Ex vivo Production of Human Red Blood Cells From Hematopoietic Stem Cells: What Is the Future in Transfusion?

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There is constant difficulty in obtaining adequate supplies of blood components, as well as disappointing performance of stabilized or recombinant hemoglobin, limited indications of oxygen transporters (perfluorocarbons), and slow development of "universal" red blood cells (RBCs). There is, therefore, a need for complementary sources of RBCs for transfusion. Thus, an attempt to generate erythroid cells in vitro makes good sense. We describe in this article a methodology permitting the massive ex vivo production of mature human RBCs having all the characteristics of native adult RBCs from hematopoietic stem cells of diverse origins: blood, bone marrow, or cord blood. This protocol allows both the massive expansion of hematopoietic stem cells/progenitors and their complete differentiation to the stage of perfectly functional mature RBCs. The levels of amplification obtained (10^5 to 2 × 10^6) are compatible with an eventual transfusion application. We discuss in this article the state of the art of this new concept and evoke possible obstacles that need to be overcome to pass from a laboratory model to clinical practice. We analyze its possible indications in the medium and long term, discuss the economic aspects, and raise the question: Can we afford the luxury of developing this approach, one that could represent a considerable advance in blood transfusion?

TRANSFUSION HAS FOR a long time been subject to the constraint of blood group compatibility being delivered as a single whole blood product, which one can collect and store for a prolonged period of several weeks.1,2 The use of blood components, made possible by the introduction of plastic materials for blood collection,3 thus marked the first step toward a certain personalization of transfusion therapy. The diverse forms of blood donation yield today 3 principal blood components: red blood cell (RBC) concentrates, platelet concentrates, and therapeutic plasma. There are many possible ways of preparing these blood components under conditions adapted to the needs of the patients and satisfying the increasingly stringent safety requirements.4 Will this ever-increasing sophistication be maintained in the coming years? The necessity to prevent risks of immunization and dangers of infection and the obligations of quality and efficacy push us to do so. Will it be possible?

Two approaches are apparent today: (i) a standard blood cell product able to meet all the requirements of emergency transfusion and (ii) a "personalized" product allowing one to resolve or avoid impossible transfusion situations.

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doi:10.1016/j.trmrv.2006.11.004
Whatever the challenge, the need for making available complementary sources of RBCs for transfusion is evident in a context of the constant difficulty in obtaining adequate supplies, lack of availability of certain phenotypes, disappointing performance of stabilized or recombinant hemoglobin (Hb), limited indications of oxygen transporters (perfluorocarbons), and slow development of universal RBCs made compatible with the ABO blood group system and/or the RH1 antigen by enzymatic treatment or antigenic masking. Consequently, an attempt to generate erythroid cells in vitro through amplification of hematopoietic stem cells (HSC) makes good sense.

In this context, the objective is threefold: (i) to induce the massive proliferation of HSC to reach quantities equal to those provided by a standard RBC concentrate (about 2 x 10^12); (ii) to obtain complete terminal maturation to the stage of mature functional RBCs; and (iii) to establish industrial production conditions compatible with transfusion requirements and the norms of Good Manufactory Practice.

We will analyze here the state of the art of this new concept, evoke the obstacles that need to be overcome to pass from the laboratory model to clinical practice, and envisage the middle- and long-term interest of what could represent a considerable advance in blood transfusion. We will discuss the economic aspects and raise the question: Can we offer ourselves the luxury of developing a new approach to transfusion?

**AVAILABILITY AND ENGINEERING OF HSC**

The development of methods of selecting immature hematopoietic progenitors and our knowledge of growth factors specifically targeting certain cell lines already make possible the ex vivo production of progenitors and HSC for grafting purposes. Cytapheresis and mobilization with granulocyte colony-stimulating factor (G-CSF) enable the easy collection of large quantities of CD34+ progenitors. Umbilical cord blood (CB) presents the advantage of being particularly rich in immature progenitors that have a greater expansion capacity. In the future, it should be possible to maintain human embryonic stem cells in culture and induce their differentiation in vitro and their expansion to provide an inexhaustible source of HSC.

Whatever their origin, these HSC could be induced to complete in vitro their maturation into functional, enucleated RBCs.

**STATE OF THE ART: A STUDY MODEL TO PROVE THE CONCEPT**

One of the major characteristics of human RBCs is that they are the only cells that have a long life span (120 days) despite the absence of a nucleus. The mechanisms of enucleation are suspected but have not been formally established because of the lack of experimental conditions permitting the massive ex vivo production of RBCs. Such conditions must in fact satisfy 3 requirements: (i) the massive amplification of primitive HSC, (ii) the controlled induction of exclusive differentiation to the erythroid line, and (iii) the completion of terminal maturation to the stage of enucleated cells.

We initially described a protocol for the expansion of HSC derived from CB in a well-defined medium and without stroma, based on the sequential addition of growth factors. Starting from CD34+ cells, this protocol allows the massive production (amplification up to 200,000 times) of pure erythroid precursors (95%-99%) containing fetal Hb. Contrary to what happens under these ex vivo conditions in the presence of growth factors alone, when such progenitors/precursors are injected into non-obese diabetic–severe combined immunodeficient (NOD-SCID) mice, they are capable of continuing to proliferate in vivo and of differentiating within 4 days to the terminal stage of enucleated cells producing adult Hb. This points to a major role of the microenvironment in terminal erythroid differentiation.

Adult human hematopoiesis is achieved in vivo through a dynamic production process situated in the bone marrow, starting from a minor population of HSC and proceeding according to a cellular hierarchy based on a pyramidal model (stem cell, progenitor, and mature cell compartments), in close contact with the microenvironment. Stromal cells play a decisive role in the secretion of soluble regulatory factors and as cells of the extracellular matrix. Contacts between cells and soluble activatory or inhibitory factors are key elements in the regulation of hematopoiesis.

On the basis of these data, we have modified our protocol to obtain the expansion and differentiation of CD34+ cells derived from blood, bone marrow, or CB in 3 steps: (i) in a liquid medium,
involving cell proliferation and induction of erythroid differentiation in the presence of stem cell factor, interleukin-3, and erythropoietin (Epo); (ii) based on a model reconstitution of the microenvironment (murine stromal cell line MS5), in the presence of Epo alone; and (iii) in the presence of the stromal cells alone, without any growth factors. This cell culture system in a well-defined medium without serum reproduces ex vivo the microenvironment existing in vivo.19

Because of this protocol, we obtained by day 15 a plateau of the mean amplification of CD34+ cells of 16,000-fold (up to 25,000) for cells from bone marrow or peripheral blood, 29,000-fold (up to 34,000) for cells obtained by leukapheresis after mobilization with G-CSF, and 140,000-fold (up to 280,000) for cells derived from CB (Fig 1).

A commitment to the erythroid lineage is morphologically evident by day 8 (95%-98% erythroblasts). The subsequent terminal differentiation is rapid, as the percentage of enucleated cells is only 1% to 5% on day 11 but 65% to 80% by day 15. At this stage, 98% ± 1% of the cells are reticulocytes with a mean cell volume of 130 ± 5 fl, a mean corpuscular hemoglobin concentration of 18 ± 1%, and a mean cell hemoglobin of 23 ± 1 pg.

Differentiation of the reticulocytes into mature RBCs continues from day 15 to day 18, as shown by the further disappearance of nuclei, progressive loss of transferrin receptor CD71 expression, and staining with laser dye styryl (LDS). At this stage, 90% to 100% of the cells are enucleated. These erythrocytes display characteristics close to those of native RBCs, namely a mean cell volume of 113 ± 3 fl, a mean cell hemoglobin of 33 ± 2 pg, and a mean corpuscular hemoglobin concentration of 29 ± 2%. The cell yield on day 18 with respect to day 15 is
77% ± 5% with a mean reticulocyte content of 18% ± 4%. This complete differentiation of vastly expanded precursors into a pure erythroid lineage is because of the targeted proliferative induction of erythroid progenitors (burst-forming unit-erythroblasts [BFU-E] and colony-forming unit-erythroblasts [CFU-E]) and occurs at the expense of granulomacrophagic progenitors. All progenitors subsequently disappear between days 8 and 11.

Interestingly, the total cell expansion attained during the first step in the presence of growth factors alone is directly related to the duration of culture. When this is prolonged for 3 additional days, the level of expansion increases dramatically to reach $2 \times 10^5$, $1.20 \times 10^5$, or $1 \times 10^5$-fold for

![Graph A](image1.png)

**Fig 2.** A, Carbon monoxide rebinding after flash photolysis of hemoglobin (Hb) from cultured RBCs (cRBCs) (gray curves with circles) and Hb from control native RBCs (black curves). The 2 samples show similar binding properties, including the allosteric transition. By varying the energy of the photolysis pulse, one can vary the total fraction of dissociated Hb and thereby probe in detail the various partially bound populations. At high photolysis levels, more singly bound tetramers are produced, which switch to the deoxy conformation (T-state) and rebind ligands more slowly. At intermediate levels (medium), one can analyze in detail the partially bound populations. At low photolysis levels, one can better probe the doubly bound tetramers, a form difficult to study by equilibrium techniques. At sufficiently low levels, the main photoproduct is triply bound tetramers that rebind ligands rapidly (R-state). B, Oxygen dissociation curve at 25°C.

![Graph B](image2.png)

![Graph C](image3.png)

**Fig 3.** Flow cytometric follow-up of CFSE-labeled cRBCs in the NOD-SCID mouse model. A, Kinetics of expression of CFSE/LDS markers in cells from peripheral blood of the mice. Horizontal axis indicates CFSE detection; vertical axis, LDS detection. Quadrant statistics are shown on each dot plot. B, On day 3, the cells were colabeled with a phycoerythrin (PE)-conjugated anti-Rh D antibody (solid histogram) or its control isotype (open histogram). Results are expressed in terms of the percentage of Rh in CFSE+ cells.
CB, peripheral blood/leukapheresis, or bone marrow products, respectively, with preservation of the terminal differentiation after steps 2 and 3 (70%-91% enucleated cells).

Functional Reticulocytes and RBCs

The reticulocytes and RBC generated ex vivo have a glucose-6-phosphate dehydrogenase content of 42 ± 1.4 units and a pyruvate kinase level of 83 ± 1.8 units/g of Hb, in keeping with the properties of a young homogenous red cell population. This indicates that they are capable of reducing glutathione and maintaining ATP levels, thus allowing a normal level of 2, 3-diphosphoglycerate. The deformability of these reticulocytes and RBCs, as evaluated by ektacytometry, is comparable to that of native erythrocytes.

The functionality of the Hb of cultured RBCs (cRBCs) is assessed by ligand-binding kinetics after flash photolysis. The bimolecular kinetics after photodissociation of carbon monoxide (CO) provides a sensitive test of Hb function. On varying the energy of the photolysis pulse, 2 phases are observed that correspond to the 2 Hb conformations (R and T states). The kinetics is thus biphasic, reflecting the 2 allosteric forms. Like native Hb, cRBC Hb is able to fix and release oxygen. Oxygen equilibrium measurements confirm the observed affinity and cooperativity. The log(P50) value is 1.2 for cRBC Hb as compared to 1.3 for control RBC Hb, and the Hill coefficients are identical (N50 of 2.28 vs 2.29) (Fig 2). The kinetic and equilibrium data therefore indicate ligand-binding properties in very close agreement with control values. Methemoglobin is not detected, which shows that cRBCs are enzymatically capable of reversing Hb oxidation.

In Vivo Fate of Cultured Human RBCs

After intraperitoneal infusion into NOD-SCID mice, CFSE-labeled cRBCs and reticulocytes obtained by apheresis persist in the circulation to the same extent as carboxyfluorescein diacetate succinimidylester (CFSE)-labeled native RBCs: CFSE+ cells are detected for 3 days in both groups of transfused animals. In vivo, the transfused reticulocytes fully mature into RBCs as shown by the appearance of CFSE+/LDS– cells: 36% ± 5% and 63% ± 7% on days 1 and 2, respectively. Strikingly, over 90% of the CFSE+ cells are mature RBCs on day 3 (Fig 3).

At this stage of development of the model, it has thus been established that it is possible to produce functional human RBCs by culture of HSC.

THE IMPERATIVES OF A PRODUCTION MODEL COMPATIBLE WITH CLINICAL REQUIREMENTS

The ultimate and decisive challenge is to design production procedures compatible with the requirements of Good Manufacture Practice. A certain number of obstacles have to be overcome: (i) to diminish by a factor of 100 the quantity of medium necessary during the culture phases by using a medical bioreactor continuously adapting the culture conditions to the cell amplification in a constant volume; (ii) to identify the soluble factor(s) inducing enucleation and hence dispense with the cellular microenvironment; (iii) to propose a rigorously defined culture medium containing no animal proteins; and (iv) to add the cytokines sequentially by means of the bioreactor, which will increase their efficacy while reducing the quantities necessary.

TRANSFUSION PERSPECTIVES OF CULTURED ERYTHROID CELLS

This technology allows the massive generation ex vivo, from HSC, of either erythroid precursors or functional RBCs. Hence, 2 transfusion products are conceivable:

Transfusion of Erythroid Precursors

Apart from the fact that the precursors obtained on day 10 can be frozen and do not lose their proliferative capacity, several elements are in favor of their possible clinical use: (i) the erythroid precursors produced in vitro in 10 days (amplification 1000-fold) could continue their proliferation (100-fold) and terminal maturation in vivo; (ii) there is no concomitant amplification of contaminating maternal cells; (iii) CD19+ B and CD3+ T lymphocytes are undetectable; (iv) only very low levels of HLA class I and HLA-DR molecules are expressed by a small percentage of cells (1.4% and 3.4%, respectively); and (v) the quantity of residual leukocytes in the amplified product on day 10 is 3 to 50 times less than in a non-deleukocyted standard product.

Transfusion of Mature RBCs

Considering on the one hand the levels of cell amplification obtained (2 × 10^5- to 2 × 10^6-fold...
for apheresis and CB with 90% to 100% maturation) and on the other hand a mean quantity of 2 to \(8 \times 10^6\) CD34+ cells/kg in an apheresis donation or 2 to \(5 \times 10^6\) CD34+ cells in a CB unit, it is the equivalent of 2 to 6 RBCs concentrates that one can theoretically produce by this procedure. As for the use of embryonic stem cells, it would allow the preparation of an almost unlimited quantity of red cell concentrates.

This type of product is of course better suited for clinical use than the precedent because, first, it permits perfect control of the dosage of the transfusion, and second, it may be considered a "pure" RBCs concentrate, in any case free of the usual contaminants such as leukocytes, platelets, and plasma.

Universal RBCs

This culture system offers a new approach to the search for universal RBCs, that is, red cells lacking membrane expression of the principal blood group system, ABO. Here, it is no longer a question of trying to eliminate the surface antigens once they have formed, but a matter of preventing their synthesis before the RBCs reach maturity. The blood group antigens ABO which are not expressed on HSC, are already present on erythroblast precursors.

Two approaches may be envisaged:

- Inhibition of gene expression in CD34+ human HSC through the use of interfering RNA (siRNA). This technique enables post-transcription inhibition of a gene in a sequence-specific manner by using double-stranded RNA to provoke degradation of the homologous messenger RNA. Such inhibition of the expression of genes has been partially achieved using antisense oligonucleotides or ribozymes, but the approach is limited by the instability of the molecules introduced.

- Biochemical intracellular inhibition of the glycosyltransferases specific for the antigens A and B.

Whatever the mechanism, this inhibition has to be initiated at the stage of CD34+ HSC and continued to that of RBCs. The methods of this approach can avoid the side effects inherent to the procedures of stripping\(^7\) or masking\(^8\) currently being tested.

**REFLECTIONS ON THE POSSIBLE INDICATIONS OF THESE NEW LABILE BLOOD PRODUCTS**

**Conventional Transfusion Applications**

Without pretending to replace "classical" transfusion, these products could at least find indications in the context of impossible transfusion situations. Such situations are encountered in the 2 circumstances of rare erythrocyte phenotypes and anti-erythrocyte polyimmunization. Moreover, certain patients dependent on blood transfusion from a very early age, like those suffering from hemoglobinop-thies and notably thalassemia, could also benefit from these products. Finally, one can further evoke the case of transfusion in intensive care.

**Rare phenotypes.** A rare phenotype is characterized by the absence of an antigen of high frequency on RBCs. Such individuals can develop antibodies against these antigens either spontaneously (subjects lacking antigen H of the ABO blood group or subjects of phenotype p of the P blood group) or after initial allogeneic stimulation during pregnancy or transfusion (anti-Vel antibodies in Vel negative subjects).

Any person with a rare phenotype must receive concentrates of identical rare RBCs. These RBC concentrates are stored frozen in small amount in Blood Banks of Rare Phenotypes. However, depending on the frequency, the reserves are not always sufficient to satisfy the demand.

In our experience of blood transfusion in France, this is notably the case for rare specificities found solely in the Afro-Antillean populations, such as certain rare phenotypes of the RH system (RH: \(-18\); RH: \(-34\)) or the blood group characterized by absence of expression of the frequent antigen MNS5 (U). The problem is magnified because repeated transfusions can be necessary among these populations, notably in patients with sickle cell disease.

One could therefore propose for these patients the constitution of stocks of rare blood obtained either from adult donors of the same rare phenotype or from CB units conserved within the framework of a bank of CBs of rare phenotype. Generally, when an individual having a rare phenotype is identified, a family study is performed to determine among other
things the possibility of finding among the relatives other individuals carrying the same particularity who would be susceptible to give blood. It would thus be entirely feasible to extend this study to a search for persons susceptible to give HSC from the blood or placenta.

**Polyimmunization.** Situations of polyimmunization constitute a second application. The case of a subject immunized against a large number of antigens resembles that of rare blood groups insofar as the blood units compatible with all the antibodies produced are few. In this situation, it would be unrealistic to look for donors presenting an antigen combination compatible with the patient and to ask them to give HSC. Conversely, one could envisage producing RBCs from the patient’s own CD34+ cells, as the patient is the best potential donor in this case.

**Hemoglobinopathies.** The idea of transfusing patients suffering from thalassemia with RBCs having a prolonged life span is an old one. Earlier studies, which made use of the fact that young RBCs have a lower density than mature or senescent RBCs, collected apheresis products that had a half-life (measured by 51Cr labeling) of 44 days as compared with 28 days for conventional RBCs concentrates. However, this technique proved to be disappointing in routine practice was thus abandoned.

The ex vivo preparation of RBCs would enable us to transfuse a cell population homogeneous in age whose life span should be close to 120 days, as compared with the mean half-life of 28 days of RBCs collected from a donor, due to the simultaneous presence of RBCs of variable age. This would reduce the number of transfusions and alleviate the inevitable iron overload, a major complication in multitransfused subjects, particularly in thalassemic patients.

**Patients transfused in intensive care.** During the conservation of RBCs, storage lesions become progressively apparent. Red blood cell concentrates are generally stored for 10 to 25 days, or even up to 50 days, before being transfused and require a delay of about 24 hours after their transfusion to recover a normalized oxygen transport function.

Red blood cell concentrates are often necessary for the management of patients in intensive care and likewise in the course of certain surgical interventions. When these patients need transfusion, they require functional RBCs as quickly as possible. Several recent studies suggest that the storage lesions can have a deleterious effect in patients transfused under these circumstances.

Use of RBCs prepared in culture under completely standardized conditions and metabolically stabilized before delivery would permit a considerable reduction of storage lesions, which increase progressively during the conservation of classical RBC concentrates. Hence, one may predict that the oxygen transport functions of these RBCs would be immediately available to the receiver. If it were shown that the storage lesions of the RBCs have a direct impact on the morbidity and mortality in intensive care and given the great difficulty of managing the stocks of RBC concentrates so as to provide products conserved for less than 7 days, the use of RBCs obtained by culture could represent a serious alternative.

**Impact on the adverse effects linked to conventional transfusion.** The suspension of cRBCs is free of leukocytes, which is of interest in that it improves the storage at 4°C of the cells because of the absence of cytokines released by senescent leukocytes and diminishes the residual risk of anti-HLA alloimmunization, at least equally as well as deleukocytation procedures.

Similarly, the use of RBCs prepared in culture should lead to a reduction of the risks linked to the presence of plasma and notably the risk of transfusion-related lung injury (TRALI), whatever the mechanism of this complication of classical blood transfusion.

**Other Possible Indications**

**Possible role in the management of sickle cell disease.** The ex vivo synthesis of hemoglobin F by cells derived from CB is related to the culture conditions because the erythroblast progenitors/precursors obtained after 10 days of culture in the absence of a microenvironment give rise in vivo, after transfusion into NOD-SCID mice, to mature RBCs containing 96% functional hemoglobin A with complete modulation of hemoglobin F (ratio 'YA'/YG of 35:65). This model is thus a new
tool to investigate the cellular and molecular mechanisms of the Hb switch.

Stimulation ex vivo of the expression of hemoglobin F in subjects with drepanocytosis could represent an interesting therapeutic approach. This activation of hemoglobin F should diminish the polymerization of hemoglobin S. One could thus propose an autologous transfusion product modified ex vivo so as to specifically amplify the synthesis of hemoglobin F.

A new medicinal vector. As RBCs have by nature an ideal biodistribution and no longer divide, such properties could be exploited to use these cells as a new type of therapeutic vector. One may cite as an example the possibility of loading cRBCs with a chemotherapeutic agent widely used in the treatment of juvenile acute leukemia, L-asparaginase. The important toxicity of this compound when it is administered by direct intravenous injection can be greatly reduced by incorporating it into RBCs, which will deliver it progressively to the organism.31

This new concept of a transporter with a finite life span and diffusion throughout the body has a considerable range of potential applications. The progenitors could likewise be genetically manipulated before the induction of erythroid differentiation to produce, for therapeutic purposes, cytoplasmic or membrane proteins having a deliberately limited duration of action.

PRODUCTION COSTS PROHIBITIVE TODAY. ....BUT TOMORROW?

Over and above the purely technological challenge of the massive production of RBCs, the veritable industrial challenge is incontestably that of the return from such an approach. If one considers the present costs of the growth factors and CD34+ selection kits, the complexity of the production of serum-free culture media, and the considerable volumes required, it is clear that cRBCs may appear to be a technological luxury.

The question is nevertheless not only raised in these terms. On the one hand, any technological innovation—including in medicine—should be analyzed in terms of the potential “market.” On the other hand, in the area of blood transfusion, it is important to foresee not only the future needs in labile blood products but also the future availability of donors.

Potential Market for cRBCs

At present, the annual requirements for RBC concentrates are of the order of 40 to 50 per 1000 inhabitants in Europe and the United States, respectively,32,33 or about 30 million RBC concentrates transfused to 6 million patients for a population of 700 million inhabitants.

If one makes the conservative hypothesis of 1% of patients in an impossible transfusion situation and/or dependent on transfusion, like thalassemic subjects, who could benefit from cRBCs, then it is more than 300,000 transfusions per year in Europe and the United States that are of immediate concern. In public health, this is...
without comparison with any other indication for cell therapy.

**Demographic Evolution and Blood Transfusion**

The demographic evolution predicted in the United States indicates an important increase in the proportion of people older than 60 years, estimated to be about 18% in 2000, 20% in 2010, 25% in 2030, and 26% in 2050. An even more rapid aging of the population is anticipated in France, where the percentage of persons older than 60 years will pass from 21% in 2004 to 23% in 2010, 29% in 2030, and 32% in 2050.

This aging of the population will have 2 important consequences for blood transfusion:

- On the one hand, it will lead to a considerable increase in malignant hematologic pathologies like, for example, acute myeloblastic leukemias, chronic myeloid leukemias, and non-Hodgkin lymphomas, the frequency of which increases with age and which have important requirements for transfusion. Hence, on the same bases as before and assuming that the incidence of these pathologies as a function of age remains stable, one may predict that their global incidence will increase by about 60% in France and 100% in the United States from now to 2050.

- On the other hand, the number of persons of age to give blood will rise much more slowly than the needs for blood components. Still, considering the demographic projections established for the United States and France, one may deduce that the population of an age to give blood will increase by only 6% in France and 35% in the United States from now to 2050, whereas the general population will grow by, respectively, 16% and 49%. These data are summarized in Figure 4.

Thus, the notion of high cost may perhaps be analyzed differently in the event of difficulty in obtaining supplies due to the lack of availability of donors. Thus, in the medium and long term, RBCs produced by culture could represent an answer satisfying the clinical requirements.

Finally, whatever the delay to its eventual use, the development of this new blood component will be conditioned by the establishment of its therapeutic efficacy and safety, like every new therapeutic product.

**CONCLUSION**

If the arrival of artificial blood has been announced for a long time, we are still waiting for authentic blood substitutes. Is this a sign that in the case of blood components, it is not easy to replace nature? The concept of the cRBCs shows that it is at least possible to imitate it. It now remains to design the technical conditions for industrial development and to prove the clinical and economic interest of this new blood components with simple characteristics: a concentrate of homogeneous RBCs having a long life span; improved storage capacity and selected phenotype, free of platelets, leukocytes, and plasma; and constant availability. The path to the production factory will be long, 5 to 10 years perhaps. In other words, tomorrow!

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