Study Protocol

PRO-CLIN-0012

A Single Center, Pilot Clinical Investigation of Surgical Bleeding in Burn Patients, and Chronically Transfused Patients With Haematologic Malignancies, Who Are Transfused With Hypoxic Red Blood Cells Manufactured With Hemanext ONE System

NCT: NCT05549232

Document Date: 16 MAR 2023

Hemanext

Clinical Investigation Plan (CIP)

"A Single Center, Pilot Clinical Investigation of Surgical Bleeding in Burn Patients, and Chronically Transfused Patients with Haematologic Malignancies, who are Transfused with Hypoxic Red Blood Cells Manufactured with Hemanext ONE system"

Type of investigation:	Clinical investigation involving a blood product (O_2/CO_2 reduced red blood cells) made with a class IIb medical device (blood container).
Registration:	Hemanext Inc. will comply with the Research Council of Norway on requirements and guidelines for registration and disclosure of medical and health-related studies involving human participants, through: www.ISRCTN.org
Identifier:	Sponsor's clinical investigation ID: CLIN-0012
Principal Investigator and Sponsor	Principal Investigator: Stian Kreken Almeland, MD Address: Haukeland University Hospital, Jonas Lies vei 65, 5021 Bergen Norway Phone: [redacted] E-mail: [redacted] Sponsor: Hemanext Inc. Address: 99 Hayden Avenue, Suite 620, Lexington, MA 02421 United States of America Chief Medical Officer Laurel Omert, MD, FACS, Telephone: [redacted] E-mail: [redacted] Clinical Project Manager Kimberly Dorsch Telephone: [redacted] Email: [redacted]

Sponsor representative	LINK Medical Research Gjerdrums vei 19 NO-0484 Oslo Norway
Medical Device:	Hemanext ONE – A blood container set used to process and store CPD/PAGGSM Red Blood Cells, Leukocytes Reduced, and O_2/CO_2 Reduced. UDI number: 0856186008TECH-0001W9
CIP Version and Date:	PRO-CLIN-0012 Rev. AB dated March 16, 2023

Revision history of amendments

Version	Comments	Changes in subject information/informed consent
Version 25 APR 2022	Final version for submission to the Ethics Committee	
Version 16 MAR 2023	The blood bank at Haukeland University Hospital in Bergen has validated the Hemanext ONE system and hypoxic RBCs will be manufactured for the study in both Bergen and Oslo	None

Signature Page(s)

ID number of the investigation:	CLIN-0012 Hemanext Inc. will comply with the Research Council of Norway on requirements and guidelines for registration and disclosure of medical and health-related studies involving human participants, through: www.ISRCTN.org
Title:	“A single center, pilot clinical investigation of surgical bleeding in burn patients, and chronically transfused patients with haematologic malignancies, who are transfused with hypoxic red blood cells manufactured with Hemanext ONE system”

The Sponsor and the Principal Investigator have approved PRO-CLIN-0012 Rev. AA dated April 25, 2022 and confirm hereby to conduct the investigation according to the CIP, the current version of the World Medical Association Declaration of Helsinki, ISO14155 norm, ICH-GCP as far as applicable, and the local legally applicable requirements.

Sponsor:

Place/Date	Signature

Principal Investigator:

Place/Date	Signature

Site:
Haukeland University Hospital,
Jonas Lies vei 65
5021 Bergen
Norway

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1. SYNOPSIS

Sponsor	Hemanext Inc. 99 Hayden Avenue, Suite 620 Lexington, MA 02421 United States of America
Title:	"A Single Center, Pilot Clinical Investigation of Surgcal Bleeding in Burn Patients, and Chronically Transfused Patients with Haematologic Malignancies, who are Transfused with Hypoxic Red Blood Cells Manufactured with Hemanext ONE system"
Short title / Investigation ID:	"Hypoxic Red Blood Cells for Burns and Hematological Malignancies at Haukeland University Hospital" CLIN-0012
Clinical Investigation Plan, version and date:	PRO-CLIN-0012 Rev. AA dated 25 April 2022
Registration:	Hemanext Inc. will comply with the Research Council of Norway on requirements and guidelines for registration and disclosure of medical and health-related studies involving human participants, through: www.ISRCTN.org
Name of the MD, Unique Device Identification (UDI), name of the manufacturer	Medical device: Hemanext ONE – A blood container set used to process and store CPD/PAGGSM Red Blood Cells, Leukocytes Reduced, and O_2/CO_2 Reduced. UDI number: 0856186008TECH-0001W9 Manufacturer: Hemanext Inc. 99 Hayden Avenue Building B, Suite 620 Lexington, MA 02421 USA
Stage of development:	Medical device: Hemanext ONE CE Marked since April 2021 (Post-Market stage) Blood Product: CPD/PAGGSM Red Blood Cells, Leukocytes Reduced, and O_2/CO_2 Reduced The Norwegian National Directorate of Health will be notified that this blood product is under evaluation. In Norway, this blood product is currently not registered with the Norwegian Directorate of Health (Helsedirektoratet).
Background and rationale:	**1. Background and rationale for hypoxic blood use:**

Red blood cells (RBCs) comprise the only tissue in the body that provides oxygen transport. Anemia results from either the acquired loss of RBCs due to disease, injury, or surgery or the inability of the patient to produce their own RBCs due inherited disorders. While the overall clinical settings of anemia can differ greatly between acute or chronic causes, the primary therapeutic goal of RBC transfusion is to increase the oxygen-carrying capacity and prevent tissue hypoxia[1]. Red blood cells can be stored for up to 42 days before transfusion but, surprisingly, there are few quality parameters that predict the efficacy and safety of a transfusion[2].

2. Consequences of Current RBC Storage Process

A review of the literature[2,3,4,5] identifies substantial evidence suggesting decreased quality of RBCs from substances that accumulate during RBC storage. These include nitric oxide (NO) scavengers and inhibitors of NO generation that affect vascular tone, procoagulant microparticles, free and non-transferrin bound iron, and bioactive lipids. Additionally, RBCs show progressively diminished deformability over time, characteristics necessary to adequately perfuse the microcirculation[4]. D'Alessandro and colleagues observed significant changes in the plasma levels of hemolytic markers, oxidized purines, plasticizers, and oxidized lipids in recipients of blood stored for 42 days, compared with 5 days[3].

Yoshida and colleagues performed an exhaustive review of the oxidative and metabolic changes that occur to RBCs during storage, summarizing elements of the RBC storage lesion from causes to associated clinical sequelae. The review identifies root causes, effects on RBCs, physiological consequences reported from in vitro experiments or animal models, and finally, potential clinical sequelae of RBC transfusion, each element being identified with its pertinent reference(s)[2].

3. Hypoxic storage and HEMANEXT blood product

Hypoxic storage, where the oxygen content of RBC units is reduced to low levels [e.g., less than 20% oxy-hemoglobin (SO_2)[6] prior to refrigeration and maintained throughout storage], was proposed as an alternative to antioxidant-based additive solutions to reduce oxidative stress during hypothermic RBC storage[5,7-11]. The rationale for implementing hypoxic storage is to reduce oxygen, the essential substrate for hemoglobin oxidation that generates the multitude of untoward physiologic and biochemical events described previously.

Hypoxic storage was also shown to counteract some metabolic impairments without requiring novel additive ingredients[5,7] independent of the reduction in oxidative stress. During pre-storage processing to reduce oxygen content in the RBC, carbon dioxide is also reduced. Carbon dioxide depletion increases cytosolic pH that was lowered from its physiological level by exposure to acidic anticoagulant and additive solutions. The resulting more neutral pH in the early phase of storage results in sustained flux through the glycolytic pathway and elevated 2,3-DPG levels, which are highly sensitive to pH and normally depleted early during hypothermic storage[7,11,12]. Additionally, deoxyhemoglobin causes metabolic modulation to release glycolytic enzymes, such as phosphofructokinase and glyceraldehyde dehydrogenase, sequestered at the band 3 binding domain, thereby enhancing overall glycolytic flux during hypoxic storage[13,14].

Metabolomics work by D'Alessandro et al has shown that hypoxic storage diminishes damaging mediators of oxidative stress and increases energy biomarkers without requiring novel additive ingredients[15]. The same group investigated the metabolomics of hypoxic storage in a two-arm cross-sectional clinical study. Hypoxic storage was found to ameliorate the metabolic phenotypes of stored RBCs (lower markers of oxidant stress such as lipids and purines and higher levels of high-energy phosphate compounds) and improve post-transfusion recovery (PTR). The study also correlated metabolic parameters to hemolysis and PTR, highlighting the role of purine and lipid oxidation (e.g., hypoxanthine and 9-HODE), so that these can be used as markers in fresh and stored units to predict end of storage blood quality[15].

Population:

The Hemanext ONE System may be used to manufacture hypoxic RBCs for any patient requiring a blood transfusion. This pilot investigation aims to study safety of hypoxic RBCs in 2 patient groups: acute burn and hematologic malignancies. Burn patients undergoing surgical excision routinely require large quantities of blood to be transfused. Those patients with hematologic malignancies often require regular transfusions.

Burn Patients

Major burn trauma has been found to trigger an inflammatory cascade of events which leads to oxidative stress and tissue damage. These injurious responses, which include complement activation, excessive histamine release, decreased blood pressure, and release of reactive oxygen species, may ultimately lead to the development of multiple organ dysfunction[16]. Early excision and grafting of large burn wounds is currently performed to attenuate the post-burn hypermetabolic state and remove the biological nidus for infection. These operative procedures can result in considerable bleeding. In one report, intraoperative blood loss was estimated at approximately 9.2% of blood volume for every 1% of total body surface area (TBSA) burn excised[20].

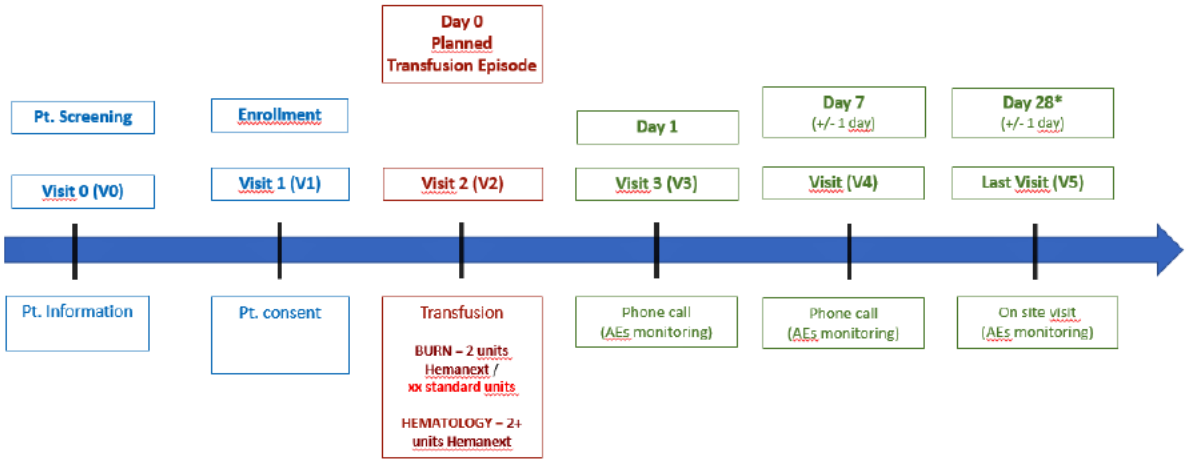
Patients with Hematological Malignancies

Hematologic malignancies such as myelodysplastic syndromes (MDS), multiple myeloma, and leukemia are increasing in prevalence in Norway. Collectively, hematologic malignancies refers to various disorders of the bone marrow which result in abnormal blood cell production. Leukemia presents with increased numbers of leucocytes in the blood and bone marrow[23]. Multiple myeloma is characterized by uncontrolled growth of plasma cells in the bone marrow. MDS consist of bone marrow diseases associated with ineffective hematopoiesis, resulting in morphologically abnormal blood cells[24]. In all cases, anemia can result from the overcrowding or ineffective production of normal blood cells, leading to inadequate oxygen delivery. Patients often require regular blood transfusions at some time during their treatment to alleviate the symptoms of severe anemia, which can include fatigue, weakness, and exacerbation of other underlying medical conditions. The treatment goal is to minimize morbidity and mortality with the lowest possible exposure[26]. Some studies show reduced survival with increased transfusion density[27]. Iron overload with organ impairment, a consequence of transfusion dependency, may be fatal in those who are heavily iron-overloaded[28,29]. Therefore, where a patient has no fatigue-induced limitation on quality of life or function at home, a more conservative approach may be preferred to avoid potential adverse effects and improve outcomes[31].

Potential B enefits of Hypoxic RBC Use

In vitro and pre-clinical data support the hypothesis that hypoxic blood carries the potential to benefit patients suffering from hematological malignancies and burn injuries. Hypoxic storage may reduce oxidative stress, as shown in metabolomics work by D'Alessandro et al highlighting diminished mediators of oxidative stress in hypoxically stored blood vs conventional[15]. The use of hypoxically stored blood may improve oxygen delivery, as evidenced in CLIN-0001 signifying that hypoxically stored blood showed improvements in ATP, 2,3 DPG, and RBC deformability[32]. The increases in 2,3 DPG levels contribute to a rightward shift of the O_2 dissociation curve, which may translate to improved oxygen delivery[34]. Additionally, hypoxically stored blood may decrease the required number of RBC transfusions. A study by D'alessandro et al demonstrates that hypoxic storage yields more viable RBCs, as indicated by greater PTR24 values[15]. The increase in viable RBCs demonstrated in the aforementioned study supports a potential 15% reduction in blood requirements for both patient groups[15]. Because transfusion-related iron overload is directly associated with the number of RBC units transfused in patient who require chronic transfusions, this reduction in blood requirements may lead to lower transfusion density and decreased risk of iron overload[5,9] in patients with hematological malignancies. Furthermore, hypoxic blood may improve resuscitation time and/or volume in burn patients, as demonstrated in a pre-clinical animal model of resuscitation from hemorrhagic shock[35].

Objective(s):	Primary Objective – for both patients groups Hypoxic RBCs safety and tolerance assessment up to 24 hours following the transfusion initiation and overall up to 7 days (+/- 1 day) after the transfusion episode (single transfusion course). Secondary Objectives : A. For both patients groups 1. Assessment of pre and post transfusion hemoglobin levels 2. Assessment of hemoglobin level before the following transfusion, if applicable 3. Assessment of AEs occurence: i. Up to 7 days (+/- 1 day) post transfusion, in comparison with historical control (including but not limited to infection, deep vein thrombosis, acute respiratory distress syndrome, transfusion-related acute lung injury, transfusion associated circulatory overload, anaphylactic shock, acute haemolytic transfusion reaction). ii. Up to the subsequent transfusion episode or up to 28 days (+/- 1 day) after the initial transfusion, whichever comes first. iii. From enrollment, up to their subsequent transfusion or 28 days (+/- 1 day) post transfusion, whichever comes first, through the assessment of patient's diary 4. Assessment of the vital signs during and up to 15 minutes after the transfusion B. For burn patients group only: 1. Assessment of arterial blood gases: i. pre-operative ii. intra-operative iii. post operative
Outcome(s):	Primary Outcome – for both patients groups Number of participants who experienced an adverse event (all types/grades) within a time frame up to 24 hours following the transfusion and overall up to 7 days (+/- 1 day) after the transfusion. The type and the grade of each adverse event will be categorized according to: • Association for the Advancement of Blood and Biotherapies (AABB) technical manual, 20th edition (2020) • Biomedical Excellence for Safer Transfusion (BEST) Collaborative review - Lancet 2016; 388: 2825–36 • Local AEs database (for reference) • ISO 14155-2020 definitions Secondary Outcomes : - for both patients groups 1. Evolution of the hemoglobin level before and after the transfusion. 2. Calculation oft he Hemoglobin increment after transfusion corrected for patient blood volume and hemoglobin dose 3. Comparison of the Hemoglobin level before the index transfusion to that prior to the

	subsequent transfusion 4. Comparison of the frequency of all AEs, including transfusion related AEs up to 7 days (+/- 1) post-transfusion compared to historical data in local patient registries (ex. The National Burn Registry) 5. Evaluation of AEs from enrollment, up to prior to the subsequent transfusion or up to 28 days (+/- 1 day) after the initial transfusion, whichever comes first. 6. Evaluation of the frequency of all AEs, from enrollment, up to their subsequent transfusion or 28 days (+/- 1 day) post-transfusion, whichever comes first, through the assessment of patient's diary, when applicable. 7. Evaluation of patient's vital signs over the course of the transfusion and up to 15 minutes post-transfusion
Design:	"A single center, pilot clinical investigation of surgical bleeding in burn patients, and chronically transfused patients with haematologic malignancies, who are transfused with hypoxic red blood cells manufactured with Hemanext ONE system". A first cohort of 5 patients – solely from the hematological malignancies (chronically transfused) group - will be enrolled, transfused and followed-up until their subsequent transfusion or 28 days (+/- 1 day) post-transfusion, whichever comes first All safety related data from this initial cohort of 5 patients will be reviewed and assessed by the clinical investigation Data Monitoring Committee (DMC). Upon favourable opinion from the clinical investigation DMC, the enrolment of the remaining patients (both groups) will resume until completion.  **NB**: * Day 28+/- 1 day or next transfusion episode, whichever happens first.
Inclusion / exclusion criteria:	INCLUSION CRITERIA Patients meeting the following criteria may be invited to participate in the clinical investigation and

	undergo the informed consent process: A. Hematological malignancies patients group: 1. Male or female patients at least 18 years of age 2. Patients expected to require ≥ 2 units of red blood cells in a single transfusion event 3. Patients who have the capacity to consent to particpate and are willing to comply with the study procedures. 4. Patients identified by a Transfusion hemoglobin trigger of less than 9 g/dL 5. Patients with a documented diagnosis of leukemia, myelomatosis or MDS requiring chronic transfusions B. Burn patients group: 1. Male or female patients at least 18 years of age 2. Patients who have the capacity to consent by themselves to participate to the clinical investigation 3. Smaller burn patients, hospitalized with a Total Body Surface Area (TBSA%) burn ≥ 10% and ≤ 50% 4. Patients expected to require ≥ 2 unit of red blood cells in a single transfusion event EXCLUSION CRITERIA Patients meeting any of the exclusion criteria may not participate in the clinical investigation. A. Both patients groups 1. Patients with any positive antibody screening test 2. Patients for whom consent has not been obtained 3. Patients with a known hemolytic anemia (congenital or acquired) 4. Patients < 18 years old 5. Patients with a known or suspected pregnancy 6. Patients with a history of major transfusion reactions 7. Patients whom the Investigator deems clinical trial participation is not in their best interest. B. Burn patients specific exclusion criteria : 1. Patients who do not have the capacity to consent by themselves to participate to the clinical investigation 2. Patients hospitalized with a Total body surface area (TBSA%) burn more than 50% 3. Patients with combined trauma in need of blood transfusions for treatment other than the burn excision
Measurements and procedures:	The following information will be collected, from enrollment throughout end of study visit, when applicable: 1. Informed consent 2. Documentaton of Inclusion/Exclusion criteria 3. Demographics 4. Medical history 5. Physical examination 6. Pregnancy test (if applicable) 7. Height 8. Weight 9. RBC unit weight and volume 10. Age (days) of the RBC unit at the time of the transfusion 11. Hemoglobin measurement - maximum 4 hours before the transfusion and, at minimum, 15 minutes before transfusion 12. Hemoglobin measurement 15 to 60 minutes after transfusion 13. Hemoglobin measurement, at a minimum, 15 minutes before the following transfusion

	14. Blood pressure every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion 15. Heart rate every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion 16. Body temperature every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion 17. Respiratory rate every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion 18. Oxygen saturation every 15 minutes and until 15 minutes after the end of the transfusion 19. Patient diary for collection of concomittant medications and changes in health status from enrollment up to their subsequent transfusion or 28 days (+/- 1 day) post-transfusion, whichever comes first 20. Routine laboratory analyses, including blood gases when applicable.
Intervention:	For both clinical investigation patient populations: • Transfusion of two units of Red Blood Cells, Leukocytes reduced, O_2/CO_2 reduced (single transfusion course) manufactured with the Hemanext ONE System, combined with PRN standard Red Blood Cells, as applicable. • Patient's diary
Control intervention (if applicable):	Not applicable
Number of subjects with rationale:	10 acute burn patients and 10 patients with haematological malignancies, who require transfusion of red cells concentrates and who fulfil all eligibility criteria will be enrolled in this clinical investigation at Haukeland University Hospital. A total of twenty patients will be sufficient to assess preliminary safety of O_2/CO_2 reduced RBCs, as the standard rate of transfusion related adverse events is usually low and these expected events are well known. If any unexpected adverse events (any type or grade) should occur, they will be identified as such.
Duration of the investigation:	This clinical investigation is estimated to last for a period of approximately 6 months.
Investigation schedule:	Anticipated Start Date: May – June 2022 Anticipated End Date: November – December 2022
Investigator(s):	Principal Investigator: Stian Almeland, MD Address: Haukeland University Hospital, Jonas Lies vei 65, 5021 Bergen Norway Phone: [redacted] E-mail: [redacted]

	Sub-Investigator: Haakon Reikvam, MD Address: Haukeland University Hospital, Jonas Lies vei 65, 5021 Bergen Norway E-mail: [redacted]
Investigational Site(s):	This clinical investigation will only involve one single clinical site: Haukeland University Hospital Jonas Lies vei 65, 5021 Bergen Norway
Blood Bank	Oslo: [redacted] Address: Department of Immunology and Transfusion Medicine Section for Blood processing Oslo University Hospital Ullevål Norway [redacted] Bergen: [redacted] Address: Haukeland University Hospital Department of Immunology and Transfusion Medicine Jonas Lies vei 65, 5021 Bergen Norway E-mail: [redacted] [redacted]
Data Monitoring Committee (DMC)	Membership: [redacted] Professor of intensive care medicine

	Haukeland University Hospital, Bergen [redacted] Chief of Burns, Plastic and reconstructive surgeon Haukeland University Hospital, Bergen [redacted] Blood Bank - Haukeland University Hospital, Bergen [redacted] Blood Bank - Haukeland University Hospital, Bergen [redacted] Blood Bank - Haukeland University Hospital, Bergen
Statistical considerations:	This clinical investigation is not statistically powered – only descriptive statistical methods will be applied
Compliance statement:	This investigation will be conducted in compliance with the CIP, the current version of the Declaration of Helsinki, ISO14155-2020, ICH-GCP (as far as applicable) as well as all national legal and regulatory requirements.

Hematogical Malignancies Group:

Investigation Periods	Screening & Enrollment Visit	Pre-Transfusion	Transfusion	Post-Transfusion	Follow Up 1	Follow-up 2	Follow-up 3
Visit	1	2	2	2	3	4	5
Time (hour, day, week)	D0	D1	D1	D1	D2 (≤ 24h)	D7 (+/- 1 day)	Day of subsequent transfusion or D28, whichever comes 1st
In- /Exclusion Criteria	x	x					
Patient Information							
Patient Information and Consent (ICF)	x						
Demographics	x						
Medical History	x						
Concomitant Medications	x	x	x	x	x	x	x
Physical Examination	x						x
Pregnancy Test	x						
Height	x						
Weight	x						
RBC Unit Weight		x					
Patient Hemoglobin		x		x			x
Blood Pressure	x	x	x	x			
Heart Rate	x	x	x	x			
Body Temperature	x	x	x	x			
Respiratory Rate	x	x	x	x			
Oxygen Saturation	x	x	x	x			
Transfusion			x				
Adverse Events, Adverse device effects	x	x	x	x	x	x	x
Review HSB (blood bag) Integrity		x	x	x			
Patient Diary	x	x	x	x	x	x	x

Burn patients Group:

Investigation Periods	Screening & Enrollment Visit	Pre-Transfusion	Transfusion	Post-Transfusion	Follow Up 1	Follow-Up 2	Follow-Up 2
Visit	1	2	2	2	3	4	5
Time (hour, day, week)	Day 0	Day 1	Day 1	Day 1	Day 2 (≤ 24 h)	Day 7 (+/- 1 day)	Day of subsequent transfusion or D28, whichever comes 1st
In- /Exclusion Criteria	x						
TBSA	x						
Patient Information							
Patient Information and Consent (ICF)	x						
Demographics	x						
Medical History	x						
Concomitant Medications	x	x	x	x	x		x
Physical Examination	x						x
Pregnancy Test	x						
Height	x						
Weight	x						
RBC Unit Weight		x					
Patient Hemoglobin		x		x			x
Blood Pressure	x	x	x	x			
Heart Rate	x	x	x	x			
Body Temperature	x	x	x	x			
Respiratory Rate	x	x	x	x			
Oxygen Saturation	x	x	x	x			
Transfusion			x				
Adverse Events, Adverse device effects	x	x	x	x	x		x
Review HSB (blood bag) Integrity		x	x	x			

2. ABBREVIATIONS

AE	Adverse Event
ADE	Adverse Device Effect
ASADE	Anticipated Serious Adverse Device Effect
ASR	Annual Safety Report
CA	Competent Authority
CEC	Competent Ethics Committee
CIP	Clinical investigation plan
CRF	Case Report Form (pCRF paper CRF; eCRF electronic CRF)
DD	Device Deficiency
DMC / DSMC	Data Monitoring Committee, Data Safety Monitoring Committee
IB	Investigator's Brochure
HSB	Hemanext Storage Bag
ICF	Informed Consent Form
ICH-GCP	International Council for Harmonisation – guidelines of Good Clinical Practice
IFU	Instruction For Use
ISF	Investigator Site File
ISO	International Organisation for Standardisation
MD	Medical Device
MDR	Medical Device Regulation (EU) 2017/745 of 5 April 2017
ORB	Oxygen Reduction Bag
PI	Principal Investigator
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SDV	Source Data Verification
SOP	Standard Operating Procedure
USADE	Unanticipated Serious Adverse Device Effect

SUMMARY OF THE REVISION HISTORY IN CASE OF AMENDMENTS

Version Nr, Version Date	Chapter	Description of change	Reason for the change
A00 11 January 2022	All	Initial release	Initial release
AA 28 April 2022	All	Based on REK letter dated March 07th, 2022	Amendment 1

3. INVESTIGATION ADMINISTRATIVE STRUCTURE

3.1 Sponsor

Chief Medical Officer
Laurel Omert, MD, FACS,
Telephone [redacted]
E-mail: [redacted]

Clinical Project Manager
Kimberly Dorsch
Telephone: [redacted]
Email: [redacted]

Address:
Hemanext Inc.
99 Hayden Avenue
Building B, Suite 620
Lexington, MA 02421
USA

Responsibilities:
Hemanext Inc, as the Sponsor of this Clinical Investigation will have the responsibility – as per ISO 14155:2020 - and liability for the initiation or implementation and management of this clinical investigation, and arranging its financial setup.

3.2 Principal Investigator(s)

Principal Investigator: Dr Stian Almeland
Address:
Haukeland University Hospital, Jonas Lies vei 65,
5021 Bergen
Norway
Phone: [redacted]
E-mail: [redacted]

Sub-Investigator: Dr Haakon Reikvam

Address:

Haukeland University Hospital, Jonas Lies vei 65,

5021 Bergen

Norway

E-mail: [redacted]

3.3 Statistician ("Biostatistician")

[redacted]
Transplantation Research Center
Brigham and Women's Hospital and Children's Hospital
Harvard Medical School,
Boston, MA
USA

[redacted]

3.4 Laboratory

Not applicable. Only standard of care laboratory assessments will be performed.

3.5 Monitoring institution

Monitoring will be performed by the Sponsor or designee.

3.6 Data Monitoring Committee

A Data Monitoring Committee (DMC) will be formed to ensure the safety of the patients participating in the investigation.

This group will provide review and adjudication of all adverse events collected during the clinical investigation. The DMC members will be selected in collaboration between the principal investigator and Hemanext before initiation of the trial.

All DMC members are free from any direct involvement with the trial. Any competing interests, both real and potential, must be declared.

A report outlining the review of adverse events will be included in the Final Study Report.

A DMC charter will be established to document the following but not limited to:

a) the responsibilities and scope of activities of the DMC;

b) the frequency, format, and documentation of meetings;

c) arrangements for handling emergency situations.

3.7 Any other relevant Committee, Person, Organisation, Institution

The hypoxic RBCs will be processed and provided to the clinical site from either of the following locations:

Department of Immunology and Transfusion Medicine
Oslo University Hospital Ullevål
Oslo, Norway

Department of Immunology and Transfusion Medicine
Haukeland University Hospital
Bergen, Norway

4. ETHICAL AND REGULATORY ASPECTS

The final positive decision of the CEC on the conduct of the investigation will be made and given in writing to the Sponsor before the investigation can begin. Additional requirements set by the CEC must be implemented.

4.1 Registration of the investigation

Registration status:

Hemanext Inc. will comply with the Research Council of Norway on requirements and guidelines for registration and disclosure of medical and health-related studies involving human participants, through: www.ISRCTN.org

4.2 Competent Ethics Committee (CEC)

The Sponsor or designee will submit the investigation to the CEC and obtain ethical committee approval before the start of the investigation. The PI ensures that approval from the CEC is obtained and filed in the Investigator site file before the investigation starts.

4.2.1 Reporting duties to the Competent Ethics Committee

CIP Amendments will be reported according to the applicable requirements and timeframes.

The regular or premature end of the investigation as well as the interruption of the investigation must be reported to the CEC within 15 days (within 24 hours if it is due to security reasons). The reasons for a premature end or an interruption must be explained.

A final report will be submitted within one year after the regular end of the investigation and within 3 months after a premature end of the investigation.

4.3 Competent Authorities (CA)

Notification of this clinical investigation and of the use of hypoxic blood will be made prior the start of this clinical investigation.

4.3.1 Reporting duties to the competent authorities

Notification of this clinical investigation and of the use of hypoxic blood will be made prior the start of this clinical investigation.

4.4 Ethical Conduct of the Investigation

The investigation will be carried out according to the CIP and with principles enunciated in the current version of the Declaration of Helsinki, the European Regulation on medical devices 2017/745 (MDR), the Norms ISO14155 and ISO14971, the ICH-guidelines of Good Clinical Practice (GCP) as applicable, and local regulatory authority's requirements. The CEC, if applicable, will receive the Annual Safety Report (ASR) and interim reports and be notified about investigation stop/end in agreement with local requirements.

4.5 Declaration of interests

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

4.6 Patient Information and Informed Consent

All potential subjects are given a Patient Information Sheet and an Informed Consent Form describing the investigation and providing sufficient information for the subject to make an informed decision about his/her participation in the investigation. The Patient Information Sheet must be provided prior to the Screening Visit. The PI must explain to each subject the nature of the investigation, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each subject must be informed that the participation in the investigation is voluntary and that he/she may withdraw from the investigation at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The subject should be informed that he/she can ask any question, and consult with family members, friends, their treating physicians or other experts before deciding about their participation in the investigation. Enough time must begiven to the subjects prior to providing informed consent.

The formal consent of a subject, using the approved consent form, is obtained before the subject is submitted to any investigation procedure. The subject should read, understand, and voluntarily agree before signing and dating the informed consent form. The consent form is signed and dated by the subject and the PI (or her/his designee). The subject must be given a copy of the signed document. The original signed and dated consent form is retained as part of the investigation records.

The subjects must be informed that authorised individuals other than their treating physician may examine his/her medical records.

4.7 Subject privacy and confidentiality

The Sponsor and the PI affirm and uphold the principle of the subjects' right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the subjects shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained because of this investigation is considered confidential and disclosure to third parties is prohibited.

For data verification purposes, authorised representatives of the Sponsor or the CEC may require direct access to parts of the medical records relevant to the investigation, including subjects' medical history.

To protect the rights and freedoms of participants about the processing of personal data, participants will be assigned a single, participant specific numerical code. Any participant records or data sets that are transferred to the sponsor will contain the numerical code only; participant names will not be transferred. All other identifiable data transferred to the sponsor will be identified by this single, participant specific code. The study site will maintain a confidential list of participants who participated in the study, linking each participant's numerical code to his or her actual identity and medical record identification. In case of data transfer, the sponsor will protect the confidentiality of participants' personal data consistent with the clinical study agreement and applicable privacy laws.

4.8 Early termination of the investigation

The Sponsor, DMC, or CEC may terminate the investigation prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient subject recruitment,
- when the safety of the subjects is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of the investigation unwise,
- early evidence of benefit or harm of the experimental intervention.

4.9 Clinical investigation plan amendments

Substantial amendments are only implemented after approval by the CEC. The use of waivers from the CIP is prohibited (Annex XV, Chapter 2, Art. 3.10 MDR).

Under emergency circumstances, deviations from the CIP to protect the rights, safety and well-being of the subjects may proceed without prior approval by the Sponsor and the CEC. Such deviations shall be documented and reported to the Sponsor and the CEC within 2 days.

All non-substantial amendments are communicated to the CEC together with the Annual Safety Report (ASR). The ASR shall include any deviations from the CIP that may have affected the rights, safety or well-being of the subject or the scientific integrity of the investigation (ISO14155:2020).

All protocol amendments will be submitted to the CEC when any revision is made to the original protocol or subsequent version of the protocol that significantly affects the safety of subjects and/or any change is made that significantly affects the scope of investigation or scientific quality of the study.

The amended protocol will be reviewed, approved and documented by the same method in which the original protocol was reviewed and approved. Once the amended protocol and all associated documents are reviewed and approved, the protocol number and effective date will be changed on the title page.

The final approved amended protocol will be distributed to participating investigator(s) at each site.

4.10 Deviation from the Clinical Investigation Plan

The use of waivers from the CIP is prohibited (Annex XV, Chapter 2, Art. 3.10 MDR).

Investigators are not allowed to deviate from the clinical investigation plan. Under emergency circumstances, deviations from the CIP to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Sponsor and the CEC. Such deviations shall be documented and reported to the Sponsor and the CEC as soon as possible, but in no event later than 5 working days after the emergency occurred.

CIP deviations are to be reported in the deviation/violation log provided with the investigator site file and the CRF. Serious CIP deviations are to be reported in writing with the corresponding log to the Sponsor and the CEC within 24 hours. Investigators will be retrained by the Sponsor or its representative. Investigators not complying with the protocol might be disqualified from participation in the clinical trial at the discretion of the Sponsor.

5. BACKGROUND AND RATIONALE

5.1 Background and Rationale for the clinical investigation

5.1.1 Background and Rationale for Hypoxic Blood:

Red blood cells (RBCs) comprise the only tissue in the body that provides oxygen transport. Anemia results from either the acquired loss of RBCs due to disease, injury, or surgery or the inability of the patient to produce their own RBCs due inherited disorders. While the overall clinical settings of anemia can differ greatly between acute or chronic causes, the primary therapeutic goal of RBC transfusion is to increase the oxygen-carrying capacity and prevent tissue hypoxia[1]. Red blood cells can be stored for up to 42 days before transfusion but, surprisingly, there are few quality parameters that predict the efficacy and safety of a transfusion[2].

5.1.2 Consequences of Current RBC Storage Process

A review of the literature[2,3,4,5] identifies substantial evidence suggesting decreased quality of RBCs from substances that accumulate during RBC storage. These include nitric oxide (NO) scavengers and inhibitors of NO generation that affect vascular tone, procoagulant microparticles, free and non-transferrin bound iron, and bioactive lipids. Additionally, RBCs show progressively diminished deformability over time, characteristics necessary to adequately perfuse the microcirculation[4]. D'Alessandro and colleagues observed significant changes in the plasma levels of hemolytic markers,

oxidized purines, plasticizers, and oxidized lipids in recipients of blood stored for 42 days, compared with 5 days[3].

Yoshida and colleagues performed an exhaustive review of the oxidative and metabolic changes that occur to RBCs during storage, summarizing elements of the RBC storage lesion from causes to associated clinical sequelae. The review identifies root causes, effects on RBCs, physiological consequences reported from in vitro experiments or animal models, and finally, potential clinical sequelae of RBC transfusion, each element being identified with its pertinent reference(s)[2].

5.1.3 Hypoxic storage and HEMANEXT product

Hypoxic storage, where the oxygen content of RBC units is reduced to low levels (e.g., less than 20% oxy-hemoglobin (SO_2)[6] prior to refrigeration and maintained throughout storage, was proposed as an alternative to antioxidant-based additive solutions to reduce oxidative stress during hypothermic RBC storage[5,7-11]. The rationale for implementing hypoxic storage is to reduce oxygen, the essential substrate for hemoglobin oxidation that generates the multitude of untoward physiologic and biochemical events described previously.

Hypoxic storage was also shown to counteract some metabolic impairments without requiring novel additive ingrdients[5,7] independent of the reduction in oxidative stress. During pre-storage processing to reduce oxygen content in the RBC, carbon dioxide is also reduced. Carbon dioxide depletion increases cytosolic pH that was lowered from its physiological level by exposure to acidic anticoagulant and additive solutions. The resulting more neutral pH in the early phase of storage results in sustained flux through the glycolytic pathway and elevated 2,3-DPG levels, which are highly sensitive to pH and normally depleted early during hypothermic storage[7,11,12]. Additionally, deoxyhemoglobin causes metabolic modulation to release glycolytic enzymes, such as phosphofructokinase and glyceraldehyde dehydrogenase, sequestered at the band 3 binding domain, thereby enhancing overall glycolytic flux during hypoxic storage[13,14].

Metabolomics work by D'Alessandro et al has shown that hypoxic storage diminishes damaging mediators of oxidative stress and increases energy biomarkers without requiring novel additive ingredients[15]. The same group investigated the metabolomics of hypoxic storage in a two-arm cross-sectional clinical study. Hypoxic storage was found to ameliorate the metabolic phenotypes of stored RBCs (lower markers of oxidant stress such as lipids and purines and higher levels of high-energy phosphate compounds) and improve post-transfusion recovery (PTR). The study also correlated metabolic parameters to hemolysis and PTR, highlighting the role of purine and lipid oxidation (e.g., hypoxanthine and 9-HODE), so that these can be used as markers in fresh and stored units to predict end of storage blood quality[15].

5.1.4 Background on Blood Use in Burn Patients:

Major burn trauma has been found to trigger an inflammatory cascade of events which leads to oxidative stress and tissue damage. These injurious responses, which include complement activation, excessive histamine release, decreased blood pressure, and release of reactive oxygen species, may ultimately lead to the development of multiple organ dysfunction[16]. Cardiac dysfunction and acute kidney injury, for example, have been well described as sequelae of burn injury and contribute to multi-organ failure[17,18,19].

Early excision and grafting of large burn wounds is currently performed to attenuate the post-burn hypermetabolic state and remove the biological nidus for infection, thereby reducing the risk of burn wound sepsis. These operative procedures can result in considerable bleeding. In one report, intraoperative blood loss was estimated at approximately 9.2% of blood volume for every 1% of total body surface area (TBSA) burn excised[20]. Burn surgeons also report progressive development of microvascular bleeding and evidence of coagulopathy requiring plasma and platelets as well as RBC transfusions[21]. Coagulopathic bleeding post-op can continue for several days, requiring additional transfusions.

Pasch and colleagues compared estimated blood loss for TBSA burns > and < 20% to provide insight for transfusion ratios for the coagulopathy of burns and also to illustrate the importance of blood loss as a benchmark in these patients to improve care[22].

Table 1

Estimated Blood loss (ml/cm^2) for Excision/Grafting		24 hours post-operative EBL Mean ml/cm^2(Std Dev)	72 hours post-operative EBL Mean ml/cm^2 (Std Dev)
Total cases	N=66	1.49 (2.16)	1.98 (2.65)
TBSA >20%	N=37	1.08 (1.52)	1.80 (1.87)
TBSA<20%	N=29	2.00 (2.64)	2.20 (3.34)
With Tourniquet	N=11	1.11 (1.34)	2.21 (1.64)
W/O Tourniquet	N=55	1.57 (2.31)	1.92 (2.83)

Table 2 below illustrates number of units of RBCs transfused per TBSA burn as published by different authors.

Table 2: Blood Loss During Excision and Grafting

Author	%TBSA	Units RBCs transfused
Posluszny and Gamelli[8]	>10% TBSA	Average 19.7 units (range of 0 to 201 units)
Vasko et al[9]	>10% TBSA	Average of 8.94 units
	>30% TBSA	Mean 17 units transfused
Palmieri et al [10]	≥20% TBSA.	Average 13.7 ± 1.1 units
	≥50% TBSA	>30 units of blood transfused per patient
Yogore et al[11]	<10% TBSA	4 ± 0.6
	11 to 19% TBSA	8 ± 1 units
	20 to 40% TBSA	12 ± 3 units
	>40% TBSA	20 ± 4 units

5.1.5 Background on Blood Use in Hematological Malignancies:

Hematologic malignancies such as myelodysplastic syndromes (MDS), multiple myeloma, and leukemia are increasing in prevalence in Norway as the population average age continues to increase. Collectively, hematologic malignancies refers to various disorders of the bone marrow which result in abnormal blood cell production. Leukemia consists of several malignant disorders that present with increased numbers of leucocytes in the blood and bone marrow[23]. Multiple myeloma is characterized by uncontrolled growth of plasma cells in the bone marrow. MDS consist of bone marrow diseases associated with ineffective hematopoiesis, resulting in morphologically abnormal blood cells[24]. In all cases, anemia can result from the overcrowding or ineffective production of normal blood cells, leading to inadequate oxygen delivery.

Patients with hematologic malignancies often require regular blood transfusions at some time during their treatment to alleviate the symptoms of severe anemia, which can include fatigue, weakness, and exacerbation of other underlying medical conditions. While treatment varies with disease severity, transfusions are given at approximately weekly intervals in intensive treatment[25]. In relying on stored donor RBCs to address anemia, the goal is not to rapidly rebuild a normal red cell mass, but to minimize morbidity and mortality with the lowest possible exposure[26]. No consensus exists on the appropriate time to commence transfusions or on the correct transfusion threshold for optimal care. Some studies show reduced survival with increased transfusion density[27]. Iron overload, a consequence of transfusion

dependency, is also independently associated with poor outcomes for patients with MDS[28,29]. Iron overload with organ impairment may be fatal in those who are heavily iron-overloaded[30]. Therefore, where a patient has no fatigue-induced limitation on quality of life or function at home, a more conservative approach may be preferred to avoid potential adverse effects and improve outcomes[31].

5.1.6 Potential Benefits of Hypoxic Blood

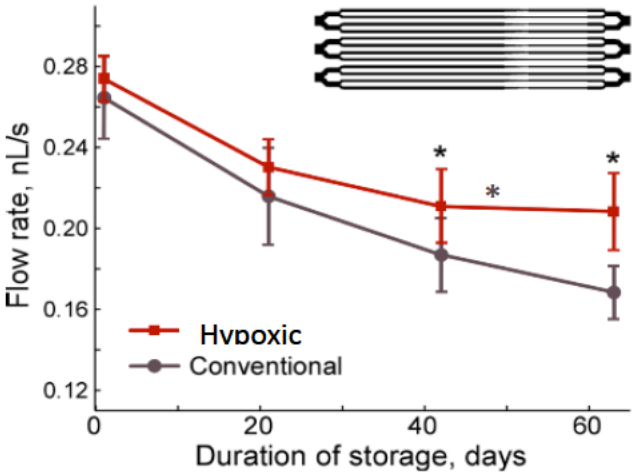
In vitro and pre-clinical data support the hypothesis that hypoxic blood carries the potential to benefit patients suffering from burn injuries.

Reduce Oxidative stress: Hypoxic storage was initially proposed as an alternative to antioxidant-based additive solutions to reduce oxidative stress during hypothermic RBC storage[5,7-11]. The rationale for implementing hypoxic storage is to reduce oxygen, the essential substrate for hemoglobin oxidation that generates the multitude of untoward physiologic and biochemical events described previously.

Metabolomics work by D'Alessandro et al has shown that hypoxic storage diminishes damaging mediators of oxidative stress and increases energy biomarkers without requiring novel additive ingredients[15]. The same group investigated the metabolomics of hypoxic storage in a two-arm cross-sectional clinical study. Hypoxic storage was found to ameliorate the metabolic phenotypes of stored RBCs (lower markers of oxidant stress such as lipids and purines and higher levels of high-energy phosphate compounds) and improve post-transfusion recovery (PTR). The study also correlated metabolic parameters to hemolysis and PTR, highlighting the role of purine and lipid oxidation (e.g., hypoxanthine and 9-HODE), so that these can be used as markers in fresh and stored units to predict end of storage blood quality[15].

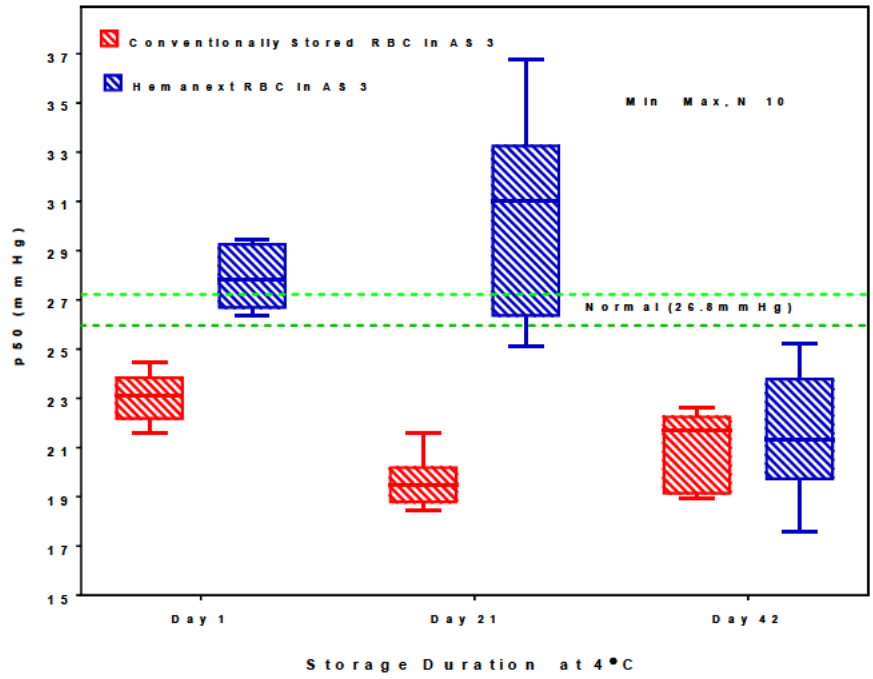
Improve Oxygen Delivery: The FDA Clinical Investigation CLIN-0001, which compared conventional blood to hypoxically stored blood at the end of storage (Day 42), demonstrated that hypoxically stored blood showed improvements in ATP, 2,3 DPG, and RBC deformability[32]. Deformability was evidenced by higher morphology scores in the hypoxic group as well as by higher flow rates by microvascular analysis[33], depicted in Figure 1.

Figure 1



The increases in 2, 3 DPG levels, which remained significantly higher than conventional blood throughout storage, contribute to a rightward shift of the O_2 dissociation curve (i.e., a higher p50), as illustrated by Whitley and colleagues below[34].

Figure 2



Reduce Resuscitation Volume/Time: A pre-clinical animal model of resuscitation from hemorrhagic shock with hypoxically stored blood demonstrated clear improvement over conventional blood resuscitation[35]. Hypoxically stored blood reduced the volume needed to achieve resuscitation by ~50% and reduced time to resuscitation. (The hypoxic group animals were resuscitated in 45 minutes compared to the conventional group animals who never reached the endpoint of 90% mean arterial pressure (MAP) after one hour) Lactate, a diagnostic and therapeutic indicator of shock and acidosis, was cleared faster, and markers of kidney damage (BUN, *u-NGAL)* were reduced with hypoxically stored blood. In summary, hypoxic storage dramatically improved resuscitation efficacy and reduced the volume capable of achieving resuscitation.

Decrease number of RBC transfusions: D'Alessandro et al[15] highlighted that Hemanext exceeded regulatory requirements for hemolysis and post-transfusion *in vivo* recovery (PTR24) by a significant margin. PTR24 values in healthy volunteers were statistically greater for Hemanext RBCs compared to control (90.2% vs 87.3%; $p < 0.05$)[15]. These data illustrate that hypoxic storage yields significantly more viable RBCs and the aforementioned PTR24 is the highest 24-hour recovery reported in the literature. The increase in viable RBCs supports a potential 15% reduction in blood requirements for chronic transfusion patients as well as potential reduction in RBC transfusions for acutely bleeding patients. Wendel et al have discussed the decrease in hemoglobin increment with RBC transfusions that occurs in patients with multiple co-morbidities who experience a systemic inflammatory response[36]. Burn patients, who are highly inflamed, may therefor experience even greater benefits from hypoxic blood.

Decrease Iron Overload: Transfusion iron overload is directly associated with the number of RBC units transfused[9]. Several studies illustrate that hypoxic storage yields more viable RBCs, as indicated by greater PTR24 values[5]. The increase in viable RBCs supports a potential reduction in blood requirements for chronic transfusion patients, potentially leading to lower transfusion density and decreased risk of iron overload.

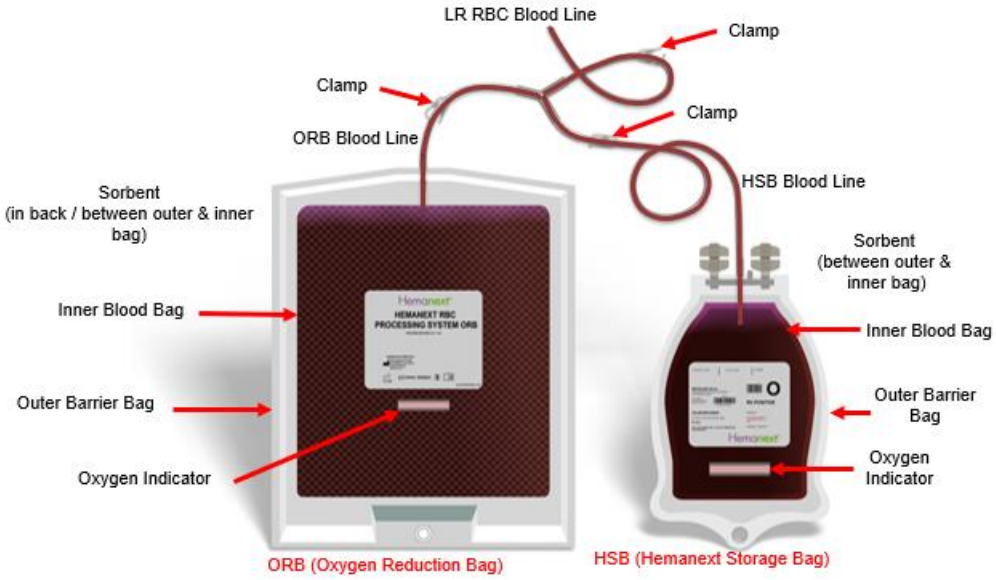
Summary: In burn patients, hypoxic RBC transfusions can be provided early in resuscitation if there is concomitant trauma, during excision and grafting, and during the post-op period of coagulopathic "oozing". Hypoxic blood presents the potential to alleviate oxidative stress, improve oxygen delivery, and improve resuscitation efficiency with fewer RBC transfusions. The clinical ramifications that may be observed include decreased organ dysfunction in these critically ill patients as well as reduction in thromboembolic events secondary to the improved deformability of the cells. In patients with hematological malignancies, hypoxic RBC transfusions can be provided in the management of anemia and resulting symptoms. Hypoxic blood presents the potential to alleviate oxidative stress and improve oxygen delivery with fewer RBC transfusions. The clinical ramifications that may be observed include decreased density of transfusions and decreased incidence of iron overload, which could improve outcomes for patients who receive chronic transfusions.

5.2 Identification and description of the Investigational Medical Device

Hemanext ONE is a blood container set used to process and store CPD/PAGGSM Red Blood Cells, Leukocytes reduced, and O_2/CO_2 Reduced. The Hemanext ONE system is an assembly of two disposables: the Oxygen Reduction Bag (ORB) and the Hemanext Storage Bag (HSB).

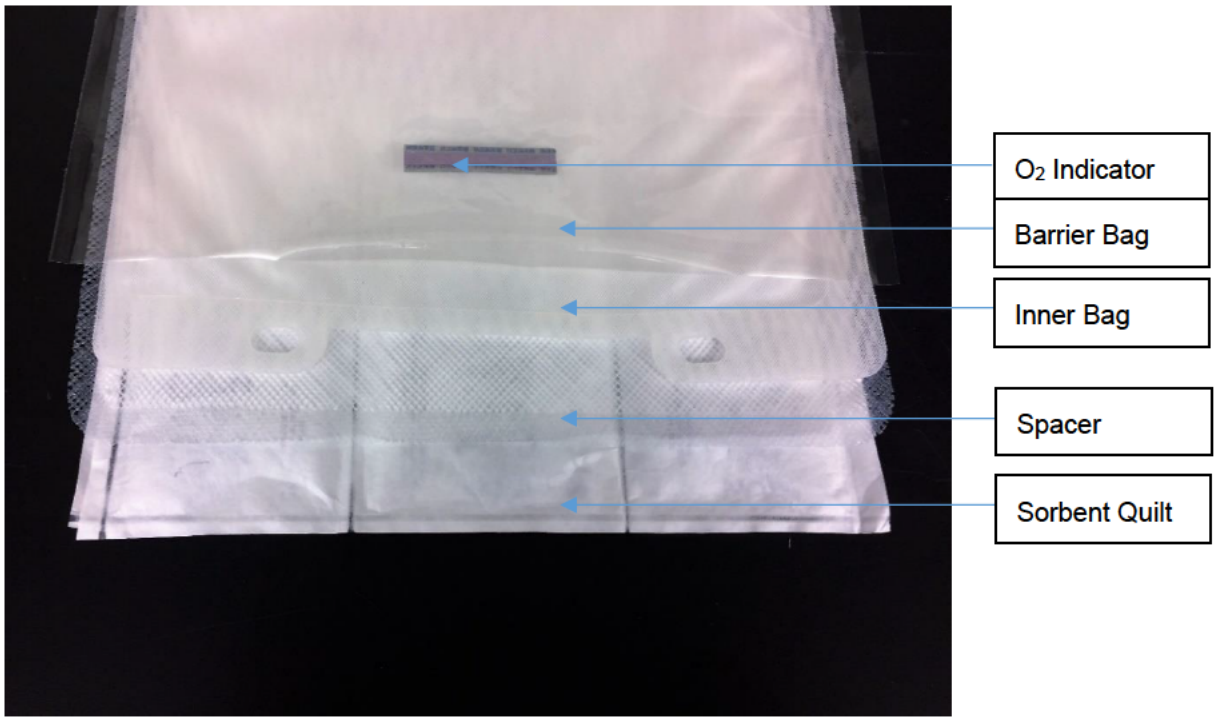
See Figure 3 below for the concept configuration. The larger bag is the ORB and the smaller bag is the HSB.

Figure 3: Hemanext ONE Processing and Storage System



The ORB consists of the following elements: (a) an inner bag; (b) outer barrier bag; (c) spacer and (d) quilt of 9 sorbent sachets. The inner bag holds the PAGGSM LR RBC and is housed in an outer barrier bag. The outer barrier bag ensures that there is no ingress of oxygen into the ORB during or post O_2 /CO_2 reduction. The inner bag is physically separated from the outer barrier bag by polyester fabric elements surrounding the outer surface of the inner bag. The materials of construction of the inner bag ensure that oxygen released from the LR RBC is transferred to the surrounding space. The sorbent quilt captures and stores oxygen released from the LR RBC. The spacer (perforated patterned polyethylene stabilizer) is placed in the space between the inner bag and the sorbent quilt to ensure that all of the surface area of the inner bag is clear and able to transfer oxygen. The oxygen indicator is designed to change color if exposed to oxygen. The ORB has tubing to allow sterile docking of the LR RBC bag (see Figure 4 below). The ORB is connected to the HSB bag via integral tubing. The connections facilitate the transfer of the oxygen reduced LR RBC blood.

Figure 4: ORB Assembly View



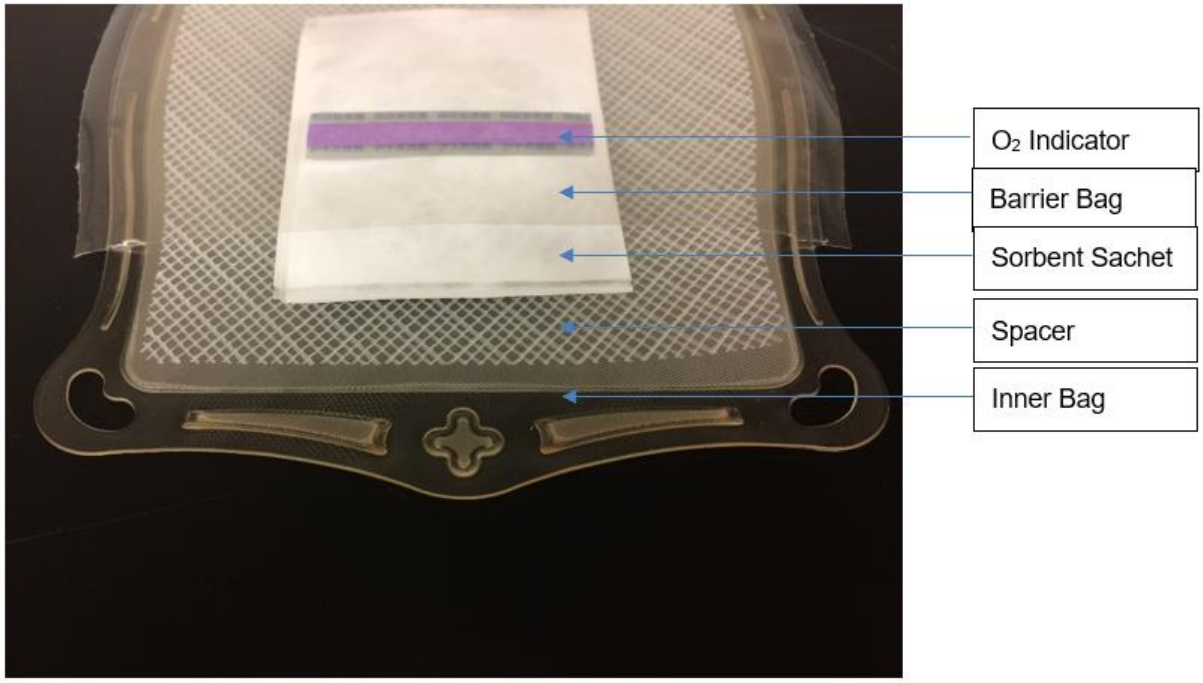
[redacted]

The HSB consists of the following elements: (a) inner bag; (b) barrier bag; (c) spacer and (d) single sorbent sachet. Materials of construction of the HSB ensure that it is capable of maintaining the appropriate low oxygen environment for additive stored LR RBC in refrigerated environments for 42 days.

The inner bag holds the blood and is housed inside the oxygen impermeable barrier bag.

A sorbent is placed in the space, between the inner and outer bags, to ensure that low oxygen levels are maintained post reduction by the HSB. See Figure 5 below.

Figure 5: HSB Assembly View



[redacted]

5.2.1 Description of Hemanext Process:

A LR RBC unit bag is connected using a sterile docking device to the ORB. The sorbent quilt between the inner bag and the barrier bag creates an O_2 /CO_2 depleted environment. LR RBC are then transferred by gravity to the ORB inner bag.

Once the full unit volume is in the ORB inner bag; the Hemanext system is transferred to a tray on an agitator (the platelet agitator should operate at 72 cycles/minute) at room temperature (22+2°C). Its contents are evenly distributed, creating a large thin layer of LR RBC contacting the inner surface of the ORB inner bag. The agitator moves in a side to side motion causing the LR RBC to travel within the inner bag in a wave-like motion at the same rate as the shaker, maintaining a constant agitation of RBC and additive solution.

The red blood cells in direct contact with the inner bag surface release oxygen through the material and into the O_2/CO_2 starved environment between the inner bag and barrier bag of the ORB. Maintaining this agitation of red blood cells and residual plasma/additive solution ensures that oxygen on the RBCs that are furthest from the walls of the bag exchange oxygen molecules with

low oxygenated solution closest to the bag surface, which in turn lose the gained oxygen by constant exposure to the oxygen-depleted environment on the outer side of the ORB inner bag.

The O_2/CO_2 absorption rate is characteristic of the oxygen reduction container material, exchange surface area, volume of RBC and agitation. At the end of the exposure time of 3 hours (+/- 15 minutes), the resulting deoxygenated LR RBC is transferred to the HSB in the kit. The HSB utilizes the same principles as the ORB to preserve a low oxygen and CO_2 environment within its contents. It utilizes a sorbent to maintain this environment and preserve the blood at low O_2 levels for its storage duration of up to 42 days. The HSB is also a transfusion ready device.

5.3 Preclinical Evidence

Study ID (Year Initiated)	Study Name	Purpose	Number of Subjects	Outcome
RESPR22 (2016)	**RESPR22:** OUS Solution Characterization with Hemanext ONE	The purpose of this work was to conduct an operational trial (feasibility study) of this new technology to mimic routine blood product processing and storage for RBC units stored in SAGM and PAGGSM. The data were useful for Hemanext to evaluate the performance of its device for the European market.	32 donors. 8 pools of four donors.	Hemanext ONE hypoxic RBC met all in vitro acceptance criteria. PAGGSM was the additive solution of choice for a CE mark trial.
RESPR27 (2016)	**RESPR27:** Hemanext ONE with 42-day Storage and in-vitro Characterization of Resultant Oxygen Reduced, Leukoreduced, Red Blood Cells in PAGGSM vs. Control.	The purpose of this pilot study was to generate data of the current design of Hemanext ONE (consisting of an oxygen reduction bag "ORB" and a Hemanext storage bag "HSB", 60" tubing, y-connector and 2 ratchet clamps) and Hemanext ONE process.	20 donors. 10 pools of two donors.	Hemanext ONE hypoxic RBC met all in vitro acceptance criteria.
CLIN-0002 (2017)	**CLIN-0002:** In Vitro Evaluation of the Hemanext ONE Red Blood Cell Processing System for Leukoreduced Red Blood Cells with CPD Anticoagulant and PAGGSM	The objective of this clinical study was to evaluate CPD/PAGGSM leukocyte-reduced RBC stored anaerobically for 49 days after deoxygenation with the Hemanext ONE	30 donors	The Hemanext ONE process passed all acceptance criteria for overnight room temperature hold with a CPD/PAGGSM set at 42 days.

	additive.	Storage System. In addition to hemolysis, a list of standard blood quality parameters were measured.		
CLIN-0001 (2019)	**CLIN-0001:** Clinical Investigation to Evaluate the Hemanext ONE Oxygen Reduction System for Leukoreduced Red Blood Cells with CP2D Anticoagulant and AS-3 Additive - Pivotal Trial	The primary endpoints that were evaluated in this clinical trial were RBC mass recovery, hemolysis and 24-hour in vivo red cell recovery.	100 donors in crossover design	The data generated in this study passed acceptance criteria to support the license of CP2D/AS-3 Leukocytes Reduced, Hypoxic Red Blood Cells under conditions of 12-hour room temperature hold.

5.4 Clinical Evidence to Date

Fourteen (14) participants received two separate infusions of approximately 10 mL of autologous radiolabeled RBCs during CLIN-0001. One of the infusions was with hypoxic RBCs in AS-3 produced using the Hemanext ONE process and the other transfusion was with conventional RBCs in AS-3. The hypoxic RBCs infused had an overall higher dual-label 24 hour *in vivo* recovery (mean: 89.3, SD: 5.81) than the conventional RBCs (mean: 85.8, SD: 6.12). None of the participants experienced any AE or SAE related to the use of the Hemanext ONE device.

5.5 Justification for the design of the clinical investigation

The Hemanext ONE System received its CE Mark in April 2021. The Hemanext ONE Post Market Clinical Evaluation Plan requires collection of safety and efficacy data for the device. This post market clinical evaluation will begin to inform on the safety of the blood product resulting from processing leukocyte reduced RBCs with the device.

The Hemanext ONE System may be used to manufacture hypoxic RBCs for any patient requiring a blood transfusion. This pilot investigation aims to study safety of hypoxic RBCs in 2 patient groups: acute burn and hematologic malignancies. Burn patients undergoing surgical excision routinely require large quantities of blood to be transfused. Those patients with hematologic malignancies often require regular transfusions.

Participants in the study will be transfused with 2 units of hypoxic blood during scheduled transfusion therapy visits. The remainder of the units required for transfusion will be drawn from traditionally processed red cells. Adverse events will be collected from the Screening visit through either discharge (burn patients), the subsequent transfusion (hematologic malignancies patient), or Day 28, whichever comes first.

Further study of the safety of hypoxic RBCs will include randomized comparator trials in several types of patients requiring blood transfusions such as surgical burn excision, hematologic malignancies, trauma, sickle cell disease and thalassemia.

5.6 Explanation for choice of comparator

Not applicable – no comparator will be used during this clinical investigation

5.7 Risk evaluation (Risk-to-Benefits rationale)

5.7.1 Potential Benefits

Hemanext has found that red cell degradation is O_2 dose dependent, and that if the O_2 concentration is reduced at the time of storage, it is possible to minimize degradation and optimize red cell quality[37]. Hemanext designed a product and process to manage a broad range of % of SO_2 values in transfused RBC for oxygen-management. This product moves the entire broad % SO_2 distribution starting with a mean of 52% ranging from 10-90% SO_2 to mean of less than 20%. This is achieved on Day 0 and the product is designed to maintain this figure for 42 days of storage[37]. Metabolomics results show improved energy and anti-oxidant status of RBC and reduced stress, and production of those agents associated with adverse events[11]. Hemanext technology may potentially improve outcomes for those patients dependent on RBC transfusions to survive by improving the following:

- Reducing transfusion volume
- Increasing transfusion interval
- Increasing hemoglobin increment
- Improving oxygen delivery
- Reducing iron overload
- Reducing sequelae of hemorrhagic shock and transfusion
- Improved quality of life

In summary, based on pre-clinical data, hypoxic red cells have the potential to improve patient outcomes and attenuate transfusion -sequelae. These improvements may be especially effective in patient populations who receive high volumes of RBCs (chronically or acutely). These patients comprise 50% of total RBC usage while only making up 20% of patients transfused[8,12].

5.8 Anticipated risks

5.8.1.1 Risks associated with the use of Hemanext ONE system

The current risk management report RMR-001 for the HEMANEXT ONE product concludes that all risks have been reduced as far as possible and no additional residual risks were identified requiring additional clinical evaluation. The documentation demonstrates that the potential benefits outweigh all residual risks.

As part of red blood cell processing and storage there are known risks which may include, but are not limited to risks related to biocompatibility, leak leading to biohazard exposure and or loss of sterility, exposure to endotoxin, hemolysis of red blood cells, and exposure of blood to non-biocompatible materials.

Based on the analysis of the benefit / risk assessment the documentation in RMR-001 supports the following conclusion:

The Hemanext ONE System was demonstrated to be substantially equivalent to commercial red cell products and to meet all requirements for red blood cell manufacture. Through product verification, in vitro, in vivo, and animal studies, the system has shown improved metrics for blood quality.

The potential clinical benefit(s) will need to be demonstrated in patients. The risks also remain equivalent to currently commercially available RBC.

5.8.1.2 Risks associated with RBCs transfusions (Non-exhaustive):

Transfusion of hypoxic RBCs has equivalent risk as transfusion of conventional RBCs, such as[38]:

- Transfusion-associated graft versus host disease
- Transfusion-related acute lung injury (TRALI)
- Post transfusion purpura
- Transfusion-associated circulatory overload (TACO)
- Transfusion-transmitted infection (TTI)
- Allergic reaction
- Transfusion-associated dyspnea (TAD)
- Febrile non-hemolytic transfusion reaction (FNHTR)
- Hypotensive transfusion reaction
- Delayed hemolytic transfusion reaction (DHTR)
- Delayed serologic transfusion reaction (DSTR)
- Acute hemolytic transfusion reaction (AHTR)
- Major cardiac event (MCE)
- Haematoma (bruise)
- Arterial puncture
- Delayed bleeding (re-bleeding)
- Nerve injury/irritation
- Localized infection/inflammation
- Deep venous thrombosis (DVT)
- Arteriovenous fistula
- Compartment syndrome
- Brachial artery pseudoaneurysm
- Vasovagal reactions

5.9 Justification of the choice of the investigation population

See sections 5.1.4, 5.1.5, and 5.1.6.

6. CLINICAL INVESTIGATION OBJECTIVES

6.1 Overall Objective

The overall objective of this study is to collect preliminary safety data on the transfusion of hypoxic RBCs, manufactured with Hemanext ONE device, in patients with burns and patients with hematological malignancies.

6.2 Primary Objective

Primary Objective – for both patients groups

Hypoxic RBCs safety and tolerance assessment up to 24 hours following the transfusion initiation and overall up to 7 days (+/- 1 day) after the transfusion episode (single transfusion course).

6.3 Secondary Objectives

Secondary Objectives :

A. For both patients groups

1. Assessment of pre and post transfusion hemoglobin levels
2. Assessment of hemoglobin level before the following transfusion, if applicable
3. Assessment of AEs occurence:
    i. Up to 7 days (+/- 1 day) post transfusion, in comparison with historical control (including but not limited to infection, deep vein thrombosis, acute respiratory distress syndrome, transfusion-related acute lung injury, transfusion associated circulatory overload, anaphylactic shock, acute haemolytic transfusion reaction).

    ii. Up to the following tranfusion episode or up to 28 days (+/- 1 day) after the initial transfusion, whichever comes first.

    iii. From enrollment, up to their subsequent transfusion or 28 days (+/- 1 day) posttransfusion, whichever comes first, through the assessment of patient's diary

4. Assessment of the vital signs during and up to 15 minutes after the transfusion

B. For burn patients group only:

1. Assessment of arterial blood gases:
    i. pre-operative
    ii. intra-operative
    iii. post operative

6.4 Safety Objectives

All safety objectives are addressed in the primary and secondary objectives.

7. CLINICAL INVESTIGATION OUTCOMES

7.1 Primary Outcome

Primary Outcome – for both patients groups

Number of participants who experienced an adverse event (all types/grades) within a time frame up to 24 hours following the transfusion and overall up to 7 days (+/- 1 day) after the transfusion.

The type and the grade of each adverse event will be categorized according to:

- Association for the Advancement of Blood and Biotherapies (AABB) technical manual, 20th edition (2020)
- Biomedical Excellence for Safer Transfusion (BEST) Collaborative review - Lancet 2016; 388: 2825–36
- Local AEs database (for reference)
- ISO 14155-2020 definitions

7.2 Secondary Outcomes

Secondary Outcomes : - for both patients groups

1. Evolution of the hemoglobin level before and after the transfusion.
2. Hemoglobin increment from transfusion corrected for patient blood volume and hemoglobin dose
3. Hemoglobin level before the following transfusion
4. Evaluate the frequency of all AEs, including transfusion related AEs up to 7 days (+/- 1) post-transfusion compared to historical data of patient registries (ex. The National Burn Registry)
5. Evaluate AEs up to 28 days after the initial transfusion, whichever comes first.
6. Evaluation the frequency of all AEs, from enrollment, up to 7 days (+/- 1 day) post transfusion, through the assessment of patient's diary, when applicable.
7. Evaluation of patient's vital signs over the course of the transfusion and up to 15 minutes post-transfusion

7.3 Other Outcomes of Interest

Not applicable.

7.4 Safety Outcomes

All safety outcomes are addressed in the primary and secondary outcomes.

8. CLINICAL INVESTIGATION DESIGN

8.1 General clinical investigation design and justification of design

This clinical investigation will be the first use of hypoxic blood manufactured with the Hemanext ONE device and is designed as a safety trial to detect any safety issues related to the hypoxic RBCs.. Subjects will receive two units of hypoxic RBCs.

8.2 Methods for minimising bias

Not applicable. This clinical investigation does not include a comparator.

8.2.1 Randomisation

Not applicable. This clinical investigation does not include a comparator.

8.2.2 Blinding procedures

Not applicable. This clinical investigation does not include a comparator.

8.2.3 Other methods for minimising bias

Not applicable. This clinical investigation does not include a comparator.

8.3 Unblinding Procedures (Code break)

Not applicable. This clinical investigation does not include a comparator.

9. CLINICAL INVESTIGATION POPULATION

9.1 Eligibility criteria

9.1.1 Inclusion Criteria

A. Hematological malignancies patients group:
   1. Patients ≥ 18 years of age
   2. Patients expected to require ≥ 2 unit of red blood cells in a single transfusion event
   3. Patients who have consented to participate
   4. Transfusion trigger less than 9 g/dL
   5. Diagnosis of leukemia, myelomatosis or MDS requiring chronic transfusions

B. Burn patients group:
   1. Patients ≥ 18 years of age
   2. Patients who have the capacity to consent by themselves to participate to the clinical investigation
   3. Smaller burn patients, hospitalized with a Total Body Surface Area (TBSA%) burn ≥ 10% less and ≤ than 50%
   4. Patients expected to require ≥ 2 unit of red blood cells in a single transfusion event

9.1.2 Exclusion Criteria – both patients groups

1. Patients with positive antibody screen
2. Patients who do not have the capacity to consent by themselves to participate to the clinical investigation
3. Patients with a known hemolytic anemia (congenital or acquired)
4. Patients for whom consent has not been obtained
5. Patients < 18 years old
6. Known or suspected pregnancy
7. Patients with a history of major transfusion reactions

Burn patients specific:

1. Total body surface area (TBSA%) burn more than 50%
2. Patients with combined trauma in need of blood transfusions other than the burn

9.2 Recruitment and screening

9.2.1 Hematological Malignancies Group

Participants will be recruited by the investigators from among the patients with hematological malignancies at the site who receive regular transfusions and who meet the inclusion/exclusion criteria. The purpose and procedures of the study will be explained to prospective participants and their written consent obtained prior to their taking part in the study. It will be emphasised to each prospective subject that, should they wish to withdraw from the study at any stage, they are free to do so without prejudicing their clinical care. Potential participants will be given the opportunity to ask any questions they may have on the design of the clinical investigation and what their participation will entail. Potential participant will be given sufficient time to decide whether they wish to participate in this clinical investigation. Once a patent has decided that they wish to participate in this clinical investigation they will be asked to sign an informed consent form.

9.2.2 Burn Group

Participants will be recruited by the investigators from among the burn patients at the site who will require transfusions and who meet the inclusion/exclusion criteria. The purpose and procedures of the study will be explained to prospective participants and their written consent obtained prior to their taking part in the study. It will be emphasised to each prospective subject that, should they wish to withdraw from the study at any stage, they are free to do so without prejudicing their clinical care.

Potential participants will be given the opportunity to ask any questions they may have on the design of the clinical investigation and what their participation will entail. Potential participants will be given the opportunity to ask any questions they may have on the design of the clinical investigation and what their participation will entail. Once a patent has decided that they wish to participate in this clinical investigation they will be asked to sign an informed consent form.

9.3 Assignment to investigation groups

Not applicable. There is only one group in this clinical investigation.

9.4 Criteria for withdrawal / discontinuation of subjects

A participant may be discontinued from the study for the following reasons:

1. Serious adverse events
2. Withdrawal of consent
3. Investigator decision
4. Death, any cause

Any participant that enrolled in the study can withdraw from the study, effectively withdrawing consent, at any time, for any reason, without prejudice or consequence. Participants are to be informed that they are free to leave the study at any time for any reason without prejudice or consequence in the informed consent document. If a participant withdraws from the study the data collected up through the time of withdrawal (including testing results) will be included within the study database. Withdrawn participants will be replaced in order to meet the sample size requirements of the study.

10. CLINICAL INVESTIGATION INTERVENTION

10.1 Identity of the medical device under investigation

All patients will receive one transfusion episode of at least two units of hypoxic RBCs that were manufactured with Hemanext ONE system.

See section 5.2 for details on the Hemanext ONE system.

See sections 11.1.1 and 11.1.2 for a detailed list of all information being collected.

10.1.1 Experimental Intervention (medical device)

Units of hypoxic RBCs will be manufactured at Oslo's University Blood Bank or Haukeland University Hospital's Blood Bank using Hemanext ONE system. The hypoxic RBCs manufactured in Oslo will be shipped to the Blood Bank at Haukeland University Hospital where they will be stored at 1-6°C for up to 42 days. Participants who would normally receive a transfusion of units of conventional RBCs will instead receive a transfusion of 2 units of hypoxic RBCs and any additional conventional RBCs as needed. All transfusions will be performed according to the site's SOPs.

10.1.2 Control Intervention (standard/routine/comparator)

Not applicable.

10.1.3 Labelling and Supply

The Hemanext ONE blood processing system (medical device) will be supplied by Hemanext to the Blood Banks after the study contract is executed. All devices will be supplied to the site before start of manufacturing.

The medical device will be a commercial product labelled with CE Mark. The hypoxic blood manufactured by Oslo's or Haukeland's Blood Bank will be labelled (on Hemanext Storage Bag / HSB) per local blood bank, Norwegian Directorate of Health and regulatory requirements, i.e. "Exclusively for Clinical Investigations".

See Appendix section for reference

Hemanext ONE blood processing system may not be used in any capacity until proof of CEC approval for the study have been received by the Sponsor and PI.

10.1.4 Storage Conditions

The Hemanext ONE kits must be stored at room temperature in a secure location. Any exposure of the kits to excessive heat or freezing must be avoided.

The hypoxic RBCs must be stored in cold storage at 1-6°C for up to 42 days.

10.2 Discontinuation or modifications of the intervention

Hemanext may suspend or prematurely terminate either a clinical investigation in an individual investigation site or the entire clinical investigation for significant and documented reasons. A Principal Investigator, DMC, CEC, or regulatory authority may suspend or prematurely terminate participation in a clinical investigation at the investigation sites for which they are responsible. If suspicion of an unacceptable risk to subjects arises during the clinical investigation, or when so instructed by the DMC, CEC, or regulatory authorities, Hemanext shall suspend the clinical investigation while the risk is assessed. Hemanext shall terminate the clinical investigation if an unacceptable risk is confirmed.

Hemanext shall consider terminating or suspending the participation of a particular investigation site or investigator in the clinical investigation if monitoring or auditing identifies serious or repeated deviations on the part of an investigator. If suspension or premature termination occurs, the terminating party shall justify its decision in writing and promptly inform the other parties with whom they are in direct communication. The Principal Investigator and Hemanext shall keep each other informed of any communication received from either the DMC, CEC, or the regulatory authority.

If, for any reason, Hemanext suspends or prematurely terminates the investigation at an individual investigation site, Hemanext shall inform the responsible regulatory authority as appropriate and

ensure that the CEC is notified, either by the Principal Investigator or by Hemanext. If the suspension or premature termination was in the interest of safety, Hemanext shall inform all other Principal Investigators.

If suspension or premature termination occurs,

- Hemanext shall remain responsible for providing resources to fulfill the obligations from the protocol and existing agreements for following up the subjects enrolled in the clinical investigation, and
- the Principal Investigator or authorized designee shall promptly inform the enrolled subjects at his/her investigation site, if appropriate.

When Hemanext concludes an analysis of the reason(s) for the suspension, implements the necessary corrective actions and decides to lift the temporary suspension, Hemanext shall inform the Principal Investigators, the DMC, the CECs, and, where appropriate, the regulatory authority of the rationale and provide them with the relevant data supporting this decision.

Concurrence shall be obtained from the CECs and, where appropriate, regulatory authorities before the clinical investigation resumes. If subjects have been informed of the suspension, the Principal Investigator or authorized designee shall inform them of the reasons for resumption.

10.3 Compliance with clinical investigation intervention

A participant being lost to follow-up will be the main risk of noncompliance in this study. If a participant is lost to follow-up, the investigator will take all necessary measures to re-establish contact with the participant to ascertain the reasons for discontinuation and the participant's state of health. The case report form (CRF) will be completed up to the participant's last visit.

10.4 Data Collection and Follow-up for withdrawn subjects

If a participant withdraws from the study, the investigator will take all necessary measures to establish contact with the participant to ascertain the participant's state of health. The case report form (CRF) will be completed up to the participant's last visit.

10.5 Clinical investigation specific preventive measures

There are no specific recommendations for preventative measures. All standard/routine measures will apply.

10.6 Concomitant Interventions (treatments)

There are no specific recommendations for concomitant interventions. All standard/routine interventions will apply.

10.7 Medical Device Accountability

The Blood Banks will be required to document 100% accountability of the Hemanext One device until all product provided has been used to process hypoxic RBCs, destroyed and/or returned to Hemanext. A "Study Product Receipt and Disposition Log" will be supplied by Hemanext in the Clinical Trial Binder at the Blood Banks for documenting each receipt and disposition (i.e., used, discarded or returned) of the medical device.

The hypoxic RBC product will be tracked via the Oslo and Bergen Blood Bank standard practice. The clinical site will record the hypoxic RBC product information in the Subject's source documents.

10.8 Return, Analysis or Destruction of the Medical Device

Unused Hemanext ONE medical device will be returned to the sponsor at the end of the trial or destroyed according to local procedures, unless other documented instructions are provided in advance to the clinical site.

In case of device deficiencies, including malfunction, usability issues, or inadequacy in the information supplied by the manufacturer including labelling, the devices will be returned to the sponsor for analysis.

11. CLINICAL INVESTIGATION ASSESSMENTS

The following information will be collected, from enrollment throughout end of study visit, when applicable:

1. Patient consent
2. Inclusion/Exclusion criteria
3. Demographics
4. Medical history
5. Physical examination
6. Pregnancy test (if applicable)
7. Height
8. Weight
9. RBC unit weight and volume
10. Age of the RCC unit at the time of the transfusion
11. Hemoglobin measurement - maximum 4 hours before the transfusion and at minimum 15 minutes before transfusion
12. Hemoglobin measurement 15 to 60 minutes after transfusion
13. Blood pressure every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion
14. Heart rate every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion
15. Body temperature every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion
16. Respiratory rate every 15 minutes during the transfusion and until 15 minutes after the end of the transfusion
17. Oxygen saturation every 15 minutes and until 15 minutes after the end of the transfusion
18. Patient diary for collection of concomittant medications and changes in health status from enrollment up to their following transfusion or 28 days (+/- 1 day) post-transfusion whichever comes first
19. Routine laboratory analyses, including blood gases when applicable.

11.1 Clinical investigation flow chart(s) / table of clinical investigation procedures and assessments

11.1.1 Hematogical Malignancies Group:

Investigation Periods	Screening & Enrollment Visit	Pre-Transfusion	Transfusion	Post-Transfusion	Follow Up 1	Follow-up 2	Follow-up 3
Visit	1	2	2	2	3	4	5
Time (hour, day, week)	D0	D1	D1	D1	D2 (≤ 24h)	D7 (+/- 1 day)	Day of subsequent transfusion or D28, whichever comes 1st
In- /Exclusion Criteria	x	x					
Patient Information							
Patient consent (ICF)	x						
Demographics	x						
Medical History	x						
Concomitant Medications	x	x	x	x	x	x	x
Physical Examination	x						x
Pregnancy Test	x						
Height	x						
Weight	x						
RBC Unit Weight		x					
Patient Hemoglobin		x		x			x
Blood Pressure	x	x	x	x			
Heart Rate	x	x	x	x			
Body Temperature	x	x	x	x			
Respiratory Rate	x	x	x	x			
Oxygen Saturation	x	x	x	x			
Transfusion			x				
Adverse Events, Adverse device effects	x	x	x	x	x	x	x
Review Device Integrity		x	x	x			
Patient Diary	x	x	x	x	x	x	

11.1.2 Burn patients Group:

Investigation Periods	Screening & Enrollment Visit	Pre-Transfusion	Transfusion	Post-Transfusion	Follow Up 1	Follow-Up 2	Follow-Up 2
Visit	1	2	2	2	3	4	5
Time (hour, day, week)	Day 0	Day 1	Day 1	Day 1	Day 2 (≤ 24 h)	Day 7 (+/- 1 day)	Day of subsequent transfusion or D28, whichever comes 1st
In- /Exclusion Criteria	x						
TBSA	x						
Patient Information							
Patient information and consent (ICF)	x						
Demographics	x						
Medical History	x						
Concomitant Medications	x	x	x	x	x		x
Physical Examination	x						x
Pregnancy Test	x						
Height	x						
Weight	x						
RBC Unit Weight		x					
Patient Hemoglobin		x		x			x
Blood Pressure	x	x	x	x			
Heart Rate	x	x	x	x			
Body Temperature	x	x	x	x			
Respiratory Rate	x	x	x	x			
Oxygen Saturation	x	x	x	x			
Transfusion			x				
Adverse Events, Adverse device effects	x	x	x	x	x		x
Review Device Integrity		x	x	x			

11.2 Assessments of outcomes

Patients will be monitored for adverse events throughout the entirety of their participation in the clinical investigation. Adverse events will be elicited from the patients, the medical record, and an adverse event diary, if applicable. Patients enrolled will undergo standard care for their disease/ illness as prescribed by the Investigators. All procedures will be performed in compliance with the site's established policies and work instructions. No additional assessments will be performed.

11.2.1 Assessment of primary outcome

Patients will be monitored for adverse events throughout the entirety of their participation in the clinical investigation. Investigators will monitor patients directly for adverse events during their transfusion visit. In addition, patients will be contacted by phone 24 hours and 7 days (+/- 1) after their transfusion visit to record any adverse events they have experienced since their transfusion visit. All adverse events will be recorded in the CRF and they will be assessed according to the instructions in section 12 of the CIP.

11.2.2 Assessment of secondary outcomes

For all secondary outcomes related to hemoglobin and vital sign measurements the sites will follow their standard practice.

For all secondary outcomes related to AE occurrence, investigators will monitor patients directly for adverse events during their transfusion visit and then patients will be contacted by phone 24 hours and 7 days (+/- 1) after their transfusion visit to record any adverse events they have experienced since their transfusion visit. The patient's chart will also be checked for any AE occurrence after their following transfusion visit or 28 days after their initial transfusion, whichever comes first. All adverse events will be recorded in the CRF and they will be assessed according to the instructions in section 12.

11.2.3 Assessment of other outcomes of interest

Not applicable.

11.2.4 Assessment of safety outcomes

See section 11.2.1 and 11.2.2 for information on assessment of safety outcomes.

11.2.4.1 Adverse events

See section 11.2.1 and 11.2.2 for information on assessment of adverse events.

11.2.4.2 Laboratory parameters

Local site staff will follow their standard procedures.

11.2.4.3 Vital signs

See section 11.2.2 for information on measurement of vital signs.

11.2.5 Assessments in subjects who prematurely stop the clinical investigation

All data from patients who withdraw/drop out of the study will be recorded up to and including their last visit before they withdraw their participation in the study.

11.2.6 Follow-up of the subjects after the regular termination of the clinical investigation

All follow up of participants will be completed before the termination of the clinical investigation.

11.3 Procedures at each visit

11.3.1 Patient Information Contact

A Patient Information Sheet will be provided to prospective study participants by the PI or his designees. No information will be collected from prospective study participants during this contact.

11.3.2 Screening Visit

The following data will be collected during this visit:

- Inclusion/exclusion criteria
- Patient/legal representative informed consent
- Demographics
- Medical history
- Physical examination
- Pregnancy test
- Height
- Weight
- Blood pressure
- Heart rate
- Body temperature
- Respiratory rate
- Oxygen saturation
- Adverse event occurrence
- Concomitant medications
- Patient Diary

11.3.3 Transfusion Visit

The patients will receive a transfusion during this visit. The following data will be collected:

- RBC unit weight
- Patient hemoglobin pre- and post-transfusion
- Blood pressure during the transfusion and for 15 minutes post-transfusion
- Heart rate during the transfusion and for 15 minutes post-transfusion
- Body temperature during the transfusion and for 15 minutes post-transfusion
- Respiratory rate during the transfusion and for 15 minutes post-transfusion
- Oxygen saturation during the transfusion and for 15 minutes post-transfusion
- Infection occurrence
- Adverse event occurrence
- Concomitant medications
- Device deficiencies
- Patient Diary

11.3.4 First Follow-Up Visit

This visit will occur 24 hours post-transfusion and will be performed over the phone. The following data will be collected:

- Infection occurrence
- Adverse event occurrence
- Concomitant medications
- Device deficiencies
- Patient Diary

11.3.5 Second Follow-Up Visit

This visit will occur 7 days (+/- 1) post-transfusion and will be performed over the phone. The following data will be collected:

- Infection occurrence
- Adverse event occurrence
- Concomitant medications
- Device deficiencies
- Patient Diary

11.3.6 Final Follow-Up

This visit will occur at the patient's subsequent transfusion visit or 28 days (+/- 1 day) after their transfusion, whichever comes first. All data from this visit will be collected from the patient's chart. The following data will be collected:

- Adverse event occurrence
- Physical examination
- Concomitant medications
- Pre-transfusion hemoglobin (if applicable)

12. SAFETY

12.1 Definition and Assessment of (Serious) Adverse Events and other safety related events

Adverse Event (AE) (Art. 2 Abs 57 MDR)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the medical device.

Serious Adverse Event (SAE) (Art. 2 Abs 58 MDR)

Any adverse event that led to any of the following:

(a) death,
(b) serious deterioration in the health of the subject that resulted in any of the following:
  (i) life-threatening illness or injury,
  (ii) permanent impairment of a body structure or a body function,
  (iii) hospitalisation or prolongation of patient hospitalisation,
  (iv) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
  (v) chronic disease,
(c) foetal distress, foetal death or a congenital physical or mental impairment or birth defect.

Note: planned hospitalization for pre-existing condition, or a procedure required by the CIP, without a serious deterioration of the health status of the subject, is not considered an SAE

Device deficiency (Art. 2 Abs 59 MDR)

Inadequacy of a medical device related to its identity, quality, durability, reliability, safety or performance, of an investigational device, including malfunction, user errors and inadequate information supplied by the manufacturer. Device deficiency data will be collected by the blood banks and the clinic staff.

The blood bank teams will use the Hemanext ONE System to manufacture hypoxic RBCs. The Oslo blood bank will ship the hypoxic RBCs to the Bergen blood bank team for storage. Bergen blood bank team will confirm blood bag integrity prior to storage, when ordered for a subject and after each transfusion.

Malfunction (ISO14155)

Failure of an investigational device to perform in accordance with its intended purpose when used in accordance with the instructions for use or the CIP.

Device deficiency with Serious Adverse Device Effect (SADE) potential (Art. 80 Abs 1 letter c MDR; ISO14155)

Any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate.

Adverse Device Effect (ADE) (ISO14155)

Adverse event possibly, probably or causally related to the use of an investigational device or procedures.

Serious Adverse Device Effect (SADE) (ISO14155)

Adverse device effect (ADE) that has resulted in any of the consequences characteristic of a serious adverse event.

Unanticipated Serious Adverse Device Effect (USADE) (ISO14155)

Serious adverse device effect (SADE) which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

Causal Relationship of SAE (MDCG 2020-10/1)

A causal relationship towards the medical device or the procedure of the investigation should be rated by the PI and the Sponsor as follows:

- **Not related:** The relationship to the device or procedures can be excluded.
- **Possible:** The relationship with the use of the investigational device is weak but cannot be ruled out completely. Alternative causes are also possible.
- **Probable:** The relationship with the use of the investigational device seems relevant and/or the event cannot reasonably be explained by another cause.
- **Causal relationship:** The serious event is associated with the investigational device or with procedures beyond reasonable doubt.

Investigational Medical Device:
A device that is assessed in a clinical investigation.
Note: An investigational device can be a non-CE marked device or a CE marked device. The definition is understood to cover also the devices investigated in post-market surveillance investigations.

Case Report Form (CRF): A printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject.

Serious Health Threat:
signal from any adverse event or device deficiency that indicates an imminent risk of death or a serious deterioration in the health in subjects, users or other persons, and that requires prompt remedial action for other subjects, users or other persons
Note: This would include events that are of significant and unexpected nature such that they become alarming as a potential serious health hazard or possibility of multiple deaths occurring at short intervals.

Unanticipated / Unexpected Serious Adverse Device Effect (USADE)

- According to ISO 14155:2020: Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current risk assessment.

12.2 Documentation and reporting in Medical Device clinical investigations

Device deficiencies and all **adverse events (AE)** including all **serious adverse events (SAE)** are collected, fully investigated and documented in the source document and appropriate CRF during the entire investigation period, i.e. from patient's informed consent until the last CIP-specific procedure, including a safety follow-up period.

- Documentation of AEs (including SAEs) by the PI includes diagnosis or symptoms, start and stop dates of event, event treatment, event resolution, assessment of seriousness and causal relationship to MD and/or investigation procedure (ISO14155).
- Documentation of DDs by the PI includes description of event, start date, investigational device information, action taken with regard to the investigational device, and whether the DD led to an AE. The Sponsor shall review all DDs and determine and document in writing whether they could have led to a SAE (DD with SADE potential) (ISO14155).

The Sponsor provides the CEC with the documentation at their request

12.3 Foreseeable adverse events and anticipated adverse device effects

There were no adverse device effects associated with the Hemanext RBC Processing System reported in the earlier in vivo pivotal human trial (cf. Investigator Brochure).

Well established blood transfusion complications (acute and delayed transfusion related effects) known to occur in medical practice using a conventional method of blood processing and storage will be considered as expected events. These effects are listed below[38]:

• Transfusion-associated graft versus host disease
• Transfusion-related acute lung injury (TRALI)
• Post transfusion purpura
• Transfusion-associated circulatory overload (TACO)
• Transfusion-transmitted infection (TTI)
• Allergic reaction
• Transfusion-associated dyspnea (TAD)
• Febrile non-hemolytic transfusion reaction (FNHTR)
• Hypotensive transfusion reaction
• Delayed hemolytic transfusion reaction (DHTR)
• Delayed serologic transfusion reaction (DSTR)
• Acute hemolytic transfusion reaction (AHTR)
• Major cardiac event (MCE)
• Haematoma (bruise)
• Arterial puncture
• Delayed bleeding (re-bleeding)
• Nerve injury/irritation
• Localized infection/inflammation
• Deep venous thrombosis (DVT)
• Arteriovenous fistula
• Compartment syndrome
• Brachial artery pseudo-aneurysm

12.4 Reporting of (Serious) Adverse Events, device deficiencies, and other safety related events

12.4.1 Reporting to the Sponsor:

The following events are to be reported to the Sponsor by the PI (or authorized designee) within 24 hours after becoming aware of the event:

- All SAEs
- Health hazards that require measures
- Device deficiencies

The Sponsor will evaluate SAEs with regard to causality and seriousness. Device deficiencies are also assessed regarding their potential to lead to an SAE (DD with SADE potential).

12.4.2 Reporting to the Competent Ethics Committee:

The following events are to be reported to the CEC promptly:

a. any serious adverse event which has a causal relation with the MD, comparator or procedure/test method or where a causal relation appears to be possible (SADE);
b. any device deficiency which, in the absence of appropriate measures or intervention or in less favourable circumstances, could have led to serious adverse events (DD with SADE potential);
c. any new information relating to an event already notified under points (a) and (b).

In order to ensure prompt notification, the Sponsor may initially submit an incomplete notification.

If safety and health hazards that require measures must be taken immediately during the conduct of the investigation, the Sponsor notifies the CEC within 2 days of these measures and the circumstances which made them necessary.

Periodic safety reporting:

An Annual Safety Report (ASR) is submitted by the Sponsor to the CEC, yearly. The ASR contains a list of all SADEs and DDs and a report on their degree of seriousness, causal relationship with the MD and procedure and on subjects' safety.

12.5 Follow-up of (Serious) Adverse Events

For serious adverse device event, and serious procedure related event the immediate report will be followed by detailed, written follow-up reports (using the SAE notification form found in the study site binder). Both the immediate and follow-up reports will identify subjects by unique code numbers assigned to the latter.

12.6 Documentation and reporting in Medical Device clinical investigations

Device deficiencies (DD) and all **adverse events (AE)** including all **serious adverse events (SAE)** are collected, fully investigated and documented in the source document and appropriate case report form (CRF) during the entire investigation period, i.e. from patient's informed consent until the last CIP-specific procedure, including a safety follow-up period.

- Documentation of AEs (including SAEs) by the PI includes diagnosis or symptoms, start and stop dates of event, event treatment, event resolution, assessment of seriousness and causal relationship to MD and/or investigation procedure (ISO14155).
- Documentation of DDs by the PI includes description of event, start date, investigational device information, action taken with regard to the investigational device, and whether the DD led to an AE. The Sponsor shall review all DDs and determine and document in writing whether they could have led to a SAE (DD with SADE potential) (ISO14155).

There were no adverse device effects associated with the Hemanext RBC Processing System reported in the earlier in vivo pivotal human trial (see Investigator Brochure).

Well established blood transfusion complications (acute and delayed transfusion related effects) known to occur in medical practice using a conventional method of blood processing and storage will be considered as expected events. These effects are listed below[38]:

- Transfusion-associated graft versus host disease
- Transfusion-related acute lung injury (TRALI)
- Post transfusion purpura
- Transfusion-associated circulatory overload (TACO)
- Transfusion-transmitted infection (TTI)
- Allergic reaction
- Transfusion-associated dyspnea (TAD)
- Febrile non-hemolytic transfusion reaction (FNHTR)
- Hypotensive transfusion reaction
- Delayed hemolytic transfusion reaction (DHTR)
- Delayed serologic transfusion reaction (DSTR)
- Acute hemolytic transfusion reaction (AHTR)
- Major cardiac event (MCE)
- Haematoma (bruise)
- Arterial puncture
- Delayed bleeding (re-bleeding)
- Nerve injury/irritation
- Localized infection/inflammation

- Deep venous thrombosis (DVT)
- Arteriovenous fistula
- Compartment syndrome
- Brachial artery pseudo-aneurysm
- Vasovagal reactions

12.7 Reporting of Safety related events

Reporting to the Sponsor:

All SAEs, device deficiencies and health hazards that require measures are reported to the Sponsor by the PI (or authorized designee) within 24 hours after becoming aware of the event. Device deficiencies are assessed regarding their potential to lead to an SAE. DD are assessed regarding their potential to lead to an SAE.

12.7.1 Reporting to the Competent Ethics Committee:

The Sponsor reports to the CEC promptly any serious adverse event which has a causal relation with the MD, comparator or procedure/test method or where a causal relation appears to be possible.

In order to ensure prompt notification, the Sponsor may initially submit an incomplete notification.

If safety and health hazards that require measures must be taken immediately during the conduct of the investigation, the Sponsor notifies the CEC within 2 days of these measures and the circumstances which made them necessary.

12.7.2 Periodic safety reporting:

An Annual Safety Report (ASR) is submitted by the Sponsor to the CEC, yearly. The ASR contains a list of all SADEs and DDs and a report on their degree of seriousness, causal relationship with the MD and procedure and on subjects' safety.

12.8 Procedure

12.8.1 Initiation of Adverse Event Reporting

The Clinical Project Manager or designee will train the investigator and staff regarding the recording and reporting of adverse events. The sponsor shall implement and maintain a system to ensure that the reporting of the reportable events will be provided by the investigator to the sponsor immediately, but not later than 3 calendar days after investigation site study personnel's awareness of the event.

Adverse events (AEs) are recorded on the CRF by the sites. The following information should be recorded:

- The description of the event with enough detail to determine if the event meets the definition of a serious adverse event
- The date of the onset of the adverse event
- Relevant medical history leading up to the event
- A description of the action to treat the adverse event
- The site will also classify the event using the scales below

The investigator shall determine the AE Severity using the following scoring system which will be included on the CRF. The severity score is necessary is used to determine if the event meets the definition of a serious adverse event. The rating may also be used to determine if an adverse device event is unanticipated. This may be the case if the severity is greater than previously identified.

Rating	Description	Definition
5	Catastrophic	Patient Death
4	Critical	Permanent impairment or life threatening injury
3	Serious	Injury requiring medical intervention to prevent permanent impairment
2	Minor	Temporary injury that does not require medical intervention
1	Negligible	Inconvenience or temporary discomfort

The relationship between the use of the medical device (including the medical – surgical procedure) and the occurrence of each adverse event shall be assessed and categorized.

During causality assessment activity, clinical judgement shall be used and the relevant documents, such as the Investigator's Brochure, the Clinical Investigation Plan or the Risk Analysis Report shall be consulted, as all the foreseeable serious adverse events and the potential risks are listed and assessed there. The presence of confounding factors, such as concomitant medication/treatment, the natural history of the underlying disease, other concurrent illness or risk factors shall also be considered.

The above considerations apply also to the serious adverse events occurring in the comparison group.

For the purpose of harmonizing reports, each SAE will be classified according to four different levels of causality:

1. Not related
2. Possible
3. Probable
4. Causal relationship

The sponsor and the investigators will use the following definitions to assess the relationship of the serious adverse event to the investigational device, the comparator or the investigation procedure.

1. Not related: Relationship to the device, comparator or procedures can be excluded when:
   - - the event has no temporal relationship with the use of the investigational device, or the procedures related to application of the investigational device;
   - - the serious adverse event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible;
   - - the discontinuation of medical device application or the reduction of the level of activation/exposure (when clinically feasible) and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious adverse event;
   - - the event involves a body-site or an organ that cannot be affected by the device or procedure;
   - - the serious adverse event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors);
   - - the event does not depend on a false result given by the investigational device used for diagnosis, when applicable;
   - In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event.

2. Possible: The relationship with the use of the investigational device or comparator, or the relationship with procedures, is weak but cannot be ruled out completely. Alternative causes are

also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases where relatedness cannot be assessed, or no information has been obtained should also be classified as possible.

3. Probable: The relationship with the use of the investigational device or comparator, or the relationship with procedures, seems relevant and/or the event cannot be reasonably explained by another cause.

4. Causal relationship: the serious adverse event is associated with the investigational device, comparator or with procedures beyond reasonable doubt when:
   - the event is a known side effect of the product category the device belongs to or of similar devices and procedures;
   - the event has a temporal relationship with investigational device use/application or procedures;
   - the event involves a body-site or organ that
     - the investigational device or procedures are applied to;
     - the investigational device or procedures have an effect on;
   - the serious adverse event follows a known response pattern to the medical device (if the response pattern is previously known);
   - the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious adverse event (when clinically feasible);
   - other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
   - harm to the subject is due to error in use;
   - the event depends on a false result given by the investigational device used for diagnosis, when applicable;

In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event.

The sponsor and the investigators will distinguish between the serious adverse events related to the investigational device and those related to the procedures (any procedure specific to the clinical investigation). An adverse event can be related both to procedures and the investigational device. Complications caused by concomitant treatments not imposed by the clinical investigation plan are considered not related. Similarly, several routine diagnostic or patient management procedures are applied to patients regardless of the clinical investigation plan. If routine procedures are not imposed by the clinical investigation plan, complications caused by them are also considered not related.

In some particular cases the event may not be adequately assessed because information is insufficient or contradictory and/or the data cannot be verified or supplemented. The sponsor and the Investigators will make the maximum effort to define and categorize the event and avoid these situations. Where an investigator assessment is not available and/or the sponsor remains uncertain about classifying the serious adverse event, the sponsor should not exclude the relatedness; the event should be classified as “possible” and the reporting not be delayed.

Particular attention shall be given to the causality evaluation of unanticipated serious adverse events. The occurrence of unanticipated events related could suggest that the clinical investigation places subjects at increased risk of harm than was to be expected beforehand. The site should determine and record if the AE was anticipated or unanticipated. If the risks for entering the study described in the informed consent / clinical brochure / clinical protocol or risk analysis reflect the nature, severity or degree of incidence of the AE, then it was anticipated. If not, then the AE is unanticipated.

12.8.2 Reporting Requirements

The following events are considered reportable events:

a) any serious adverse event that has a causal relationship with the investigational device, the comparator or the investigation procedure or where such causal relationship is reasonably possible;

b) any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate;

c) any new findings in relation to any event referred to in points a) and b).

All causality assessments should be made using the guidance in the section above. Only causality level 1 (i.e. “not related”) is excluded from reporting. If either the sponsor or the investigator has assigned a higher causality level than "not related", the event should be reported.

12.8.3 Reporting Timelines

The sponsor must report to the CEC and as applicable, to all NCAs where the clinical investigation is authorized to start:

- For all reportable events as described in section 6.4 which indicate an imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for other patients/subjects, users or other persons or a new finding to it: **Immediately, but not later than 2 calendar days after awareness by sponsor of a new reportable event or of new information in relation with an already reported event.**
    - This includes events that are of significant and unexpected nature such that they become alarming as a potential public health hazard. It also includes the possibility of multiple deaths occurring at short intervals. These concerns may be identified by either the NCA or the manufacturer.
- Any other reportable events as described in section 6.4 or a new finding/update to it: **Immediately, but not later than 7 calendar days following the date of awareness by the sponsor of the new reportable event or of new information in relation with an already reported event.**

12.9 List of foreseeable adverse events and anticipated adverse device effects, together with their likely incidence, mitigation or treatment

There are currently no adverse device effects associated with the Hemanext RBC Processing System reported in the earlier in vivo and pivotal human trial as reported in the Investigator Brochure. Hemanext RBC Processing System related potential Adverse Device Effects (including potential Serious Adverse Effects) may include, but are not limited to risks related to:

- Lack of sickle cell trait testing of RBC unit before processing and storage in the Hemanext RBC Processing System leading to high hemolysis and sickled red blood cells being transfused
    - A false negative on sickle trait testing of RBC unit before processing and storage in the Hemanext RBC Processing System leading to high hemolysis and sickled red blood cells being transfused
- Undetected leak in the HSB inner bag leading to a loss of sterility of the RBC unit. The product labeling is for fluid path sterility only.
- Use of a non-indicated additive solution with the Hemanext RBC Processing System leading to negatively affected quality of RBCs

The incidence is unknown. Mitigation of associated risk is achieved via continuous pharmacovigilance activities conducted during the study.

Well established blood transfusion complications (acute and delayed transfusion related effects) known to occur in medical practice using a conventional method of blood processing and storage will be considered as expected events. These effects are listed below[18]:

- Transfusion-associated graft versus host disease

- Transfusion-related acute lung injury (TRALI)
- Post transfusion purpura
- Transfusion-associated circulatory overload (TACO)
- Transfusion-transmitted infection (TTI)
- Allergic reaction
- Transfusion-associated dyspnea (TAD)
- Febrile non-hemolytic transfusion reaction (FNHTR)
- Hypotensive transfusion reaction
- Delayed hemolytic transfusion reaction (DHTR)
- Delayed serologic transfusion reaction (DSTR)
- Acute hemolytic transfusion reaction (AHTR)
- Major cardiac event (MCE)
- Haematoma (bruise)
- Arterial puncture
- Delayed bleeding (re-bleeding)
- Nerve injury/irritation
- Localized infection/inflammation
- Deep venous thrombosis (DVT)
- Arteriovenous fistula
- Compartment syndrome
- Brachial artery pseudo-aneurysm
- Vasovagal reactions

12.10 Assessment, notification and reporting on the use of radiation sources

Not Applicable.

13. STATISTICAL METHODS

13.1 Hypothesis

Study is not powered so there is no hypothesis in this clinical investigation.

13.2 Determination of Sample Size

10 burn patients and 10 patients with haematological malignancies, who require transfusion of red cell concentrate and who fulfil all eligibility criteria will be enrolled in this clinical investigation at Haukeland University Hospital.

A total of twenty patients will be sufficient to assess preliminary safety as the rate of transfusion related adverse events is usually low and if unexpected adverse events (any type or grade) should occur, this will be identified.

13.3 Statistical criteria of termination of the investigation

There are no statistical criteria for the termination of the investigation.

13.4 Planned Analyses

A descriptive analysis will be performed on all quantitative data: means and standard deviations will be calculated for quantitative data, medians and interquartile intervals for discrete data, performance and qualitative variables. A listing of all AEs will be provided.

13.4.1 Datasets to be analysed, analysis populations

Data from all patients will be included in the analysis. This includes data from patients who withdraw or drop out of the study up to and including their last visit.

13.4.2 Primary Analysis

This clinical investigation is not powered to support a quantitative analysis of the primary endpoint.

13.4.3 Secondary Analyses

The analysis of quantitative secondary endpoints will be limited to descriptive statistics.

13.4.4 Interim analyses

There will be no interim analysis of the study endpoints.

13.4.5 Deviation(s) from the original statistical plan

All deviations from the statistical plan will be reported and if necessary the statistical plan will be updated.

13.5 Handling of missing data and drop-outs

Investigators will attempt to contact any patients who received a transfusion during the investigation and who subsequently dropped out of the study in order to ensure that they did not experience any adverse events either in the 7 days (+/- 1) following their transfusion, or beyond 7 days (+/-1) until their following transfusion or 28 days post-transfusion, whichever comes first. Any patients who drop out of the investigation before receiving a transfusion will be replaced in order to meet the sample size requirements.

14. QUALITY ASSURANCE AND CONTROL

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must ensure that the CRFs are securely stored at the study site in encrypted electronic and/or paper form and are password protected or secured in a locked room to prevent access by unauthorized third parties.

The investigator must permit study related monitoring, audits, ethics committee review, and regulatory agency inspections and provide direct access to source data documents. This verification may also occur after study completion. It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

Monitoring details describing strategy, methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques, are provided in the monitoring plan.

The sponsor or designee is responsible for the data management of this study, including quality checking of the data.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ISO 14155:2020, ICH GCP, and all applicable regulatory requirements.

The investigator(s) will notify the sponsor immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with the sponsor to prepare the investigator site for the inspection and will allow the sponsor, whenever feasible, to be present during the inspection. The investigator site and investigator will promptly resolve any discrepancies that are identified between the study data and the participant's medical records. The investigator will promptly provide copies of the inspection findings to the sponsor or its agent. Before response submission to the regulatory authorities, the investigator will provide the sponsor or its agents with an opportunity to review and comment on responses to any such findings.

14.1 Data handling and record keeping / archiving

Records and documents pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor. The investigator must ensure that the records continue to be stored securely for as long as they are maintained.
When participant data are to be deleted, the investigator will ensure that all copies of such data are promptly and irrevocably deleted from all systems.

14.1.1 Case Report Forms

All original source data will be contained in original laboratory printouts, site laboratory notebooks, site data sheets and/or study-specific source documents. Data transmittal to the Sponsor will be accomplished using paper Case Report Forms (CRFs). Original source data will not be entered directly on the CRFs. Original data is to be transcribed from source data records to the CRFs by site study staff. Data entered onto the CRFs will then be verified against the source data during Sponsor on-site monitoring visits. At the study site visits the verified and corrected CRFs must be reviewed and

electronically signed by the respective Principal Investigator to finalize the data. Investigative site study data and records for this protocol are subject to monitoring or inspection by the Sponsor, and notified body.

14.1.2 Specification of source data and source documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator site.

Data reported on the CRF or entered in the CRF that are from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

14.1.3 Archiving of essential clinical investigation documents

See section 14.1.

14.2 Data management

For all subjects enrolled in the study, site staff will promptly complete and allow Hemanext and/or its designees' access to all case report forms (CRFs) required for the study in the form supplied by the Sponsor or its designee. Site staff will not be required to disclose information in CRFs which would permit identification of a subject enrolled in, or a candidate for, the study. CRFs will be provided to Hemanext or its designees in a medium specified by Hemanext or its designee. At the request of Hemanext or its designees, sites will promptly correct any errors and/or omissions to the CRFs for the study and will make available to the Sponsor and/or its designees the corrected CRFs and supporting records for further verification. Investigator agrees to sign/e-sign a statement in each subject's study records or CRF attesting to his/her review of the CRF and verifying that the information included on such form is accurate and includes the treatment, care, and events surrounding such subject's involvement in the study.

Sites will retain organized subject, laboratory, and study device inventory records relating to the study for the period of time required by applicable federal law or regulation. The site will not destroy such records without giving the Sponsor prior written notice and the opportunity to further store such records, at the Sponsor's cost and expense. Case Report Forms will be retained for a minimal period of 15 years after completion of the study. Clinical records kept by the Sponsor will be held in locked cabinet.

14.2.1 Data Management System

Refer to Data Management Plan.

14.2.2 Data security, access and back-up

Refer to Data Management Plan.

14.2.3 Analysis and archiving

Refer to Data Management Plan.

14.2.4 Electronic and central data validation

Refer to Data Management Plan.

14.3 Monitoring

Monitoring will be conducted by the sponsor or delegate according to clinical monitoring SOPs to assure that the protocol is followed, any adverse events are reported, data collected are scientifically sound, and that the study is conducted in compliance with ICH Good Clinical Practice, ISO, and MDR guidelines. On-site monitoring visits will occur at study initiation, during the study performance as needed (minimum one interim visit) and at the study close.

On-site monitoring visits will take place at each center at study initiation, during the course of the study (as long as there are patients in follow up at the site) at the frequency defined in the monitoring plan, and for the final visit at the close of the study. The initiation visit is intended to provide an opportunity for the monitor to review the Investigational Plan with the Investigators and to ensure that the Investigators:

1. have appropriate training, facilities, patient load, time, and willingness to comply with study requirements;
2. have the approval of the supervising CEC for the Investigational Plan;
3. have all study documentation and required records on site; and
4. assume responsibility for the investigation at their center.

Visits during the study are intended to assess Investigators' adherence to the Investigational Plan, maintenance of records, reports and investigational devices, and review of source documents for accuracy, completeness, and legibility. During these study visits, the monitor is required to assess the progress of the study toward meeting study objectives, and to identify any concerns that stem from observations of device performance and/or review of the Investigator's patient records, study management documents, and informed consent documents, and to ensure accountability of all patients that have been treated under the study.

The monitor's final on-site visit at completion of the study is intended to assure that all the data have been properly completed and to have a closing meeting with the Investigators and their staff members. Reports of the on-site visits will be made by the monitor and should include resolution of concerns, completion of appropriate follow-up activities, completion of assigned tasks, and any necessary corrective actions. At the close of the study, the monitor will prepare a final report.

14.4 Audits and Inspections

The sponsor retains the right to audit any investigational site for the duration of this clinical investigation.

All investigators do guarantee access to their site and source data for monitors, auditors, or inspectors of the competent administrative authority.

14.5 Confidentiality, Data Protection

All parties will comply with all applicable laws, including laws regarding the implementation of organizational and technical measures to ensure protection of participant data.

Participants' personal data will be stored at the study site in encrypted electronic and/or paper form and will be password protected or secured in a locked room to ensure that only authorized study staff have access. The study site will implement appropriate technical and organizational measures to ensure that the personal data can be recovered in the event of disaster. In the event of a potential personal data breach, the study site will be responsible for determining whether a personal data breach has in fact occurred and, if so, providing breach notifications as required by law.

To protect the rights and freedoms of participants with regard to the processing of personal data, participants will be assigned a single, participant-specific numerical code. Any participant records or data sets that are transferred to the sponsor will contain the numerical code; participant names will not be transferred. All other identifiable data transferred to the sponsor will be identified by this single, participant-specific code. The study site will maintain a confidential list of participants who participated in the study, linking each participant's numerical code to his or her actual identity and medical record identification. In case of data transfer, the sponsor will protect the confidentiality of participants' personal data consistent with the clinical study agreement and applicable privacy laws.

14.6 Storage of biological material and related health data

Not applicable.

No biological material and/or related health data from the participants will be stored for the purpose of this clinical investigation.

15. PUBLICATION AND DISSEMINATION POLICY

The results of this study may be published or presented at scientific meetings by the investigator after publication of the overall study results or 1 year after the end of the study (or study termination), whichever comes first.

The investigator agrees to refer to the primary publication in any subsequent publications, such as secondary manuscripts, and submits all manuscripts or abstracts to the sponsor 30 days before submission. This allows the sponsor to protect proprietary information and to provide comments and the investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any study or Hemanext intervention related information necessary for the appropriate scientific presentation or understanding of the study results.

For all publications relating to the study, the investigator will comply with recognized ethical standards concerning publications and authorship, including those established by the International Committee of Medical Journal Editors.

The sponsor will comply with the requirements for publication of the overall study results covering all investigator sites.

Authorship of publications for the overall study results will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

If publication is addressed in the clinical study agreement, the publication policy set out in this section will not apply.

16. FUNDING AND SUPPORT

16.1 Funding

As the sponsor of the study, Hemanext, Inc. will provide all the funding for this investigation.

16.2 Other Support

Not applicable.

17. INSURANCE

Insurance will be provided by the Sponsor. A copy of the certificate is filed in each investigation site file and the sponsor's Trial Master File.

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19. APPENDICES

19.1 **Investigator Brochure**

Document provided separately.

19.2 **Hemanext Storage Bag (HSB) labelling**

EXCLUSIVELY FOR CLINICAL INVESTIGATIONS

Sponsor : Hemanext Inc.

Clinical Investigation reference : CLIN-0012

Principal Investigator : Dr Stian Almeland, Aukeland University Hospital, Bergen (Norway)

19.3 **Hemanext ONE Instructions for Use**

IFU Reference: LBL-194-0057 revision E01