primary transplanted animals, nodules consisting of polygonal cells arranged in sheets in the mediastinum, macrophages pneumonia, regional hyperkeratosis in the non-glandular portion of the stomachs, and focal gastric ulceration were regarded related to age and immune compromised status of these NSG mice.

In all groups of secondary transplanted animals, for example low medullar myeloid/erythroid ratio, probably related to the lack of antigenic stimulation of these NSG mice, and lung aggregates/atelectasis/hemorrhages and extramedullary myelopoiesis, consistent with the method of euthanasia, were regarded unrelated to treatment.

Thus, transplantation of NSG mice with UM171-expanded cells was not associated with any histopathological changes attributed to the test article up to 24 weeks (primary transplant) and 16 weeks (secondary transplant) post-administration.

Transplantation studies to evaluate the short-term and long-term repopulating cell types were conducted as described in study **IRIC/2014-05-005**. In short, human CD34+ umbilical cord cells were expanded for 7 or 12 days (3F + LDLs with 38 nM UM171) and subsequently transplanted IV at $8.3x10^3$ to $7.5x10^4$ (7 days) or at $4.6x10^4$ to $4.1x10^5$ (12 days) into sub-lethally irradiated female NSG mice with analysis until week 24 post-transplantation. Human cell engraftment in mice was 40-50% in primary and 0.3 to 22.9% in secondary mice. The study design and pharmacological data are summarized and discussed in the pharmacology section 3.2.2.

Study **IRIC/2014-07-006** is presenting the histopathological examination of the above-described treated animals. There were two unscheduled deaths. One mouse (treated with $3.46x10^6$ cells expanded in DMSO) died at week 18, and one mouse (treated with $3.46x10^6$ UM171-expanded cells) became sick and died of neurological problems at week 13. Deaths were considered incidental, which can be accepted, although it is not well understood why tissues were not preserved and/or histopathological analysis was not conducted for these animals.

Occasional perivascular lymphoid infiltrates (liver, colon, lung, and pancreas) in several mice and a granulomatous infiltrate in the bone marrow of one mouse, were regarded related to immune system (NSG mice) defects at such age. A moderate to massive expansion of their splenic white pulp, reflecting the success of the transplantation, was observed, but clonal expansion was ruled out by immunohistochemistry, TCR and Ig rearrangement experiments. A hepatocellular adenoma with lipidosis, a benign lesion of mouse origin (i.e. not derived from UM171-expanded cells), was observed and is occasionally reported in rodents and not regarded treatment related.

The data from this study demonstrate that administration of high doses of UM171-expanded cells, up to $5.54x10^6$ per animal, were not associated with any adverse histopathological changes to 28 weeks after administration of the cells.

Study **IRIC/2015-04-001** is submitted as part of the pharmacology studies to evaluate the impact of addition of IL-6 (4F) compared to the 3F culture condition on long-term engraftment of UM171-cultured HSCs (pharmacology section 3.2.2) using $4.07x10^5$ – $1.32x10^6$ cells for 3F conditions and $1.22x10^6$ – $4.08x10^6$ cells for 4F condition. Safety data from this study is summarized and discussed below. In short, human CD34+ umbilical cord cells were expanded for 12 days (3/4F with 35 nM UM171), subsequently transplanted IV at three different doses into sub-lethally irradiated mice and engraftment was analysed at week 2 and every 4 weeks until week 24 post-transplantation. At the time of the necropsy (12 or 24 weeks), findings in bone marrow, spleen and lung related to immune defects in NSG mice at such age, were observed, but no adverse findings could be attributed to the UM171 treated cells. Although these findings have been acquired from a limited number of animals as not all tissues of the animals were suitable for analysis.

Study **ECT-2018-12-001** is submitted as part of the pharmacology studies to evaluate engraftment of their product following cryopreservation, as this preservation is also used for the clinical product (pharmacology section). Safety data in this study is summarized and discussed below. In short, human CD34+ CB cells expanded for 7 days (4F with 35 nM UM171, thus clinical/commercial manufacturing protocol) either or not cryopreserved, were transplanted IV at $8.1x10^4$ to $1.7x10^5$ into sub-lethally irradiated female NSG mice. Mice were sacrificed 20 weeks post-transplant and histopathology of liver, spleen, kidneys, lungs and femur was performed. Review of the gross pathology exams and histological analyses showed that all groups displayed normal histological features or show some minimal deviation from normal, which are reported below. These findings are not regarded of significance and are not treatment related.

2.5.4.2. Repeat dose toxicity

Repeat dose studies were not conducted and are not relevant for the product as it is applied only once.

2.5.4.3. Genotoxicity

The validation of the HPLC method for the quantification of UM171 in dose formulations can be accepted.

An AMES and a micronucleus test were conducted to assess the genetic safety of UM171 and these tests are summarized in Table 1.

Table 1: Overview of genotoxicity studies

Type of test/study ID/GLP	**Test system**	**Concentrations/ Concentration range/ Metabolising system**	**Results positive/negative/equivocal**

Gene mutations in bacteria	S. typhimurium TA98	0.50, 0.16, 0.050, 0.016, 0.0050 and 0.0016 mg/plate; +/- S9	Negative
	S. typhimurium TA100, TA1535 and TA1537:	0.16, 0.050, 0.016, 0.0050, 0.0016 and 0.00050 mg/plate; +/- S9	Negative
	E. coli WP2 uvrA:	1.6, 0.50, 0.16, 0.050, 0.016 and 0.0050 mg/plate; +/- S9	Negative
Gene mutations in mammalian cells	CHO-cells, HGPRT-locus	- 9.0, 6.0, 4.0, 2.7, 1.8, 1.2, 0.79, 0.53 and 0.35 µg/mL (4 hours in the absence of S9), - 13.5, 9.0, 6.0, 4.0, 2.7, 1.8 and 1.2 µg/mL (4 hours in the presence of S9), - 6.0, 4.0, 2.7, 1.8, 1.2, 0.79, 0.53, 0.35 and 0.23 µg/mL (22 hours in the absence of S9) +/- S9	No increases in either the frequency of micronuclei (mean %MN) or hypodiploidy (mean %HD) compared the concurrent negative controls (% MN and %HD values were all < 3-fold or < 7-fold neg. ctrl) not induce chromosome breaks or loss in cultured CHO cells

UM171 was tested in an in vitro AMES mutagenicity using in absence and presence of S9 at the following UM171 concentrations for S. typhimurium and E. coli strains. Heavy precipitation for UM171 was observed as well as cytotoxicity for all salmonella strains, limiting the test concentration of UM171. Therefore, in the main assay, lower concentrations were used as is noted below.

• S. typhimurium TA98: 0.50, 0.16, 0.050, 0.016, 0.0050 and 0.0016 mg/plate;

• S. typhimurium TA100, TA1535 and TA1537: 0.16, 0.050, 0.016, 0.0050, 0.0016 and 0.00050 mg/plate; and

• E. coli WP2 uvrA: 1.6, 0.50, 0.16, 0.050, 0.016 and 0.0050 mg/plate

Using the plate incorporation and preincubation versions of the bacterial reverse mutation test, no increase in the mean number of revertant counts was ≥ 3.0 (TA1535 and TA1537) or ≥ 2.0 (TA98, TA100, and WP2 uvrA) times the mean concurrent negative control following treatment with UM0128171. Therefore, the criteria for a positive response were not met, and the test item was considered to be negative, i.e., not mutagenic in this test system.

UM171 was also tested in a non-GLP AMES assay with Salmonella typhimurium histidine auxotroph strains TA98 (frameshift mutagens) and TA100 (frameshift and base-pair substitution mutations). UM171. In the GLP AMES test, the used UM171 concentration was lower due to cytotoxicity. Cytotoxicity was not mentioned for this non-GLP AMES assay. Therefore, the results of the GLP-AMES test are taken into consideration. UM171 was evaluated for its potential to induce micronuclei (clastogenic response) or hypodiploidy (aneugenic response) in cultured Chinese Hamster Ovary (CHO) cells in the absence and presence of metabolic activation (S9).

For the micronucleus assay, dose-dependent cytotoxicity was observed in all treatment conditions following treatment with UM171. The highest analysable (i.e., non-cytotoxic) concentrations were 4.0 µg/mL (4 hours in the absence of S9), 9.0 µg/mL (4 hours in the presence of S9) and 2.7 µg/mL (22 hours in the absence of S9), with RICC values of 40, 47 and 71%, respectively. Under the conditions tested, there were no biologically relevant increases in either the frequency of micronuclei (mean %MN) or in the frequency of hypodiploidy (mean %HD) compared to the concurrent negative control for any concentration of UM171. The mean %MN and %HD values were all < 3-fold or < 7-fold the concurrent negative controls, respectively, and were within the laboratory historical negative control

range. UM171 was considered to be negative, i.e. the test item did not induce chromosome breaks and/or loss in cultured CHO cells. But the relationship of the study conditions with the clinical culturing condition with UM171 is not clear (see discussion section).

Cytogenic analysis of UM171 treated (12-day culture) using metaphase preparations (G banding) and chromosomal breakage analyses (Giemsa staining) were conducted in 2013 (DMSO control and 3F condition, both n=1) and 2015 (3F and 4F conditions, both n=3). Conventional cytogenetic analysis was performed, and the number and type of structural chromosomal abnormalities were scored on 50 or 100 Giemsa-stained metaphases. According to the applicant, the average number of aberrations per cell reported in UM171-expanded cells was like that of controls, and cells cultured in 4F conditions did not show more breaks than cells cultured in 3F conditions. However, in 2015 no control treatment was taken along. Strictly spoken, only 3F and 4F can be compared in this experiment. Furthermore, the dorocubicel is also frozen in liquid nitrogen, prior to use, which was also not considered in this experiment.

However, the largest hesitation with this experiment is the resolution. The CB cells are treated with UM171 to preserve or enhance the stem cell characteristics of the cells. This is achieved by epigenetic influences of UM171. The intended epigenetic mechanism of the UM171 is addressed in the pharmacological part of the dossier and this report. However, whether there are unintended epigenetic changes is not addressed. The applicant set out to study the cytogenetic effects, but with methods that might be far too insensitive and not adequate to evaluate potentially occurring (epi)genetic changes that could result in unintended effects. In the absence of real long-term clinical data, this is of vital importance for risk estimation. Therefore, additional information is requested. See section 3.2.6 for further discussion of this issue in the secondary pharmacology and the toxicology part of that section.

2.5.4.4. Carcinogenicity

Carcinogenicity studies were not submitted, which can be agreed.

2.5.4.5. Reproductive and developmental toxicity

No reproductive and developmental toxicity studies were presented.

2.5.4.6. Toxicokinetic data

Regarding toxicokinetics after single dosing of UM171 to mice, rat and cynomolgus monkey, only exposure values were determined. For more PK parameters such as clearance and volume of distribution, be referred to pharmacokinetics section 3.2.3. As UM171 was given intravenously, the C_{max} is anticipated to be like the given dose. The limited toxicokinetic evaluation can be agreed also because only traces of UM171 will enter the human body.

For interspecies comparison data from single dosing UM171 PK studies in mice, rat and cynomolgus monkey was used. The animal to human multiples were based on Human Equivalent Doses as the traces of UM171 can only be measured in the final drug product but cannot be determined after administration to human. Therefore, AUC and C_{max} are not determined in human. Consequently, a comparison based on HED can be agreed.

2.5.4.7. Local tolerance

Not applicable.

2.5.4.8. Other toxicity studies

Extractable studies

Extractables from Origen BioMedical CS50N Cryostore freezing bag and Transfer pack were studied by extracting samples using methods at pH 3.0, pH 10.0 and with EtOH. Extracted samples were analysed for volatile, semi-volatile, non-volatile organic compounds and inorganic elements by HS-GC-FID/MS, GC-FID/MS, LC-DAD/MS, and ICP-MS, respectively. For cyclohexanone, Benzyl Alcohol, Benzoic acid, Dibutyl Phthalate and Bis(2-ethylhexyl) phthalate (DEHP) the applicant provided a risk analysis resulting in a safe dose of 2, 5, 3.4, 0,066 and 0,25 mg/kg/day respectively based on human and animal data. The applicant concludes that '*given the acute exposure of drug product to the cryobag and transfer bag, the limited single dose exposure of patients to the drug product, the cytotoxicity of standard of care for many hematological malignancies prior to use of the adjuvant drug product indicated, the preliminary risk of exposure to extractables identified originating from the primary container closure system CCS is negligible'.* This conclusion is accepted.

ICP-MS did not detect any inorganic elements above the LOQ relative to control. The elemental impurity concentrations were below the ICH Q3D (R2) limits for parenteral drug products.

The applicant refers to an actual use leachable study identifying compounds of concern with consideration of the Analytical Evaluation Threshold (AET) identified in study SGS-EL-07-ExCellThera-23-006R.

2.5.5. Ecotoxicity/environmental risk assessment

The active pharmaceutical ingredients (APIs) of both components of Zemcelpro consist of human hematopoietic stem and progenitor cells:

- ECT-001-CB-DP1 or dorocubicel *(CD34+ cells from cord blood, cultured with UM171 for 7 days),* and

- ECT-001-CB-DP2 *(unexpanded CD34- (CD3+) cells from the cord blood)*,

The ERA presented consisted of a justification for not submitting ERA studies, based on the nature of the active substances (naturally present in the environment).

Due to the origin of the hematopoietic stem and progenitor cells from human cord blood, and their fast degradation in the environment, the environmental risk is considered negligible.

Environmental studies on unaltered cells of human origin were therefore not be performed.

An ERA is not required for impurities in the drug product, such as traces of UM171.

2.5.6. Discussion on the non-clinical aspects

Pharmacology

The applicant has focused on DP1 in the non-clinical dossier and has not provided sufficient information about the cellular composition of DP2 and its role in reconstitution. It is mentioned in the Quality module on DS that dorocubicel (which is DP1, not DP2) contains a considerable amount of CD34- cells (such as DCs and mast cells) which could have immunomodulatory effects. The applicant claimed that this CD34- fraction within the CD34+ cell population is of importance for the efficacy and/or safety of the product. Nevertheless, the applicant has non-clinically only evaluated the impact of UM171 on CD34+ cell (sub)populations. In addition, the dossier does not include standard primary or secondary pharmacological target binding screening assays, such as receptor binding or enzyme inhibition assays,

to identify potential on- and off-targets of UM171 and to determine IC50/EC50 values. Although the amount of UM171 transferred to the patient is minimal, and its potential *in vivo* off-target effects are considered negligible, it is noted that during culture, several different types of CD34- cells in DP1, primarily dendritic cells and mast cells, are exposed to UM171.

The applicant has justified the absence of standard receptor binding, enzyme inhibition, and off-target screening assays and mentioned that there is no evidence of any off-target effect of UM171 (e.g. on CD34- cells) at the dose of 35 nM. Although no UM171-specific binding/interaction studies have been conducted nor any information on the functionality of CD34- cells in DP1 has been provided, the applicant has sufficiently explained UM171's mechanism-of-action and no major clinical issues have occurred so far that warrant additional non-clinical investigation.

Data collected from 97 clinical productions demonstrate that the DP2 composition is more or less similar to unmanipulated umbilical cord blood, although no batch information on the exact composition of DP2 has been provided in the quality and non-clinical parts of the dossier. The applicant mentioned that clinical batches of DP2 primarily consists of CD4+ and CD8+ T cells, B cells and NK cells. The T cells play a major role in the clinical outcome of Zemcelpro, as T cells in DP2 can expand *in vivo* and thereby add to the short-term reconstitution of these cells and contribute to the GvL effect. In contrast, the other lymphoid cell types have a more limited role in the contribution to clinical benefit, which is related to e.g. the absence of certain maturation/stimulation factors *in vivo* and the low numbers in the final dosing. Long(er)-term reconstitution of T cells, as well as B and NK cells, is related to *de novo* production from transplanted stem cells from DP1 instead of proliferation from DP2 cells. It is therefore also unlikely that DP2 cells have a considerable impact on persistence of Zemcelpro cells.

The applicant further explains that there is no relation between the dose of T cells (in DP2) and the clinical outcomes (such as engraftment, event-free survival, adverse events). It should, however, be mentioned that the study population was small and the T cell dose per patient was relatively low, thus the added value of a higher T cell dose could not be truly determined. Taken together, the applicant has provided acceptable insight in the constitution of DP2 and the role of the (primarily T) cells in the functionality of Zemcelpro. Long-term reconstitution and persistence of the combination of DP1 and DP2 is only assessed in clinical studies, which is acceptable.

A sequential usage approach of both products was not applied non-clinically, while the residual amount of UM171 in DP1 (although at low concentrations) could have an impact on the cells in DP2. No PD and safety studies have been conducted to evaluate any possible unexpected effects related to DP2 infusion. The applicant has justified the absence of a study with DP2 stating that 1) the CD34- cells in this fraction are minimally manipulated and very closely resemble the starting material, and 2) *in vivo* transplantation cannot be performed with DP2 due to an expected GvHD reaction in the NSG mice caused by mature immune cells in this fraction. In addition, as DP1 and DP2 are not administered simultaneously in patients, it will be difficult to evaluate the combination of DP1 and DP2 in non-clinical (*in vitro*) studies. Therefore, the lack of such studies is endorsed. Moreover, the applicant is of the opinion that residual levels of UM171 in the blood are not expected to impact the DP2. Indeed, the safety of UM171 is likely more related to the potential *ex vivo* impact on the epigenetics of UCB cells (DP1) in a 7-day culture than to the potential *in vivo* impact on DP2 by very low amounts of this compound. Therefore, the absence of information/data on the impact of UM171 on DP2 is considered acceptable.

Dorocubicel (ECT-001-CB-DP1)

Animal model

All *in vivo* studies have been conducted with female NSG mice. It is well-known that sex-associated factors play an important role in the survival, proliferation, and self-renewal of HSCs in mouse models (e.g. Notta et al., Blood, 2010[44]). Human HSCs engraft poorly in adult male mice. Efficacy data from males should therefore come from clinical studies.

The used NSG mice have only been mildly irradiated as they already lack several immune cell types. In contrast, patients will be pretreated with a stronger myeloablation regimen to eliminate both haematopoietic (stem) cells and tumour cells, and to allow for donor cell engraftment. The applicant explained that the regimen used non-clinically is still of relevance, because the murine studies were intended to evaluate engraftment and multilineage reconstitution of UM171-expanded human cells and potential toxicities arising with this treatment. The approach of the preconditioning regimen in mice can be accepted to show the proof-of-concept of the product, however, the quantitative engraftment and lineage reconstitution data obtained in mice cannot be directly translated to patients.

Process adaptations evaluated non-clinically

Between 2013 and 2022, the applicant has adapted their manufacturing process several times and the changes were to be substantiated with non-clinical data. Except for study ECT/2018-12-001, none of the submitted non-clinical studies have been conducted with the clinical/commercial manufacturing process using 35 mM UM171, 4F and no LDLs in a 7-day culture. No thorough non-clinical study has been conducted to evaluate the final clinical process culture conditions all together. As such, the following culture conditions used in the clinical/commercial process have not been sufficiently substantiated with non-clinical data:

1) UM171 concentration: the selection of the optimal UM171 concentration was based on a single *in vitro* study (CD34+ CD45RA- expansion) in a 12-day culture with 3F instead of 4F and without *in vivo* confirmation. Recent work suggests that a higher UM171 concentration may be more beneficial for (*in vitro*) expansion of the more primitive cell compartment and better *in vivo* engraftment, based on the mode of action of this molecule (Tellechea et al., Blood, 2023, poster session 501 abstract 2681[45]).

2) Culture duration: although a shorter duration of the culture appeared to result in better engraftment in NSG mice (study IRIC/2016-04-001), this was based on a cell culture with 3F + LDLs instead of 4F.

3) Growth factor composition: the 4F condition was selected based on a 12-day culture (study CRM_2015_001 and IRIC/2015-04-001) and no clear *in vivo* evidence for better engraftment with the addition of IL-6 was provided. It is not clear why the applicant has chosen the 4F regimen as condition for the clinical and commercial manufacturing process. Furthermore, it has not been discussed by which (anticipated) mechanism IL-6 would provide an advantage with 4F over 3F.

The difference in *in vivo* engraftment but not in *in vitro* phenotyping of the cells (following 7- versus 12-day culture, study IRIC/2016-04-001 and ECT/2018-12-003) indicates that product evaluation based on phenotypic analysis only is insufficient and long-term culturing assays and/or *in vivo* studies would be needed to determine the most appropriate UM171 concentration and optimal culture conditions for UCB cell expansion and long-term engraftment. Therefore, the applicant could have evaluated the presence and proliferative functionality of early/primitive progenitors, e.g. in an LTC-IC

[44] Notta F, Doulatov S, Dick JE. Engraftment of human hematopoietic stem cells is more efficient in female NOD/SCID/IL-2Rgc-null recipients. Blood. 2010 May 6;115(18):3704-7. doi: 10.1182/blood-2009-10-249326. Epub 2010 Mar 5. PMID: 20207983.

[45] Maria Florencia Florencia Tellechea, PhD1*, Jalila Chagraoui, PhD2*, Nadine Mayotte, MS2* and Guy Sauvageau, MD/PhD2,3,4,5, Deciphering the Boundaries of KBTBD4-CoREST1 Axis Modulation to Maximally Expand Human HSCs, Clinically Relevant Abstract ASH Symposium Hematopoietic Stem and Progenitor Cells and Hematopoiesis: Basic and Translational Progra, Poster session 501, abstract 2681

assay (Liu et al., Methods Mol Biol, 2013[46]; Hao et al., Blood, 1996[47]). Although this assay was used in study IRIC/2014-05-003, a 12-day culture duration and 3F condition was used here. In the other non-clinical studies, long-term HSC evaluation was only based on phenotypic markers (which are not always clearly mentioned in the study reports) instead of a specific culturing assay (see below sub-section 'Proof-of-concept and mechanism-of-action').

During clinical development, the applicant has adapted their product formulation from fresh to cryopreserved cells. To substantiate comparability of both formulations, a non-clinical engraftment study was conducted (ECT/2018-12-001), using cells produced with the clinical/commercial culture conditions. Although the applicant claims that cryopreservation of dorocubicel has no negative impact on phenotypical and reconstituting capacities of the UM171-expanded CD34+ cells, the study results indicate that mean long-term engraftment was lower with cryopreserved cells (approximately 0.3% and 0.6% at week 12 and 20) compared to fresh cells (approximately 2% and 1.7% at week 12 and 20). The clinical significance of the *in vivo* differences in engraftment between cryopreserved and fresh cells are unknown. It should be mentioned that there were considerable individual variations between the mice and between the donor cell batches used. It would be valuable to have additional *in vitro* data, evaluating more in depth and with less variation the impact of cryopreservation on early/primitive progenitors (i.e. real long-term progenitor cells) in the product. The applicant was therefore requested to incorporate a functional assay, specifically the Long-Term Culture-Initiating Cell (LTC-IC) assay, to assess the impact of cryopreservation on the frequency and functionality of long-term engrafting cells within dorocubicel.

The applicant did not evaluate the impact of cryopreservation of dorocubicel via LTC-IC, but via *in vivo* transplantation into NSG mice. The applicant asserts that this method is the gold standard for evaluating the *in vivo* biological activity of hematopoietic stem cells. According to the applicant, the engraftment data showed that long-term progenitor cells remained functional following cryopreservation. In addition, the applicant has re-analysed the data from study ECT-2018-12-001 in report RPT-0015 and also added clinical sample data and short-term engraftment data from NSG-SGM3 mice.

From report RPT-0015, it can be concluded that cryopreservation did not have a significant effect on the LT-HSC frequency in dorocubicel and on the engraftment of human cells within NSG mice. Considering the explanation from the applicant regarding potential issues in the cell handling within the ECT-2018-12-001 study, the results from study RPT-0015 seem more reliable. The non-significance of the difference in % human CD45 when using fresh or cryopreserved cells (both from PD runs and clinical samples), in combination with the considerable variation between animals (independent of freezing status of the cells), results in the conclusion that from a non-clinical perspective, there seems to be no clear impact of cryopreservation on the long-term engraftment. The absence of an LTC-IC assay can be endorsed for this comparability approach in study RPT-0015.

Proof-of-concept and mechanism-of-action

With their non-clinical PD program, the applicant has shown that UM171 has the ability to further expand cord blood-derived progenitor cells and could provide *in vivo* engraftment. As such, there is a proof-of-concept to use UM171 in the manufacturing process to produce umbilical cord-derived progenitor cells that can be transplanted into patients. However, although long-term engraftment was shown with UM171-expanded cells in NSG mice, effect of UM171 (and of cryopreservation) on the expansion of the cell types that really matter in long-term reconstitution (i.e. early/primitive

[46] Liu M, Miller CL, Eaves CJ. Human long-term culture initiating cell assay. Methods Mol Biol. 2013;946:241-56. doi: 10.1007/978-1-62703-128-8_15. PMID: 23179836.
[47] Hao QL, Shah AJ, Thiemann FT, Smogorzewska EM, Crooks GM. A functional comparison of CD34 + CD38- cells in cord blood and bone marrow. Blood. 1995 Nov 15;86(10):3745-53. PMID: 7579341.

progenitors) was not evaluated, e.g. in an LTC-IC assay. Moreover, the only non-clinical study conducted with the clinical/commercial culturing protocol suggests that transplantation of uncultured cells resulted in an overall better long-term engraftment (i.e. increase in % engraftment between week 3 and 20) compared to cells cultured with UM171. Taken together, data on the efficacy of Zemcelpro to result in appropriate long-term engraftment should come from patients.

The applicant has shown for both cord blood cells and peripheral blood cells that a) all cytokines (NB: 3F condition) are needed to achieve maximal fold expansion and UM171 cannot induce CD34+(CD45RA-) cell proliferation in the absence of cytokines, b) the extent of proliferative response to UM171 is depending on the intrinsic activation status and proliferation potential of the stem cells (i.e. related to type of progenitors in sample, thus donor dependent), c) the proliferative response to UM171 is AhR pathway independent, and that d) the effect of UM171 on progenitor cells is reversible. Although the conditions used in most of the studies are not fully in line with the clinical culture process, the study data and literature data together implicate that neonatal cord blood cells and adult peripheral blood cells behave comparably in their proliferative response to UM171.

The applicant conducted two mechanistic studies to evaluate the intracellular and molecular actions of UM171. In the first study, UM171 was shown to induce signalling via NF-κB and in the meantime induce an EPCR-dependent anti-inflammatory response. However, several experiments in this study were conducted with clinically-irrelevant culture conditions (with high UM171 concentrations, 3F and LDLs, variable culture durations) and cells (AML cell line instead of cord blood cells) that may have impacted the outcome of the UM171 pathway analysis. Although the function of UM171 on a cellular level has more or less been clarified non-clinically, the mechanism-of-action on a molecular/pathway level remains uncertain because of the described study limitations.

The data from the second study indicate that UM171 acts on an ubiquitin-ligase complex to enhance the degradation of the LSD1/CoREST complex. Nevertheless, it is not yet clear whether the ubiquitin-ligase complex can affect additional protein complexes that include LSD1. Moreover, recent literature from the same group indicates that treatment with UM171 can also lead to preservation of HSC regenerative capacities via distinct pathways, such as degradation of MYC (Chagraoui et al., Blood, 2024[48]). This information was, however, not provided to substantiate the mode of action of UM171. Furthermore, the applicant has only focused on the epigenetic changes that would occur in the presence of UM171 but increase in the CoREST complex with HSC attrition (once UM171 is washed away and dorocubicel is infused in patients) was not evaluated. Thus far, it remains unclear what the long-term effect of temporary (*ex vivo*) CoREST complex degradation may be on (*in vivo*) HSC functionality, stability, and persistence.

Clearly, only part of the mechanism-of-action of UM171 has been elucidated so far, but this was achieved using clinically irrelevant high UM171 concentrations (e.g. 250 nM), different culture conditions (3F with LDLs) and AML cell lines instead of cord blood cells in several experiments. Considering that the culture conditions and cell type used may impact the level/activity and type of genes and proteins that are affected by UM171 treatment, it appears that the mechanism-of-action of UM171 at clinically relevant culture conditions (7-day culture with 4F, 35 nM UM171 and without LDLs) has not been sufficiently elucidated. Although it can be concluded from the mechanism-of-action studies that UM171 treatment - at least- results in intracellular and epigenetic changes that lead to enhanced stem cell expansion, the exact pathways by which UM171 acts remain to be elucidated, including the identification of UM171 primary molecular target(s). A comprehensive and up-to-date summary of the current understanding of the mechanism-of-action is described below under Secondary PD.

[48] Chagraoui J, Girard S, Mallinger L, Mayotte N, Tellechea MF, Sauvageau G. KBTBD4-mediated reduction of MYC is critical for hematopoietic stem cell expansion upon UM171 treatment. Blood. 2024 Mar 7;143(10):882-894. doi: 10.1182/blood.2023021342. PMID: 38207291.

Secondary PD studies

Based on the primary PD studies, the mechanism of action (and thus primary or secondary PD) of UM171 has not been fully elucidated. Although a study to the mechanism of UM171 resulting in HSC expansion have been published recently (Chagraoui J et al, Blood, 2024), this was not discussed by the applicant. Also, other available in vitro UM171 data seem to be present in the public domain (Tellechea et al., Blood, 2023, poster session 501, abstract 2681; Subramaniam et al., Blood, 2020[49]; Chagraoui et al., Cell Stem Cell, 2021[50]), which is not discussed (in much detail) by the applicant. As such it cannot be concluded that effects of UM171 are confined to HSC expansion without safety concerns. Thereby, it was observed by Hu and colleagues that UM171 next to its role in HSC expansion, also has an anti-leukemic effect (Hu, A et al., Cell Death Discov. 2022[51]). In addition, reversibility was shown for 'one' functional effect of UM171, but not on molecular level or on other potential effects. It is considered relevant that the presence of UM171 during culture will not lead to any (epigenetic) changes in the expanded cells (different 'programming') that might be more permanent and/or can have an impact on their *in vivo* safety. This is also considered of importance because of the absence of long-term safety data in the clinic

The UM171-related mechanism on an epigenetic level was investigated in study IRIC/2022-01-002 (see Primary Pharmacology section above). UM171 would act on KBTBD4 (in a ubiquitin-ligase complex, $CRL3^{KBTBD4}$) to mediate enhanced degradation of the LSD1/CoREST epigenetic complex, leading to epigenetic changes to retain the expansive capacity of HSCs. Nevertheless, it was not clear whether the $CRL3^{KBTBD4}$ complex could affect additional protein complexes that include LSD1. Moreover, recent literature indicated that treatment with UM171 can also lead to preservation of HSC regenerative capacities via distinct pathways, such as degradation of MYC. Furthermore, the applicant had only focused on the epigenetic changes that would occur in the presence of UM171 but increase in the CoREST complex with HSC attrition (once UM171 is washed away and dorocubicel is infused in patients) was not evaluated. Therefore, the applicant further discussed the MoA of UM171 on the molecular level as well as the cellular consequences of this effect, indicating that loss of CoREST1-related silencing of the chromatin results in increased NFkB signalling and EPCR expression. Loss of MYC-related chromatin binding results in e.g. reduced ROS accumulation in cells. Both pathways result in maintenance of HSC functionality and protection from oxidative stress.

At the proposed concentration of 35 nM, UM171 appears to primarily (or only) target KBTBD4. KBTBD4 seems only to interact with CUL3-based E3 ubiquitin ligases for protein ubiquitination. Although the downstream pathways of KBTBD4-related protein degradation are likely broader than the investigated CoRest1 and MYC complexes, additional pathways have not (yet) been reported in literature. Whether unintended mechanisms are also involved in UM171's pharmacology remains to be determined. For now, the applicant has provided the most up-to-date understanding of the intracellular and (epi)genetic mechanisms of action of UM171.

The applicant mentions that the effect of UM171 is concentration-dependent and that concentrations with e.g. 1 µM would not be desirable. However, Tellechea et al. (2023, Blood, poster abstract) mentions that an UM171 concentration of 125 nM would result in maximal RCOR1 degradation and maximal primitive cell expansion. *In vivo*, cells cultured with 125 nM UM171 showed better engraftment compared to cells cultured with 35 nM UM171. Based on these data, a higher UM171 would still act via degradation

[49] Subramaniam A, Žemaitis K, Talkhoncheh MS, Yudovich D, Bäckström A, Debnath S, Chen J, Jain MV, Galeev R, Gaetani M, Zubarev RA, Larsson J. Lysine-specific demethylase 1A restricts ex vivo propagation of human HSCs and is a target of UM171. Blood. 2020 Nov 5;136(19):2151-2161. doi: 10.1182/blood.2020005827. PMID: 32582923; PMCID: PMC7645986.

[50] Chagraoui J, Girard S, Spinella JF, Simon L, Bonneil E, Mayotte N, MacRae T, Coulombe-Huntington J, Bertomeu T, Moison C, Tomellini E, Thibault P, Tyers M, Marinier A, Sauvageau G. UM171 Preserves Epigenetic Marks that Are Reduced in Ex Vivo Culture of Human HSCs via Potentiation of the CLR3-KBTBD4 Complex. Cell Stem Cell. 2021 Jan 7;28(1):48-62.e6. doi: 10.1016/j.stem.2020.12.002. PMID: 33417871.

[51] Hu A, Gao J, Varier KM, Gajendran B, Jiang F, Liu W, Wang C, Xiao X, Li Y, Zacksenhaus E, Ali S, Ben-David Y. UM171 cooperates with PIM1 inhibitors to restrict HSC expansion markers and suppress leukemia progression. Cell Death Discov. 2022 Nov 5;8(1):448. doi: 10.1038/s41420-022-01244-6. PMID: 36335089; PMCID: PMC9637110.

of the CoREST1 complex but may also involve other pathways that are not (or differently) affected when using 35 nM UM171. This has not been further investigated. Considering that in the current clinical manufacturing process 35 nM UM171 is used, no additional investigations related to UM171 mechanisms at higher concentrations are warranted.

The applicant further states that UM171 primarily acts as a temporary regulator of gene expression (amongst others via CoREST degradation) and cellular phenotype, without causing permanent genetic or epigenetic changes although latter data have not been presented in the dossier, and that stem cell properties are preserved by UM171. This would be reassuring for the long-term safety of short-term UM171-expanded cells. In the *in vitro* reversibility studies (study reports RPT-0019 and -0022), removal of UM171 resulted in a decrease in % CD34+ (CD45RA-) cells and an increase in RCOR1 protein level, suggesting that cells will turn into their differentiation program. However, it was not shown whether downstream targets of e.g. RCOR1/CoREST (such histone modifications) were also reversed. See the Toxicology -Dorocubicel/ECT-001-CB-DP1 section for further analysis assessing the reversibility of UM171.

Strictly spoken, while it has not been shown non-clinically that after culturing with and removal of UM171, sufficient CD34+ cells will retain their stemness in culture for long-term repopulation of the bone marrow, available in vivo data suggest that stemness is preserved in dorocubicel. Clinically, so far there is only one patient with graft failure. Among all patients with follow-up beyond one year (and up to >5 years) no cases of late cytopenia, secondary graft failures, skewed haematopoiesis or post-transplant leukaemia or myelodysplasia have been reported, even in recipients of high cell dose products. Nevertheless, there may also be other/milder signs of decreased bone marrow engraftment, which have not been presented clinically. In addition, the number and follow-up time of patients treated with UM171-expanded cells is still limited. Nevertheless, patients will remain closely monitored for the next years.

Pharmacokinetics

The applicant did not study the distribution of ECT-001-CB-DP1 / dorocubicel or ECT-001-CB-DP2, but instead studied pharmacokinetics (absorption and metabolism) of UM171, the compound used in 7-day culture of donor umbilical cord blood (UCB) to generate dorocubicel. However, only 8 ng/kg (or 0.114 ng/mL plasma) residual UM171 is expected to be found in patients. The evaluation of the pharmacokinetics of UM171 may thus be regarded of limited relevance for extrapolation to human, although it is reassuring to known that it seems to be rapidly eliminated. In vivo PK studies in NSG mice, rats, and cynomolgus monkeys all demonstrated fairly rapid clearance of UM171 from the blood stream within several hours tested up to Human Equivalent Dose (HED) dose levels 20,000-fold (mice and rat) or 8,000-fold (cynomolgus monkey) as compared to human (8 ng/kg based on 70 kg individual).

The in vitro liver metabolism studies of this product indicated rapid clearance in liver microsomes for mice, rat and human without evidence for human metabolites.

Toxicology

<u>UM171</u>

Single dose toxicity and mutagenicity/ genotoxicity was evaluated separately for UM171, the compound used in 7-days culture of donor umbilical cord blood (UCB) to generate dorocubicel, and of UM171 treated cells/ dorocubicel, which can be agreed. However, as only traces of UM171 (8 ng/kg based on a 70 kg weighing individual) is expected to be transferred to the patient, a value that is lower than the TTC, the evaluation of the single dose toxicity and genotoxicity of UM171 may thus be regarded of limited relevance for extrapolation to human.

Intravenous administration at doses 8,000-20,000 times higher than the estimated human exposure to UM171 demonstrated no toxicity in rats (single-dose study) or cynomolgus monkeys (administrated 3 times with a 7-day washout period before testing the next dose). Histopathology was examined in relevant organs without any adverse findings.

The single dose toxicity study in rats and the "repeat dose toxicity study" (3x administration with a 7-day washout period) in non-human primates were not in accordance with GLP. The applicant asserts that the samples for each study were evaluated impartially, and the final study reports were reviewed to ensure an accurate representation of the raw data, results, and conclusions. Although it would have been desirable to have a more systematic and detailed account of the areas lacking GLP, it is uncertain what impact this would have on the overall non-clinical safety assessment of the product. Based on a 'weight of evidence approach,' it is therefore not deemed relevant to raise questions about this and the submitted discussion is considered sufficient. Since the presence of UM171 in the product overall is minimal, the studies themselves and a more detailed assessment of GLP are considered to have little significance for the overall safety evaluation.

Dorocubicel

Genotoxicity of UM171 was evaluated in a non-GLP AMES test at first with no mutagenic response observed. After receiving scientific advice from EMA the applicant conducted an additional GLP compliant AMES test in accordance with ICH S2 and OECD guidance with no mutagenic response observed. The applicant was also advised to include data on genetic impact of UM171-treated cells and discuss potential proliferative signals after infusion of the cells (i.e., whether expansion increased risk of cancer).

Genotoxicity of UM171-treated cells was further assessed in a GLP micronucleus assay (Study 391175). In the micronucleus assay with UM171, micronuclei (mean %MN) or hypodiploidy (mean %HD) values were all < 3-fold or < 7-fold the concurrent negative controls, respectively, and were within the laboratory historical negative control range. The assay was performed using Chinese Hamster Ovary (CHO) cells cultured in the presence of high concentrations (up to 13.5 µg/ml, 850-fold higher than the concentration of UM171 used in culture) of UM171. However, this concentration was not analysable due to cytotoxicity reasons. Thereby, the level of cytotoxicity elevated also with increasing incubation time. However, as an incubation time of 7 days was not tested in this assay, it is not clear which UM171 concentration is cytotoxic to the CHO cells. The relevance of this micronucleus test to support genetic safety of UM171 treated Cord Blood cells is thus unclear. The applicant argued that the test is mainly to investigate the chromosome damaging potential of the compound. The applicant explains that the stringent conditions, like the high UM171 concentration for 22 hr are sufficient to inform on the absence of clastogenicity and aneugenicity of UM171. It can indeed be considered that together with the negative outcome of the Ames test, this negative outcome of the micronucleus test supports the genetic safety of UM171 with regard to genotoxicity.

The genetic safety of the UM171 7-days cultured cells was analysed by means of Giemsa staining and G-banding, methods that are mostly capable of evaluating large scale chromosome rearrangements or aberrations. The average number of aberrations per cell reported in UM171-expanded cells was in general similar to that of controls, and cells cultured in 4F conditions did not show more breaks than cells cultured in 3F conditions, confirming the absence of genetic instability of UM171-expanded cells in this study. There was also no indication of neoplasia in the in vivo toxicological studies of UM171 expanded cells in NSG mice. Nonetheless, there is uncertainty regarding whether this analysis is sufficiently sensitive to detect all relevant effects on chromosomal integrity, as highlighted in a previous Scientific Advice (SA).

The applicant acknowledged that the cytogenetic analysis performed using the standard G-band karyotyping method is limited in its sensitivity to detect all potential effects on chromosomal integrity.

However, the applicant presents the following arguments to support the safety profile of UM171: 1) the applicant referred to published data demonstrating that UM171 reduces the levels of culture-induced reactive oxygen species (ROS), which are known to cause DNA damage, in phenotypic long-term hematopoietic stem cells (LT-HSCs). These findings align with observations of reduced DNA damage in hematopoietic stem cells (HSCs) cultured for seven days with UM171 compared to controls. 2) It is further argued that UM171 restricts the cell division of cultured CD34+CD45RA– cord blood (CB) cells thereby reducing the replicative stress to which stem cells are subjected during ex vivo culture. 3) according to the applicant, UM171 enhances the robustness of the stem cell pool by increasing clonal diversity *in vivo* This is described as providing an "additional safety level" against concerns related to clonal selection following expansion.4) No neoplastic findings have been observed in 116 patients treated with UM171 expanded cells.

Taking these considerations into account, alongside the absence of clinical findings indicative of neoplasia, the overall safety profile is considered acceptable. However, it is worth noting that the applicant could have chosen to use more sensitive methodologies. A combination of the proposed karyotyping approach with additional molecular/optical assays—either targeted or whole-genome—could have been employed to comprehensively detect potential effects on chromosomal integrity.

Dorocubicel / ECT-001-CB-DP1

Toxicity of dorocubicel was addressed in the pharmacology studies by histopathological analysis of relevant organs. This approach can be agreed. No adverse effects were observed.

The standard genotoxicity studies are not considered suitable to evaluate for the occurrence of (un)intended UM171 related epigenetic changes.

The applicant was asked to provide an epigenetic analysis to provide evidence that UM171 could be regarded safe in respect to unintended epigenetic editing. The applicant referred to report RPT-0019, related to the maintenance of low levels of RCOR1 during expansion of CD34+ cord blood cells under clinically relevant conditions, with a rapid increase in the RCOR1 protein level and release of the CD34+ cell differentiation program after UM171 removal. However, this phenotypic reversibility is no direct proof of the reversibility at an epigenetic level. It was expected that the applicant would provide data on acetylation and methylation of the DNA to compare epigenetics of cells before, during and after culture with UM171 and compare that to DMSO cultured cells. However, the applicant took a different approach and continued their investigation on the level of RCOR1 and the effects of its temporary degradation and its effects due to the use of UM171 in culturing, which was already initiated as part of the pharmacology studies for this therapy. The applicant used 4 batches of cryopreserved UM171 (7 days, 35 nm) cultured cells, which were placed back in culture and cultured another 7 days with either DMSO, reflecting 'Washout', or cultured in presence of UM171, reflecting the 'UM171-culture' condition. Although it was requested to analyse samples before, during and after culturing with UM171, considering the nature of UM171, to preserve HSPCs in their stem-cell-ness state, for instance low transcriptionally active, we can agree with the approach taken by the applicant.

The applicant used these UM171 cultured and the washout samples to analyse 4 different parameters that altogether support, next to the pharmacologic nature of UM171, also the safety of the compound to the cells in its current use (7 days of culture at 35 nM). The four 'read-outs' are summarized below.

Histone acetylation: H3K27ac levels were decreased in the CD34+ and CD34+CD45RA- populations after UM171 washout (at D9 and D10, 2 and 3 days of washout) compared to the UM171-cultured cell condition. This indicates that the UM171-induced repression of the deacetylase function is reversible.

Myc signalling: UM171 leads to a reduced expression of MYC target genes, including CD71. CD71 surface expression is used as a surrogate for MYC signalling. Surface expression of CD71 is increased

in HSPCs at D9 and significantly at D10 (D2 and D3 after washout), suggesting that UM171-induced MYC degradation in stem cells is reverted upon withdrawal of UM171, which is endorsed.

Lineage output: megakaryocyte/erythrocyte differentiation: 7 days upon UM171 washout, cells expressing CD71 +/-CD235a (corresponding to erythroid cells at different stage of maturation) re-emerged, as well as a small number of CD41/CD61 (megakaryocytic markers) cells, which suggests the reactivation of the erythroid differentiation program. However, in the UM171-cultured cell sample, these populations were undetectable. These data suggest that UM171 retains the cells in a stem-cell state and that multilineage differentiation is initiated upon removal of UM171 from the culture conditions. This is also confirmed *in vivo* upon transplantation of the cultured cells (see next subsection).

Unbiased RNA sequencing: RNA sequencing (RNA-Seq) was used to assess the functional consequences and potential reversibility of epigenetic alterations. Through high-resolution quantification of transcriptome-wide gene expression changes, RNA-Seq serves as an essential tool for assessing whether epigenetic states are functionally maintained or reversed. The signature upon 7 days UM171 culture is characterized by high proportion of stem cells and progenitors, and the rarity of erythroid and megakaryocyte progenitors, which remains present in the additional 7 days of UM171 culture. However, in the washout situation (UM171 is removed from culture after the initial presence of 7 days) a proportion of HSPC decreases and megakaryocyte/erythrocyte populations emerge, indicating resumption of cellular differentiation at the expanse of HSPC expansion, supporting reversibility of UM171 effect at the molecular level.

Clinical data: The applicant noted that among all patients with follow-up beyond one year (and up to >5 years) no cases of late cytopenia, secondary graft failures, skewed haematopoiesis or post-transplant leukaemia or myelodysplasia have been reported, which would have been expected when UM171-cultured HSCs would have been functionally impaired or at risk for transformation. Instead, immune reconstitution remained robust, supporting the sustained functional capacity of UM171-expanded HSCs. And, despite UM171's *in vitro* bias against megakaryopoiesis, patients achieved timely platelet recovery, indicating that any lineage skewing is reversible upon *in vivo* engraftment, further supporting the safety and functional integrity of UM171-expanded cells.

Taken together, although the applicant took a different approach to indicate the safety of the UM171-cultured cells than requested, the risk for safety issues related to UM171-induced (unwanted) epigenetic changes seems low and patients will remain closely monitored for the next years.

The applicant did not perform any carcinogenicity studies, which is in line with ICH S9 since the product is to be used in patients with advanced cancer.

No reproductive and developmental toxicity studies on either UM171 or dorocubicel were conducted by the applicant. The applicant contended that such studies are unnecessary as ECT-001-CB is not recommended for use in pregnant women, and only minimal residual amounts of UM 171 were detected in dorocubicel. The absence of such studies is considered acceptable.

No juvenile studies were performed by the applicant, which is considered acceptable given that Zemcelpro is indicated to adults only.

No local tolerance was performed by the applicant, which is considered acceptable given that Zemcelpro is administrated intravenously.

ERA

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, Zemcelpro is not expected to pose a risk to the environment. An ERA is not needed.

The applicant submitted a justification for not submitting ERA studies on un-altered cells of human origin. Although only traces of UM171 may still be present in the API, the environmental risk presented by UM171 was assessed by the applicant. The PECsw value of UM171 was below the action limit of 0.01 mcg/l. With a calculated log Kow of 3.53 for UM171 (EPISUITE v4.11, KOWWIN v1.68) according to the applicant, the trigger value for a phase II assessment of 4.5 is not met by UM171. A further PBT assessment is considered unnecessary due to the log Kow of 3.53.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.5.7. Conclusion on the non-clinical aspects

Pharmacology

With the submitted *in vitro* and *in vivo* non-clinical PD studies (only evaluating dorocubicel, thus ECB-001-CB-DP1), the applicant has provided a proof-of-concept that UM171 appears able to enhance umbilical cord-derived CD34+ cell expansion (when compared to untreated CD34+ cells), which could lead to long-term engraftment in patients. However, the molecular and epigenetic mechanisms of UM171 have not been fully elucidated and the impact of UM171 (and cryopreservation) on real long-term progenitors (i.e. early/primitive progenitor cells) and on CD34- cells within the DP1 fraction has not been considered in the non-clinical program. Moreover, adaptations in the production process related to culture of the cells with UM171 have not been fully substantiated with thorough non-clinical data. Based on the submitted clinical data, there are no concerns. However, long term safety and efficacy will be followed up in the post-marketing studies.

It appears that the applicant has studied the evaluation process of the pharmacodynamics of Zemcelpro and the impact of different culture conditions in a rather random manner. Thereby, it seems more or less coincidental which culture conditions (UM171 concentration, growth factors, culture duration) have been selected for the clinical and commercial manufacturing process. Taken together, no firm conclusions can be drawn from the non-clinical primary PD data and therefore information on the efficacy of Zemcelpro should come from clinical studies.

A hERG safety pharmacology screening assay with UM171 was also performed. The lack of GLP compliance is acceptable due to the negligible residual levels of UM171 in dorocubicel.

Pharmacokinetics

The applicant studied pharmacokinetics (absorption and metabolism) of UM171, the compound used in 7-day culture of donor umbilical cord blood (UCB) to generate dorocubicel. However, only 8 ng/kg (or 0.114 ng/mL plasma) residual UM171 is expected to be found in patients. The evaluation of the pharmacokinetics of UM171 indicate that any trace of UM171 in the final Drug Product will be rapidly cleared from the bloodstream upon administration, without producing any major metabolites.

Toxicology

Single dose toxicity and mutagenicity/ genotoxicity was evaluated separately for UM171, the compound used in 7-days culture of donor umbilical cord blood (UCB) to generate dorocubicel, and of UM171 treated cells/ dorocubicel, which can be agreed. UM171 did not show signs of adverse toxicity nor any evidence of genotoxicity as evaluated in an Ames and a micronucleus test. However, as only traces of UM171 (8 ng/kg based on a 70 kg weighing individual) is expected to be transferred to the patient, a value that is lower than the TTC, the evaluation of the single dose toxicity and genotoxicity of UM171 may thus be regarded of limited relevance for extrapolation to human.

Toxicity of ECT-001-CB-DP1/dorocubicel was addressed in the pharmacology studies by histopathological analysis of relevant organs. This approach can be agreed. No adverse effects were

observed. No increase in the number or type of structural chromosomal abnormalities were detected in UM171 7-days cultured cells that were analysed by means of Giemsa staining and G-banding, methods that are mostly used for evaluating large scale chromosome rearrangements or aberrations. This method is not considered suitable to evaluate the occurrence of (un)intended UM171 related epigenetic changes. However, in the pharmacology section the applicant was asked to give a more comprehensive overview of the primary (intended) and secondary (unintended) pharmacologic mechanism of action of UM171 from own and public data as well as to provide an epigenetic analysis of CB-cells before and after the 7 days culture with UM171 including a discussion of the functional consequences and long term safety effects. Although the applicant took a different approach to indicate the safety of the UM171-cultured cells than requested, based on the provided information it can be agreed that the risk for safety issues related to UM171-induced (unwanted) epigenetic changes seems low and patients will remain closely monitored for the next years.

ERA

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, Zemcelpro is not expected to pose a risk to the environment. An ERA is not needed.

Overall, the non-clinical part of the dossier is acceptable.

The CHMP endorse the CAT conclusions on the non-clinical aspects as described above.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

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

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

- **Tabular overview of clinical studies**

Study number/ Jurisdiction	Indication	Study design	Status
ECT-001-CB.001 (Study 001) (formerly known as UM0128171-001) Clinicaltrials.gov: NCT02668315 Canada	Patients with life-threatening haematologic malignancies and lacking an HLA matched donor	Phase I/II, Multicentre, single-arm, open-label. Primary endpoints: • Feasibility • Safety • Kinetics of neutrophil and platelet recovery • Determine minimal cord blood unit cell dose that results in prompt engraftment as a single cord transplant	Completed Cohen et al. (2020) Enrolled: 27 / Infused: 26
ECT-001-CB.002 (Study 002) Clinicaltrials.gov: NCT03913026 Canada	High-risk leukaemia/MDS	Phase II, Single site, single-arm, open-label. Primary endpoints: • TRM at Day 100- and 1-year post transplant • RFS at 1- and 2-years post-transplant	Recruitment completed in August 2022, 3-year follow-up ongoing. Enrolled: 31 / Infused: 30
ECT-001-CB.003 (Study 003) Clinicaltrials.gov: NCT03441958 Canada	High-risk Multiple Myeloma	Phase I/II, Single site, single-arm, open-label. Primary endpoints: • Safety • Feasibility of the procedure • Kinetics of donor lymphoid and myeloid cells recovery • Incidence and grade of chronic GvHD at 1- and 2-year post transplant	Recruitment completed in November 2021, 3-year follow-up ongoing. Enrolled: 19 / Infused: 18
ECT-001-CB.004 (Study 004) Clinicaltrials.gov: NCT04103879 EudraCT No. 2022-002458-26 USA / The Netherlands	High-risk leukaemia/MDS	Phase II, Single site, single-arm, open-label. Primary endpoints: • Safety and feasibility • RFS at 1- and 2-years post-transplant	Recruitment completed in December 2023, 2-year follow-up ongoing. Enrolled: 33 / Infused: 30
ECT-001-CB.007 (Study 007) Clinicaltrials.gov: NCT04990323 USA	Paediatric high risk myeloid malignancies	Phase I/II, Single site, single-arm, open-label Primary endpoints: • Safety • Incidence of relapse at 1-year post transplant	Recruitment initiated in 2021, still ongoing. Enrolled: 13 / Infused: 12

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

No dedicated PK-PD studies have been conducted because the standard clinical pharmacology studies such as dose escalation/dose range finding, absorption, distribution, metabolism and excretion, drug-drug interaction and special population studies are not considered feasible for cell therapy products.

Characterization of the CD34+ cells in dorocubicel and CD3+ cells in ECT-001-CB-DP2 in the final product using quantitative flow cytometry assessment was performed as part of release testing for each lot.

An overview of the measured product attributes is provided in Table . Cell product quantity was measured by the dose of CD34+ or CD3+ cells per kg of patient body weight. The purity of dorocubicel was measured by the percentage of viable CD34+ cells and the purity of ECT-001-CB-DP2 was tested by the percentage of viable CD3+ cells of the total viable white blood cells (CD45+). Potency of the cells was measured through the proportion of viable cells within the CD34+ and CD3+ cells in dorocubicel and ECT-001-CB-DP2, respectively. The fold expansion of viable CD34+ cells during the 7-day culture was also used as a measure of potency for dorocubicel.

Assessed population are:
Modified FAS (mFAS): A subset population consisting of adult patients pooled from Studies 001, 002 and 004, actually treated (per-protocol) with single ECT-001-CB derived from a small CB unit (n=38).

Supportive modified FAS (smFAS): Subset population consisting of all adult patients, pooled from Studies 001, 002 and 004, actually treated (per protocol) with single ECT-001-CB regardless of the size of the cord blood (CB) unit used as starting material (n=82).

Both fractions (ECT-001-CB-DP1 and ECT-001-CB-DP2) of the final product are cryopreserved. As cryopreservation leads to a decrease in cell dose and cell viability, this product attribute was evaluated both pre- and post-cryopreservation.

Table 2: Parameters used for assessment of correlation between product attributes and cellular kinetics

Product attributes		Cellular kinetics
Dorocubicel:	vs	Neutrophil recovery
Dose of vCD34+ cells (quantity, pre-cryo)		Platelet recovery
Dose of vCD34 cells (quantity, post-cryo)		Donor chimerism in different cell subsets
% vCD34+ cells (purity)		Immune reconstitution: IgG and CD4 T cell levels
Viability of CD34 cells (potency, pre-cryo)		
Viability of CD34 cells (potency, post-cryo)		
Expansion of vCD34+ cells (potency)		
ECT-001-CB-DP2:		
Dose of vCD3 cells (quantity, pre-cryo)		
Dose of vCD3 cells (quantity, post-cryo)		
% vCD3+ cells (purity)		
Viability of CD3 cells (potency, pre-cryo)		
Viability of CD3cells (potency, post-cryo)		

Table provides an overview of the product attributes.

Table 3: Quantity, purity and potency of ECT-001-CB-DP1 (dorocubicel) and ECT-001-CB-DP2 fraction

	smFAS (n=82)	mFAS (n=38)
ECT-001-CB-DP1 (dorocubicel)		
Manufacturing process		
N	82	38
Fresh (n, %)	24 (29.3%)	14 (36.8%)
Cryopreservation (n, %)	58 (70.7%)	24 (63.2%)
vCD34+ cell dose (×10⁶/kg) *Quantity – pre-cryopreservation*		
E N	82	38
Mean (SD)	3.34 (1.52)	2.46 (1.18)
Median (range)	3.41 (0.56-6.24)	2.56 (0.58-4.64)
vCD34+ cell dose (×10⁶/kg) *Quantity – post-cryopreservation*		
N	58	24
Mean (SD)	2.96 (1.51)	2.11 (1.04)
Median (range)	2.78 (0.30-6.38)	2.14 (0.40-4.04)
Percent vCD34+ cells (%)		

	smFAS (n=82)	mFAS (n=38)
Purity – pre-cryopreservation		
N	82	38
Mean (SD)	76.4 (7.0)	76.0 (7.1)
Median (range)	77.0 (55.0-93.1)	76.9 (55.0-85.4)
Viability CD34+ cells (%) *Potency – pre-cryopreservation*		
N	82	38
Mean (SD)	97.6 (1.67)	97.8 (1.4)
Median (range)	98.0 (92-100)	98.0 (94 – 100)
Viability CD34+ cells (%) *Potency – post-cryopreservation*		
N	58	24
Mean (SD)	86.3 (14.1)	93.5 (8.87)
Median (range)	93.5 (45-100)	96.0 (58-100)
vCD34+ cell expansion (fold) *Potency*		
N	82	38
Mean (SD)	46.2 (17.5)	46.8 (18.0)
Median (range)	44.0 (18.0 – 107.4)	44.9 (18.0 – 86.8)
ECT-001-CB-DP2		
vCD3+ cell dose ($\times 10^6$/kg) *Quantity – pre-cryopreservation*		
N	82	38
Mean (SD)	2.69 (1.27)	2.15 (0.80)
Median (range)	2.49 (0.97 – 6.75)	2.01 (0.97 – 4.14)
vCD3+ cell dose ($\times 10^6$/kg) *Quantity – post-cryopreservation*		
N	58	24
Mean (SD)	2.32 (1.37)	1.58 (0.69)
Median (range)	1.93 (0.52-6.45)	1.40 (0.52-3.39)
Percent vCD3+ cells (%) *Purity – pre-cryopreservation*		
N	82	38
Mean (SD)	30.7 (7.0)	31.5 (7.6)
Median (range)	29.5 (17.0-58.5)	31.0 (18.0-58.5)
Viability CD3+ cells (%) *Potency – pre-cryopreservation*		
N	82	38
Mean (SD)	93.7 (6.1)	94.1 (5.7)
Median (range)	96 (68 – 100)	96 (79 – 100)
Viability CD3+ cells (%) *Potency – post-cryopreservation*		
N	58	24
Mean (SD)	80.5 (13.2)	77.5 (16.0)
Median (range)	84 (36-97)	82 (36-96)

Dose proportionality and time dependencies

Please refer to *section 2.6.5.1 Dose response studies*.

Special populations

No dedicated clinical studies have been conducted in special populations. The impact of intrinsic factors (including age, gender, race, ethnicity) and extrinsic (HLA-matching, conditioning regimen) on cellular kinetics have been evaluated in the pooled dataset of patients from Studies 001, 002 and 004, referred to as smFAS.

Age

Transplant patients within the study were adults up to the age of 66 years old. Patients were separated based on their age, i.e. <40 (n=30), 40-54 (n=29) and ≥55 years old (n=23). Time to neutrophil and platelet recovery was similar between the three groups. There was no significant difference between T cell donor chimerism at 0.5 months post-transplant. Younger patients showed higher IgG levels at 3 months post-transplant compared to the two older groups (p = 0.0015 vs 40-54 yrs old, p = 0.056 vs 55+ yrs old). IgG levels were not significantly different between the three groups at 12 months post-transplant.

Gender

The smFAS consisted of 51 males and 31 females. Time to neutrophil and platelet recovery was similar between the two groups. There was no significant difference between T cell donor chimerism at 0.5 months. IgG levels were similar at 3 months and 12 months post-transplant.

Ethnic factors

Patients were separated into two groups based on race (White, n=65, vs Others, n=14) and two groups based on ethnicity (Hispanics, n=9, vs Others, n=70). Three patients did not have any race or ethnicity reported. Race and ethnicity did not impact time to neutrophil or platelet recovery, T cell donor chimerism at 0.5 month or IgG levels at 3 months. There was no significant difference in IgG levels between Hispanic and non-Hispanic patients at 12 months.

Weight

The median weight in the mFAS population was 87.5 (range 59.0 – 144.0) kg. Patients were separated into two groups based on weight (<80 kg n=41; ≥80 kg, n=39). Time to neutrophil and platelet recovery were similar between the two groups. There was no significant difference between T cell donor chimerism at 0.5 months and IgG levels at 3 months post-transplant in the 2 groups. IgG levels at 12 months post-transplant were higher for recipients under 80 kg (p=0.0055).

HLA matching

The impact of HLA matching (5/8 versus ≥6/8) was studied. The HLA matching level of the graft of patients in the smFAS population was 5/8 (n=48) or ≥6/8 (n=34). Time to neutrophil and platelet recovery were similar between the two groups. There was no significant difference between T cell donor chimerism at 0.5 months and IgG levels at 3 and 12 months posttransplant in the two groups.

Conditioning regimen

The impact of conditioning regimen (high versus intermediate intensity according to Spyridonidis A et al. 2020 was studied (see section *3.3.7.1. Patient exposure*). Time to neutrophil and platelet recovery was similar between the high (n=22) versus intermediate (n=58) intensity conditioning regimens. There was no significant difference between T cell donor chimerism at 0.5 months or IgG levels at 3 and 12 months.

Pharmacokinetic interaction studies

No interaction studies have been performed.

Pharmacokinetics using human biomaterials

Not applicable.

Comparability study (fresh versus cryopreserved)

Study SRT-ECT-011 was performed to assess the impact of manufacturing process changes including the introduction of cryopreservation. Data from 88 lots were re-analysed at release, 24 lots manufactured pre-change, and 64 lots manufactured post-change, representing 69% and 97% of the full pre-change and post-change lots manufactured for clinical use, respectively. This included patients from study 001, 002, 003, 004 and 007. Study 003 was performed in high-risk multiple myeloma and study 007 in paediatric high risk myeloid malignancies.

The impact of the manufacturing process change on CD34+ characteristics was assessed. These are measured after 7-day culture at release, so pre-cryopreservation for cryopreserved lots. All lots had CD34+ dose and CD34+ viability within release criteria. CD34+ dose in lots manufactured post-change is higher than that of lots manufactured pre-change.

In addition to CD34+ parameters at release (pre-cryopreservation), the impact of cryopreservation was tested by measuring CD34+ parameters post-cryopreservation. Cryopreservation led to a decrease in CD34+ cell dose and viability post-cryopreservation, as well as a decrease in percent and concentration of CD34+ cells.

Evaluation of clinical efficacy

To further supplement analytical comparability data, and as cryopreservation leads to a decreased dose, viability, concentration and percentage of CD34+ cells, comparison of clinical outcomes was performed between patients treated with fresh or cryopreserved ECT-001-CB-DP1. The impact of a lower CD34+ dose, CD34+ viability and CD34+ recovery after cryopreservation on clinical efficacy were also evaluated.

ECT-001-CB is being studied in a diverse patient population with heterogeneous diseases and disease risks. For this analysis, a more homogeneous patient cohort was used consisting of adult patients treated with ECT-001-CB for leukaemia, lymphoma and myelodysplasia, i.e., patients included in the ECT-001-CB.001, .002 and .004 trials only. Patients included in the analyses were selected according to the following criteria:

Inclusion criteria

1. Patients treated with a single expanded ECT-001-CB product; and

2. Adult patients treated for acute leukemias, lymphomas or myelodysplasias following a myeloablative or reduced intensity conditioning regimen.

Exclusion criteria

1. Patients who were treated with double CB transplant as per protocol (i.e., with one expanded ECT-001-CB and one unmanipulated CBU);

2. Patients with documented high titers of donor-specific anti-HLA antibodies;

3. Patients who did not receive the ECT-001-CB grafts;

4. Pediatric patients, i.e., under 18 years of age at time of transplant;

5. Patients with a major protocol deviation; and

6. Patients with less than 3 months of follow-up.

Baseline data

A total of 69 patients were included from study ECT-001-CB.001 (n=22 fresh), ECT-001-CB.002 (n=2 fresh; n=26 cryopreserved) and ECT-001-CB.004 (n=19 cryopreserved). Patients in the two cohorts had similar demographic characteristics, with no significant differences between patients' age and weight, distribution of Karnofsky performance score (KPS), sex and disease type and status, as well as conditioning regimens used.

Almost all patients (22/24, 92%) in the fresh cohort were enrolled in the ECT-001-CB.001 trial, which included patients with haematological malignancies without a suitable donor. In contrast, all patients in the cryopreserved cohort were enrolled in the ECT-001-CB.002 and ECT-001-CB.004 trials, which included patients with high-risk haematological malignancies. Larger CBU units with more HLA-mismatches were selected as starting material for manufacture of ECT-001-CB in the ECT-001-CB.002 and ECT-001-CB.004 trials. This led to a higher proportion of 5/8 HLA matching in the cryopreserved cohort (78%) compared to the fresh group (21%, $p = 7.27 \times 10^{-6}$). The selection of larger CBU in the ECT-001-CB.002 and ECT-001-CB.004 trials also led to a higher CD34+ dose at release in the cryopreserved group (median of 4.83×10^6/kg) compared to the fresh group (median of 3.22×10^6/kg, $p = 6.46 \times 10^{-3}$). However, there was no significant difference between the dose of viable CD34+ cells that patients received in the cryopreserved group (i.e. CD34+ dose evaluated after cryopreservation of ECT-001-CB-DP1) with that received by the patients in the fresh group (i.e. evaluated at release, without cryopreservation of ECT-001-CB-DP1). Finally, a portion of the fresh cohort received cyclosporine A and mycophenolate mofetil (MMF) as immunosuppressive regimen at the beginning of the ECT-001-CB.001 trial. However, due to a high incidence of severe engraftment/pre-engraftment syndrome observed in the first patients, it was decided to switch to an immunosuppressive regimen consisting of Tacrolimus and MMF instead.

Outcomes

There was no significant difference in cumulative incidence of neutrophil recovery between patients treated with fresh vs cryopreserved ECT-001-CB-DP1 (p = 0.498, Figure).

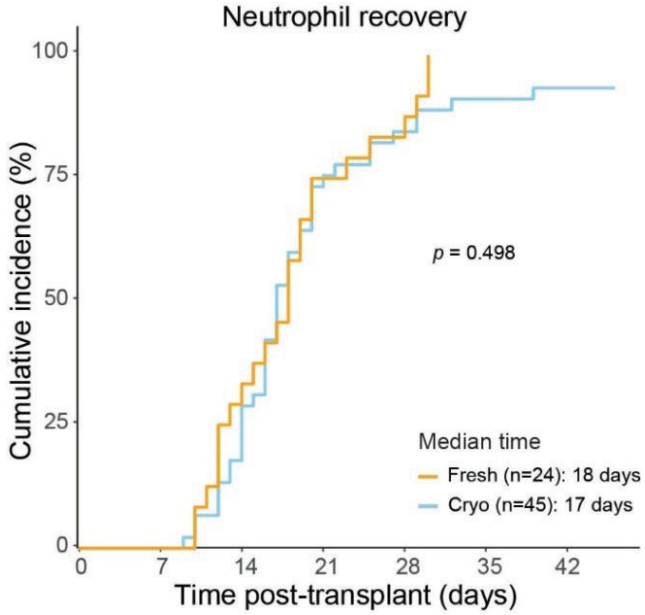

Figure 1: Kinetics of neutrophil recovery.

The clinical outcomes for patients with fresh versus cryopreserved ECT-001-CB-DP1 are shown in Table .

Table 4: Clinical outcomes fresh versus cryopreserved

	Fresh (N=24)	**Cryopreserved (N=45)**
Median follow-up (months)	36.0 (IQR 35.0 – 38.3)	19.7 (IQR 3.2 – 35.9)
Neutrophil recovery (days)	18 (range 10-30)	18 (range 10-30)
Platelet recovery (days)	42 (range 27-63)	37.5 (range 9-39)
Grade III-IV acute GVHD (at 12 months)	8.3%	17.8%
Moderate to severe chronic GVHD (at 12 months)	0%	5%
TRM (at 12 months)	4.2%	17%
OS (at 12 months)	83.3% (95%CI: 70-100%)	76.0% (95%CI: 64-90%)
PFS (at 12 months)	70.8% (95%CI: 55-92%)	74.0% (95%CI: 62-89%)

A subgroup analysis was performed in lots with a low CD34+ viability post-cryopreservation. The cohort of patients that received a cryopreserved ECT-001-CB-DP1 was split in a low CD34+ viability group, i.e. <70% post-cryopreservation, compared to those with higher CD34+ viability post-cryopreservation (i.e. ≥70%). Over the 43 lots of cryopreserved ECT-001-CB-DP1, 9 (21%) had a CD34+ viability measured post-cryopreservation below 70%, which is the minimal CD34+ viability required for release of the product prior to cryopreservation of the cells.

Patients who received a cryopreserved ECT-001-CB-DP1 fraction with a CD34+ viability <70% post-cryopreservation had a median time to neutrophil recovery of 17 days, compared to a median of 17 days for patients who received a cryopreserved ECT-001-CB-DP1 fraction with a CD34+ viability ≥70% post cryopreservation.

2.6.2.2. Pharmacodynamics

Mechanism of action

ECT-001-CB is a cell therapy product manufactured by expanding allogeneic cord blood (CB)- derived HSCs ex vivo. The active ingredients are the cells contained in the two fractions of ECT- 001-CB:

- the fraction of 7-day expanded CD34+ cells (CD34+ cell fraction; pINN dorocubicel; ECT-001-CB-DP1)
- the fraction of remaining CD34- (CD3+) cells from the CB, which contains immune cells from the donor, including T lymphocytes (unexpanded CD34- cells, sometimes referred to as ECT-001-CB-DP2)

The ECT-001 expansion technology combines the use of the proprietary small molecule UM171, a pyrimido-indole derivative which preserves hematopoietic stem cells functions and prevents their differentiation in vitro, and a fed-batch culture system, which leads to the reduction of endogenously produced negative regulators of stem cell function.

The primary mechanism of action of dorocubicel lies in promoting hematopoietic recovery and immune reconstitution through the activity of expanded CD34+ hematopoietic stem cells.

The unexpanded CD34- cells, consisting primarily of CD3+ T cells, play a complementary role by supporting immune reconstitution and providing graft-versus-leukemia (GVL) effects post-transplantation.

Hematopoietic stem/progenitor cells from Zemcelpro migrate to the bone marrow where they divide, mature, and differentiate in all haematological cell lineages. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin.

By expanding HSC, the use of UM171 allows the expansion of small cord units that hold the best HLA matched CB to increase accessibility to CB transplant, minimize complications, and ensure long-term in vivo engraftment for positive clinical outcomes.

Primary and secondary pharmacology

T cell reconstitution (SRT-ECT-040)

Study SRT-ECT-040 investigated T cell reconstitution in patients included in study 001 (ECT-001-CB.001) and compared the result to a retrospective cohort of patients receiving unmanipulated CB.

Twenty-two patients were included in study 001. Two patients were excluded because of progressive disease or death of TRM within 3 months post-HSCT, therefore 20 patients from study 001 were included in study SRT-ECT-040. The unmanipulated CB cohort consisted of 12 consecutive patients who consented to research sample collection and were transplanted with either single (n=10) or double (n=2) unmanipulated CBs following similar myeloablative or reduced toxicity myeloablative regimens at the same institution. Patients in the two cohorts received either a myeloablative conditioning regimen or reduced toxicity myeloablative conditioning regimen and no patient received ATG as part of the conditioning regimen. Patient characteristics are provided in Table 5:.

Table 5: Patients characteristics (SRT-ECT-040)

	UM171-expanded CB (n=20)	Unmanipulated CB (n=12)	*p*
Median age, years (range)	45 (19-63)	49 (26-61)	0.40
Sex, n (%)			
Male	13 (65%)	8 (67%)	1.0
Female	7 (35%)	4 (33%)	
Number of CB units, n (%)			
Single	20 (100%)	10 (83%)	0.13
Double	-	2 (17%)	
HLA matching, n (%)			
Single CB transplant			
5/8	3 (15%)	3 (25%)	1.9e-4
≥6/8	17 (85%)	4 (33%)	
4/6	-	1 (8%)	
5/6	-	2 (17%)	
Double CB transplant			
4/8 & 5/8	-	1 (8%)	
6/8 & 6/8	-	1 (8%)	
Conditioning regimen, n (%)			
Cyclophosphamide, 12GyTBI, Fludarabine	8 (40%)	5 (42%)	1.0
Cyclophosphamide, Thiotepa, 4GyTBI, Fludarabine	12 (60%)	7 (58%)	
Anti-thymocyte globulin in regimen, n (%)	-	-	-
CMV status before transplant			
Seropositive	9 (45%)	5 (42%)	1.0
Seronegative	11 (55%)	7 (58%)	
Acute GVHD, n (%)			
Grade II-IV	14 (70%)	6 (50%)	0.58
Grade III-IV	2 (10%)	1 (8%)	0.88
Chronic GVHD, n (%)			
All	3 (15%)	2 (17%)	0.96
Moderate to severe	0	0	-

Values are presented as number (%) unless otherwise indicated.
TBI indicates total body irradiation; —, none.
Gy: Gray, TBI: total body irradiation.

T cell reconstitution following UM171-expanded (ECT-CB-001) CB transplantation and unmanipulated CB transplantation is shown in Figure . Patients in both groups received a similar dose of total nucleated cells (TNC). Patients receiving ECT-CB-001 had a 17-fold greater CD34+ cell dose and a 2-fold lower CD3+ T cell dose. Patients in study 001 received ECB-CB-001-DP1 (dorocubicel) fresh, before the manufacturing process change. The ECT-CB-001-DP2 fraction was cryopreserved. The CD3+ T cell dose was measured before cryopreservation. As cryopreservation results in cell loss, the estimated CD3+ T cell dose infused is expected to be lower.

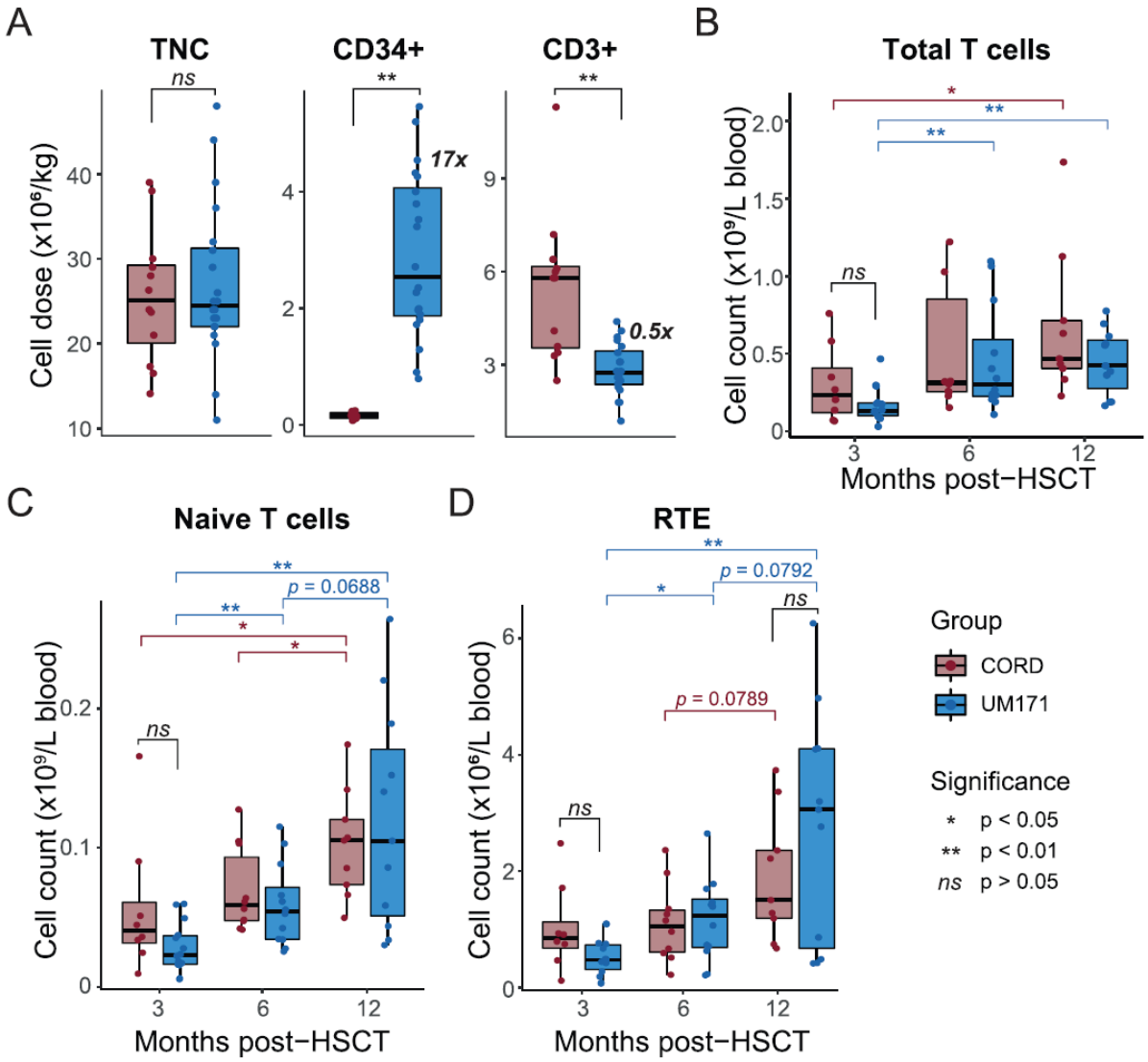

Figure 2: T cell reconstitution following UM171-expanded (ECT-CB-001) CB transplantation and unmanipulated CB transplantation. (A) Total nucleated cells, CD34+, and CD3+ cell dose infused in UM171-expanded (blue) and unmanipulated (red) CB graft. CD3+ cell dose is an upper estimate in UM171-expanded CB graft, as additional cell loss occurring during final wash, formulation, and cryopreservation is not considered here (evaluated to be an additional ~30% loss). Count of (B) total T cells, (C) naive T cells, and (D) RTEs post transplantation in patients treated with UM171-expanded (blue) or unmanipulated (red) CB grafts.

There were no significant differences in the total T cell count at any time points between patients treated with ECT-CB-001 (UM171) and patients treated with unmanipulated CB (CORD). There were no significant differences in cell increase in naïve T cell and RTE cells from 3 months to 6 and 12 months post-HSCT.

No significant differences were observed between the two cohorts for both CD4+ and CD8+ T cells, as well as naïve, central memory, effector memory and CD45RA+ effector memory T cell subsets (data not shown). Patients who underwent transplantation with UM171-expanded CB showed greater T-cell receptors (TCR) diversity post-transplantation.

Dose-efficacy

A weak negative correlation was found between the dose of CD34+ cell dose in dorocubicel and the time to neutrophil recovery both for the CD34+ cell dose measured pre- and post-cryopreservation. There was a weak negative but not significant correlation between time to neutrophil engraftment and the dose of CD34 cells originally contained in the CBU used as starting material in the manufacture of ECT-001-CB. There was no correlation between the dose of CD3+ cells post-cryopreservation and the time to neutrophil engraftment.

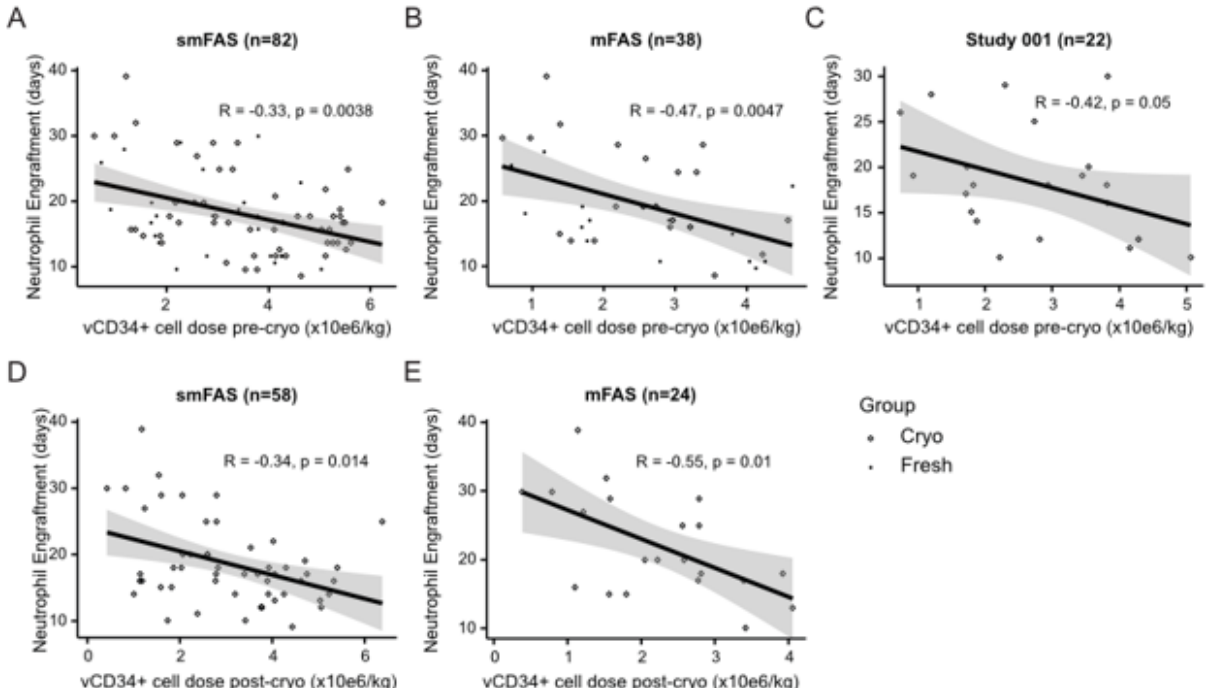

Figure 1 Correlation between pre-cryopreservation CD34+ cell dose and neutrophil recovery for the in the smFAS (A), mFAS (B) and study 001 (C) and the post-cryopreservation vCD34+ cell dose in the smFAS (D) and mFAS (E).

There was a weak correlation between the pre-cryopreservation vCD34 dose and time to platelet recovery in the smFAS population while no correlation was observed between the post-cryoprservation vCD34 dose and time to platelet recovery (Figure 2). The dose of CD3+ cells in ECT-001-CB-DP did not correlate with the time to platelet recovery.

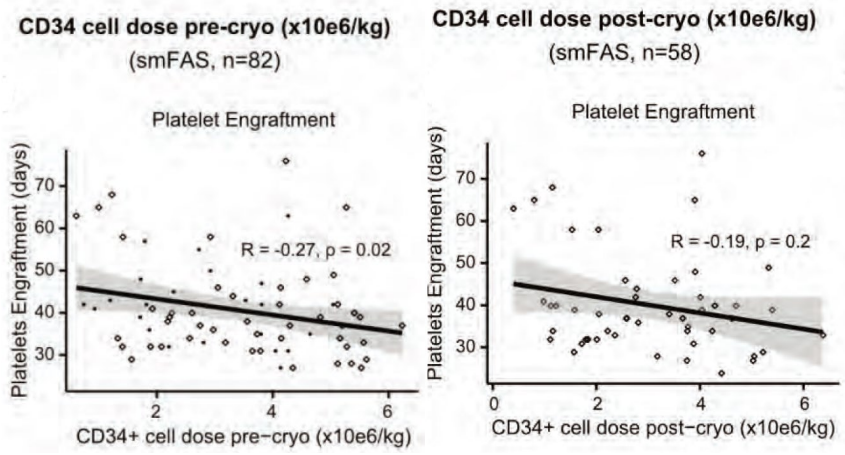

Figure 2 Correlation between the dose of CD34+ cells and time to platelet engraftment, pre-cryopreservation (left) and post-cryopreservation (right)

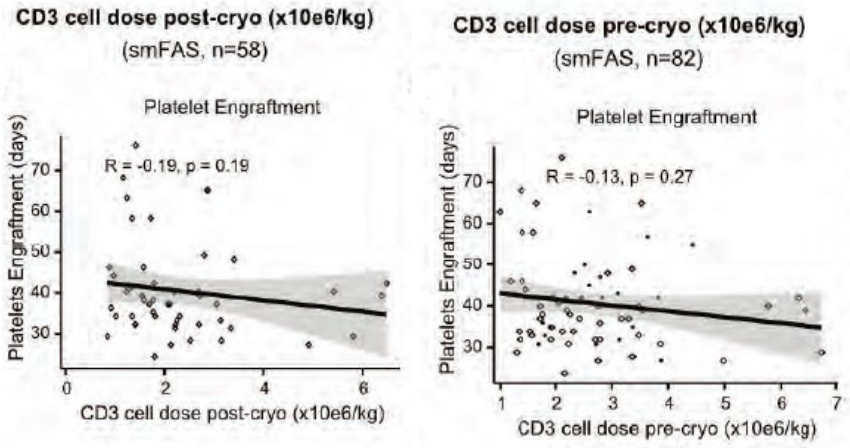

Figure 3 Correlation between the dose of CD3+ cells and time to platelet engraftment, pre-cryopreservation (left) and post-cryopreservation (right).

There was one patient in the mFAS population with primary graft failure and no patients with secondary graft failure.

At 1-year post-transplant, 35/38 (92.1%) patients had either completed 1 year of follow-up (n=21) or were discontinued early (n=14) due to relapse (n=9), non-relapse mortality (NRM, n=4) or graft failure (n=1) in the mFAS population. Patients with insufficient follow-up time (i.e., <12 months) at time of data extract were excluded from this analysis (n=3/38; 7.9%).

There was no statistical difference in the dose of CD34+ cells infused to patients who achieved EFS at 1 year (i.e. were alive, disease-free and had not suffered graft failure) compared to that of patients who relapsed or progressed, died of NRM or had graft failure before 1 year post- transplant (unpaired bilateral Student T test, p = 0.69)

There was also no statistical difference in the dose of CD3+ cells infused to ECT-001-CB treated patients who achieved EFS at 1 year compared to that of patients who relapsed or progressed, died of NRM or had graft failure in the first year post-HSCT, both when considering pre- and post-cryopreservation cell doses.

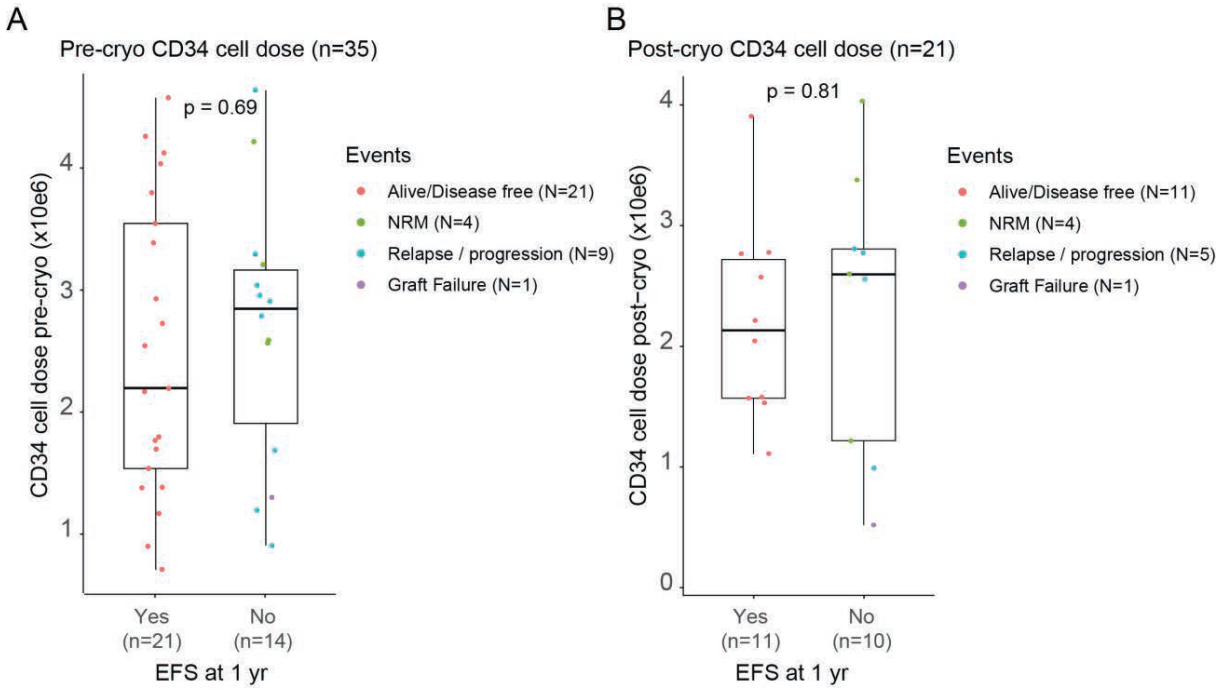

Figure 4 CD34+ cell dose as measured pre-cryotherapy (A) and post-cryotherapy (B) in patients who achieved EFS at 1 year

At 2 years, 31/38 (81.6%) ECT-001-CB treated patients had either completed 2 years of follow-up (n=16) or were discontinued early (n=15) due to relapse (n=10), NRM (n=4) or graft failure (n=1). Patients with insufficient follow-up time (i.e., <24 months) at time of data extract were excluded from this analysis (n=7/38, 18.4%). There was no statistical difference in the dose of CD34+ cells infused to patients who achieved EFS at 2 years compared to that of patients who relapsed or progressed, died of NRM or had graft failure in the first 2 years post-HSCT for both pre- and post-cryopreservation CD34+ cell doses. There was also no statistically significant difference for the CD3+ cell dose infused.

Dose-safety

Acute GVHD

In the mFAS, 26/38 (68.4%) patients had grade II-IV acute GVHD, and 9/38 (23.7%) had grade III-IV acute GVHD. There was no statistically significant difference between the CD34+ cell dose received by patients who had or didn't have acute GHVD or grade II-IV or III-IV (*Figure 5*).

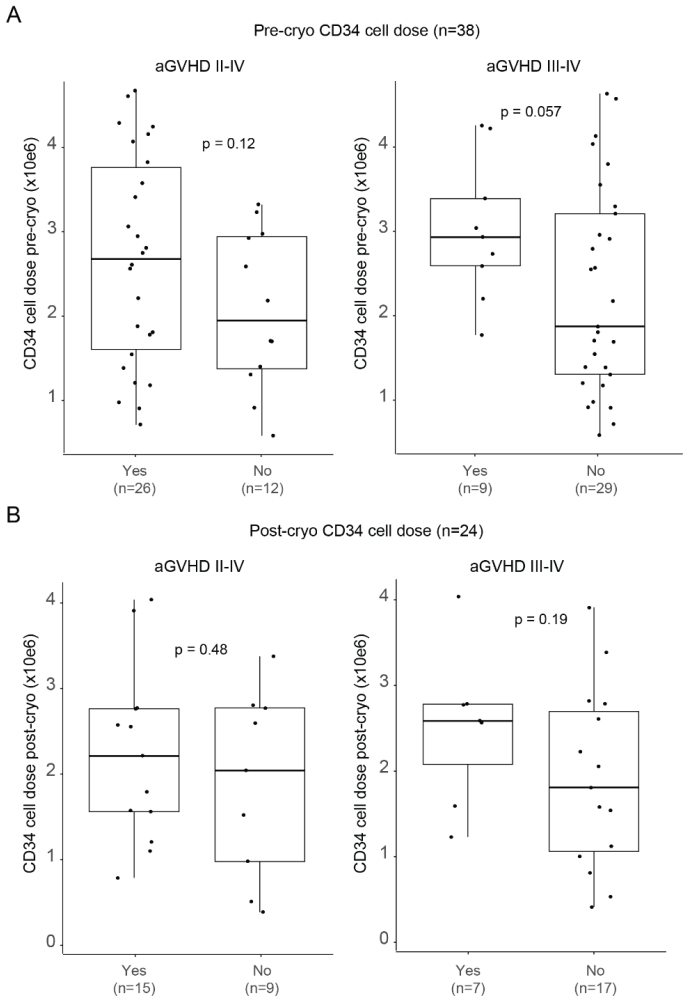

Figure 5 CD34+ dose infused to patients with or without acute GVHD. CD34+ cell dose as measured pre-cryopreservation (A) and post-cryopreservation (B).

There was also no significant difference between the dose of CD3+ cells, either pre- or post-cryopreservation, in patients who had or did not have acute GHVD or grade II-IV or III-IV after transplantation.

Chronic GvHD

The rate of chronic GvHD following ECT-001-CB in the mFAS was 7/38 (18.4%). Of these 7 patients, 4 had mild and 3 had moderate chronic GVHD. There was no statistically significant difference between the CD34+ cell dose and CD3+ cell dose received by patients who had or did not have chronic GvHD (*Figure 6*).

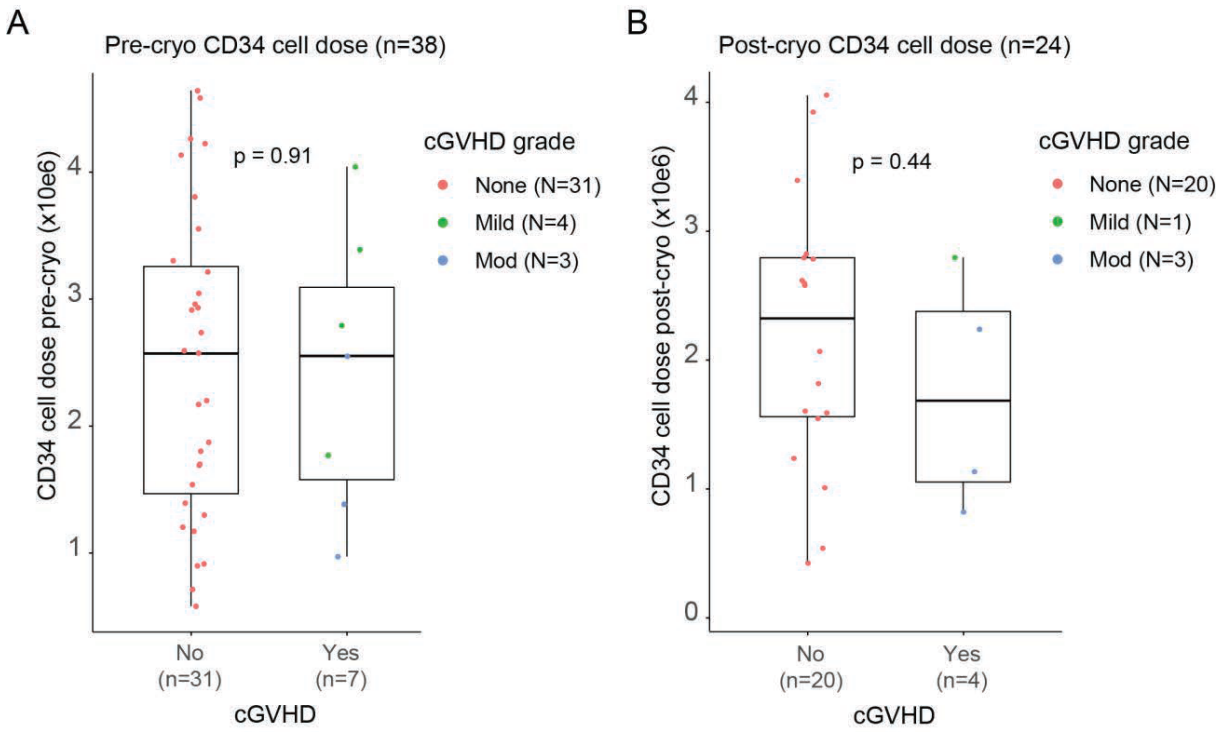

Figure 6 CD34+ cell dose infused to patients with or without chronic GVHD

Infectious events

In the mFAS, 28/38 (73.7%) patients had a total of 68 infectious events of grade ≥3. There was no statistically significant difference between the CD34+ cell dose received by patients who had at least one infectious event of grade ≥3 or not (unpaired bilateral Student T test, p = 0.8992, *Figure 7*).

Four patients had at least one infectious event with a maximal grade of 4 (life-threatening) and five with a maximal grade of 5 (leading to death). There was no statistically significant difference between the CD34+ cell dose received by patients who had at least one infectious event of grade ≥4 or not (unpaired bilateral Student T test, p = 0.5891).

There was no statistically significant difference between the CD3+ cell dose (as measured both pre- and post-cryopreservation) and infectious events of grade ≥3 or ≥4.

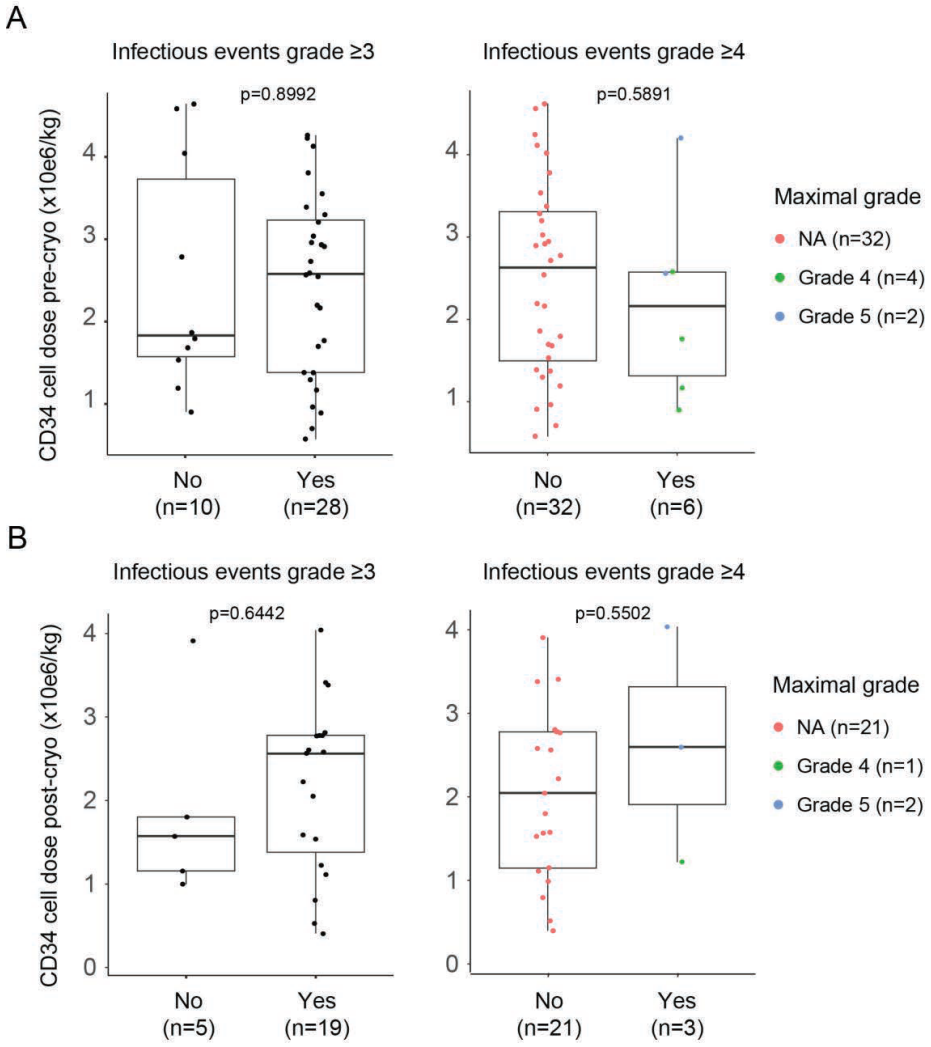

Figure 7 CD34+ dose infused to patients with or without at least one infectious event of grade ≥3 or ≥4. CD34+ cell dose as measured pre-cryopreservation (A) and post-cryopreservation (B).

2.6.3. Discussion on clinical pharmacology

Methods

No dedicated PK-PD studies were conducted which is acceptable considering the type of medicinal product. The assessment of PK and PD was based on combined data from three clinical studies (study 001, 002 and 004). The pooling of data is agreed as pooling leads to a larger evaluable dataset. The mFAS, limited to patients with ECT-001-CB derived from a small CB unit, is the most relevant population and was used for the main analysis of clinical pharmacology. Data from the smFAS, which included patients regardless of the size of the CB unit, were provided as supportive evidence.

The final product ECT-001-CB consists of dorocubicel and the ECT-001-CB-DP2 fraction. Characterization of the CD34+ cells in dorocubicel and CD3+ cells in ECT-001-CB-DP2 in the final product using quantitative flow cytometry assessment was performed as part of release testing for each lot. Pharmacokinetics was investigated by assessment of the correlation between CD34+ and CD3+ product attributes (quantity, purity and potency) and cellular kinetics (neutrophil recovery,

platelet recovery, donor chimerism and immune reconstitution). Pharmacodynamics was studied by assessment of dose-response for the efficacy endpoints time to neutrophil recovery, primary and secondary graft failure and EFS at 1 year after infusion. Safety endpoints included in the dose-response assessment were acute GVHD, chronic GVHD and the number and severity of infectious events. Dose-response was studied for both dorocubicel and ECT-001-CB-DP2.

In addition, a comparability study (SRT-ECT-011) to assess fresh versus cryopreserved use of dorocubicel and a T cell reconstitution study (SRT-ECT-040) were performed

Fresh versus cryopreserved (study SRT-ECT-011)

At the initiation of the clinical development dorocubicel was infused fresh, however the manufacturing process was changed in 2019 and cryopreservation was introduced to extend the shelf life of dorocubicel and to make ECT-001-CB available multinational. Besides the introduction of cryopreservation, the manufacturing change also included a change in formulation, storage conditions, storage duration, shipping and in-use process. All lots had a CD34+ cell dose and CD34+ viability within release criteria *before* cryopreservation. As expected, cryopreservation resulted in a decreased dose, viability, concentration and percentage recovery of CD34+ cells.

The clinical outcomes pre-change (fresh) and post-change (cryopreservation) were compared using lots from study 001, 002 and 004. Neutrophil recovery and platelet recovery were comparable pre-change and post-change. Toxicity in terms of grade III-IV acute GVHD, moderate to severe chronic GVHD and TRM was substantially higher in the cryopreserved group. However, the value of the comparability study is limited as no conclusion can be drawn based on the comparison of post-change (cryopreserved) data to historical (fresh) data due to differences in patient population, in particular differences in HLA-matching and CB size. To enable assessment of the efficacy and safety of cryopreserved dorocubicel, a data-update was provided for patients who received cryopreserved dorocubicel derived from a small sized cord. This is discussed in more detail in the clinical efficacy and clinical safety sections, and in brief the presented results suggested similar neutrophil and platelet engraftment kinetics and proportions with cryopreserved ECT-001-CB derived from a small cord compared to the old FAS.

In the comparability study there was a large variability in recovery of CD34+ cells after cryopreservation. In response to the LoQ, the dosing strategy was updated to base the dose administered to the patient on post-cryopreservation testing of cryovials. Post-cryopreservation testing of CD34+ is supported.

New post- instead of pre-cryopreservation release acceptance criteria for both CD34+ and CD3+ were proposed. For the CD34+ cell dose, the lower limit of the release acceptance criteria was based on the minimum dose where clinically acceptable neutrophil engraftment was achieved within acceptable time, resulting in a lower limit of 0.40×10^6 vCD34+ cells/kg. Further dosing data to confirm the CD34+ cell dose range will be provided as part of the proposed confirmatory trials ECT-001-CB.011 and ECT-001-CB.012.

The justification of the proposed target post-cryopreservation minimum CD3+ cell dose is considered acceptable. DP2 has been cryopreserved since the beginning of the product development and similar efficacy and safety was observed in patients treated with a relatively low CD3+ dose. Further post-cryopreservation CD3+ cell dose data will be provided by the confirmatory trials ECT-001-CB.011 and ECT-001-CB.012, which have been imposed as Specific Obligations.

Pharmacokinetics

Conventional pharmacokinetic assessment of absorption, distribution, metabolism and excretion (ADME) are not applicable for this type of cell-based medicinal product. An overview of

pharmacokinetics was provided. Cryopreservation resulted in a reduction of the quantity and potency of both dorocubicel and ECT-001-CB-DP2.

There was a weak negative correlation between the CD34+ cell dose and the time to neutrophil recovery, meaning that a higher CD34+ cell dose results in faster neutrophil recovery. This is expected, as faster neutrophil recovery in recipients of higher CD34+ cell dose is also reported for recipients of unmanipulated CB. There was a weak negative correlation between time to neutrophil engraftment and the dose of CD34 cells originally contained in the CBU used as starting material, however this was not significant. There was no correlation between the purity (percentage CD34+ cells) and potency (viability and expansion) of CD34+ cells and cellular kinetics. This could be caused by the small dynamic range for CD34+ cell viability, since all lots had >90% CD34+ cell viability at release which is presumably measured pre-cryotherapy. The range in viability is expected to be higher post-cryotherapy, as cryotherapy results in a decrease in viability. The correlation between post-cryopreservation CD34+ cell dose and viability with cellular kinetics was similar to the pre-cryopreservation CD34+ cell dose and viability.

High donor chimerism was reached rapidly. Full donor chimerism in the T cell subset was achieved slightly later compared to myeloid cells.

The ECT-001-CB-DP2 fraction contains the remaining CD34- cells from the CB quantified by the CD3+ cell dose. There were no correlations between the CD3+ cell dose, percentage of CD3+ cells and viability of CD3+ cells and cellular kinetics in the mFAS population.

There was no effect of age, weight, gender, race, ethnicity, HLA matching level and conditioning regimen intensity on time to neutrophil recovery. Younger patients (<40 years old) showed higher IgG levels at 3 months post-transplant, while there was no difference at 12 months post-transplant. This might be caused by a more rapid B cell reconstitution in younger patients. No data were provided for the paediatric population, which is acceptable as no paediatric indication is claimed.

T-cell reconstitution (Study SRT-ECT-040)

Study SRT-ECT-040 investigated T cell reconstitution in patients included in study 001 (ECT-001-CB.001, n=20) compared to a retrospective cohort of patients receiving unmanipulated CB (n=12). Patients receiving ECT-CB-001 had a 17-fold greater CD34+ cell dose and a 2-fold lower CD3+ T cell dose. No significant differences in T cell reconstitution were observed over time. Although this was an unmatched comparison and the number of studied patients was small, the study provided some reassurance that immune reconstitution is similar to patients treated with unmanipulated CB and that the lower CD3+ cell dose in ECT-CB-001 compared to unmanipulated CB does not affect immune reconstitution. A limitation of study SRT-ECT-040 is that only patients treated with fresh ECT-CB-001 were investigated. As cryopreservation results in cell loss, it is unclear whether this has an effect on T cell reconstruction.

PK/PD

There was a weak correlation between the quantity of CD34+ cells and neutrophil recovery, with a higher dose leading to faster neutrophil recovery. This is consistent with the literature, where a high CD34+ cell dose has been associated with faster neutrophil recovery and supports the rationale for the use of expansion technology. There was no correlation between the dose of CD3+ cells post-cryopreservation and the time to neutrophil and platelet recovery. The requested target dose is 5×10^5 to 7.5×10^6 CD34+ cells/kg and the neutrophil recovery was fast also among the lower dose range. Since only one patient experienced graft failure, these data are to limited to draw any conclusions. There was no statistical difference in the dose of CD34+ and CD3+ cells infused and EFS at 1 year, however the number of EFS events was low.

No association was reported between the CD34+ and CD3+ cell dose and grade II-IV acute GVHD, while the number of patients with grade III-IV acute GVHD (n=9) was too low for assessment of association with cell dose. The same holds for the incidence of chronic GVHD (n=7).

A higher CD34+ cell dose was weakly correlated with a faster neutrophil recovery, however this did not translate into an association between CD34+ cell dose and infection. There was also no relation between CD3+ cell dose and infection. Of note, in all protocols of ECT-001-CB, only AEs of grade ≥3 were reported, therefore the number of patients with low grade infectious events is unknown.

2.6.4. Conclusions on clinical pharmacology

The correlation between product attributes and cellular kinetics was sufficiently described. The dose-response for dorocubicel and ECT-001-DP2 is generally acceptable.

The current application for Zemcelpro is acceptable from a clinical pharmacology point of view.

The CHMP endorse the CAT assessment regarding the conclusions on the clinical pharmacology as described above.

2.6.5. Clinical efficacy

Main efficacy data is derived from a pooled analysis of efficacy (ISSE) of three single arm open-label studies (Phase I/II Study 001 and Phase II studies 002 and 004) of ECT-001-CB following a myeloablative conditioning regimen for haematological malignancies. Two of these studies reflect the target indication and investigated the intended commercial cryopreserved product, i.e., Studies 002, and 004. Patients from study 001 have been included in supportive pooled analyses.

Context for interpretation of the outcomes derived from the ECT-001-CB clinical trials is provided through three formal matched controlled comparisons of patients treated with ECT-001-CB in Study 001 to matched patients identified from the European Society for Blood and Marrow Transplantation (EBMT) and the Centre for International Blood and Marrow Transplant Research (CIBMTR) registries in separate matched-controlled studies.

Supportive data is derived from studies 003 and 007 and a few patients treated in a compassionate use program.

2.6.5.1. Dose response study(ies)

Dose de-escalation Phase I/II Study 001 was designed to first document engraftment of expanded CB cells (Part 1), and then to determine the minimal size of CB units prior to expansion that would result in prompt engraftment after expansion (Part 2).

In part 1 of the study, patients received double cord transplants comprised of one ECT-001-CB and one unmanipulated CB unit. Once expanded cell engraftment was demonstrated in at least 2 patients, single ECT-001-CB transplant was permitted in part 2 of the study.

In Part 2, three dose cohorts were planned*Table 6*. Dose reduction was allowed once 3 patients had shown prompt neutrophil engraftment within 18 days or less, from small (i.e. containing < 2.0×10^5 CD34+ cells/kg at thaw, before expansion) single expanded CB units. The third cohort was never

opened as CB units with good HLA matching (≥6/8 HLA match) could always be found within the pool of CB units available with cell criteria from cohort 2.

For safety reasons, a non-manipulated cord was infused if the expanded cord did not meet all of the following criteria:

1. Viability of total cells upon initial thaw was ≥ 70% of cord to be expanded (viability measured on total cells with 7-aminoactinomycine D [7-AAD]).
2. ≥ 0.5 x 10^6/kg viable CD34+ cells (measured at end of expansion culture, in dorocubicel),
3. ≥ 1 x 10^6/kg viable CD3+ cells (measured prior to cryopreservation of CD34- component, in ECT-001-CB-DP2)

Table 6: Patient cohorts Study 001 Part 2

Cohort	Pre-thaw CB cell count prior to manipulation	Number of patients
1	TNC dose ≥ $2.0x10^7$/kg and CD34+ ≥ $1.0x10^5$/kg at cryopreservation	Minimum 3
2	TNC dose ≥ $1.5x10^7$/kg and CD34+ ≥ $0.5x10^5$/kg at cryopreservation	Minimum 3
3	TNC dose $1.25x10^7$/kg and CD34+ ≥ $0.25x10^5$ at cryopreservation	-
Patient weight at time of cord selection was used. If weight increased by > 5% at time of admission and cell dose no longer met minimum criteria, PI approval was required to proceed. Cell dose data provided by the cord bank were used for cord selection.		

Following expansion of CD34+ cells, minimal cell content required at formulation (prior to cryopreservation) for single ECT-001-CB transplantation were:

- CD34+ dose contained in dorocubicel: ≥ 0.5 x 10^6/kg
- CD3+ dose contained in ECT-001-CB-DP2: ≥ 1.0 x 10^6/kg

Efficacy

In total, 27 CB units were used to manufacture ECT-001-CB in Study 001, and 26 (96.3%) were successfully expanded and infused. Infusion was not performed for 1 (3.7%) patient due to expansion failure. One patient (1/27, 3.7%) required a back-up cord infusion.

In Part 1 of the study, 4 patients received a double cord (1 expanded, 1 non-expanded) transplantation. One case of primary graft failure was reported. This patient was the first to receive an expanded CB, had several risk factors for graft failure and received a 12-day rather than a 7-day expanded CB. Of the three patients who engrafted, 1 achieved full donor chimerism with the expanded unit, 1 with the non-manipulated unit, and 1 with both CBs.

In Part 2 of the study, 22 patients received a single cord transplantation. All 22 patients achieved full donor chimerism with the expanded CB unit. Among these patients, the probability of progression free survival (PFS) at 1 and 3 years post-transplant was 86% and 58%, respectively with a 1 and 3 year overall survival (OS) probability of 96% and 63%, respectively.

The smallest CB unit to achieve prompt neutrophil engraftment (defined as 18 days or less) was a CD34+ cell dose of $0.66x10^5$/kg at cryopreservation (prior to thawing for expansion).

Safety

The most commonly reported adverse events were anaemia, leukopenia, neutropenia, and lymphopenia. No cases of moderate to severe chronic GvHD were reported. One patient died (no relapse) due to alveolar haemorrhage of the lungsTransplanted-related mortality (TRM) was 0-5%.

A maximum CD34+ cell dose of 4.0 x 10^6/kg was implemented after observation of high incidence of severe engraftment syndrome with higher doses of CD34+ cells infused in the first part of Study 001, along with a change of GvHD prophylaxis treatment from Cyclosporine A to Tacrolimus. The maximal dose of CD34+ cells was subsequently increased to 5.0 x 10^6 CD34+ cells/kg when cells were given fresh or 5.75 x 10^6 CD34+ cells/kg when cryopreservation of dorocubicel was introduced in 2019 to account for cell loss occurring during cryopreservation. As there seemed to be an association between the occurrence of alloimmune reaction (either acute GvHD or ES/pre-engraftment syndrome [PES]) and a lower risk of relapse within 2 years post-transplant in Study 001 the maximum dose was subsequently increased to 7.5 x 10^6 CD34+ cells/kg. This in order to infuse 6.4 x 10^6/kg viable CD34+ cells/kg post-thaw (i.e. accounting for the ~15% cell loss during the last freeze-thaw cycle).

All patients included in all clinical trials with ECT-001-CB (Studies 001, 002, 003, 004 and 007) received doses of:

- CD34+ dose contained in dorocubicel: between 0.5 and 7.5 x 10^6 CD34+ cells/kg
- CD3+ dose contained in ECT-001-CB-DP2: ≥ 1.0 x 10^6 CD3+ cells/kg

Approved posology

In the clinical trials with ECT-001-CB the dose was based on pre-cryopreservation release acceptance criteria for both CD34+ and CD3+ while post-approval this will be based on post-cryopreservation release acceptance criteria. For the CD34+ cell dose, the lower limit of the release acceptance criteria was based on the minimum dose where clinically acceptable neutrophil engraftment was achieved within acceptable time, resulting in a lower limit of 0.40 x 10^6 vCD34+ cells/kg. Further dosing data to confirm the CD34+ cell dose range will be provided as part of the proposed confirmatory trials ECT-001-CB.011 and ECT-001-CB.012. Please refer to section *2.6.2 on Clinical Pharmacology*.

2.6.5.2. Main studies

Pooled analysis (ISSE)

Main efficacy data is derived from a pooled analysis of clinical outcomes of ECT-001-CB following a myeloablative regimen in a subgroup of patients from two studies which reflect the target indication and investigated the intended commercial cryopreserved product, i.e. Studies 002, and 004. Patients from study 001 have been included in supportive pooled analyses.

Study-001 is a prospective, single-arm, multicentre, open-label phase I-II dose de-escalation study of ECT-001-CB in patients who need an allogeneic hematopoietic stem cell transplant but lack a suitable donor. The study was conducted at 2 study sites in Canada and is completed.

Study 002 is an ongoing single arm, single centre, open-label phase II study of ECT-001-expanded cord blood transplantation in patients with high-risk acute leukaemia/myelodysplasia conducted at 1 study site in Canada. Recruitment is completed.

Study 004 is an ongoing single arm, multicentre, open-label Phase II study in patients with high or very high risk acute leukaemia/myelodysplasia conducted in the USA and 1 site in Europe (the Netherlands). Recruitment is completed.

Methods

- **Study Participants**

Pooled analysis (ISSE)

For the new pivotal FAS population, results for a subgroup of patients from Study 002 and 004 were pooled. For the supportive 'old' FAS, all patients from studies 001, 002 and 004 were included, except for the first four patients in Study 001 Part 1 who received double CB transplantation with one ECT-001-CB unit and one unmanipulated CB unit. Eligibility criteria per study are described below.

Study 001

Key inclusion criteria

Eligible patients were to be aged 3–64 years, who weighed 12 kg or more, had a haematological malignancy (excluding primary myelofibrosis) with an indication for allogeneic hematopoietic stem cell transplant, did not have a suitable 8/8 HLA-matched donor, had adequate organ function, had a Karnofsky performance status (KPS) score of 70% or more and had signed the informed consent. Additionally, all patients had to have at least two CB units available (with a minimal HLA match of 4/6 when DRB1 is performed at the allele level and A, B at antigen resolution (intermediate resolution) and ≥ 4/8 HLA match when A, B, C and DRB1 are performed at the allele level), one unit with sufficient cell dose for expansion and one for unmanipulated CB transplant (or two back-up units if one was not available).

Key exclusion criteria

Primary myelofibrosis never treated with chemotherapy, any haematologic disease never treated with chemotherapy and planned conditioning regimen did not include 12Gy total body irradiation, creatinine clearance less than 60 mL/min per 1.73 m^2, a left ventricular ejection fraction of less than 40%, forced vital capacity, forced expiratory volume in 1 sec, and diffusion lung capacity (corrected for haemoglobin) less than 50% predicted, aspartate aminotransferase and alanine aminotransferase concentrations more than 2.5 times the upper limit of normal, bilirubin more than 2 times the upper limit of normal, an autologous HSCT in the previous 6 months or myeloablative allogeneic HSCT in the previous 12 months before enrolment, planned use of antithymocyte globulin in conditioning regimen, availability of a CB with a CD34+ cell count at cryopreservation of $\geq 5x10^5$/kg or a 6/6 matched related donor and participation in a trial with an investigational agent within 30 days prior to study entry. Pregnancy, breastfeeding, or unwillingness to use appropriate contraception are also excluded.

Study 002

Key inclusion criteria

Presence of high-risk acute leukaemia/myelodysplasia defined as one of the following:

- Acute Myeloid Leukaemia: a) Primary induction failure b) Chemorefractory relapse c) Relapse after allogeneic or autologous transplant d) High risk AML in CR1: i) any adverse genetic abnormality as defined by European Leukemia Net (ELN) excluding FLT3 mutation; ii) secondary or therapy related AML excluding good risk genetic abnormalities (as defined by ELN); or iii) any other poor risk feature known to be associated with a PFS or DFS ≤40% at 2 years after conventional transplantation. e) CR2 excluding good risk genetic abnormalities defined by ELN. f) ≥CR3

- Acute Lymphoid Leukaemia: a) Primary induction failure b) Chemorefractory relapse c) Relapse after allogeneic or autologous transplant d) High risk ALL in CR1: Philadelphia-like ALL or any other poor risk feature known to be associated with an PFS or DFS ≤40% at 2 years after conventional transplantation. e) ≥CR2 f) MRD+ within 1 month of start of conditioning regimen.

- Myelodysplastic syndrome: a) Relapse after allogeneic or autologous transplant b) ≥10 % blasts within 1 month of start of conditioning regimen c) Very poor cytogenetics (>3 abnormalities) d) Any poor risk feature known to be associated with a PFS or DFS ≤40% at 2 years after conventional transplantation e) TP53 mutation f) ≥40 years old and RAS or JAK2 mutation. g) CMML with HCT-specific CPSS score high or intermediate h) Stable disease after 6 cycles of azacitidine (or another demethylating agent). i) Progressive disease while on azacitidine (or another demethylating agent).

Patients had to be aged 18-70 years old, a CB unit for expansion and back-up unit for unmanipulated CB transplant in case of manufacturing failure (both with a minimal HLA match of 4/6 when DRB1 is performed at the allele level and A, B at antigen resolution (intermediate resolution) and ≥ 4/8 HLA match when A, B, C and DRB1 are performed at the allele level), the cord to be expanded had to have a CD34+ cell count >0.5 x 10^5/kg and TNC>1.5 x 10^7/kg (all pre-freeze), patients had to have adequate organ function, adequate comorbidity (haematopoietic cell transplantation specific comorbidity index, HCT-CI) score, a KPS score of 70% or more and had signed the informed consent.

Key exclusion criteria

Patients never treated with cytotoxic chemotherapy and planned conditioning regimen does not include 12 Gy TBI, an allogeneic myeloablative or autologous HSCT within 6 months, planned use of antithymocyte globulin in condition regimen, planned use of an HLA matched CB (8/8 allele matched), HLA antibodies with significant titers directed towards expanded CB, uncontrolled infection, malignancy other than the one for which CB transplant is being performed, with expected survival estimated to be less than 75% at 5 years, seropositivity for HIV, hep B or C infection with measurable viral load, liver cirrhosis, active central nervous system involvement, chloroma >2 cm, ≥50% blasts in marrow in an evaluable marrow sample (>25% of normal cellularity for age) collected less than one month prior to start of conditioning regimen, peripheral blasts >1000/mm^3, participation in a trial with an investigational agent within 30 days prior to study entry, Pregnancy, breastfeeding, or unwillingness to use appropriate contraception are also excluded.

Study 004

Key in- and exclusion criteria

Study 002 and study 004 are sister trials conducted in Canada and in the USA, respectively, and target the same population of adult patients with high and very high risk acute leukaemia or myelodysplasia.

- **Treatments**

ECT-001-CB transplant

Patients included in studies 001 Part 2, 002 and 004 were to receive a CB transplantation with expanded CD34+ cells and an unexpanded CD34- fraction (ECT-001 CB transplant). The CD34- fraction was infused at least 4 hours after the CD34+ expanded fraction.

Conditioning regimen prior to transplantation

Patients included in Studies 001, 002 and 004 received a high or intermediate intensity conditioning regimen prior to infusion of ECT-001-CB.

CB selection

The starting material for manufacture of ECT-001-CB was performed identically for all studies. The CB unit to be expanded had to meet the following criteria:

- HLA match ≥ 4/6,

- Pre-cryopreservation (pre-expansion) CD34+ cell dose ≥ 0.5 x 10^5/kg,
- Pre-cryopreservation (pre-expansion) TNC dose ≥ 1.5 x 10^7/kg,
- Needs to be erythrodepleted by bank prior to cryopreservation, and
- Must comply with local site regulations and:
    - in the USA, come from a cord bank that is FACT (Foundation for the Accreditation of Cellular Therapy) or AABB (American Association of Blood Banks) accredited, FDA approved or eligible for NMDP Investigational New Drug (IND),
    - in Europe come from a cord bank that is FACT, or AABB accredited, or complying with quality standards of the JACIE (Joint Accreditation Committee ISCT-Europe & EBMT) or European National Authority.

- **Objectives**

The primary objective for the ISSE pooled analysis was to establish adequate hematopoietic reconstitution of patients with haematological diseases who are medically indicated for allogeneic HSCT but lack a readily available suitable donor.

Secondary objectives of the pooled analysis were to characterise the successful outcome of the transplant, and to assess outcomes on patient-relevant, time-dependent outcomes and safety.

- **Outcomes/endpoints**

Primary endpoint*

Time to neutrophil engraftment, defined as the time from transplant and first day of three consecutive days with absolute neutrophil count (ANC) ≥ 0.5 x 10^9/L.

Secondary endpoints*

- *Time to platelet engraftment* - defined as the time between transplant and first day of a sustained platelet count ≥ 20 x 10^9/L with no platelet transfusion in the preceding 7 days.

- *Proportion reaching neutrophil engraftment by Day 42* - The proportion of patients achieving neutrophil engraftment by 42-day post-transplant.

- *Proportion reaching platelet engraftment by Day 100* - The proportion of patients achieving platelet engraftment by 100-day post-transplant.

- *Non-relapse mortality (NRM)* - defined as death due to any cause other than malignant relapse occurring after the commencement of the conditioning regimen that could be related to the transplantation procedure. If the participant received another stem cell source as treatment for graft failure, NRM was considered as death at any time unrelated to relapse until 4 months post 2nd transplant. If death occurs ≥ 4 months after the 2nd transplant from a transplant related complication, it was not considered NRM related to the trial.

- *Overall survival (OS)* – defined as the time of transplant until death from any cause or last follow-up.

- *Progression-free survival (PFS)* – defined as the time of transplant until relapse/progression, death or last follow-up.

- *Graft versus host disease (GvHD)-free and relapse-free survival (GRFS)* - defined as the absence of grade 3-4 acute GvHD, moderate or severe chronic GvHD, relapse/progression, death or last follow-up.

- *Chronic GvHD-free and relapse-free survival (CRFS)* – defined as the absence of moderate to severe chronic GvHD, relapse or /progression, death or last follow-up.

- *Acute GvHD* - the incidence of acute GvHD using NIH criteria (Harris et al. 2016[52]).

- *Chronic GvHD - t*he incidence of chronic GvHD using NIH criteria (Jagasia et al. 2015[53]).

* Of note: all timings were defined as time from infusion.

- **Sample size**

Data cut-off for the pooled analysis was planned on November 30th, 2022, at which time it was expected that over 100 patients would have been transplanted using ECT-001-CB from studies 001, 002, 003, 004 and 007. This cut-off date has been selected to ensure an adequate safety database is available for assessment.

- **Randomisation and Blinding (masking)**

Not applicable.

- **Statistical methods**

Analysis sets pooled analysis

All pooled analysis sets have been defined post-hoc. In response to the CAT/CHMP list of questions, the pivotal analysis set and first supportive analysis set for the efficacy analysis have been narrowed to only the patients who received a cryopreserved product. These patients were derived from Study 002 and 004.

Pivotal Full Analysis Set (Pivotal FAS): All patients from Studies 002 and 004 who were intended to be transplanted (intent-to-treat, **ITT**) with a **cryopreserved** ECT-001-CB derived from the expansion of a **small** CB unit (defined as containing <1.5 X 10^5 CD34+ cells/kg or <2.5 X 10^7 TNC/kg pre-cryopreservation, before expansion) (n=25).

Supportive analysis sets

Cryo supportive FAS (Cryo sFAS): All patients from Studies 002 and 004 who were intended to be transplanted with single **cryopreserved** ECT-001-CB (**ITT**) **regardless of the size** of the cord blood (CB) unit used as starting material (n=62).

(Old) Full analysis Set (FAS): Primary analysis set including a subset of adult patients from Studies 001, 002 and 004 who were intended to be transplanted (intent-to-treat, **ITT**) with ECT-001-CB derived from the expansion of a **small** CB unit. This analysis set includes patients who were enrolled in a trial but did not receive ECT-001-CB (e.g. due to expansion failure). ECT-001-CB in this analysis set and the other supportive analysis sets below could be cryopreserved or fresh.

[52] Harris AC, Young R, Devine S, et al. (2016) International, multicentre standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai Acute GVHD International Consortium. Biology of Blood and Marrow Transplantation;22(1):4-10.
[53] Jagasia MH, Greinix HT, Arora M, et al. (2015) National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant,21(3):389- 401.

Modified FAS (mFAS): A subset of the FAS consisting of adult patients pooled from Studies 001, 002 and 004, actually treated (**per protocol**) with single ECT-001-CB derived from a **small** CB unit (n=38).

Supportive FAS (sFAS): All patients from Studies 001, 002 and 004 who were intended to be transplanted with single ECT-001-CB (**ITT**) **regardless of the size** of the cord blood (CB) unit used as starting material (n=87).

Supportive modified FAS (smFAS): Subset of the sFAS consisting of all adult patients, pooled from Studies 001, 002 and 004, actually treated (**per protocol**) with single ECT-001-CB **regardless of the size** of the cord blood (CB) unit used as starting material (n=82).

Analysis methods of efficacy endpoints

Neutrophil and platelet engraftment

To assess the incidence of neutrophil and platelet engraftment from day of transplant, cumulative incidence curves were computed along with a 95% confidence interval. The median time to neutrophil and platelet engraftment were examined. Proportion of patients having achieved neutrophil or platelet engraftment by day 42 and 100 were also computed.

Non-Relapse Mortality

The NRM was estimated using a cumulative incidence curve, with relapse considered as a competing risk. Failure to receive transplant was also considered a competing risk in the analysis of the FAS. The cumulative incidence curve was computed throughout the study, and in particular at 100 days, 1- and 2-years post-transplant along with a 95% confidence interval.

OS, PFS, GRFS and CRFS

OS, PFS, GRFS and CRFS distributions were estimated by the Kaplan-Meier curve. All participants were to be followed for a maximum of 2 years post-transplant for progressive disease and mortality. Survival estimates for each endpoint were reported at 1- and 2-years post-transplant, along with 95% confidence intervals.

Acute and chronic GVHD

To assess the incidence of acute and chronic GVHD from the day of transplant, a cumulative incidence curve was computed throughout the study, along with a 95% confidence interval with death and relapse considered as competing risks. Failure to receive transplant was also considered a competing risk in the analysis of the FAS.

Multiplicity control

There was no multiplicity control in this single arm trial. However, there were interim stopping guidelines in place for safety (failure to expand, graft failure, TRM, Acute GVHD grade 3-4) based on an intermediate form of the Pocock and O'Brien-Flemming bounds (Ivanova et al, Biometrics 2005[54]).

Handling missing data

Where partial (day and/or month) start dates are missing for medication administration, partially missing current medications start dates will be imputed as occurred at the first day and/or month of a given year.

[54] Ivanova, A.; Qaqish, B. F.; Schell, M. J., Continuous toxicity monitoring in phase II trials in oncology. Biometrics 2005, 61 (2), 540-5. 10.1111/j.1541-0420.2005.00311.x

Results

- **Participant flow**

In the pivotal subset of patients enrolled for infusion of a single ECT-001-CB cryopreserved graft derived from a small CB unit (pivotal FAS; n=25), 24 (96.0%) patients were infused. One patient (4.0%) discontinued before receiving ECT-001-CB due to shipping failure.

Of the 25 patients enrolled, 7 patients (28.0%) were ongoing at time of data cut-off, 6 (24.0%) had completed their study follow-up period, and 12 (48.0%) had been prematurely discontinued (6 relapse, 5 NRM and the shipping failure).

The patient flow in the pivotal FAS and supportive populations of the pooled analysis (i.e. patients enrolled for transplantation with *cryopreserved* ECT-001-CB derived from any size cords – Cryo sFAS; patients enrolled for transplantation with ECT-001-CB derived from a small CB unit – (old) FAS; patients who actually received ECT-001-CB derived from a small CB unit – mFAS; patients enrolled for transplantation with ECT-00-CB derived from any size cords – sFAS; and patients actually transplanted with ECT-00-CB derived from any size cords – smFAS) is shown in Figure 8.

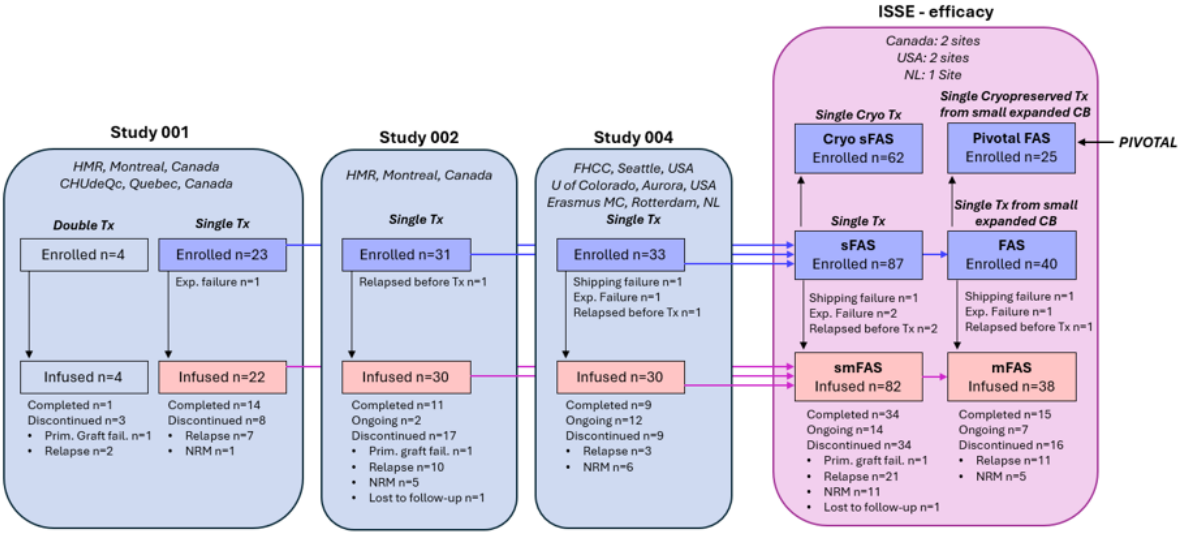

Figure 8 Patient disposition in pivotal and supportive datasets for efficacy evaluation

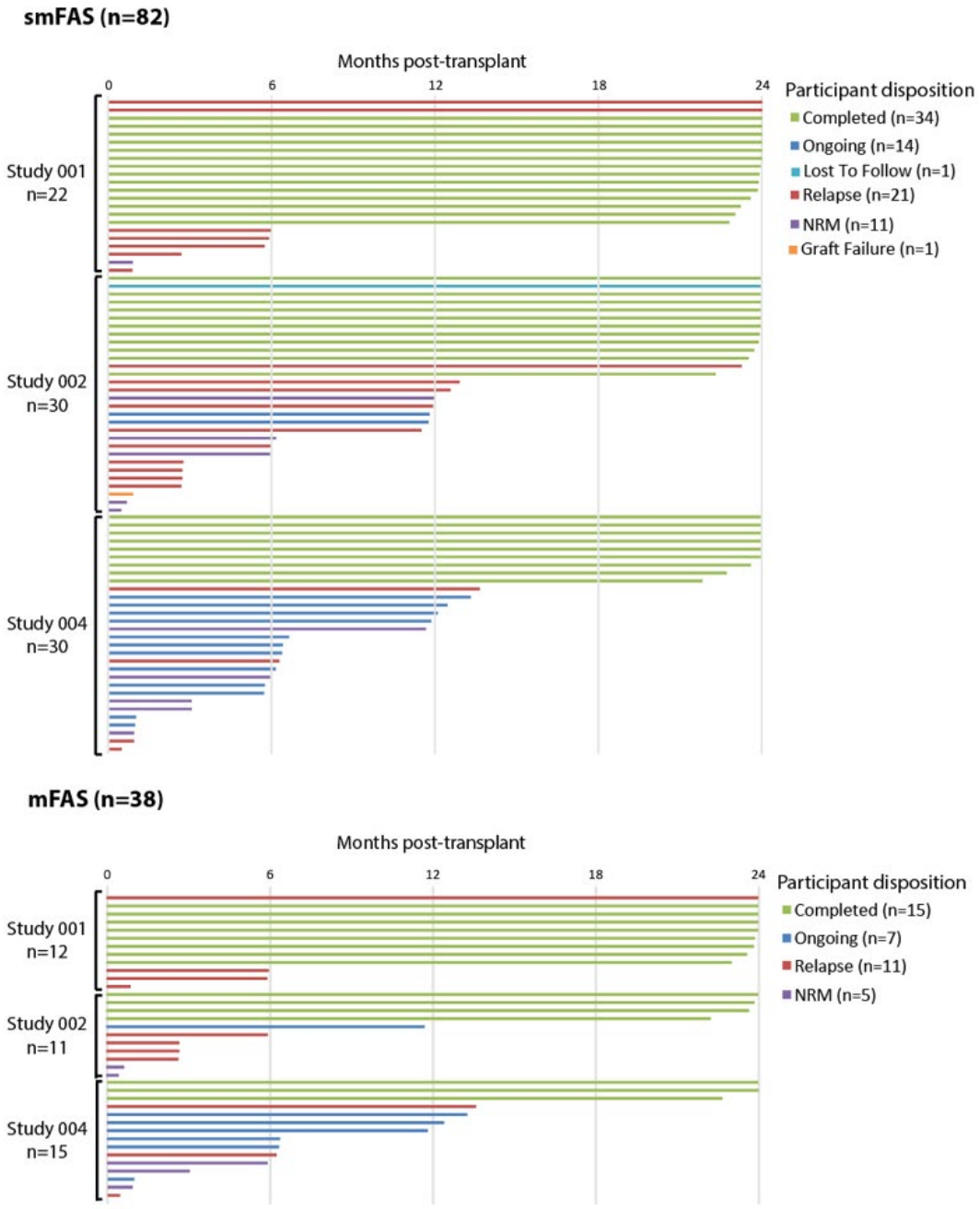

Figure 9 Participant disposition in supportive analysis sets smFAS and mFAS.

The median follow-up of patients ongoing or having completed the study was 13.27 months in the pivotal population and 23.87-24.02 months in the supportive population subsets (Table).

Table 7. Participants disposition in ISSE

	FAS (ITT, N=40)	mFAS (PP, N=38)	sFAS (ITT, N=87)	smFAS (PP, N=82)	Pivotal FAS (ITT, N=25)	Cryo sFAS (ITT, N=62)
Enrolled	40 (100%)	38 (100%)	87 (100%)	82 (100%)	25 (100%)	62 (100%)
Transplanted	38 (95.0%)	38 (100%)	82 (94.3%)	82 (100%)	24 (96.0%)	58 (93.5%)
Participants who reached 6 months						
Yes	28 (70%)	28 (73.7%)	65 (74.7%)	65 (79.3%)	16 (64.0%)	45 (72.6%)
No	12 (30%)	10 (26.3%)	22 (25.3%)	17 (20.7%)	9 (36.0%)	17 (27.4%)
Participants who reached 12 months						
Yes	21 (52.5%)	21 (55.3%)	51 (58.6%)	51 (62.2%)	11 (44.0%)	34 (54.8%)
No	19 (47.5%)	17 (44.7%)	36 (41.4%)	31 (37.8%)	14 (56.0%)	28 (45.2%)
Participants who reached 24 months						
Yes	16 (40%)	16 (42.1%)	38 (43.7%)	38 (46.3%)	6 (24.0%)	21 (33.9%)
No	24 (60%)	22 (57.9%)	49 (56.3%)	44 (53.7%)	19 (76.0%)	41 (66.1%)
Participant Status						
Completed the study	15 (37.5%)	15 (39.5%)	34 (39.1%)	34 (41.5%)	6 (24.0%)	19 (30.6%)
Prematurely discontinued	18 (45%)	16 (42.1%)	39 (44.8%)	34 (41.5%)	12 (48.0%)	29 (46.8%)
Ongoing	7 (17.5%)	7 (18.4%)	14 (16.1%)	14 (17.1%)	7 (28.0%)	14 (22.6%)
Discontinued before transplant	18 (45%)	16 (42.1%)	39 (44.8%)	34 (41.5%)	12 (48.0%)	29 (46.8%)
Reasons for discontinuation before transplant						
Product Arrived Compromised	1 (2.5%)	0 (0.0%)	1 (1.1%)	0 (0.0%)	1 (4.0%)	1 (1.6%)
Expansion Failure	1 (2.5%)	0 (0.0%)	2 (2.3%)	0 (0.0%)	0 (0.0%)	1 (1.6%)
Patient relapsed prior to transplant	0 (0.0%)	0 (0.0%)	2 (2.3%)	0 (0.0%)	0 (0.0%)	2 (3.2%)
Reasons for early discontinuation after transplant						
Relapse/progression	11 (61.1%)	11 (68.8%)	21 (53.8%)	21 (61.8%)	6 (50.0%)	13 (44.8%)
NRM	5 (27.8%)	5 (31.2%)	11 (28.2%)	11 (32.4%)	5 (41.7%)	10 (34.5%)
Primary graft failure	0 (0.0%)	0 (0.0%)	1 (2.6%)	1 (2.9%)	0 (0.0%)	1 (3.4%)
Lost to follow up	0 (0.0%)	0 (0.0%)	1 (2.6%)	1 (2.9%)	0 (0.0%)	1 (3.4%)
Median duration of follow up (mo) (IQR)	23.87 (0.92-38.18)	23.87 (0.92-38.18)	23.99 (0.92-38.6)	23.99 (0.92-38.6)	13.27 (0.92-38.18)	24.02 (0.92-38.6)
Number of patients per study, n(%)						
Study 001	13 (32.5%)	12 (31.6%)	23 (26.4%)	22 (26.8%)	0 (0.0%)	0 (0.0%)
Study 002	11 (27.5%)	11 (28.9%)	31 (35.6%)	30 (36.6%)	9 (36.0%)	29 (46.8%)
Study 003	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Study 004	16 (40.0%)	15 (39.5%)	33 (37.9%)	30 (36.6%)	16 (64.0%)	33 (53.2%)
Study 007	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

ITT: Intent-to-treat, PP: Per protocol
ECT-001-CB transplant derived from a small CB: FAS: Full analysis set (pivotal, ITT)
mFAS: Full analysis set (supportive, PP)
ECT-001-CB transplant: sFAS: Supportive analysis set (supportive, ITT)
smFAS: Full analysis set (supportive, PP)

- **Recruitment**

Data for the pooled analysis were extracted from the individual studies with a data cut-off of March 15th, 2024.

Study 001 – The first patient was recruited in Jan 2016, and the last study visit was completed in May 2021. A total of 33 months were necessary to enroll the targeted sample size. All patients were followed for 3 years post-transplant.

Study 002 - The first patient was recruited in May 2019, and the last study visit is targeted in Oct 2025. The study is still ongoing (all patients transplanted, currently in follow-up phase). At the time of the data cut-off, 31 patients were enrolled and 30 infused. All patients will be followed for 3 years post-transplant.

Study 004 - The first patient was recruited in Jan 2020, and the last study visit is targeted in Sep 2025. The study is still ongoing (all patients transplanted, currently in follow-up phase). At the time of the data cut-off, 33 patients were enrolled and 30 infused. All patients will be followed for 2 years post-transplant.

- **Conduct of the study**

Several major changes to the planned conduct of study have been made in the clinical trials that contributed to the pooled analysis data set. Most important changes pertained to the drug product and cell dose. From Study 002 onwards CD34+ cells were administered after cryopreservation and thawing, instead of fresh. The dose was first capped at 4.0 x 10^6/kg due to the occurrence of severe engraftment syndrome and subsequently increased after a change in GvHD prophylaxis and accounting for cell loss due to cryopreservation. In addition, viability criteria for CD34- cells changed during Study 001.

- **Baseline data**

Pooled analysis

The median age was 47 (24-64) years and the majority of patients were male (72%) in the FAS population (Table). Most participants suffered from AML (44%), ALL (44%) or MDS (12%) as their underlying disease and 28% had received a prior allogeneic transplant.

Table 8. Participant demographics and disease history in ISSE

	FAS (ITT, N=40)	mFAS (PP, N=38)	sFAS (ITT, N=87)	smFAS (PP, N=82)	Pivotal FAS (ITT, N=25)	Cryo sFAS (ITT, N=62)
Age (years)						
Mean (SD)	44.7 (12.36)	44.3 (12.55)	43.1 (13.73)	43.1 (13.94)	46 (11.92)	43.2 (13.76)
Median (min-max)	46 (20-64)	45.5 (20-64)	44.5 (19-66)	44.5 (19-66)	47 (24-64)	43 (19-66)
Sex n(%)						
Male	28 (70%)	27 (71.1%)	54 (62.1%)	51 (62.2%)	18 (72.0%)	38 (61.3%)
Female	12 (30%)	11 (28.9%)	33 (37.9%)	31 (37.8%)	7 (28.0%)	24 (38.7%)
Race n(%)						
American Indian or Alaska Native	1 (2.5%)	1 (2.6%)	3 (3.4%)	3 (3.7%)	1 (4.0%)	3 (4.8%)
Asian	1 (2.5%)	1 (2.6%)	2 (2.3%)	2 (2.4%)	1 (4.0%)	2 (3.2%)
Black	3 (7.5%)	3 (7.9%)	5 (5.7%)	5 (6.1%)	1 (4.0%)	3 (4.8%)
Native Hawaiian or Pacific Islander	2 (5%)	2 (5.3%)	4 (4.6%)	2 (5%)	2 (5.3%)	4 (4.6%)
White	30 (75%)	28 (73.7%)	69 (79.3%)	65 (79.3%)	17 (68.0%)	46 (74.2%)
Other	3 (7.5%)	3 (7.9%)	4 (4.6%)	4 (4.9%)	3 (12.0%)	4 (6.5%)
Ethnicity n(%)						
Hispanic or Latino	4 (10%)	3 (7.9%)	10 (11.5%)	9 (11%)	4 (16.0%)	10 (16.1%)
Not Hispanic or Latino	21 (52.5%)	21 (55.3%)	48 (55.2%)	46 (56.1%)	19 (76.0%)	46 (74.2%)
Unknown	13 (32.5%)	12 (31.6%)	26 (29.9%)	24 (29.3%)	0 (0.0%)	3 (4.8%)
Diagnosis category n(%)						
Acute Myeloid Leukaemia (AML)	20 (50%)	18 (47.4%)	45 (51.7%)	41 (50%)	11 (44%)	34 (54.8%)
Acute Lymphoid Leukaemia (ALL)	13 (32.5%)	13 (34.2%)	28 (32.2%)	27 (32.9%)	11 (44%)	20 (32.3%)
Myelodysplastic Syndrome (MDS)	3 (7.5%)	3 (7.9%)	8 (9.2%)	8 (9.8%)	3 (12%)	8 (12.9%)
Chronic Myelogenous Leukaemia: patients who progressed to blast crisis	0 (0.0%)	0 (0.0%)	1 (1.1%)	1 (1.2%)	0 (0.0%)	0 (0.0%)
Hodgkin Lymphoma	1 (2.5%)	1 (2.6%)	1 (1.1%)	1 (1.2%)	0 (0.0%)	0 (0.0%)

Table continued. Participant demographics and disease history in ISSE

	FAS (ITT, N=40)	mFAS (PP, N=38)	sFAS (ITT, N=87)	smFAS (PP, N=82)	Pivotal FAS (ITT, N=25)	Cryo sFAS (ITT, N=62)
Non-Hodgkin Lymphoma, aggressive lymphoma: Diffuse large B cell lymphoma	1 (2.5%)	1 (2.6%)	2 (2.3%)	2 (2.4%)	0 (0.0%)	0 (0.0%)
Adult T-cell Leukaemia/Lymphoma	1 (2.5%)	1 (2.6%)	1 (1.1%)	1 (1.2%)	0 (0.0%)	0 (0.0%)
Chronic Lymphocytic Leukaemia and transformation to Hodgkin lymphoma	1 (2.5%)	1 (2.6%)	1 (1.1%)	1 (1.2%)	0 (0.0%)	0 (0.0%)
Participants who received previous allogeneic HSCT n(%)	11 (27.5%)	10 (26.3%)	27 (31.0%)	25 (30.5%)	7 (28.0%)	20 (32.3%)
Participant serology at screening						
CMV antibodies positive n(%)	21 (52.5%)	20 (52.6%)	43 (49.4%)	42 (51.2%)	15 (60.0%)	32 (51.6%)
VZV positive n(%)	39 (97.5%)	37 (97.4%)	84 (96.6%)	81 (98.8%)	24 (96.0%)	59 (95.2%)
Toxoplasma antibody positive n(%)	6 (15.0%)	6 (15.8%)	13 (14.9%)	13 (15.9%)	3 (12.0%)	7 (11.3%)
HSV positive n(%)*	16 (40.0%)	15 (39.5%)	41 (47.1%)	40 (48.8%)	6 (24.0%)	21 (33.9%)
HSV-1 positive n(%)*	12 (30.0%)	11 (28.9%)	26 (29.9%)	25 (30.5%)	12 (48.0%)	26 (41.9%)

Studies 001 and 002 collected Herpes Simplex Virus (HSV) overall whereas Study 004 collected Herpes Simplex Virus-1 (HSV-1) and Herpes Simplex Virus-2 (HSV-2) separately.

Source: RPT-0018, Table 2-4

Approximately 70% of participants received a conditioning regimen of intermediate intensity and ~30% a conditioning regimen of high intensity prior to ECT-001-CB transplantation. GVHD prophylaxis consisted of cyclosporine A and MMF for 7 patients (in Study 001), and Tacrolimus and MMF for 66 patients in the smFAS subset.

Approximately 40% of patients received a ≥6/8 HLA matched ECT-001-CB graft with other patients receiving a 5/8 HLA matched ECT-001- CB graft.

Fresh versus cryopreserved

When clinical trials with ECT-001-CB were launched, cells contained in the dorocubicel (ECT-001-CB-DP1) component were manufactured and used fresh directly after culture, without cryopreservation of the cells. In order to extend the shelf life of dorocubicel and to make ECT-001-CB available to treat patients at additional clinical sites (multinational), the cryopreservation of dorocubicel was introduced in July 2019. The number of patients receiving fresh or cryopreserved ECT-001-CB are shown in Table 9.

*Table 9. Number of patients (per-protocol) receiving fresh or cryopreserved ECT-001-CB by size of CBU as starting material**

	Small size CBU as starting material		Standard size CBU as starting material		Total
	Fresh	**Cryopreserved**	**Fresh**	**Cryopreserved**	
Study 001	12	0	10	0	22
Study 002	2	9	0	19	30

Study 004	0	15	0	15	30
Total	14[2]	24[1]	10	34	82

**Table composed by assessor based on the report of Biopharmaceutic evaluations for ECT-001-CB, Appendix 1, Supplementary Table S1. [1]Included in the new pivotal FAS*

Expansion

Expansion of small CB units (defined as containing <1.5 X 10^5 CD34+/kg or <2.5 X 10^7 TNC/kg pre-cryopreservation, before expansion) resulted in the production of the targeted amount of cells (above 0.50 x 10^6 CD34+ cell/kg) in 39/40 (97.5%) lots across studies ECT-001-CB.001, ECT-001-CB.002, and ECT-001-CB.004. One lot did not achieve sufficient cell dose due to low expansion (<10 fold) and was not released for clinical use. The median number of viable CD34+ cells contained in dorocubicel manufactured from a small CB unit was 2.56 x 10^6 CD34+ cells/kg, compared to 4.26 x 10^6 CD34+ cells/kg in dorocubicel manufactured from a standard CB unit.

Infused dose

The median dose of CD34+ cells was ~2.6 x 10^6 CD34+ cells/kg in the FAS and mFAS, and ~3.4 x 10^6 CD34+ cells/kg in the sFAS and smFAS subsets. Similarly, the median dose of CD3+ cells was slightly lower at ~1.7 x 10^6 CD3+ cells/kg in the FAS and mFAS, compared to ~2.2 x 10^6 CD3+ cells/kg in the sFAS and smFAS subsets.

- **Numbers analysed**

The number of patients included in the 6 analyses sets and individual studies is displayed in Table . For the number of patients treated with fresh or cryopreserved dorocubicel derived from a small or standard size cord are displayed in Table.

- **Outcomes and estimation**

Primary endpoint - Pooled Analysis

Time to neutrophil engraftment

A total of 21/25 (84%) of patients in the pivotal FAS population reached neutrophil engraftment at the time of data cutoff (March 15th, 2024). The median time to neutrophil engraftment was 20 days (range 10-39; Table) for patients who have reached neutrophil engraftment.

For patients who did not reach neutrophil engraftment (e.g. participants who were enrolled but did not receive the product or who did not engraft for any reason including NRM and relapse before engraftment), a worst-case scenario of engraftment at 42 days was inferred. In this scenario, the median time to neutrophil engraftment increased by 5 days in the Pivotal FAS population (25 d, range 10-42) and 1-2 days in the other populations.

Of the 4 patients who did not reach engraftment, 1 was discontinued before receiving ECT-001-CB transplant, 2 patients died of NRM and 1 patient had disease progression prior to neutrophil engraftment (competing risks).

Similar neutrophil engraftment kinetics were observed in the other pooled subsets and individual studies.

In the pivotal population, no patient had primary graft failure. In the population of patients treated with ECT-001-CB regardless of the size of the CB unit used as starting material (sFAS and smFAS), one patient had primary graft failure related to the presence of high titer donor-specific anti-HLA

antibodies. No secondary graft failure has been observed in adult patients transplanted with single ECT-001-CB for haematological malignancies.

Table 10. Tabular summary of efficacy across studies

Efficacy parameters	FAS pivotal (ITT, N=25)	Old FAS (ITT, N=40)	mFAS (PP, N=38)	sFAS (ITT, N=87)	smFAS (PP, N=82)	Cryos FAS (ITT, N=62)	Study 001 (PP, N=22)	Study 002 (PP, N=30)	Study 004 (PP, N=30)
Neutrophil recovery									
Evaluable patients, n	**25**	40	38	87	82	62	22	30	30
Median time to neutrophil engraftment (days), median (range) for patients who have reached neutrophil engraftment	**20 d (10-39)**	18 d (10-39)	18 d (10-39)	17 d (9-39)	17 d (9-39)	17 d (9-39)	18 d (10-30)	17 d (10-39)	18 d (9-32)
Median time to neutrophil engraftment (days), median (range) for all patients[1]	**25 d (10-42)**	20 d (10-42)	19.5 d (10-42)	18 d (9-42)	18 d (9-42)	18 d (9-42)	NR	NR	NR
Proportion achieving neutrophil engraftment by day 42, n (%)	**21 (84.0%)**	35 (87.5%)	35 (92.1%)	77 (88.5%)	77 (93.9%)	53 (85.5%)	22 (100%)	28 (93.3%)	27 (90.0%)
Short-term secondary endpoint									
Evaluable patients for platelet recovery*, n	**24**	39	37	85	80	60	22	30	28
Median time to platelet engraftment (days), median (range) for patients who have reached platelet engraftment	**40 d (29-175)**	42 d (27-175)	42 d (27-175)	39 d (24-175)	39 d (24-175)	37 d (24-175)	42 d (27-63)	37 d (27-68)	39 d (24-175)
Median time to platelet engraftment (days), median (range) for all patients[2]	**48 d (29-175)**	43.5 d (27-175)	42.5 d (27-175)	41 d (24-175)	40 d (24-175)	40 d (24-175)	NR	NR	NR
Proportion achieving platelet engraftment by day 100, n (%)	**19 (79.2%)**	33 (84.6%)	33 (89.2%)	72 (84.7%)	72 (90.0%)	49 (81.7%)	21 (95.5%)	27 (90.0%)	24 (85.7%)

Table continued. Tabular summary of efficacy across studies

Efficacy parameters		FAS pivotal (ITT, N=25)	Old FAS (ITT, N=40)	mFAS (PP, N=38)	sFAS (ITT, N=87)	smFAS (PP, N=82)	Cryos FAS (ITT, N=62)	Study 001 (PP, N=22)	Study 002 (PP, N=30)	Study 004 (PP, N=30)
Long-term secondary endpoints										
Non-relapse mortality, cumulative incidence (95%CI)	12 months	**21.2% (4–38)**	12.9% (2–24)	13.6% (2–25)	11.0% (4–18)	11.7% (4–19)	14.2% (5–24)	4.5% (0–14)	13.6% (1–26)	15.6% (1–30)
	24 months	**21.2% (4–38)**	12.9% (2–24)	13.6% (2–25)	13.9% (6–22)	14.8% (7–23)	18.9% (8–30)	4.5% (0–14)	17.4% (3–32)	22.2% (4–41)
OS, Kaplan-Meier estimates (95% CI)	12 months	**66.0% (49–89)**	71.3% (58–87)	72.3% (59–89)	75.7% (67–86)	79.1% (70–89)	72.2% (61–85)	86.4% (73–100)	76.0% (62–93)	77.3% (63–95)
	24 months	**51.4% (32–82)**	57.4% (43–77)	61.4% (47–81)	62.1% (52–74)	66.3% (56–79)	60.1% (48–76)	72.7% (56–94)	60.6% (45–82)	70.3% (53–93)
PFS, Kaplan-Meier estimates (95% CI)	12 months	**52.8% (36-78)**	58.2% (44-76)	61.3% (47–80)	67.1% (58–78)	71.3% (62-82)	66.9% (56-80)	72.7% (56–94)	69.1% (54–88)	72.7% (57–93)
	24 months	**45.3% (28-74)**	54.7% (41-74)	57.7% (43–77)	56.6% (46–69)	60.1% (50–73)	50.1 (38-67)	72.7% (56–94)	53.8% (38–76)	52.0% (33–82)
GRFS, Kaplan-Meier estimates (95% CI)	12 months	**29.1% (15–55)**	38.3% (26–57)	40.4% (27–60)	53.3% (44–65)	56.7% (47–69)	50.8% (39–66)	63.6% (46–87)	52.2% (37–74)	57.6% (42–79)
	24 months	**NR**	30.6% (18–51)	32.3% (20–53)	41.0% (31–54)	43.5% (33–57)	32.8% (22–50)	59.1% (42–84)	36.1% (22–60)	34.6% (18–68)
CRFS, Kaplan-Meier estimates (95% CI)	12 months	**45.2% (29–71)**	53.5% (40–72)	56.3% (42–75)	63.5% (54–75)	67.4% (58–79)	61.6% (50–76)	72.7% (56–94)	62.2% (47–83)	69.2% (53–90)
	24 months	**30.2% (15–62)**	46.8% (33–66)	49.3% (35–69)	50.1% (40–63)	53.2% (43–66)	42.6% (31-59)	68.2% (51–91)	42.8% (28–66)	49.5% (31–79)
Acute GVHD grade III-IV at 12 months, cumulative incidence (95%CI)		**28.5% (10–47)**	22.8% (10–36)	24.0% (10–38)	16.3% (8–24)	17.3% (9–26)	19.6% (10–30)	9.1% (0–21)	13.3% (0–26)	27.7% (11–45)
Moderate to severe chronic GVHD at 24 months, cumulative incidence (95%CI)		**17.4% (0–38)**	8.7% (0–19)	9.2% (0–20)	5.3% (0–10)	5.6% (0–11)	8.0% (0–16)	-	10.8% (0–23)	3.9% (0–12)
* One patient from mFAS and two patients from smFAS did not reach sufficient follow up time (<100 days) and were removed from the per protocol analysis sets (mFAS and smFAS).										

[1] Inferring the worst-case scenario, i.e. 42 days, to subject who did not reach ANC>=500/µl (participants who were enrolled but did not receive the product or who did not engraft for any reason including NRM and relapse before engraftment)

[2] Inferring the worst-case scenario, i.e. 100 days, to subject who did not reach platelets>=20,000/µl (participants who were enrolled but did not receive the product or who did not engraft for any reason including NRM and relapse before engraftment)

NR: not reported; CI: confidence interval.
N.B.: No case of moderate to severe chronic GVHD were reported at time of data cut-off in Studies 001.

Source: M2.7.3 Summary of Clinical Efficacy, Sections 3.2.1 and 3.2.2, RPT-0018 Tables 5-18.

Pooled Analysis – Secondary endpoints

Time to platelet engraftment

Platelet engraftment at day 100 posttransplant had been achieved by 17/25 (68%) of patients in the FAS (including 1 patient who was not transplanted due to shipment failure). The median (range) time to platelet engraftment (platelet count ≥ 20000/µl) was 40 (29-175) days for patients who have reached platelet engraftment.

For patients who did not reach platelet engraftment (e.g. participants who were enrolled but did not receive the product or who did not engraft for any reason including NRM and relapse before engraftment), a worst-case scenario was inferred (i.e. 100 days). In this scenario, the median time to neutrophil engraftment increased by 8 days in the Pivotal FAS population (48 d, range 29-175) and by 1-3 days in the other populations.

Similar kinetics of platelet recovery were observed in the other population subsets and individual studies.

Proportion reaching neutrophil engraftment by Day 42

See time to neutrophil engraftment endpoint above.

Proportion reaching platelet engraftment by Day 100

See time to platelet engraftment endpoint above.

Non-relapse mortality (NRM)

In the pivotal FAS population, the cumulative incidences (95%, CI) of NRM were 12.2% (0-26) at 100 days and 21.2% (4-38) at both 12 and 24 Months. In the Cryo sFAS population, the cumulative incidences of NRM were 4.9% (0-10) and 18.9% (8-30), respectively.

In the old FAS population, 5/40 (12.5%) patients died of NRM. The cumulative incidences of NRM were 7.6% (0-16) at 100 days and 12.9% (2-24) at both 12 and 24 Months.

Median cumulative probability of NRM was not (yet) reached in the analysis populations.

Overall survival (OS)

Median OS was not (yet) reached. The probability of OS was 66.0% (95% CI: 49-89) and 51.4% (95% CI: 32-82) in the FAS at 1 and 2-year posttransplant, respectively (Figure 10). Results were similar or slightly higher in the other population subsets.

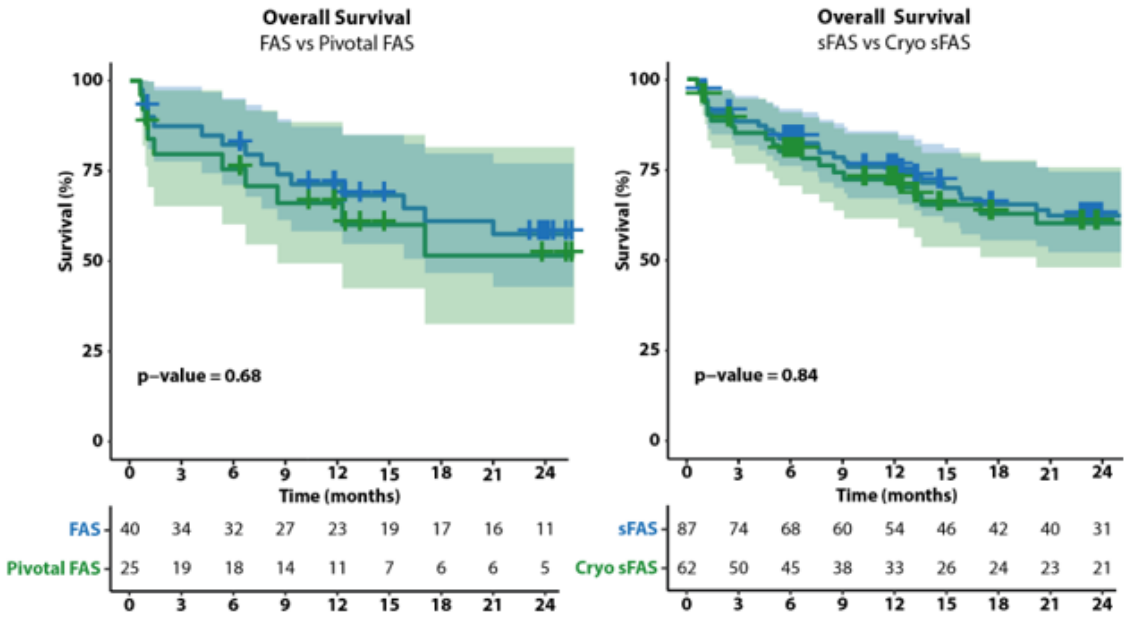

Figure 10. KM estimates of OS in Pivotal FAS, FAS, sFAS and Cryo sFAS populations

Progression-free survival (PFS)

Median PFS was not (yet) reached. Probabilities of PFS (95% CI) in the pivotal FAS population were 79.8% (65-97) at 100 days, 52.8% (36-78) at 12 Months and 45.3% (28-74) at 24 Months (Figure 11). PFS was similar or slightly higher in the other population subsets. Decrease in PFS was driven mostly by relapse in all population subsets.

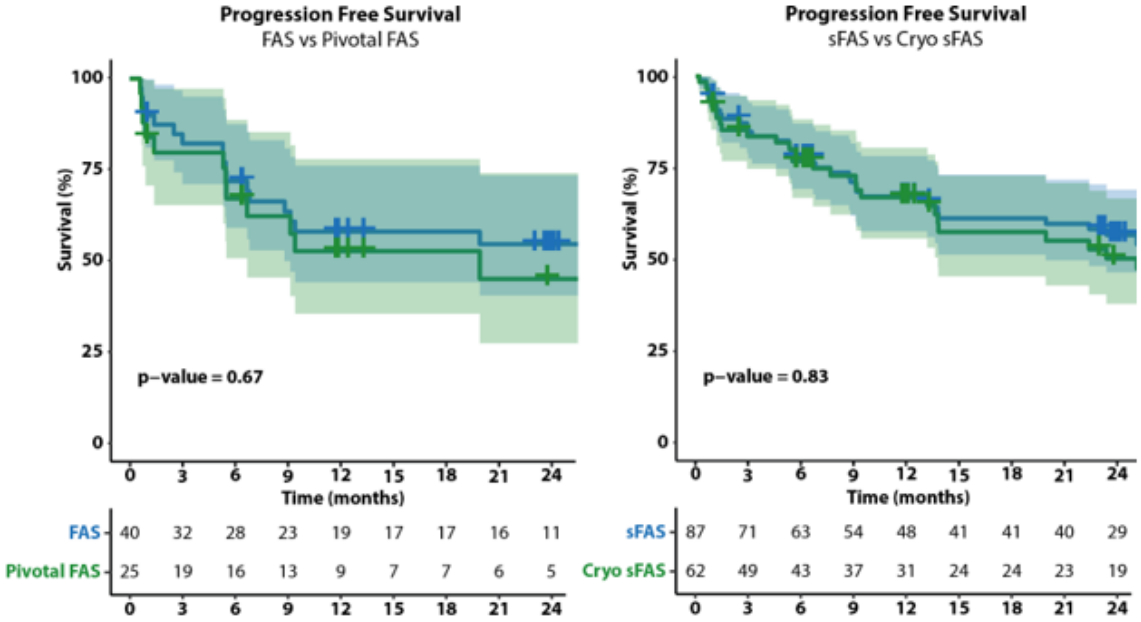

Figure 11. KM estimates of PFS in Pivotal FAS, FAS, sFAS and Cryo sFAS populations

Graft versus host disease (GvHD)-free and relapse-free survival (GRFS)

At Month 12, the probability of GRFS (95% CI) was 29.1% (15-55) for patients in the pivotal FAS and 50.8% (39-66) for the Cryo sFAS. At Month 24, the probability of GRFS (95% CI) was 32.8% (22-50) for Cryo sFAS. No GFRS data were available for the pivotal FAS population past 21 months.

Decrease in GRFS was driven mostly by grade III-IV acute GVHD and relapse in all population subsets.

Chronic GvHD-free and relapse-free survival (CRFS)

The probability of CRFS (95% CI) was 45.2% (29-71) at Month 12 and 30.2% (15-62) at Month 24 in the pivotal FAS population. For the Cryo sFAS, these probabilities were 61.6% (50-76) and 42.6% (31-59) at 12 at Month 12 and 24, respectively. Results were similar or higher in the other analysis populations. Decrease in CRFS was driven mostly by relapse in all population subsets.

Acute GvHD

In total 15/25 (60%) and 39/62 (62.9%) of patients had acute GVHD (aGVHD) in the pivotal FAS and Cryo sFAS population subsets. Most patients had Grade II acute GvHD (~32-43% across populations) and 2-4% of patients had Grade IV GvHD.

The cumulative incidence (95% CI) of grade II-IV aGVHD at 12 Months was 62.7% (42-83) in the pivotal FAS population and 65.4% (53-78) in the Cryo sFAS population. The cumulative incidence (95% CI) of grade III-IV aGVHD at 12 Months was 28.5%% (10-47) and 19.6% (10-30), respectively.

Chronic GvHD

In total, 4/25 (16%) and 6/62 (9.7%) of patients had chronic GVHD in the pivotal FAS and Cryo sFAS population subsets.

The cumulative incidence (95% CI) of all grade chronic GVHD was 12.4% (0-26) in the pivotal FAS population at 12 Months, and 33.5% (4-63) at 24 Months. The cumulative incidence of moderate-severe chronic GvHD was 8.4% (0-20) at 12 Months and 17.4% (0-38) at 24 Months.

In the Cryo sFAS population, the cumulative incidence of all grade chronic GvHD was 13.8% (5-23) at 12 Months and 18.9% (8-30), at 24 Months. And for moderate-severe chronic GvHD the cumulative incidence was 4.8% (1-9) and 6% (1-11), respectively.

- **Ancillary analyses**

Subgroup analyses

Subgroup analyses were planned and performed on the smFAS subset (n=73) with data cut-off November 30th 2022 for comparison of efficacy endpoints according to several extrinsic (conditioning regimen, HLA matching) and intrinsic factors (sex, age, race, ethnicity, disease type, disease risk and prior allogeneic transplant).

The following intrinsic and extrinsic factors had impact on some of the efficacy endpoints:

- *HLA matching (5/8 vs. ≥6/8)*:The proportion of participants achieving platelet engraftment was lower in participants treated with 5/8 HLA matched ECT-001-CB (35/42 or 83.3%) vs 6+/8 (29/29 or 100%, p = 0.036), although they had faster engraftment (median time to platelet engraftment at 36 days vs 42 days for 5/8 vs 6+/8, respectively, p = 0.002). Also, the incidence of NRM was higher in participants treated with 5/8 HLA-matched ECT-001-CB at 24 months (8/44 or 23.79% CI in 5/8, vs. 1/29 or 4.37% CI in 6+/8).
- *Condition regimen (high vs. intermediate* intensity: all NRM events occurred in participants treated with regimens of intermediate intensity.
- *Age (18-39, 40-54 and ≥55 years old)*: The maximum age of included patients in the Pooled Analysis is 66 years. All NRM events occurred in participants 40 years or older (9/48 or 18.8%), leading to a significantly higher incidence of NRM in older participants. This translated into a lower OS, PFS, GRFS and CRFS in participants 40-54 years of age, decreasing further with the highest age group.
- *Disease type (AML, ALL, MDS or others)*: Patients with AML reported lower OS, PFS and CRFS rates vs other disease types.

The impact of intrinsic and extrinsic factors on neutrophil and platelet recovery was re-evaluated using updated data as of March 15, 2024, with no change to the original conclusions.

Time from enrolment to transplantation

The time required from enrolment of a patient to the availability of the graft at clinical site and to infusion of ECT-001-CB was evaluated for the two studies that were conducted outside of Canada (Study 004 and supportive Study 007). A total of 42 lots from the two studies, including 3 US sites and 1 European site, which best reflect the logistics required for the treatment of patients in countries of the European Union, were evaluated.

The median time between enrolment of patients and arrival of product at clinical site was 25 days (Figure 12). All ECT-001-CB lots arrived at clinical site in <2 months (60 days) after enrolment. The median time between enrolment of patients and infusion of product was 36.5 days.

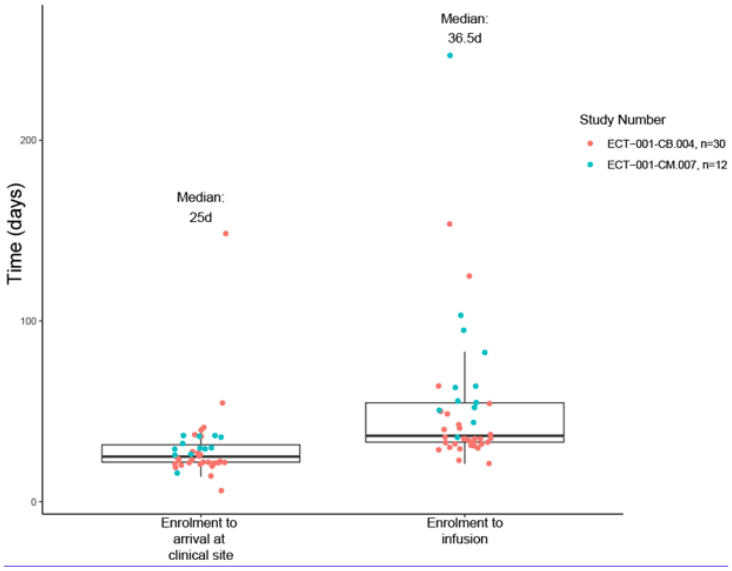

Figure 12. Time from patient enrolment to arrival of ECT-001-CB to clinical site, and to patient infusion for patients treated in studies .004 and .007.

Summary of main efficacy results

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

Table 11. Summary of Efficacy for the integrated efficacy analysis of pooled ECT-001-CB clinical trials

Title: Integrated efficacy analysis of pooled ECT-001-CB clinical trials		
Study identifier	Pooled analysis of ECT-CB 002 and ECT-CB-004 ()	
Design	Pooled analysis of two prospective, single-arm, open-label Phase 2 studies in 5 study sites (3 USA, 1 Canada, 1 EU).	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	Study 002: May 2019 – fu ongoing Study 004: Jan 2020 – fu ongoing Not applicable Not applicable
Hypothesis	Exploratory: No hypothesis defined, study performed with the overall objective to present precise and relevant information on the pooled safety and efficacy parameters in patients transplanted with ECT-001-CB.	
Treatments groups	Pivotal FAS (ITT - all participants with a cryopreserved ECT-001-CB derived from expansion of a small CB unit)	Cryopreserved ECT-001-CB transplant with expanded CD34+ cells and unexpanded CD34- fraction following a conditioning regimen with a TCI score of 2.5 and above
	Cryopreserved supportive FAS (Cryo sFAS; ITT– all participants with a cryopreserved ECT-001-CB regardless of the size of the CB used as starting material)	Similar to FAS

Title: Integrated efficacy analysis of pooled ECT-001-CB clinical trials			
Study identifier	Pooled analysis of ECT-CB 002 and ECT-CB-004 ()		
Endpoints and definitions	Primary endpoint	Time to neutrophil engraftment	Time from transplant at first day of three consecutive days with absolute neutrophil count (ANC) ≥ 0.5 x 10^9/L.
	Secondary endpoint	Time to platelet engraftment	Time between transplant and first day of a sustained platelet count ≥ 20 x 10^9/L with no platelet transfusion in the preceding 7 days
	Secondary endpoint	aGvHD	The incidence of acute GvHD using NIH criteria
	Secondary Endpoint	cGvHD	The incidence of chronic GvHD using NIH criteria
Database lock	15 March 2024		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat populations consisting of patients (intented to be) treated with single ECT-001-CB derived from a small CB unit (pivotal FAS) or any size CB unit (Cryopreserved supportive FAS population).		
Descriptive statistics and estimate variability	Treatment group	Pivotal FAS	Cryo supportive FAS
	Number of subject	25	62
	Time to neutrophil engraftment (median)	20 d (for patients who reached engraftment) 25 d (for all patients)*	17 d (for patients who reached engraftment) 18 d (for all patients)*
	range	10-39 10-42	9-39 9-42
	Time to platelet engraftment	40 d (for patients who reached engraftment) 48 d (for all patients)*	37 d (for patients who reached engraftment) 40 d (for all patients)*
	range	29-175 29-175	24-175 24-175
	aGVHD grade II-IV	60%	62.9%
	cGVHD	16%	9.7%

Title: Integrated efficacy analysis of pooled ECT-001-CB clinical trials	
Study identifier	Pooled analysis of ECT-CB 002 and ECT-CB-004 ()
Notes	Abbreviations: d: days, pivotal FAS: Full Analysis Set, fu: follow-up, Cryo sFAS: Cryopreserved supportive FAS FAS, ITT: intent-to-treat, TCI: transplant conditioning intensity The mFAS analysis set (n=34) included a subset of the FAS (n=36) consisting of adult patients actually treated (per protocol) with single ECT-001-CB derived from a small CB unit. Two patients of the FAS (ITT) were not infused due to expansion failure (n=1) and shipping failure (n=1). *Worst-case scenario analysis, patients who did not reach neutrophil engraftment by Day 42 or platelet engraftment by Day 100 post-transplant, including the patients who were not transplanted, or failed to engraft for any reason (NRM or relapse prior to engrafting) were inferred to have failed at Day 42, or Day 100, respectively

2.6.5.3. Clinical studies in special populations

Elderly patients: Clinical studies of ECT-001-CB did not include a sufficient number of subjects aged 65 years and over to determine whether they respond differently than younger subjects. The maximum age of included patients in the Pooled Analysis is 66 years. Subgroup analysis for patients aged (18-39, 40-54 and ≥55 years old) are presented in the *Ancillary analysis - Subgroup analyses* section of this report.

Paediatric patients: Although the target population comprises adults only, 12 paediatric patients were treated in ongoing Study 007 (see section *supportive data* below).

Patients with hepatic and renal impairment: Clinical studies of ECT-001-CB did not include patients with hepatic or renal impairment and it could therefore not be determined whether they respond differently than patients without hepatic or renal impairment.

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

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable.

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

The pooled analysis of clinical studies (001,) 002 and 004 is the pivotal efficacy analysis and discussed in the main sections above.

2.6.5.6. Supportive studies

Comparative matched-controlled studies

EBMT registry matched analyses

In two EBMT matched-controlled studies of similar design, ECT-001-CB treated patients were matched to six separate registry control cohorts identified from the EBMT observational database:

- Patients undergoing single or double cord blood (CB) transplantation without antithymocyte globulin (ATG) in the conditioning regimen
- Patients receiving peripheral blood stem cells (PBSC) from a MUD (10/10 HLA-matched).
- Patients receiving bone marrow cells (BM) from a MUD (10/10 HLA-matched).
- Patients transplanted with stem cells from 9/10 mismatched unrelated donors (MMUD, PBSC or BM), regardless GvHD prophylaxis.
- T-replete haplo-identical donors (PBSC or BM).
- Matched sibling donors (MSD, PBSC or BM).

The aim of the first study (Study 1) was to perform a matched-controlled analysis, comparing outcomes of 22 patients with high-risk hematologic malignancies from the Phase I/II single arm trial (ECT-001-CB.001) of a single ECT-001-expanded cord blood unit for transplantation, those of the six real-world cohorts.

In the context of the MAA assessment, a supplemental retrospective cohort study of similar design (Study 2) was also conducted to compare 24 infused patients from Studies ECT-001-CB.002 and 004 who received cryopreserved ECT-001-CB derived from the expansion of a small CB unit and allografted patients from six separate cohorts of the EBMT registry. Unlike Study 001, Studies 002 and 004 were specifically conducted in patients with high- and very high-risk acute leukemia and myelodysplasia populations known to have a poorer prognosis and higher likelihood of relapse. In contrast, Study 001 included patients with a broader range of disease risk. At the time of the data cut-off, Study 001 was completed while Studies 002 and 004 were still ongoing, with ~13 months of median follow-up.

The primary objectives in both studies were neutrophil and platelet recovery (both described at 30, 42, 60 and 100 days post-transplant, when available), NRM, PFS, OS and GRFS. For matched EBMT Study 2, CRFS was included as primary objective as well. With regard to neutrophil recovery, the 42 day's timepoint was chosen for neutrophil engraftment because primary graft failure is defined as absence of neutrophil engraftment by day +42 post-transplant in CB transplantation as per Kharfan-Dabaja et al. 2021.

In both EBMT matched analyses, patients were matched to controls according to disease and status at transplant, previous allogeneic HSCT, age at transplant, KPS (<90, 90-100) and conditioning regimen. In the first Study for 10/10 MUD PBSC recipient, exact matching was used for disease and status at transplant, previous allograft, KPS and conditioning regimen, and nearest neighbour was used for patient age at transplant. For all other control groups in Study 1 and all of Study 2, exact matching was used for disease and status at transplant, and propensity score matching for the other variables.

Results

A tabular summary of results for EBMT matched Study 1 is presented in Table and for Study 2 in Table . The matched analysis of patients treated with cryopreserved ECT-001-CB and the main control group of patients who underwent single or double cord blood transplantation is shown in Table.

Neutrophil engraftment

- In Study 1, neutrophil engraftment was significantly faster among patients with ECT-001-CB (median of 18 days) compared to non-manipulated CBU (22 days) and 10/10 MUD - BM recipients (23 days), with 100% of ECT-001-CB patients achieving neutrophil recovery at 42 days compared to 91.7% and 93.5% for the respective control groups. No significant difference was found in incidence of and time to neutrophil recovery between ECT-001-CB and other control groups.
- In Study 2, neutrophil engraftment was attained in 87.5% in patients treated with ECT-001-CB, with a median time to engraftment of 20 days compared to 25 days for non-manipulated CBU transplant. In other donor sources the median time was 16-21 days.

Platelet recovery

- In Study 1, platelet recovery (median time to recovery 43 days) among patients with ECT-001-CB was similar to that of non-manipulated CBU transplant (40 days), and slower than other stem cell sources (14-26 days).
- In Study 2, platelet recovery (median time to recovery 40 days) was also similar to that of non-manipulated CBU transplant (36 days), and slower than other stem cell sources (13-27 days). Results in the control cohorts were considered not reliable due to the high number of missing values in the EBMT registry (between 10% and 20%).

NRM and PFS

- In Study 1, NRM and PFS among patients with ECT-001-CB were similar to that of other stem cell sources, with a trend towards better outcomes for ECT-001-CB for NRM compared to 9/10 MMUD and haploidentical donors, and for PFS compared to haploidentical donors and MSD, although not reaching significance ($p < 0.1$).
- In Study 2, NRM and PFS were similar (NRM) and numerically lower (PFS) compared to other stem cell sources (e.g. NRM was 20% vs. 21% in the non-manipulated CBU control group at 2 years and PFS 47.1% vs. 58%, respectively).

OS

- In Study 1, OS among patients with ECT-001-CB was higher compared to transplantation using haploidentical donors with a trend towards better outcomes for ECT-001-CB compared to 9/10 MMUD, although not reaching significance ($p < 0.1$).
- In Study 2, OS with ECT-001-CB was comparable or numerically slightly lower to other stem cell sources (e.g. 57% at 2 year vs. 60.9% in the unmanipulated CBU control group).

GFRS

- In Study 1, GRFS with ECT-001-CB was significantly higher compared to non-manipulated CBU, 10/10 MUD - PBSC controls, 9/10 MMUD, haploidentical and MSD cohorts, and appeared higher compared to 10/10 MUD - BM controls without reaching significance ($p < 0.1$).
- In Study 2, a higher incidence of grade II-IV acute GVHD in the ECT-001-CB cohort often lead to a numerically lower GRFS when compared to other sources. The cumulative incidence at 24 Months was 23.3% with ECT-001-CB-cryo vs. 39.8% in the CBU control group.

Incidence of acute GVHD Grade III-IV

- In Study 1, the incidence of acute GVHD grade III-IV with ECT-001-CB was similar to that of other stem cell sources.

- In Study 2, the incidence of acute GVHD grade III-IV with ECT-001-CB at 180 days was 35% vs. 15.2% in the CBU control group.

Incidence of moderate to severe chronic GvHD

- In Study 1, the incidence of moderate to severe cGvHD (0%) with ECT-001-CB was significantly lower compared to 10/10 MUD - PBSC controls, 10/10 MUD - BM controls and MSD cohorts, and appeared lower compared to non-manipulated CBU and haploidenticalcontrols without reaching significance ($p < 0.1$).
- In Study 2, the incidence of moderate to severe chronic GVHD (0%) with ECT-001-CB was lower compared to other control cohorts.

CRFS was only analysed in Study 2, and the incidence with ECT-001-CB was similar to that of other stem cell sources.

Table 12. Tabular summary of results of EBMT matched analysis (Study 1)

Comparison of outcomes	Study 001[4] (n=22)	EBMT					
		CBU (n=36)	10/10 PBSC (n=59)	10/10 BM (n=31)	9/10 MMUD (n=62)	Haplo (n=59)	MSD (n=61)
Median time to neutrophil recovery	**18 d**	22 d p<0.001	17 d p=0.94	23 d p=0.014	17 d p=0.98	19 d p=0.09	18 d p=0.83
% neutrophil engraftment at D42	**100%**	91.7% p<0.001	98.3% p=0.69	93.5% p=0.015	98.4% p=0.98	93.2% p=0.14	95.1% p=0.81
Median time to platelet recovery (days)	**43 d**	40 d p=0.41	15 d p<0.0001	20 d p<0.0001	16.5 d p<0.0001	26 d p<0.0001	14 d p<0.0001
NRM[1] HR [95% CI]	**4.4%**	0.22 [0.02-2.08] p=0.19	0.25 [0.03-2.47] p=0.24	0.24 [0.03-1.65] p=0.15	0.16 [0.02-1.19] p=0.073	0.13 [0.02-1.06] p=0.056	0.44 [0.11-1.77] p=0.25
OS[1] HR [95% CI]	**85.3%**	0.83 [0.32-2.15] p=0.7	0.51 [0.21-1.28] p=0.15	0.81 [0.38-1. 7] p=0.57	0.48 [0.18-1.3] p=0.15	0.41 [0.19-0.92] p=0.03	0.57 [0.28-1.18] p=0.13

PFS[1] HR [95% CI]	**72.3%**	0.74 [0.29-1.91] p=0.53	0.49 [0.19-1.27] p=0.14	0.72 [0.31-1.67] p=0.45	0.39 [0.14-1.07] p=0.068	0.42 [0.19-0.95] p=0.038	0.48 [0.21-1.09] p=0.08
Grade III-IV acute GVHD (%)[2]	**8.9%**	17.1% p=0.52	16.4% p=0.45	10% p=0.85	17% p=0.41	8.9% p=0.6	15.8% p=0.49
Mod. to sev. Chronic GVHD (%)[3]	**0%**	16.3% p=0.07	16.0% p=0.04	30.6% p=0.015	7.3% p=0.21	12.5% p=0.10	26.9% p=0.01
GRFS[1] HR [95% CI]	**63.6%**	0.55 [0.23-1.31] p=0.18	0.40 [0.17-0.95] p=0.037	0.57 [0.27-1.21] p=0.14	0.40 [0.16-0.98] p=0.046	0.45 [0.2-1] p=0.049	0.37 [0.16-0.82] p=0.014

[1]HR calculated over a 2-year follow-up period.
[2]Endpoints reported at 180 days. P-value are Gray test. Cox model (with cluster) cannot be used as there is not enough events in the ECT-001-CB group.
[3] Endpoints reported at 2 years. P-value are Gray test. Cox model (with cluster) cannot be used as there is not enough events in the ECT-001-CB group.
[4] Results for Study 001 presented in this table were derived from ECT-001-CB.001 CSR.
N.B: Dark green shows better outcome for ECT-001-CB with $p < 0.05$; Light green shows a trend for better outcome for ECT-001-CB with $p < 0.1$ and > 0.05. Dark orange shows better outcome for control with $p < 0.05$.

Table 13: Tabular summary of results of the EBMT matched analysis (Study 2)

Comparison of outcomes	ECT-001-CB (n=24)	EBMT					
		CBU (n=41)	10/10 PBSC (n=69)	10/10 BM (n=41)	9/10 MMUD (n=66)	Haplo (n=72)	MSD (n=68)
Median time to neutrophil recovery (min-max)	20 d (10-39)	25 d (7-69)	16 d (8-35)	21 d (2-40)	17 d (10-37)	20 d (11-53)	16 d (9-47)
Neutrophil engraftment at D42 [95% CI]	87.5% [60.5-94.1]	85.4% [69.3-93.4]	98.6% [83.4-99.9]	98.4% [76.3-99.9]	95.5% [84.9-98.7]	95.8% [86.1-98.8]	97.1% [86.2-99.4]
Median time to platelet recovery (min-max)	40 d (29-175)	36 d (4-69)	14 d (1-42)	20 d (3-43)	14.5 d (8-62)	27 d (9-79)	13 d (1-33)
NRM[1] HR [95% CI]	20%	1.1 [0.34-3.58] p=0.88	1.84 [0.42-7.97] p=0.42	2.7 [0.69-10.58] p=0.15	0.8 [0.3-2.13] p=0.65	1.31 [0.34-5.07] p=0.7	2.83 [0.68-11.68] p=0.15
OS[1] HR [95% CI]	57%	1.31 [0.57-2.98] p=0.53	1.28 [0.52-3.17] p=0.59	1.14 [0.46-2.87] p=0.77	1.03 [0.47-2.26] p=0.93	1.23 [0.49-3.1] p=0.66	1.79 [0.77-4.17] p=0.18
PFS[1] HR [95% CI]	47.1%	1.45 [0.78-2.7] p=0.24	1 [0.47-2.15] p=0.99	1.11 [0.47-2.63] p=0.81	0.97 [0.5-1.86] p=0.92	1.19 [0.49-2.9] p=0.71	1.88 [0.9-3.9] p=0.091
Relapse HR [95% CI]	32.9%	1.82 [0.68-4.9] p=0.24	0.77 [0.31-1.9] p=0.57	0.86 [0.31-2.36] p=0.77	1.17 [0.4-3.39] p=0.78	1.12 [0.42-2.95] p=0.83	1.54 [0.6-3.96] p=0.38
Grade III-IV acute GVHD [95% CI][2]	35%	15.2% [5.4-29.5]	8.8% [3.2-18]	2.1% [0.2-9.9]	16.8% [8.2-27.9]	6.8% [2.2-15.1]	15.7% [7.3-27.1]
Mod. to sev. Chronic GVHD [95% CI][3]	0%	19.5% [7.7-35.2]	9.7% [3-21.2]	12% [4.3-23.9]	13.8% [5.5-25.8]	7.6% [2.4-16.8]	19.2% [9.3-31.7]
GRFS[1] HR [95% CI]	23.3%	1.7 [0.81-3.57] p=0.16	1.65 [0.86-3.14] p=0.13	1.61 [0.75-3.46] p=0.23	1.49 [0.88-2.52] p=0.14	1.99 [0.94-4.25] p=0.074	2.09 [1.17-3.73] p=0.013
CRFS HR [95% CI]	47.1%	1.08 [0.57-2.04] p=0.81	0.9 [0.43-1.88] p=0.78	0.84 [0.37-1.92] p=0.68	0.84 [0.44-1.61] p=0.6	1.03 [0.46-2.31] p=0.94	1.19 [0.66-2.14] P=0.57

[1] HR calculated over 2-year follow-up period.
[2] Endpoints reported at 180 days. P-value are Gray test. Cox model (with cluster) cannot be used as there is not enough events in the ECT-001-CB group.
[3] Endpoints reported at 2 years. P-value are Gray test. Cox model (with cluster) cannot be used as there is not enough events in the ECT-001-CB group.
Source: EBMT Matched-Control Analysis, 23 December 2024

Table 14. EBMT matched analysis cord blood control cohort vs. cryopreserved ECT-001-CB (Study 2)

		EBMT (Cord blood) N=41	ECT-001-CB-cryo N=22
Neutrophil engraftment Incidence	At 42 days [95% CI] (Pts at risk)	85.4% [69.3-93.4] (n=4)	86.4% [58.2-96.1] (n=0)
Platelet engraftment Incidence	At 100 days [95% CI] (Pts at risk)	83.3% [62.9-93.1] (n=3)	72.2% [42.7-88.3] (n=2)
Overall Survival	At 24 months [95% CI] (Pts at risk)	60.9% [46.3-80] (n=17)	52.5% [32.9-83.7] (n=4)
Progression-free Survival	At 24 months [95% CI] (Pts at risk)	58% [43.4-77.5] (n=16)	41.7% [23.2-74.7] (n=4)
Relapse Incidence	At 24 months [95% CI] (Pts at risk)	21% [9.1-36.3] (n=16)	36.1% [13.2-59.9] (n=4)
Non-Relapse Mortality	At 24 months [95% CI] (Pts at risk)	21% [9.1-36.3] (n=16)	22.2% [6.5-43.6] (n=4)
Acute GVHD grade II-IV	At 180 days [95% CI] (Pts at risk)	45.5% [27.8-61.5] (n=11)	61.1% [33.4-80.1] (n=3)

Acute GVHD grade III-IV	At 180 days [95% CI] (Pts at risk)	15.2% [5.4-29.5] (n=21)	33.3% [13-55.4] (n=7)
Chronic GVHD	At 24 months [95% CI] (Pts at risk)	42.1% [24.3-59] (n=8)	27.8% [3.9-60.2] (n=1)
GvHD-Relapse-Free Survival	At 24 months [95% CI] (Pts at risk)	39.8% [26.2-60.6] (n=12)	22.2% [7.9-62.4] (n=2)
Chronic GvHD-Relapse-Free Survival	At 24 months [95% CI] (Pts at risk)	45.8% [31.6-66.3] (n=13)	41.7% [23.2-74.7] (n=4)

CIBMTR registry matched analysis

The same 22 ECT-001-CB treated patients from study ECT-001-CB.001 as matched with the first EBMT registry matched analysis described above, were matched to two patient cohorts identified from the CIBMTR registry, representative of North American real-world clinical data. The two CIBMTR cohorts pertained:

- Patients who underwent single- or double- umbilical cord blood (UCB) transplantation without anti-thymocyte globulin (ATG)/alemtuzumab in the conditioning regimen or as GvHD prophylaxis
- Matched unrelated donor (8/8 human leukocyte antigen [HLA] matched) peripheral blood stem cells (PBSC) allogeneic hematopoietic cell transplant (alloHCT) recipients with or without ATG/alemtuzumab in the conditioning regimen or as GVHD prophylaxis

The participants identified from the CIBMTR database were matched to the 22 participants treated with single ECT-001-CB transplantation in Study 001 using disease, refined disease index, disease status for second allogeneic HSCT only (intermediate, advanced), for AML only (CIBMTR-modified ELN cytogenetic risk stratification (favourable, intermediate, poor), age, HCT-CI and KPS.

A tabular summary of results is presented in Table .

Table 15: Tabular summary of results of the CIBMTR matched analysis

Comparison of outcomes	ECT-001-CB	CIBMTR	
	Study 001 (n=22)	CBU (n=67)	8/8 MUD PBSC/ BM (n=70)
Median time to neutrophil recovery	**18 d**	21 d p<0.01	13 d p<0.01
Primary graft failure	**0%**	5.1% p=0.28	0 p=NE
Median time to platelet recovery (days)	**42 d**	42 d p=0.84	18 d p<0.01
NRM[1] HR [95% CI]	**4.5%**	0.13 [0.02-0.89] p=0.04	0.20 [0.04-1.10] p=0.06
PFS[2] Probability % [95% CI]	**72.7%**	45.6% [33.5-58] p=0.02	45.4 [33.3-57.7] p=0.02
OS[2] Probability % [95% CI]	**72.7%**	53.6% [41.3-65.7] p=0.09	59.2 [47.1-70.8] p=0.23
GRFS[1] HR [95% CI]	**63.6%**	0.37 [0.02-0.89] p=0.02	0.27 [0.12-0.60] p<0.01
Relapse/ progression[3] Probability % [95% CI]	**22.7%**	24.3% [14.3-36.0] p=0.88	35.2 [23.9-47.4] p=0.26
Grade II-IV acute GvHD[4] Probability % [95% CI]	**63.6%**	48.2 [36.2-60.3] p=0.21	51.6% [39.8-63.3] p=0.33
Grade III-IV acute GvHD[4] Probability % [95% CI]	**9.1%**	16.7% [8.7-26.6] p=0.33	30.1% [19.9-41.4] p=0.01
Chronic GVHD all grade Probability % [95% CI]	**13.6%**	24.8 [14.6-36.6] p=0.24	55.2 [42.6-67.5] p<0.01

[1]HR calculated over a 2-year follow-up period.
[2] Endpoints reported at 2 years, estimates calculated using Kaplan-Meier function.
[3] Endpoints reported at 2 years, estimates calculated using cumulative incidence function.
[4] Endpoints reported at 1 year, estimates calculated using cumulative incidence function.
NE = not evaluated
N.B: Dark green shows better outcome for ECT-001-CB with $p < 0.05$; Light green shows a trend for better outcome for ECT-001-CB with $p < 0.1$ and > 0.05. Dark orange shows better outcome for control with $p < 0.05$.

Study 003/04X

Study 003 is a Phase I/II open-label study of reduced intensity allogeneic transplant of ECT-001-CB in patients with newly diagnosed high-risk multiple myeloma (after induction treatment).

In total 12/20 (60%) of the CB units used for expansion did not meet the minimum cell dose requirements of ASCT for single unmanipulated CB transplant.

Overall, 20 patients were enrolled and 19 infused. Overall, 13 patients were discontinued early, mostly due to relapse (n=8).

Eighteen of the 19 evaluable patients (94.7%) engrafted at a median of 10.5 days (range, 4-18 days). One subject had full primary graft failure after receiving a low intensity conditioning regimen that proved insufficient for donor engraftment and led to primary graft rejection. All evaluable patients (n=18) had a platelet recovery at a median of 36 days (range 25–61).

The estimated cumulative incidence of NRM was 5.3% at 1-year and 15.8% at both 2- and 3-year post-transplant. The probability of 1-, 2- and 3-year post-transplant OS was 89.5%, 73.7% and 68.4%m respectively. PFS at 1-, 2- and 3-year post-transplant was 68.4%, 52.6% and 47.4%, respectively.

The cumulative incidence of grade II-IV acute GVHD was 68.4% at 6- and 12-month post-transplant; and of Grade III-IV 5.3% at both timepoints. The cumulative incidence of chronic GVHD at 1-, 2- and 3-year post-transplant was estimated at 15.8%, of which 10.5% moderate-severe cases. GRFS at 1-, 2- and 3-year post-transplant was estimated at 57.9%, 42.1% and 36.8%, respectively. CRFS was estimated at 63.2%, 47.4% and 42.1%, respectively.

Study 007

Study 007 is a Phase I/II open-label, single-arm, single-centre study of ECT-001-expanded cord blood transplantation in paediatric and young adult (<21 years) patients with high-risk and very high-risk myeloid malignancies. Topline results were shown based on a data cut-off of October 22nd 2024.

In total 3/13 (23.1%) of the CB units used for expansion did not meet the minimum cell dose requirements of ASTCT for single unmanipulated CB transplant.

Twelve of 13 patients enrolled received ECT-001-CB. One patient did not receive ECT-001-CB due to favourable disease evolution prior to transplantation and therefore removed from a per protocol analysis. Eight patients were prematurely discontinued due to primary graft failure (n=1), NRM (n=1) or relapse (n=6).

Of the 12 patients transplanted with ECT-001-CB, 11 engrafted at a median of 19 days (range 12-30 days) with one patient not achieving neutrophil engraftment due to primary graft failure. There was no case of secondary graft failure.

One patient died of NRM, resulting in a cumulative incidence of 0.0% and 12.5% at 6- and 12- months, respectively. The probability of 6-, 12 and 24-month post-transplant OS was 90.9%, 64.9% and 32.5%, respectively. PFS at 6-, 12 and 24-months post-transplant was 73.3%, 50.3% and 16.8%, respectively.

No case of grade III-IV acute GVHD or chronic GVHD has been reported so far. In absence of grade III-IV acute GVHD and chronic GVHD, the composite endpoints GRFS and CRFS were similar to PFS estimates.

2.6.6. Discussion on clinical efficacy

Initially, a conditional marketing authorisation was requested for adult patients with haematological malignancies requiring an allogeneic hematopoietic stem cell transplantation who lack a readily available suitable donor. In response to the list of questions (LoQ), the applicant has reworded the indication to clearly specify the last resort setting, i.e. 'for whom no other type of suitable donor cells is available'. Moreover, the proposed indication is adjusted to reflect that all studied patients received a myeloablative conditioning regimen. In only 0.8% of allogeneic HCTs in adults in Europe a CBU is used, while a suitable standard size CBU is available in 81-96% of patients depending on race and ethnicity. Therefore, the number of patients that lack access to any type of suitable donor (including double-cord) is expected to be very limited in clinical practice.

Design and conduct of clinical studies

Posology cryopreserved dorocubicel –During the clinical development the manufacturing process of dorocubicel has changed from using the CD34+ cells *fresh* directly after culture to a *cryopreserved* formulation. The additional cryopreservation step results in a lower CD34+ cell count and viability. The commercial dosing strategy will be based on a post-cryopreservation CD34+ viable cell concentration, to prevent underestimation of the CD34+ cell dose, with potential lack of efficacy. For the CD34+ cell dose, the lower limit of the release acceptance criteria was based on the minimum dose where clinically acceptable neutrophil engraftment was achieved within acceptable time, resulting in a lower limit of 0.40 x 10^6 vCD34+ cells/kg. Further dosing data to confirm the CD34+ cell dose range will be provided as part of the proposed confirmatory trials ECT-001-CB.011 and ECT-001-CB.012. Please refer to section 2.6.2 on Clinical Pharmacology.

During the conduct of the clinical studies the preferred GvHD prophylaxis was changed to tacrolimus + MMF and the maximum recommended CD34+ cell dose after expansion was capped in order to limit the occurrence of engraftment syndrome. In response to the LoQ, the preferred GvHD prophylaxis is reflected in SmPC section 4.2. Additional analyses have been presented in which no correlation between engraftment syndrome and CD34 cell dose could be detected. Altogether, the proposed post-cryopreservation upper threshold of 7.5 x 10^6 CD34 cells/kg is acceptable and even unlikely to be reached in clinical practice in the proposed target population 'for whom no other type of suitable donor cells is available ' and therefore only have a small sized cord available.

The CD34+ fraction (ECT-001-CB-DP1) contains the expanded CD34+ cells along with a mixture of immune cells, including hematopoietic stem and progenitor cells which are essential for prompt engraftment and regeneration of the blood system of the recipient. Donor T cells arising from engrafted CD34+ hematopoietic stem cells are important for long lasting graft versus tumour effect. The donor T cells present in the CD34- fraction (ECT-001-CB-DP2) are important for the immediate graft vs. tumour effect in stem cell transplantation. The number of CD3+ cells in DP2 is lower compared to unmanipulated cord blood as donor source, due to the small starting size of the selected CBU and cell loss because of the CD34+/CD34- cell selection and cryopreservation. Based on additional justification presented in response to the LoQ, the proposed post-cryopreservation minimum CD3+ cell dose is however considered acceptable (please refer to PD section and quality assessment report).

Design pooled analysis – Main efficacy data was initially derived from a pooled analysis (ISSE) of three single arm open-label Phase I-II studies (Study 001, 002 and 004) of ECT-001-CB following a myeloablative conditioning regimen in patients with haematological malignancies. A new pooled analysis was performed to account for the additional cryopreservation step in the manufacturing process. This analysis included only patients from Study 002 and 004 who received cryopreserved ECT-001-CB derived from a small sized cord. Both pooled analyses were post-hoc with no type I error control and no preplanning. Some of the risks for resulting biases could be mitigated as the analysis methods used are standard for the field. In addition, the registry statisticians developed the matching methods and performed the registry comparison independently of the applicant.

In terms of the proposed setting (i.e. patients 'for whom no other type of suitable donor cells is available '), randomisation against the preferred active treatment is by definition impossible and from this perspective, the applicant's choice for a single arm trial design can be understood. By inclusion criteria, however, some patients, in particular in studies 002 and 004 which did not specifically targeted patients without suitable donors, had a single or double back-up CB that did or did closely satisfy the normal cord blood transplantation criteria of the hospital where study 001 was conducted. Therefore, randomisation seems to have been possible in these latter studies.

The primary endpoint of the pooled analysis, i.e. time to neutrophil engraftment (recovery), was agreed in previous scientific advice. It is an acceptable first step to establish efficacy of ECT-001-CB,

as it reduces bias due to heterogeneity in the patient population. Time to neutrophil engraftment is considered to be able to isolate treatment effect (EMA/CHMP/564424/2021), as spontaneous and fast recovery of neutrophils without transplant is unlikely and (time to) engraftment can be objectively assessed.
The long term clinical time to event outcomes (NRM, PFS, OS, GFRS, time to chronic GvHD and to lesser extent time to short GvHD) cannot be interpreted in a single arm trial. In addition, time to neutrophil engraftment is not predictive for OS, PFS or relapse related mortality and interpretation of long term time to event outcomes is hampered by the heterogeneity in diseases included and small numbers in each disease. Therefore, these long-term outcomes may not refer to 'the' survival rate of a well-described population. However, they are meaningful in comparison to the matched registry data to assess whether a detrimental effect compared to normal (-sized cord blood) transplantation in the long term time to event outcomes is present.

Additional to the problems because of the single arm trial designs, all time related endpoints were defined as time from infusion, which is considered problematic as it ignores the time needed to manufacture, ship and apply the product. Limited data on the impact of time zero on the endpoints has been presented. It is acceptable that further information will become available post approval with planned RCT Study ECT-001-CB.011. Moreover, while the initial analyses for time to engraftment considered only subjects with successful engraftment, in response to the list of questions, additional analyses including all subjects were presented.

Supportive real world data - Context for interpretation of the efficacy outcomes derived from the single-arm clinical trials is provided through formal registry matched controlled comparisons. As the reason for not having a readily available donor is unrelated to the prognosis, the registry matched controlled comparison with outcomes of patients that do have a donor available is acceptable. The EBMT unmanipulated CB cohort is considered of most relevance in the context of the different donor sources comparisons in the real-world registry data. However, several biases (e.g. assessment bias [except for OS]; bias due to lack of preplanning and variability in disease history) render the use of these real world data not fully suitable for contextualization.

Additional supportive data is derived from Study 003 and 007 and a few patients treated in a compassionate use program. These studies have not been included in the pooled analysis due to differences in study population (different conditioning regimen and age group).

Generalizability to EU population- No efficacy data have been presented for patients included in clinical studies in the EU, the study population mainly consists of patients from Canada. Study 001 and 002 were conducted at 1 centre in Canada, and Study 004 in the US. The Canadian population is similar to the European Union population in terms of diversity and disease prevalence. Clinical treatment practice guidelines for haematological diseases or allogeneic HSCT used in Canada, as well as CB selection criteria used throughout the studies are in line with current European practice. Generalizability of the study results obtained in the US and Canada to European clinical practice is considered sufficiently justified.

Efficacy data and additional analyses

Study population- Despite differences in disease characteristics between the 3 main clinical studies, and not all haematological malignancies being studied, it is the transplant procedure which ties the primary results of the trials together. Hence, it is acceptable that not for all haematological malignancies a high number of patients was included and several (in particular rare types) were not investigated. Besides, additional information will be collected post-approval via a registry and (ongoing) clinical studies.

Follow-up – The new pivotal FAS comprised only 25 patients (24 transplanted). The median duration of follow-up was 13.27 months at the updated data cut-off (March 15th 2024). Follow-up for 7 patients (28%) in study 002 and 004 is still ongoing. Longer follow-up is available for the previous defined pooled analysis including non-cryopreserved ECT-001-CB (median 23.87 months). In the context of a CMA, longer follow-up data for cryopreserved ECT-001-CB will be presented post-approval.

Engraftment - After transplantation, neutrophil recovery was prompt (median time 20 days in engrafted patients, 25 days for all patients), robust (84% of patients achieved engraftment by day 42 post-HSCT) and sustained (no secondary graft failure occurred up to date) in the pooled study population who received cryopreserved ECT-001-CB derived from a small size cord (pivotal FAS, n=25). Platelet recovery occurred within a median time of 40 days for patients who reached platelet engraftment (48 days when analysed in all patients) and was achieved by 68% of patients by Day 100 post-HSCT.

Long term efficacy – Next to engraftment results, it should be demonstrated that graft versus tumour effect is maintained with a transplant related mortality and side effects within an acceptable range. The cumulative incidence of NRM was found to be 21.2% (95% CI: 4 – 38) at both 12 and 24 months in the pivotal FAS. At 1- and 2-years post- transplantation, OS was estimated at 66% (95% CI 49-89) and 51.4% (95% CI 32-82) in the pivotal FAS. The estimated PFS at 1- and 2-year was 52.8% (95% CI: 36-78) and 45.3% (95% CI 28-74), respectively. The proportion of patients with relapse was ~35.7% after 12 months in the pivotal FAS. While slightly higher non-relapse mortality (NRM) and lower PFS and OS rates were observed in the new cryopreserved subsets compared to the old FAS, these differences might reflect the baseline risk of the included patients (limited to those with high or very high-risk disease risk). It can however not be excluded that the differences were (also) caused by the cryopreservation step and/or small sized cord. The proportions of relapsed patients remained comparable among the study groups at each analysed time point.

Isolation of long-term treatment effect is not possible based on single arm trials. Main contextualisation of engraftment and time-dependent endpoints is provided by EBMT matched analyses (Study 2). Given possible biases for long term clinical outcomes because of the single arm trial vs. registry comparison and the lack of prespecification of what constitutes a similar effect, a firm conclusion on a similar/superior effect could not be drawn. In the main comparison with unmanipulated CB, neutrophil engraftment was attained in 86.4% of patients treated with cryopreserved ECT-001-CB derived from a small cord, with a median time to engraftment of 20 days compared to 85.4% and 25 days for EBMT control group who received an unmanipulated CBU transplant and seemed similar to other donor sources. Results for matched analyses of time dependent endpoints NRM, PFS, OS and CRFS between patients treated with cryopreserved ECT-001-CB derived from a small cord and the EBMT matched cohorts seem numerically slightly worse for ECT-001-CB. No statistically significant differences were observed, however, interpretation of p-values is hampered by the small number of patients and limited follow-up in the ECT-001-CB cohort. The higher incidence of acute GvHD in the ECT-001-CB cohort often led to a lower GRFS. Results for chronic GvHD seem better for the ECT-001-CB cohort, but these outcomes are even more hampered by the limited follow-up time. With additional matching on comorbidity index HCT-CI, OS results seemed comparable between EBMT unmanipulated cord and cryopreserved ECT-001-CB.

Overall, considering the high risk disease study population and last resort setting without available other donor source, results are still considered promising and not indicative of a longer term detrimental effect of cryopreserved ECT-001-CB. This is acceptable for a CMA, but further confirmation is needed post-approval. This will be obtained via the clinical studies proposed as specific obligations.

Subgroups - Subgroup analyses are based on the smFAS population (n=82), which is acknowledged considering the limited number of patients in the pivotal FAS population (n=25). However, with

updated data, the sample size might be sufficient to at least rule out the most critical differences. The influence of the intrinsic and extrinsic factors on efficacy results in the target population remains unknown. This will be further analysed post-marketing in the planned randomised trial and registry.

HLA matching impacted some efficacy parameters (platelet engraftment and NRM). Patients with 5/8 HLA matched ECT-001-CB had a higher incidence of NRM vs. 6+/8 matched ECT-001-CB at 24 months (8/44 or 23.79% CI in 5/8, vs 1/29 or 4.37% CI in 6+/8). It is agreed that every additional mismatch increases the risk of NRM and a confounding factor might consist of participants with high-risk disease recruited in Study 002 and 004, who receive in majority 5/8 HLA-matched transplants.

All NRM events occurred in patients who received an intermediate instead of high intensity conditioning regimen. It is acknowledged that a confounding factor consisted of older participants receiving intermediate conditioning regimen intensity. All NRM events in the efficacy cohort occurred in patients 40 years or older. This translated into a lower OS, PFS, GRFS and CRFS in patients 40 years or older. This is not unexpected as older age is an important risk factor for NRM.

Supportive studies 003 and 007 - High level (interim) results have been presented for supportive Studies 003 and 007. High level engraftment results for Study 003 in patients with multiple myeloma) generally supported efficacy of ECT-001-CB in the pooled analysis. There was 1 primary graft failure in a patient who received a low intensity conditioning regimen (which is currently not recommended in combination with ECT-001-CB in the SmPC).

Study 007 investigated paediatric patients, and the number of infused patients is still very limited (n=12). Consequently, this study is of limited support to the efficacy of ECT-001-CB in adults. Available data is adequately reflected in SmPC section 5.1.

Additional expert consultation

Zemcelpro was selected for early dialogue with healthcare providers (EHA). The healthcare providers described the different sources for allogeneic HSCT. About 20 to 30% of the patients will have an HLA identical sibling as potential stem cell donor. An alternative is a HLA-compatible unrelated donor. The likelihood of finding a HLA-compatible unrelated donor in international registries for the Caucasian population is about 80%, while this is significantly reduced to less than 50% for other ethnic populations (e.g. Caribbean, African etc.).
The use of haploidentical stem cell transplantation by using novel graft-versus-host disease prophylactic treatment has become the most frequently used stem cell source for patients lacking an HLA-identical sibling or completely matched unrelated donor and is available in about 90% of the patients.
Cord blood transplantation is an alternative stem cell source for those who are lacking an HLA-identical sibling or unrelated donor. However, the most recent development of haploidentical stem cell transplantation suggests a better outcome in comparison to standard (unexpanded) cord blood transplantation. Currently the number of cord blood transplantations in stem cell registries in Europe and US is steadily declining, but there are still some indications, especially if expansion leads to faster engraftment and the suggested stronger graft-versus-leukemia effect by retrospective studies can be confirmed.

Assessment of paediatric data on clinical efficacy

The target population comprises adults only. Supportive Study 007 investigates efficacy in paediatric patients (see Section 2.6.5.6 Supportive studies).

Additional efficacy data needed in the context of a conditional MA

Taking into consideration the single arm trial design, the limited number of patients enrolled in the pivotal studies and the lack of long-term follow-up of the treated patients, the committees considered that additional efficacy data should be provided to satisfy the incomprehensiveness of the dossier. In the context of a conditional MA approval, the applicant shall:

- Submit the final results of the pivotal single-arm trials (CT-001-CB.002 and 004),
- Provide additional data from patients aged 18-21 years included in the paediatric study.
- Provide additional efficacy data in the target population by a real-world registry study from patients treated with marketed Zemcelpro in the approved indication in Europe.
- Provide confirmation of the efficacy by conducting and submitting the results of a randomised phase 3 study comparing Zemcelpro with best available allogeneic stem cell source in adult patients with high-risk leukaemia/myelodysplasia.

2.6.7. Conclusions on the clinical efficacy

Overall, considering the high risk disease targeted and last resort clinical setting of patients without available donor source, efficacy results are considered promising and acceptable for a conditional MA. However further confirmation is needed post-approval via the post-authorisation clinical studies included as specific obligations, in particular to address uncertainties regarding the dosing, contextualization of time-dependent endpoints, lack of comparator, limited sample size, and long-term effect.

The CAT considers the following measures (Specific Obligations) necessary to address the missing efficacy data in the context of a conditional MA:

- In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall submit the final results from Study ECT-001-CB.002: A Phase II Open-label Study of ECT-001-expanded Cord Blood Transplantation in Patients with High Risk Acute Leukemia/myelodysplasia.

- In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall submit the final results from Study ECT-001-CB.004: Phase II Open-Label Study of ECT-001-Expanded Cord Blood Transplantation in Patients with High and Very High-Risk Acute Leukemia/Myelodysplasia.

- In order to confirm the efficacy and safety of Zemcelpro in patients aged 18-21 years with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall conduct and submit the results of the subgroup analysis of patients aged 18-21 years from study ECT-001-CB.010: A Prospective Randomized Phase II Trial of Allogeneic SCT with ECT-001-CB Expanded Cord Blood Transplant Without Serotherapy Versus Other Stem Cell Source in Pediatric Patients with High risk/refractory/relapsed Acute Myeloid Leukaemia, according to an agreed protocol.

- In order to confirm the efficacy and safety of Zemcelpro, and to further evaluate the dose parameters used in adult patients with high-risk and very high-risk acute leukaemia/MDS, the MAH shall submit the results of study ECT-001-CB.011: A Multicenter, Prospective,

Randomized, Open-Label Phase III Study of ECT-001-CB (ECT-001-Expanded Cord Blood) Transplantation versus Best Alternative Allogeneic Stem Cell Source Transplantation (Haplo, MMUD) in Patients with High-Risk Acute Leukemia/Myelodysplasia which is conducted according to an agreed protocol.

- In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall conduct and submit the results of a prospective, non-interventional study based on data from a registry, and evaluate dose parameters collected for Zemcelpro lot manufactured for each patient enrolled in the study, according to an agreed protocol.

The CHMP endorses the CAT conclusion on clinical efficacy as described above.

2.6.8. Clinical safety

To provide a sufficient sample size for safety evaluation, safety analysis has been performed on the totality of patients treated with ECT-001-CB drug product across all clinical trials. The Safety Analysis Set (SAS, n=116) at the March 15th, 2024 cutoff date consists of all patients who received an ECT-001-CB transplant with single dose intravenous administration of ECT-001-CB included in the five single-arm open-label clinical trials with ECT-001-CB (from studies ECT-001-CB.001, ECT-001-CB.002, ECT-001-CB.003,ECT-001-CB.004 and ECT-001-CB.007).

2.6.8.1. Patient exposure

The patient population included in the SAS is heterogenous as patients were treated for several types of haematological malignancies, including acute leukaemias, myelodysplasia, lymphomas, and multiple myeloma. Moreover, various conditioning regimens were used, from regimens of high and intermediate intensities in Studies 001, 002, 004 and 007 and low to intermediate intensities in Study 003. As several studies were still ongoing at time of data cut-off, only 18 patients had completed their respective study follow-up, 18 patients were ongoing, and 58 patients were discontinued early. Reasons for early discontinuation included relapse (n=38, 32.8%), NRM (n=14, 12.1%), noncompliance (n=1, 0.9%) and primary graft failure (n=4, 3.4%). Median follow-up for the SAS was 22.49 months (range 0.89-48.4 months), with 67 (57.8%) and 48 (41.1%) patients reaching 12 and 24 months, respectively (Table).

Table 16: Overall participant disposition in the SAS pool as of November 30th 2022.

Disposition Reason	**Study 003*** n (%)	**ISSE data** n (%)	**SAS pool*** n (%)
ECT-001-CB infused	18	116	116
Study follow-up completed	4 (22.2%)	36 (31.0%)	40 (34.5%)
Study follow-up ongoing	0 (0.0%)	18 (15.5%)	18 (15.5%)
Discontinued early	14 (77.8%)	44 (37.9%)	58 (50.0%)
Relapsed	10 (55.6%)	28 (24.1%)	38 (32.8%)
NRM	2 (11.1%)	12 (10.3%)	14 (12.1%)
Primary graft failure	1 (5.6%)	3 (2.6%)	4 (3.4%)
Noncompliance	1 (5.6%)	0 (0.0%)	1 (0.9%)
Lost to follow-up	0 (0.0%)	1 (0.9%)	1 (0.9%)
Missing	0 (0.0%)	18 (15.5%)	0 (0.0%)
Patients reaching 12 months**	11 (61.1%)	56 (48.3%)	67 (57.8%)

Patients reaching 24 months**	8 (44.4%)	40 (34.5%)	48 (41.4%)
*Missing data for 18 participants' disposition in Study 003 in the ISSE Addendum-2024 analysis was completed using data extracted from the applicant internal database. ** patients reaching 12 or 24 months without an event that would have led to early discontinuation (includes relapse, NRM and primary graft failure) Source: ISSE Addendum-2024 Table 1, RPT-0023 Supplementary Table S2.			

Conditioning regimens

In the SAS, all patients received a preconditioning regimen after enrolment and prior to infusion of ECT-001-CB. The list of the different conditioning regimens used in the five studies contributing to the evaluation of safety is shown in Table .

Table 17: Description of conditioning regimens intensity

Code	Conditioning regimen description	Intensity score*	Studies	SAS n=116 n (%)
Cy-TBI13.2Gy-Flu or Cy-TBI12Gy-Flu	- Fludarabine 75 mg/m^2 - Cyclophosphamide 120 mg/kg - TBI 12 or 13.2 Gy	High (4.5)	001, 002 and 004	23 (19.8%)
Clo-Flu-Bu	- Clofarabine 120 mg/m^2 - Fludarabine 40mg/m^2 - Busulfan, IV weight based with PK analysis – target of 90mg*h/L x 4 days	High (4.0)	007	8 (6.9%)
Bu-TT-Flu	- Busulfan 12 mg/kg PO or 9.6 mg/kg IV - Thiotepa 10 mg/kg - Fludarabine 150 mg/m^2	Intermediate (3.5)	002	3 (2.6%)
Flu-Cy-TT-TBI4Gy (MIDI) or Flu-Cy-TT-TBI2Gy	- Fludarabine 150 mg/m^2 - Cyclophosphamide 50mg /kg - Thiotepa 10mg/kg - TBI 200 or 400 cGy	Intermediate (3.0)	001, 002, 004 and 007	64 (55.2%)
Cy-Flu-TBI4Gy	- Cyclophosphamide 50 mg/kg - Fludarabine 200 mg/m^2 - TBI 400 cGy	Intermediate (2.5)	003	9 (7.8%)
Cy-Flu-TBI2Gy	- Cyclophosphamide 50 mg/kg - Fludarabine 150 mg/m^2 - TBI 200 cGy	Low (2.0)	003	7 (6.0%)
Flu-TBI2Gy	- Fludarabine 90 mg/m^2 - TBI 200 cGy	Low (1.5)	003	2 (1.7%)
*Regimen intensity score calculated according to Spyridonidis et al, 2020.				

GVHD prophylaxis and supportive care

All patients received GVHD prophylactic treatment. At the initiation of Study 001, GVHD prophylaxis consisted of Cyclosporine A and mycophenolate mofetil (MMF).

- Cyclosporine was administered from day -3 (prior to infusion of ECT-001-CB) at 1.5-3.2 mg/kg intravenously every 12 hours with the aim of maintaining a trough level of 200 to 500 µg/L. Full dose of Cyclosporine A was administered until day +100 post-transplant. In the absence of GVHD, the dose was tapered by 10% per week and discontinued by day +180.
- MMF was also started on day -3 (prior to infusion of ECT-001-CB) and continued until day +35 at which time it is discontinued without taper. Dose is 15 mg/kg intravenously every 8 hours.

However, 2 cases of severe (grade 4) engraftment syndrome occurred in patients who receive this GVHD prophylactic treatment. As cyclosporine was previously identified as a risk factor for engraftment syndrome (Kanda et al, 2013[55]), the GVHD prophylaxis was changed from Cyclosporine A to

[55] Kanda J et al (2013). Pre-engraftment syndrome after myeloablative dual umbilical cord blood transplantation: risk factors and response to treatment. Bone Marrow Transplant. 48(7):926-31. doi: 10.1038/bmt.2012.279.

Tacrolimus in 2017. Following this change, all other patients enrolled in all ECT-001-CB studies received Tacrolimus and MMF:

- Tacrolimus was administered from day -3 (prior to infusion of ECT-001-CB) at 0.03 mg/kg intravenously daily by continuous infusion with the aim of maintaining a trough level of 10-15 µg/L.
- MMF is also started on day -3 (prior to infusion of ECT-001-CB) and continued until day +35 at which time it is discontinued without taper. Dose is 15 mg/kg intravenously every 8 hours.

In addition to GVHD prophylaxis, granulocyte colony stimulating factor (G-CSF) was administered at 5 µg/kg/day by subcutaneous injections starting on day +2 to all patients until absolute neutrophil count (ANC) reaches 1.5 x 10^9/L. Similar to supportive care provided with standard-of-care for HSCT, patients also received antibacterial, antifungal, antiviral and anti-parasite prophylaxis, transfusional support and post-transplant vaccination per the respective study protocols.

Premedication for infusion related reactions are recommended in the SmPC section 4.2. with antipyretics, histamine antagonists, and corticosteroids, or equivalent medicinal products, according to local institutional guidelines, be administered 30-60 minutes before the infusion of Zemcelpro to reduce the possibility of an infusion reaction.

ECT-001-CB

The clinical dose of CD34+ and CD3+ cells administered for all studies with ECT-001-CB were within the following:

- vCD34+ cells in dorocubicel: 0.5 to 7.5 x 10^6/kg vCD34/kg, and
- vCD3+ cells in ECT-001-CB-DP2: ≥1.0 x 10^6/kg vCD3/kg

In the SAS, all patients received doses of vCD34+ and vCD3+ cells within the target range. The median dose of vCD34 cells was 3.85 x 10^6/kg (range 0.58 to 7.50 x 10^6/kg) and the median dose of vCD3+ cells was 2.60 x 10^6/kg (range 0.97 to 16.5 x 10^6/kg) (Table 1).

Table 1 ECT-001-CB administration in the SAS (safety set).

Description	Study 001 *N=26*	Study 002 *N=30*	Study 003 *N=18*	Study 004 *N=30*	Study 007 *N=12*	SAS *N=116*
vCD34 cell dose (x10^6/kg)						
Mean (SD)	2.97 (1.56)	4.49 (1.55)	4.25 (1.19)	3.24 (1.67)	2.60 (1.26)	3.69 (1.67)
Median	2.88	4.86	4.75	3.07	2.60	3.85
Min-Max	0.59-6.30	1.35-7.50	2.07-5.76	0.58-5.75	1.34-3.86	0.58-7.50
vCD3 cell dose (x10^6/kg)						
Mean (SD)	2.97 (0.81)	2.85 (1.33)	2.80 (0.92)	2.43 (1.41)	15.12 (1.46)	2.98 (2.07)
Median	2.87	2.68	2.55	2.08	15.12	2.60
Min-Max	1.23-4.49	1.32-6.75	1.09-4.38	0.97-6.45	13.66-16.57	0.97-16.57

Characteristics of the safety population

The SAS population consisted of patients of 1 to 66 years of age, including 63.8% male and 36.2% female. All patients had a Karnofsky performance status of at least 70, with 38.8% of patients having KPS of 70-80 and 61.2% of ≥90 (see tables below).

Table 19: Participant Demographics at Baseline in the SAS Population (Safety set)

Participants demographics	SAS (N=116)
Age (years)	
Mean (SD)	41.3 (16.6)
Median (Min-Max)	44 (1-66)
Sex, n (%)	
Male	74 (63.8%)
Female	42 (36.2%)
Race, n (%)	
American Indian or Alaska Native	3 (2.6%)
Asian	3 (2.6%)
Black	7 (6.0%)
Native Hawaiian or Other Pacific Islander	3 (2.6%)
White	93 (80.2%)
Other	7 (6.0%)
Ethnicity, n (%)	
Hispanic or Latino	11 (9.5%)
Not Hispanic or Latino	56 (48.3%)
Unknown	2 (1.7%)
Not reported	3 (2.6%)
Missing	44 (37.9%)
KPS, n (%)*	
70-80	45 (38.8%)
90-100	71 (61.2%)
*Missing data for 003 extracted from the applicant internal database.	

Most participants included into this analysis suffered from acute myeloid leukemia as their underlying disease, followed by acute lymphoid leukemia, multiple myeloma and myelodysplastic syndrome (Table).

Table 20: Participant Primary Disease History in the SAS population (Safety set)

Primary Disease History	SAS (N=116)
Disease Category	
Acute Myeloid Leukaemia (AML)	50 (43.1%)
Acute Lymphoid Leukaemia (ALL)	28 (24.1%)
Myelodysplastic Syndrome (MDS)	14 (12.1%)
Chronic Myelogenous Leukaemia: patients who progressed to blast crisis	1 (0.9%)
Hodgkin Lymphoma	1 (0.9%)
Non-Hodgkin Lymphoma, aggressive lymphoma: Diffuse large B cell lymphoma	2 (1.7%)
Adult T-cell Leukaemia/Lymphoma	1 (0.9%)
Chronic Lymphocytic Leukaemia and transformation to Hodgkin lymphoma	1 (0.9%)
Multiple Myeloma	18 (15.5%)
Received previous allogenic HSCT in the past	
Yes	31 (26.7%)
No	85 (73.3%)

2.6.8.2. Adverse events

In all protocols of ECT-001-CB, only AEs of grade ≥3 (i.e. severe, life-threatening or fatal) or highly unusual grade 2 AEs were reported, as such a complete adverse drug event profile of all CTCAE grade AEs cannot be generated for ECT001. Association or relatedness of AEs to the study agent were assessed by the investigator according to the categories definite, probable, possible, unlikely, unrelated. Adverse drug reactions were AEs with a definite, probable or possible relation to the study agent. All grade 3-5 toxicity (CTCAE) events and highly unusual grade 2 AEs were compiled at the start of the conditioning regimen through to the time of discharge from the transplant centre (approximately 3M/Day 100) and thereafter for the 6, 12 and 24 months timepoints. Per protocol, grade 1 and 2 safety events were systematically not reported throughout the different trials.

Whereas investigator-initiated Studies 001, 002 and 003 reported all AEs whether intended or not, ExCellThera-initiated Study 004 and 007 limited AE reporting to unintended events, i.e., haematological events or abnormal laboratory values directly related to conditioning regimens were not reported as AEs in these 2 trials. As several haematological AEs reported in Studies 001, 002 and 003 are caused by the conditioning regimen received as part of the treatment plan prior to infusion of the cells, the frequency of haematological AE has also been reported specifically for Studies 004 and 007 combined, which only reported unintended AEs. In addition, acute and chronic GVHD were evaluated as part of the efficacy endpoints in all ECT-001-CB trials using the NIH criteria (Jagasia et al, 2015, Harris et al. 2016) and as safety endpoint using the NCI CTCAE.

A total of 115 participants (99.1%) suffered from one or more AEs during the conduct of their study (see Table *2*). All of these events were considered to be either severe, life-threatening or fatal – only those three types of severity grades were analysed and displayed for the purposes of the safety analysis. Of all the participants, 103 participants had an AE that was considered related to study treatment (88.8%, adverse drug reaction).

Table 2 Overall Summary of Adverse Events

	SAS (N=116)
Number of participants with any AEs of grade ⩾3	115 (99.1%)
Number of participants with any serious AEs	61 (52.6%)
Number of participants with any AEs related to study treatment*	103 (88.8%)

*AEs related to study treatment were the AEs that have possible, probable, and definite relationship to study drug.

The most frequently reported system organ classes were 'blood and lymphatic disorders' (82.8%), followed by 'infections and infestations' (76.7%), 'metabolism and nutrition disorders' (69.8%), 'gastrointestinal disorders' (50.9%), 'vascular disorders' (42.2%), 'immune system disorders' (37.9%), 'general disorders and administration site conditions' (35.3%) and 'investigations' (35.3%) (Table).

Table 22: Adverse Events (grade ≥3) Post-ECT-001-CB Transplant, Regardless of Study Drug Relationship, By System Organ Class (Safety Set)

System Organ Class		**SAS (N=116)**
Number of participants with any AEs, n (%)	**115**	**(99.1%)**
Blood and lymphatic system disorders*	96	(82.8%)
Infections and infestations	89	(76.7%)
Metabolism and nutrition disorders	81	(69.8%)
Gastrointestinal disorders	59	(50.9%)
Vascular disorders	49	(42.2%)
Immune system disorders	44	(37.9%)
General disorders and administration site conditions	41	(35.3%)
Investigations	41	(35.3%)

System Organ Class		SAS (N=116)
Respiratory, thoracic and mediastinal disorders	24	(20.7%)
Musculoskeletal and connective tissue disorders	20	(17.2%)
Renal and urinary disorders	19	(16.4%)
Nervous system disorders	19	(16.4%)
Skin and subcutaneous tissue disorders	16	(13.8%)
Cardiac disorders	13	(11.2%)
Psychiatric disorders	13	(11.2%)
Injury, poisoning and procedural complications	8	(6.9%)
Hepatobiliary disorders	5	(4.3%)
Neoplasms benign, malignant and unspecified	3	(2.6%)
Eye disorders	2	(1.7%)
Reproductive system and breast disorders	2	(1.7%)
Congenital, familial and genetic disorders	2	(1.7%)
Ear and labyrinth disorders	1	(0.9%)
Endocrine disorders	1	(0.9%)
Surgical and medical procedures	1	(0.9%)

AEs were classified into SOC and preferred term using MedDRA Version 23.1 or later.
A participant with multiple events coding to the same primary SOC was counted only once for that primary SOC.
*: include the occurrence of intended events reported in the investigator-initiated Studies 001/002/003 protocols.

Whereas investigator-initiated Studies 001, 002 and 003 reported all AEs whether intended or not, ExCellThera-initiated Study 004 and 007 limited AE reporting to unintended events, i.e., haematological events or abnormal laboratory values directly related to conditioning regimens were not reported as AEs in these 2 trials. The Summary of Product Characteristics (SmPC) reports all safety events whether they were intended or not.

Table 23. Adverse Events (grade ≥3) post-ECT-001-CB Transplant with Frequency ≥5%, Regardless of Study Drug Relationship, by Preferred Term and Maximal Toxicity Grade (Safety Set)

Preferred Term	SAS (N=116)							
	Grade 3		Grade 4		Grade 5		All grades	
Number of participants with any AEs , n(%)	**27**	**(23.3%)**	**71**	**(61.2%)**	**17**	**(14.7%)**	**115**	**(99.1%)**
Neutropenia*	5	(4.3%)	70	(60.3%)	0	(0.0%)	75	(64.7%)
Thrombocytopenia*	0	(0.0%)	74	(63.8%)	0	(0.0%)	74	(63.8%)
Leukopenia*	0	(0.0%)	73	(62.9%)	0	(0.0%)	73	(62.9%)
Lymphopenia*	1	(0.9%)	70	(60.3%)	0	(0.0%)	71	(61.2%)
Anaemia*	62	(53.4%)	4	(3.4%)	0	(0.0%)	66	(56.9%)
Hypertension	44	(37.9%)	0	(0.0%)	0	(0.0%)	44	(37.9%)
Febrile Neutropenia*	41	(35.3%)	0	(0.0%)	0	(0.0%)	41	(35.3%)
Hyperglycaemia	34	(29.3%)	0	(0.0%)	0	(0.0%)	34	(29.3%)
Decreased Appetite	33	(28.4%)	0	(0.0%)	0	(0.0%)	33	(28.4%)
Nausea	29	(25.0%)	0	(0.0%)	0	(0.0%)	29	(25.0%)
Acute Graft Versus Host Disease[#]	23	(19.8%)	2	(1.7%)	2	(1.7%)	27	(23.3%)
Hypogammaglobulinaemia	22	(19.0%)	0	(0.0%)	0	(0.0%)	22	(19.0%)
Pneumonia	20	(17.2%)	1	(0.9%)	1	(0.9%)	22	(19.0%)
Diarrhoea	20	(17.2%)	0	(0.0%)	0	(0.0%)	20	(17.2%)
Sepsis	16	(13.8%)	2	(1.7%)	2	(1.7%)	20	(17.2%)
Mucosal Inflammation	19	(16.4%)	0	(0.0%)	0	(0.0%)	19	(16.4%)
Hypervolaemia	16	(13.8%)	0	(0.0%)	0	(0.0%)	16	(13.8%)
Stomatitis	16	(13.8%)	0	(0.0%)	0	(0.0%)	16	(13.8%)
Alanine Aminotransferase Increased	13	(11.2%)	1	(0.9%)	0	(0.0%)	14	(12.1%)
Engraftment Syndrome	11	(9.5%)	2	(1.7%)	0	(0.0%)	13	(11.2%)
Hypokalaemia	13	(11.2%)	0	(0.0%)	0	(0.0%)	13	(11.2%)
Pyrexia	13	(11.2%)	0	(0.0%)	0	(0.0%)	13	(11.2%)
Acute Kidney Injury	12	(10.3%)	0	(0.0%)	0	(0.0%)	12	(10.3%)
Hypophosphataemia	12	(10.3%)	0	(0.0%)	0	(0.0%)	12	(10.3%)
Rash Maculo-Papular	12	(10.3%)	0	(0.0%)	0	(0.0%)	12	(10.3%)
Blood Creatinine Increased	11	(9.5%)	0	(0.0%)	0	(0.0%)	11	(9.5%)

Preferred Term	SAS (N=116)							
	Grade 3		Grade 4		Grade 5		All grades	
CD4 Lymphocytes Decreased	9	(7.6%)	2	(1.7%)	0	(0.0%)	11	(9.5%)
Epstein-Barr Virus Infection Reactivation	10	(8.6%)	0	(0.0%)	0	(0.0%)	10	(8.6%)
Bacteraemia	10	(8.6%)	0	(0.0%)	0	(0.0%)	10	(8.6%)
Coronavirus infections	9	(7.8%)	0	(0.0%)	1	(0.9%)	10	(8.6%)
Device related infection	9	(7.8%)	0	(0.0%)	0	(0.0%)	9	(7.8%)
Headache	9	(7.8%)	0	(0.0%)	0	(0.0%)	9	(7.8%)
Hypomagnesaemia	9	(7.8%)	0	(0.0%)	0	(0.0%)	9	(7.8%)
Clostridium Difficile Colitis	8	(6.9%)	0	(0.0%)	0	(0.0%)	8	(6.9%)
Fatigue	7	(6.0%)	0	(0.0%)	0	(0.0%)	7	(6.0%)
Epstein-Barr Viraemia	7	(6.0%)	0	(0.0%)	0	(0.0%)	7	(6.0%)
Hyponatraemia	5	(4.3%)	0	(0.0%)	1	(0.9%)	6	(5.2%)
Transplant failure	0	(0.0%)	6	(5.2%)	0	(0.0%)	6	(5.2%)
Syncope	6	(5.2%)	0	(0.0%)	0	(0.0%)	6	(5.2%)

AEs were classified into SOC and preferred term using MedDRA Version 23.1 (or later).
A participant with multiple events coding to the same preferred term within a primary SOC was counted only once for the preferred term within that primary SOC.
*: include the occurrence of intended events reported in the investigator-initiated Studies 001/.002/.003 protocols. If limited to unintended events as in ECT-001-CB.004/.007 trials, the incidence of neutropenia, thrombocytopenia, leukopenia, lymphopenia becomes lower than 5.0%, that of anaemia 23.8% and that of febrile neutropenia 33.3%.
#: as per CTCAE.Source: *ISSE Addendum-2024, Post-Text Table 14.3.2 and 14.3.1.11., RPT-0023 Supplementary Table S4.*

2.6.8.3. Adverse drug reactions

The majority of participants reported at least one adverse drug reaction (n=103 or 88.8% of the SAS population). The maximal grade of ADR experienced was severe for n=37 (31.9%), life-threatening for n=57 (49.1%), and fatal for n=9 participants (7.8%, Table).

Table 24. Adverse Reactions (grade ≥3) Post-ECT-001-CB Transplant by Maximal Toxicity Grade (Safety Set)

System Organ Class	Preferred Term	Maximal Toxicity Grade	SAS (N=116)	
Overall	Overall	Total	103	(88.8%)
		Severe	37	(31.9%)
		Life threatening	57	(49.1%)
		Death	9	(7.8%)

AEs were classified into SOC and preferred term using MedDRA Version 23.1 (or later).
A participant with multiple events coding to the same preferred term within a primary SOC was counted only once for the preferred term within that primary SOC.

The most frequently occurring events suspected to be related to study drug (≥ 10% all patients; grade ≥3) were: lymphopenia (46.6%), anaemia (44.0%), neutropenia (35.3%), thrombocytopaenia (31.9%), leukopenia (29.3%), acute GVHD (22.4%), hypogammaglobulinaemia (18.1%), febrile neutropenia (15.5%), engraftment syndrome (11.2%), hypertension (12.9%), pneumonia (11.2%). Fatal ADRs included sepsis (0.9%), pulmonary alveolar haemorrhage (PAH) (1.7%), acute GVHD (1.7%), enterococcal infection (0.9%), idiopathic pneumonia syndrome (0.9%), organizing pneumonia (0.9%), pneumonia (0.9%), and pulmonary hypertension (0.9%, see Table).

Table 25. Adverse Reactions (grade ≥3) Post-ECT-001-CB Transplant of frequency ≥5% by Preferred Term and Maximal Toxicity Grade (Safety Set)

Preferred Term	SAS (N=116)							
	Grade 3		Grade 4		Grade 5		All grades	
Number of participants with any ADRs, n (%)	37	(31.9%)	57	(49.1%)	9	(7.8%)	103	(88.8%)
Lymphopenia*	5	(4.3%)	49	(42.2%)	0	(0.0%)	54	(46.6%)
Anaemia*	50	(43.1%)	1	(0.9%)	0	(0.0%)	51	(44.0%)
Neutropenia*	8	(6.9%)	33	(28.4%)	0	(0.0%)	41	(35.3%)
Thrombocytopenia*	2	(1.7%)	35	(30.2%)	0	(0.0%)	37	(31.9%)
Leukopenia*	2	(1.7%)	32	(27.6%)	0	(0.0%)	34	(29.3%)
Acute Graft Versus Host Disease [#]	22	(19.0%)	2	(1.7%)	2	(1.7%)	26	(22.4%)
Hypogammaglobulinemia	21	(18.1%)	0	(0.0%)	0	(0.0%)	21	(18.1%)
Febrile Neutropenia*	18	(15.5%)	0	(0.0%)	0	(0.0%)	18	(15.5%)
Hypertension	15	(12.9%)	0	(0.0%)	0	(0.0%)	15	(12.9%)
Engraftment Syndrome	11	(9.5%)	2	(1.7%)	0	(0.0%)	13	(11.2%)
Pneumonia	11	(9.5%)	1	(0.9%)	1	(0.9%)	13	(11.2%)
Cd4 Lymphocytes Decreased	9	(7.8%)	2	(1.7%)	0	(0.0%)	11	(9.5%)
Epstein-Barr Virus Infection Reactivation	10	(8.6%)	0	(0.0%)	0	(0.0%)	10	(8.6%)
Decreased Appetite	10	(8.6%)	0	(0.0%)	0	(0.0%)	10	(8.6%)
Diarrhoea	10	(8.6%)	0	(0.0%)	0	(0.0%)	10	(8.6%)
Nausea	10	(8.6%)	0	(0.0%)	0	(0.0%)	10	(8.6%)
Rash Maculo-Papular	9	(7.8%)	0	(0.0%)	0	(0.0%)	9	(7.8%)
Sepsis	6	(5.2%)	1	(0.9%)	1	(0.9%)	8	(6.9%)
Epstein-Barr Viraemia	6	(5.2%)	0	(0.0%)	0	(0.0%)	6	(5.2%)
Pyrexia	6	(5.2%)	0	(0.0%)	0	(0.0%)	6	(5.2%)

ADRs were classified into preferred term using MedDRA Version 23.1 (or later).
A participant with multiple events coding to the same preferred term within a primary SOC was counted only once for the preferred term within that primary SOC.
*: include the occurrence of intended ADRs reported in the investigator-initiated Studies 001/.002/.003 protocols.
[#]: as per CTCAE

2.6.8.4. Serious adverse event/deaths/other significant events

ADRs of special interest

To verify that ex vivo expansion did not affect the safety profile normally observed with unmanipulated CB transplantation, AESIs were selected based on those normally expected in this context and included engraftment syndrome, pulmonary alveolar haemorrhage (PAH), posttransplant lymphoproliferative disorder (PTLD), pneumonitis (including cryptogenic organising pneumonia (COP), idiopathic pneumonia syndrome (IPS)) and infections, and were closely monitored in patients transplanted with ECT-001-CB across the clinical development program (Table).

Table 26. Adverse events of special interest post-ECT-001-CB infusion, regardless of study drug relationship

Preferred Term	SAS (N=116)							
	Grade 3		Grade 4		Grade 5		All grades	
Infections	61	(60.4%)	6	(5.9%)	7	(6.9%)	87	(75.0%)
Engraftment Syndrome	11	(9.5%)	2	(1.7%)	0	(0.0%)	13	(11.2%)
Cryptogenic Organising Pneumonia	0	(0.0%)	3	(2.6%)	1	(0.9%)	4	(3.4%)
Idiopathic Pneumonia Syndrome	2	(1.7%)	0	(0.0%)	1	(0.9%)	3	(2.6%)
Pneumonitis	1	(0.9%)	0	(0.0%)	0	(0.0%)	1	(0.9%)
Pulmonary Alveolar Haemorrhage	0	(0.0%)	1	(0.9%)	2	(1.7%)	3	(2.6%)
Post-Transplant Lymphoproliferative Disorder	1	(0.9%)	2	(1.7%)	0	(0.0%)	3	(2.6%)
GVHD*	**SAS (n=116)**							
Acute Graft Versus Host Disease	**Grade II**		**Grade III**		**Grade IV**		**All grades**	
	62	(53.4%)	14	(12.1%)	1	(0.9%)	77	(66.4%)
Chronic Graft Versus Host Disease	**Mild**		**Moderate**		**Severe**		**All grades**	
	10	(8.6%)	7	(6.0%)	0	(0.0%)	17	(14.7%)

AEs were classified into SOC and preferred term using MedDRA Version 23.1 (or later). A participant with multiple events coding to the same preferred term within a primary SOC was counted only once for the preferred term within that primary SOC.
*GVHD events were graded according to the NIH consensual criteria.

Serious adverse drug reactions

A total of 61 participants (52.6%) reported at least one SAE during the conduct of their study. (see Table). The SOC most frequently reported was 'infections and infestations' (21.6%), followed by 'immune system disorders' (12.9%), 'respiratory, thoracic and mediastinal disorders' (8.6%), 'general disorders and administration site conditions' (7.8%) and 'blood and lymphatic system disorders' (5.2%). The SAE preferred terms most frequently reported were 'acute graft versus host disease' (11.2%), 'pneumonia' (5.2%), 'sepsis' (5.2%) and 'transplant failure' (5.2%).

Table 27. Serious Adverse Reactions Post-ECT-001-CB Transplant by SOC and Preferred Term (Safety Set)

System Organ Class Preferred Term	SAS (N=116)	
Number of participants with any SAEs, n (%)	**61**	**(52.6%)**
BLOOD AND LYMPHATIC SYSTEM DISORDERS	**6**	**(5.2%)**
Neutropenia	5	(4.3%)
Hypofibrinogenemia	1	(0.9%)
CARDIAC DISORDERS	**3**	**(2.6%)**
Myocardial Infarction	1	(0.9%)
Pericardial Effusion	1	(0.9%)
Pericarditis	1	(0.9%)
CONGENITAL, FAMILIAL AND GENETIC DISORDERS	**2**	**(1.7%)**
Aplasia	1	(0.9%)
Cytogenetic Abnormality	1	(0.9%)
GASTROINTESTINAL DISORDERS	**5**	**(4.3%)**
Nausea	2	(1.7%)
Abdominal pain	1	(0.9%)
Gastrointestinal Conditions Nec	1	(0.9%)
Jejunal Perforation	1	(0.9%)
Mouth Haemorrhage	1	(0.9%)
Vomiting	1	(0.9%)

System Organ Class Preferred Term	SAS (N=116)	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	**9**	**(7.8%)**
Disease Recurrence	3	(2.6%)
Pyrexia	2	(1.7%)
Multiple Organ Dysfunction Syndrome	2	(1.7%)
Complication Associated With Device	1	(0.9%)
Systemic Inflammatory Response Syndrome	1	(0.9%)
IMMUNE SYSTEM DISORDERS	**15**	**(12.9%)**
Acute Graft Versus Host Disease	13	(11.2%)
Engraftment Syndrome	2	(1.7%)
INFECTIONS AND INFESTATIONS	**25**	**(21.6%)**
Pneumonia	6	(5.2%)
Sepsis	6	(5.2%)
Pneumocystis Jirovecii Pneumonia	3	(2.6%)
Septic Shock	2	(1.7%)
Adenovirus Infection	1	(0.9%)
Adenovirus Reactivation	1	(0.9%)
Bacteraemia	1	(0.9%)
Citrobacter Sepsis	1	(0.9%)
Coronavirus Infections	1	(0.9%)
Cytomegalovirus Infection Reactivation	1	(0.9%)
Device Related Infection	1	(0.9%)
Enterococcal Infection	1	(0.9%)
Epstein-Barr Virus Infection	1	(0.9%)
Gastrointestinal Candidiasis	1	(0.9%)
Human Herpesvirus 6 Infection	1	(0.9%)
Metapneumovirus Infection	1	(0.9%)
Myelitis	1	(0.9%)
Pharyngeal Abscess	1	(0.9%)
Respiratory Tract Infection	1	(0.9%)
Rhinovirus Infection	1	(0.9%)
Staphylococcal Bacteraemia	1	(0.9%)
Upper Respiratory Tract Infection	1	(0.9%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	**7**	**(6.0%)**
Transplant Failure	6	(5.2%)
Transfusion Reaction	1	(0.9%)
METABOLISM AND NUTRITION DISORDERS	**2**	**(1.7%)**
Hyperglycaemia	1	(0.9%)
Hyponatraemia	1	(0.9%)
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)	**3**	**(2.6%)**
Post Transplant Lymphoproliferative Disorder	3	(2.6%)
NERVOUS SYSTEM DISORDERS	**3**	**(2.6%)**
Syncope	2	(1.7%)
Encephalopathy	1	(0.9%)
PSYCHIATRIC DISORDERS	**2**	**(1.7%)**
Depression	1	(0.9%)
Suicidal Ideation	1	(0.9%)
RENAL AND URINARY DISORDERS	**1**	**(0.9%)**
Acute Kidney Injury	1	(0.9%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	**10**	**(8.6%)**
Pulmonary Alveolar Haemorrhage	3	(2.6%)
Respiratory Failure	2	(1.7%)
Asthma	1	(0.9%)
Idiopathic Pneumonia Syndrome	1	(0.9%)
Lung Infiltration	1	(0.9%)
Organising Pneumonia	1	(0.9%)
Pulmonary Embolism	1	(0.9%)
Pulmonary Hypertension	1	(0.9%)
Stridor	1	(0.9%)

System Organ Class Preferred Term	SAS (N=116)	
VASCULAR DISORDERS	**1**	**(0.9%)**
Hypertension	1	(0.9%)

SAEs were classified into SOC and preferred term using MedDRA Version 23.1 (or later).
A participant with multiple events coding to the same preferred term within a primary SOC was counted only once for the preferred term within that primary SOC.

The maximal grade of SAE experienced was severe for n=19 (16.4%), life-threatening for n=25 (21.6%) and fatal for n=17 participants (14.7%, see Table).

Table 28. Serious Adverse Reactions Post-ECT-001-CB Transplant by Maximal Toxicity Grade (Safety Set)

System Organ Class	***Preferred Term***	***Maximal Toxicity Grade***	***SAS (N=116)***	
Overall	Overall	Total	61	(52.6%)
		Severe	19	(16.4%)
		Life threatening	25	(21.6%)
		Death	17	(14.7%)

SAEs were classified into SOC and preferred term using MedDRA Version 23.1 (or later).
A participant with multiple events coding to the same preferred term within a primary SOC was counted only once for the preferred term within that primary SOC.

Deaths

Among the 116 patients transplanted with ECT-001-CB, 42 (36.2%) deaths were reported during ECT-001-CB trials' follow-up. The most frequent cause of death was disease progression/relapse (n=26). One patient died from pneumonia ~1 year after transplant failure-related study discontinuation and 15 patients (12.9%) died during their study follow-up of non-relapse-related events. Their causes of death come from 4 SOCs: 'infections and infestations' (n=8, 6.9%), 'respiratory, thoracic and mediastinal disorders' (n=6, 5.2%), 'immune system disorders' (n=2, 1.7%) and 'general disorders and administration site conditions' (n=1, 0.9%). Actual causes of death include 8 infections and various respiratory or multiorgan complications (n=7), such as PAH, pneumonitis, pulmonary hypertension, respiratory failure and multiorgan failure (*Table*).

Table 29. Incidence of Deaths Unrelated to Relapse Post-ECT-001-CB Transplant (Safety Set)

System Organ Class	***Preferred Term***	***SAS (N=116)***	
Overall	**Overall**	**15**	**(12.9%)**
General disorders and administration site conditions	**Overall**	**1**	**(0.9%)**
	Multiple Organ Dysfunction Syndrome	1	(0.9%)
Immune system disorders	**Overall**	**2**	**(1.7%)**
	Acute Graft Versus Host Disease	2	(1.7%)
Infections and infestations	**Overall**	**8**	**(6.9%)**
	Sepsis	2	(1.7%)
	Septic Shock	2	(1.7%)
	Citrobacter Sepsis	1	(0.9%)
	Coronavirus Infections	1	(0.9%)
	Enterococcal Infection	1	(0.9%)
	Metapneumovirus Infection	1	(0.9%)

System Organ Class	***Preferred Term***		***SAS (N=116)***
	Pneumocystis Jirovecii Pneumonia	1	(0.9%)
	Pneumonia	1	(0.9%)
Respiratory, thoracic and mediastinal disorders	**Overall**	**6**	**(5.2%)**
	Pulmonary Alveolar Haemorrhage	2	(1.7%)
	Idiopathic Pneumonia Syndrome	1	(0.9%)
	Organising Pneumonia	1	(0.9%)
	Pulmonary Hypertension	1	(0.9%)
	Respiratory failure	1	(0.9%)

Table reports the incidence of death prior to study discontinuation (does not include death related to relapse of disease).
Several grade 5 events could be reported for the same participant.

Hospitalizations and last day of fever before engraftment

All patients were hospitalized at the start of conditioning regimen administration. Of all participants in the SAS population (N=116), 83 participants provided data on hospitalization, and data was not available for the remainder (i.e. for Study 003, n=18). The median duration of the initial hospitalization was 34 days, ranging from 16 days to 130 days (n=83). A total of 4/55 (7.1%) required a stay at the intensive care unit (ICU) during initial hospitalization, with a median duration of 12 days.

A total of n=36/83 (43.4%) participants required subsequent re-hospitalization. The mean (SD) duration of the subsequent hospitalization was 19.0 (56.99) days. Of these 27 participants were re-hospitalized within the first 100 days post-transplant. The mean number of days in the hospital within the first 100 days post-transplant was 13.1 (SD: 20.33) days.

2.6.8.5. Laboratory findings

Laboratory assessments (e.g., lumbar puncture, BM biopsy, immunoglobulin) and clinical evaluations (e.g. chest X-ray, electrocardiogram [ECG], cardiac and pulmonary function evaluation) are mandatory steps in the determination of HSCT eligibility for the inclusion of patients into ECT-001-CB trials, result for these assessments were not systematically recorded into the clinical trial databases in the ECT-001-CB trials. Clinically significant abnormalities were reported as AEs.

Measures for haematological assessment included haematocrit, haemoglobin, white blood cell (WBC), red blood cell (RBC), platelets, differentials for neutrophils, lymphocytes, monocytes, eosinophils and basophils. Results at screening, conditioning, at transplant, and on days 7, 14, 21 and 28 and months 3, 6, 12 and 24 are reported for the SAS population. Clinically significant haematologic abnormalities were reported as AEs. Recovery of neutrophils and platelets after infusion of ECT-001-CB constitute efficacy endpoints.

Clinical chemistry: Measures included ALT, AST, total bilirubin, direct bilirubin, alkaline phosphatase, BUN, uric acid, serum creatinine, total protein, albumin, globulin, A/G ratio, calcium, sodium, potassium, chloride, phosphate and magnesium. Results at screening, conditioning, at transplant, and on days 7, 14, 21 and 28 and months 3, 6, 12 and 24 are reported for the SAS population. Clinically significant chemistry abnormalities were reported as AEs.

Immunoglobulin levels: A total of 45.5% of participants in the SAS population had their immunoglobulins checked at screening and pre-transplant, respectively. The mean (SD) Immunoglobulin G (IgG) levels were 785.5 (506.49) mg/dL at screening and 805.4 (328.19) mg/dL at

pre-transplant, respectively. None of the immunoglobulin levels were considered clinically significant at screening or pre-transplant.

Immunoglobulin assessments were performed at Day 14, 28, 42, 56, 70 and 84, Month 3, 6, 12, 18 and 24. For the SAS population, three participants (3.0%) had a clinically significant result at Day 14, two (2.0%) at Day 56, two (2.0%) at Day 70, one (1.0%) at Day 84, two (2.0%) at Month 6 and two (2.0%) at Month 12, with other visits producing no clinically significant results.

Bone Marrow Aspiration and Biopsy: Bone Marrow aspirations and biopsies were analysed separately by studies 001/002 and studies 004/007. Measures of aspiration and biopsy included morphology, follow cytometry, cytogenetics. For studies 001 and 002 at screening, 94.6% of aspirations were performed and 37.7% were abnormal and 43.9% of biopsies were also considered to be abnormal. At Month 12, 51.8% of aspiration were performed, and 13.8% were considered abnormal. 15.8% of biopsies were also considered abnormal.

For studies 004 and 007 at screening, 96.3% were performed. 34.6% and 44.4% of aspirations and biopsies were considered abnormal, respectively. At month 12, 29.6% of aspiration were performed, and none were considered abnormal. None of the biopsies were considered abnormal either.

Viral serology assessments: Baseline data on the patient serology prior to transplantation included the results of the following tests: HIV-1,2 antibody, hepatitis B antigen (HBsAg), hepatitis C virus (HCV), hepatitis C antibody (HepCAb), CMV antibody, EBV, HSV, varicella-zoster virus (VZV), HTLV-1, HTLV-2, toxoplasma antibody, syphilis, aspergillosis. Any viral infectious events of grade ≥3 post-transplant was documented as an AE.

2.6.8.6. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.7. Safety in special populations

No patients over 65 years and no hepatically or renal impaired patients were included in the ECT001-CB studies.

No formal studies with ECT-001-CB cells have been conducted in pregnant women. No preclinical reproductive studies have been conducted with ECT-001-CB to assess whether it may cause foetal harm when administered to a pregnant woman.

Investigation on potential impact of sex, age, race, ethnicity, disease type, prior allogeneic HSCT, HLA matching between donor and recipient, and conditioning regimen intensity on safety outcomes were analyzed in the SAS subset (n=101, all patients that received ECT-001-CB in the five completed or ongoing studies with ECT-001-CB, DCO November 30th 2022). In general, the subgroup analyses did not reveal major differences, although power was not sufficient to allow for proper analysis in all cases. An integrated safety analysis by age stratification (18–39, 40–54, and ≥55 year) was performed on the SAS subset, with a data cut-off of March 15th, 2024 (n=116) and data was consistent with the known association between increasing age and higher non-relapse mortality risk (*Sorror et al, 2014*). Additionally, the applicant does show that the conditioning regimen and haematological malignancies does not lead to a differential safety profile, which is important for the target indication.

2.6.8.8. Immunological events

Immunogenicity was not studied. As umbilical CB is associated with low immunogenicity this is agreed.

Graft failure:

In the SAS population, 6/116 (5.2%) patients suffered from primary graft failure. In Study 001, one case of graft failure developed in a patient receiving a double CB transplantation with one ECT-001-CB and one unmanipulated CB unit. This patient was the first to receive ECT-001-CB, had several risk factors for graft failure, and received a 12-day, rather than a 7-day expanded CB (*Study 001 CSR, section 11.4.2.1*). In Study 002, one patient had primary graft failure due to the presence of high titre donor-specific anti-HLA antibodies (*ISSE CSR, section 14.2.1*). In Study 003, the first two patients received a low intensity conditioning regimen that proved insufficient for donor engraftment and led to complete or partial primary graft rejection (*SRT-ECT-045, section 6.3*). In Study 004, one patient had a very poor graft function with <10% bone marrow cellularity and >95% donor chimerism at BM aspirate when she died on Day +42 post-transplant of acute respiratory failure. Finally, a patient of Study 007 failed to engraft (*SRT-ECT-041).*

2.6.8.9. Safety related to drug-drug interactions and other interactions

No pharmacokinetic drug interaction studies with ECT-001-CB have been performed as they are not applicable to a cell based therapy.

Two drugs known to interfere with hematopoietic reconstitution following unmanipulated CB transplantation might also impact engraftment of ECT-001-CB and their use has been considered based on historical use of unmanipulated CB transplantation rather than specific nonclinical studies with dorocubicel.

- In vivo T cell depletion with agents such as ATG were shown to lead to delayed T cell reconstitution and subsequent outcomes in the context of CB transplantation, including higher frequency of lethal infections and increased risk of relapse (De Koning et al, 2017[56]). Therefore, their use is not recommended with ECT-001-CB, especially in the pre-transplant and early post-transplant period. No ATG was used in conditioning regimens in clinical trials with ECT-001-CB.
- G-CSF is often used to accelerate engraftment in the context of CB transplantation in accordance with current clinical practice by stimulating stem cell proliferation. Per clinical trial protocols G-CSF was administered to all patients transplanted with ECT-001-CB. G-CSF was administered at a dosage of 5 µg/kg/day from day +1 or +2 of transplant until absolute neutrophil counts (ANC) reached 1.5 x 10^9/L and if ANC subsequently decreases below 1.0 x 10^9/L before day +100 at the same dosage until recovery.

2.6.8.10. Discontinuation due to adverse events

Not applicable.

2.6.8.11. Post marketing experience

Not applicable.

[56] De Koning C, Admiraal R, Nierkens S, & Boelens JJ. Immune reconstitution and outcomes after conditioning with anti-thymocyte-globulin in unrelated cord blood transplantation; the good, the bad, and the ugly. Stem Cell Investigation. 2017;4:38.

2.6.9. Discussion on clinical safety

Safety is presented from a pooled analysis (SAS) of all patients transplanted with ECT-001-CB (n=116; studies ECT-001-CB.001, ECT-001-CB.002, ECT-001-CB.003, ECT-001-CB.004 and ECT-001-CB.007). The single-arm design of the studies and required concomitant treatment, makes it difficult to disentangle the precise contribution of ECT-001-CB on top of the well-known risks of an allogenic transplantation. The SAS differs from the pooled efficacy analysis as the safety analysis also includes patients from ECT-001-CB.003 with high risk multiple myeloma (n=18) and ECT-001-CB.007 in pediatric patients with high risk myeloid malignancies (n=12) and consists of patients receiving either the fresh or cryopreserved product. The safety has not been separately presented for patients who received cryopreserved dorocubicel derived from a small sized cord, this is agreed as the sample size is small, the baseline risk of the included patients differs, and the efficacy (NRM and GvHD) and non-clinical data do not suggest that dorocubicel cryopreservation impacts the safety. As such presentation of the pooled safety analysis over 5 clinical studies is agreed. The size of the safety database with 116 patients infused is limited but acceptable in light of a request for CMA.

The SAS pool has a DCO of 15 March 2024. The median follow-up for the SAS was 22.49 months (range 0.89-48.4 months), with 67 (57.8%) and 48 (41.1%) patients reaching 12 and 24 months, respectively. The median duration of follow-up is sufficient to identify the earlier and immediate AEs. Long term safety follow-up will be provided as part of the specific obligations (registry study).

The studied SAS population presented with a median age of 41.3 years, the youngest patient enrolled was 1 years old, the oldest 66 years. Most patients in the SAS pool had AML, ALL and MDS. The applicant refers to the unmet medical need for patient that do not have a donor available, especially for patients with rare HLA genotypes for whom finding a matched donor is challenging. The studied population consisted of >80% of white patients with an underrepresentation of rare HLA genotypes. In general, the studied population is in line with the target indication.

Safety data

A total of 115 participants (99.0%) suffered from one or more severe, life-threatening or fatal AEs, a total of 61 participants (52.6%) had one or more SAEs. Of all the participants, 103 participants had an AE ≥ Grade 3 that was considered related to study treatment (88.8%, adverse drug reaction).

Within the SAS pool adverse events of special interest (AESIs) ≥ Grade 3 include infections (75.0%), acute GvHD (66.4%), engraftment syndrome (11.2%), chronic GvHD (14.7%), and and less frequent events (each <5%) such as cryptogenic organizing pneumonia, idiopathic pneumonia syndrome, post-transplant lymphoproliferative disorder, and pulmonary alveolar hemorrhage.

Infections are expected following conditioning and HSCT, 75% of the SAS population reported a grade ≥3 ECT-001-CB post-transplant infection. The proportion of patients reporting bacterial and viral infections were 52.6% and 45.7%, respectively. Few patients reported fungal (9.5%) or unspecified (8.6%) infectious events. Most participants reporting an infectious event had a maximal toxicity grade of 3 (n=72), eight suffered from a fatal infection (grade 5).

The incidence of acute GvHD of grade II-IV was high with 66.4% of which most patients had grade II acute GvHD (53.4%). The incidence of chronic GvHD was lower with 14.7% (mild, moderate, severe). GvHD (acute and chronic, scoring per NIH criteria) was an efficacy endpoint for the clinical studies but is also reflected as ADR in the SmPC. Please refer to the clinical efficacy section for further contextualization of the data on GvHD.

Among the SAS population (n=116), 42 (36.2%) deaths were reported, with the most frequent cause of death disease progression (n=26). Fatal adverse reactions occurred in 7.8% of patients treated with Zemcelpro, including infections (2.6% including sepsis (0.9%), enterococcal infection (0.9%),

pneumonia (0.9%), acute GvHD (1.7%), PAH (1.7%), IPS (0.9%), COP (0.9%), and pulmonary hypertension (0.9%). The causes of death were to be expected from a stem cell transplantation, although longer follow up is needed in the context of the CMA post marketing.

During the conduct of the clinical studies the dose of ECT-001-CB changed multiple times in order to limit the occurrence of engraftment syndrome (ES). Upon recommendation to use tacrolimus and MMF for GVHD prophylactic treatment and the maximal CD34+ cell dose infused with ECT-001-CB capped at 4.0 x 10^6/kg in July 2017, the incidence and severity of ES decreased. The incidence and severity of engraftment syndrome declined in the following patients after introduction of these changes. To ensure prompt engraftment and immune reconstitution, the maximal dose of CD34+ cells was subsequently increased. Initially a high percentage of patients had ES (36.4% patients) which significantly decreased (10% cases grade ≥3, with no grade 4 occurring since) with the use of tacrolimus and MMF. There were no deaths due to ES.

Multiple subgroup analyses have been presented (conditioning regimen, disease type, gender, age, race, ethnicity, prior alloSCT, HLA matching). In general, the subgroup analyses did not reveal major differences, although power was not sufficient to allow for proper analysis in all cases. In support of the proposed indication, the applicant shows that the conditioning regimen and haematological malignancies does not lead to a differential safety profile.

While the safety of the final product mainly reflects class specific effects, an additional layer of safety concerns could be posed by the treatment of HSCs with UM171. Short term safety of UM171 was assessed in nonclinical studies and this did not raise any safety concerns. As any unwanted effects on the cells could lead to the development of clonal hematopoiesis, which has been associated with an increased risk of malignancies, clonal hematopoiesis is listed as an important potential risk in the Risk management plan.

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

Additional safety data needed in the context of a conditional MA

Taking into consideration the single arm trial design of the pivotal studies, the limited number of patients enrolled and the short time follow up available of the treated patients, the CAT and CHMP considered that long-term safety data is lacking. The following measures (Specific Obligations) are necessary to address the missing safety data in the context of a conditional MA:

- Provision of the final study results of the pivotal studies CT-001-CB.002 and 004.
- In order to compare the efficacy of infusing Zemcelpro to standard of care allogeneic HSCT and to evaluate the safety of Zemcelpro in patients aged 18-21 years included in the high-risk paediatric AML prospective, interventional study, the MAH shall conduct and submit the results of the subgroup analysis in patients aged 18-21 years.
- Conduct and submission of the results of a prospective, randomized, open-label phase 3 study of Zemcelpro versus best available allogeneic stem cell source (haplo, MMUD) in adult patients with high risk acute leukemia/MDS, to provide confirmatory evidence of the safety and efficacy of Zemcelpro.
- Conduct and submission of the results of a prospective registry-based study to collect real-world safety and effectiveness data on patients that have received commercially available Zemcelpro.

2.6.10. Conclusions on the clinical safety

The safety of ECT-001-CB has been presented from 116 patients treated within 5 clinical studies. The reported AEs were as expected from an allogeneic SCT and are expected to be adequately managed with proper recommendations in the SmPC as treatment is administered within qualified treatment centres for cord blood transplantation. Uncertainties with respect to safety are the single arm study design, and short follow-up duration of the clinical studies. Uncertainties can be addressed with specific obligations in the context of a CMA.

Overall, Zemcelpro is approvable based on the safety profile described.

The CAT considers the following measures necessary to address the missing safety data in the context of a conditional MA:

- In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall submit the final results from Study ECT-001-CB.002: A Phase II Open-label Study of ECT-001-expanded Cord Blood Transplantation in Patients with High Risk Acute Leukemia/myelodysplasia.
- In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall submit the final results from Study ECT-001-CB.004: Phase II Open-Label Study of ECT-001-Expanded Cord Blood Transplantation in Patients with High and Very High-Risk Acute Leukemia/Myelodysplasia.
- In order to confirm the efficacy and safety of Zemcelpro in patients aged 18-21 years with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall conduct and submit the results of the subgroup analysis of patients aged 18-21 years from study ECT-001-CB.010: A Prospective Randomized Phase II Trial of Allogeneic SCT with ECT-001-CB Expanded Cord Blood Transplant Without Serotherapy Versus Other Stem Cell Source in Pediatric Patients with High risk/refractory/relapsed Acute Myeloid Leukaemia, according to an agreed protocol.
- In order to confirm the efficacy and safety of Zemcelpro, and to further evaluate the dose parameters used in adult patients with high-risk and very high-risk acute leukaemia/MDS, the MAH shall submit the results of study ECT-001-CB.011: A Multicenter, Prospective, Randomized, Open-Label Phase III Study of ECT-001-CB (ECT-001-Expanded Cord Blood) Transplantation versus Best Alternative Allogeneic Stem Cell Source Transplantation (Haplo, MMUD) in Patients with High-Risk Acute Leukemia/Myelodysplasia which is conducted according to an agreed protocol.
- In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall conduct and submit the results of a prospective, non-interventional study based on data from a registry, and evaluate dose parameters collected for Zemcelpro lot manufactured for each patient enrolled in the study, according to an agreed protocol.

The CHMP endorses the CAT conclusion on clinical safety as described above.

2.7. Risk Management Plan

2.7.1. Safety concerns

Safety Concerns	
Important potential risks	• Clonal haematopoiesis
Missing information	• Use in patients over 65 years of age

2.7.2. Pharmacovigilance plan

No additional pharmacovigilance activities are required.

The following studies imposed as specific obligations in the context of a conditional marketing authorisation for efficacy reasons will address, as well, the safety of the product:

Study ECT-001-CB.002: A phase II open-label study of ECT-001-expanded cord blood transplantation in patients with high-risk acute leukemia/myelodysplasia (Canada), final CSR due 28 February 2026

Study ECT-001-CB.004: A Phase II open-label study of ECT-001-expanded cord blood transplantation in patients with high and very high-risk acute leukemia/myelodysplasia (USA/EU), final CSR due 31 August 2026

ECT-001-CB.010: A Prospective Randomized Phase II Trial of Allogeneic SCT with ECT-001-CB Expanded Cord Blood Transplant without Serotherapy versus other Stem Cell Source in Paediatric Patients with High Risk/Refractory/Relapsed Acute Myeloid Leukaemia (CAML Study), final CSR due 30 June 2030

ECT-001-CB.011: A Multicentre, Prospective, Randomized, Open-Label Phase III Study of Dorocubicel (ECT-001-Expanded Cord Blood) Transplantation versus Best Available Allogeneic Stem Cell Source Transplantation in Adult Patients with High-Risk Acute Leukaemia/Myelodysplasia, final CSR due 30 June 2030

ECT-001-CB.012: Prospective registry study in a real-world setting in Europe assessing the effectiveness and safety of Zemcelpro in patients with haematological malignancies requiring an allogeneic stem cell transplantation following myeloablative conditioning for whom no other type of suitable donor cells is available, final CSR due 31 December 2031

2.7.3. Risk minimisation measures

Safety Concern	**Risk Minimisation Measures**	**Pharmacovigilance Activities**
Important potential risks		
Clonal haematopoiesis	**Routine risk minimization measures:** Clinical setting: IV product that can only be administered in a qualified transplant centre under the direction of and supervised by a healthcare professional experienced in HSCT Pack size: not applicable Legal status: prescription only medicine	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** None

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
	Additional risk minimisation measures: None	**Additional pharmacovigilance activities:** None
Use in patients over 65 years of age	**Routine risk minimization measures:** SmPC Section 4.2 Pack size: not applicable Legal status: prescription only medicine **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** None **Additional pharmacovigilance activities:** None

2.7.4. Conclusion

The CAT considers that the risk management plan version 1.0 is acceptable.

The CHMP endorses the CAT conclusion on the RMP as described above.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3), point (ia), of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

The applicant requested the use of English only for the particulars on the outer container (cryo-shipper), intermediate packaging (metal cassette), according to Art. 63(1); and as per Art 63(3) the applicant also requested a full translation exemption for the small immediate packaging units (infusion bag) and package leaflet so that these materials, when printed, would only appear in English.

The QRD Group agreed to the use of English only for outer and inner labels for Zemcelpro. In relation to the package leaflet, it was found not acceptable to use English only therefore, the applicant should provide a printed package leaflet in the national language along with the pack; alternatively, the use of a QR code to provide a platform where patients can download the package leaflet would also be acceptable. Additionally, the Group recommended to use modular cryogenic pack(s) containing the cassettes in order to label it/them with the outer label, instead of labelling the cryoshipper.

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zemcelpro (Dorocubicel / Allogeneic umbilical cord-derived CD34- cells, unexpanded) is included in the additional monitoring list as it contains a new active substance and it is approved under a conditional marketing authorisation.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Treatment of adult patients with haematological malignancies requiring an allogeneic haematopoietic stem cell transplantation following myeloablative conditioning for whom no other type of suitable donor cells is available.

3.1.2. Available therapies and unmet medical need

The treatment of patients with haematological malignancies requiring an allogeneic haematopoietic stem cell transplantation for whom no suitable donor cells is available (including double CB or standard size CB unit), represents a clinical challenge considering the dismal prognosis. An alternative cell therapy approach in some haematological malignancies includes treatment with CAR-T cells, although superiority over HSCT has not been demonstrated yet and novel treatment options are needed.

In only 0.8% of allogeneic HCTs in adults in Europe a CBU is used, while a suitable standard size CBU is available in 81-96% of patients depending on race and ethnicity. Therefore, the number of patients that lack access to any type of suitable donor cells (including double-cord) is expected to be very limited. There is nevertheless an unmet medical need for patients that lack access to any type of suitable donor cells.

3.1.3. Main clinical studies

The main evidence of efficacy submitted is a pooled analysis (ISSE) of two single arm open-label studies (Phase II studies ECT-001-CB.002 and ECT-001-CB.004) of ECT-001-CB following a myeloablative conditioning regimen in patients with high-risk leukaemia/MDS. Patients who received cryopreserved ECT-001-CB transplantation derived from a small CB unit (pivotal Full Analysis Set, FAS) form the main pooled efficacy population and comprised 25 patients (24 transplanted).

The primary objective for the ISSE pooled analysis was to establish adequate hematopoietic reconstitution of patients with haematological diseases who are medically indicated for allogeneic HSCT but lack a readily available suitable donor. Secondary objectives of the pooled analysis were to characterise the successful outcome of the transplant, and to assess outcomes on patient-relevant, time-dependent outcomes and safety.

Safety is presented from a pooled analysis (SAS) of adult and pediatric patients treated with a single dose of ECT-001-CB included in the 5 single arm open label clinical studies (n=116; studies ECT-001-CB.001, ECT-001-CB.002, ECT-001-CB.003, ECT-001-CB.004 and ECT-001-CB.007). The SAS also includes patients with ECT-001-CB derived from standard size CB units and patients with high risk multiple myeloma from ECT-001-CB.003 (n=18) and paediatric patients with high risk myeloid malignancies from ECT-001-CB.007 (n=12).

3.2. Favourable effects

Expansion of small CB units using UM171 led to a manufactured cell dose above 0.5 x 10^6 CD34+ cell/kg in 39/40 (97.5%) lots across studies ECT-001-CB.001, ECT-001-CB.002 and ECT-001-CB.004.

Pooled analysis – pivotal FAS

After transplantation with cryopreserved ECT-001-CB derived from a small CB unit, the median (range) time to neutrophil recovery was 20 (10-39) days. In total 21/25 (84%) patients achieved engraftment by day 42 post-HSCT and no secondary graft failure occurred up to the data cut-off in the FAS.

Platelet engraftment occurred within a median (range) time of 40 (29-175) days and was achieved by 17/25 patients (68%) by day 100 post-HSCT in the FAS.

Supportive real-world data - matched analysis EBMT Study 2

Neutrophil engraftment at Day 42 was attained in 86.4% of matched patients (n=22) treated with ECT-001-CB, with a median time to engraftment of 20 days compared to 85.4% and 25 days for the EBMT control group who received an unmanipulated CBU transplant (n=41). For other donor sources, neutrophil engraftment at Day 42 was attained in 96-99% and the median time to recovery was 16-21 days.

Platelet engraftment at Day 100 was attained in 72.2% of matched patients (n=22) with a median time to platelet recovery of 40 days compared to 83.3% and 36 days in the CBU cohort (n=41; median 13-27 days with other donor sources).

3.3. Uncertainties and limitations about favourable effects

- The pivotal study population of the pooled analysis consists of a very small number of patients. Engraftment results (as measured by time to neutrophil and platelet recovery) of ECT-001-CB derived from a small sized cord are based on 24 patients (infused patients). As mitigation measure, enlarged

data sets and long term efficacy data will be collected in the frame of the CMA and will provide comprehensive data for the dossier.

- Comprehensive data regarding (long term) clinical efficacy of cryopreserved dorocubicel is missing. The single arm trial designs of the pooled studies hamper interpretation of time-dependent endpoints.

- The lower range of the proposed posology for CD34+/CD3+ cell dose was studied in a very limited number of patients. Comprehensive data is expected from the confirmatory studies (ECT-001-CB.011 and EC-001-CB.012) in which dose parameters will be collected.

- All NRM events occurred in patients 40 years or older. This translated into a lower OS, PFS, GRFS and CRFS in these patients. This is not unexpected as older age is an important risk factor for NRM.

3.4. Unfavourable effects

A total of 115 participants (99.1%) suffered from one or more severe, life-threatening or fatal AEs, a total of 61 participants (52.6%) had ≥1 SAE. Of all the participants, 103 participants had an AE ≥ Grade 3 that was considered related to study treatment (88.8%, adverse drug reaction). The most frequently (>10%) occurring adverse reactions of ≥ Grade 3 were lymphopenia (46.6%), anaemia (44.0%), neutropenia (35.3%), thrombocytopenia (31.9%), leukopenia (29.3%), acute GVHD (22.4%), hypogammaglobulinaemia (18.1%), febrile neutropenia (15.5%), hypertension (12.9%), engraftment syndrome (11.2%), and pneumonia (11.2%).

Adverse events of special interest (AESIs) ≥ Grade 3 in the SAS include infections (75.0%), acute GvHD (66.4%), engraftment syndrome (11.2%), chronic GvHD (14.7%), cryptogenic organizing pneumonia (COP; 3.4%), idiopathic pneumonia syndrome (IPS; 2.6%), post-transplant lymphoproliferative disorder (PTLD; 2.6%), and pulmonary alveolar hemorrhage (PAH; 2.6%).

In the SAS population, 6/116 (5.2%) patients suffered from primary graft failure, no cases of secondary graft failure were observed.

The overall incidence of grade ≥3 post-transplant infections was 75.0% of which 8 were fatal. Most infections were bacterial (52.6%) and viral (45.7%) origin.

Among the SAS population (n=116), 32 (36.2%) deaths were reported during the follow-up period of ECT-001-CB trials by the DCO on 15 March 2024. Fatal adverse reactions occurred in 7.8% of patients treated with Zemcelpro, including infections (2.6% including sepsis (0.9%), enterococcal infection (0.9%), pneumonia (0.9%), acute GvHD (1.7%), PAH (1.7%), IPS (0.9%), COP (0.9%), and pulmonary hypertension (0.9%). and 7 respiratory or multiorgan complications

Multiple subgroup analyses have been presented (conditioning regimen, disease type, gender, age, race, ethnicity, prior alloSCT, HLA matching). In general the results from the subgroup analyses is supportive of the proposed indication.

3.5. Uncertainties and limitations about unfavourable effects

- The median follow-up for the SAS was 22.49 months (range 0.89-48.4 months), with 67 (57.8%) and 48 (41.1%) patients reaching 12 and 24 months, respectively. Thus, long-term duration of follow-up is limited.

- The single-arm design of the studies and required concomitant treatment, makes it difficult to disentangle the precise contribution of ECT-001-CB on top of the well-known risks of a transplantation.

3.6. Effects Table

Table 3. Effects Table for ECT-001-CB for the treatment of adult patients with haematological malignancies requiring an allogeneic hematopoietic stem cell transplantation following myeloablative conditioning for whom no other type of suitable donor cells is available (data cut-off efficacy: 30 Nov 2022; Safety Analysis Pool (SAS): 15 March, 2024)).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Neutrophil recovery	Time to neutrophil engraftment (median; range)	Days	20 (10-39)	N/A	- Key efficacy results are based on a subset (n=20) of a small study population (n=25) derived from single arm trials. - Confirmation of long term treatment effect to be provided post-approval.	Study 002 and 004 (1)
Platelet recovery	Time to platelet engraftment (median; range)	Days	40 (29-175)	N/A	See above.	same as above
Unfavourable Effects						
Adverse drug reaction	Grade ≥3 AE considered related to the study treatment	%	88.8	N/A	- AE profile appears as expected in post transplantation setting. - Data is derived from small single arm studies. - Long term duration of follow-up is limited.	(2)
Deaths	Proportion of patients who died any time post infusion	%	36.2	N/A	Fifteen patients (12.9%) died of non-relapse-related events.	(2)
Infections	Proportion of patients with grade ≥3 post-transplant infections	%	73.3	N/A	Infections were mostly of bacterial (50.5%) and viral (48.5%) origin as expected in the post transplantation setting.	(2)
Acute GvHD	Proportion of patients with acute GvHD grade II-IV	%	66.4	N/A		(2)
Chronic GvHD	Proportion of patients with chronic GvHD (mild, moderate, severe)	%	14.7	N/A		(2)

Abbreviations: FAS: Full Analysis Set, ISSE: integrative study of safety and efficacy.
Notes: (1) Efficacy results are based on the FAS population (n=25) of the cryoFAS (2) Safety results are based on the SAS population (n=116).

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

A conditional approval is requested for adult patients with haematological malignancies requiring an allogeneic haematopoietic stem cell transplantation for whom no other type of suitable donor cells is available.

In treatment lines where alternative donors are available, a randomized controlled trial would have been necessary to establish relative efficacy and safety. In the current last resort setting where no suitable donor is available, demonstration of engraftment of ECT-001-CB derived from a small CB unit, with maintained graft versus tumour effect and transplant related mortality and side effects within an acceptable range based on the pooled single arm trials is considered clinically relevant.

As there are concerns about the effect of cryopreservation on the viable cell dose, the dose administered is based on post-cryopreservation testing of cryovials. Further confirmation of the CD34+ and CD3+ cell dose range will be provided post-approval as part of the confirmatory studies (ECT-001-CB.011 and ECT-001-CB.012), as selection of the CD34+ and CD3+ release criteria is based on limited data.

The pivotal clinical efficacy data are supported by a set of 25 patients who received cryopreserved dorocubicel from a small sized cord (derived from study 002 and 004). After transplantation, neutrophil and platelet recovery was prompt, robust and sustained (no secondary graft failure occurred up to date) in the pivotal study population. While slightly higher NRM and slightly lower PFS and OS rates were observed in the cryopreserved subsets compared to the population receiving small cord irrespective of cryopreservation, these differences might reflect the baseline risk of the included patients (limited to those with high or very high-risk disease risk in the new pivotal FAS, whereas the data set irrespective of cryopreservation also included patients with lower risk disease from Study 001). It can, however, not be excluded that the differences were (also) caused by the cryopreservation step and/or small sized cord. The proportions of relapsed patients remained comparable among the study groups at each analysed time point.

Isolation of long-term treatment effect is not possible based on single arm trials. Contextualisation of engraftment and time-dependent endpoints was proposed using EBMT matched analyses. Given possible biases for long term clinical outcomes because of the single arm trial vs. registry comparison, the lack of pre-specification of what constitutes a similar effect, and the small sample size and limited follow-up, a firm conclusion on a similar/superior effect could however not be drawn. Nevertheless results suggest similar engraftment kinetics between cryopreserved ECT-001-CB and the main EBMT control group with an unmanipulated CBU transplant.Results for matched analyses of time dependent endpoints NRM, PFS, OS and CRFS seem numerically slightly worse for ECT-001-CB.

The safety has not been separately presented for patients who received cryopreserved dorocubicel derived from a small sized cord; this is agreed as the sample size is small, the baseline risk of the included patients differs, and the efficacy (type of NRM events and GvHD) and non-clinical data do not suggest that dorocubicel cryopreservation impacts the safety. Altogether, the safety of ECT-001-CB was based on 116 patients treated within 5 clinical studies (SAS pool); this is limited but can be accepted in the context of a CMA. Safety is based on analyses of single arm studies only. As the scope

of the application is for patients that lack any available other allogeneic cell sources, this is considered acceptable. However, single-arm design of the studies and required concomitant treatment, make it difficult to disentangle the precise contribution of ECT-001-CB on top of the well-known risks of allogenic transplantation.

Within the SAS pool only half of the patients have a follow-up of more than 12 months and 41.1% of 24 months. Thus, limited information is available on long-term and late adverse events, such as non-relapse mortality, incidence of chronic graft versus host disease and secondary graft failure. This will require long-term follow-up in a patient registry (study 012, SOB) as proposed by the applicant and in line with guidelines (please refer to *3.7.3. Additional considerations on the benefit-risk balance*).

A limitation of the clinical development program of ECT-001-CB is that only AEs of grade ≥3 (i.e. severe, life-threatening or fatal) or highly unusual grade 1-2 AEs were collected and reported. Due to this selective reporting additional common (and uncommon) AEs/ADRs of low grade were not considered ADRs by the applicant. The applicant committed to the collection of grade 1 and 2 adverse events in the planned post marketing studies.

Overall, considering the high risk disease study population and last resort clinical setting of patients without available donor source, results are considered acceptable for a conditional MA. However further confirmation is needed post-approval via the post-authorisation clinical studies included as specific obligations, to address uncertainties regarding the dosing, contextualization of time-dependent endpoints, lack of comparator, limited sample size, and long-term efficacy and safety profile.

3.7.2. Balance of benefits and risks

Based on the main efficacy data (proven neutrophils and platelets engraftment) and the acceptable safety profile in the context of a SCT, the final benefit/risk balance of ECT-001 is positive in the context of a conditional marketing authorisation.

3.7.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

As discussed above, uncertainties remain due to the limited size and follow-up of the pivotal studies population treated with cryopreserved ECT-CB-001, the single arm trial designs of the pivotal studies hampering interpretation of time-dependent endpoints, and the limited reliability of the contextualization of time-dependent endpoints derived from EBMT matched analysis.

Consequently the data cannot be considered comprehensive, but the efficacy and safety data provided are regarded as sufficient for a favourable benefit-risk conclusion in the context of a Conditional Marketing Authorisation under Article 14-a of Regulation (EC) No 726/2004. Post-authorisation studies are imposed to diminish uncertainties and contribute to data comprehensiveness. These studies will provide longer term data as well as further comparative efficacy and safety data in patients with an available allogeneic stem cell source (MMUD, haplo). The provision of this data post-authorisation will complement the data available so far in order to have a comprehensive understanding of efficacy and safety and confirm the positive benefit-risk balance of the product in the approved indication.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the treatment a seriously debilitating and life-threatening disease (as per Article 2, point 1, of Regulation (EC) No 507/2006). In addition, the product is designated as an orphan medicinal product.

Furthermore, the CAT considers that the product fulfils the requirements for a conditional marketing

authorisation:

- The benefit-risk balance is positive, as discussed.
- It is likely that the applicant will be able to provide comprehensive data post-authorisation with the 5 Specific Obligations (SOB) proposed.

  Two of the proposed SOBs entail the submission of follow-up results from studies 002 (by 28 February 2026) and 004 (by 31 August 2026) that will provide additional evidence on efficacy and safety of ECT-001-CB based on longer follow-up data.

Furthermore, studies 010, 011 and 012 are proposed as SOBs to support data comprehensiveness.

- ECT-001-CB.010: In order to compare the efficacy of infusing Zemcelpro to standard of care allogeneic HSCT and to evaluate the safety of Zemcelpro in patients aged 18-21 years included in the high-risk paediatric AML prospective, interventional study, the MAH shall conduct and submit the results (by 30 June 2030) of the subgroup analysis in patients aged 18-21 years (ECT-001-CB.010). The study is expected to provide relevant data (i.e. an additional independent data set to increase the sample size of patients treated with ECT-001-CB) in support of the adult indication. A subgroup analysis in a maximum of 20 patients aged 18-21 will be provided within the CSR, as this patient population will contribute to the comprehensiveness of the clinical data. While controlled data will be derived from this study, these will likely provide limited contextualisation for long-term efficacy of ECT-001-CB due to the limited number of adult patients included. As stated above, this study is mainly intended to provide an additional independent dataset to increase the number of patients treated with ECT-001-CB.
- A prospective, randomized, open-label phase III study (ECT-001-CB.011) of Zemcelpro versus best available allogeneic stem cell source (haplo, MMUD) in adult patients with high risk acute leukemia/MDS to provide confirmatory evidence of the safety and efficacy of ECT-001-CB. Although there are several concerns related to the study protocol that still need to be addressed within 3 months after approval, this study is expected to address uncertainties associated with the lack of comparator, limited sample size and follow-up in the clinical trials that formed the basis for this CMA. The planned RCT will include patients representing a broader target population because patients with an available allogeneic stem cell source (MMUD, haplo) are eligible for inclusion. By excluding patients with a standard size or double cord blood unit as best available allogeneic stem cell source, no comparison can be made between expanded cord blood (Zemcelpro) and unexpanded cord blood. Although this is considered a limitation of the study, the study design is suitable to confirm the efficacy and safety of Zemcelpro in the indication patient population. The primary endpoint is EFS and the sample size was increased to 208 patients which is welcome. However, Study 011 is designed as a superiority trial, and for fulfilment of the SOB it is considered sufficient to show non-inferiority to available SOC (here MMUD/haplo). A revised study protocol including a non-inferiority margin should be submitted within 3 months after approval. Final results of the study are to be submitted by 30 June 2030.
- A prospective registry-based study (ECT-001-CB.012) to collect real-world safety and effectiveness data on patients that have received commercially available ECT-001-CB to further define the risk-benefit profile of ECT-001-CB. The proposed registry is expected to address concerns associated with the limited sample size and follow-up of the clinical trials that formed the basis for the CMA. As only 112 UCB transplantations in adults were performed in Europe in 2022 and the indication is for a last resort setting, the applicant

was requested to provide justification for the feasibility of the projected number of patients (30 patients in 2 years) which they did and was confirmed by various experts and an independent market research. The study protocol of study 011 was amended in order to collect all grades of AEs (not limited to grade 3 and higher) as well as the collection of haematological AEs (which was not foreseen) to allow for an accurate short and long term safety profile and AE incidence rate associated with the treatment of ECT-001. Final results of this study are to be submitted by 30 June 2031.

- Unmet medical needs will be addressed for patients with haematological malignancies who do not have access to any other type of transplant (due to absence of HLA-matched siblings, HLA-matched unrelated donors or haploidentical donors or CB of sufficient cell quantity) in the absence of other products authorised in the EU for this indication. Nevertheless, the targeted patient population is expected to be very small.
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. Zemcelpro would provide adult patients with haematological malignancies requiring an allogeneic hematopoietic stem cell transplantation following myeloablative conditioning, 'for whom no other type of suitable donor cells is available' (including double cord or standard size single cord blood graft) access to a potentially curative therapy. Therefore, it is agreed that the benefits to public health of immediate availability would outweigh the risks inherent in the fact that additional data are still required.

Additional expert consultation

Zemcelpro was selected for early dialogue with healthcare providers (EHA). The healthcare providers described the different sources for allogeneic HSCT. About 20 to 30% of the patients will have an HLA identical sibling as potential stem cell donor. An alternative is a HLA-compatible unrelated donor. The likelihood of finding a HLA-compatible unrelated donor in international registries for the Caucasian population is about 80%, while this is significantly reduced to less than 50% for other ethnic populations (e.g. Caribbean, African etc.).
The use of haploidentical stem cell transplantation by using novel graft-versus-host disease prophylactic treatment has become the most frequently used stem cell source for patients lacking an HLA-identical sibling or completely matched unrelated donor and is available in about 90% of the patients.
Cord blood transplantation is an alternative stem cell source for those who are lacking an HLA-identical sibling or unrelated donor. However, the most recent development of haploidentical stem cell transplantation suggests a better outcome in comparison to cord blood transplantation. Currently the number of cord blood transplantations in stem cell registries in Europe and US is steadily declining, but there are still some indications, especially if expansion leads to faster engraftment and the suggested stronger graft-versus-leukemia effect by retrospective studies can be confirmed.

The CHMP endorses the CAT conclusion on conditional marketing authorisation as described above.

3.8. Conclusions

The overall benefit/risk balance of Zemcelpro is positive, subject to the conditions stated in section 'Recommendations'.

The CHMP endorse the CAT conclusion on Benefit-Risk balance as described above

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit- risk balance of Zemcelpro is favourable in the following indication:

Zemcelpro is indicated for the treatment of adult patients with haematological malignancies requiring an allogeneic haematopoietic stem cell transplantation following myeloablative conditioning for whom no other type of suitable donor cells is available.

The CAT therefore recommends the granting of the conditional marketing authorisation subject to the below conditions.

Based on the draft opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit- risk balance of Zemcelpro in the treatment of adult patients with haematological malignancies requiring an allogeneic haematopoietic stem cell transplantation following myeloablative conditioning for whom no other type of suitable donor cells is available, is favourable and therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

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

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall submit the final results from **study ECT-001-CB.002**, A Phase II Open-label Study of ECT-001-expanded Cord Blood Transplantation in Patients with High Risk Acute Leukemia/myelodysplasia.	28 February 2026
In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall submit the final results from study **ECT-001-CB.004**, A Phase II Open-Label Study of ECT-001-Expanded Cord Blood Transplantation in Patients with High and Very High-Risk Acute Leukemia/Myelodysplasia.	31 August 2026
In order to confirm the efficacy and safety of Zemcelpro in patients aged 18-21 years with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall conduct and submit the results of the subgroup analysis of patients aged 18-21 years from study **ECT-001-CB.010**: A Prospective Randomized Phase II Trial of Allogeneic SCT with ECT-001-CB Expanded Cord Blood Transplant Without Serotherapy Versus Other Stem Cell Source in Pediatric Patients with High risk/refractory/relapsed Acute Myeloid Leukaemia, according to an agreed protocol.	30 June 2030
In order to confirm the efficacy and safety of Zemcelpro, and to further evaluate the dose parameters used in adult patients with high-risk and very high-risk acute leukaemia/MDS, the MAH shall submit the results of study **ECT-001-CB.011**: A Multicenter, Prospective, Randomized, Open-Label Phase III Study of ECT-001-CB (ECT-001-Expanded Cord Blood) Transplantation versus Best Alternative Allogeneic Stem Cell Source Transplantation (Haplo, MMUD) in Patients with High-Risk Acute Leukemia/Myelodysplasia, according to an agreed protocol.	30 June 2030
In order to confirm the efficacy and safety of Zemcelpro in adult patients with haematological malignancies requiring an allogeneic HSCT following myeloablative conditioning for whom no other type of suitable donor cells is available, the MAH shall conduct and submit the results of a prospective, non-interventional study based on data from a registry, and evaluate dose parameters collected for Zemcelpro lot manufactured for each patient enrolled in the study, according to an agreed protocol.	30 June 2031

The CHMP endorses the CAT conclusion on the specific obligation to complete post-authorisation measures for the conditional marketing authorisation as described above.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

The CHMP endorse the CAT conclusion on the conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

New Active Substance Status

Based on the review of available data on the active substance, the CAT considers that dorocubicel is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorses the CAT conclusion on the new active substance status claim.