# From Stem Cell to Red Blood Cells In Vitro: "The 12 Labors of Hercules"

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**KEYWORDS**

• Artificial blood • Stem cells • Hemoglobin
• Transfusion medicine



In the context of the constant difficulty of obtaining supplies of blood products, the interest of disposing of complementary sources of red blood cells (RBCs) for transfusion is evident. The development of chemical or natural molecules that would replace hemoglobin is proving difficult. Artificial blood is still unattainable. Hence, instead of replacing what is made by nature, why not copy it? For these reasons, attempting to generate red blood cells in the laboratory makes sense. This article describes the research in progress that will permit the large-scale production of human red blood cells from hematopoietic stem cells. It also discusses the state of the art of this concept, suggests the obstacles to be overcome to pass from the laboratory model to clinical practice, and analyzes the possible indications in the medium and long term. The potential interest of pluripotent stem cells as an unlimited source of RBCs is considered. If it succeeds, this new approach could mark a considerable advance in the field of transfusion.

## WHY SEARCH FOR NEW SOURCES OF RBCS?

### *Demographic Demands for Transfusion Medicine*

The demographic evolution predicted in the United States[1] indicates an important increase in the proportion of people over 60 years of age, 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,[2] where the percentage of persons over 60 years will go from 21% in 2004 to 23% in 2010, 29% in 2030, and 32% in 2050. This aging of the population will have two important consequences for blood transfusion. On the one hand, it will lead to a considerable increase in malignant hematologic conditions; for example, acute myeloblastic leukemias, chronic myeloid leukemias,

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and non-Hodgkin's lymphomas, the frequency of which increases with age[3] and which have important requirements for transfusion. Hence, on the same basis as before and assuming that the incidence of these conditions as a function of age remains stable, one can 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 an age to give blood will rise much more slowly than will the need 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 16% and 49% respectively. Therefore, whether we wish it or not, it will be mandatory to find alternatives to conventional transfusion practices.

### *The Challenge of Oxygen Carrier Substitutes*

The idea of artificial blood, universal and safe, has been in circulation for more than 50 years. There is no hope, even in the long term, of replacing the white cells that defend us against infections or the platelets that initiate blood coagulation in the event of bleeding. The dream of artificial blood has to be limited to the red cells—primordial cells having essentially only one function, to transport oxygen and deliver it to all the tissues of the body. This vital function is ensured by a special pigment, hemoglobin. Hence, when one speaks of artificial blood, one is simply talking about replacing this hemoglobin.

Can we replace such a refined cellular model such as the RBC? At first sight, that blood cell might seem extremely uninteresting, a simple bag which has lost the vital elements, like its nucleus. It is, in reality, a cell that has pushed its specialization, oxygen transport, to the point of eliminating all that is not useful.

However, this cell knows how to be unique. At its surface, there exist more than thirty blood-group families, which prohibit the transfusion of any indiscriminant type of RBC to any indiscriminate receiver. By liberating hemoglobin from this bag or replacing it with a totally synthetic molecule, while still conserving its oxygen transport capacity, one would attain the objective of an artificial blood. A recent meta-analysis recently reported a 30% increased risk of myocardial infarction in patients who received hemoglobin substitutes.[4]

One can turn to artificial oxygen transporters such as the perfluorocarbon molecules, but these synthetic molecules are unstable in the blood stream. Hence, they cannot carry out their role of oxygenation for very long—24 to 48 hours at the most—and they can in no way provide long-term transfusion support for a patient who lacks RBCs.

Nothing has yet led to a concrete therapeutic application. Much time has gone by. It is now several decades that we have been looking for a substitute for RBCs. In vain! Nature is not so easy to replace.

### *RBCs from Stem Cells*

If one cannot replace nature, why not "simply" copy her? We indeed have sufficient knowledge of the biology of hematopoietic stem cells (HSC) to hope to generate human RBCs in the laboratory. One may reasonably predict that it will soon be possible to produce enough to transfuse "cultured" red blood cells (cRBC).[5]

HSCs represent 1 cell in 10,000 in the bone marrow. In close contact with the medullar microenvironment, they proliferate and differentiate according to a well-defined hierarchy to give rise to the different cell lines of the blood.[6] We have known since the 1980s that HSC are very numerous in umbilical cord blood.[7] One can also make them pass

from the bone marrow to the blood by injecting specific growth factors, which facilitates their collection. The medullar microenvironment is composed of different cells, grouped under the generic name of stromal cells. These cells secrete soluble factors which regulate the production of HSC and facilitate their interactions.[8,9]

This fundamental knowledge of hematopoiesis has enabled us to improve the practice of bone marrow grafting and to widen the concept to the grafting of HSC from peripheral blood or umbilical cord blood. We have been trying for about 10 years to improve the grafting of HSC by increasing the numbers of these cells in the grafts. One study focused on ex vivo expansion.[10] While working on this problem, it was tempting to force the cells to differentiate specifically to the RBC line, known as the erythroid line. This cell line nevertheless has an essential particularity: at the end of its maturation in the bone marrow, the erythroid cell expels its nucleus before entering the blood stream. This is the birth of the RBC, the only cell of the body to have a long life span, 120 days, despite the absence of a nucleus. In this context, an attempt to generate erythroid cells in vitro through amplification of stem cells makes good sense.

We can let our scientific imagination run wild for an instant and dream of the ideal transfusion of tomorrow: an automated production of universal RBC from an infinite source of HSC, replacing the present system based on volunteer donors. A so-called blood-farming program would aim at developing new technologies to enable the in vitro production of RBCs that are pure, readily available, and free of storage lesions. The ultimate goal of such a program would be the development of an automated cell culture and packaging system capable of generating transfusable amounts of universal donor (type O/Rh negative) RBC using human stem cells as the starting material. The RBC produced by the system would be the functional equivalent of donor-derived RBC and induce no greater responses than normal donor-derived RBC. The result would be an automated culture system that would (1) maintain a self-renewing progenitor cell population, (2) support the differentiation, separation, and packaging of transfusable RBC, and (3) be ready for submission to worldwide regulation agencies for all applicable device and transfusable cell product approvals.

To achieve these goals, revolutionary advances in research areas such as the control of progenitor cell expansion or differentiation and the development of automated bioreactors capable of automated cell manipulation and purification would be necessary. Just an impossible dream? Let us analyze the obstacles to be overcome—something like the twelve labors of Hercules.

Overcoming these obstacles means creating in vitro the experimental conditions satisfying three requirements: stimulation of the proliferation of primitive HSC, induction of the exclusive commitment to the erythroid line, and completion of their terminal maturation to the stage of enucleated cells.



## LABOR #1: PROLIFERATION AND ERYTHROID COMMITMENT

Neildez-Nguyen and colleagues[11] initially described a protocol for the expansion of HSC derived from cord blood in a well-defined medium without stroma, based on the sequential addition of growth factors. Starting from CD34+ cells, this protocol allowed the massive production (amplification up to 200,000 times) of pure erythroid precursors (up to 99%) containing fetal hemoglobin (HbF). Contrary to what happens under these ex vivo conditions in the presence of growth factors alone, when such progenitors or precursors are injected into NOD-SCID mice, they were capable of continuing to proliferate in vivo and of differentiating within 4 days to the terminal stage of enucleated cells producing adult hemoglobin.

The first step is overcome since we are able to induce an exclusive erythroid differentiation with a level of amplification compatible with transfusion medicine

requirements. That first observation demonstrates that if a large level of expansion could be reached in vitro, full differentiation only occurred in vivo pointing to a major role of the microenvironment in terminal erythroid differentiation.

## LABOR #2: PROLIFERATION AND TERMINAL MATURATION

The need for an in vitro reconstitution of the bone marrow microenvironment became obvious. On the basis of these data, investigators subsequently modified the protocol to obtain the expansion and differentiation of CD34+ cells derived from blood, bone marrow, or cord blood in three steps[12]: (1) in a liquid medium, involving cell proliferation and induction of erythroid differentiation in the presence of stem cell factor, interleukin-3, and erythropoietin (Epo); (2) based on a model reconstitution of the microenvironment (murine stromal cell line MS5), in the presence of Epo alone; (3) 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 (**Fig. 1**).

Using this protocol, we obtain, after 15, days a plateau of the mean amplification of CD34+ cells of 20,000-fold for cells from bone marrow or peripheral blood, 30,000-fold for cells obtained by leukapheresis after mobilization with G-CSF, and 200,000-fold for cells derived from cord blood. A total commitment to the erythroid lineage is morphologically evident after 8 days. 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 (volume and haemoglobin content).

On an immunologic basis, cRBCs express at their surface the very same blood group antigens compared with native cells (**Fig. 2**). We further tested more than 25 blood-group antigen families without noting any difference.

## LABOR # 3: MATURATION AND CONSERVATION OF RETICULOCYTES

The population generated in vitro is mainly composed of reticulocytes. The main feature is that the experimental conditions are not favorable to maturation up to final

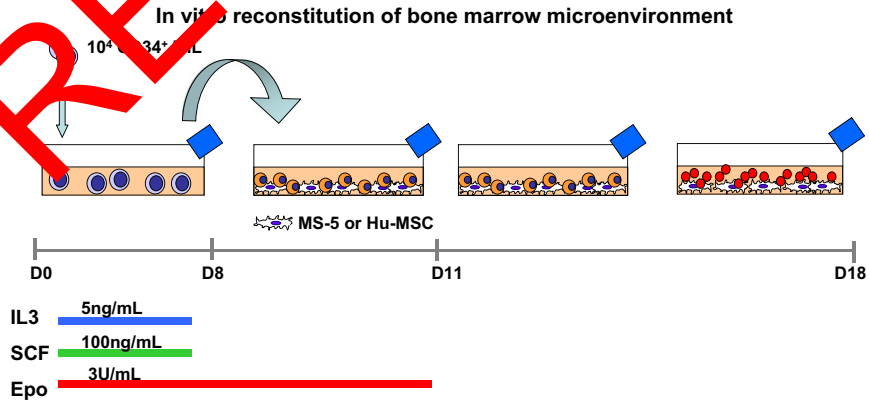


**Fig. 1.** Amplification of erythroid cells human CD34$^+$ cells from cord blood, bone marrow, or peripheral blood are cultured in a liquid medium on a layer of stromal cells of murine origin (MS5) according to a three-phase protocol.

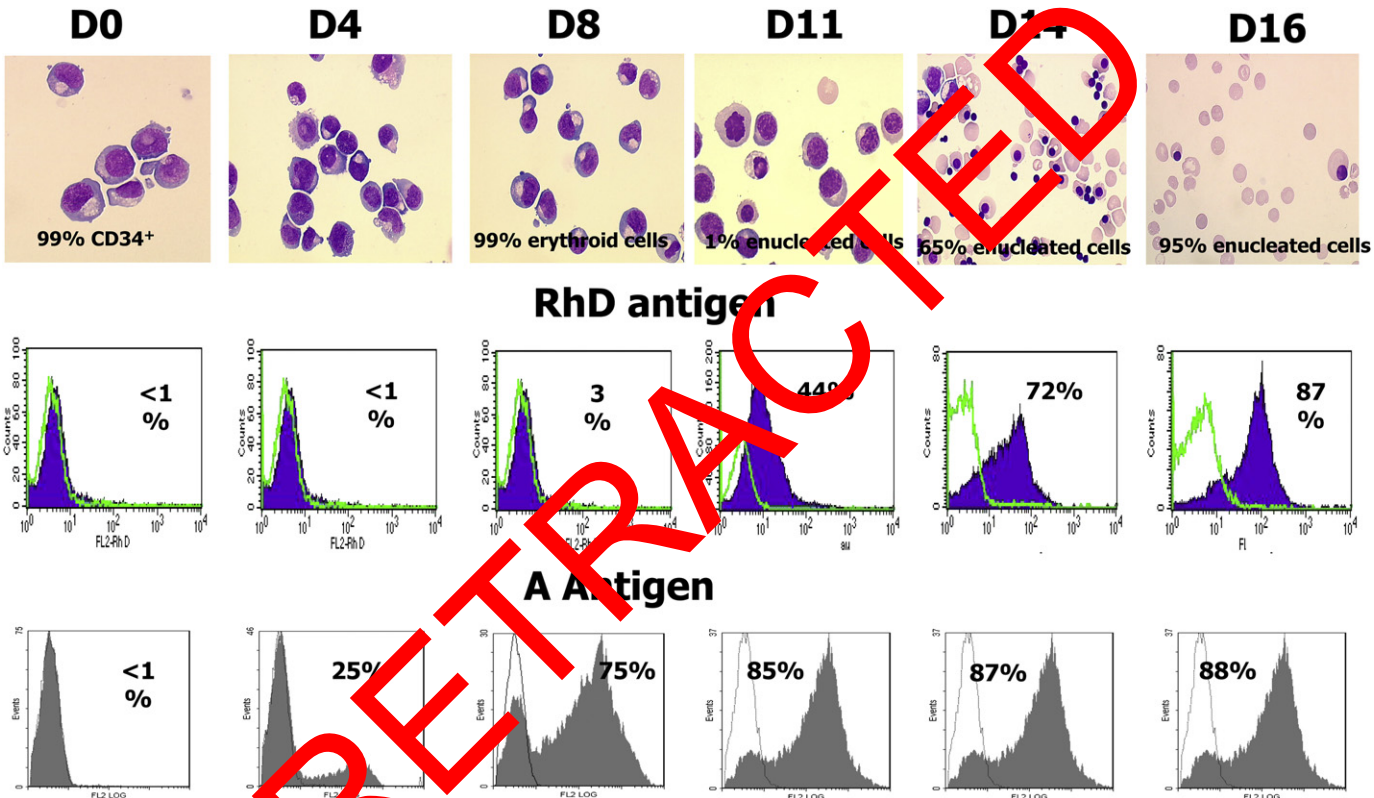




**Fig. 2.** Blood group antigen expression along the culture.

RBCs. Once produced, reticulocytes have to be transferred into a defined medium unless they hemolyze. That point is crucial for further clinical use if full maturation is required. Fortunately, as described below, in vivo maturation occurs.

The conservation of cRBCs is possible up to at least 30 days in vitro. It is, however, mandatory to design conditions dedicated to the status of reticulocytes, that is, different from the ones commonly used for native RBCs (eg, Sagmanitol).

### LABOR #4: TERMINAL DIFFERENTIATION INTO ADULT AND FUNCTIONAL HEMOGLOBIN

The reticulocytes and cRBCs generated ex vivo have to be functionally equivalent to donor-derived RBC. That is the case because:

1. They have glucose-6-phosphate dehydrogenase and pyruvate kinase levels in keeping with the properties of a young homogenous RBC population. This indicates that they are capable of reducing glutathione and maintaining ATP levels and, therefore, have a normal level of 2, 3-diphosphoglycerate.
2. Their deformability, as evaluated by ektacytometry, is comparable to that of native erythrocytes.
3. The functionality of the hemoglobin present in cRBC is assessed by ligand-binding kinetics after flash photolysis. The bimolecular kinetics after photodissociation of CO provides a sensitive test of hemoglobin function. On varying the energy of the photolysis pulse, two phases are observed which correspond to the two hemoglobin conformations (R and T states). The kinetics are thus biphasic, reflecting the two allosteric forms.
4. Like native hemoglobin, cRBC hemoglobin 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 hemoglobin as compared with 1.3 for control RBC hemoglobin and the Hill coefficients are identical (N50 of 2.28 vs 2.29). The kinetic and equilibrium data, therefore, indicate ligand-binding properties in very close agreement with control values. Methemoglobin is not detected, which shows that cRBC are enzymatically capable of reversing hemoglobin oxidation.

Interestingly, starting from BM or LK CD34+, the in vitro generated RBCs contained a great majority of adult hemoglobin. On the contrary, starting from cord blood CD34+ cells, they contained a great majority of HbF, probably secondary to the proliferating status of these various sources.

### LABOR #5: SIMPLIFICATION OF THE PROCEDURE TO AVOID MICROENVIRONMENT RECONSTITUTION

In an attempt to simplify the culture process, Miharada and colleagues[13] developed a stroma-free protocol for ex vivo production of enucleated RBCs. Although they successfully demonstrated that stromal elements were not an absolute requirement for enucleation, expansion was only 700,000-fold with a degree of enucleation of 77.5% compared with the 98% previously reported by Giarratana and colleagues.[12] These data were in accordance with the author's observation that, although contact between stromal cells and erythroid cells is useful for erythroid proliferation, it is not mandatory for enucleation. Indeed, in this system, elimination of the direct contact between MS-5 cells and erythroid cells by a 0.45 μm transwell did not abrogate all enucleation. Interestingly, stromal cells are highly involved in the nuclei phagocytosis. These results are strong arguments for the fact that stromal cells produce soluble factors permitting erythroblast maturation up to enucleation.

The key success, therefore, relies on designing a define culture medium that mimics the microenvironment but avoiding the complexity of a coculture system. It was shown that reticulocytes generated in vitro in the presence of a defined culture medium and three growth factors (stem cell factor, interleukin-3, and Epo), but in the absence of coculture with stromal cells, have all the necessary functional characteristics in terms of enzymatic content, membrane deformability, and the capacity of hemoglobin to fix and release oxygen.

Attempts to improve the system have been looked for. For instance, Fujimi and colleagues[14] proposed to cultivate cord blood CD34+ cells on a telomerase gene transduced (hTERT) human stromal cell line, followed by a period of monoculture, subsequently succeeded by a period in coculture with macrophages derived from parallel cocultures of CD34+ cells (from a different donor) on hTERT stromal cells in the presence of macrophage colony stimulating factor and granulocyte colony stimulating factor. In a final phase, enucleation was achieved as described by Miharada and colleagues.[13] Given that the yield per umbilical cord blood donation was no greater than that achieved previously,[12] the added complexity and associated costs of this method would be difficult to justify in a manufacturing environment.



## LABOR #6: IN VIVO LIFESPAN STUDY

After intraperitoneal infusion into NOD-SCID mice, CFSE-labeled cRBC and reticulocytes obtained by apheresis persist in the circulation to the same extent as CFSE-labeled native RBC: CFSE+ cells are detected for 3 days in both groups of transfused animals. In vivo, the transfused reticulocytes fully maturate into RBC as shown by the appearance of CFSE+/LDS- cells. Over 90% of the CFSE+ cells are mature RBCs by day 3. The next step will be to demonstrate in vivo in humans that cRBC have a normal lifespan of close to 120 days, work which is currently in progress.

Of major interest for cRBC transfusion is the fact that it should enable the infusion of a cell population homogeneous in age with a life span close to 120 days compared with the mean half-life of 28 days of heterogeneous normal donor RBCs. This minimizes the number of transfusions required by serially transfused patients, reducing the potential of iron overload or the development of alloimmunization.

## LABOR #7: QUANTITATIVE ASPECT—$2000\times10^9$ RBCS OR $10^9$ PER PACKED-RBC UNIT

If the objective is to generate RBCs from HSC for the purpose of transfusion, we immediately face the problem of the quantity of cells to be produced: one unit of conventional packed RBC contains more or less 2000 billions of cells! The challenge is therefore to exploit at the maximum the proliferation or differentiation capacity of HSC up to exhaustion to reach terminal maturation as much as possible.

In the author's experience, the best source for that purpose clearly is cord blood derived HSC. They generate 5- to 10-fold more RBCs than HSC derived from peripheral blood, in relation with an increased proliferating capacity while the enucleation capacity is similar.

In experimental conditions, in presence of a defined culture medium in a stroma-free system, combining a first phase of HSC amplification followed by a second phase of erythroid differentiation, enable generation of 4 to 22 million RBC from one cord blood CD34+ cell. Extrapolating these data to an average cord blood containing 5 million CD34+ cells, that is the equivalent of 10 to 50 packed RBC units, which could be produced in vitro from only one cord blood unit!

The benchmark is reached. Now the challenge is the industrial production.

## LABOR #8: SPECIFIC INDICATIONS FOR AUTOLOGOUS SUPPORT

One potentially major indication of cRBCs concerns patients with myelodysplastic syndrome. They indeed require long-term RBC transfusion support, with major alloimmunization and infectious risks. In that situation, they would draw benefit from autologous cRBCs generated from their own peripheral hematopoietic progenitor cells. One of the key question deals with the capacity of these erythroid precursors to reach terminal maturation, that is, enucleation, because myelodysplastic syndrome is classically reported as a qualitative rather than quantitative erythroid defect. Using the model of in vitro generation of mature RBC from human HSC to the 5q(del) syndrome, it is shown that (1) the erythroid commitment of the pathologic clones is not altered, (2) their terminal differentiation capacity is preserved since they can achieve final erythroid maturation up to the stage of enucleation, and (3) the drop in RBC production is secondary to the decrease in the pool of erythroid progenitor cells and the alteration of their proliferative capacity.[15] These data open the way to the indication requiring our capacity to dramatically increase cell proliferation.

Patients with sickle cell disease are the second major candidates. A high level of HbF within the sickle RBCs is correlated with a decrease of sickling. Therefore, an autologous cRBC product engineered to have a high level of HbF could be designed. Work is in progress to evaluate the feasibility and interest of this approach based on functional properties: increase in the level of HbF, diminution of sickle cell formation, diminution of adherence, and modification of the metabolomic profile. Preliminary results for RBC derived from peripheral CD34+ cells of homozygous patients support this hypothesis: tripling the level of HbF in sickle RBC reduced sickle cell formation by one-half.

## LABOR #9: UNIVERSAL RBCS

This culture system offers a new approach to the search for "universal" cRBCs, that is, RBCs lacking membrane expression of the two principal blood group systems, ABO and RHD. 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 cRBCs reach maturity. The blood group antigens ABO and RH, which are not expressed on HSCs, are already present on erythroblast precursors. Two approaches may be envisioned:

1. Inhibition of gene expression in CD34+ human HSCs through use of interfering RNA (siRNA). This technique enables posttranscription inhibition of a gene in a sequence-specific manner by employing doubled 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.
2. 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+ HSCs and continued to that of cRBCs. The methods of this approach can in fact avoid the side effects inherent to the procedures of antigen stripping or antigen masking currently being tested.

A new laboratory tool: focusing on the Kidd blood group system that relies on expression of hUT-B1 glycoprotein under the Jka or Jkb antigenic configurations, Bagnis and colleagues[16] demonstrated that hematopoietic progenitors could be genetically modified to exhibit a chosen Kidd phenotype.

Beyond production of atypical Kidd phenotypes, this genetic strategy could allow generation of rare blood phenotypes from hematopoietic stem cells regardless of initial donor phenotype. Potential applications for genetically modified blood include production of control samples for immunohematologic testing and for resolution of antibody detection in multiple transfused patients.

Finally, the simplest way to provide universal O Rh negative cRBCs would be to collect cord blood cells from donors with that specific phenotype. That represents the most common and easiest unlimited source of stem cells.

## LABOR #10: UNLIMITED SOURCE OF STEM CELLS—THE PROMISES OF PLURIPOTENT STEM CELLS

As in many other therapeutic applications, human embryonic stem cells (hESCs) and related technologies, for example, induced pluripotent stem cells (iPSCs), could provide an essentially unlimited and well-characterized supply of starting material from which to manufacture RBCs. Several studies have shown that hESC cultures can be induced to undergo hematopoietic differentiation, which leads to the generation of primitive (nucleated) RBCs.[17] More recently, Lu and colleagues[18] were able to obtain $3 \times 10^{10}$ primitive RBCs from a single six-well plate of hESCs, equal to an approximately 3000-fold expansion. These investigators also described the generation of mature enucleated RBCs from hESCs by a divergent process. However, a numerical expansion of only 30- to 50-fold was achieved, with an enucleation frequency of 40%. A much better understanding and reproduction of the embryonic and fetal processes leading to adult HSCs will be required before hESCs can replace HSCs as a starting material. Nevertheless, this result demonstrates that it is possible to obtain enucleated RBCs from hESC cultures.

The generation of RBCs from reprogrammed human adult stem cells, or iPSCs,[19] is to be shown yet. This would open the way to putative new developments since iPSCs could provide allogeneic as well as autologous sources of cultured RBCs. However, the same limitations exist as with hESCs concerning the amplification capacity of these cells and significant improvements in proliferative capacity will be a prerequisite to their potential use for transfusion purposes. In any case, one of the main interests of RBCs derived from hESCs or iPSCs is the very low risk of teratogenicity in the final product, since RBCs are enucleated cells easy to purify by conventional filtration. Although the underlying biology of RBC expansion and differentiation has yet to be fully elucidated, achievements over the past few years are promising. It seems likely that hESCs or iPSCs and related technologies will, in time, surpass umbilical cord blood and other donated material as a source of starting material, in many respects eliminating supply constraints.

## LABOR #11: INDUSTRIAL DEVELOPMENT FOR CLINICAL APPLICATION

The ultimate and decisive challenge is to design production procedures compatible with the requirements of good manufacturing practices. A certain number of obstacles have to be overcome: (1) reduction by a factor of 100 of the quantity of medium necessary during the culture phases through use of a medical bioreactor continuously adapting the culture conditions to the cell amplification in a constant volume, (2) development of a rigorously defined culture medium containing no animal proteins, and (3) sequential addition of the cytokines by means of the bioreactor, which will increase their efficacy while reducing the quantities necessary.

If these technical developments allow us to overcome the quantitative limits, at least theoretically, the practical question of the manipulation of such culture volumes

remains unresolved. Static culture methods using flasks, dishes, or gas permeable bags are not feasible. Innovative bioreactor technologies enabling more compact geometries and higher cell densities are therefore required.

The tools needed include bioreactors. Several types of bioreactor have been used to culture hematopoietic cells.[20,21] The typical approach to scale up a cell culture bioprocess is to move from a two-dimensional surface to a stirred three-dimensional tank reactor, which permits efficient mass transport even on very large scales.

Bioreactors allow control of several major parameters. First, they allow the automated monitoring and control of environmental factors such as pH, temperature, and dissolved oxygen concentration. These parameters have a significant influence on culture performance and their careful optimization is mandatory to reach clinically significant cell yields. This is notably true for hematopoietic cell culture. Control of oxygen tension[22,23] regulates both the expansion kinetics and the commitment of cultured hematopoietic cells,[14] while pH control acts similarly.

The key question is how to overcome the cell density limitation inherent to static systems. Dynamic systems using membrane perfusion bioreactors will probably increase this limit by up to a factor of 100.

Although bioreactors have been successfully employed for the culture of hematopoietic cells, they have not yet been used to manufacture standard donor products such as RBCs. Due to the very large number of cells required, the existing bioreactor technologies are insufficient to meet the demands of routine RBC manufacture and new breakthroughs in related technologies will be necessary.

An automated cell culture system capable of maintaining a self-renewing progenitor population, which provides an environment for efficient erythroid differentiation and allows sorting or purification and packaging of the end product RBC in a manner directly suitable for transfusion, does not yet exist. It also has to be kept in mind that an automated culture and packaging system must operate with minimal user intervention. To achieve these aims, revolutionary advances will be necessary in areas such as the control of progenitor cell expansion or differentiation and the development of bioreactors capable of automated cell manipulation and purification.



## LABOR #12: TO FIND THE MARKET

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 two circumstances: the need for rare RBC phenotypes and anti-RBC antigen poly-immunization. Moreover, certain patients are dependent for life on RBC transfusions from a very early age, such as those suffering from hemoglobinopathies, notably thalassemia, could also benefit from these products. Finally, they could be used in the case of such cRBC transfusions in intensive care.

## SUMMARY: TRANSFUSION IN THE YEAR 2020

Let us imagine a possible scenario for cRBC production in 2020 as illustrated in **Fig. 3**. If this scenario becomes reality sometime during the 21st century, a simple skin biopsy could be used to generate an iPSC master-cell bank from an adult with aplastic anemia or myeloid leukemia. These cells could then be amplified and induced to differentiate into mesodermal cells and subsequently into HSCs for cRBC production. An important step toward this futuristic goal was recently achieved in a mouse model of sickle cell anemia by transplanting hematopoietic cells generated by iPSC methods after correction of the sickle cell abnormality by homologous recombination.[24]

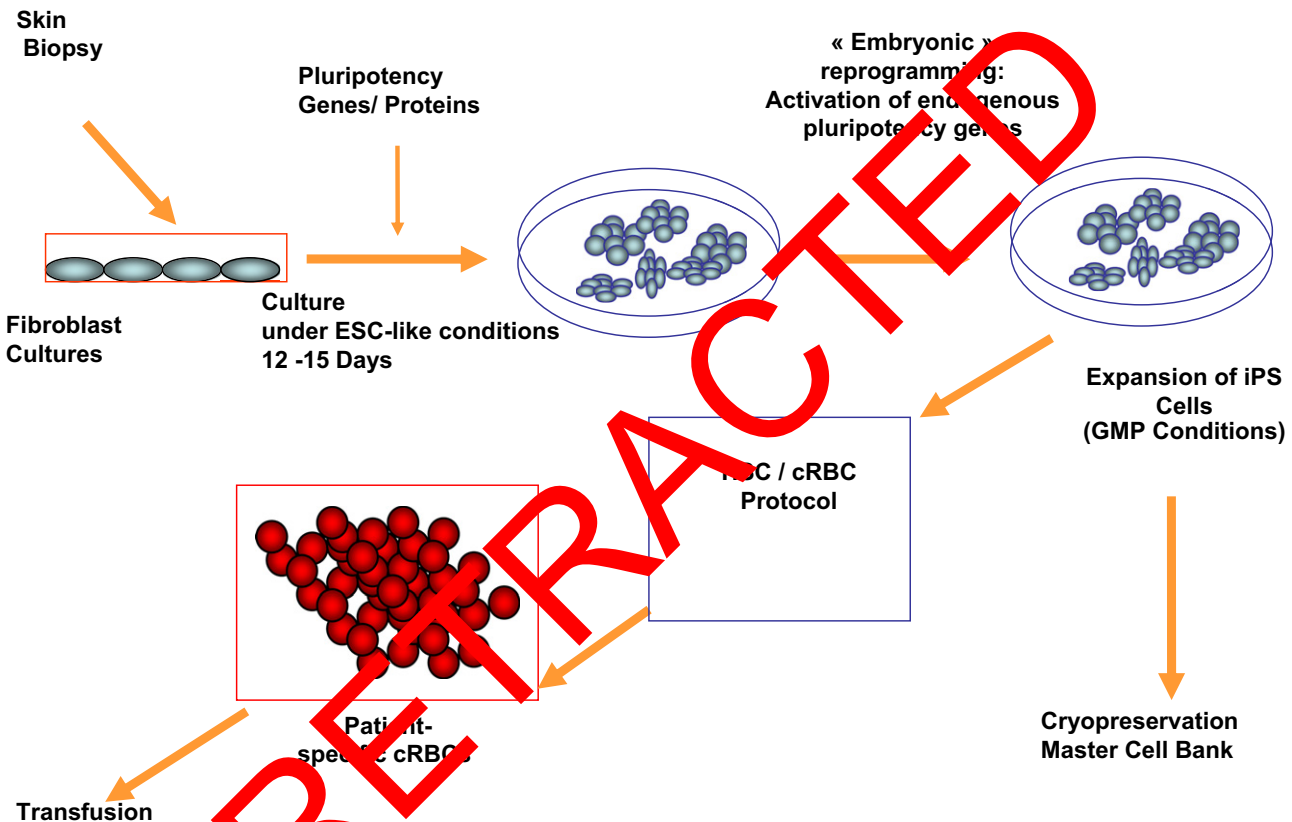




**Fig. 3.** Autologous or allogeneic cRBC generation from iPSCs. cRBC, cultured red blood cell; ESC, embryonic stem cell; GMP, good manufacturing practices; HSC, hematopoietic stem cells; iPS, induced pluripotent stem cells.

Although the arrival of artificial blood has been announced for a long time (more than 5 decades), we are still waiting for authentic blood substitutes. Is this an indication that in the case of cultured blood components it will not be easy to replace nature? The concept of the cultured RBC shows that it is at least possible to imitate nature. It now remains to create the technical conditions for the industrial development of cRBCs and to demonstrate the clinical and economic interest of this new blood product with simple characteristics: a concentrate of homogeneous cRBCs having a long life span, improved storage capacity, and selected phenotype, free of platelets, leukocytes, and plasma and constantly available. The path to the clinical production unit will be long, 5 to 10 years perhaps. In other words—tomorrow!



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