INVESTIGATIONAL MEDICINAL PRODUCT DOSSIER

SR1-expanded umbilical cord blood-derived hematopoietic stem and progenitor cells

VERSION 1.0

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ABBREVIATIONS

(in alphabetical order)

AE	Adverse event
AhR	Aryl hydrocarbon receptor
API	Active pharmaceutical ingredient
ATMP	Advanced therapy medicinal product
CD	Cluster of differentiation
DMSO	Dimethylsulfoxide
EDTA	Ethylenediaminetetraacetic acid
FACS	Fluorescence activated cell sorting
Flt3L	FMS-like tyrosine kinase 3 ligand
GMP	Good manufacturing practice
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSA	Human serum albumin
HSC	Hematopoietic stem cells
HSPC	Hematopoietic stem and progenitor cells
IgG	Immunoglobulin G
JACIE	Joint Accreditation Committee ISCT & EBMT
LTC-IC	Long-term culture-initiating cell
MMIZ	Erasmus MC: Afdeling Medische Microbiologie en Infectieziekten
NK	Natural Killer cell
PBS	Phosphate buffered saline
QA	Quality assurance
QC	Quality control
QP	Qualified Person
SCF	Stem cell factor
SCGM	Stem cell growth medium
SCT	Stem cell transplantation
SFT	SCF + Flt3L + TPO
SOP	Standard operating procedure
SR1	StemRegenin-1
TPO	Thrombopoietin
UCB	Umbilical cord blood
WBC	White blood cells
WMDA	World marrow donor association

CHEMICAL PHARMACEUTICAL AND BIOLOGICAL DATA

1. INTRODUCTION

Bone marrow and mobilized peripheral blood are the most common hematopoietic stem and progenitor cell (HSPC) sources for transplantation. Yet, a suitable HLA-matched donor is not available for all patients eligible for allogeneic stem cell transplantation (SCT). Umbilical cord blood (UCB) is a valuable alternative stem cell source for patients in need of SCT but lacking a matched sibling or unrelated stem cell donor. After single umbilical cord blood transplantation (UCB-SCT), the time to neutrophil recovery and immune reconstitution is delayed and the incidence of graft failure is higher as compared to matched unrelated stem cell transplantation, especially in adult patients, which is generally attributed to the low number of hematopoietic stem and progenitor cells (HSPC) present in a single UCB unit (1,2). Graft failure, delayed myeloid engraftment and the profound delay in immune reconstitution lead to increased morbidity and mortality. Double UCB-SCT has been developed to improve engraftment (3). Double UCB-SCT is now commonly used in the adult setting and demonstrated a significant reduction in the risk of graft failure. However, double UCB-SCT did not result in faster neutrophil recovery or immune reconstitution despite doubling the cell dose (4,5).

Ex vivo expansion of HSPC is a promising approach that may favorably alter the kinetics of neutrophil and platelet recovery and immune reconstitution after UCB-SCT, depending on the expansion conditions. Ex vivo expansion protocols using cocktails of hematopoietic growth factors only, selectively expand more mature committed HPC at the expense of immature self-renewing long-term repopulating HSC (6,7). Transplantation of ex vivo expanded UCB-HSPC skewed towards more committed progenitors may be clinically exploited when combined with an unmanipulated UCB. The safety and clinical feasibility of this approach is currently assessed in both single and a double UCB-SCT settings (8). In the single UCB-SCT setting, ex vivo expansion is performed on only a portion of a UCB product. The ex vivo expanded product is then combined with the unmanipulated portion of the same UCB for transplantation. In the double UCB-SCT setting, an ex vivo expanded UCB-HSPC product is combined with a second unmanipulated UCB. Clinical data showed that the rapid initial hematopoietic reconstitution is primarily derived from the ex vivo expanded product, demonstrating the enhanced capacity of the expanded cells to provide rapid myeloid recovery, whereas long-term sustainable hematopoiesis is derived from the unmanipulated UCB product (9). In order to expand not only the more committed progenitor compartment but also the multipotent self-renewing HSC, expansion protocols are used in which hematopoietic growth factors, that promote both proliferation and differentiation of HSPC, are combined with differentiation-inhibiting factors. Factors that attenuate the differentiation of ex vivo expanded HSPC include the Notch ligand Delta1, nicotinamide, the copper chelator tetraethylenepentamine (TEPA) and the aryl hydrocarbon receptor antagonist StemRegenin1 (SR1) (7, 10-13). Results from the closed and ongoing Phase I/II clinical trials with these “non-skewed” ex vivo expanded UCB-HSPC also demonstrate a more rapid recovery of neutrophils that are almost exclusively derived from the ex vivo expanded graft (14-17). Sustained

long-term engraftment was derived from the ex vivo expanded unit in more than half of the patients in the clinical trials with nicotinamide- and SR1-expanded units (14, 17).

We have developed an efficient cytokine and SR1-based ex vivo expansion system for UCB-derived $CD34^+$ HSPC that results in a marked increase in absolute number of committed hematopoietic progenitor cells (HPC) and multi-potent hematopoietic stem cells (HSC). In preclinical studies, the expanded cell product was capable of long-term multi-lineage repopulation in immunodeficient mice (18). These preclinical studies served as the foundation for process development activities to prepare a GMP-compliant expansion protocol for clinical application. Frozen UCB products are thawed, washed and $CD34^+$ HSPC are selected using the CE-marked clinical grade CliniMACS technology. The selected $CD34^+$ HSPC are cultured in expansion medium with hematopoietic cytokines (SCF, FLT3-L and TPO) and SR1 in a closed system using gas-permeable culture bags for 10 days. Like the non-manipulated UCB products, the final SR1-expanded UCB-derived HSPC product contains a mixture of mature hematopoietic cells, lineage-restricted hematopoietic progenitors as well as multi-lineage phenotypic hematopoietic stem cells but in much higher numbers.

The SR1-expanded UCB-derived HSPC product will be used in a clinical phase I/II trial in which patients with hematological malignancies eligible for UCB-SCT are transplanted with the expanded HSPC product (see clinical protocol). The primary aim of the clinical study is to evaluate feasibility of single UCB-SCT using one SR1-expanded UCB-derived HSPC product. Secondary objectives includes the evaluation of the contribution of the progeny of the expanded product to the short- and long-term hematopoietic recovery and the kinetics of neutrophil and platelet recovery and immune reconstitution after transplantation.

2.1.S DRUG SUBSTANCE

Not applicable.

2.1.P MEDICINAL DRUG PRODUCT

2.1.P.1 Description and Composition of the Cell Therapy Medicinal Product

Nomenclature

SR1-expanded Umbilical cord blood (UCB)-derived hematopoietic stem and progenitor cells (HSPC).

General properties

The cell therapy medicinal product consists of SR1-expanded UCB-derived HSPC that contains a mixture of mature hematopoietic cells, hematopoietic progenitor cells (HPC) as well as phenotypic hematopoietic stem cells (HSC). The product contains at least 20 x 10^6 expanded $CD34^+$ HSPC. Upon transplantation, both HPC and HSC have the ability to home to their natural habitat in the bone marrow and exhibit proliferation and differentiation capacity. The in vivo proliferation and

differentiation of HSPC is orchestrated by soluble and cell-bound signals derived from bone marrow niche cells. HPC exhibit lineage-restricted differentiation potential and contribute mainly to early hematopoietic reconstitution following transplantation. HSC possess self-renewal capacity, have multi-lineage differentiation potential and contribute mainly to long-term hematopoietic recovery following transplantation.

2.1.P.2 Pharmaceutical Development

2.1.P.2.1 Components used in the generation of the Medicinal Product

Control of Starting Material

UCB units are obtained from public cord blood banks that are affiliated with international donor registries. The acceptance criteria of cryopreserved UCB units for ex vivo expansion and UCB units for direct infusion are identical (see Table 1). The procedure for receipt, acceptance and storage of UCB products is described in SOP CK10.4313 and falls under the license as Tissue Establishment of the Transplantation laboratory. Upon receipt, staff of the Transplantation laboratory checks the identity and integrity of the UCB unit, the transport temperature, the results of the communicable disease testing of the mother and the sterility testing of the product commissioned by the Cord Blood Bank and the presence of the final donor clearance by the Cord Blood Bank before acceptance of the frozen UCB product and temporary storage in a cryogenic storage device for cell therapy products of the Transplantation laboratory.

Table 1. Specification of the cellular starting material

Test	Method	Release criteria
Identity of UCB product	Comparison of unique product code on label of UCB bag and documentation of UCB-bank and Matchis	Product code on UCB bag and documentation match.
Integrity of frozen UCB product bag	Visual examination	No visible breaks or other abnormalities
Maternal communicable disease testing	Antibody and antigen tests on blood of the mother less than 30 days prior to collection of UCB	Negative for HIV-1, HIV-2, HBV, HCV and Treponema pallidum
Sterility of UCB product	Microbial cultures	Negative for bacterial and fungal contamination
Final donor clearance by Cord Blood Bank	Documentation	Present
Transport temperature	Temperature sensor in dryshipper	< -120 °C

Control of ancillary materials,

The manufacturing process of SR1- expanded UCB-derived HSPC starts with a minimal manipulation (thawing, washing and $CD34^+$ cell selection) which falls under the Tissue Establishment license of the Transplantation laboratory. The ancillary materials used during the minimal manipulation (see Table 2) are released by technicians of the Transplantation laboratory according to procedure CK06.2110. The ancillary materials used in the minimal manipulation are either registered medicinal products or CE-marked components approved for processing of human hematopoietic HSPC.

The manufacturing process continues with substantial manipulation (ex vivo expansion of the selected $CD34^+$ cells) which falls under the Manufacturing license for Cell- and Gene-therapy medicinal products of the Transplantation laboratory. For each ancillary material used during the substantial manipulation (see Table 2), except registered medicinal products, an ancillary material dossier is prepared (see appendix D for an example). The ancillary material dossier includes information about the producer and the production process of the ancillary material, a copy of a certificate of analysis (see appendix B), release criteria, a procedure for aliquoting (when applicable) and the theoretical dilution factor in the end product. The ancillary material dossier is approved by the QP. Before release, ancillary materials are quarantined. The release of these ancillary materials is performed by the head QA, according to procedure CK06.2120, based on the Certificate of Analysis..
The ancillary materials used in the manufacturing process are listed in Table 2.

Table 2. Ancillary materials

Product	Manufacturer	Source	Qualification	Theoretical dilution factor in end product
Minimal manipulation (Tissue Establishment license)				
CliniMACS® PBS/EDTA buffer	Miltenyi Biotec	CE-marked component	Certificate of Analysis	
CliniMACS® CD34 reagent	Miltenyi Biotec	CE-marked component	Certificate of Analysis	
$MgSO_4$ solution	Pharmachemie	Chemical	Registered Medicinal Product RVG 51948	
Pulmozyme® : DNAse	Roche	Recombinant protein	Registered Medicinal Product RVG 16734	
Cealb: Human serum albumin (HSA)	Sanquin	Donor blood	Registered Medicinal Product RVG16910	
GammaQuin: Normal human immunoglobulin (IgG)	Sanquin	Donor blood	Registered Medicinal Product RVG 16941	
Substantial manipulation (GMP license)				
SCGM serum-free medium	CellGenix	Human and recom-binant proteins GMP-grade	QA release statement / CoA	125.000
SCF	CellGenix	Recombinant Protein. GMP-grade	QA release statement / CoA	125.000
Flt3L	CellGenix	Recombinant Protein. GMP-grade	QA release statement / CoA	125.000

TPO	CellGenix	Recombinant Protein. GMP-grade	QA release statement / CoA	125.000
SR-1	Chem-Connection	Chemical GMP-grade	QA release statement / CoA	125.000
0.9% NaCl	Baxter	-	Registered Medicinal Product RVG 27512	None (= also excipient)
Cealb: Human serum albumin	Sanquin	Donor blood	Registered Medicinal Product RVG16910	None (= also excipient)

Control of excipients for administration

The excipients used for resuspension of the expanded cells are 0,9 % NaCl and Cealb/human serum albumin. Both excipients are registered medicinal products.

Table 3. Excipients for administration

Product	Manufacturer	Source	Qualification
0.9% NaCl	Baxter	Chemical GMP-grade	Registered Medicinal Product RVG 27512
Cealb: Human serum albumin	Sanquin	Donor blood	Registered Medicinal Product RVG16910

2.1.P.3 Manufacture

2.1.P.3.1 Manufacturer(s)

Manufacturing facility

Transplantation Laboratory
Department of Hematology
Erasmus University Medical Center
Wytemaweg 80
3015 CN Rotterdam
The Netherlands

Qualified Person (QP)

Dr. V. Lorenzi, Department of Hospital Pharmacy, Erasmus MC.
Prof. dr. A.G. Vulto, Department of Hospital Pharmacy, Erasmus MC.

Description of manufacturing facility

The SR1-expanded UCB-derived HSPC product will be produced in the Erasmus MC Core facility for Cell- and Gene Therapy which is localized within the department of Hematology. The Transplantation Laboratory of the Department of Hematology is responsible for the supervision and management and of the Erasmus MC Core Facility for Cell- and Gene-Therapy. A comprehensive quality management system is operational, ensuring that all applicable quality and safety rules are met. It includes: internal

audits; registration of errors, accidents and adverse events; validation of key equipment and procedures; maintenance of supplies, reagents and equipment, calibration of equipment and participation in external proficiency testing programs. There are written policies and procedures regarding testing, processing, storage, release and transport of cell- therapy products.

The cleanroom facility comprises two clean-rooms for non-substantial manipulation of HSPC-, T cell- and investigational products and three separate clean-rooms for substantial manipulation, i.e. the production of ATMP cell-therapy medicinal products, for clinical application. The clean-rooms (GMP class C) are equipped with class A laminar flow cabinets. According to GMP Annex 1, aseptic processing steps should occur in a class A laminar flow cabinet in a class B cleanroom. To fulfill the GMP requirements, we validated that class A conditions in the laminar flow cabinet are maintained in our class C ATMP production cleanrooms during aseptic processing procedures (CK.VAL.2011.04).

The Transplantation laboratory of the department of Hematology of the Erasmus MC holds a license as a Tissue Establishment and a manufacturing license and GMP certificate for the production of cell-therapy medicinal products from the competent authorities in the Netherlands (see Table 4 & appendix C).

Table 4. Overview of accreditations and licenses of the Transplantation laboratory, department Hematology, Erasmus MC.

Type of accreditation/license	Accreditation/license Start / expiration year	Accreditation/license number
RvA-CCKL accreditation	2006 / 2019	153
JACIE accreditation	2006 / 2020	537
Tissue Establishment license	2006 / -	5512 L/EO
Manufacturing license for the production of ATMP Cell- and Gene Therapy products GMP certificate	2012 / - 2015 / -	108517F NL/H/15/1004371

2.1.P.3.2 Description of Manufacturing Process and Process Controls

Collection and transport of cellular starting material

Umbilical Cord Blood (UCB) is collected at specialised independent cord blood collection facilities and subsequently processed, cryopreserved and stored in public cord blood banks world-wide. The department of Hematology, Erasmus MC is not involved in the collection and storage of umbilical cord blood units. The search for suitable UCB units that meet the criteria for HLA-matching and total nucleated cell number, for direct transplantation or ex vivo expansion prior to transplantation, for an individual patient is performed by Stichting Matchis (formerly Europdonor) in Leiden in collaboration with the department of Hematology, Erasmus MC. UCB units are obtained from public cord blood banks that are affiliated with international donor registries, that are either accredited by the World

Marrow Donor Association (WMDA) or work according WMDA standards. Preferably, UCB units are selected from NETCORD/FACT accredited cord blood banks. The cryopreserved UCB units are shipped in a dry-shipper container. The cryopreserved UCB units and accompanying documentation are transported by a qualified courier and delivered at the Transplantation laboratory, department Hematology, Erasmus MC. The procedure for receipt, acceptance and storage of UCB products is described in SOP CK10.4313 and in section 2.1.P.2.1. Since 2006, the Transplantation laboratory has received more than 200 UCB units from public cord blood banks worldwide for direct infusion in patients included in the Hovon 106 and Hovon 115 studies. The UCB product is accepted for non-substantial manipulation using the criteria in Table 1.

Manufacturing procedure

The manufacturing process of SR1-expanded UCB-derived HSPC is performed in the Erasmus MC cleanroom facility for Cell- and Gene-Therapy. The production process is performed according to SOP's of the Transplantation laboratory that are authorized by the head of the Transplantation laboratory Dr. E. Braakman. A flow chart of all successive steps in the production process is provided in Appendix A. The ancillary materials/ excipients and the respective steps in which they are used in the production process are also shown in this flow chart. The in-process (IP) control tests and the critical steps in the production process after which they are performed are shown in the same flow chart.

Thawing, washing and isolation of $CD34^+$ HSPC from cryopreserved UCB (SOP CK10.4234)

Cryopreserved UCB units are thawed at 37 $^{\circ}$C and diluted 1:1 in thawing medium (see for composition Table 5) and incubated for 30 minutes at room temperature (RT). Subsequently, cells are centrifuged and washed once with washing medium (see for composition Table 5).

Table 5. Composition of media used in generation of SR1-expanded UCB-derived HSPC

Medium	Composition
Thawing medium	0,9 % NaCl containing 5% human serum albumin, 3.5 mM $MgSO_4$ and 100 U/ml Pulmozyme®
Washing medium	0,9 % NaCl containing 0.5% human serum albumin
CliniMACS® PBS/EDTA buffer	Phosphate buffered saline, pH 7.2, supplemented with 1 mM EDTA and 0,5 % human serum albumin
Expansion medium	SCGM serum-free medium containing 50 ng/ml SCF, 50 ng/ml Flt3L, 50 ng/ml TPO and 1 µg/ml SR-1
Infusion medium	0.9% NaCl containing 5% human serum albumin

To isolate $CD34^+$ HSPC, washed UCB cells are resuspended in 8 ml washing buffer, 0.75 ml CliniMACS CD34 reagent and 1 ml Gammaquin are added. Cells are incubated for 30 minutes at RT.

After incubation, UCB cells are washed and resuspended in 100 ml washing medium. The cell bag is docked to the CliniMACS tubing set assembled on the CliniMACS instrument. The $CD34^+$ cell selection is performed using the automated $CD34^+$ cell selection program on the CliniMACS instrument. The CliniMACS instrument, CliniMACS disposable tubing set, CliniMACS CD34 reagent and CliniMACS PBS/EDTA buffer are all CE-marked and routinely used in stem cell transplantation laboratories for immuno-magnetic positive selection of $CD34^+$ HSPC for transplantation. After the selection procedure, the CD34-positive cell fraction and CD34-negative fractions are collected. Samples are taken from both fractions and the absolute cell number and number and frequency of $CD34^+$ cells are determined. Finally, the CD34-positive cell fraction, named HPC, CORD BLOOD CD34-enriched product, when meeting the release criteria (see Table 6), is released for further processing into an ATMP by the Responsible Person of the Tissue Establishment. The CD34-negative fraction, which is a source of donor T cells, is cryopreserved and stored in the vapor phase of liquid nitrogen until the day of transplantation, when it is thawed and infused 1 day after the administration of the SR1-expanded UCB-derived HSPC product.

Table 6 Release criteria for HPC, CORD BLOOD CD34-enriched product for further processing into an ATMP

Release criteria	Specification
Product identification	Product ID on product bag and release form are identical
Screening for communicable diseases (HBV, HCV, HIV 1 / 2, Treponema)	- Screening < 30 days prior to collection - No signs of any active infection - Final donor clearance by UCB-bank is present
Visual inspection	Product looks normal, bag is intact
Number of viable $CD34^+$ cells	≥ 0,5 x 10^6
% viable $CD34^+$ cells within viable $CD45^+$ cells	≥ 80 %
Viability	≥ 50 %

Ex vivo expansion of HPC, CORD BLOOD CD34-enriched product (SOP CK10.4235)

The HPC, CORD BLOOD CD34-enriched product is centrifuged and resuspended in serum-free expansion medium (see for composition Table 5) at a cell concentration of 1,0 x 10^5 cells/ml. The resuspended cells are transferred into a VueLife® 290-AC cell gas permeable culture bag. The culture bag with cells is incubated in a 37 °C , 5% CO_2 humidified incubator. An equal volume of expansion medium is added to the cultures at day 2, 4, 7 and 9 to achieve the desired HSPC expansion. Cultures are visually examined for signs of microbial contamination and cell morphology at each medium addition. At day 7, the concentration and number of WBC and viable $CD34^+$ cells is determined to check whether the cell concentration is still within the optimal range for expansion (≤ 1,0 x 10^6 WBC/ml). The cell density is guiding in the decision on the volume of the expansion medium

to be added at day 7, to stay within the optimal cell concentration range. The in-process controls, analysis methods and product specifications are tabulated in Table 7.

Preparation of the SR1-expanded UCB-derived HSPC product for infusion

After 10 days of culture, the SR1-expanded UCB-derived HSPC are harvested. Cells are centrifuged, resuspended and washed twice with washing medium. After washing, cells are resuspended in 100 ml infusion medium (see for composition Table 5). Final product release is based on the product specifications including sterility, the number of WBC, viability and the number of viable $CD34^+$ HSPC cells. The release criteria for the SR1-expanded UCB-derived HSPC are shown in Table 9. In addition and for information only, the SR1-expanded UCB-derived HSPC product is also analyzed for the frequencies of phenotypic HSC ($CD34^+CD38^{low}CD45RA^-CD90^+$) and mature T-, B- and NK-cells (see Table 7).

Administration of cell therapy medicinal product

The final SR1-expanded UCB-derived HSPC product, when meeting the release criteria, is conditionally released by the QP. The released product is transported to the ward by staff of the Transplantation laboratory for direct infusion into the patient. No final dilution or concentration steps occur outside the Erasmus MC Core facility for Cell- and Gene-therapy. Definitive release of the SR1-expanded UCB-derived HSPC product occurs by the QP after confirmation that the microbial cultures of the final product are negative.

Batch Size

Each SR1-expanded UCB-derived HSPC product is prepared for a single administration in an individual patient. Thus, the batch size is 1. The product identification number of a batch is a serial number assigned by the Transplantation laboratory.

2.1.P.3.3 Controls of Critical Steps and Intermediates

At several critical points during the production process of SR1-expanded UCB-derived HSPC, in process controls are performed (see Appendix A and Table 7), on basis of which process decisions are made. Some in process controls are performed for monitoring purposes only and are not used for process decisions. All in process controls are performed by qualified personnel. The analysis procedures are described in SOP's of the Transplantation Laboratory except for the sterility testing which is outsourced to the department of Medical Microbiology and Infectious Diseases (MMIZ) of the Erasmus MC. The sterility test is described in SOP an050 from MMIZ. MMIZ is ISO 15189:2012 accredited (accreditation number M132) and holds a license as Donor Test laboratory from the competent authorities in The Netherlands (license number 108505 L/VD). The quality requirements and the respective roles and responsibilities of the departments of MMIZ and Hematology in the outsourced microbiology testing are laid down in a Service Level Agreement (DVO MMIZ-HEMA).

Please be referred to Table 1 and Table 6 for control of the cellular starting material for the non-substantial and the substantial manipulation respectively.

After thawing and washing of the cryopreserved Cord Blood unit, a sample is taken for sterility testing, WBC/RBC counts, viability testing and $CD34^+$ cell enumeration. Testing for bacterial and fungal contamination is performed using Bactec™ PEDS plus culture bottles by MMIZ. WBC/RBC count is measured using an Act Diff hematology analyzer in the cleanroom facility. Determination of the percentage of viable cells is performed manually using the Trypan blue dye exclusion method.
Enumeration of viable $CD34^+$ cells is performed using flowcytometric analysis on a FacsCanto flowcytometer in the quality control laboratory of the Transplantation laboratory that is situated next to the cleanroom facility.

After the $CD34^+$ cell selection procedure, samples are taken from the CD34-positive and CD34-negative cell fraction for sterility testing, WBC count, viability testing and $CD34^+$ cell, T-, B- and NK cell enumeration. In view of the low number of cells in the positive fraction WBC count is performed manually using a hemocytometer and microscope. The other in process tests are performed as described above.

After washing the $CD34^+$ cell fraction and resuspension in expansion medium, a WBC count is performed just prior to initiation of the expansion culture.
Before each medium addition, the culture bag with cells is visually inspected for signs of microbial contamination. From day 7/8 of culture onwards, a sample is taken before each medium addition for WBC count, viability testing and $CD34^+$ cell enumeration.

After harvesting and washing the final ex vivo expanded cell product, the final cell product is resuspended in infusion medium (see Table 5). A sample is taken for sterility testing, WBC count, viability testing and enumeration of viable $CD34^+$ HSPC and phenotypic HSC, mature T-, B- and NK-cells.

Table 7. In-process control tests and process decisions

Intermediate product	In process test	Analysis method	Laboratory site	Criteria	Out of range process decision
A) Thawing, washing and CD34$^+$ cell selection of UCB product					
Thawed and washed UCB unit (Day 0)	Sterility	Microbial culture BacTec™ PEDS culture bottle	Dept. of MMIZ, Erasmus MC	No bacterial or fungal contamination.	Stop production.
	WBC / RBC count	Act Diff Hematology analyzer	Transplantation laboratory	None	-
	Viability	Trypan Blue staining	Transplantation laboratory	None	-
	Viable CD34+ cell enumeration	Flow cytometry	Transplantation laboratory	≥ 0.5 x 10^6 CD34+ cells	No start of positive selection of CD34+ cells
CD34-positive cell fraction (Day 0)	Sterility	Microbial culture BacTec™ PEDS culture bottle	Dept. of MMIZ, Erasmus MC	No bacterial or fungal contamination.	Stop production.
	WBC count	Bürker counting chamber	Transplantation laboratory	None	-
	Viability	Trypan Blue staining	Transplantation laboratory	≥ 50 %	-
	Viable CD34+ cell enumeration	Flow cytometry	Transplantation laboratory	≥ 0.5 x 10^6 CD34+ cells	No initiation of ex vivo expansion.
B) Ex vivo expansion of CD34-positive Fraction (=HPC, CORD BLOOD CD34-enriched)					
Washed CD34-positive cell fraction (Day 0)	WBC count	Bürker counting chamber	Transplantation laboratory	≥ 80 % of WBC count before wash	-
Intermediate expanded cell product (Days 2,4,7,9)	Sterility	Visual examination	Transplantation laboratory	No bacterial or fungal contamination.	Stop production.
Intermediate expanded cell product (Day 7)	WBC count	Bürker counting chamber	Transplantation laboratory	None	-
	Viability	Trypan Blue staining	Transplantation laboratory	None	-
	Viable CD34+ cell enumeration	Flow cytometry	Transplantation laboratory	None	-
Final SR1-expanded UCB-derived HSPC product (Day 10)	Sterility	Microbial culture BacTec™ PEDS culture bottle	Dept. of MMIZ, Erasmus MC	No bacterial or fungal contamination.	No definitive release of product.
	WBC count	Bürker counting chamber	Transplantation laboratory	WBC ≥ 50 x 10^6 cellen	No release of product.
	Viability	Trypan Blue staining	Transplantation laboratory	≥ 70 % trypan blue negative	No release of product.
	Viable CD34$^+$ cell enumeration	Flow cytometry	Transplantation laboratory	Viable CD34$^+$ cells > 20 x 10^6 cells	No release of product.
	Enumeration of viable HSPC subsets, T-, B- and NK cells	Flow cytometry	Transplantation laboratory	None	-

2.1.P.3.4 Manufacturing Process Development, Batch analysis, Justification of Release Criteria and Process Validation

Summary of preclinical development of the manufacturing process.

In the past three years, the ex vivo expansion protocol of UCB-derived CD34$^+$ HSPC has been developed and optimized by researchers of the department of Hematology, Erasmus MC in close collaboration with researchers from Glycostem Therapeutics and the Laboratory of Hematology, Radboud UMC. Previously, our collaborators developed a highly efficient two-step expansion and differentiation protocol for the generation of large numbers of functional NK cells from UCB-derived CD34$^+$ HSPC (19, 20). In the first step, CD34$^+$ HSPC are induced to expand for two weeks in

Glycostem's GBGM medium supplemented with early-acting hematopoietic growth factors (SCF, TPO and Flt3L) and late-acting cytokines (G-CSF, GM-CSF, IL-6, LIF and MIP1α) and serum (complete GBGM medium). Subsequently, the expanded progenitor cells are induced to differentiate into NK cells. The first step of the Glycostem / Radboud UMC NK cell expansion/differentiation protocol served as the starting point for the preclinical development of this ex vivo expansion protocol.

Culture of purified UCB-derived $CD34^+$ HSPC in complete GBGM medium resulted in a 30 to 90-fold increase in total number of viable nucleated cells after one week of culture. However, the robust proliferation was accompanied by massive differentiation of $CD34^+$ HSPC into more mature $CD34^-$ cells leading to only a 5-10-fold increase in number of $CD34^+$ HSPC after 2 weeks of culture. To reduce the massive differentiation while maintaining the robust proliferation, we evaluated the effects of serum removal and the removal of late acting cytokines from the medium. Removal of serum reduced the overall expansion 2 to 3-fold, but did not affect the fold increase in number of $CD34^+$ HPC, due to the delayed differentiation of $CD34^+$ HSPC into $CD34^-$ cells. Removal of late acting cytokines from serum-free medium did not affect the overall expansion of nucleated cells nor the expansion of $CD34^+$ HSPC as compared to complete serum-free medium.

In accordance with recent literature (13), we demonstrated that the aryl hydrocarbon receptor (AhR) antagonist, StemRegenin 1 (SR1), promotes SCF, Flt3L and TPO (SFT)-driven $CD34^+$ HSPC expansion by inhibition of HSPC differentiation (18). Addition of SR1 to the SFT-expansion medium resulted in no significant difference of CD45+ cells after 14 days of culture in either SFT or SFT+SR1 medium (Fig1A, $p=0.23$). During expansion, the frequency of $CD34^+$ cells gradually declined due to concomitant differentiation of the expanding cells. Addition of SR1 delayed the decline in frequency of $CD34^+$ cells, resulting in a higher frequency of $CD34^+$ cells at both 7 and 14 days compared to SFT medium alone (Fig1B, $p<0.0001$ for both time points), suggesting inhibition of differentiation by SR1. The increased frequency of $CD34^+$ cells led to an increased expansion of $CD34^+$ cells after 1 and 2 weeks of culture (Fig1C, $p=0.0083$ and $p=0.006$ respectively). A 55-fold (SD ± 44.5) expansion of $CD34^+$ cells upon addition of SR1 after 2 weeks of culture was observed, compared to a 23-fold (SD ± 20.8) expansion upon culture in SFT medium alone.

$CD34^+$ cells are a heterogeneous population, of which the most immature HSC are only a minor subset. Next, we evaluated whether the expanded $CD34^+$ cells still contained cells with the most immature phenotype, i.e. $Lin^-CD34^+CD38^{low}CD45RA^{low}CD90^+$ cells (referred to as "$CD34^+CD90^+$ cells" hereafter), which are highly enriched for HSC (21). Before culture, $CD34^+CD90^+$ comprised 4.0% (SD ±2.1) of the $CD34^+$ cell population (Fig1D). After 14 days of culture, the frequency of $CD34^+CD90^+$ cells within the $CD34^+$ cell population remained stable in both culture conditions (Fig1D, $p=0.38$ for SFT; $p=0.18$ for SFT+SR1). The number of cells with the most immature phenotype expanded 26.9-fold (SD ±9.6) in the presence of SR1 and 8.9-fold (SD ±9.5) in the absence of SR1 (Fig1E, $p=0.0003$).

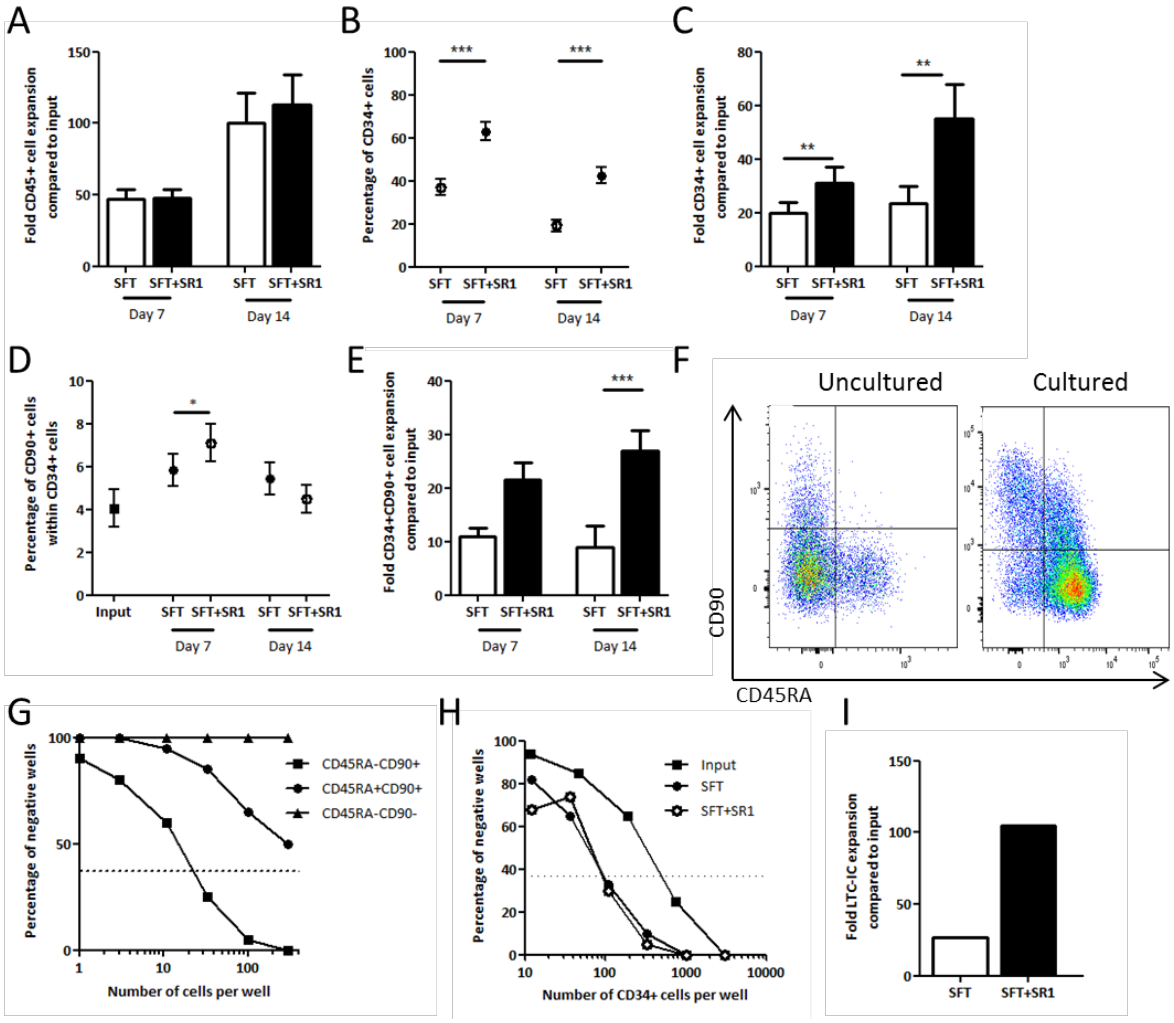

Figure 1. StemRegenin1 promotes growth factor-driven expansion of UCB-derived CD34$^+$ cells. *UCB-derived CD34$^+$ cells were cultured in serum free SFT medium with or without the addition of SR1. Cells were analyzed using flow cytometry at 7 and 14 days of culture. Shown are (A) the CD45$^+$ cell expansion compared to input (n=10), (B) the frequency of CD34$^+$ cells within the CD45$^+$ cell population (n=10), (C) the expansion of CD34$^+$ cells compared to input (n=10), (D) the frequency of CD90$^+$ cells within the CD34$^+$ cell population (n=6) and (E) expansion compared to input of CD34$^+$CD90$^+$ cells (n=6).(F) Shows the expression of CD45RA and CD90 in either uncultured (left panel) or cultured (right panel) Lin$^-$CD34$^+$CD38^{low} cells (representative out of 6). (G) and (H) show the frequency of negative wells versus the number of cells plated in a LTC-IC assay. In (G), LTC-IC frequency for several sorted population upon SFT+SR1 culture are shown. (H) shows the LTC-IC frequency in uncultured and cultured CD34$^+$ cells. (I) Fold expansion of LTC-IC after 14 days of culture compared to input. * p<0.05, ** p<0.01, *** p<0.001*

During expansion, a population of CD45RA$^+$CD90$^+$ cells appeared within CD34$^+$ cells that was not present in unexpanded CD34$^+$ cells (Fig1F). To assess whether after ex vivo expansion in the presence of SR1, the functionally most immature cells were primarily present within the phenotypic most immature CD45RA^{low}CD90$^+$ subset or also present in the new CD45RA$^+$CD90$^+$ subset, 3 populations of cells were sorted, namely Lin$^-$CD34$^+$CD38^{low}CD45RA^{low}CD90$^+$ cells, Lin$^-$CD34$^+$CD38^{low}CD45RA^{low}CD90^{low} cells and Lin$^-$CD34$^+$CD38^{low}CD45RA$^+$CD90$^+$ and long term culture-initiating (LTC-IC) assays were performed. At present, the LTC-IC assay is the best in vitro surrogate assay for primitive HSPC. The highest frequency of LTC-IC was found in the CD45RA^{low}CD90$^+$ population (Fig1G, 1 in 23.3 cells), indicating that after culture, the phenotypically most immature cells

are still enriched for LTC-IC. The new $CD45RA^{+}CD90^{+}$ subset contained a much lower frequency of LTC-IC (~1/400), whereas the $CD90^{low}$ population contained no detectable LTC-IC. To quantify the increase in LTC-IC in expansion cultures in the presence and absence of SR1, we determined LTC-IC frequencies upon 14 days of culture in either SFT or SFT+SR1 medium, without prior cell sorting. Upon 14-day culture in either the absence or presence of SR1, an increase in the frequency of LTC-IC in $CD34^{+}$ cells was observed (Fig1H, input: 1 in 466 cells; SFT: 1 in 109 cells; SFT+SR1: 1 in 90 cells), resulting in a 105-fold expansion of LTC-IC upon culture in SFT+SR1 medium (Fig1I). Taken together, our data show that SR1 not only enhances growth factor driven expansion of $CD34^{+}$ cells, but also of the phenotypically most immature $Lin^{-}CD34^{+}CD38^{low}CD45RA^{low}CD90^{+}$ cells and the functionally most immature cells as assessed by the in vitro LTC-IC assay.

To evaluate the engraftment potential and repopulation ability of SR1-expanded UCB-derived $CD34^{+}$ cells, sub lethally irradiated NSG mice were transplanted with either 10^{5} purified unmanipulated $CD34^{+}$ cells or the input-equivalent (i.e. the number of expanded cells that is derived from 10^{5} $CD34^{+}$ cells) after expansion in the presence or absence of SR1. In time, the percentage human chimerism in blood increased (Fig 2A). All mice engrafted in the bone marrow, with human chimerism levels 72.9% for non-expanded cells and 33.3% and 28.2% for SFT- and SFT+SR1 expanded cells, respectively (Fig 2B). Multi-lineage engraftment was assessed both in the peripheral blood and bone marrow of all transplanted mice (Fig 2C). In all engrafted mice more than 80% of human cells in blood were $CD19^{+}$ B-cells, but $CD33^{+}$ myeloid cells, $CD3^{+}$ T cells and $CD56^{+}$ NK-cells were also detected, albeit in low levels (Fig 2D). Multi-lineage engraftment was also found in bone marrow of mice transplanted with either input CD34+ cells or CD34+ cells expanded with or without SR1 (Fig2E).

In conclusion, we have developed an efficient serum-free, cytokine- and SR1-based ex vivo expansion system for UCB-derived $CD34^{+}$ HSPC that results in a marked increase in absolute number of $CD34^{+}$ HSPC, including the phenotypic most immature HSC subpopulation.

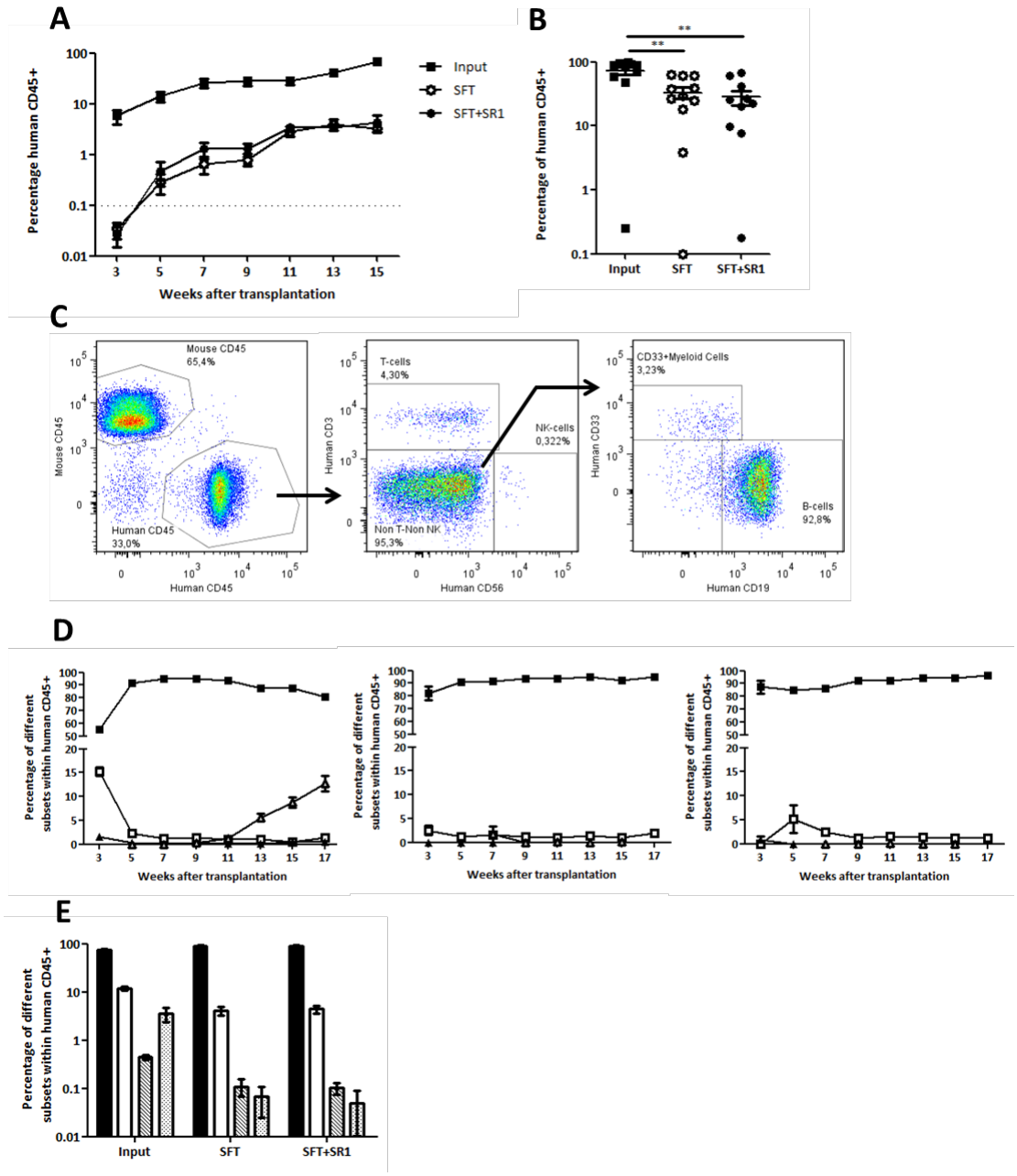

Figure2. Engraftment potential and hematopoietic repopulation ability of SR1-expanded UCB-derived CD34$^+$ cells. *10^5 Selected, non-expanded CD34$^+$ cells and the input-equivalent of those cells upon 8 days of culture in SFT of SFT+SR1 medium were intravenously transplanted into NSG mice (input: n=25; SFT: n=18; SFT+SR1; n=15). Short-term repopulation was measured in the peripheral blood every 2 weeks, starting 3 weeks after transplantation. At week 15 or 17, mice were sacrificed and long-term human chimerism was measured in the bone marrow. Shown are (A) levels of human chimerism in the peripheral blood (Y-axis) over time (X-axis), (B) levels of human chimerism at 15 or 17 weeks post-transplantation in the bone marrow , (C) representative FACS plots showing the gating strategy determining the different subsets within the human CD45$^+$ population, (D) the representation of CD19$^+$ B cells (black squares), CD33$^+$ myeloid cells (white squares), CD56$^+$ NK cells (black triangles) and CD3$^+$ T cells (white triangles) within the human CD45$^+$ population in the peripheral blood over time upon transplantation of non-expanded (left panel), SFT-expanded (middle panel) or SFT+SR1-expanded cells (right panel) and (E) the distribution of CD19$^+$ B cells (black bar), CD33$^+$ myeloid cells (white bar), CD56$^+$ NK cells (striped bar) and CD3$^+$ T cells (dotted bar) in the bone marrow 15 or 17 weeks after transplantation. ** p<0.01*

Translation of the research expansion protocol into a GMP-compliant expansion protocol.

The preclinical development studies described above served as the foundation for process development activities to prepare a GMP-compliant expansion protocol for clinical application.

In the preclinical studies, we noted that serum-free GBGM expansion medium, in contrast to serum-containing GBGM expansion medium showed batch to batch variability in their potency to support $CD34^+$ HSPC expansion. Therefore three commercially available serum-free HSPC-expansion media were compared: StemSpan SFEM medium, StemSpan ACF medium and CellGro SCGM medium. StemSpan SFEM medium is a research grade expansion medium produced by Stemcell Technologies. The same company produces the StemSpan ACF medium, which is not manufactured in compliance with GMP guidelines but which is used in FDA-approved cell therapy trials in Canada and the USA. The CellGro SCGM medium is manufactured in compliance with GMP guidelines by CellGenix in Germany. We compared the potency of these 3 media to support hematopoietic stem and progenitor cell expansion ex vivo and found the three media to be more potent than GBGM medium. The 3 media were more or less equally potent. Based on these results and the fact that the CellGro SCGM medium is manufactured according to GMP guidelines, we decided to use the Cellgro SCGM medium in our GMP-compliant expansion protocol. Overall we observed a stable and reproducible expansion of $CD34^+$ cells of at least 30-fold in SCGM medium.

Next, we performed upscaling studies using Vuelife® 290AC culture bags instead of plates and flasks. The fold expansion in bags was also comparable to that in wells/flasks. Finally, we changed the research-grade SR1 from Abcam Medchem which was used in the preclinical experiments, into the GMP-grade SR1 from ChemConnection in the expansion cultures. The fold expansion with research- and GMP-grade SR1 were found to be similar.

Validation of manufacturing process

To demonstrate that the GMP-compliant expansion protocol reproducibly yield the required expansion of $CD34^+$ cells, 8 validation runs were performed in which clinically relevant numbers of UCB-derived $CD34^+$ cells (i.e. > 0,5 x 10^6 $CD34^+$ cells) were expanded in Vuelife® 290AC culture bags in SCGM medium with GMP-grade cytokines (SCF, Flt3L and TPO) and SR1. Table 8 summarizes the yields of WBC and $CD34^+$ cells and the fold expansion of WBC and $CD34^+$ cells in these expansion cultures.

Table 8. Validation of expansion protocol

Exp. nr.	Cell number at start of culture (x 10^6)		Cell number at end of culture (x 10^6)		Fold expansion	
	WBC	CD34+ cells	WBC	CD34+ cells	WBC	CD34+ cells
1	1,84	1,70	421	172	229	101
2	2,14	1,93	488	175	227	91
3	1,77	1,74	399	149	225	86
4	0,83	0,82	105	37	127	45
5	2,01	2,00	184	74	92	37

Exp. nr.	Cell number at start of culture (x 10^6)		Cell number at end of culture (x 10^6)		Fold expansion	
	WBC	CD34+ cells	WBC	CD34+ cells	WBC	CD34+ cells
6	2,09	2,01	375	183	180	91
7	2,27	2,20	157	86	69	39
8	2,67	2,60	679	261	254	100

Exp. 1, 2 and 6 were started with selected $CD34^+$ cells from a fresh UCB product and exp. 3, 4, 5, 7 and 8 were started with selected CD34+ cells from a cryopreserved UCB product.

The results of these 8 validation runs demonstrate that the end-product of the GMP-compliant expansion protocol always met the predetermined release criteria (WBC ≥ 50 x 10^6 cells and $CD34^+$ cells ≥ 20 x 10^6) (see table 9). These 8 validation runs were performed in the research lab. We will perform 3 additional validation runs in a clean room of the Erasmus MC Core Facility for Cell- and Gene-Therapy before the start of the clinical trial.

Justification of release criteria (see Table 9)

It is well established that the dose of $CD34^+$ cells infused is related to patient survival and time required for engraftment (2, 22). In the past 10 years, we have thawed more than 200 UCB products derived from public cord blood banks world-wide for double UCB-SCT. The median dose of viable $CD34^+$ cells in a thawed UCB product was found to be 1,86 x 10^6 (range 0,24 x 10^6 – 13 x 10^6). The release criterion of ≥ 20 x 10^6 viable $CD34^+$ cells ensures that the dose of ex vivo expanded viable $CD34^+$ cells is at least 10-fold higher than the median dose of viable $CD34^+$ cells in a thawed UCB product. On basis of the published results of clinical trials with ex vivo expanded UCB-derived HSPC (10, 14-17), a dose of more than 20 x 10^6 viable $CD34^+$ cells is expected to result in a more rapid recovery of neutrophils.

2.1.P.4 Control of Excipients

Please be referred to paragraph 2.1.P.2.1 (Components used in the generation of the Medicinal Product).

2.1.P.5 Control of Cell Therapy Medicinal Product

2.1.P.5.1 Specification(s)

The analytical procedures used to control the intermediate cell products and the ex vivo expanded HSPC end-product and the specifications are described in paragraph 2.1.P.3.3. and included in Table 7.

The QC tests on intermediate cell products and the end-product are performed by qualified ATMP production personnel except for the sterility tests that are performed by qualified personnel from the department of MMIZ. It is the responsibility of the head QA/QC to verify that the analyses have been performed correctly and to report the results of the QC tests on the release form (appendix E). The

release of the UCB-derived ex vivo expanded HSPC end-product is performed by one of the QPs for cell- therapy products, Dr. V. Lorenzi or Prof Dr. A Vulto. The release of the HSPC end-product is based on results of control tests from both intermediate cell products and the end-product. The SR1-expanded UCB-derived HSPC end-product will only be released when the following release criteria are met (Table 9).

Table 9. Release criteria for UCB-derived ex vivo expanded HSPC product

In process test	Analysis method	Criteria
Release of UCB-derived $CD34^+$ cell fraction for ex vivo expansion by the Responsible Person of Tissue Establishment	-	Present.
Appearance (intermediate and end-products)	Visual examination	The product shows no signs of contamination (not cloudy), no clumps and the bag is intact.
Sterility (end-product)	Microbial culture: BacTec™ PEDS culture bottle	No bacterial or fungal contamination.
Viability (end-product)	Trypan Blue exclusion	≥ 70 % life cells (trypan blue negative).
WBC count (end-product)	Bürker counting chamber	≥ 50 x 10^6 WBC.
$CD34^+$ cell enumeration (end-product)	Flow cytometry	≥ 20 x 10^6 $CD34^+$ cells.

The results from all HSPC end-product control tests, except for the microbial culture of the end product, are available prior to release of the HSPC end-product. When all other release criteria are met, the QP will conditionally release the HSPC end-product for delivery to the clinic for administration to the patient. The result of the sterility test on the HSPC end-product is available after approximately one week. In case the sterility test result is negative, i.e. no signs of bacterial or fungal contamination, the QP will definitively release the HSPC end-product (see appendix E for the ATMP release form). In case the sterility test result is positive, the contaminant micro-organism will be identified and an antibiogram will be established. These results are immediately reported to the head of production, the PI and the hematologist of the patient.

2.1.P.5.2 Validation of Analytical Procedures

Analysis of microbial contamination is performed using the Bactec automated blood culture and detection system by the department MMIZ. The Bactec automated sterility test has been validated according to Ph. Eur. 2.6.27 (23-25). The automated Bactec system is more sensitive, faster in time to detection and less prone to false positive results than the manual CFR method.

The manual determination of nucleated cell count and viability is performed by a haemocytometer and the trypan blue exclusion method (CK10.4001) in accordance with Ph. Eur. 2.7.29.

The automated determination of nucleated cell count is performed with an Act Diff Hematology analyzer in accordance with Ph. Eur. 2.7.29. The automated cell counting using the Act Diff

Hematology analyzer has been validated by the Transplantation laboratory (documents CK.VAL.2010.01 and CK.VAL. 2014.01).

The enumeration of $CD34^+$ HSPC, $CD3^+$ T cells, $CD19^+$ B cells and $CD3^-CD16/56^+$ NK-cells using flowcytometric analysis on a FacsCanto is performed according to SOPs CK10.4022 and CK10.4024, in accordance with Ph. Eur. 2.7.23 and 2.7.24. The flowcytometric analysis has been validated by the Transplantation laboratory (CK.VAL2011.01). The Transplantation laboratory participates in the UKNEQAS proficiency testing program for enumeration of $CD34^+$ cells and lymphocyte subsets.

2.1.P.5.3 Characterization of Impurities

Traces of medium and other ancillary materials will be present in the end-product. At the end of the expansion culture, the SR1-expanded UCB-derived HSPC product is harvested and extensively washed with 0.9 % NaCL and 0,5 % HSA. After washing, the expanded HSPC are resuspended in a volume of 100 ml infusion buffer. The calculated reduction factor of medium and ancillary materials used during the ex vivo expansion is at least 10.000-fold. Based on the ≥ 10.000-fold reduction factor, we calculate that the end-product contains less than 1 ng SCF, Flt3L and TPO and less than 10 ng SR1.

The reduction factor of the DMSO in the initial cryopreserved UCB product and the ancillary materials used in the preceding non-substantial manipulation is even much higher due to the CD34-selection procedure on the CliniMACS and the subsequent washing step. Based on this, we can confirm that DMSO levels in the end-product will be compliant with the requirements in the ICH-Q3 guideline.

2.1.P.6 Container Closure System

The UCB-derived ex vivo expanded HSPC end-product is packaged in a 150 ml Baxter Fenwal Transfer Pack™ container. The transfer pack is designed to be a single use, sterile, non-pyrogenic fluid path device that is used for the collection, transfer and processing of blood and blood components. The transfer pack is made of PVC plastic and sterilized by radiation. An example of the label on the SR1-expanded UCB-derived HSPC end-product is shown in appendix F.

2.1.P.7 Stability, Storage Conditions, Transport and Logging

The SR1-expanded UCB-derived HSPC end-product is intended for direct infusion. The storage temperature for the SR1-expanded UCB-derived HSPC end-product is 1-8 °C and the expiry time is 6 hours after the resuspension of the washed cells in infusion medium. After release by the QP on the day of harvest, the expanded HSPC product will transported at 1-8 °C in an outer shipping container by staff of the Transplantation laboratory to the clinic according to procedure CK09.4210. During transport, the temperature is monitored using an ACR Temp data logger (CK07.2416). A record of the temperature during shipment is maintained with the production records of the batch.

2.2 NON-CLINICAL DATA AND TOXICOLOGY

Please be referred to the clinical protocol and § 2.1.P.3.4.

2.3 CLINICAL DATA

Please be referred to the clinical protocol and § 2.4.

2.4 OVERALL RISK AND BENEFIT ASSESSMENT

Risk assessment of raw materials:

In addition to the SCGM culture medium, four biologically active raw materials (the growth factors SCF, Flt3L, TPO and the chemical compound StemRegenin1) are used during the production of the ex vivo expanded UCB-derived HSPC product.

The culture medium SCGM and the growth factors SCF, Flt3L and TPO are manufactured, tested and released in compliance with the relevant GMP guidelines by CellGenix GmbH and are intended for clinical ex vivo use. No animal or human-derived materials were used during the manufacturing of the cytokines SCF, Flt3L and TPO. The SCGM medium does not contain animal derived components (xeno-free). Human proteins present in SCGM medium have been collected from healthy donors at the time of collection, and samples of their donations were tested individually and found to be negative for viral diseases (HIV1/HIV2, HBV, HCV, Parvovirus B19). CellGenix is certified according to ISO 9001:2008.

The GMP-batch StemRegenin1 has been manufactured, tested and released by ChemConnection and complies with EU GMP guidelines and the Guidance for Industry, Q7 GMP practice guidelines for Active Pharmaceutical Ingredients. The materials used for the manufacturing of the StemRegenin1 batch do not originate from animal products. ChemConnection has extensive experience with cGMP production of API for large pharmaceutical companies and holds a pharmaceutical license to produce API (Registernummer 6303 F).

At the end of the expansion culture, the end product is extensively washed resulting in a more than 10.000 fold reduction of the raw materials in the end product (see grondstofdossiers for the calculation of the fold reduction). Based on the concentration of the biologically active raw materials in the production process and the theoretical fold reduction factor, we calculated that the residual amount of growth factors in the end product is less than 1 ng . These levels are below the detection level of growth factor-specific sensitive ELISA's. The residual amount of SR1 in the end product is calculated to be less than 10 ng. These trace amounts of raw materials will be rapidly further diluted *in vivo* after intravenous infusion. In view of all the above, we do not expect these trace amounts of raw materials to induce any adverse reactions in patients.

Risk assessment in connection with sterility:

The cellular starting material (both UCB product and the selected $CD34^+$ cell fraction) and all raw materials have been tested for bacterial and fungal contamination and are required to be negative. The expansion of UCB-derived HSPC occurs in an almost closed production process. Only at the start of the culture an aseptic manufacturing step is performed in which the $CD34^+$ cell fraction is pelleted before resuspension in expansion medium and transfer to the culture bag. The aseptic manufacturing step occurs in a Class A biohazard cabinet in a class C cleanroom of the Erasmus MC Core facility for Cell- and Gene-Therapy. The production personnel have received specific training on aseptic manufacturing including the requirement to successfully perform a bouillon process simulation test as part of their qualification. At all steps of the expansion protocol, the cell suspension is visually examined for signs of contamination. The microbial culture of the end product is not available prior to

release of the of the HSPC end-product. When all other release criteria are met, the QP will conditionally release the HSPC end-product for delivery to the clinic for administration to the patient. In case the sterility test result is negative, i.e. no signs of bacterial or fungal contamination, the QP will definitively release the HSPC end-product (see appendix E for the ATMP release form). In case the sterility test result is positive, the contaminant micro-organism will be identified and an antibiogram will be established. These results are immediately reported to the head of production, the PI and the hematologist of the patient.

Additional risk assessment considerations:

Mycoplasma

The end product will not be tested for contamination with mycoplasma because the risk of mycoplasma contamination occurring during the culture is considered to be extremely low in view of the GMP-grade of the raw materials, the closed culture system and the short culture period. Moreover, cellular products containing hematopoietic stem cells, derived from blood, bone marrow or cord blood, that are used for stem cell transplantation are never tested for mycoplasma. There are no indications that infusion of stem grafts results in mycoplasma-related complications. The FACT-JACIE International standards for hematopoietic cellular therapy product collection, processing and administration (6^{th} edition, March 2015) do not require stem cell grafts to be tested for contamination with mycoplasma.

Presence of prions, animal viruses and human viruses

The risk of contamination of the end product with prions, animal- or human viruses is considered to be almost non-existent. No animal-derived products are used in the production process and the human proteins present in the SCGM medium are derived from donors that have been tested to be negative for viral diseases (HIV1/HIV2, HBV, HCV, Parvovirus B19). The release of the UCB unit by the UCB bank includes negative results of communicable disease testing of the mother less than 30 days prior to collection of the UCB.

Malignant transformation

There are no cases reported of malignant transformation of ex vivo expanded UCB-derived $CD34^+$ cells in patients in any of the clinical trials performed so far (9, 14-17). In addition, there is no evidence for any leukemic transformation in immuno-deficient mice engrafted with human cells after transplantation of ex vivo expanded UCB-derived $CD34^+$ cells (10-13,18).

Transfusion reactions

The incidence of infusion related toxicities in patients receiving unmanipulated or *ex vivo* expanded UCB products is low. Recently, the adverse events (AE) associated with the infusion of unmanipulated and *ex vivo* expanded UCB products occurring within 24 hours has been systematically examined in 137 patients (26). A total of three grade 2 and two grade 3 infusion reactions occurred resulting in an overall AE rate of 3.7 % (2.2 % grade 2 and 1.5 % grade 3). The

majority of AEs manifested as signs of hypertension and shortness of breath. The ex vivo expansion of UCB-derived $CD34^+$ cells did not increase the incidence of AE in patients as compared to patients that received unmanipulated UCB units. In conclusion, the infusion of *ex vivo* expanded UCB derived HSPC is a safe procedure associated with a low probability of inducing severe reactions.

For benefits and the overall risk/benefit assessment, please be referred to the clinical protocol.

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2.6 APPENDICES

Appendix A: Flowchart of SR1-expanded UCB-derived HSPC production process

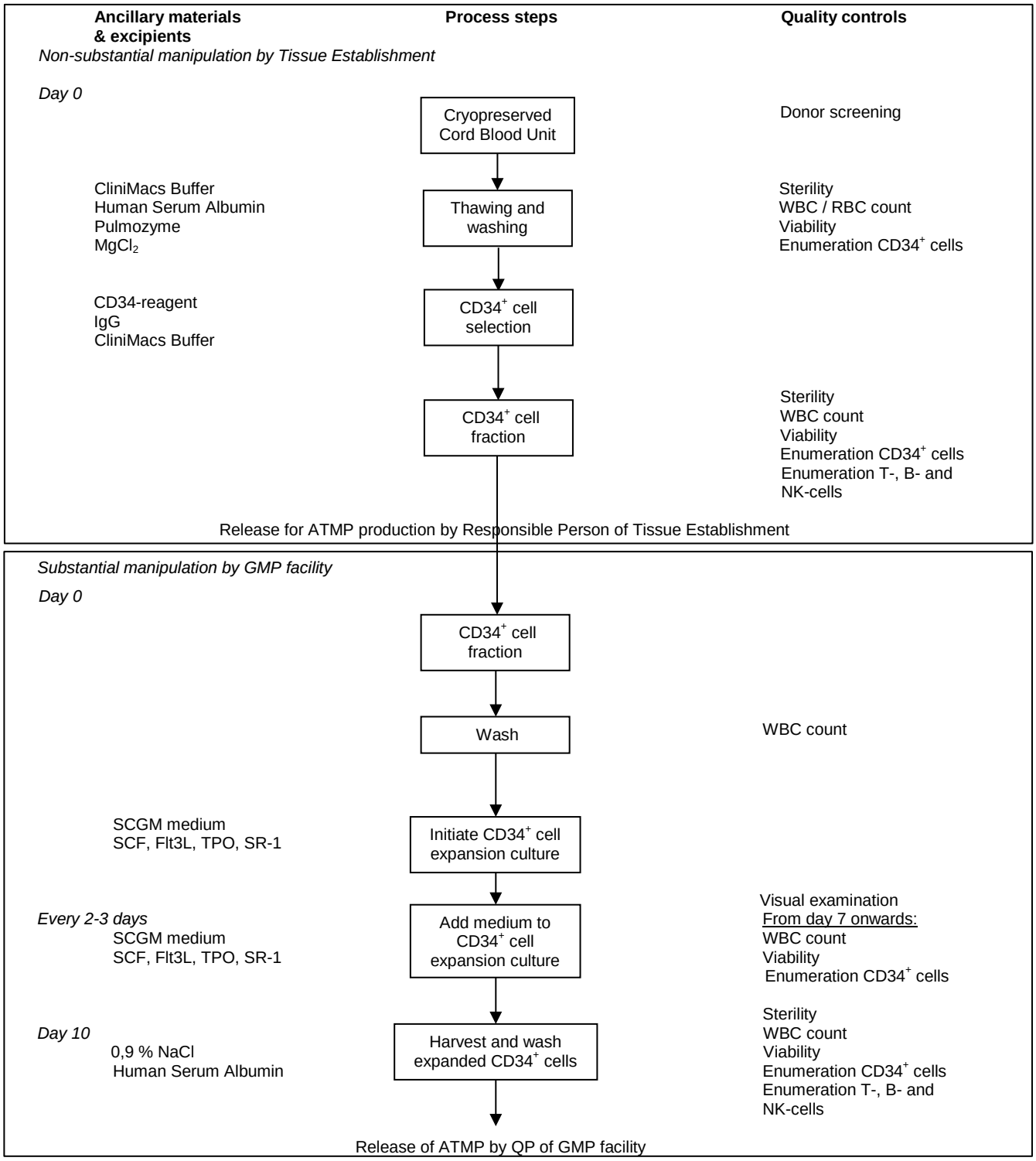


Fig. 1. Flowchart of the production process of the SR1-expanded UCB-derived HSPC product including ancillary materials and excipients and in process controls.

Appendix B: Certificates of analysis of ancillary materials

CELLGRO® CellGenix

Certificate of Analysis

Product:	**GMP Recombinant Human Flt3-Ligand (rh Flt-3L)**		
Intended use:	For clinical *ex vivo* use		
Order number	1015-050	**Lot:**	1015ID43
Production:	08/2015	**Expiry:**	08/2018
Source:	The methionyl form of the *E. coli* expressed truncated human Flt-3L contains 162 amino acid residues (including a C-terminal His-6-Tag) and has a molecular mass of approximately 18 kDa.		
Formulation:	Lyophilized from a 0.2 µm -filtered solution in PBS		

Release Testing	Test Method	Specification	Lot Result
Identity	DNA sequencing (*end-of-production* cells)	Complies with reference sequence	Complies
Identity	N-terminal protein sequencing (Edman)	Complies with reference sequence	Complies
Purity	SDS-PAGE, Coomassie stain	≥ 97%	> 99%
Product-related proteins	SDS-PAGE, Coomassie stain	≤ 5% oligomers	< 1%
Specific activity	Proliferation of OCI-AML5 cells, calibration against NIBSC 96/532	0.5 – 2.0 x10^6 IU/mg	1.5 x 10^6 IU/mg
Host-cell DNA	Fluorimetric assay	≤ 200 ng/mg	< 100 ng/mg
Host cell protein	ELISA	≤ 1.0 µg/mg	< 0.3 µg/mg
Endotoxin	Gel clot assay (Ph. Eur.)	≤ 1000 EU/mg	< 100 EU/mg
Sterility	Inoculation method (Ph. Eur.)	Sterile	Complies
Mass per vial	Spectrophotometrical measurement at 280 nm	43 - 57 µg	Complies

Page 1 of 2 *ZY-CA-1015d*

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel. +49 761 88889-0 | Fax +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

CELLGRO®

CellGenix

Handling Instructions:

Reconstitution:	Recommended in 200 µl of sterile H_2O to a final concentration of 250 µg/ml.
Dilution:	Recommended in CellGro® / CellGenix™ serum-free media. For dilution with PBS or protein free medium, a carrier protein (0.1-1% albumin or 1-10 % appropriate serum) has to be included. Failure to dilute product according to these instructions may result in loss of activity.
Storage and Stability:	Store material at -20°C or below. Store reconstituted material at -20°C or below. Avoid multiple freeze/thaw cycles.

Quality Statement:

This product is manufactured, tested and released in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered products" has been considered in the design of this product.

08.03.2016 i.V. Kloack

Date *Quality Manager*

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel. +49 761 88889-0 | Fax +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

CELLGRO®

CellGenix

Certificate of Analysis

Product:	**GMP Recombinant Human Stem Cell Factor (rh SCF)**		
Intended use:	For clinical ex vivo use		
Order number:	1018-050	**Lot:**	1018LH11
Production:	08/2014	**Expiry:**	03/2018
Source:	The methionyl form of the *E.coli* expressed mature human SCF contains 171 amino acid residues (including a C-terminal His6-tag) and has a molecular mass of 19 kDa.		
Formulation:	Lyophilized from a 250 µg/ml, 0.2µm sterile-filtered solution in 25 mM Na-phosphate, containing 150 mM NaCl and 1 mM EDTA		

Release Testing	Test Method	Specification	Lot Result
Identity	DNA sequencing *(plasmid from end-of-production cells)*	Complies with reference sequence	Complies
Identity	N-terminal protein sequencing (Edman)	Complies with reference sequence	Complies
Purity	SDS-PAGE, Coomassie stain	≥ 97%	> 99%
Product-related proteins	SDS-PAGE, Coomassie stain	≤ 10% oligomers ≤ 5% isomers	< 1% 3 %
Specific activity	Proliferation of TF1 cells, calibration against NIBSC 91/682	0.5 – 2.0 x10^6 IU/mg	1.2 x 10^6 IU/mg
Host-cell DNA	Fluorimetric assay	≤ 200 ng/mg	< 40 ng/mg
Host cell protein	ELISA	≤ 1 µg/mg	< 0.1 µg/mg
Endotoxin	Gel clot assay (Eur.Pharm.)	≤ 1000 EU/mg	< 100 EU/mg
Sterility	Inoculation method (Eur.Pharm.)	Sterile	Complies
Mass per vial	Spectrophotometrical measurement at 280 nm	43 - 57 µg	Complies

Page 1 of 2 *ZY-CA-1018c*

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel. +49 761 88889-0 | Fax +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

CELLGRO®

CellGenix

Handling Instructions:

Reconstitution:	Recommended in 200 µl of sterile H_2O to a final concentration of 250 µg/ml.
Dilution:	Recommended in CellGro® / CellGenix™ serum-free media. For dilution with PBS or protein free medium, a carrier protein (0.1-1% albumin or 1-10 % appropriate serum) has to be included. Failure to dilute product according to these instructions may result in loss of activity.
Storage and Stability:	Store material at -20°C or below. Store diluted material at -20°C or below. Avoid multiple freeze/thaw cycles.

Quality Statement:

This product is manufactured, tested and released in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered products" has been considered in the design of this product.

18.7.2015 D. Becker

Date *Quality Manager*

ZY-CA-1018c

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel. +49 761 88889-0 | Fax +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

CELLGRO®

CellGenix

Certificate of Analysis

Product: **GMP Recombinant Human Thrombopoietin (rh TPO)**

Intended use: For clinical *ex vivo* use

Order Number: 1017-050 **Lot:** 1017OB11

Production: 03/2015 **Expiry:** 07/2017

Source: The methionyl form of the *E.coli* expressed truncated human TPO_{174} contains 181 amino acid residues (including an N-terminal His-6-tag) and has a molecular mass of 19.6 kDa.

Formulation: Lyophilized from a 0.2 µm-filtered aqueous solution

Release Testing	Test Method	Specification	Lot Result
Identity	DNA sequencing (end-of-production cells)	complies with reference sequence	complies
Identity	N-terminal protein sequencing (Edman)	complies with reference sequence	complies
Purity	SDS-PAGE, Coomassie stain	≥ 98%	> 99%
Product-related proteins	SDS-PAGE, Coomassie stain	≤ 2% fragments ≤ 10% oligomers	< 1% < 5%
Specific activity	Proliferation of MO7e cells, calibration against an in-house reference standard	$10 \times 10^6 - 40 \times 10^6$ U/mg	20×10^6 U/mg
Host cell DNA	Fluorimetric assay	≤ 200 ng/mg	< 55 ng/mg
Endotoxin	Gel clot assay (Ph. Eur.)	≤ 1000 EU/mg	< 100 EU/mg
Host cell protein	ELISA	≤ 1 µg/mg	< 0.1 µg/mg
Sterility	Inoculation method (Ph. Eur.)	sterile	complies
Mass per vial	BCA-Assay	43 – 57 µg	complies

Page 1 of 2 *ZY-CA-1017c*

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel. +49 761 88889-0 | Fax +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

CELLGRO® CellGenix

Handling Instructions:

Reconstitution:	It is recommended to reconstitute with 200 µl sterile water.
Dilution:	Recommended in CellGro® / CellGenix™ serum-free media. For dilution with protein free medium, a carrier protein (0.1-1% albumin or 1-10% appropriate serum) has to be included. Failure to dilute product according to these instructions may result in loss of activity.
Storage and Stability:	Store material at -20°C or below. Store reconstituted material at -20°C or below. Avoid multiple freeze/thaw cycles.

Quality Statement:

This product is manufactured, tested and released in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered products" has been considered in the design of this product.

17.05.15 D. Blocker

Date *Quality Manager*

Page 2 of 2 *ZY-CA-1017c*

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel. +49 761 88889-0 | Fax +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

CELLGRO®

CellGenix

Certificate of Analysis

Product:	**Stem Cell Growth Medium (SCGM)**		
Intended use:	For clinical ex vivo use		
Order Number:	20802 / 20902	**Lot:**	1023D
Production:	08/2015	**Expiry:**	08/2017
Applications:	Cultivation of hematopoietic stem and progenitor cells, Expansion of NK cells		

Microbial, Chemical and Physical Testing:	**Specification**	**Lot Result**
Sterility (Ph.Eur.)	sterile	complies
pH (Ph.Eur.)	7.2 – 7.5	7.4
Osmolality (Ph.Eur.)	290 – 350 mOsm/kg H_2O	309 mOsm/kg H_2O
Endotoxin (Ph.Eur.)	≤ 1.0 EU/ml	0.1 EU/ml
Mycoplasma (Ph.Eur.)	not detectable	complies
Colour	red	complies
Appearance	clear	complies

Bioassay (Expansion of $CD34^+$ cells, 7 days):	**Specification**	**Lot Result**
Viability	≥ 80 %	100 %
Fold increase	≥ 10	18
$CD34^+$ cells	≥ 20 %	38 %

Handling Instructions:

Storage and stability: Store at +2°C to +8°C. Light protection recommended.

Quality and Raw Material Statement:

This product is manufactured in compliance with the relevant GMP-guidelines. USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered products" has been considered in the design of this product. The formulation does not contain animal derived components (xeno-free). Human proteins have been collected from healthy donors at the time of collection, and samples of their donations were tested individually and found negative for viral diseases by approved methods (HIV1/HIV2, HBV, HCV, Parvovirus B19).

11.01.2016 i.V.

Date *Quality Manager*

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel. +49 761 88889-0 | Fax +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

ME-CA-2002a

CHEMCONNECTION

CC-CA-2016.286 v01

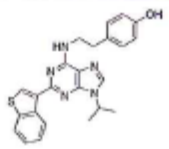
Title: Certificate of Analysis

1. General information

Manufacturer:	ChemConnection BV Kloosterstraat 9 5349 AB, Oss The Netherlands P: +31 (0)412 846024 E: info@chemconnection.nl W: www.chemconnection.eu

2. Batch information

Compound name:	StemRegenin1
Batch code:	JB0241A
Molecular Formula:	$C_{24}H_{23}N_5OS$
Molecular Weight:	429.5



Manufacturing date:	04 January 2016
Batch size:	16.9 g
Retest date (if applicable):	07 June 2017
Storage conditions:	≤ -15°C
Precautions:	Not applicable

This Certificate of Analysis is authorized by QA	
By:	T. Seeven
Signature:	
Date:	07 Jun 2016

CHEMCONNECTION

CC-CA-2016.286 v01

Title: Certificate of Analysis **page 2 of 4**

3. Test results

Test	Test method	Criterion (if appropriate)	Result
Appearance	Visual inspection	Solid	Solid
Identity by NMR	NMR	Complies with structure	Complies with structure
Identity by mass spectroscopy	MS	Complies with structure	Complies with structure
Assay by NMR	NMR	≥ 97% (m/m)	98% (m/m)
Related impurities: Unspecified impurities Each individual ≥ 0.05 %a/a: – RRT 1.17 – RRT 2.13	CC-AM-2016.131 v02	 Report result % (a/a), No impurity > 1.0% (a/a)	 0.11% a/a 0.09% a/a
Total impurities ≥ 0.05 %a/a by HPLC	CC-AM-2016.131 v02	≤ 2.5% (a/a)	0.21% a/a
Residual solvents by GC-HS – Ethanol – Tetrahydrofuran	CC-AM-2014.034 v03	 ≤ 5000 ppm ≤ 720 ppm	 3039 ppm < 100 ppm
Residual solvents by GC-FID – Dimethylacetamide	CC-AM-2015.677 v01	 ≤ 1090 ppm	 < 100 ppm
Water content	CC-AM-2014.122 v02	Report result % (m/m)	< 0.10% (m/m)
Heavy metals – Arsenic – Cadmium – Lead – Mercury – Palladium	ICP-OES/MS	 ≤ 1.5 ppm ≤ 0.5 ppm ≤ 0.5 ppm ≤ 3.0 ppm ≤ 5 ppm	 < 0.05 ppm < 0.01 ppm 0.01 ppm < 0.05 ppm 0.44 ppm
Inorganic residues	Sulphate ash	≤0.1% (m/m)	< 0.1%(m/m)
Endotoxins	EP 2.6.14	< 0.25 EU/mg	< 0.058 EU/mg
Bioburden: – Total aerobic microbial count – Total yeast mould count	EP 2.6.12	 <100 CFU/g, report result <100 CFU/g, report result	 10 CFU/g < 10 CFU/g

page 2 of 4 Confidential

CHEMCONNECTION

CC-CA-2016.286 v01

Title: Certificate of Analysis **page 3 of 4**

4. Certificate of Compliance statement

This GMP batch StemRegenin1 J80241A, has been manufactured and tested by ChemConnection and complies with:

- The Rules Governing Medicinal Products in the European Union Volume 4; Good Manufacturing Practice; Medicinal Products for Human and Veterinary Use, Part I: Basic Requirements for Medicinal Products and Part II: Basic Requirements for Active Substances used as Starting Materials
- Guidance for Industry, Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients

CHEMCONNECTION

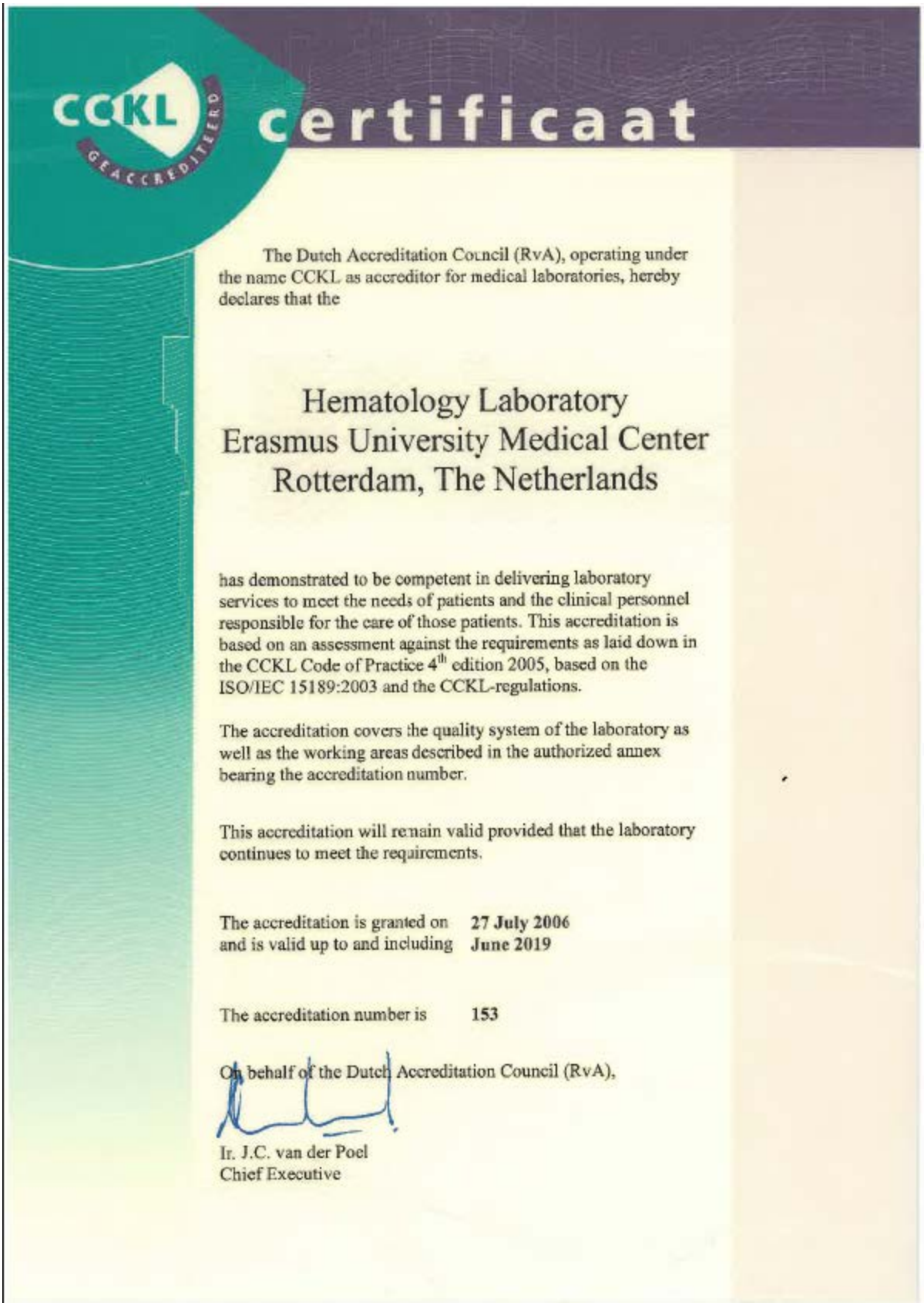
CC-CA-2016.286 v01

Title: Certificate of Analysis page 4 of 4

Revision History

Current version	CC-CA-2016.286 v01
Previous version	N/A, First version
Reason for update	N/A
Changes	N/A
Ref.	Specification: CC-SP-2016.049 v01 Analytical Raw Data: ARF01263

Appendix C: Accreditations and licenses of the Transplantation laboratory



CCKL GEACCREDITEERD

certificaat

The Dutch Accreditation Council (RvA), operating under the name CCKL as accreditor for medical laboratories, hereby declares that the

Hematology Laboratory
Erasmus University Medical Center
Rotterdam, The Netherlands

has demonstrated to be competent in delivering laboratory services to meet the needs of patients and the clinical personnel responsible for the care of those patients. This accreditation is based on an assessment against the requirements as laid down in the CCKL Code of Practice 4th edition 2005, based on the ISO/IEC 15189:2003 and the CCKL-regulations.

The accreditation covers the quality system of the laboratory as well as the working areas described in the authorized annex bearing the accreditation number.

This accreditation will remain valid provided that the laboratory continues to meet the requirements.

The accreditation is granted on **27 July 2006**
and is valid up to and including **June 2019**

The accreditation number is **153**

On behalf of the Dutch Accreditation Council (RvA),

Ir. J.C. van der Poel
Chief Executive

CCKL GEACCREDITEERD

Hematology Laboratory
Erasmus University Medical Center
Rotterdam, The Netherlands

Description of the working areas for which the accreditation is granted:

Haematology

Stem Cell Processing

This annex is valid from **29 Aril 2015** up to and including **June 2019**.

An actual overview of accredited institutions can be found on the website of CCKL (www.cckl.nl).

On behalf of the Dutch Accreditation Council (RvA),

Ir. J.C. van der Poel
Chief Executive

Date: 29 April 2015 Accreditation number **153** Annex

The Joint Accreditation Committee ISCT-EBMT (JACIE)

hereby declares that

Erasmus MC-Daniel Cancer Institute,
Erasmus Medical Center
Rotterdam, Netherlands

has been found to meet the standards as set out in the FACT-JACIE International Standards for Cellular Therapy, edition 5 in the following area(s):

Autologous & Allogeneic Transplantation in Adult Patients
Collection of HPC, Apheresis
Cell Processing - minimally manipulated

Programme Director: J.J. Cornelissen

John Snowden
JACIE Medical Director, Chair JACIE Committee

Maria Vittoria Gazzola
Chair, JACIE Accreditation Committee

537 25/01/2016 24/01/2020

ERKENNING ALS ORGAANBANK

Ons kenmerk
Farmatec-BMC/JZ-15496

Registernummer 5512 L/EO

Ons kenmerk
Farmatec-BMC/JZ-15496

Den Haag
29 oktober 2015

De Minister van Volksgezondheid, Welzijn en Sport,

Gelezen het advies van Inspectie voor de Gezondheidszorg inzake de erkenning van Academisch Ziekenhuis Rotterdam, h.o.d.n. Erasmus MC te Rotterdam (dossiernummer 24485070, vermeld op het uittreksel uit het handelsregister van de Kamer van Koophandel), ontvangen op 29 oktober 2015.

Gelet op artikel 9 van de Wet veiligheid en kwaliteit lichaamsmateriaal;

BESLUIT:

1. Academisch Ziekenhuis Rotterdam, h.o.d.n. Erasmus MC, 's-Gravendijkwal 230 te Rotterdam , een erkenning als orgaanbank op grond van de Wet veiligheid en kwaliteit lichaamsmateriaal te verlenen.
2. Deze erkenning geldt, voor de in de bijlage genoemde eenheid, type lichaamsmateriaal, vermelde handelingen, doelen en verantwoordelijke persoon.

De Minister van Volksgezondheid, Welzijn en Sport,
namens deze,

Afdelingshoofd
Farmatec

Dhr. M.J. van de Velde

Pagina 3 van 4

CIBG
Ministerie van Volksgezondheid, Welzijn en Sport

> Retouradres Postbus 16114 2500 BC Den Haag

WVKL erkenning als orgaanbank
Eenheid: Transplantatielaboratorium, afdeling Hematologie
Registernummer: 5512 L/EO

Verantwoordelijke personen
De heer E. Braakman

Type lichaamsmateriaal	Pre/postmortaal		Handelingen	Doelen	Aanwijzingen
Cellen verkregen uit beenmerg of perifeer bloed[1]	☒	☐	1,2,4	A,B	-
Stamcellen uit beenmerg	☒	☐	1,2,4	A	-
Stamcellen uit navelstrengbloed	☒	☐	1,2	A	-
Stamcellen uit perifeer bloed	☒	☐	1,2,4	A	-

Handelingen
1. bewaren
2. bewerken
3. distribueren[2]
4. in ontvangst nemen na verkrijgen

Doelen
A. directe toepassing op de mens
B. verdere verwerking tot geneesmiddel

Aanwijzingen
I. Exporteren
II. Importeren
III. Ontvangen uit een andere EU-lidstaat

[1] Bestemde donatie: = Designated donations: A unit collection form a donor called by the facility to provide product (for example, HLA-compatible) to be used by a specific recipient (or for cellular therapy products, possibly a small group of products). Referentie: ICCBAA, ISBT 128 implementation guide, use of the product code data structure [003], Cellular Therapy, March 2012, version 1.0.0.

[2] Distribueren = Lichaamsmateriaal, dat is vrijgegeven voor directe toepassing op de mens (dus klinisch gebruik), verzenden en afleveren aan de instelling die het lichaamsmateriaal gaat toepassen. Indien het lichaamsmateriaal fysiek binnen de eigen instelling/organisatie wordt gebruikt (dezelfde rechtspersoon), valt dit buiten de definitie distribueren.

Pagina 4 van 4

Health Care Inspectorate - Pharmaceutical Affairs and Medical Technology

CERTIFICATE NUMBER: ***NL/H/15/1004371***

CERTIFICATE OF GMP COMPLIANCE OF A MANUFACTURER [1, 2]

Part 1

Issued following an inspection in accordance with :
Art. 15 of Directive 2001/20/EC

The competent authority of Netherlands confirms the following:
The manufacturer: ***Academisch Ziekenhuis Rotterdam, h.o.d.n. Erasmus MC***
Site address: ***'s-Gravendijkwal 230, ROTTERDAM, 3015CE, Netherlands***

Has been inspected under the national inspection programme in connection with manufacturing authorisation no. ***108517 F*** in accordance with Art. 13 of Directive 2001/20/EC transposed in the following national legislation:
Art. 100 of the Medicines Act

From the knowledge gained during inspection of this manufacturer, the latest of which was conducted on ***2015-03-13*** , it is considered that it complies with :

- The principles and guidelines of Good Manufacturing Practice laid down in Directive 2003/94/EC [3]

This certificate reflects the status of the manufacturing site at the time of the inspection noted above and should not be relied upon to reflect the compliance status if more than three years have elapsed since the date of that inspection. However, this period of validity may be reduced or extended using regulatory risk management principles by an entry in the Restrictions or Clarifying remarks field. This certificate is valid only when presented with all pages and both Parts 1 and 2. The authenticity of this certificate may be verified in EudraGMP. If it does not appear, please contact the issuing authority.

[1] *The certificate referred to in paragraph 111(5) of Directive 2001/83/EC and 80(5) of Directive 2001/82/EC, shall also be required for imports coming from third countries into a Member State.*

[2] *Guidance on the interpretation of this template can be found in the Help menu of EudraGMDP database.*

[3] *These requirements fulfil the GMP recommendations of WHO.*

Online EudraGMP, Ref key: 28320 Issuance Date: 2015-03-13 Signatory: Drs Y. v. Kooij Page 1 of 2

Part 2

Human Investigational Medicinal Products

1 MANUFACTURING OPERATIONS	
1.1	**Sterile products**
	1.1.1 Aseptically prepared (processing operations for the following dosage forms)
	1.1.1.4 Small volume liquids
1.3	**Biological medicinal products (list of product types)**
	1.3.1 Biological medicinal products (list of product types)
	1.3.1.3 Cell therapy products
	1.3.2 Batch Certification (list of product types)
	1.3.2.3 Cell therapy products
1.6	**Quality control testing**
	1.6.1 Microbiological: sterility
	1.6.2 Microbiological: non-sterility
	1.6.3 Chemical/Physical
	1.6.4 Biological

2015-03-13

Name and signature of the authorised person of the Competent Authority of Netherlands

Inspectie voor de Gezondheidszorg
Ministerie van Volksgezondheid, Welzijn en Sport
Rijksoverheid

Drs Yolanda van Kooij
Health Care Inspectorate - Pharmaceutical Affairs and Medical Technology
Tel: ***+31 88 1205000***
Fax: ***+31 88 1205001***

Online EudraGMP, Ref key: 28320 Issuance Date: 2015-03-13 Signatory: Drs Y. v. Kooij Page 2 of 2

Appendix D: Example of an ancillary material dossier (SCF)

CK06.2141 versie 0.3 / 20-07-2016	Paraaf:		Geldig tot: .... - .... - 2018	pag 1/pag 8

Grondstofdossier voor ATMP producten

GMP Recombinant Human Stem Cell Factor (rhSCF)

Producent/Leverancier : CellGenix - GmbH - CellGro
Productnummer : 1018-050
Bewaartemperatuur : -20ºC tot -80ºC (vermijd herhaald vriezen-ontdooien)
Houdbaarheid product : 3,5 jaar houdbaar na productiedatum
Gebruikt bij : Expansie van hematopoietische stam- en voorlopercellen
Risicocategorie : Low risk / ~~medium risk~~ / ~~high risk~~

Risicoanalyse:

Producent: CellGenix is gecertificeerd volgens het kwaliteitsmanagementsysteem ISO 9001:2008 voor de ontwikkeling, productie en distributie van recombinante humane cytokines voor ex-vivo gebruik voor klinische cellulaire therapieën en regeneratieve medicijnen.

Productinformatie:

Algemeen: GMP Recombinant Human Stem Cell Factor (rhSCF) wordt geproduceerd door *E.coli* bacteriën die getransfecteerd zijn met een DNA-sequentie die codeert voor het SCF eiwit.

Productieproces: rhSCF wordt geproduceerd, getest en vrijgegeven volgens relevante GMP richtlijnen. Er zijn geen materialen van dierlijke of humane oorsprong gebruikt tijdens de productie. De United States Farmacopoea (USP) hoofdstuk <1043> '*ancillary materials for cell, gene and tissue-engineered products*' is als richtlijn gebruikt tijdens de productie. Van elke batch wordt de identiteit, zuiverheid, specifieke activiteit, hoeveelheid gastheer DNA en gastheer eiwit, endotoxine gehalte en steriliteit bepaald volgens de op het Certificate of Analysis (CoA) en datasheet vermelde methoden. De resultaten van deze QC testen staan vermeld op het CoA van de batch en zijn vrijgifte criteria. Batches rhSCF worden vrijgegeven door de Quality Manager van CellGenix.

Productspecificaties: GMP rhSCF wordt geleverd als een gevriesdroogd product in een ampul met 50 µg ± 15% en is exclusief bestemd voor "clinical *ex vivo* use". Voor de precieze productspecificaties wordt verwezen naar de CellGro GMP rhSCF Data Sheet van CellGenix (zie bijlage 2). Het CoA van iedere batch rhSCF bevat de resultaten van deze testen (zie bijlage 3).

Toepassing:

rhSCF wordt gebruikt voor de expansie van hematopoietische stam- en voorlopercellen afkomstig uit navelstrengbloed. Het oplossen en uitvullen van rhSCF zal gebeuren volgens het CoA. Eerst wordt het product opgelost in 200 µl steriel water tot een concentratie van 250 µg/ml. Dit wordt verder verdund tot een concentratie van 20 µg / ml in CellGro® SCGM medium met 1% humaan serum albumine en uitgevuld in ampullen met 250 µl per ampul en opgeslagen bij -80°C. De gebruiksconcentratie in het expansiemedium is 50 ng/ml.

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Grondstofdossier voor ATMP producten

Acceptatiecriteria:

Nr.	Specificatie	Acceptatiecriterium
	Batch vrijgifte door QA CellGenix	Aanwezig
	Certificate of Analysis	Aanwezig
	Visuele inspectie batch	Geen bijzonderheden
	Houdbaarheid product	≥ 6 maanden na inkeuring
1	Identiteit	Voldoet
2	Zuiverheid	≥ 97 %
3	Specifieke activiteit	0.5 - 2.0 x 10^5 IU/mg
4	Gastheer DNA	≤ 200 ng/ml
5	Gastheer Eiwit	≤ 1 µg/ml
6	Endotoxine (EU/mg)	≤ 1000 EU/mg
7	Steriliteit (EP)	Steriel

Goedkeuring van de risicoanalyse en de acceptatiecriteria van de grondstof door QP:

Naam QP : .....

Datum : .....

Paraaf QP :

Bijlagen:

1) CellGenix - GmbH - CellGro ISO 9001:2008 Certificate
2) Data Sheet - GMP rhSCF
3) Certificate of Analysis - GMP rhSCF
4) Stroomschema productieproces met de berekening van de theoretische maximale hoeveelheid rhSCF in het eindproduct "SR1-geëxpandeerde HSPC".

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Grondstofdossier voor ATMP producten

Inkeuring:

GMP Recombinant Human Stem Cell Factor (rhSCF)

Producent/Leverancier : CellGenix - GmbH - CellGro
Productnummer : 1018-050
Batchnummer :
Grootte batch :
Datum ontvangst :
In ontvangst name door :
Datum inkeuring :
Bewaartemperatuur : -20ºC tot -80ºC (vermijd herhaald vriezen-ontdooien)
Houdbaarheidsdatum :

Nr.	Specificatie	Acceptatiecriterium	Inkeuring
	Batch vrijgifte door QA CellGenix	Aanwezig	
	Certificate of Analysis	Aanwezig	
	Visuele inspectie batch	Geen bijzonderheden	
	Houdbaarheid product	≥ 6 maanden na inkeuring	
1	Identiteit	Voldoet	
2	Zuiverheid	≥ 97 %	
3	Specifieke activiteit	0.5 - 2.0 x 10^5 IU/mg	
4	Gastheer DNA	≤ 200 ng/ml	
5	Gastheer Eiwit	≤ 1 µg/ml	
6	Endotoxine (EU/mg)	≤ 1000 EU/mg	
7	Steriliteit (EP)	Steriel	

Grondstof is goedgekeurd / afgekeurd voor gebruik in ATMP productieproces (doorhalen wat niet van toepassing is).

Opmerkingen: .....

.....

.....

Locatie opslag: .....

Vrijgifte van de grondstofbatch door hoofd quality assurance (HQA):

Naam HQA : .....
Datum : .....
Paraaf HQA :

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Grondstofdossier voor ATMP producten

Bijlage 1: CellGenix – GmbH – CellGro - ISO 9001:2008 Certificate

Certificate

Standard **ISO 9001:2008**

Certificate Registr. No. 01 100 120139

Certificate Holder:

CellGenix

CellGenix GmbH
Am Flughafen 16
D - 79108 Freiburg

Scope: Development, production and distribution of high quality reagents for clinical cell therapy and regenerative medicine.

Proof has been furnished by means of an audit that the requirements of ISO 9001:2008 are met.

Validity: The certificate is valid from 2015-07-03 until 2018-07-02.
First certification 2012

2015-05-15

TÜV Rheinland Cert GmbH
Am Grauen Stein · 51105 Köln

www.tuv.com

DAkkS

TÜVRheinland®
Precisely Right.

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Grondstofdossier voor ATMP producten

Bijlage 2: Data Sheet GMP rhSCF

CellGenix

Data Sheet: CellGenix™
GMP Recombinant Human Stem Cell Factor (rh SCF)
Order No.: 1018-050

Source	A DNA sequence encoding SCF protein was expressed in *E. coli*
Molecular mass	The methionyl form of the *E. coli* expressed human SCF contains 171 amino acid residues (including a C-terminal His6-tag) and has a molecular mass of 19 kDa.
Identity	Confirmed by DNA-sequencing of the expression plasmid in end of production (EOP)-cells and N-terminal sequencing of the final product
Purity	≥ 97%, as determined by SDS-PAGE (under reducing and non-reducing conditions) and visualized by Coomassie staining
Product-related proteins	Oligomers ≤ 10% Isomers ≤ 5% as determined by SDS-PAGE (under reducing and non-reducing conditions) and visualized by Coomassie staining
Endotoxin	≤ 1000 EU/mg, as determined by LAL gel clot test according to Ph.Eur.; typically < 100 EU/mg
Residual host-cell DNA	≤ 200 ng/mg, as determined with a fluorimetric assay
Residual host-cell protein	≤ 1 µg/mg, as determined by ELISA
Activity	0.5 - 2.0 x 10^6 IU/mg calibrated with the 1st International Standard NIBSC, # 91/682 Measured in a cell proliferation assay using a human factor-dependent cell line, TF-1
Sterility	Sterility test according to Ph.Eur. of the vialed product (direct inoculation)
Formulation	Lyophilized from a 250 µg/ml, 0.2µm sterile-filtered solution in 25mM Na-phosphate, containing 150mM NaCl and 1mM EDTA
Mass per vial	43 - 57 µg, as determined by spectrophotometrical measurement at 280 nm using PBS containing 1 mM EDTA as reference (A_{280} = 0.53 for a concentration of 1 mg/ml SCF in formulation buffer)
Transport	Ambient temperature
Storage at	-20°C or below. Avoid repeated freeze-thaw cycles
Shelf life	Minimum 6 months from date of shipping
Intended use	**For clinical ex vivo use. Not intended for human in vivo application**
Quality statement	This product is manufactured, tested and released in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered products" has been considered in the design of this product

Manufacturer

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ZY-DS-1018c-USA 17 March 2015

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Grondstofdossier voor ATMP producten

Bijlage 3: Certificate of Analysis GMP rhSCF

CELLGRO® CellGenix

Certificate of Analysis

Product:	**GMP Recombinant Human Stem Cell Factor (rh SCF)**		
Intended use:	For clinical ex vivo use		
Order number:	1018-050	**Lot:**	1018LH11
Production:	08/2014	**Expiry:**	03/2018
Source:	The methionyl form of the *E.coli* expressed mature human SCF contains 171 amino acid residues (including a C-terminal His6-tag) and has a molecular mass of 19 kDa.		
Formulation:	Lyophilized from a 250 µg/ml, 0.2µm sterile-filtered solution in 25 mM Na-phosphate, containing 150 mM NaCl and 1 mM EDTA		

Release Testing	Test Method	Specification	Lot Result
Identity	DNA sequencing *(plasmid from end-of-production cells)*	Complies with reference sequence	Complies
Identity	N-terminal protein sequencing (Edman)	Complies with reference sequence	Complies
Purity	SDS-PAGE, Coomassie stain	≥ 97%	> 99%
Product-related proteins	SDS-PAGE, Coomassie stain	≤ 10% oligomers ≤ 5% isomers	< 1% 3 %
Specific activity	Proliferation of TF1 cells, calibration against NIBSC 91/662	0.6 – 2.0 x10^6 IU/mg	1.2 x 10^6 IU/mg
Host-cell DNA	Fluorimetric assay	≤ 200 ng/mg	< 40 ng/mg
Host cell protein	ELISA	≤ 1 µg/mg	< 0.1 µg/mg
Endotoxin	Gel clot assay (Eur.Pharm.)	≤ 1000 EU/mg	< 100 EU/mg
Sterility	Inoculation method (Eur.Pharm.)	Sterile	Complies
Mass per vial	Spectrophotometrical measurement at 280 nm	43 - 57 µg	Complies

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Manufacturer

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Grondstofdossier voor ATMP producten

CELLGRO® CellGenix

Handling Instructions:

Reconstitution:	Recommended in 200 µl of sterile H_2O to a final concentration of 250 µg/ml.
Dilution:	Recommended in CellGro® / CellGenix™ serum-free media. For dilution with PBS or protein free medium, a carrier protein (0.1-1% albumin or 1-10 % appropriate serum) has to be included. Failure to dilute product according to these instructions may result in loss of activity.
Storage and Stability:	Store material at -20°C or below. Store diluted material at -20°C or below. Avoid multiple freeze/thaw cycles.

Quality Statement:

This product is manufactured, tested and released in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered products" has been considered in the design of this product.

18.7.2015 D. Rödel

Date *Quality Manager*

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Manufacturer
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Grondstofdossier voor ATMP producten

Bijlage 4: Stroomschema en maximale hoeveelheid rhSCF in eindproduct

Grondstof	Processtap	concentratie rhSCF	volume	hoeveelheid rhSCF
rhSCF	Start HSPC expansie kweek met CD34$^+$ geselecteerde cellen.	50 ng/ml	10-30 ml	
	Check expansiekweken iedere 2/3 dagen. Voeg expansie medium toe.	50 ng/ml	variabel	
	Op dag 10-12: einde van de expansiekweek	50 ng/ml	max. 300 ml	15.000 ng
	Transfer celsuspensie naar 600 ml transferzak en vul aan tot 600 ml met 0,9 % NaCl + 0,5 % HSA. Was de cellen door de zak te centrifu-geren, minstens 580 ml supernatant te verwijderen en de cel pellet van max. 20 ml aan te vullen tot 600 ml met 0,9 % NaCl + 0,5 % HSA	0,83 ng/ml	600 ml	500 ng
	Was de cellen door de zak te centrifugeren minstens 580 ml supernatant te verwijderen en de cel pellet van max. 20 ml aan te vullen tot 600 ml met 0,9 % NaCl + 5 % HSA	27,8 pg/ml	600 ml	16,7 ng
	Centrifugeer de celsuspensie en verwijder ten minste 580 ml supernatant.	27,8 pg/ml	20 ml	0.55 ng
	Vul de 20 ml celsuspensie aan tot het gewenste infusievolume.			

Maximum hoeveelheid rhSCF aan einde expansie kweek is 300 ml x 50 ng/ml = 15 µg

Maximum hoeveelheid rhSCF in eindproduct is 20 ml x 27,8 pg/ml = 0,55 ng

Totale verdunningsfactor 27 x 10^3 maal.

Appendix E: QP release form "SR1-expanded UCB-derived HSPC"

CK.FORM.061 versie 1 / 23-08-2016	Bekrachtigd door: Dr. E. Braakman	Paraaf: Datum:	Geldig tot: .... - .... - 2018	pag 1/pag 1

QP Release: SR1-geëxpandeerde Cord Blood HSPC

Patiënt gegevens

Naam : ..... M/V

Geboortedatum : .....

Patiëntnummer : .....

Product gegevens

Product identificatie :.....

Datum / tijd eind productie : ..... / ..... uur

QP release criteria

Resultaten ingevuld door: ..... **Paraaf: .....**

Specificatie	Criterium	Resultaat	Paraaf QP
Product identificatie	Product identificatie op productzak, werk- en release-formulier komen overeen		
Vrijgave van HPC, CORD BLOOD CD34-enriched product voor verdere verwerking tot ATMP door VP van orgaanbank	Aanwezig		
Microbiologische contaminatie van HPC, CORD BLOOD CD34-enriched product bij start kweek	Microbiologische kweek is negatief voor contaminatie met bacteriën en schimmels		
Geen afwijkingen gerapporteerd tijdens de productie van het ATMP die invloed kunnen hebben op de kwaliteit van het ATMP	Werkformulier voor akkoord getekend door hoofd productie		
Visuele inspectie	Het product vertoont geen tekenen van contaminatie (niet troebel), geen klontjes en de zak is intact		
Opbrengst	Totaal aantal WBC $\geq 50 \times 10^8$ cellen		
Opbrengst	Totaal aantal $CD34^+$ cellen $\geq 20 \times 10^8$ cellen		
Viability	≥ 70% levende cellen (bepaald met trypaan blauw kleuring)		
Etikettering	Inhoud etiket klopt met voorbeeld etiket, houdbaarheid = 6 uur na eind productie		

Voorlopige vrijgave van product ID ..... ja / nee*

(uitslag van bacteriologisch onderzoek van eindproduct nog niet bekend)

Naam QP ..... Datum ..... Tijd ..... Paraaf .....

Bacteriologische controle van eindproduct (dag 10) ID..... : geen groei / groei* ☐

Definitieve vrijgave van product ID ..... ja / nee*

Naam QP ..... Datum ..... Tijd ..... Paraaf .....

* Doorhalen wat niet van toepassing is.

Appendix F: Label on SR1 expanded UCB derived HSPC end-product

INVESTIGATIONAL PRODUCT
SR1-expanded UCB-derived HSPC
Studie: NLXXXXXXXXXX Studienummer: XXXX

Product ID nummer :
PID nummer donor :
HPC(CB) ID :
Volume product : ml
Aantal $CD34^+$ cellen : x 10^6
Patiëntnaam :
PID nummer patiënt :
Datum uitgifte :
Houdbaar tot : uur
Bewaartemperatuur product: tussen 1-8 ° C

Product alleen geschikt voor toediening bij deze patiënt
Alleen voor intraveneuze toediening
Identificeer nauwkeurig de bedoelde ontvanger en product
product niet bestralen en geen leukoreductiefilter gebruiken
Uitsluitend voor gebruik binnen klinische trial

Investigator:
Erasmus MC
Prof. Dr. JJ Cornelissen / Dr. E. Braakman
Wytemaweg 80, 3015 CN, Rotterdam
Transplantatielaboratorium Hematologie,
Ee1369, 010-7043952

Appendix G: Overview of product-specific SOP's and forms

Grondstofdossiers

ATMP.HE.06.2140	Grondstofdossier Flt3L
ATMP.HE.06.2141	Grondstofdossier SCF
ATMP.HE.06.2142	Grondstofdossier TPO
ATMP.HE.06.2143	Grondstofdossier SCGM medium
ATMP.HE.06.2144	Grondstofdossier StemRegenin1

SOP's Productie proces

CK10.4234	Ontdooien en $CD34^+$ cel selectie van HPC, Cord Blood en vrijgave van $CD34^+$ cel fractie voor verdere verwerking tot ATMP
ATMP.HE.10.4235	Expansie van $CD34^+$ cellen in een Vuelife® 290-AC zak

SOP's in process QC

CK07.2491	Apparatuurvoorschrift Coulter® Ac •T bloedcel-counter
CK10.4001	Het tellen van cellen in de Bürker telkamer
CK10.4022	Bepaling van het absolute aantal viabele $CD34^+$ cellen mbv flowcytometrie
CK10.4024	Bepaling van T-, B-, NK en hematopoiëtische voorlopercellen m.b.v. flowcytometrie

Werk-, release- en uitgifte-formulieren

CK.FORM.059	Werkformulier: Ontdooien van HPC, Cord Blood en isolatie van $CD34^+$ cellen
CK.UITG.013	Vrijgifte & Uitgifte HPC, Cord Blood CD34-enriched product t.b.v. verdere verwerking tot ATMP
ATMP.HE.FORM.001	Werkformulier: Bereiden en uitvullen van stockoplossingen van Flt3L, SCF, TPO en SR1
ATMP.HE.FORM.002	Werkformulier: Expansie van $CD34^+$ cellen in een Vuelife® 290-AC zak
ATMP.HE.FORM.003	QP Release formulier: SR1 geëxpandeerde Cord Blood HSPC
ATMP.HE.FORM.004	Uitgifte- / infusieformulier: SR1 geëxpandeerde Cord Blood HSPC

Labels

CK.ETIK.617	Etiketten voor tussen- en eindproducten van SR1 geëxpandeerde Cord Blood HSPC