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Safety of hypoxic red blood cell administration in patients with transfusion-dependent hematological malignancies: An interim analysis

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ABSTRACT

Anemia is a common symptom of hematological malignancies and red blood cell (RBC) transfusion is the primary supportive treatment, with many patients becoming transfusion dependent. Hemanext Inc. (Lexington, MA, United States) has developed a CE mark certified device to process and store RBCs hypoxically – citrate-phosphatedextrose (CPD)/phosphate-adenine-glucose-guanosine-saline-mannitol (PAGGSM) RBCs, leukocytes-reduced (LR), O_2/CO_2 reduced – with the aim of improving RBC quality for transfusion. This interim analysis describes the first patients to receive hypoxic RBCs, administered as part of a pilot post-marketing study in Norway. The primary outcome was adverse events (AEs) within 24 h of transfusion initiation and overall up to 7 days (± 1 day) post-transfusion. Secondary outcomes included changes in hemoglobin levels post-transfusion. Five patients with hematological malignancies were included (80 % male, mean age 69.8 [SD ± 19.3] years). Prior to the study, patients had been receiving conventional RBC transfusions every two weeks. Patients received 2 units of hypoxic RBCs over 2 h without complication. One mild AE (rhinovirus) was reported two days post-treatment and was deemed unrelated to treatment. The mean ± SD pre-transfusion hemoglobin level was 7.7 ± 0.5 g/dL, evolving to 9.0 ± 0.9 g/dL following administration of hypoxic RBCs; an increase of 17 %. This interim analysis showed that transfusion with hypoxic RBCs processed with the CPD/PAGGSM LR, O_2/CO_2 reduced system was effective and well tolerated in patients with hematologic malignancies. The overall clinical program will assess whether the use of hypoxic RBCs can reduce transfusion interval versus conventional RBCs in patients requiring acute and chronic transfusions.

1. Introduction

Hematological malignancies, including myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), are blood disorders that present with cytopenias, which are instigated by mutations in hematopoietic stem cells [1]. These mutations lead to the proliferation of abnormal or 'dysplastic' myeloid blasts in bone marrow, as well as in peripheral blood [2]. MDS is often a precursor to secondary AML, which develops in around 30 % of patients [3]. In MDS, dysplastic blasts displace mature cells in the blood stream. This leads to lower counts of platelets, causing major bleeding; leukocytes, compromising the immune system; and red blood cells (RBCs), causing anemia [1]. Many patients are, therefore, transfusion dependent, requiring regular RBC transfusions [4]. Although a necessary treatment for many patients with MDS, regular transfusion is associated with many complications, such as transfusion-related adverse events (AEs) and iron overload [4]. Furthermore, patient quality of life (QoL) may be negatively affected by frequent transfusions [5].

Hypothermic storage has been shown to cause both metabolic and oxidative damage to RBCs, which is referred to as the storage lesion [6]. Hemanext Inc. (Lexington, MA, United States) has developed a CE mark certified device to process and store hypoxic RBCs –

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citrate-phosphate-dextrose (CPD)/phosphate-adenine-glucose-guanosine-saline-mannitol (PAGGSM), leukocytes reduced (LR), O_2/CO_2 reduced. Hypoxic processing aims to improve the safety and efficacy of transfusion therapy by allowing storage without the need for novel additives. Hypoxic storage has been shown to improve energy and redox metabolism of RBCs [7]. In animal models, it was demonstrated that lower volumes of hypoxic RBCs were sufficient to resuscitate from hemorrhagic shock and maintain hemodynamics versus conventional RBCs and resulted in a lower incidence of organ injury [8]. A crossover study with blood donated by 100 healthy volunteers compared the percentage of RBCs recovered at the end of storage (Day 42) from conventionally and hypoxically stored RBCs. Findings showed that post-transfusion in vivo recovery values were greater in hypoxically stored RBCs than in conventionally stored RBCs (89.3 % versus 85.5 %; $p < 0.05$) [9]. The larger number of viable RBCs in hypoxically stored blood may particularly benefit those who require frequent RBC transfusions by increasing the interval between transfusions. Use of hypoxic RBCs over standard RBCs may also reduce the release of free iron into circulation and, therefore, iron overload. This interim safety analysis describes the administration of hypoxic RBCs to five patients with hematological malignancies as part of a pilot postmarketing surveillance study.

2. Methods

2.1. Study design and patients

Patients received hypoxic RBCs as part of a pilot clinical postmarketing surveillance study in Norway investigating the safety of single administrations of hypoxic RBCs. The study was carried out according to the clinical investigation plan and principles of the Declaration of Helsinki, the European Regulation on medical devices 2017/745 (MDR), the Norms ISO14155 and ISO14971, the International Council for Harmonization Guidelines of Good Clinical Practice, as applicable, and local regulatory authority requirements. The study protocol was approved by Regionale Komiteer for Medisinsk og Helsefaglig Forskningsetikk (REK), Southeastern Norway. Potential study patients were provided with a patient information sheet prior to the screening visit and written informed consent was provided by each patient prior to any investigation procedures.

The study protocol included plans to administer hypoxic RBCs to 10 patients with hematological malignancies and 10 patients with burns; the study design is detailed in Fig. 1. The present report details the results of a scheduled interim safety analysis of the first five hematological malignancy patients to receive hypoxic RBCs. Patients were included in the hematological malignancies group if they were aged ≥ 18 years with a diagnosis of leukemia, multiple myeloma or MDS requiring chronic transfusions and had a hemoglobin (Hb) level ≤ 9 g/dL requiring ≥ 2 units of RBCs in a single transfusion event. Patients were excluded if they had any positive antibody screening test, a known hemolytic anemia (congenital or acquired), known or suspected pregnancy, or had a history of major transfusion reactions.

2.2. Study assessments

Demographic and clinical characteristics were recorded for each patient at enrollment.

All patients received one transfusion episode of two units of hypoxic RBCs that were produced at the Oslo University Hospital Blood Bank using the CPD/PAGGSM LR, O_2/CO_2 reduced system and stored at 1–6 °C for up to 42 days. Participants who would normally have received a transfusion of conventional RBCs were instead given a transfusion of two units of hypoxic RBCs. No standard premedication was administered prior to transfusion. All transfusions were performed according to the site's standard operating procedures.

Vital signs were measured every 15 min during the transfusion and until 15 min post-transfusion. Patients were provided with a diary for collection of concomitant medications and changes in health status from enrollment to 28 days post-transfusion. Investigators monitored patients directly for AEs during their transfusion visit and patients were contacted by telephone 24 h and 7 ± 1 days after their transfusion visit to record any AEs experienced since their transfusion visit (Fig. 1). Each patient returned to the clinic for a final visit at 28 ± 1 days or just prior to their subsequent transfusion, whichever occurred first.

Blood samples were obtained at a maximum of 4 h and a minimum of 15 min pre-transfusion, then 15–60 min post-transfusion, as well as at study exit. Hematological laboratory parameters measured included Hb, white blood cells, neutrophils, monocytes, thrombocytes, reticulocytes, mean corpuscular volume, ferritin and creatinine.

2.3. Study outcomes

The primary outcome of the study was the number of participants who experienced an AE (all types/grades) up to 24 h following the transfusion initiation and overall up to 7 days (± 1 day) after the transfusion.

Secondary outcomes included assessment of Hb levels pre- and post-transfusion and before the subsequent transfusion, if applicable; AE occurrence up to 7 days (± 1 day) post-transfusion compared with historical data published in local registries; AEs up to 28 days post-transfusion or until the subsequent transfusion, whichever occurred first; AEs reported in patient diaries from enrollment up to 28 days post-transfusion or until the subsequent transfusion, whichever occurred first; and assessment of vital signs during the transfusion and up to 15 min post-transfusion.

For all secondary outcomes related to Hb and vital sign measurements, the site followed their standard practice. For all secondary outcomes related to AE occurrence, the above procedure was followed. In addition, the patient's chart was also checked for any AE occurrence.

2.4. Statistical analysis

To compare patient characteristics, descriptive statistics were calculated. The safety analysis was conducted by calculating the incidences of AEs. All patients were included in the safety set.

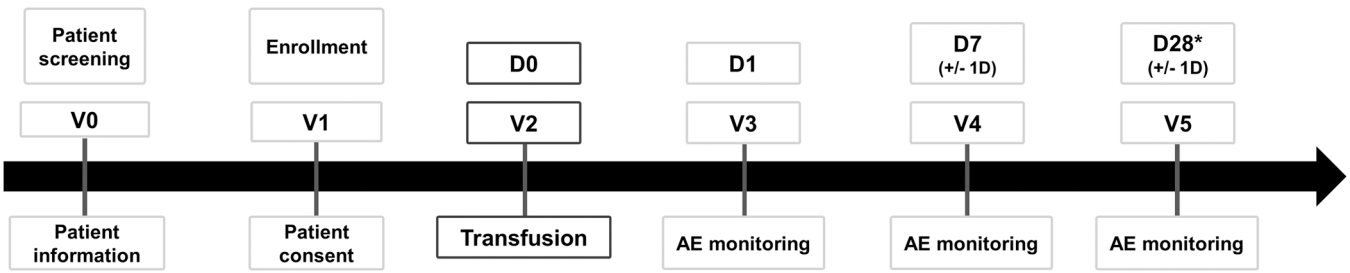

Fig. 1. Study design. *Day 28 ± 1 day or next transfusion episode, whichever happens first.AE, adverse event; D, day; V, visit.

3. Results

3.1. Patients

Between 30 August 2022 and 30 November 2022, five patients with hematological malignancies were enrolled and included in this interim analysis. No patients were excluded from the safety analysis. The baseline characteristics of patients are shown in Table 1. The mean ± standard deviation (SD) age was 69.8 ± 19.3 years, and 80 % were male. Mean ± SD body mass index was 24.8 ± 3.4 kg/m^2. Three patients (60 %) had a primary diagnosis of MDS, two with ring sideroblasts and one with a del5q mutation. Of the two remaining patients, one had a primary diagnosis of AML, while the other had primary myelofibrosis. The mean ± SD time since diagnosis was 6.6 ± 5.3 years. The mean ± SD pre-transfusion Hb level was 7.7 ± 0.5 g/dL and patients were transfused with a mean ± SD of 478.0 ± 20.5 mL of hypoxic RBCs with a mean ± SD age of 20.8 ± 12.3 days.

3.2. Adverse events

One AE (rhinovirus) was recorded within 7 days of the transfusion (primary endpoint), occurring two days post-treatment. This event was mild in severity and was deemed unrelated to the treatment. The event resolved prior to study exit 28 days post-transfusion. No AEs occurred within 28 days of the transfusion (secondary endpoint) outside of the above-mentioned event. There were also no occurrences of the other secondary endpoint (AEs reported in patient diaries), and given the limited data, no comparison with local registries was performed for this interim safety analysis.

3.3. Hemoglobin levels

The mean ± SD Hb level evolved from 7.7 ± 0.5 g/dL prior to the transfusion to 9.0 ± 0.9 g/dL post-transfusion, representing an increase of 17%. Patients had a subsequent transfusion a mean ± SD of 22.8 ± 6.4 days after the first transfusion, at which point the mean ± SD Hb level was 8.2 ± 1.7 g/dL.

3.4. Laboratory findings and vital signs

Blood test values pre-transfusion and at study exit are shown in Table 2. Although most values remained similar during this time period, there was an increase in mean white blood cell count and a reduction in the mean reticulocyte count; however, these were not clinically significant. No changes in vital signs were noted during the course of the study.

4. Discussion

This interim safety analysis in five patients with hematological malignancies showed that transfusion with hypoxic RBCs processed with the CPD/PAGGSM LR, O_2/CO_2 reduced system was effective and well tolerated in patients with hematological malignancies. No treatment-related adverse events were reported up to 28 days after transfusion and there were no changes in vital signs. Mean Hb increased by 17 % as a direct result of the transfusion, with levels remaining higher than pre-transfusion levels at subsequent transfusion. The majority of the other blood test parameters remained similar from pre-transfusion to study exit, although mean white blood cell count increased and mean reticulocyte count decreased during this period. However, the increase in white blood cells was predominantly based on one patient with an already elevated white blood cell count, and the reduction in reticulocytes was affected by one patient whose pre-transfusion count was well above the reference value, but whose reticulocyte count was not determined at study exit.

Table 1
Baseline characteristics.

Variable	
Age, years, mean ± SD	69.8 ± 19.3
Sex, n (%)	
Male	4 (80.0)
White, n (%)	5 (100.0)
BMI, kg/m^2, mean ± SD	24.8 ± 3.4
Time since diagnosis of hematological malignancy, years, mean ± SD	6.6 ± 5.3

BMI, body mass index; SD, standard deviation.

Table 2
Blood test before first transfusion and at study exit.

Parameter	Pre-transfusion	Study exit	Reference value
Hemoglobin, g/dL	7.7 ± 0.5	8.2 ± 1.7	13.4–17.0
WBC, cells x 10^9/L	25.0 ± 44.0	35.9 ± 65.6	4.1–9.8
Neutrophils, cells x 10^9/L	1.6 ± 0.7[a]	1.1 ± 0.6[a]	1.8–6.9
Monocytes, cells x 10^9/L	0.5 ± 0.1[b]	0.4 ± 0.3[a]	0.3–0.9
Thrombocytes, cells x 10^9/L	102 ± 69	94 ± 74	145–348
Reticulocytes, cells x 10^{12}/L	1.61 ± 3.10	0.07 ± 0.04[a]	0.03–0.10
MCV, fL	97 ± 8	98 ± 8[a]	82–98

Values are mean ± SD.
MCV, mean corpuscular volume; SD, standard deviation; WBC, white blood cells.
[a] n = 4;
[b] n = 3.

Hematological malignancies, such as MDS, are increasing in prevalence in Norway as the population average age continues to increase [10]. MDS are heterogeneous myeloid disorders characterized by inefficient hematopoiesis, which leads to peripheral blood cytopenias [11,12], primarily anemia [13]. RBC transfusion alleviates the symptoms of anemia, which include fatigue and weakness, and is a major component of supportive care for patients with MDS [14]. Patients with transfusion-dependent MDS require frequent use of hospital outpatient services to receive RBC transfusions [15] and, in intensive treatment, RBC transfusions may be given at weekly intervals [16]. Frequent transfusions are associated with a heavy burden on patients, including transfusion reactions [4], reductions in QoL [5] and iron overload [4], which can be fatal [17]. There are also substantial cost implications for healthcare systems [18], and evidence to suggest that increased transfusion density reduces overall survival [19].

The primary therapeutic goal of RBC transfusion is to increase the oxygen-carrying capacity and prevent tissue hypoxia [20]. The introduction of additives allows RBCs to be stored for up to 42 days before transfusion [21], but there are several disadvantages associated with this length of storage. Evidence suggests that patients who receive RBCs at the upper limit of this time frame have an increased risk of morbidity and mortality due to a high storage lesion burden [6,21]. There are also significant changes in the plasma levels of hemolytic markers, oxidized purines, plasticizers, and oxidized lipids [22] and, over time, the deformability of RBCs also diminishes, which affects perfusion in the microcirculation [23].

Within our transfusion service, RBCs range from 14 to 20.5 days old; A+ and O+ RBCs are most commonly used and these have an average age of 14–15 days old. Although the hypoxic RBCs administered in the present study were of a similar age (20.8 ± 12.3 days old), hypoxic storage confers biochemical advantages that may translate into clinical benefits. Hypoxic storage of RBCs reduces oxidative stress and has been shown to counteract some metabolic impairments, reducing the need for novel additive ingredients [24,25]. Hypoxic storage has also been shown to improve post-transfusion recovery over conventionally stored RBCs, and the use of hypoxically stored blood may improve oxygen delivery [9]. Additionally, hypoxically stored blood may decrease the required number of RBC transfusions [9]. Therefore, this reduction in blood requirements may lead to lower transfusion dose density and decreased

risk of iron overload [24,26] in patients with hematological malignancies.

The CPD/PAGGSM LR, O_2/CO_2 reduced system is a blood container set used to process and store hypoxic RBCs for any patient requiring a blood transfusion. A clinical investigation demonstrated that the CPD/PAGGSM LR, O_2/CO_2 reduced system exceeded United States regulatory requirements for hemolysis and post-transfusion in vivo recovery (PTR24) by a significant margin, and that hypoxic storage yields more viable RBCs than conventionally stored RBCs [9]. This increase in viable RBCs supports a potential 15% reduction in blood requirements for transfusion-dependent patients [9] and will be investigated in a future randomized, prospective trial.

This pilot postmarketing surveillance study aims to collect preliminary safety data on the transfusion of hypoxic RBCs, produced with the CPD/PAGGSM LR, O_2/CO_2 reduced system, in two patient groups: acute burn and hematological malignancies. This interim safety analysis has demonstrated that hypoxic RBCs are well tolerated in patients with hematological malignancies, and five additional subjects with hematological malignancies will subsequently be enrolled and transfused. Safety and tolerability of hypoxic RBCs in 10 patients with burn injury will then be studied and reported in addition to a summary report of the full study cohort. Further studies investigating the efficacy of hypoxic RBC administration in patients with thalassemia in Italy, MDS in Germany, and sickle cell disease in the US are planned.

The overall clinical program will aim to establish if there is a benefit to transfusion interval with hypoxic RBCs, as well as any improvements in other outcomes, including patient QoL and reduction in ferritin levels over time in patients who require chronic RBC transfusion compared with conventional RBCs.

5. Conclusion

This interim safety analysis showed that transfusion with hypoxic RBCs processed with the CPD/PAGGSM LR, O_2/CO_2 reduced system was effective and well tolerated in patients with hematological malignancies. Hypoxic storage yields more viable RBCs than conventional storage, facilitating a potential reduction in blood requirements for transfusion-dependent patients, and thus reducing the associated healthcare and cost burden.

CRediT authorship contribution statement

Håkon Reikvam: Conceptualization, investigation, writing – reviewing and editing; **Geir Hetland**: Resources, writing – reviewing and editing; **Farshid Ezligini**: Resources, writing – reviewing and editing; **Kim Dorsch**: Conceptualization, methodology, writing – reviewing and editing; **Laurel Omert**: Conceptualization, methodology, writing – reviewing and editing; **Andrew Dunham**: Formal analysis, writing – reviewing and editing; **Stian K Almeland**: Conceptualization, methodology, investigation, writing – reviewing and editing.

Role of the funding source

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Conflicts of interest

HR, GH, FE and SA declare no conflicts of interest. KD, LO, and AD report employment with and stock in Hemanext, Inc.

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