

## Advances in cellular technology in the hematology field: What have we learned so far?

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**Author contributions:** All the authors contributed to this paper.

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Telephone: +55-32-40095100

Received: July 25, 2014

Peer-review started: July 26, 2014

First decision: August 28, 2014

Revised: September 14, 2014

Accepted: September 18, 2014

Article in press: December 16, 2014

Published online: January 26, 2015

### Abstract

Despite the advances in the hematology field, blood transfusion-related iatrogenesis is still a major issue to be considered during such procedures due to blood antigenic incompatibility. This places pluripotent stem cells as a possible ally in the production of more suitable blood products. The present review article aims to provide a comprehensive summary of the state-of-the-art concerning the differentiation of both embryonic stem cells and induced pluripotent stem cells to hematopoietic cell lines. Here, we review the most recently published protocols to achieve the production of blood cells for future application in hemotherapy, cancer therapy and basic research.

**Key words:** Hematopoietic stem cells; Progenitor; Induced pluripotent stem cells; Reprogramming; Embryonic stem cells

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**Core tip:** In the past few years, considerable advance has been made possible in the field of cellular therapy, both in its basic and clinically applied form. Stem cells may have important future applications in hematology, with the possibility of supplying the blood needed for transfusion with the use of blood products produced *in vitro*. Thus, in this paper, we summarize the recently established protocols for differentiating both embryonic and induced pluripotent stem cells into blood cells for possible future use for hemotherapy.

Souza GT, Maranduba CP, Souza CM, Amaral DLAS, Guia FC, Zanette RSS, Rettore JVP, Rabelo NC, Nascimento LM, Pinto ÍFN, Farani JB, Neto AEH, Silva FS, Maranduba CMC, Atalla A. Advances in cellular technology in the hematology field: What have we learned so far? *World J Stem Cells* 2015; 7(1): 106-115 Available from: URL: <http://www.wjgnet.com/1948-0210/full/v7/i1/106.htm> DOI: <http://dx.doi.org/10.4252/wjsc.v7.i1.106>

## INTRODUCTION

Hemotherapy is considered to be the collection of techniques in which whole blood or its fractions are transfused into a patient in order to re-establish hemostasis and to treat certain conditions. It may be carried out using stocked whole blood or by using processed concentrates, red blood cells (RBCs), platelets or plasma, according to the needs of the patient. The blood transfusion, however, is closely related to causing iatrogenic events as a consequence of predictable or unpredictable reasons. Transfusion-related acute lung injury and hemolytic transfusion reaction (HTR) are the more commonly related conditions following blood transfusions which hinder the execution of this procedure with the life-threatening nature of these consequences<sup>[1]</sup>.

In order to minimize the inherent risks, blood transfusion requires immunological compatibility between the blood of the donor and recipient to avoid HTR and to ensure the therapeutic efficacy of the RBC concentrates. Identifying the blood group-related antigens in the surface of blood cells to be infused and the determination of hematological and immune status of the recipient serum are mandatory procedures before the blood transfusion. Many scientific works have made efforts towards the production of universal RBCs which may be transfused regardless of the antigenic phenotype of the recipient. These efforts tend to focus on management of cells by treating the AB antigens with polyethylene glycol or by erasing the AB epitopes by enzymatic digestion with glycosidase<sup>[2,3]</sup>. However, these strategies have limited benefits and do not apply to silencing phenotypes other than the AB antigens or to impose the expression of a specific antigen in the cells.

The previously cited immunological compatibility between donor and receiver is due to the presence of membrane antigens on the donor cells that are bound to elicit an immune response in the receiver. More commonly discussed are the AB antigens that define the ABO group to which the individual belongs, so as to be used as guidance in blood transfusions, and are also aimed at when attempting to diminish eliciting an immune response of donor cells<sup>[2,3]</sup>. However, transfusion-related iatrogenesis still happens despite using ABO compatible blood. Considerations about other possible antigens are necessary due to the occurrence of immune response that is not connected to the AB antigens. Thus, the phenotype of antigens of individuals may be classified into two clinically relevant groups, defined according to the frequency in which those antigens appear: public

antigens, the collection of antigens present in the great majority of the population; private antigens, those found in very few individuals<sup>[4]</sup>. In order to prevent immune response, it is intuitively necessary to ensure compatibility of the greatest number of antigens possible. Matching donor and recipient bloods correctly would be highly unlikely due to the numerous phenotypes of blood cell antigens, rendering it necessary to perform immunological testing on the serum of the recipient in order to screen for the presence of antibodies against the most common antigens, namely public antigens. However, attainment of RBCs expressing a negative phenotype for a public antigen or a positive phenotype for a private antigen is difficult. Circumvention of this problem may only be done reliably by the installation of worldwide facilities for banking rare bloods<sup>[4]</sup>, which is logistically complicated and would add high costs to blood transfusions<sup>[5]</sup>.

It is necessary to consider the context of where the production of blood cells that induce no or less immune response is in order to achieve safety of the blood transfusion processes. The development of *in vitro* protocols capable of generating functional antigen-controlled cells is mandatory, as well as the compilation of those protocols. The use of stem cells characterizes an attempt to engineer blood cells which could serve the purpose of supplying the need of health systems for considerably voluminous amounts of safer blood products for transfusion, as well as for other applications in research that may culminate in future therapeutic protocols, including the production of cancer-targeted lymphocytes. Both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been reported to be successfully differentiated *in vitro* to cells constituting blood products<sup>[6-8]</sup>.

## DIFFERENTIATION OF ESCS TOWARDS BLOOD CELL PRODUCTION

ESCs may provide an inexhaustible and donorless source of cells for human hemotherapy, with the possibility of being indefinitely propagated in appropriate culture conditions. In addition to the proliferation competence of ESCs, these cells also display potentiality to differentiate into all tissues found in an individual, including hematopoietic differentiation. The possibility of manipulating the expression of antigen genes by homologous recombination is another feature that makes ESCs a suitable tool to generate blood cells of interest<sup>[9]</sup>. Thus arises great interest in using human ESCs in order to supply the need for blood products. *In vitro* hematopoietic differentiation of ESCs has already been well documented along with the hematopoietic precursors involved, erythroid, myeloid, macrophage, megakaryocytic and lymphoid<sup>[10-17]</sup>. Nevertheless, large-scale production of functioning blood cells is still in development. Substantial technological advances have been made in engineering mature hematopoietic tissue from murine ESCs; publications by Kitajima *et al.*<sup>[18]</sup> (2003), Kennedy *et al.*<sup>[19]</sup> (2003) and Fraser *et al.*<sup>[20]</sup> (2003) display the practical aspects of murine cell differentiation<sup>[18-21]</sup>.

Here we have chosen to review the protocols being established in order to differentiate human ESCs into the various cell lineages of mature blood cells, including the differentiation to megakaryocytes through which platelets may be acquired, as well as to analyze the results obtained by the most recent advances.

### **Production of erythrocytes**

The *in vitro* generation of RBCs is of particular interest as an alternative to classic transfusion in the sense that it could provide cells of a particular phenotype circumventing the problems related to immune response upon transfusion and, in addition, it would diminish the risk of infection by blood-borne pathogens<sup>[22-24]</sup>. However, the viability of using the produced cells depends on their functionality and the capability of the method of producing enough quantity of blood product, factors still being developed by ongoing research.

Various protocols intended to achieve satisfactory erythrocytic differentiation of ESCs have been developed. As a consensus, the protocols rely on appropriate culture conditions and the use of cytokines that will be discussed later. Erythropoietin (Epo), responsible for activating anti-apoptotic pathways and stimulating hemoglobin synthesis, and stem cell factor (SCF) act mainly to promote proliferation of the erythroid progenitor cells and seem to be the two central factors in this differentiation<sup>[25]</sup>; nevertheless, more recent research has been able to perform erythrocytic differentiation independently of Epo<sup>[26]</sup>, as detailed later.

The underlying regulatory molecular mechanism involved in the differentiation discussed in this topic requires alteration in expression of transcription factors of the GATA family. GATA1 is closely related to hematopoietic differentiation, including the erythroid lineages, and is mostly expressed during the final steps of the pathway by which RBCs are formed. GATA2, however, is responsible for maintaining the less differentiated status of the cells and proliferating<sup>[27]</sup>.

### **Production of erythrocytes via hemangioblasts:**

Although extensively investigated, *in vitro* hematopoietic differentiated RBCs were not reported to successfully carry oxygen until the studies by Lu *et al.*<sup>[28]</sup> in 2008, in which hemangioblasts were used as an intermediate for differentiation. Despite the success, the RBCs derived from *in vitro* differentiation still displayed structural differences concerning the globin chains expressed in the cells<sup>[28]</sup>.

Hemangioblasts are considered to be bipotential cells which differentiate into both hematopoietic cells and endothelial cells, placing them as an alternative for generating functional blood cells. Several research groups have already attempted to produce a significant amount of hemangioblasts which could be differentiated to erythrocytes as a final aim with clinical applications<sup>[28-30]</sup>. However, the production of hemangioblasts is still considered to be insufficient due to its high costs and low

quantity of cells of interest produced.

In 2007, Lu *et al.*<sup>[31]</sup> issued two publications in which a cheaper and significantly more efficient previously established protocol to produce hemangioblasts was detailed and tested. Also, the oxygen-carrying capability of the erythroid cells later produced was tested<sup>[28,32]</sup>. The protocol established by Lu *et al.*<sup>[31]</sup> is summarized in Tables 1 and 2.

Characterization of the cells produced after the steps in Table 1 displayed that the cells were nucleated and significantly larger than erythrocytes but showed an abundance of hemoglobin, whereas the cells obtained after the protocol in Table 2 were more similar to RBCs when morphologically compared as this protocol promoted enucleation of the cells.

The number of cells obtained with the delineated protocol was 800% higher than in previous studies<sup>[31]</sup>. In addition, most of the cells produced expressed the RBC marker membrane proteins and did not express myelomonocytic or megakaryocytic antigens, 75% were CD 71 positive and 30% were CD 253a positive. Moreover, the erythroid cells successfully functioned as an oxygen carrier. Thus, the protocol employed by Lu *et al.*<sup>[31]</sup> and summarized in Tables 1 and 2 is an advance in the attempts to produce RBCs.

### **Production of erythrocytes independently of erythropoietin:**

More recently, Kim *et al.*<sup>[26]</sup> proposed a method by which a substantially higher RBC production from ESC was possible without the use of Epo as a differentiation inducer<sup>[26]</sup>.

In this study, erythrocytic differentiation was achieved by transfecting the gene for the F36V-MPL protein with lentiviral vector which consists of the intracellular domain MPL and the drug binding extracellular domain F36V<sup>[33]</sup>. The normal MPL protein is activated by thrombopoietin, while a small molecule, AP20187, may also activate the F36V-MPL protein, homodimerizing the MPL domain that activates erythropoiesis. MPL domain activation was found to up-regulate the expression of GATA1, commonly related to differentiation to erythroid cells. Additionally, the protein transduced in the cells does not suffer internalization as a negative feedback mechanism when the ligand binds to the external domain. These cited features show that the protocol used by the group has the capability of producing apparently superior results compared to previous studies<sup>[26]</sup>.

In the study by Kim *et al.*<sup>[26]</sup>, differentiation occurred satisfactorily and more efficiently. Globin chain expression and enucleation was higher than in previous studies using Epo, with the conclusion that the homodimerization of the transfected protein intracellular domain is more potent at promoting erythropoiesis than induction with Epo<sup>[26]</sup>. Although it signifies a great step forward in the understanding of the underlying mechanisms of this differentiation, the use of lentiviral vectors poses a severe hindrance in its utilization for therapy due to the augmented possibility of malignant transformation of the

**Table 1 Steps taken to obtain erythroid cells from human embryonic stem cells by the formation of hemangioblasts**

Step-Days	Procedure
1-3.5 prior to step 2	EB formation is stimulated by plating hESCs on ultra-low attachment plates with serum-free medium supplemented with BMP-4, VEGF <sub>165</sub> and basic FGF; 48 h later, half of the medium renewed and accreted with SCF, thrombopoietin and FLT3 ligand; EBs are formed and induced to differentiate to hemangioblasts
2-d 0 to 10	EBs formed are dissociated with trypsin and a single cell suspension is obtained; The cells are re-suspended in appropriate medium and further put in contact with BGM with added FGF and t-PTD-HoxB4 fusion protein, where they are expanded for 10 d; The hemangioblasts are expanded enough after this period of 10 d
3- d 11 to 20	Differentiation to erythroid cells may be achieved by culturing the hemangioblasts obtained in step 2 for 5 d with the addition of BGM supplemented with Epo to the medium used in step 2; The cells are then expanded for up to seven more days on Stemline II-based medium supplemented with SCF, Epo and methylcellulose
4-d 21	Erythroid cells obtained after step 3 were suspended in IMDM with 0.5% BSA in order to be collected by centrifugation The cells are washed with IMDM and BSA and normally plated so that non-erythroid cells adhere, allowing the non-adherent to be separated by collecting the medium and centrifugation

hESCs: Human embryonic stem cells; FGF: Fibroblast growth factor; BMP-4: Bone morphogenetic protein-4; SCF: Stem cell factor; BGM: Blast-colony growth media; Epo: Erythropoietin; IMDM: Iscove modified Dulbecco medium; BSA: Bovine serum albumin.

**Table 2 Enucleation of Hemangioblasts. The technique here summarized starts by taking the hemangioblasts at the 7<sup>th</sup> day of step 2, clarified in Table 1**

Step	Procedure
1	Hemangioblasts obtained on day 7 of the procedures described in Table 1 are filtered and plated with Stemline II medium supplemented with inositol, folic acid, monothioglycerol, transferrin, insulin, ferrous nitrate, ferrous sulphate and BSA, along with penicillin-streptomycin solution
2	For 7 d, the medium described in step 1 had hydrocortisone, SCF, interleukin-3 and Epo added; Cell density was kept at 10 <sup>6</sup> cells/mL
3	After the 7th day, SCF and interleukin-3 were no longer added to the medium
4	The cells were co-cultured with human mesenchymal stem cells or OP9 mouse stromal cells at different days between days 19 and 36; Medium was composed as described in Step 1 with the addition of Epo

SCF: Stem cell factor; Epo: Erythropoietin; BSA: Bovine serum albumin.

transfected cells.

### Production of platelets

The genesis of platelets, both *in vivo* and *in vitro*, depends on the formation of the megakaryocytes as an intermediate. Megakaryocytes are polyploidy cells that underwent successive DNA replications without mitosis. Platelets are the result of the maturation of megakaryocytes in which they are fragmented<sup>[34]</sup>. Efforts in understanding the dynamics of megakaryocytic differentiation as well as the final maturation to platelets are important in order to make advances in the *in vitro* production of this blood product that may be used for research about platelets and coagulation and eventually for thrombocytopenia treatment.

In 2002, Eto *et al.*<sup>[35]</sup> described the production of large quantities of megakaryocytes from murine ESCs. The ESCs were co-cultured with OP9 stromal cells and the differentiation was induced by thrombopoietin, IL-6 and IL-11 in order to obtain polyploid megakaryocytes and subsequently pro-platelets. The results were confirmed by the expression of  $\alpha$ IIb $\beta$ 3 and platelet glycoprotein Ib $\alpha$  as the cells obtained could successfully bind to fibrinogen by the  $\alpha$ IIb $\beta$ 3 integrin responding to platelet

agonists. No hematopoietic stem cell, erythrocyte and leucocyte markers were found to be expressed after differentiation<sup>[35]</sup>.

Years later, further research by Gaur *et al.*<sup>[36]</sup> managed the production of megakaryocytes starting with human ESCs, which also displayed properties of mature cells, responding to platelet agonists and binding to fibrinogen. Cell culture was also performed with OP9 cells and differentiation was induced by human thrombopoietin<sup>[36]</sup>.

Although Mks have been successfully produced by successful differentiation of both mouse<sup>[35,37,38]</sup> and human ESCs<sup>[12,39-42]</sup>, a great part of the protocols employed required co-culture with stromal cells, imposing difficulties in determining the exact requirements for direct differentiation of ESC to megakaryocytes.

### Production of mast cells

Mast cell differentiation using ESCs has been well documented by Kovarova *et al.*<sup>[43,44]</sup> since 2001. The process follows three steps: firstly, Ebs are formed after placing the ESCs in ultra-low attachment plates; secondly, the EBs are then transferred to Matrigel-treated plates where induction to differentiation starts by supplementation with SCF, IL-6, IL-3 and Fms-like tyrosine kinase 3 ligand (Flt-

3L) which yields non-adherent hematopoietic progenitors; and thirdly, the progenitors collected in the second step are further differentiated by adding fetal bovine serum (FBS), IL-6 and SCF<sup>[43,44]</sup>. Although mast cells are not documented in the literature as prone to transfection, the *in vitro* differentiation of mast cells is relevant in the studies on inflammatory diseases<sup>[44]</sup>.

**Production of macrophages:** Mature macrophages can be classified into two groups, M1, which mainly mediate defence against pathogens and inflammatory response, and M2, more connected to wound healing and tissue remodelling. Fetal macrophages present with a M2-like phenotype that accounts for the scar-free wound healing in embryos. Thus, studies have been performed in order to study the properties of ESC-derived macrophages and their applications<sup>[45]</sup>. Evidence was found that in spite of the M2 properties *in vivo* in embryos, the macrophages obtained from ESC differentiation do not promote better cutaneous tissue healing<sup>[45]</sup>. In spite of these findings, the *in vitro* production of macrophages is still important for providing a cellular model for infections as these cells are the specific hosts for certain pathogens, namely, HIV-1, dengue virus, *Leishmania* and *Mycobacterium tuberculosis*<sup>[46]</sup>.

van Wilgenburg *et al.*<sup>[46]</sup> provided the most recent detailed protocol for macrophage *in vitro* production, rendering the macrophage differentiation protocol more efficient and predictable<sup>[47]</sup>. Two protocols were developed by the group: a “quick protocol”, in which EBs were formed by culturing the ESCs on a layer of mouse feeder cells before mechanical dissociation of the bodies that were then cultured on ultra-low attachment plates where differentiation was stimulated by IL-3 and macrophage colony-stimulating factor (M-CSF); and the “defined protocol”, where no feeder layer or serum was applied, EBs were formed by the spinning method<sup>[48]</sup> and the medium used was the XVIVO™ serum-free medium<sup>[45]</sup>.

### Production of T lymphocytes

de Pooter *et al.*<sup>[49]</sup> successfully differentiated mouse ESCs into T lymphocyte lineages following the culture conditions previously established by Nakano *et al.*<sup>[50]</sup> where the stem cells were co-cultured with OP9 feeder cells and differentiation was induced by the flt-3 ligand and IL-7.

*In vitro* T-cell differentiation was reported to happen after ESCs were differentiated to hematopoietic stem cells (HSC), as described by Vodnyak *et al.*<sup>[51]</sup>. When culturing the ESCs on a feeder layer of stromal cells and supplemented with Flt-3L, IL-7 and SCF, it was possible to attain a substantial number of hematopoietic progenitor cells (HPCs) which were later committed to the differentiated T lymphocytes<sup>[52]</sup>. However, a detailed protocol to achieve efficient human T lymphocyte differentiation from ESCs has not yet been devised.

### Production of B lymphocytes

In 2003, the production of functional B lymphocytes was described by Cho *et al.*<sup>[53,54]</sup>. Flt-3L and IL-7 were

added to the medium on defined days; Flt-3L was added on the fifth day and IL-7 on the eighth day, when pre-B cells were obtained. Fully differentiated and functional B lymphocytes were produced after stimulation with lipopolysaccharide (LPS). The B cells produced expressed the markers that indicate commitment to this lineage, including IgM which had its expression enhanced by LPS stimulation<sup>[54]</sup>.

In the most recent publication on the subject, the induction of lymphopoietic differentiation of ESCs was found to yield two HPC phenotypes: CD34+CD45RA+CD7- and CD34+CD45RA+CD7+. CD7- had its potential turned more to the production of B cells but could differentiate into natural killer (NK) and T cells, while CD7+ was more likely to generate NK and T cells<sup>[55]</sup>.

### Production of eosinophils

Hamaguchi-Tsuru *et al.*<sup>[56]</sup> published a detailed protocol to differentiate eosinophils from murine ESCs and evidence of their functionality in 2004. The differentiation was achieved by cultivating the cells on OP9 stromal cells with stimulation of IL-5 and either IL-3 or granulocyte-macrophage colony stimulating factor (GM-CSF). Eotaxin was also employed in an attempt to differentiate the cells which was only achieved when combined with either IL-3 or IL-5. During the differentiation process, the level of expression of GATA1 transcription factor was found to increase the relationship between GATA1 and hematopoiesis, briefly explained earlier in this review. In addition, the expression of CCAAT binding proteins, IL-3 receptor, GM-CSF receptor and major basic proteins was also augmented<sup>[56]</sup>.

So far, no definite and detailed protocol has been established for the differentiation of eosinophils from human ESCs.

### Production of neutrophils

A neutrophil differentiation protocol was documented and investigated by Lieber *et al.*<sup>[57,58]</sup> in which mouse ESCs were used. The differentiation protocol followed was performed in three steps: firstly, ESCs were cultured on an OP9 feeder layer under conditions that elicited EB formation until the 8<sup>th</sup> day; secondly, the day 8 EBs were dissociated and placed in a new medium with added basic fibroblast growth factor (FGF), IL-6, kit ligand (KL), IL-11 and leukemia inhibitory factor (LIF); and the third step promoted the final differentiation into mature neutrophils by transferring the cells to a new medium supplemented with G-CSF, GM-CSF and IL-6<sup>[57,58]</sup>.

A neutrophil production protocol for human ESCs was more recently published. EB formation was stimulated by bone morphogenetic protein-4 (BMP-4), SCF, FTL-3L, IL-6, IL-6 receptor fusion protein and thrombopoietin. Further differentiation required G-CSF in order to attain mature neutrophils which expressed the lineage markers<sup>[59]</sup>.

### Production of dendritic cells

Standard well-defined protocols for producing dendritic

**Table 3 Summary and comparison of protocols employed for differentiation of embryonic stem cells and induced pluripotent stem cells**

Hematopoietic lineage	Culture conditions for ESCs	Culture conditions for iPSCs	Cytokines used in ESC differentiation	Cytokines used in iPSC differentiation	Ref.
Erythrocytes	Cells grown initially on mitomycin-treated Mouse Embryonic Fibroblasts; BGM and Stemline II media are used; Final step was in co-culture with OP9 cells or human mesenchymal stem cells	Cells are grown in co-culture with human fetal liver-derived feeder layer	BMP-4, VEGF <sub>165</sub> , FGF, thrombopoietin, FLT-3L, t-PTD-HoxB4 fusion protein, Epo, IL-3	IL-3, SCF, Epo, BMP-4, Insulin-like growth factor-1	[28,31,32,74]
Megakaryocytes	Cells are grown in co-culture with OP9 stromal cells	Feeder-free culture in serum-free Stemline medium or in OP9 or C3H10T1/2	IL-6, IL11 and thrombopoietin	BMP-4, VEGF, FGF, SCF, thrombopoietin, FLT-3L, IL-11	[35,75,76]
Mast cells	Cells are grown in co-culture with OP9 stromal cells	Mice iPSCs were initially co-cultured in on mitomycin-treated Mouse Embryonic Fibroblasts with LIF added. In the final step, OP9 cells were used as feeder layer	SCF, IL-6, IL-3 and FLT-3L	IL-3, IL-6 and SCF	[43,44,77]
Macrophages	Cells are grown in co-culture with OP9 stromal cells, but in an alternative method, no feeder layer was used, EBs were produced by the spinning method	Cells are grown in co-culture with OP9 stromal cells	IL-3 and M-CSF	FGF, BMP-4, VEGF, IL-3, IL-6, IL-11, SCF	[45,78]
T lymphocytes	Cells are grown in co-culture with OP9 stromal cells	Initial culture was done on irradiated SNL76/7 cells and further steps on OP9-DL1 cells	FLT-3L, IL-7 and SCF	FLT-3L, IL-5	[49-52,79]
B lymphocytes	Cells are grown in co-culture with OP9 stromal cells	Cells are grown in co-culture with OP9 stromal cells	FLT-3L, IL-7	FLT-3L, IL-3, IL-7 and SCF	[53-55,80]
Eosinophils	Cells are grown in co-culture with OP9 stromal cells	No established protocol	IL-5, IL-3, GM-CSF and Eotaxin	No established protocol	[56]
Neutrophils	Cells are grown in co-culture with OP9 stromal cells	Cells are grown in co-culture with OP9 stromal cells	BMP-4, SCF, FLT-3L, IL-6, IL-6 receptor fusion protein, thrombopoietin and G-CSF	VEGF, IL-3, SCF, thrombopoietin and G-CSF	[59,81]
Dendritic cells	Cells are grown in co-culture with OP9 stromal cells	Cell culture was feeder free, initially on mTeSR1 medium and posteriorly using X-VIVO 15 medium	GM-CSF, IL-4, TNF- $\alpha$	BMP-4, VEGF, SCF, GM-CSF, IL-4	[60,61,82]
Natural killer Cells	Cells are grown in co-culture with M210-B4 cells initially and AFT024 cells in the final step	Cells are grown in co-culture with M210-B4 cells initially and AFT024 cells in the final step	IL-3, IL-7, IL-15, SCF and FLT-3L	IL-3, IL-7, IL-15, SCF and FLT-3L	[63,83]

ESCs: Embryonic stem cells; iPSCs: Induced pluripotent stem cells; FGF: Fibroblast growth factor; BMP-4: Bone morphogenetic protein-4; SCF: Stem cell factor; BGM: Blast-colony growth media; Epo: Erythropoietin; BSA: Bovine serum albumin.

cells (DC) from human ESCs are available. The procedure involves formation of EBs on an OP9 cell monolayer and requires the use of GM-CSF, IL-4 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) as cytokines to induce differentiation. The process requires the formation of HPC before a final step that gives rise to a mature DC due to the presence of GM-CSF and IL-4<sup>[60,61]</sup>.

### Production of natural killer cells

Natural killer (NK) cells have particular importance in the cytotoxic response of effective immunity. The modulation of these cells has been investigated as they may be useful tools in the treatment of neoplasms, which has been recently been explored<sup>[62]</sup>.

NK cell differentiation was done by culturing the ESCs initially on a monolayer of M210-B4, a murine bone marrow lineage, as feeder cells. CD34+ and CD45+ were isolated from the first step and differentiated to NK when co-cultured with another feeder cell, AFT024, on medium

supplemented with  $\beta$ -mercaptoethanol, ascorbic acid and the cytokines IL-3, IL-7, IL-15, SCF and FLT-3L<sup>[63]</sup>.

## DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TOWARDS BLOOD CELL PRODUCTION

iPSCs was the term coined to describe the first cell lineage produced by reverting the differentiated status of somatic cells by using defined factors and four transcription factors were found to be necessary to reprogram fibroblasts, oct-4, Sox-2, Klf-4 and c-Myc<sup>[64]</sup>. The cells acquired could successfully differentiate into cells of the three germ lines and resembled the ESCs in several other morphological aspects as well as in the gene expression signature<sup>[64]</sup>. The understanding of the underlying mechanisms of cellular reprogramming provided by Takahashi *et al.*<sup>[64]</sup> allowed further studies to develop protocols to reprogram other

somatic cell lines and produce iPSCs<sup>[8,65,66]</sup>. The advances in the iPSC subsidized studies focused on their use for tissue production through cellular differentiation. Several following works managed to develop differentiation protocols to be applied to iPSCs, including hematopoietic differentiation<sup>[67-69]</sup>. Additionally, cellular reprogramming readily encouraged considerable advances in tissue engineering since the development and use of iPSCs is not bound by the same hindrances imposed by the ethical constraints inherent to ESCs<sup>[64]</sup>.

The differentiation of iPSCs may also circumvent the problems related to immunological incompatibility upon engraftments and transfusion as iPSCs can be produced in a patient-specific manner<sup>[64,70,71]</sup>. However, the production of these cells still is highly costly and widely inefficient with the proportion of cells which are successfully reprogrammed. Safety issues also have to be taken into consideration since lentiviral vectors were employed by Raya *et al*<sup>[68]</sup> and most of the following attempts to produce iPSCs. Nevertheless, several steps have been recently taken towards a safer and more efficient production of iPSCs<sup>[64-66]</sup>.

Considering the lack of ethical hindrances, the possibility of patient-specific cellular reprogramming and the great differentiation potential of iPSCs, they are a great candidate for use in obtaining donor-free blood products<sup>[67-69]</sup>.

In 2009, Lengerke *et al*<sup>[72]</sup> cultured fibroblast-derived iPSCs under conditions similar to those used for ESC procedures, enabling expansion of a long period. In addition, when differentiation was induced by BMP-4, EB formation was evidenced from the first day and flow cytometry provided evidence of the hematopoietic profile of the cells obtained after day 17<sup>[72]</sup>.

Protocols for hematopoietic differentiation of iPSCs are currently being devised and tested for functionality and resemble the protocols used in ESCs. The already established protocols for differentiating iPSCs into hematopoietic cell lineages were recently reviewed by Lim *et al*<sup>[73]</sup>. In order to summarize the state-of-the-art on the matter, Table 3 displays the culture conditions and cytokines employed in order to achieve differentiation as well as to compare the protocols used for ESCs.

Apart from the previously stated advantages of employing iPSCs for the production of blood products, the cells obtained with cellular reprogramming technology have been reported as useful tools for progression in the field of immunotherapy.

In 2013, Themeli *et al*<sup>[84]</sup> successfully produced tumor-targeting T lymphocytes by differentiating iPSCs. Differentiated NK cells were also investigated for cancer immunotherapy<sup>[85]</sup>. Although little has yet been produced, the available data regarding iPSC aspects of differentiation potential as well as the possibility of using patient-specific cells make iPSCs a promising alternative to generate immunotherapeutic blood products<sup>[86,87]</sup>.

Besides the applications cited above, the knowledge of cellular reprogramming may also be applied to cancer cells in order to produce iPSCs that can potentially predict the progression of the disease and serve as an *in vitro*

model<sup>[88,89]</sup>. Miyoshi *et al*<sup>[89]</sup> published the attainment of cancer stem cells by transfecting defined transcription factors to gastrointestinal neoplastic cells in 2009. More recently, iPSCs were produced starting from pancreatic carcinoma cells<sup>[90]</sup>. Considering that pluripotency has also been achieved from hematopoietic somatic cells<sup>[91-93]</sup>, reprogramming is a promising technology for studying the development of hematological cancers which are of great importance in the population<sup>[94]</sup>.

## CONCLUSION

From what could be perceived from data in the literature, differentiation of blood cells from either ESCs or iPSCs is an on-going field of studies from which various applications can be investigated. Most hematopoietic cells are already being successfully produced from pluripotent stem cells; however, there is an ever-progressing search for more efficient protocols in order to achieve sufficient production in the number of cells for transfusion through a safe protocol that also yields functioning blood cells.

Apart from the enterprise towards acquiring blood products through ESC and iPSC differentiation, the hematopoietic differentiation of these cells can also be applied for other purposes. T and NK cell-based immunotherapy seems to be an alternative to cancer treatment with future applications, as well as the reprogramming of cancer cells that may be of great use in determining the pathways through which carcinogenesis occurs.

## REFERENCES

- 1 **Goodnough LT**, Levy JH, Murphy MF. Concepts of blood transfusion in adults. *Lancet* 2013; **381**: 1845-1854 [PMID: 23706801 DOI: 10.1016/S0140-6736(13)60650-9]
- 2 **Liu QP**, Sulzenbacher G, Yuan H, Bennett EP, Pietz G, Saunders K, Spence J, Nudelman E, Levery SB, White T, Neveu JM, Lane WS, Bourne Y, Olsson ML, Henrissat B, Clausen H. Bacterial glycosidases for the production of universal red blood cells. *Nat Biotechnol* 2007; **25**: 454-464 [PMID: 17401360 DOI: 10.1038/nbt1298]
- 3 **Tan Y**, Qiu Y, Xu H, Ji S, Li S, Gong F, Zhang Y. Decreased immunorejection in unmatched blood transfusions by attachment of methoxypolyethylene glycol on human red blood cells and the effect on D antigen. *Transfusion* 2006; **46**: 2122-2127 [PMID: 17176324 DOI: 10.1111/j.1537-2995.2006.01038.x]
- 4 **Bagnis C**, Chapel S, Chiaroni J, Bailly P. A genetic strategy to control expression of human blood group antigens in red blood cells generated in vitro. *Transfusion* 2009; **49**: 967-976 [PMID: 19175544 DOI: 10.1111/j.1537-2995.2008.02078.x]
- 5 **Shander A**, Hofmann A, Gombotz H, Theusinger OM, Spahn DR. Estimating the cost of blood: past, present, and future directions. *Best Pract Res Clin Anaesthesiol* 2007; **21**: 271-289 [PMID: 17650777 DOI: 10.1016/j.bpa.2007.01.002]
- 6 **Choi KD**, Vodyanik M, Slukvin II. Hematopoietic differentiation and production of mature myeloid cells from human pluripotent stem cells. *Nat Protoc* 2011; **6**: 296-313 [PMID: 21372811 DOI: 10.1038/nprot.2010.184]
- 7 **Inoue-Yokoo T**, Tani K, Sugiyama D. Mesodermal and hematopoietic differentiation from ES and iPS cells. *Stem Cell Rev* 2013; **9**: 422-434 [PMID: 22684542 DOI: 10.1007/s12015-012-9388-1]
- 8 **de Souza GT**, de Souza CM, da Costa Maranduba CM.

- Perspectives on the production of pluripotent cells from hematopoietic cells. *Rev Bras Hematol Hemoter* 2014; **36**: 305-306 [PMID: 25031174]
- 9 **Olsen AL**, Stachura DL, Weiss MJ. Designer blood: creating hematopoietic lineages from embryonic stem cells. *Blood* 2006; **107**: 1265-1275 [PMID: 16254136 DOI: 10.1182/blood-2005-09-3621]
  - 10 **Kaufman DS**, Hanson ET, Lewis RL, Auerbach R, Thomson JA. Hematopoietic colony-forming cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 2001; **98**: 10716-10721 [DOI: 10.1073/pnas.191362598]
  - 11 **Lu SJ**, Li F, Vida L, Honig GR. CD34+CD38- hematopoietic precursors derived from human embryonic stem cells exhibit an embryonic gene expression pattern. *Blood* 2004; **103**: 4134-4141 [PMID: 14962900 DOI: 10.1182/blood-2003-10-3575]
  - 12 **Vodyanik MA**, Bork JA, Thomson JA, Slukvin II. Human embryonic stem cell-derived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. *Blood* 2005; **105**: 617-626 [PMID: 15374881 DOI: 10.1182/blood-2004-04-1649]
  - 13 **Wang L**, Menendez P, Shojaei F, Li L, Mazurier F, Dick JE, Cerdan C, Levac K, Bhatia M. Generation of hematopoietic repopulating cells from human embryonic stem cells independent of ectopic HOXB4 expression. *J Exp Med* 2005; **201**: 1603-1614 [PMID: 15883170 DOI: 10.1084/jem.20041888]
  - 14 **Chadwick K**, Wang L, Li L, Menendez P, Murdoch B, Rouleau A, Bhatia M. Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. *Blood* 2003; **102**: 906-915 [PMID: 12702499 DOI: 10.1182/blood-2003-03-0832]
  - 15 **Woll PS**, Martin CH, Miller JS, Kaufman DS. Human embryonic stem cell-derived NK cells acquire functional receptors and cytolytic activity. *J Immunol* 2005; **175**: 5095-5103 [PMID: 16210613 DOI: 10.4049/jimmunol.175.8.5095]
  - 16 **Zambidis ET**, Peault B, Park TS, Bunz F, Civin CI. Hematopoietic differentiation of human embryonic stem cells progresses through sequential hemoendothelial, primitive, and definitive stages resembling human yolk sac development. *Blood* 2005; **106**: 860-870 [PMID: 15831705 DOI: 10.1182/blood-2004-11-4522]
  - 17 **Chang KH**, Nelson AM, Cao H, Wang L, Nakamoto B, Ware CB, Papayannopoulou T. Definitive-like erythroid cells derived from human embryonic stem cells coexpress high levels of embryonic and fetal globins with little or no adult globin. *Blood* 2006; **108**: 1515-1523 [PMID: 16645170 DOI: 10.1182/blood-2005-11-011874]
  - 18 **Kitajima K**, Tanaka M, Zheng J, Sakai-Ogawa E, Nakano T. In vitro differentiation of mouse embryonic stem cells to hematopoietic cells on an OP9 stromal cell monolayer. *Methods Enzymol* 2003; **365**: 72-83 [PMID: 14696338 DOI: 10.1016/S0076-6879(03)65005-6]
  - 19 **Kennedy M**, Keller GM. Hematopoietic commitment of ES cells in culture. *Methods Enzymol* 2003; **365**: 39-59
  - 20 **Fraser ST**, Yamashita J, Jakt LM, Okada M, Ogawa M, Nishikawa S, Nishikawa S. In vitro differentiation of mouse embryonic stem cells: hematopoietic and vascular cell types. *Methods Enzymol* 2003; **365**: 59-72 [PMID: 14696337 DOI: 10.1016/S0076-6879(03)65004-4]
  - 21 **Nakayama N**, Lee J, Chiu L. Vascular endothelial growth factor synergistically enhances bone morphogenetic protein-4-dependent lymphohematopoietic cell generation from embryonic stem cells in vitro. *Blood* 2000; **95**: 2275-2283 [PMID: 10733496]
  - 22 **Migliaccio AR**, Whitsett C, Migliaccio G. Erythroid cells in vitro: from developmental biology to blood transfusion products. *Curr Opin Hematol* 2009; **16**: 259-268 [PMID: 19444099 DOI: 10.1097/MOH.0b013e32832bcaa2]
  - 23 **Douay L**, Lapillonne H, Turhan AG. Stem cells—a source of adult red blood cells for transfusion purposes: present and future. *Crit Care Clin* 2009; **25**: 383-398, Table of Contents [PMID: 19341915 DOI: 10.1016/j.ccc.2008.12.008]
  - 24 **Douay L**, Andreu G. Ex vivo production of human red blood cells from hematopoietic stem cells: what is the future in transfusion? *Transfus Med Rev* 2007; **21**: 91-100 [PMID: 17397760 DOI: 10.1016/j.tmr.2006.11.004]
  - 25 **Muta K**, Krantz SB, Bondurant MC, Wickrema A. Distinct roles of erythropoietin, insulin-like growth factor I, and stem cell factor in the development of erythroid progenitor cells. *J Clin Invest* 1994; **94**: 34-43 [PMID: 7518834 DOI: 10.1172/JCI117327]
  - 26 **Kim WS**, Zhu Y, Deng Q, Chin CJ, He CB, Grieco AJ, Dravid GG, Parekh C, Hollis RP, Lane TF, Bouhassira EE, Kohn DB, Crooks GM. Erythropoiesis from human embryonic stem cells through erythropoietin-independent AKT signaling. *Stem Cells* 2014; **32**: 1503-1514 [PMID: 24677652 DOI: 10.1002/stem.1677]
  - 27 **Moriguchi T**, Yamamoto M. A regulatory network governing Gata1 and Gata2 gene transcription orchestrates erythroid lineage differentiation. *Int J Hematol* 2014; **100**: 417-424 [PMID: 24638828 DOI: 10.1007/s12185-014-1568-0]
  - 28 **Lu SJ**, Feng Q, Park JS, Vida L, Lee BS, Strausbauch M, Wettstein PJ, Honig GR, Lanza R. Biologic properties and enucleation of red blood cells from human embryonic stem cells. *Blood* 2008; **112**: 4475-4484 [PMID: 18713948 DOI: 10.1182/blood-2008-05-157198]
  - 29 **Wang L**, Li L, Shojaei F, Levac K, Cerdan C, Menendez P, Martin T, Rouleau A, Bhatia M. Endothelial and hematopoietic cell fate of human embryonic stem cells originates from primitive endothelium with hemangioblastic properties. *Immunity* 2004; **21**: 31-41 [PMID: 15345218 DOI: 10.1016/j.immuni.2004.06.006]
  - 30 **Kennedy M**, D'Souza SL, Lynch-Kattman M, Schwantz S, Keller G. Development of the hemangioblast defines the onset of hematopoiesis in human ES cell differentiation cultures. *Blood* 2007; **109**: 2679-2687 [PMID: 17148580 DOI: 10.1182/blood-2006-09-047704]
  - 31 **Lu SJ**, Feng Q, Caballero S, Chen Y, Moore MA, Grant MB, Lanza R. Generation of functional hemangioblasts from human embryonic stem cells. *Nat Methods* 2007; **4**: 501-509 [PMID: 17486087 DOI: 10.1038/nmeth1041]
  - 32 **Lu SJ**, Luo C, Holton K, Feng Q, Ivanova Y, Lanza R. Robust generation of hemangioblastic progenitors from human embryonic stem cells. *Regen Med* 2008; **3**: 693-704 [PMID: 18729794 DOI: 10.2217/17460751.3.5.693]
  - 33 **Richard RE**, Wood B, Zeng H, Jin L, Papayannopoulou T, Blau CA. Expansion of genetically modified primary human hemopoietic cells using chemical inducers of dimerization. *Blood* 2000; **95**: 430-436 [PMID: 10627446]
  - 34 **Deutsch VR**, Tomer A. Megakaryocyte development and platelet production. *Br J Haematol* 2006; **134**: 453-466 [PMID: 16856888 DOI: 10.1111/j.1365-2141.2006.06215.x]
  - 35 **Eto K**, Murphy R, Kerrigan SW, Bertoni A, Stuhlmann H, Nakano T, Leavitt AD, Shattil SJ. Megakaryocytes derived from embryonic stem cells implicate CalDAG-GEFI in integrin signaling. *Proc Natl Acad Sci USA* 2002; **99**: 12819-12824 [PMID: 12239348 DOI: 10.1073/pnas.202380099]
  - 36 **Gaur M**, Kamata T, Wang S, Moran B, Shattil SJ, Leavitt AD. Megakaryocytes derived from human embryonic stem cells: a genetically tractable system to study megakaryocytopoiesis and integrin function. *J Thromb Haemost* 2006; **4**: 436-442 [PMID: 16420577 DOI: 10.1111/j.1538-7836.2006.01744.x]
  - 37 **Berthier R**, Prandini MH, Schweitzer A, Thevenon D, Martin-Sisteron H, Uzan G. The MS-5 murine stromal cell line and hematopoietic growth factors synergize to support the megakaryocytic differentiation of embryonic stem cells. *Exp Hematol* 1997; **25**: 481-490 [PMID: 9197325]
  - 38 **Uzan G**, Prandini MH, Rosa JP, Berthier R. Hematopoietic differentiation of embryonic stem cells: an in vitro model to study gene regulation during megakaryocytopoiesis. *Stem Cells* 1996; **14** Suppl 1: 194-199 [PMID: 11012221 DOI:

- 10.1002/stem.5530140725]
- 39 **Fujimoto TT**, Kohata S, Suzuki H, Miyazaki H, Fujimura K. Production of functional platelets by differentiated embryonic stem (ES) cells in vitro. *Blood* 2003; **102**: 4044-4051 [PMID: 12920021 DOI: 10.1182/blood-2003-06-1773]
  - 40 **Takayama N**, Nishikii H, Usui J, Tsukui H, Sawaguchi A, Hiroyama T, Eto K, Nakauchi H. Generation of functional platelets from human embryonic stem cells in vitro via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors. *Blood* 2008; **111**: 5298-5306 [PMID: 18388179 DOI: 10.1182/blood-2007-10-117622]
  - 41 **Klimchenko O**, Mori M, Distefano A, Langlois T, Larbret F, Lecluse Y, Feraud O, Vainchenker W, Norol F, Debili N. A common bipotent progenitor generates the erythroid and megakaryocyte lineages in embryonic stem cell-derived primitive hematopoiesis. *Blood* 2009; **114**: 1506-1517 [PMID: 19478046 DOI: 10.1182/blood-2008-09-178863]
  - 42 **Lu SJ**, Li F, Yin H, Feng Q, Kimbrel EA, Hahm E, Thon JN, Wang W, Italiano JE, Cho J, Lanza R. Platelets generated from human embryonic stem cells are functional in vitro and in the microcirculation of living mice. *Cell Res* 2011; **21**: 530-545 [PMID: 21221130 DOI: 10.1038/cr.2011.8]
  - 43 **Kovarova M**, Koller B. Differentiation of mast cells from embryonic stem cells. *Curr Protoc Immunol* 2012; **Chapter 22**: Unit 22F.10.1-22F.1016 [PMID: 22470136 DOI: 10.1002/0471142735.im22f10s97]
  - 44 **Kovarova M**, Latour AM, Chason KD, Tilley SL, Koller BH. Human embryonic stem cells: a source of mast cells for the study of allergic and inflammatory diseases. *Blood* 2010; **115**: 3695-3703 [PMID: 20200352 DOI: 10.1182/blood-2009-08-237206]
  - 45 **Dreymueller D**, Denecke B, Ludwig A, Jahnen-Dechent W. Embryonic stem cell-derived M2-like macrophages delay cutaneous wound healing. *Wound Repair Regen* 2013; **21**: 44-54 [PMID: 23126541 DOI: 10.1111/j.1524-475X.2012.00858.x]
  - 46 **van Wilgenburg B**, Browne C, Vowles J, Cowley SA. Efficient, long term production of monocyte-derived macrophages from human pluripotent stem cells under partly-defined and fully-defined conditions. *PLoS One* 2013; **8**: e71098 [PMID: 23951090 DOI: 10.1371/journal.pone.0071098]
  - 47 **Moore KJ**, Fabunmi RP, Andersson LP, Freeman MW. In vitro-differentiated embryonic stem cell macrophages: a model system for studying atherosclerosis-associated macrophage functions. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1647-1654 [PMID: 9763539 DOI: 10.1161/01.ATV.18.10.1647]
  - 48 **Ng E**, Davis R, Hatzistavrou T, Stanley E, Elefanta A. Directed Differentiation of Human Embryonic Stem Cells as Spin Embryoid Bodies and a Description of the Hematopoietic Blast Colony Forming Assay. *Curr Protoc Stem Cell Biol* 2008; **Chapter 1**: Unit 1D.3 [PMID: 18770631 DOI: 10.1002/9780470151808.sc01d03s4]
  - 49 **de Pooter RF**, Cho SK, Carlyle JR, Zúñiga-Pflücker JC. In vitro generation of T lymphocytes from embryonic stem cell-derived prehematopoietic progenitors. *Blood* 2003; **102**: 1649-1653 [PMID: 12738664 DOI: 10.1182/blood-2003-01-0224]
  - 50 **Nakano T**, Kodama H, Honjo T. Generation of lymphohematopoietic cells from embryonic stem cells in culture. *Science* 1994; **265**: 1098-1101 [PMID: 8066449]
  - 51 **Vodyanik MA**, Thomson JA, Slukvin II. Leukosialin (CD43) defines hematopoietic progenitors in human embryonic stem cell differentiation cultures. *Blood* 2006; **108**: 2095-2105 [PMID: 16757688 DOI: 10.1182/blood-2006-02-003327]
  - 52 **Timmermans F**, Velghe I, Vanwalleghem L, De Smedt M, Van Coppernelle S, Taghon T, Moore HD, Leclercq G, Langerak AW, Kerre T, Plum J, Vandekerckhove B. Generation of T cells from human embryonic stem cell-derived hematopoietic zones. *J Immunol* 2009; **182**: 6879-6888 [PMID: 19454684 DOI: 10.4049/jimmunol.0803670]
  - 53 **Cho SK**, Zúñiga-Pflücker JC. Development of lymphoid lineages from embryonic stem cells in vitro. *Methods Enzymol* 2003; **365**: 158-169 [PMID: 14696344]
  - 54 **Cho SK**, Webber TD, Carlyle JR, Nakano T, Lewis SM, Zúñiga-Pflücker JC. Functional characterization of B lymphocytes generated in vitro from embryonic stem cells. *Proc Natl Acad Sci USA* 1999; **96**: 9797-9802 [PMID: 10449774]
  - 55 **Larbi A**, Mitjavila-Garcia MT, Flamant S, Valogne Y, Clay D, Usunier B, l'Homme B, Féraud O, Casal I, Gobbo E, Divers D, Chapel A, Turhan AG, Bennaceur-Grisicelli A, Haddad R. Generation of Multipotent Early Lymphoid Progenitors from Human Embryonic Stem Cells. *Stem Cells Dev* 2014; **23**: 2983-2995 [PMID: 24955741]
  - 56 **Hamaguchi-Tsuru E**, Nobumoto A, Hirose N, Kataoka S, Fujikawa-Adachi K, Furuya M, Tominaga A. Development and functional analysis of eosinophils from murine embryonic stem cells. *Br J Haematol* 2004; **124**: 819-827 [PMID: 15009071]
  - 57 **Lieber JG**, Keller GM, Worthen GS. The in vitro differentiation of mouse embryonic stem cells into neutrophils. *Methods Enzymol* 2003; **365**: 129-142 [PMID: 14696342]
  - 58 **Lieber JG**, Webb S, Suratt BT, Young SK, Johnson GL, Keller GM, Worthen GS. The in vitro production and characterization of neutrophils from embryonic stem cells. *Blood* 2004; **103**: 852-859 [PMID: 14525782]
  - 59 **Yokoyama Y**, Suzuki T, Sakata-Yanagimoto M, Kumano K, Higashi K, Takato T, Kurokawa M, Ogawa S, Chiba S. Derivation of functional mature neutrophils from human embryonic stem cells. *Blood* 2009; **113**: 6584-6592 [PMID: 19321863 DOI: 10.1182/blood-2008-06-160838]
  - 60 **Silk KM**, Tseng SY, Nishimoto KP, Lebkowski J, Reddy A, Fairchild PJ. Differentiation of dendritic cells from human embryonic stem cells. *Methods Mol Biol* 2011; **767**: 449-461 [PMID: 21822895 DOI: 10.1007/978-1-61779-201-4\_33]
  - 61 **Mohib K**, Wang L. Differentiation and Characterization of Dendritic Cells from Human Embryonic Stem Cells. *Curr Protoc Immunol* 2012; **Chapter 22**: Unit 22F.11 [PMID: 22855358 DOI: 10.1002/0471142735.im22f11s98]
  - 62 **Shin JH**, Zhang L, Murillo-Sauca O, Kim J, Kohrt HE, Bui JD, Sunwoo JB. Modulation of natural killer cell antitumor activity by the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* 2013; **110**: 12391-12396 [PMID: 23836658 DOI: 10.1073/pnas.1302856110]
  - 63 **Woll PS**, Grzywacz B, Tian X, Marcus RK, Knorr DA, Verneris MR, Kaufman DS. Human embryonic stem cells differentiate into a homogeneous population of natural killer cells with potent in vivo antitumor activity. *Blood* 2009; **113**: 6094-6101 [PMID: 19365083 DOI: 10.1182/blood-2008-06-165225]
  - 64 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]
  - 65 **Robinton D**, Daley G. The promise of induced pluripotent stem cells in research and therapy. *Nature* 2012; **481**: 295-305 [PMID: 22258608 DOI: 10.1038/nature10761]
  - 66 **Yamanaka S**. Induced pluripotent stem cells: past, present, and future. *Cell stem cell* 2012; **10**: 678-684 [DOI: 10.1016/j.stem.2012.05.005]
  - 67 **Panopoulos AD**, Belmonte JC. Induced pluripotent stem cells in clinical hematology: potentials, progress, and remaining obstacles. *Curr Opin Hematol* 2012; **19**: 256-260 [PMID: 22555392 DOI: 10.1097/MOH.0b013e328353c78f]
  - 68 **Raya A**, Rodríguez-Pizà I, Guenechea G, Vassena R, Navarro S, Barrero MJ, Consiglio A, Castellà M, Río P, Sleep E, González F, Tiscornia G, Garreta E, Aasen T, Veiga A, Verma IM, Surrallés J, Bueren J, Izpisua Belmonte JC. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* 2009; **460**: 53-59 [PMID: 19483674 DOI: 10.1038/nature08129]
  - 69 **Hanna J**, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R. Treatment of sickle cell anemia mouse model with

- iPS cells generated from autologous skin. *Science* 2007; **318**: 1920-1923 [PMID: 18063756 DOI: 10.1126/science.1152092]
- 70 **Ohnuki M**, Takahashi K, Yamanaka S. Generation and characterization of human induced pluripotent stem cells. *Curr Protoc Stem Cell Biol* 2009; **Chapter 4**: Unit 4A.2 [PMID: 19536759 DOI: 10.1002/9780470151808.sc04a02s9]
- 71 **Beltrão-Braga PC**, Pignatari GC, Maiorka PC, Oliveira NA, Lizier NF, Wenceslau CV, Miglino MA, Muotri AR, Kerkis I. Feeder-free derivation of induced pluripotent stem cells from human immature dental pulp stem cells. *Cell Transplant* 2011; **20**: 1707-1719 [PMID: 21457612 DOI: 10.1002/9780470151808.sc04a02s9]
- 72 **Lengerke C**, Grauer M, Niebuhr NI, Riedt T, Kanz L, Park IH, Daley GQ. Hematopoietic development from human induced pluripotent stem cells. *Ann N Y Acad Sci* 2009; **1176**: 219-227 [PMID: 19796250 DOI: 10.1111/j.1749-6632.2009.04606.x]
- 73 **Lim WF**, Inoue-Yokoo T, Tan KS, Lai ML, Sugiyama D. Hematopoietic cell differentiation from embryonic and induced pluripotent stem cells. *Stem Cell Res Ther* 2013; **4**: 71 [PMID: 23796405 DOI: 10.1186/scrt222]
- 74 **Chang CJ**, Mitra K, Koya M, Velho M, Desprat R, Lenz J, Bouhassira EE. Production of embryonic and fetal-like red blood cells from human induced pluripotent stem cells. *PLoS One* 2011; **6**: e25761 [PMID: 22022444 DOI: 10.1371/journal.pone.0025761]
- 75 **Nakamura S**, Takayama N, Hirata S, Seo H, Endo H, Ochi K, Fujita K, Koike T, Harimoto K, Dohda T, Watanabe A, Okita K, Takahashi N, Sawaguchi A, Yamanaka S, Nakauchi H, Nishimura S, Eto K. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell* 2014; **14**: 535-548 [PMID: 24529595 DOI: 10.1016/j.stem.2014.01.011]
- 76 **Takayama N**, Eto K. In vitro generation of megakaryocytes and platelets from human embryonic stem cells and induced pluripotent stem cells. *Methods Mol Biol* 2012; **788**: 205-217 [PMID: 22130710 DOI: 10.1007/978-1-61779-307-3\_15]
- 77 **Yamaguchi T**, Tashiro K, Tanaka S, Katayama S, Ishida W, Fukuda K, Fukushima A, Araki R, Abe M, Mizuguchi H, Kawabata K. Two-step differentiation of mast cells from induced pluripotent stem cells. *Stem Cells Dev* 2013; **22**: 726-734 [PMID: 23045993 DOI: 10.1089/scd.2012.0339]
- 78 **Grigoriadis AE**, Kennedy M, Bozec A, Brunton F, Stenbeck G, Park IH, Wagner EF, Keller GM. Directed differentiation of hematopoietic precursors and functional osteoclasts from human ES and iPS cells. *Blood* 2010; **115**: 2769-2776 [PMID: 20065292 DOI: 10.1182/blood-2009-07-234690]
- 79 **Lei F**, Haque R, Xiong X, Song J. Directed differentiation of induced pluripotent stem cells towards T lymphocytes. *J Vis Exp* 2012; **(63)**: e3986 [PMID: 22617911 DOI: 10.3791/3986]
- 80 **Carpenter L**, Malladi R, Yang CT, French A, Pilkington KJ, Forsey RW, Sloane-Stanley J, Silk KM, Davies TJ, Fairchild PJ, Enver T, Watt SM. Human induced pluripotent stem cells are capable of B-cell lymphopoiesis. *Blood* 2011; **117**: 4008-4011 [PMID: 21343609 DOI: 10.1182/blood-2010-08-299941]
- 81 **Morishima T**, Watanabe K, Niwa A, Fujino H, Matsubara H, Adachi S, Suemori H, Nakahata T, Heike T. Neutrophil differentiation from human-induced pluripotent stem cells. *J Cell Physiol* 2011; **226**: 1283-1291 [PMID: 20945397 DOI: 10.1002/jcp.22456]
- 82 **Leishman A**, Fairchild P. Differentiation of Dendritic Cells from Human Induced Pluripotent Stem Cells. *Stem Cells and Cancer Stem Cells* 2004; **12**: 29-37 [DOI: 10.1007/978-94-017-8032-2\_3]
- 83 **Ni Z**, Knorr DA, Clouser CL, Hexum MK, Southern P, Mansky LM, Park IH, Kaufman DS. Human pluripotent stem cells produce natural killer cells that mediate anti-HIV-1 activity by utilizing diverse cellular mechanisms. *J Virol* 2011; **85**: 43-50 [PMID: 20962093 DOI: 10.1128/JVI.01774-10]
- 84 **Themeli M**, Kloss C, Ciriello G, Fedorov V, Perna F, Gonen M, Sadelain M. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. *Nat Biotechnol* 2013; **31**: 928-933 [PMID: 23934177 DOI:10.1038/nbt.2678]
- 85 **Knorr DA**, Ni Z, Hermanson D, Hexum MK, Bendzick L, Cooper LJ, Lee DA, Kaufman DS. Clinical-scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy. *Stem Cells Transl Med* 2013; **2**: 274-283 [PMID: 23515118 DOI: 10.1038/nbt.2678]
- 86 **Sachamitr P**, Hackett S, Fairchild PJ. Induced pluripotent stem cells: challenges and opportunities for cancer immunotherapy. *Front Immunol* 2014; **5**: 176 [PMID: 24860566 DOI: 10.3389/fimmu.2014.00176]
- 87 **Jiang Z**, Han Y, Cao X. Induced pluripotent stem cell (iPSCs) and their application in immunotherapy. *Cell Mol Immunol* 2014; **11**: 17-24 [PMID: 24336163 DOI: 10.1038/cmi.2013.62]
- 88 **Ramos-Mejia V**, Fraga MF, Menendez P. iPSCs from cancer cells: challenges and opportunities. *Trends Mol Med* 2012; **18**: 245-247 [PMID: 22521522 DOI: 10.1016/j.molmed.2012.04.001]
- 89 **Miyoshi N**, Ishii H, Nagai K, Hoshino H, Mimori K, Tanaka F, Nagano H, Sekimoto M, Doki Y, Mori M. Defined factors induce reprogramming of gastrointestinal cancer progression. *Proc Natl Acad Sci USA* 2010; **107**: 40-45 [PMID: 20018687 DOI: 10.1073/pnas.0912407107]
- 90 **Kim J**, Hoffman JP, Alpaugh RK, Rhim AD, Reichert M, Stanger BZ, Furth EE, Sepulveda AR, Yuan CX, Won KJ, Donahue G, Sands J, Gumbs AA, Zaret KS. An iPSC line from human pancreatic ductal adenocarcinoma undergoes early to invasive stages of pancreatic cancer progression. *Cell Rep* 2013; **3**: 2088-2099 [PMID: 23791528 DOI: 10.1016/j.celrep.2013.05.036]
- 91 **Ye Z**, Zhan H, Mali P, Dowey S, Williams DM, Jang YY, Dang CV, Spivak JL, Moliterno AR, Cheng L. Human-induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders. *Blood* 2009; **114**: 5473-5480 [PMID: 19797525 DOI: 10.1182/blood-2009-04-217406]
- 92 **Okabe M**, Otsu M, Ahn DH, Kobayashi T, Morita Y, Wakiyama Y, Onodera M, Eto K, Ema H, Nakauchi H. Definitive proof for direct reprogramming of hematopoietic cells to pluripotency. *Blood* 2009; **114**: 1764-1767 [PMID: 19564635 DOI: 10.1182/blood-2009-02-203695]
- 93 **Loh YH**, Agarwal S, Park IH, Urbach A, Huo H, Heffner GC, Kim K, Miller JD, Ng K, Daley GQ. Generation of induced pluripotent stem cells from human blood. *Blood* 2009; **113**: 5476-5479 [PMID: 19299331 DOI: 10.1182/blood-2009-02-204800]
- 94 **Smith A**, Howell D, Patmore R, Jack A, Roman E. Incidence of haematological malignancy by sub-type: a report from the Haematological Malignancy Research Network. *Br J Cancer* 2011; **105**: 1684-1692 [PMID: 22045184 DOI: 10.1038/bjc.2011.450]

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