

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Umbilical Cord Blood Hematopoietic Stem and Progenitor Cell Expansion for Therapeutic Use

Suzanne M Watt and Peng Hua

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/64851>

Abstract

Hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for severe hematological malignancies and other severe disorders of the blood, immune system, and bone marrow. It is the most successful regenerative therapy to date, with 2013 marking the one millionth HSCT and the 25th anniversary of the first umbilical cord blood (UCB) HSCT. UCB has most often been used for allogeneic HSCT when a matched bone marrow or peripheral blood donor is unavailable. Recently, novel genome editing technologies to correct inherited gene disorders or to modulate biomarkers/receptors on HSC and the potential use of HSCT for a variety of other nonmalignant conditions have led to a surge of interest in autologous HSCT, with the HSC source depending on the condition to be treated. UCB HSCs may be used to generate red blood cells, granulocytes, or platelets *ex vivo* for transfusion into difficult-to-transfuse patients. Alternatively, UCB may be reprogrammed to induced pluripotent stem cells or used to generate cell lines, which can then be differentiated into different cell lineages for transfusion or used as diagnostic reagents. Disadvantages of UCB are its restricted cell numbers and delayed hematological engraftment. Here, UCB HSC expansion/manipulation *ex vivo* and clinical applications are addressed.

Keywords: cord blood, microenvironmental niche, human hematopoietic stem/progenitor cells, lineage hierarchy, *ex vivo* expansion, clinical trials

1. Introduction

Hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for severe hematological malignancies and other severe disorders of the blood, immune system, and bone marrow. Its usage has increased rapidly and substantially since the first HSCT in 1957, almost

60 years ago, and with the one millionth HSCT being performed in 2013 [1]. Over 40,000 HSCT were performed in Europe in 2014 for more than 36,000 individuals for all disease indications, with 58% being autologous and the remainder receiving HSCs sourced from related and unrelated allogeneic donors of bone marrow (BM), peripheral blood after induced mobilization (mPB) or umbilical cord blood (UCB) [2]. The main indications for these HSCTs were leukemias, lymphoid neoplasias, solid tumors, and nonmalignant diseases [2].

The year 2013 also marked the 25th anniversary of the first UCB HSCT in a child with Fanconi's anemia using an human leukocyte antigen (HLA)-identical sibling UCB donation [3]. The recipient remains alive and well, having achieved full donor chimaerism. Worldwide, there are estimated to be over 730,000 unrelated UCB units banked for public use in more than 160 international cord blood banks, and more than 35,000 have been transplanted [3, 4]. In 2014, UCB accounted for 2% of allogeneic HSCT in Europe, with substantially more in the USA and Japan [2]. From available worldwide data, the Center for International Blood and Bone Marrow Research (CIBMTR) reported that UCB accounted for 8% of allogeneic HSCTs [2–6]. In their international survey report of 2015, the CIBMTR recorded that 32% of pediatric and 10% of adult-unrelated donor HSCTs used UCB as the HSC source [6]. Indications for allogeneic UCB HSCT have included hematological malignancies, bone marrow failure, severe anemias, metabolic storage diseases, immune-deficiencies, and some cancers. Studies in December 2014 estimated that there were over 4 million UCB units banked for private/family use (see [4]) of which at least 1015 (530 autologous and 485 allogeneic) had been transplanted by December 2013 (see [4]). Indications for family UCB HSCTs have included the severe hemoglobinopathies and the treatment of brain injury (see [4]), and these and other indications will be discussed further.

UCB has several advantages over bone marrow or mobilized peripheral blood as the HSCT cell source. It is noninvasive, can be collected, tested, HLA-typed and banked ahead of use, is readily available for urgent HSCTs, and for black and minority ethnic recipients who do not have a matched bone marrow or peripheral blood donor, demonstrates less-associated graft versus host disease (GvHD) in the allogeneic setting, particularly where less rigorous HLA matching is possible and is not subject to donor attrition (reviewed in Refs. [7–10]). Its main disadvantages are its limited cell dose, delayed hematological engraftment (early neutrophil and platelet and long term immune reconstitution), lack of additional donor lymphocytes for infusion and interbank variability in viable hematopoietic stem and progenitor cell (HSPC) content on product release. It may also be subject to changing accreditation and quality control standards between the time of banking and its use, and the UCB donor may have immunological or hematopoietic disorders that are not manifested at the time of donation or during transplant follow-up (reviewed in Refs. [7–10]). UCB HSPC can also be used for other purposes. These include generating adequate supplies of mature blood cells from the HSPCs *ex vivo* for transfusion into difficult-to-transfuse patients or modulating resistance to specific acquired infections and correcting monogenic gene disorders, in dividing HSC, using new genome editing technologies [11–14].

A single UCB unit generally has sufficient HSCs for pediatric, but not adult HSCT [11–14]. Different approaches have been used to enhance the HSPC numbers in UCB grafts so that their

use can be extended to adult HSCTs, to develop further uses for banked cord blood units in other transfusion and transplant therapies or as diagnostic or research reagents, or to improve their engraftment in the bone marrow hematopoietic stem cell niche. These include the use of double UCB units for transplant without or with ex vivo manipulation of cells to enhance their HSPC dose or improve engraftment. The readouts following the manipulation and expansion of HSPCs include assessing the levels of defined hematopoietic progenitor cell subsets using specific markers and using functional assays to demonstrate hematopoietic repopulation capacity in vivo in surrogate animal models (see Refs. [15, 16]). The majority of HSPCs in human UCB are found in the CD133+ and CD34+ fractions of CD45+ cells [17–27] and these progenitors can be further segregated into or enriched for HSC or their immediate myeloid and lymphoid progeny with the discriminatory marker sets identified by Notta et al. [15].

In this review, we will describe these lineage hierarchies in human UCB, the procedures used to expand or manipulate the engraftment of these HSPCs, and the established and potential clinical uses of both unmanipulated and ex vivo manipulated autologous and allogeneic UCB units.

2. Indications for hematopoietic stem cell transplants and the use of umbilical cord blood

The indications for which allogeneic and autologous HSCTs are most often used have recently been provided as guidelines [28] from the American Society of Blood and Marrow Transplantation (ASBMT). **Table 1** summarizes those for allogeneic HSCT based on the published ASBMT disease categories and recommendations with more specific details discussed in, but without reference to, HSC source. This does not necessarily exclude their use for autologous HSCT and, where this is appropriate, this is also described in Ref. [28].

Indication and disease status	Pediatric <18 years	Adult ≥18 years
Acute myeloid leukemia		
Complete response (CR)1 intermediate and high risk and CR2+	Yes	Yes
Not in remission	Yes	Yes
Acute promyelocytic leukemia		
CR2, molecular remission or not in molecular remission		Yes
CR3+		Yes
Not in remission		Yes
Relapse	Yes	Yes
Acute lymphoblastic leukemia		
CR1, standard risk	No	Yes
CR1, high risk, CR2, CR3+	Yes	Yes
Not in remission	Yes	Yes

Indication and disease status	Pediatric <18 years	Adult ≥18 years
Chronic myeloid leukemia		
Chronic phase	Yes	Yes
Accelerated phase	Yes	Yes
Blast phase	Yes	Yes
Myelodysplastic syndromes		
Low risk	Yes	Yes
High risk	Yes	Yes
Juvenile myelomonocytic leukemia	Yes	
Therapy related	Yes	Yes
Myelofibrosis and myeloproliferative diseases		
Primary, low risk, and intermediate/high risk		Yes
Secondary		Yes
Hypereosinophilic syndromes, refractory		Yes
Plasma cell disorders		
Myeloma, initial response		In development
Myeloma, sensitive relapse		Yes
Myeloma, refractory		Yes
Plasma cell leukemia		Yes
Relapse after autologous transplant		Yes
T cell non-Hodgkin lymphoma		
CR1, high risk	Yes	
CR2	Yes	
CR3 ⁺	Yes	
Not in remission	Yes	
T cell lymphoma		
CR1		Yes
Primary refractory, sensitive		Yes
Primary refractory, resistant		Yes
First relapse, sensitive		Yes
First relapse, resistant		Yes
Second or greater relapse		Yes
Relapse after autologous transplant		Yes
Diffuse large B cell lymphoma		
Primary refractory, sensitive		Yes
Primary refractory, resistant		Yes
First relapse, sensitive		Yes
First relapse, resistant		Yes
Second or greater relapse		Yes
Relapse after autologous transplant		Yes

Indication and disease status	Pediatric <18 years	Adult ≥18 years
Lymphoblastic B cell non-Hodgkin lymphoma (non-Burkitt)		
CR1, high risk	Yes	
CR2	Yes	
CR3+	Yes	
Not in remission	Yes	
Burkitt's lymphoma		
First remission	Yes	Yes
First or greater relapse, sensitive	Yes	Yes
First or greater relapse, resistant	Yes	Yes
Relapse after autologous transplant		Yes
Hodgkin lymphoma		
Primary refractory, sensitive	Yes	Yes
Primary refractory, resistant	Yes	Yes
First relapse, sensitive	Yes	Yes
First relapse, resistant	Yes	Yes
Second or greater relapse	Yes	Yes
Relapse after autologous transplant		Yes
Anaplastic large cell lymphoma		
Primary refractory, sensitive	Yes	
Primary refractory, resistant	Yes	
First relapse, sensitive	Yes	
First relapse, resistant	Yes	
Second or greater relapse	Yes	
Follicular lymphoma		
Primary refractory, sensitive		Yes
Primary refractory, resistant		Yes
First relapse, sensitive		Yes
First relapse, resistant		Yes
Second or greater relapse		Yes
Transformation to high grade lymphoma		Yes
Relapse after autologous transplant		Yes
Mantle cell lymphoma		
CR1/PR1		Yes
Primary refractory, sensitive		Yes
Primary refractory, resistant		Yes
First relapse, sensitive		Yes
First relapse, resistant		Yes
Second or greater relapse		Yes
Relapse after autologous transplant		Yes

Indication and disease status	Pediatric <18 years	Adult ≥18 years
Lymphoplasmacytic lymphoma		
Primary refractory, resistant		Yes
First or greater relapse, sensitive		Yes
First or greater relapse, resistant		Yes
Relapse after autologous transplant		Yes
Cutaneous T cell lymphoma		
Relapse		Yes
Relapse after autologous transplant		Yes
Plasmablastic lymphoma		
CR1		Yes
Relapse		Yes
Chronic lymphocytic leukaemia		
High risk, first, or greater remission		Yes
T cell prolymphocytic leukaemia		Yes
B cell, prolymphocytic leukaemia		Yes
Transformation to high-grade lymphoma		Yes
Solid tumors		
Germ cell tumor, relapse	In development	
Germ cell tumor, refractory	In development	
Ewing's sarcoma, high risk or relapse	In development	
Soft tissue sarcoma, high risk or relapse	In development	
Neuroblastoma, high risk or relapse	In development	
Breast cancer, metastatic		In development
Renal cancer, metastatic		In development
Nonmalignant diseases		
Severe aplastic anemia, new diagnosis	Yes	Yes
Severe aplastic anemia, relapse/refractory	Yes	Yes
Fanconi's anemia	Yes	Yes
Dyskeratosis congenita	Yes	Yes
Diamond-Blackfan anemia	Yes	Yes
Sickle cell disease	Yes	Yes
Thalassemia	Yes	In development
Congenital amegakaryocytic thrombocytopenia	Yes	
Mast cell diseases		Yes
Severe combined immunodeficiency	Yes	
T cell immunodeficiency, SCID variants	Yes	
Common variable immunodeficiency		Yes
Wiskott–Aldrich syndrome	Yes	Yes
Hemophagocytic disorders	Yes	Yes

Indication and disease status	Pediatric <18 years	Adult ≥18 years
Lymphoproliferative disorders	Yes	
Severe congenital neutropenia	Yes	Yes
Chronic granulomatous disease	Yes	Yes
Other phagocytic cell disorders	Yes	
IPEX syndrome	Yes	
Juvenile rheumatoid arthritis	In development	
Systemic sclerosis	In development	
Other autoimmune and immune dysregulation disorders	Yes	
Mucopolysaccharidosis (MPS-I and MPS-VI)	Yes	
Other metabolic diseases	Yes	
Osteopetrosis	Yes	
Globoid cell leukodystrophy (Krabbe)	Yes	
Metachromatic leukodystrophy	Yes	
Cerebral X-linked adrenoleukodystrophy	Yes	

SCID, Severe combined immunodeficiency; IPEX, Immune dysregulation, polyendocrinopathy, enteropathy, X-linked; MPS, mucopolysaccharidosis.
 (Adapted with permission from [28]).

Table 1. ASBMT guidelines for indications for allogeneic HSCT in pediatric and adult patients.

Allogeneic UCB HSCT may be used to treat blood disorders, cancers including hematological malignancies, and metabolic and immune disorders. **Table 2** shows a list of 95 diseases in adult and pediatric patients, which Cord:Use defines as being treatable with UCB HSCT [29]. A potential curative option for β -thalassemia is allogeneic HSCT from an HLA-matched sibling donor with reported disease-free survival of 65% in adults and 88% in children (reviewed in reference [30]). For severe sickle cell disease, similar transplants are reported to result in 85–90% disease-free survival in children [31]. While allografts are usually curative for young patients with an HLA-matched sibling donor, this is not an option for the vast majority of patients with sickle cell disease. HLA-matched sibling directed UCB HSCTs (with or without preimplantation genetic HLA-matching), therefore, can provide curative therapies for children suffering from hemoglobinopathies [32, 33]. Better outcomes have also been reported in children transplanted with UCB HSCs in such metabolic disorders as Hurler syndrome, metachromatic leukodystrophy and Krabbe disease, and for congenital bone marrow failure and immunodeficiencies [4, 34–38].

Cancers

Hematological

- Acute lymphocytic leukemia
- Acute myeloid leukemia
- Acute biphenotypic leukemia

- Acute undifferentiated leukemia
- Juvenile chronic myeloid leukemia
- Chronic lymphocytic leukemia
- Chronic myeloid leukemia
- Juvenile myelomonocytic leukemia
- Adult T cell leukemia/lymphoma
- Hodgkin's lymphoma
- Non-Hodgkin's lymphoma
- Lymphoma
- Mantle cell lymphoma
- Burkitt's lymphoma
- EBV lymphoproliferative disease
- Multiple myeloma
- Juvenile chronic myelogenous leukemia
- Myeloid/natural killer cell precursor acute leukemia
- Prolymphocytic leukemia
- Plasma cell leukemia
- Chronic myelomonocytic leukemia
- Thymoma
- Waldenstrom's macroglobulinemia

Other

- Ewing sarcoma
- Neuroblastoma
- Rhabdomyosarcoma
- Wilms tumor

Other blood disorders

- β -thalassemia major
- Sickle cell anemia
- Diamond-Blackfan anemia
- Fanconi's anemia
- Severe aplastic anemia
- Congenital dyserythropoietic anemia
- Essential thrombocythemia
- Polycythemia vera
- Pure red cell aplasia
- Leukocyte adhesion deficiency syndrome
- Paroxysmal nocturnal hemoglobinuria
- Congenital amegakaryocytic thrombocytopenia
- Congenital cytopenia
- Glanzmann's thrombasthenia

- Refractory anemia with excess blasts
- Refractory anemia with excess blasts in transition
- Refractory anemia with ringed sideroblasts
- Myelodysplasia
- Acute myelofibrosis
- Shwachman-Diamond syndrome
- Dyskeratosis congenita
- Agnogenic myeloid metaplasia
- Amyloidosis

Immune and metabolic disorders

- Kostmann syndrome
- Congenital neutropenia
- Chronic granulomatous disease
- Chediak-Higashi syndrome
- Adenosine deaminase deficiency
- Bare lymphocyte syndrome
- Mannosidosis
- Reticular dysgenesis
- Langerhans cell histiocytosis
- Hemophagocytic lymphohistiocytosis
- Neuronal ceroid lipofuscinosis
- Sanfilippo Syndrome
- Sly Syndrome
- X-linked agammaglobulinemia
- Evans syndrome
- X-linked lymphoproliferative disease
- X-linked hyper IgM syndrome
- Immunodysregulation polyendocrine enteropathy-X-linked (IPEX)
- Severe combined immunodeficiency (SCID)
- Wiskott-Aldrich syndrome (WAS)
- Nezelof's syndrome (thymic dysplasia with normal immunoglobulins)
- DiGeorge syndrome
- IKK gamma deficiency
- Omenn syndrome (resembles GvHD)
- Congenital erythropoietic porphyria
- Gaucher's disease (glucocerebrosidase/acid beta-glucosidase deficiency)
- Purine nucleoside
- Hurler's disease (mucopolysaccharidosis type I)
- Hurler-Scheie disease (mucopolysaccharidosis type I)
- Hunter syndrome (mucopolysaccharidosis type II)

- Sanfilippo disease (mucopolysaccharidosis type III)
- Morquio syndrome (mucopolysaccharidosis type IV)
- Maroteaux-Lamy disease (mucopolysaccharidosis type VI)
- Fucosidosis
- Myelokathexis
- Phosphorylase deficiency
- X-linked adrenoleukodystrophy
- Metachromatic leukodystrophy
- Krabbe disease (galactosylceramide lipidosis)
- Inclusion-cell disease (mucopolipidosis II)
- Wolman disease (acid lipase deficiency)
- Neimann-Pick Disease (sphingomyelin lipidosis, sphingomyelinase deficiency)
- Lesch-Nyhan syndrome (hypoxanthine guanine phosphoribosyl transferase/HPRT deficiency)
- Sandhoff disease (hexosaminidase A and B deficiency)
- Tay-Sachs disease (hexosaminidase A deficiency)

Table 2. Disease indications that may be treated with allogeneic cord blood transplantation based on information in [3, 5, 7, 29].

Graft failure and delayed immune reconstitution in UCB HSCT with myeloablative therapy are not without risk, and only 25% of the patients have a matched sibling donor. Lower UCB HSC engraftment rates have been observed where resistance to engraftment occurs (e.g., hemoglobinopathies, chronic myeloid leukemia, and acquired aplastic anemia, see Ref. [4]). Autologous UCB HSCTs have been less common than allogeneic transplants, but the recent development of novel genome editing technologies opens the way to using this new technology to correct certain inherited or acquired gene disorders in autologous HSCs and sourced from UCB at birth or alternatively from mobilized peripheral blood and bone marrow as appropriate, and to then transplant these cells into the affected individual to correct the disease. HLA matching of these grafts and hence GvHD has not, to date, been a problem for these autologous transplants. However, the use of myeloablative conditioning creates a substantial risk. Recently, studies in mice suggest that the risk of myeloablative conditioning can be greatly reduced by using CD45-saporin toxin conjugated antibody treatment to make space in the bone marrow for transplanted cells to treat sickle cell disease in the autologous setting without significant adverse effects on graft recovery [39], but this has not been conducted in the human. However, earlier studies using rat CD45 antibodies produced in Cambridge, UK [40, 41] have demonstrated the safety and efficacy of an ^{111}In -labeled CD45 conjugate in bone marrow transplant patients with acute leukemia [42]. This may then provide a safer approach with gene-modified HSCTs for treating the β -globin-associated severe hemoglobinopathies, as well as congenital immunodeficiencies and HIV AIDS. While α -thalassemia affects the production of the α -globin chain in β -thalassemia and sickle cell disease, mutations in the β -globin gene result in absent or reduced β -globin and abnormal hemoglobin structure, respectively [43–45]. Importantly, the inherited hemoglobin disorders, the thalassemias, and sickle cell disease constitute the most common monogenic disorders worldwide [43–47]. Around 300,000

children are born with sickle cell disease each year [43–47], and there are around 36.7 million people infected with HIV [48]. These conditions result in a reduced life expectancy and quality of life [48–53].

An analysis of European trends to 2014 [2] suggests a peak of allogeneic UCB HSCTs in 2012 and a slight decline since this time paralleling an increase in haploidentical HSCTs when combined with posttransplant cyclophosphamide prophylaxis. Two parallel Phase II clinical trials using haploidentical versus double UCB HSCTs in a reduced intensity conditioning regime setting indicate that the 1-year disease-free survival is similar (see [4]). A comparative trial of these two approaches is currently recruiting. In a recent review, Kurtzberg [54] cites higher relapse rates with haploidentical HSCTs for hematological malignancies in a limited number of studies.

The UK guidelines for alternative donor selection, dosages, and matching have recently been published [10] for pediatric and adult malignancy and bone marrow failure as well as pediatric immune deficiencies and metabolic disorders. Where UCB HSCTs are done, single UCB units are recommended unless there are insufficient cells when double UCB HSCT are considered, but with each unit having a total nucleated cell count of $>1.5 \times 10^7$ for each unit per kg recipient body weight or a total CD34 cell dose $>1.8 \times 10^5/\text{kg}$, as viable cell dose infused is associated with engraftment outcomes [7, 8, 10]. There is no requirement for inter-UCB unit HLA-matching in the double UCB HSCT scenario at this time. Recent studies from Brunstein et al. [55] reviewed in Ref. [54] indicate that greater allele-level HLA mismatching of UCB HSCT between donor and recipient in a significant number of patients with hematological malignancy undergoing double UCB HSCTs could protect against disease relapse without affecting engraftment, GvHD, and nonrelapse mortality. In a further recent survey of UCB HSCT in older patients (50 years) presenting principally with acute myeloid leukemia, myelodysplasia, and non-Hodgkin lymphoma in Europe and North America, Rafii et al. [5] confirmed the efficacy of UCB HSCTs with reduced intensity conditioning in these patients. Further studies on donor selection are warranted. However, leukemic relapse is a major cause of mortality in HSCT recipients, and this must be taken into account in donor selection strategies.

Although there is the potential for autologous UCB HSCTs to rise substantially with new technological developments in the treatment of inherited monogenic diseases and acquired immunodeficiencies such as HIV and AIDS, current indications for autologous UCB use are low or are in development. The main use of autologous UCB grafts (82%) has been for brain injury, and as described in Ref. [4], this includes cerebral palsy, ataxia, apraxia, traumatic brain injury, hypoxic ischemic encephalopathy, and periventricular leukomalacia [56, 57]. Autologous UCB transplants (7%) have been used in clinical trials to treat type 1 diabetes but responses were transient [4, 58, 59].

3. Other uses of UCB

UCB hematopoietic stem and progenitor cells (HSPCs) may also be used to generate red blood cells, granulocytes, or platelets *ex vivo* for transfusion [11, 60]. Alternatively, and although

initial studies have used fibroblasts [61–63], UCB may be reprogrammed to induced pluripotent stem (iPS) cells [64], which can then be differentiated into different cell lineages, e.g., to generate red blood cells and platelets. As these end cells lack nuclei, they may allay certain safety concerns with respect to iPS cells and tumorigenicity (provided that enucleated cells can survive transplantation). Currently, however, these strategies do not replicate the production of over 3×10^{11} blood cells that are generated in adults per day. Other cell types may be isolated, used, expanded, or manipulated from UCB or the umbilical cord (UC) to enhance engraftment, to eradicate malignancies, to prevent GvHD, or prevent infections. The strategies to improve UCB engraftment and immune reconstitution are listed in **Table 3**. This is updated from data presented in [65].

1. Increasing cell dose

Improved collection and processing of cord blood

Infusion of two cord blood units (double cord blood transplantation) in adults

Ex vivo expansion of cord blood HSC/HPC

Infusion of cord blood with third-party donor cells (haploidentical graft)

2. Improving delivery and homing/retention of HSC

Direct intrabone infusion of cord blood

Increased stromal-derived factor-1 (SDF-1) (CXCL12)/CXCR4 interaction (e.g., inhibition of CD26 peptidase; treatment of UCB HSC with dmPGE2)

Ex vivo fucosylation of HSC/HPC

3. Improving selection of cord blood units

Enhanced HLA-matching in some clinical settings

Detection of donor-specific anti-HLA antibodies

4. Modifying UCB transplant regimens

Using reduced-intensity conditioning

Using T-replete protocols

Using CD45-toxin conjugates for autologous UCB HSCT

5. Expanding specific cell populations (ex vivo or in vivo)

Natural killer (NK) cells

T cells/pathogen-specific T cells (CMV, EBV, adenovirus)

Regulatory T cells (Tregs)

Neutrophils

6. Coinfusing cord blood with accessory cells

Mesenchymal stem cells (MSC)

7. Improving thymopoiesis

Interleukin-7 (IL-7), interleukin-2 (IL-2), and interleukin-15 (IL-15)

Reducing sex steroid hormones (androgen, estrogen)

Growth hormone (GH), insulin-like growth factor 1 (IGF-1)

Keratinocyte growth factor (KGF)

Tyrosine kinase inhibition (sunitinib)
FMS-like tyrosine kinase receptor III ligand (Flt3L)
Stem cell factor (SCF)
Inhibition of p53 (pifithrin- β (PFT- β))

Key: HSC, hematopoietic stem cells; HPC, hematopoietic progenitor cells; UCB, umbilical cord blood; HLA, human leukocyte antigen.

Based on and updated from Danby and Rocha [65].

Table 3. Potential methods to improve engraftment and immune reconstitution in UCB transplantation.

4. Assessing the quality and potency of UCB cells

UCB dosages are often 5–10% of those obtained for BM and mPB and as many as 10–20% of UCB HSCTs can result in graft failure [65, 66]. The total nucleated cell (TNC) and CD34+ cell counts have most often been used in selecting UCB units for transplantation as a particular threshold dosage of these cells in a graft correlates with better engraftment and better clinical outcomes [3]. Poorer outcomes of HLA-mismatched UCB HSCTs are reported with dosages of CD34+ cells and TNCs of less than 1.7×10^5 cells/kg and 2.5×10^7 cells/kg recipient body weight, respectively, and much improved outcomes with median TNCs of 10×10^7 cells/kg [66–69]. Although double cord blood transplants increase cell numbers in the graft, the time for engraftment does not increase in comparison with single UCB HSCTs that are appropriately dosed [70].

Because the potency of the unmanipulated UCB unit following cryopreservation has been cited as the most important parameter in predicting engraftment [71], Kurtzberg and colleagues have developed an Apgar Score for enhancing the quality and hence the potency of UCB HSPCs at the time of collection and banking [72]. For Caucasoid babies, they predict the best quality UCBs are likely to be obtained if birth weights are >3500 g, deliveries are between 34 and 38 weeks of gestation, and the UCB units are processed within 10 h of collection. This is because both CD34+ cells and progenitor cell number decrease at and after 40 weeks gestation, even though TNCs increase, and also due to loss in cell viabilities with delays in processing and cryopreservation postcollection [72, 73]. It is of note that in the USA, African-American UCB units contain 30% less TNC after processing and therefore, may not meet the criteria for TNC numbers that have been set for Caucasoid donors in some banks [72]. Although there are moves by some cord blood banks to bank UCB units with $>1.75 \times 10^9$ TNCs, Kurtzberg's studies provide a note of caution and suggest that this would limit banking of UCB units collected to 5% of UCBs collected, adding to cost, but more importantly, potentially compromising the potency of UCBs for transplantation since as many as 25% of such units would be predicted to have insufficient progenitors for successful engraftment.

Measuring the HSC content of UCB presents some difficulty as the gold standard is in vivo transplantation over an individual's lifespan or alternatively in surrogate nonhuman primate models, which are likely to be closer to the human situation than in vivo immune-deficient

murine models [3, 16, 74]. However, this may be particularly important in the genome-editing context. Viability of banked UCB units is generally assessed using in vitro colony forming unit (CFU) content. Although this is not a measure of the repopulating HSCs, CFU content correlates with neutrophil engraftment and posttransplant survival and can be performed in 2–3 weeks [75, 76]. In human UCB, aldehyde dehydrogenase (ALDH)^{bright} cells as assessed by flow cytometry of viable CD45⁺CD34⁺ or CD45⁺CD133⁺ cells have been strongly correlated with CFU content and may represent a more rapid surrogate potency assay for predicting at least early neutrophil engraftment [76] provided that the thaw-wash protocol is followed after thawing cryopreserved UCB units. The coexpression of MA6 on CD34⁺ cells has been reported recently as predictor of platelet recovery [77].

More complex phenotyping of HSCs is not generally carried out in the clinical setting. However, combinations of biomarkers have been used to identify and segregate human HSCs from their immediate progeny and to define the HSPC lineage hierarchy of unmanipulated UCB units in the research setting [15]. The classical hematological hierarchy comprises rare, durable long-term repopulating HSC, which give rise to short-term repopulating HSC and multipotent progenitors (MPP) and subsequently to oligopotent, and finally, unipotent progenitors that differentiate into more than 10 hematopoietic lineages. Based on this hierarchical lineage tree, experimental studies have demonstrated that UCB HSCs can be enriched in the Lineage (Lin)⁻, CD133⁺, CD34⁺ or CD34⁻, CD90⁺, CD38^{lo/-}, CD45RA⁻, and CD49f⁺ subsets [19–27, 47, 78–86]. The CD90⁻ fraction that contains multipotent progenitors (MPPs) also contains HSCs and, when measured by 20-week repopulation in NSG mice, 1 in 20 of the CD90⁺ and 1 in 100 of the CD90⁻ UCB cells were defined as HSCs [15]. Both the CD90⁺ and CD90⁻ subsets could be serially transplanted at least for a further 14–16 weeks [15]. Notably, the CD90⁺ (50–70%) and CD90⁻ (10–20%) cells that expressed CD49f (CD49^{+/high}), and those CD90⁻ cells that become CD90⁺ after in vitro culture on OP9 stroma retained their 20-week long-term repopulating ability in NSG mice [15]. When the CD90⁺ UCB cells were further segregated on the basis of CD49f expression, only the CD90⁺CD49f^{+/high} cells could be serially transplanted [15]. Notta et al. [15] defined the MPPs that were CD90⁻CD49f⁻ as transiently engrafting cells (2–4 weeks in NSG mice) or short-term repopulating HSCs and concluded that most HSCs reside in the CD90⁺ fraction, but 1 in 5.5 UCB HSCs lacked CD90 expression, and approximately 10% of the UCB CD90⁺CD49f⁺ cells fraction are HSCs [15]. More recent experiments from Notta et al. [86] have added further to our understanding of the human hematopoietic lineage and the developmental changes it undergoes from fetal liver to UCB to bone marrow hematopoiesis. Importantly, these investigators developed an in vitro single-cell assay that exclusively assesses myeloid, including erythroid and megakaryocytic, lineage potential of individual CD34⁺ cells by combining MS-5 stromal cultures with LDL and eight cytokines, stem cell factor, thrombopoietin, FMS-like tyrosine kinase 3 ligand, interleukin 6, interleukin 3, interleukin 11, granulocyte macrophage colony stimulating factor and erythropoietin (SCF, TPO, Flt3L, IL6, IL3, IL11, GM-CSF and Epo respectively) and found that 72% of the enriched UCB CD49f⁺ HSCs gave rise to such clones [86]. Of these, approximately half formed high proliferative potential (HPP)-CFU [86]. This level of clonogenic potential was not observed in semisolid methocel cultures used to assay CFU content.

Cell subset	Biomarkers (epitope)	% of CD34+ cells in UCB (mean±SEM)
HSC	CD34+CD38-/loCD45RA-CD90+CD49f+CD71-CD110(BAH1)-	1.52 ±0.21
MPP F1	CD34+CD38-/loCD45RA-CD90-CD49f-CD71-CD110(BAH1)-	2.05 ±0.29
MPP F2	CD34+CD38-/loCD45RA-CD90-CD71+CD110(BAH1) -	0.29 ±0.07
MPP F3	CD34+CD38-/loCD45RA-CD90-CD71+CD110(BAH1)+	0.20 ±0.04
CMP F1	CD34+CD38+CD10-CD45RA-CD135+CD71-CD110(BAH1)-	21.94 ±3.66
CMP F2	CD34+CD38+CD10-CD45RA-CD135+CD71+CD110(BAH1)-	0.78 ±0.25
CMP F3	CD34+CD38+CD10-CD45RA-CD135+CD71+CD110(BAH1)+	1.80 ±0.38
MEP F1	CD34+CD38+CD10-CD45RA-CD135-CD71-CD110(BAH1)-	2.26 ±0.34
MEP F2	CD34+CD38+CD10-CD45RA-CD135-CD71+CD110(BAH1)-	1.43 ±0.38
MEP F3	CD34+CD38+CD10-CD45RA-CD135-CD71+CD110(BAH1)+	4.28 ±0.98
GMP 7+	CD34+CD38+CD10-CD45RA+CD135+ CD7+	1.81 ±0.18
GMP 7-	CD34+CD38+CD10-CD45RA+CD135+CD7-	8.58 ±2.26
Lymphoid	CD34+CD38+CD10+	4.96 ±1.42

Results are summarized from those presented by Notta et al [86].

Table 4. Biomarkers that segregate human UCB HSPC subsets.

Oligopotent or bipotent myeloid progenitors, as the offspring of HSCs, have previously been categorized into common myeloid progenitors (CMP), granulocyte-macrophage progenitors (GMP), and megakaryocyte-erythroid progenitors (MEP) on the basis of differential expression on CD34+CD38+ cells of CD123 [15, 86] or CD135 [87, 88] and CD45RA [84]. Using additional biomarkers to those described above or in Ref. [15], particularly CD110 and CD71, but not lineage markers (used in [15]) as shown in **Table 4** [86], has allowed progeny of HSCs to be segregated further (e.g., into 3 MPP, 3 MEP, 3 CMP, and 2 GMP subsets), with the erythroid/megakaryocytes now being shown to originate from the HSC compartment predominantly and with megakaryocytes emerging from the multipotent HSC and MPP compartments, rather than the CMP subset. This again redefines the lineage relationships in UCB and has implications for expanding distinct HSPC subsets ex vivo.

5. Improving UCB HSPC grafts with cytokine and small molecule treatments ex vivo

Different approaches to improve the efficacy of UCB units clinically have been and are being taken. Whether UCB is used in the related, unrelated, or autologous HSCT settings described above or to generate blood cells ex vivo, enhancing UCB HSC self-renewal or graft content, improving delayed hematological reconstitution, and improving UCB homing to and engraftment in patient bone marrow niches are important issues to address and some of the approaches are described below.

5.1. Biologics to enhance UCB homing to and engraftment in the bone marrow niche

UCB HSCs demonstrate a defect in homing to the bone marrow [87, 89–97]. This is related to the expression of homing receptors and adhesion molecules on UCB HSC and their progeny. Fucosylated selectins are required for HSCs to roll on bone marrow sinusoidal endothelium [87, 89–91], before being attracted into the bone marrow niche via specific chemokines. CXCL12 is a key chemotactic factor controlling HSC homing to and more specifically engraftment and retention in the bone marrow niche [92–94]. Its cognate receptor, CXCR4, is expressed on HSC and their progeny including pre-B and T lymphoid cells [92–96]. Coreceptors or other factors that regulate CXCR4 signaling in response to CXCL12 on HSCs include CD26 (DPPIV), endolyn, JAM-A, VCAM-1, thrombin, fibrinogen, hyaluronic acid, and C3a [17, 95–100].

Clinical trials have been based on double or single UCB HSCTs and are exemplified in the following findings:

- i. Systemic infusion of stigliptin, a CD26 inhibitor, aimed at enhancing the HSC homing/engraftment response to the key chemokine CXCL12 by inhibiting CXCL12 degradation. The mechanism of CD26 action has been studied and found not only to degrade CXCL12 but also to truncate a number of other growth factors such as GM-CSF, IL3, M-CSF, EPO, Flt3L, and SCF. Truncation alters their growth factor activity. For example, truncated EPO blocks the activity of the full-length EPO molecule, while truncated GM-CSF binds to its receptor with higher affinity [96, 97]. Two multicentre Phase II trials have commenced where Stigalipitin is given orally for 3 days in the myeloablative conditioning, single UCB HSCT setting. Although initial outcomes indicated that oral treatment with Stigalipitin showed a median time to neutrophil engraftment of 21 days, if the UCB unit were red-cell depleted, neutrophil engraftment correlated with DPPIV suppression [98]. This approach does not require ex vivo manipulation of the UCB graft, but dosage of Stigalipitin needs further investigation for this treatment to be more effective [99].
- ii. Priming of UCB grafts with C3a. This complement pathway protein is produced by bone marrow MSCs and interacts with the C3aR on UCB HSPCs to enhance CXCL12-mediated migration [100, 101]. A Phase I clinical trial involving C3a priming of a one of the two UCB units in the nonmyeloablative conditioning double cord blood setting [101] did not, however, demonstrate preferable neutrophil recovery in the manipulated UCB unit in most cases, although the CD3 content of the graft correlated with engraftment. Lund et al. [102] have reviewed this trial recently and concluded that the study has added value for designing further clinical trials using manipulated and unmanipulated double UCB units.
- iii. Ex vivo treatment of one of the two UCB units using prostaglandin E2 (dmPGE2) aimed at enhancing HSC homing by upregulating CXCR4 and HSC survival (FT1050; ProHema) [102–106].
- iv. Ex vivo treatment of one of the two UCB units using fucosyl transferases, fucT-VI, or fucT-VII, aimed at enhancing HSC entry to the bone marrow niche by fucosylation of homing receptors, e.g., the selectins (ASC101) [75, 87, 107].

The latter two approaches [83, 102–107] require relatively short exposures of UCB cells to fucosyl transferases or dmPGE2 prior to transplant. dmPGE2 was first identified as a potential agent for expanding HSC in a high throughput screen in zebrafish [105]. The initial clinical study on nine patients receiving nonmyeloablative conditioning and double UCB HSCTs (with 1 unit primed with dmPGE2) did not demonstrate improved engraftment [104]. In a subsequent similar clinical trial, but for which the dmPGE2 treatment of the UCB cells was first optimized, improved time to neutrophil recovery (median 17.5 days) was observed when compared with historical controls (21 days) and with the dmPGE2 primed UCB unit engrafting in over 80% of the patients. This has preceded to a Phase II clinical trial [106], and recent studies suggest that dmPGE2 may modulate Wnt signaling in UCB T cells and enhance immune reconstitution posttransplant [103]. The effects of dmPGE2 in patients undergoing myeloablative conditioning are unknown. Two Phase II clinical trials are being conducted to examine the effects of CD34+ cell fucosylated on engraftment. Double UCB HSCTs in which patients receive myeloablative conditioning for high-risk hematological malignancies and where 1 UCB CD34+ cell graft is fucosylated for 30 min prior to HSCT demonstrate a median time to neutrophil and platelet engraftment of 17 and 35 days, respectively, compared with 26 and 45 days for historical controls [87, 107]. However, the fucosylated UCB unit contributed to engraftment in approximately half of the patients. Biologics that specifically modulate homing and engraftment activities of UCB grafts thus warrant further investigation and optimization.

5.2. Ex vivo expansion of UCB units prior to infusion

It has been predicted that a fourfold expansion of HSCs in UCB would allow the majority of banked UCB units to be used for single UCB HSCT. Currently, the principal practice is to transplant an unmanipulated UCB unit with an ex vivo expanded UCB unit, with the former generally engrafting longer term and the latter contributing to early neutrophil engraftment. The preferred aim is to move to a single UCB unit where a portion of the UCB unit is expanded and transplanted with the unexpanded portion or to expand a single UCB unit ensuring that the manipulated cells can enhance short-term engraftment without compromising long-term engraftment. Approaches have used cytokines with or without small molecules or MSCs to expand UCB units.

5.2.1. Classical cytokine expansion

Current expansion protocols for UCB HSPCs are still in development and evolving continually with improvements in understanding the bone marrow niches and advances in cell and molecular technologies. However, they have evolved from many studies conducted over the past four decades or more commencing with studies in the 1960s and 1970s on in vitro cultures of murine hematopoietic stem/progenitor cells [108, 109] and the identification of monoclonal antibodies [110] to define specific cell surface biomarkers on HSPC subsets. Notably, some of the initial basic cytokine cocktails, such as SCF, TPO, and Flt3L, are still used and are supplemented with new cytokines or factors or better characterized supportive cells and factors either in static or perfusion bioreactor culture conditions in the presence of extracellular matrix molecules that amplify their efficacy ([18, 80, 111–117] and references therein). Notably,

removal of inhibitory factors is also beneficial in promoting human UCB HSPC expansion using a fed-batch approach [111, 112]. Our own unpublished studies also indicate that expansion of HSPCs is a multistage process in which exposure to different cytokine combinations over time influences HSC self-renewal and differentiation *ex vivo*. In the initial clinical trials using cytokine-based expansion, in which part of the graft was unmanipulated and CD34+ cells from part of the same UCB unit were expanded in a limited number of cytokines (e.g., SCF, granulocyte-colony stimulating factor (G-CSF) and megakaryocyte growth and differentiation factor (MGDF) for 10 days or in a perfused bioreactor with Flt3L, Epo, and GM-CSF-IL3 fusion protein for 12 days) and then both manipulated and unmanipulated cells transplanted, no improvements in neutrophil or platelet recovery were observed [66]. These studies, however, provided the impetus for the identification of new factors for HSPC expansion and for the design of further clinical trials.

5.2.2. Further cytokine and small molecule addition to expand UCB HSPCs

With an improving knowledge of stem cell niches and microenvironments [118, 119] and technological advances, numerous factors have been identified that regulate HSPC proliferation and differentiation and some may potentially also control HSC self-renewal. Here, we will restrict our discussion to UCB expansion *ex vivo* and, as appropriate, discuss clinical applications, while other approaches to generate and assay (in xenograft *in vivo* models, e.g., in zebrafish) patient-specific HSPCs derived from ES or iPS cells have recently been reviewed and will not be discussed further [118].

Additional factors or small molecules that enhance UCB HSPC proliferation or function include the Notch Delta-like ligand 1 (DLL1), StemRegenin1 (SR 1), the copper chelator tetraethylenepentamide (TEPA), GSK3 β inhibitors of WNT signaling, p18—a specific inhibitor of cyclin-dependent kinase (CDK), pyrimidoinole derivatives such as UM171, specific miRNAs, and epigenetic modifiers such as histone deacetylase (HDAC) inhibitors and nicotinamide, as well as additional growth factors such as the designer cytokine hyper-IL-6, oncostatin M, IL-11, angiopoietin-like 5, and other angiopoietin-like molecules and IGFBP2 [120–139]. Coculture of UCB cells with mesenchymal stromal cells has also been examined [140].

Human UCB CD34+CD38– HSPCs cultured in SCF, Flt3L, TPO, IL6, and IL3 and with Fc immobilized DLL1 Notch ligand over several weeks *in vitro* demonstrate a tenfold increase in CD34+ cells with enhanced repopulating ability in immunodeficient mice [124, 125]. Similar to Notch signaling, another key developmental signaling pathway, Wnt, also serves as a potential target for maintaining the HSPC multipotency during *ex vivo* expansion. Increased early engraftment and chimaerism levels in immunodeficient mice were observed when UCB CD34+ cells were expanded in cytokines supplemented with an inhibitor of glycogen synthase kinase-3 β (GSK3 β), BIO [141]. Another GSK3 β inhibitor CHIR99021 also appears to maintain HSC functionality, at least when tested on murine HSPCs [142]. A higher level of chimaerism was observed 4 months postsecondary HSC transplantation for UCB cells expanded with CHIR99021 and the mTOR inhibitor rapamycin for a week [142]. Thus activating Wnt/ β -catenin signaling and inhibiting mTOR may serve to increase UCB HSC numbers in future studies.

Wnt and TGF β pathways also have opposing roles in regulating the balance between HSC self-renewal, quiescence, and differentiation [143], and these are controlled by miRNAs. By regulating the balance of these two signaling pathways, the miR-99a/100~125b tricistronic miRNAs are reported to promote human HSCs expansion and to favor megakaryocytic differentiation [143]. Additionally, miR-126 regulates HSC proliferation and differentiation by targeting PI3K/AKT/mTOR signaling [131]. Regulating these miRNAs may serve as a potential strategy to modulate HSPCs by targeting multiple functional, but opposing, signaling pathways.

p18 [144] as a specific inhibitor of cyclin-dependent kinase (CDK) is a potential direct target of cell cycle regulation. Two small-molecule compounds P18IN003 and P18IN011 were identified in this study as being able to enhance the proliferation of mouse HSC cells and increase the reconstitution to bone marrow by at least threefold. This may have a potential for human UCB HSC expansion.

Fares et al. screened 5289 small molecules for expansion of mPB CD34+CD45RA $^-$ cells, and this and subsequent synthesis of derivatives of one compound UM729 led to the identification of UM171, a pyrimidoindole derivative, which expanded human UCB CD34+ cells over 100-fold with limited differentiation in 12-day fed-batch cultures supplemented with three essential cytokines, SCF, TPO, and Flt3L [122, 145]. Cells expanded with UM171 show improved hematological reconstitution in NSG mice for at least 18 weeks postsecondary transplantation (13-fold higher than DMSO control). By comparing the RNAseq profiling of cells treated with DMSO or UM171 at different concentrations, UM171 suppressed erythroid/megakaryocyte transcripts. Of note, TMEM183A and PROCR (CD201) encoding genes were found to be significantly upregulated after UM171 treatment. Both TMEM183A and PROCR are cell surface molecules, with the latter being expressed highly on murine HSCs [146]. A direct comparison [122] has been made between the effects of UM171 and the purine derivative SR 1, which was also identified by a high throughput screen on mPB CD34+ cells in the presence of SCF, TPO, Flt3L, and IL6 and which functions as an aryl hydrocarbon receptor antagonist. SR 1 was reported to expand UCB CD34+ and immunodeficient mouse in vivo repopulating cells by 670- and 17-fold, respectively, over 3 weeks of culture [123]. Fares et al. [122] have suggested, however, that SR 1 expands less durable engrafting cells than UM171.

Other small molecules used for HSPC expansion include nicotinamide and TEPA. The vitamin, nicotinamide, generates oxidized nicotinamide adenine dinucleotide (NAD) that regulates the function of the sirtuins (SIRT1). As well as generating oxidized NAD, nicotinamide acts as a specific inhibitor of SIRT-1, and when it is added to human UCB cultures containing SCF, TPO, Flt3L, and IL6 for 3 weeks, then expansion of in vivo (in NOD/SCID mice) repopulating HSCs occurs [129, 132]. When UCB CD133+ cells were cultured in TEPA with SCF, TPO, Flt3L, and IL6 for 3 weeks, an 89-fold increase in CD34+ cells was observed, together with increases in in vivo NOD/SCID repopulating cells [132].

Poycomb group (PcG) genes, identified as global epigenetic transcriptional repressors, have been demonstrated to work through negatively regulating Hox genes [147]. Genes in the Hox families appear to be highly expressed in murine long-term repopulating HSCs [148]. This supports the idea that HSPC expansion can be regulated epigenetically. Histone deacetylases

(HDACs) are classed as important epigenetic modifiers of the eraser type. There are 11 HDAC family members that function as zinc dependent deacetylases of histone and nonhistone proteins. These are divided into four classes. Class I comprises HDAC1-3 and HDAC8, class II consists of HDAC4-7 and HDAC9-10, class III are the sirtuins SIRT1-7 that require NAD as a cosubstrate for their activity, and class IV comprises HDAC11 [149]. Elizalde et al. [150] demonstrated HDAC3 as a potential target for regulating CD34+ cells. Hoffman and colleagues subsequently tested eight HDAC inhibitors, which inhibit class I and II HDACs and found that VPA had a robust influence in promoting the expansion of human UCB CD34⁺C90⁺CD184⁺CD49f⁺ CD45A⁻ HSCs, which expressed key biomarkers such as CD90, CD49f, and CXCR4 and were ALDH^{high} [121]. To evaluate the repopulating activity of expanded cells, sublethally irradiated NSG mice were injected with cells expanded in cytokines (SCF, IL-3, Flt-3, and TPO) and HDAC inhibitors for 7 days. Assessment of the human CD45+ cell chimaerism 13–14 weeks posttransplant demonstrated higher levels of chimaerism in grafts expanded with VPA plus cytokines (32.2±11.3%) than grafts with cytokines alone (13.2±6.4%). The former cells showed secondary transplantation activity (measured at 15–16 weeks posttransplant). Additionally, when compared with the uncultured cells, VPA with cytokines generated 36-fold more SCID-repopulating cells (SRCs) ex vivo. Using limiting dilution analyses, VPA-expanded grafts were also found to contain significantly more SRCs (1 in 31) than control primary grafts (1 in 1115) or cytokine alone expanded grafts (1 in 9223). However, although three of eight HDAC inhibitors were more effective in improving HSC expansion, further studies are required to more accurately define the mode of action of these HDAC inhibitors in HSC expansion. As indicated above, it has been suggested that HDAC3 serves as a target for regulating HSC expansion [150], but not all HDAC inhibitors, and particularly not VPA, target HDAC3 only. Further investigations are warranted to determine the mechanism of action by which HDAC inhibitors provide improved UCB HSC expansion.

5.2.3. Clinical trials of cytokine and small molecule expanded UCB HSPCs

In these clinical trials, a second, unmanipulated UCB unit with adequate cell numbers and/or the unmanipulated CD34⁻ or CD133⁻ fraction of the expanded UCB unit are generally cotransplanted to ensure the presence of durable long-term engrafting HSCs. Outcomes have generally reported improved times to early neutrophil engraftment.

Clinical trials which have been or are being progressed include the following:

- i. Notch ligand (DLL1) enhanced expansion of HSC/HPC ex vivo of one of the two UCB units prior to transplant. Delaney et al. [151] expanded CD34⁺ UCB with SCF, Flt3L, TPO, IL6, IL3, and DLL1 and noted an improved median neutrophil engraftment of 16 days in a double UCB transplant setting. T cells were not expanded, whereas myeloid cells (CD33⁺, CD14⁺) from the expanded UCB unit predominated. Over 3 weeks, TNC expansion averaged 562-fold and CD34⁺ cell expansion averaged 164-fold. Longer-term engraftment (of <1 year) was observed for two of the nine patients evaluated, but in one patient, this was not maintained and in the second, the patient died from sepsis at 6 months posttransplant [102].

- ii. Ex vivo expansion of one of the two UCB units prior to transplant using bone marrow-derived MSCs from a third party haploidentical family member or purified bone marrow Stro3+ MPCs protected under patent to Mesoblast. For the Mesoblast clinical trial [140], where Stro3+ MSCs were cocultured with UCB CD34+ cells for 2–3 weeks in the presence of SCF, G-CSF, FL, TPO, median TNC, and CD34+ cell expansions were c.12- and 30-fold, respectively. Expansion of the HSPC generated more myeloid cells, while NK cells were preserved. Neutrophil engraftment was enhanced (median 15d) in patients receiving HSCT with myeloablation, and the MSCs were equally efficient if used as an off-the-shelf product or sourced as a haploidentical product. Chimaerism from both expanded and unmanipulated UCB units was 46% on days 21–30; this reduced to 13% at 6 months, and by 1 year, the unmanipulated UCB unit had engrafted. The expanded cells, therefore, contributed to early neutrophil recovery, and the unmanipulated UCB unit to longer-term repopulation.
- iii. Tetraethylenepentamide (TEPA) enhanced expansion of HSPC ex vivo of part of an UCB unit prior to transplant of the treated and untreated UCB unit (StemEx, Gamida Cell). In this clinical trial [152], the smaller part of the UCB unit was expanded and transplanted with the remaining unmanipulated fraction of the UCB unit. For expansion, UCB CD133+ cells were cultured in SCF, TPO, Flt3L, IL6, and TEPA for 3 weeks and infused 24 h after the unmanipulated UCB unit. However, the median time to neutrophil and platelet engraftment did not appear to be enhanced.
- iv. Nicotinamide (pyridine-3-carboximide) enhanced expansion of HSC/HPC ex vivo of one of the two UCB units prior to transplant (NiCord, Gamida Cell). Gamida Cell used nicotinamide to expand 1 UCB CD133+ selected unit over 3 weeks. Cells that were CD133– were cryopreserved and subsequently infused with the expanded and nonexpanded UCB units into patients with myeloablative HSCTs for hematological malignancies [129]. Results show that median time to neutrophil engraftment was 13 days. T cell recovery was similar to double UCB transplants, and the Nicord unit generally engrafted as assessed with a median follow-up of 21 months posttransplant (8/11 engrafted with the Nicord unit). There was one nonengraftment and two patients engrafted with the unmanipulated UCB unit. This was the first trial to demonstrate longer-term engraftment of the expanded UCB unit. A new trial has commenced recruiting with planned HSCT of a single Nicord expanded unit. The first two grafts have reported median neutrophil recoveries by day 10/11.
- v. SR 1—inhibiting the aryl hydrocarbon receptor with its antagonist StemRegenin 1 (SR 1) to prevent HSC differentiation and used in a double UCB setting with CD34– cells also being infused (HSC835, Novartis). SR1 with SCF, IL6, TPO, and FL cytokines has been used to expand 1 UCB CD34+ cells and to cotransplant this with an unmanipulated UCB graft in patients undergoing myeloablative therapy for hematological malignancies [134]. This has been modified to also infuse the UCB CD34– fraction. CD34 expression persisted over 3 weeks with a 670-fold CD34+ cell expansion and 17- and 12-fold expansions in NSG repopulating cells for first versus second NSG transplants. A Phase I/II clinical trial has been completed, and this showed 330-fold

expansion of UCB CD34+ cells with SR 1 and cytokines. The median time to neutrophil recovery for 17 patients has been reported as being 15 days. Further studies are planned based on infusion of the expanded unit only. Recent *in vitro* studies [153] indicate that SR 1 promotes the production of megakaryocyte precursors from CD34+ cells with 90% reaching the proplatelet stage with TPO addition, thereby potentially contributing to the *ex vivo* production of platelets from normal cells for transfusion.

- vi. A Phase I/II clinical trial (NCT02668315) involving UM171-expanded UCB HSCs produced in a fed-batch culture system is recruiting patients with hematological malignancies from January 2016.

With most of these clinical studies, the common denominator is that it is possible to reduce the time to neutrophil engraftment to 15–17 days and possibly down to 10–11 days. Thus, improved early neutrophil engraftment is possible with current expansion protocols and the key question going forward relates to whether it is possible to maintain or promote long-term hematopoietic engraftment, particularly where genome editing is applied and where there is a need to expand blood cells *ex vivo* for difficult-to-transfuse patients.

6. Conclusions

The key considerations in expanding human UCB HSC include (i) cost to health providers of GMP cell products and clinical trials, (ii) the number of clinical trials that UCB recipients can be entered into and alternative protocols for HSCT, (iii) restrictions in the use of some compounds or protocols related to intellectual property rights, (iv) defining which UCB units will engraft long term and why 1 UCB unit will engraft in preference to another, (v) addressing variability in donor cell response, particularly related to platelet engraftment and long-term reconstitution of the expanded UCB unit, (vi) the availability of licensed facilities in which to expand cells, and (vii) the optimal development of the best and cheapest protocols to allow rapid engraftment of neutrophils and platelets, longer-term lymphoid reconstitution, and expansion of the HSCs without their differentiation for long-term hemopoietic reconstitution at affordable costs to the healthcare provider and for transplant recipients.

Clinical trials to date have demonstrated improved homing or retention in the bone marrow niche and improved early neutrophil engraftment. Further research and development is required to regulate the self-renewal of the HSC without significant differentiation in order to facilitate novel genome engineering studies and the development of this into a cost-effective GMP-grade process. Ensuring that the cells can effectively home to and engraft in the bone marrow after this manipulation may require the addition of small molecules (e.g., fucTVI, dmPGE2) for a short period prior to transplant after expansion with specific growth factor cocktails. In the short term, better characterization and refinement of newer and existing UCB products will result in shorter hospital stays and improved prognosis for HSC transplant recipients as to-time-to-hematological-reconstitution shortens, the incidence of graft failure reduces and treatment of residual malignant or cure for acquired or inherited disease improves. Better characterization of UCB self-renewal and differentiation should also ex-

pand the treatment choices available and will be a valuable resource to patients in ethnic minority and other groups where it can be difficult to find a matched graft or where graft engineering and genome editing offer the best choice for a cure. In the longer term, a full understanding of the molecular mechanisms that govern HSC commitment and differentiation, the homing of HSCs to bone marrow, and the control, retention, and engraftment of normal HSCs within the specialized bone marrow niches will lead to achievable and cost effective translation of a great deal of research into effective clinical practice for many millions of individuals worldwide.

Acknowledgements

The authors have no conflicts of interest to declare. We would like to acknowledge NHS Blood and Transplant, a University of Oxford RDM award, and Innovate UK for their support. We would like to thank Mrs Wendy Slack for proof reading the manuscript.

Author details

Suzanne M Watt* and Peng Hua

*Address all correspondence to: suzanne.watt@nhsbt.nhs.uk

Stem Cell Research, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford and NHS Blood and Transplant, Oxford, UK

References

- [1] Gratwohl A, Pasquini MC, Aljurf M, Atsuta Y, Baldomero H, Foeken L, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. *Lancet Haematol.* 2015;2:e91–100. doi:10.1016/s2352-3026(15)00028-9.
- [2] Passweg JR, Baldomero H, Bader P, Bonini C, Cesaro S, Dreger P, et al. Hematopoietic stem cell transplantation in Europe 2014: more than 40 000 transplants annually. *Bone Marrow Transplant.* 2016;51:786–92. doi:10.1038/bmt.2016.20.
- [3] Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood.* 2013;122(4):491–8. doi:10.1182/blood-2013-02-453175.
- [4] Ballen KK, Verter F, Kurtzberg J. Umbilical cord blood donation: public or private? *Bone Marrow Transplant.* [Review]. 2015;50:1271–8. doi:10.1038/bmt.2015.124.
- [5] Rafii H, Ruggeri A, Volt F, Brunstein CG, Carreras J, Eapen M, et al. Changing trends of unrelated umbilical cord blood transplantation for hematologic diseases in patients

- older than fifty years: a Eurocord-Center for International Blood and Marrow Transplant Research Survey. *Biol Blood Marrow Transplant*. 2016. doi:10.1016/j.bbmt.2016.05.015.
- [6] Pasquini MC ZX. Current uses and outcomes of hematopoietic stem cell transplantation: CIBMTR summary slides. 2015; Available from: <http://www.cibmtr.org>.
- [7] Wagner JE, Eapen M, Carter S, Wang Y, Schultz KR, Wall DA, et al. One-unit versus two-unit cord-blood transplantation for hematologic cancers. *New Engl J Med*. 2014;371(18):1685–94. doi:10.1056/NEJMoa1405584.
- [8] Michel G, Galambrun C, Sirvent A, Pochon C, Bruno B, Jubert C, et al. Single versus double-unit cord blood transplantation for children and young adults with acute leukemia or myelodysplastic syndrome. *Blood*. 2016. doi:10.1182/blood-2016-01-694349.
- [9] Watt SM. *Umbilical Cord Blood Banking*. MJ Caplan, editor-in-chief. Elsevier Press; Kidlington, UK. 2016, Chapter 99811.
- [10] Hough R, Danby R, Russell N, Marks D, Veys P, Shaw B, et al. Recommendations for a standard UK approach to incorporating umbilical cord blood into clinical transplantation practice: an update on cord blood unit selection, donor selection algorithms and conditioning protocols. *Brit J Haematol*. 2016;172:360–70. doi:10.1111/bjh.13802.
- [11] Engert A, Balduini C, Brand A, Coiffier B, Cordonnier C, Dohner H, et al. The European Hematology Association Roadmap for European Hematology Research: a consensus document. *Haematologica*. 2016;101:115–208. doi:10.3324/haematol.2015.136739.
- [12] Genovese P, Schirotti G, Escobar G, Di Tomaso T, Firrito C, Calabria A, et al. Targeted genome editing in human repopulating haematopoietic stem cells. *Nature*. 2014;510:235–40. doi:10.1038/nature13420.
- [13] Lombardo A, Naldini L. Genome editing: a tool for research and therapy: targeted genome editing hits the clinic. *Nat Med*. 2014;20:1101–3. doi:10.1038/nm.3721.
- [14] Pernet O, Yadav SS, An DS. Stem cell-based therapies for HIV/AIDS. *Adv Drug Deliv Rev*. 2016. doi:10.1016/j.addr.2016.04.027.
- [15] Notta F, Doulatov S, Laurenti E, Poepl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science*. 2011;333(6039):218–21. doi:10.1126/science.1201219.
- [16] Cheung AMS, Nguyen LV, Carles A, Beer P, Miller PH, Knapp DJHF, et al. Analysis of the clonal growth and differentiation dynamics of primitive barcoded human cord blood cells in NSG mice. *Blood*. 2013;122:3129–37. doi:10.1182/blood-2013-06-508432.
- [17] Chang C-H, Hale SJ, Cox CV, Blair A, Kronsteiner B, Grabowska R, et al. Junctional Adhesion Molecule-A is highly expressed on human hematopoietic repopulating cells

and associates with the key hematopoietic chemokine receptor CXCR4. *Stem Cells*. 2016;34:1664–78. doi:10.1002/stem.2340.

- [18] van der Garde M, van Hensbergen Y, Brand A, Slot MC, de Graaf-Dijkstra A, Mulder A, et al. Thrombopoietin treatment of one graft in a double cord blood transplant provides early platelet recovery while contributing to long-term engraftment in NSG mice. *Stem Cells Dev*. 2015;24(1):67–76. doi:10.1089/scd.2014.0294.
- [19] Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood*. 1997 Dec 15;90(12):5002–12. dx.doi.org/.
- [20] de Wynter EA, Buck D, Hart C, Heywood R, Coutinho LH, Clayton A, et al. CD34+AC133+ cells isolated from cord blood are highly enriched in long-term culture-initiating cells, NOD/SCID-repopulating cells and dendritic cell progenitors. *Stem Cells*. 1998;16:387–96. doi: 10.1002/stem.160387.
- [21] Corbeil D, Roper K, Hellwig A, Tavian M, Miraglia S, Watt SM, et al. The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. *J Biol Chem*. 2000;275(8):5512–20. doi: 10.1074/jbc.275.8.5512.
- [22] McGuckin CP, Pearce D, Forraz N, Tooze JA, Watt SM, Pettengell R. Multiparametric analysis of immature cell populations in umbilical cord blood and bone marrow. *Eur J Haematol*. 2003;71:341–50. doi: 10.1034/j.1600-0609.2003.00153.x.
- [23] Gorgens A, Radtke S, Mollmann M, Cross M, Durig J, Horn PA, et al. Revision of the human hematopoietic tree: granulocyte subtypes derive from distinct hematopoietic lineages. *Cell Rep*. 2013;3:1539–52. doi:10.1016/j.celrep.2013.04.025.
- [24] Gorgens A, Radtke S, Horn PA, Giebel B. New relationships of human hematopoietic lineages facilitate detection of multipotent hematopoietic stem and progenitor cells. *Cell Cycle*. 2013;12:3478–82. doi: 10.4161/cc.26900.
- [25] Takahashi M, Matsuoka Y, Sumide K, Nakatsuka R, Fujioka T, Kohno H, et al. CD133 is a positive marker for a distinct class of primitive human cord blood-derived CD34-negative hematopoietic stem cells. *Leukemia*. 2014;28:1308–15. doi: 10.1038/leu.2013.326.
- [26] Radtke S, Gorgens A, Kordelas L, Schmidt M, Kimmig KR, Koninger A, et al. CD133 allows elaborated discrimination and quantification of haematopoietic progenitor subsets in human haematopoietic stem cell transplants. *Br J Haematol*. 2015;169(6):868–78. doi: 10.1111/bjh.13362.
- [27] Pepperell EE, Watt SM. A novel application for a 3-dimensional time lapse assay that distinguishes chemotactic from chemokinetic responses of hematopoietic CD133+ stem/progenitor cells. *Stem Cell Res*. 2013;11:707–20. doi: 10.1016/j.scr.2013.04.006.

- [28] Majhail NS, Farnia SH, Carpenter PA, Champlin RE, Crawford S, Marks DI, et al. Indications for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2015;21:1863–9. doi:10.1016/j.bbmt.2015.07.032.
- [29] CORD:USE | Cord Blood Bank 2015; Available from: <http://corduse.com/>.
- [30] Negre O, Eggimann AV, Beuzard Y, Ribeil JA, Bourget P, Borwornpinyo S, et al. Gene therapy of the beta-hemoglobinopathies by lentiviral transfer of the beta(A(T87Q))-globin gene. *Hum Gene Ther*. 2016;27(2):148–65. doi: 10.1089/hum.2016.007.
- [31] Talano JA, Cairo MS. Hematopoietic stem cell transplantation for sickle cell disease: state of the science. *Eur J Haematol*. 2015;94:391–9. doi:10.1111/ejh.12447.
- [32] Smythe J, Armitage S, McDonald D, Pamphilon D, Guttridge M, Brown J, et al. Directed sibling cord blood banking for transplantation: the 10-year experience in the National Blood Service in England. *Stem Cells*. 2007;25:2087–93. doi: 10.1634/stemcells.2007-0063.
- [33] Locatelli F, Rocha V, Reed W, Bernaudin F, Ertem M, Grafakos S, et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood*. 2003;101:2137–43. doi: 10.1182/blood-2002-07-2090.
- [34] Prasad VK, Kurtzberg J. Cord blood and bone marrow transplantation in inherited metabolic diseases: scientific basis, current status and future directions. *Brit J Haematol*. 2010;148:356–72. doi: 10.1111/j.1365-2141.2009.07974.x.
- [35] Boelens JJ, Aldenhoven M, Purtill D, Ruggeri A, Defor T, Wynn R, et al. Outcomes of transplantation using various hematopoietic cell sources in children with Hurler syndrome after myeloablative conditioning. *Blood*. 2013;121:3981–7. doi: 10.1182/blood-2012-09-455238.
- [36] Staba SL, Escolar ML, Poe M, Kim Y, Martin PL, Szabolcs P, et al. Cord-blood transplants from unrelated donors in patients with Hurler's Syndrome. *New Engl J Med*. 2004;350:1960–9. doi: 10.1056/NEJMoa032613.
- [37] Prasad VK, Mendizabal A, Parikh SH, Szabolcs P, Driscoll TA, Page K, et al. Unrelated donor umbilical cord blood transplantation for inherited metabolic disorders in 159 pediatric patients from a single center: influence of cellular composition of the graft on transplantation outcomes. *Blood*. 2008;112:2979–89. doi: 10.1182/blood-2008-03-140830.
- [38] Escolar ML, Poe MD, Provenzale JM, Richards KC, Allison J, Wood S, et al. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. *New Engl J Med*. 2005;352:2069–81. doi: 10.1056/NEJMoa042604.
- [39] Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, et al. Nongonotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat Biotech*. 2016; advance online publication.

10.1038/nbt.3584. <http://www.nature.com/nbt/journal/vaop/ncurrent/abs/nbt.3584.html#supplementary-information>.

- [40] Swirsky DM, Watt SM, Gilmore DJ, Hayhoe FG, Waldmann H. The characterisation of monoclonal antibodies against haemopoietic cells: comparison of an immunoperoxidase method with fluorescence activated cell sorting. *J Immunol Methods*. 1983;61:171–82. doi: 10.1016/0022-1759(83)90160-6.
- [41] Hale G, Hoang T, Prospero T, Watt SM, Waldmann H. Removal of T cells from bone marrow for transplantation. Comparison of rat monoclonal anti-lymphocyte antibodies of different isotypes. *Mol Biol Med*. 1983;1:305–19.
- [42] Glatting G, Muller M, Koop B, Hohl K, Friesen C, Neumaier B, et al. Anti-CD45 monoclonal antibody YAML568: A promising radioimmuno conjugate for targeted therapy of acute leukemia. *J Nucl Med*. 2006;47:1335–41.
- [43] Fucharoen S, Weatherall DJ. Progress toward the control and management of the thalassemias. *Hematol Oncol Clin North Am*. 2016;30:359–71. doi: 10.1016/j.hoc.2015.12.001.
- [44] Piel FB, Weatherall DJ. The alpha-thalassemias. *New Engl J Med*. 2014;371:1908–16. doi: 10.1056/NEJMra1404415.
- [45] Piel FB, Weatherall DJ. Sickle-cell disease: a call to action. *Trans R Soc Trop Med Hyg*. 2015;109(6):355–6. doi: 10.1093/trstmh/trv035.
- [46] Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Org*. 2008;86:480–7. doi: 10.2471/blt.06.036673.
- [47] Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet*. 2013;381:142–51. doi: 10.1016/s0140-6736(12)61229-x.
- [48] WHO. 2016; Available from: www.who.int.
- [49] Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease – life expectancy and risk factors for early death. *New Engl J Med*. 1994;330:1639–44. doi:10.1056/NEJM199406093302303.
- [50] Telfer PT, Warburton F, Christou S, Hadjigavriel M, Sitarou M, Kolnagou A, et al. Improved survival in thalassemia major patients on switching from desferrioxamine to combined chelation therapy with desferrioxamine and deferiprone. *Haematologica*. 2009;94(12):1777–8. doi: 10.3324/haematol.2009.009118.
- [51] Borgna-Pignatti C. The life of patients with thalassemia major. *Haematologica*. 2010;95(3):345–8. doi: 10.3324/haematol.2009.017228
- [52] Roseff SD. Sickle cell disease: a review. *Immunohematology*. 2009;25:67–74.

- [53] Taylor LEV, Stotts NA, Humphreys J, Treadwell MJ, Miaskowski C. A review of the literature on the multiple dimensions of chronic pain in adults with sickle cell disease. *J Pain Sympt Manage*. 2010;40:416–35. doi: 10.1016/j.jpainsymman.2009.12.027.
- [54] Kurtzberg J. To match or not to match in cord blood transplantation: a modern look at a recurring question. *Biol Blood Marrow Transpl*. 2016;22:398–9. doi: 10.1016/j.bbmt.2016.01.020.
- [55] Brunstein CG, Fuchs EJ, Carter SL, Karanes C, Costa LJ, Wu J, et al. Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood*. 2011;118:282–8. doi: 10.1182/blood-2011-03-344853.
- [56] De La Peña I, Sanberg PR, Acosta S, Lin SZ, Borlongan CV. G-CSF as an adjunctive therapy with umbilical cord blood cell transplantation for traumatic brain injury. *Cell Transpl*. 2015;24:447–57. doi: 10.3727/096368915x686913.
- [57] Cotten CM, Murtha AP, Goldberg RN, Grotegut CA, Smith PB, Goldstein RF, et al. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. *J Pediatr*. 2014;164:973–9. doi: 10.1016/j.jpeds.2013.11.036.
- [58] Haller MJ, Wasserfall CH, Hulme MA, Cintron M, Brusko TM, McGrail KM, et al. Autologous umbilical cord blood transfusion in young children with type 1 diabetes fails to preserve C-peptide. *Diabetes Care*. 2011;34(12):2567–9. doi: 10.2337/dc11-1406.
- [59] Huang CJ, Butler AE, Moran A, Rao PN, Wagner JE, Blazar BR, et al. A low frequency of pancreatic islet insulin-expressing cells derived from cord blood stem cell allografts in humans. *Diabetologia*. 2011;54:1066–74. doi: 10.1007/s00125-011-2071-2.
- [60] Darghouth D, Giarratana M-C, Oliveira L, Jolly S, Marie T, Boudah S, et al. Bio-engineered and native red blood cells from cord blood exhibit the same metabolomic profile. *Haematologica*. 2016;44:23. doi: 10.3324/haematol.2015.141465.
- [61] Carpenter L, Malladi R, Yang CT, French A, Pilkington KJ, Forsey RW, et al. Human induced pluripotent stem cells are capable of B-cell lymphopoiesis. *Blood*. 2011;117:4008–11. doi:10.1182/blood-2010-08-299941.
- [62] Moreau T, Evans AL, Vasquez L, Tijssen MR, Yan Y, Trotter MW, et al. Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. *Nat Commun*. 2016;7:11208–23. doi:10.1038/ncomms11208.
- [63] Feng Q, Shabrani N, Thon Jonathan N, Huo H, Thiel A, Machlus Kellie R, et al. Scalable generation of universal platelets from human induced pluripotent stem cells. *Stem Cell Rep*. 2014;3:817–31. doi:10.1016/j.stemcr.2014.09.010.

- [64] Yang CT, French A, Goh PA, Pagnamenta A, Mettananda S, Taylor J, et al. Human induced pluripotent stem cell derived erythroblasts can undergo definitive erythropoiesis and co-express gamma and beta globins. *Brit J Haematol.* 2014;166:435–48. doi: 10.1111/bjh.12910
- [65] Danby R, Rocha V. Improving engraftment and immune reconstitution in umbilical cord blood transplantation. *Front Immunol.* 2014;5:68. doi:10.3389/fimmu.2014.00068.
- [66] Mehta RS, Rezvani K, Olson A, Oran B, Hosing C, Shah N, et al. Novel techniques for ex vivo expansion of cord blood: clinical trials. *Front Med.* 2015 2:89. doi: 10.3389/fmed.2015.00089.
- [67] Brunstein CG, Miller JS, McKenna DH, Hippen KL, DeFor TE, Sumstad D, et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: kinetics, toxicity profile, and clinical effect. *Blood.* 2016;127:1044–51. doi: 10.1182/blood-2015-06-653667.
- [68] Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood.* 2002;100:1611–8. doi: 10.1182/blood-2002-01-0294.
- [69] Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood.* 2010;115:1843–9. doi: 10.1182/blood-2009-07-231068.
- [70] Scaradavou A, Brunstein CG, Eapen M, Le-Rademacher J, Barker JN, Chao N, et al. Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. *Blood.* 2013;121:752–8. doi: 10.1182/blood-2012-08-449108.
- [71] Webb S. Banking on cord blood stem cells. *Nat Biotechnol.* 2013;31:585–8. Doi: 10.1038/nbt.2629.
- [72] Page KM, Mendizabal A, Betz-Stablein B, Wease S, Shoulars K, Gentry T, et al. Optimizing donor selection for public cord blood banking: influence of maternal, infant, and collection characteristics on cord blood unit quality. *Transfusion.* 2014;54:340–52. Doi: 10.1111/trf.12257.
- [73] Guttridge MG, Soh TG, Belfield H, Sidders C, Watt SM. Storage time affects umbilical cord blood viability. *Transfusion.* 2014;54:1278–85. doi: 10.1111/trf.12481.
- [74] Kim S, Kim N, Presson AP, Metzger ME, Bonifacino AC, Sehl M, et al. Dynamics of HSPC repopulation in nonhuman primates revealed by a decade-long clonal-tracking study. *Cell Stem Cell.* 2014;14:473–85. doi: 10.1016/j.stem.2013.12.012.
- [75] Beksac M, Preffer F. Is it time to revisit our current hematopoietic progenitor cell quantification methods in the clinic? *Bone Marrow Transplant.* 2012;47:1391–6. doi: 10.1038/bmt.2011.240.

- [76] Shoulars K, Noldner P, Troy JD, Cheatham L, Parrish A, Page K, et al. Development and validation of a rapid, aldehyde dehydrogenase bright-based cord blood potency assay. *Blood*. 2016;127:2346–54. doi: 10.1182/blood-2015-08-666990.
- [77] Simmons PJ, Robinson SN, Munsell MF, Thomas MW, Javni JA, Brouard N, et al. Expression of a surface antigen (MA6) by peripheral blood CD34+ cells is correlated with improved platelet engraftment and may explain delayed platelet engraftment following cord blood transplantation. *Stem Cells Dev*. 2015;24:1066–72. doi: 10.1089/scd.2014.0439.
- [78] Civin CI. CD34 stem cell stories and lessons from the CD34 wars: the Landsteiner Lecture 2009. *Transfusion*. 2010;50:2046–56. doi: 10.1111/j.1537-2995.2010.02729.x.
- [79] Watt SM, Karhi K, Gatter K, Furley AJ, Katz FE, Healy LE, et al. Distribution and epitope analysis of the cell membrane glycoprotein (HPCA-1) associated with human hemopoietic progenitor cells. *Leukemia*. 1987;1:417–26.
- [80] Piacibello W, Sanavio F, Severino A, Dane A, Gammaitoni L, Fagioli F, et al. Engraftment in non-obese diabetic severe combined immunodeficient mice of human CD34(+) cord blood cells after ex vivo expansion: evidence for the amplification and self-renewal of repopulating stem cells. *Blood*. 1999;93:3736–49.
- [81] Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. *PNAS USA*. 1992;89:2804–8.
- [82] Craig W, Kay R, Cutler RL, Lansdorp PM. Expression of Thy-1 on human hematopoietic progenitor cells. *J Exp Med*. 1993;177:1331–42.
- [83] Hao QL, Shah AJ, Thiemann FT, Smogorzewska EM, Crooks GM. A functional comparison of CD34 + CD38– cells in cord blood and bone marrow. *Blood*. 1995;86:3745–53.
- [84] Mayani H, Dragowska W, Lansdorp PM. Characterization of functionally distinct subpopulations of CD34+ cord blood cells in serum-free long-term cultures supplemented with hematopoietic cytokines. *Blood*. 1993;82:2664–72.
- [85] Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. *Cell Stem Cell*. 2007;1:635–45. doi:10.1016/j.stem.2007.10.001.
- [86] Notta F, Zandi S, Takayama N, Dobson S, Gan OI, Wilson G, et al. Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. *Science*. 2016;351: aab2116. doi: 10.1126/science.aab2116.
- [87] Robinson SN, Thomas MW, Simmons PJ, Lu J, Yang H, Parmar S, et al. Fucosylation with fucosyltransferase VI or fucosyltransferase VII improves cord blood engraftment. *Cytherapy*. 2014;16:84–9. doi:10.1016/j.jcyt.2013.07.003.

- [88] Goardon N, Marchi E, Atzberger A, Quek L, Schuh A, Soneji S, et al. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. *Cancer Cell*. 2011;19:138–52. doi:10.1016/j.ccr.2010.12.012.
- [89] Xia L, McDaniel JM, Yago T, Doeden A, McEver RP. Surface fucosylation of human cord blood cells augments binding to P-selectin and E-selectin and enhances engraftment in bone marrow. *Blood*. 2004;104:3091–6. doi:10.1182/blood-2004-02-0650.
- [90] Hidalgo A, Frenette PS. Enforced fucosylation of neonatal CD34+ cells generates selectin ligands that enhance the initial interactions with microvessels but not homing to bone marrow. *Blood*. 2005;105:567–75. doi:10.1182/blood-2004-03-1026.
- [91] Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science*. 1999;283:845–8.
- [92] Ohno N, Kajiume T, Sera Y, Sato T, Kobayashi M. Short-term culture of umbilical cord blood-derived CD34 cells enhances engraftment into NOD/SCID mice through increased CXCR4 expression. *Stem Cells Dev*. 2009;18(8):1221–6. doi:10.1089/scd.2008.0298.
- [93] Kahn J, Byk T, Jansson-Sjostrand L, Petit I, Shivtiel S, Nagler A, et al. Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. *Blood*. 2004;103(8):2942–9. doi:10.1182/blood-2003-07-2607.
- [94] Lai CY, Yamazaki S, Okabe M, Suzuki S, Maeyama Y, Iimura Y, et al. Stage-specific roles for CXCR4 signaling in murine hematopoietic stem/progenitor cells in the process of bone marrow repopulation. *Stem Cells*. 2014;32:1929–42. doi:10.1002/stem.1670.
- [95] Forde S, Tye BJ, Newey SE, Roubelakis M, Smythe J, McGuckin CP, et al. Endolyn (CD164) modulates the CXCL12-mediated migration of umbilical cord blood CD133+ cells. *Blood*. 2007;109:1825–33. doi:10.1182/blood-2006-05-023028.
- [96] Broxmeyer HE, Capitano M, Campbell TB, Hangoc G, Cooper S. Modulation of hematopoietic chemokine effects In vitro and in vivo by DPP-4/CD26. *Stem Cells Dev*. 2016;25:575–85. doi:10.1089/scd.2016.0026.
- [97] Ou X, O'Leary HA, Broxmeyer HE. Implications of DPP4 modification of proteins that regulate stem/progenitor and more mature cell types. *Blood*. 2013;122(2):161–9. doi:10.1182/blood-2013-02-487470.
- [98] Farag SS, Srivastava S, Messina-Graham S, Schwartz J, Robertson MJ, Abonour R, et al. In vivo DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. *Stem Cells Dev*. 2013;22:1007–15. doi:10.1089/scd.2012.0636.
- [99] Velez de Mendizabal N, Strother RM, Farag SS, Broxmeyer HE, Messina-Graham S, Chitnis SD, et al. Modelling the sitagliptin effect on dipeptidyl peptidase-4 activity in

- adults with haematological malignancies after umbilical cord blood haematopoietic cell transplantation. *Clin Pharm*. 2014;53:247–59. doi:10.1007/s40262-013-0109-y.
- [100] Reza R, Mastellos D, Majka M, Marquez L, Ratajczak J, Franchini S, et al. Functional receptor for C3a anaphylatoxin is expressed by normal hematopoietic stem/progenitor cells, and C3a enhances their homing-related responses to SDF-1. *Blood*. 2003;101:3784–93. doi:10.1182/blood-2002-10-3233.
- [101] Brunstein CG, McKenna DH, DeFor TE, Sumstad D, Paul P, Weisdorf DJ, et al. Complement fragment 3a priming of umbilical cord blood progenitors: safety profile. *Biol Blood Marrow Transpl*. 2013;19:1474–9. doi:10.1016/j.bbmt.2013.07.016.
- [102] Lund TC, Boitano AE, Delaney CS, Shpall EJ, Wagner JE. Advances in umbilical cord blood manipulation—from niche to bedside. *Nature Rev Clin Oncol*. 2015;12:163–74. doi:10.1038/nrclinonc.2014.215.
- [103] Li L, Kim HT, Nellore A, Patsoukis N, Petkova V, McDonough S, et al. Prostaglandin E2 promotes survival of naive UCB T cells via the Wnt/beta-catenin pathway and alters immune reconstitution after UCBT. *Blood Cancer J* 2014;4:e178. doi:10.1038/bcj.2013.75.
- [104] Cutler C, Multani P, Robbins D, Kim HT, Le T, Hoggatt J, et al. Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. *Blood*. 2013;122:3074–81. doi:10.1182/blood-2013-05-503177.
- [105] North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature*. 2007;447:1007–11. doi:10.1038/nature05883.
- [106] Hagedorn EJ, Durand EM, Fast EM, Zon LI. Getting more for your marrow: boosting hematopoietic stem cell numbers with PGE2. *Exp Cell Res*. 2014;329:220–6. doi:10.1016/j.yexcr.2014.07.030.
- [107] Popat U, Mehta RS, Rezvani K, Fox P, Kondo K, Marin D, et al. Enforced fucosylation of cord blood hematopoietic cells accelerates neutrophil and platelet engraftment after transplantation. *Blood*. 2015;125:2885–92. doi:10.1182/blood-2015-01-607366.
- [108] Bradley TR, Metcalf D. The growth of mouse bone marrow cells in vitro. *Aus J Exp Biol Med Sci*. 1966;44:287–99.
- [109] Dexter TM, Allen TD, Lajtha LG, Schofield R, Lord BI. Stimulation of differentiation and proliferation of haemopoietic cells in vitro. *J Cell Physiol*. 1973;82:461–73. doi:10.1002/jcp.1040820315.
- [110] Kohler G, Milstein C. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur J Immunol*. 1976;6:511–9. doi:10.1002/eji.1830060713.
- [111] Csaszar E, Chen K, Caldwell J, Chan W, Zandstra PW. Real-time monitoring and control of soluble signaling factors enables enhanced progenitor cell outputs from human cord blood stem cell cultures. *Biotech Bioeng*. 2014;111:1258–64. doi:10.1002/bit.25163.

- [112] Csaszar E, Kirouac DC, Yu M, Wang W, Qiao W, Cooke MP, et al. Rapid expansion of human hematopoietic stem cells by automated control of inhibitory feedback signaling. *Cell Stem Cell*. 2012;10:218–29. doi:10.1016/j.stem.2012.01.003.
- [113] Rappold I, Watt SM, Kusadasi N, Rose-John S, Hatzfeld J, Ploemacher RE. Gp130-signaling synergizes with FL and TPO for the long-term expansion of cord blood progenitors. *Leukemia*. 1999;13:2036–48.
- [114] Gordon MY, Riley GP, Watt SM, Greaves MF. Compartmentalization of a haematopoietic growth factor (GM-CSF) by glycosaminoglycans in the bone marrow microenvironment. *Nature*. 1987;326:403–5. doi:10.1038/326403a0.
- [115] Gullo F, van der Garde M, Russo G, Pennisi M, Motta S, Pappalardo F, et al. Computational modeling of the expansion of human cord blood CD133+ hematopoietic stem/progenitor cells with different cytokine combinations. *Bioinformatics*. 2015;31:2514–22. doi:10.1093/bioinformatics/btv172.
- [116] Haylock DN, Horsfall MJ, Dowse TL, Ramshaw HS, Niutta S, Protopsaltis S, et al. Increased recruitment of hematopoietic progenitor cells underlies the ex vivo expansion potential of FLT3 ligand. *Blood*. 1997;90(6):2260–72.
- [117] Horwitz ME, Frassoni F. Improving the outcome of umbilical cord blood transplantation through ex vivo expansion or graft manipulation. *Cytotherapy*. 2015;17:730–8. doi:10.1016/j.jcyt.2015.02.004.
- [118] Rowe RG, Mandelbaum J, Zon LI, Daley GQ. Engineering hematopoietic stem cells: lessons from development. *Cell Stem Cell*. 2016;18:707–20. doi:10.1016/j.stem.2016.05.016.
- [119] Yu VW, Scadden DT. Heterogeneity of the bone marrow niche. *Curr Opin Hematol*. 2016;23:331–8. doi:10.1097/moh.0000000000000265.
- [120] Baron F, Ruggeri A, Nagler A. Methods of ex vivo expansion of human cord blood cells: challenges, successes and clinical implications. *Expert Rev Hematol*. 2016;9:297–314. doi:10.1586/17474086.2016.1128321.
- [121] Chaurasia P, Gajzer DC, Schaniel C, D'Souza S, Hoffman R. Epigenetic reprogramming induces the expansion of cord blood stem cells. *J Clin Invest*. 2014;124:2378–95. doi:10.1172/JCI70313.
- [122] Fares I, Chagraoui J, Gareau Y, Gingras S, Ruel R, Mayotte N, et al. Cord blood expansion. pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. *Science*. 2014;345:1509–12. doi:10.1126/science.1256337.
- [123] Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science*. 2010;329:1345–8. doi:10.1126/science.1191536.
- [124] Delaney C, Varnum-Finney B, Aoyama K, Brashem-Stein C, Bernstein ID. Dose-dependent effects of the Notch ligand Delta1 on ex vivo differentiation and in vivo

- marrow repopulating ability of cord blood cells. *Blood*. 2005;106(8):2693–9. doi:10.1182/blood-2005-03-1131.
- [125] Dahlberg A, Woo S, Delaney C, Boyle P, Gnirke A, Bock C. Notch-mediated expansion of cord blood progenitors: maintenance of transcriptional and epigenetic fidelity. 2015;29:1948–51. doi:10.1038/leu.2015.61.
- [126] Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med*. 2010;16:232–6. doi:10.1038/nm.2080.
- [127] Drake AC, Khoury M, Leskov I, Iliopoulou BP, Fragoso M, Lodish H, et al. Human CD34+ CD133+ hematopoietic stem cells cultured with growth factors including Angptl5 efficiently engraft adult NOD-SCID IL2rgamma-/- (NSG) mice. *PLoS One*. 2011;6:e18382. doi:10.1371/journal.pone.0018382.
- [128] Famili F, Brugman MH, Taskesen E, Naber BE, Fodde R, Staal FJ. High levels of canonical Wnt signaling lead to loss of stemness and increased differentiation in hematopoietic stem cells. *Stem Cell Rep*. 2016;6:652–9. doi:10.1016/j.stemcr.2016.04.009.
- [129] Horwitz ME, Chao NJ, Rizzieri DA, Long GD, Sullivan KM, Gasparetto C, et al. Umbilical cord blood expansion with nicotinamide provides long-term multilineage engraftment. *J Clin Invest*. 2014;124:3121–8. doi:10.1172/jci74556.
- [130] Jiang J, Zhao M, Zhang A, Yu M, Lin X, Wu M, et al. Characterization of a GSK-3 inhibitor in culture of human cord blood primitive hematopoietic cells. *Biomed Pharm*. 2010;64:482–6. doi:10.1016/j.biopha.2010.01.005.
- [131] Lechman ER, Gentner B, Ng SW, Schoof EM, van Galen P, Kennedy JA, et al. miR-126 regulates distinct self-renewal outcomes in normal and malignant hematopoietic stem cells. *Cancer Cell*. 2016;29:214–28. doi:10.1016/j.ccell.2015.12.011.
- [132] Peled T, Mandel J, Goudsmid RN, Landor C, Hasson N, Harati D, et al. Pre-clinical development of cord blood-derived progenitor cell graft expanded ex vivo with cytokines and the polyamine copper chelator tetraethylenepentamine. *Cytotherapy*. 2004;6:344–55. doi:10.1080/14653240410004916.
- [133] Piacibello W, Gammaitoni L, Bruno S, Gunetti M, Fagioli F, Cavalloni G, et al. Negative influence of IL3 on the expansion of human cord blood in vivo long-term repopulating stem cells. *J Hematother Stem Cell Res*. 2000;9:945–56. doi:10.1089/152581600750062408.
- [134] Wagner JE, Jr., Brunstein CG, Boitano AE, DeFor TE, McKenna D, Sumstad D, et al. Phase I/II trial of StemRegenin-1 expanded umbilical cord blood hematopoietic stem cells supports testing as a stand-alone graft. *Cell Stem Cell*. 2016;18:144–55. doi:10.1016/j.stem.2015.10.004.
- [135] Zhang CC, Kaba M, Iizuka S, Huynh H, Lodish HF. Angiopoietin-like 5 and IGFBP2 stimulate ex vivo expansion of human cord blood hematopoietic stem cells as assayed

- by NOD/SCID transplantation. *Blood*. 2008;111:3415–23. doi:10.1182/blood-2007-11-122119.
- [136] Oostendorp RA, Gilfillan S, Parmar A, Schiemann M, Marz S, Niemeyer M, et al. Oncostatin M-mediated regulation of KIT-ligand-induced extracellular signal-regulated kinase signaling maintains hematopoietic repopulating activity of Lin-CD34+CD133+ cord blood cells. *Stem Cells*. 2008;26:2164–72. doi:10.1634/stemcells.2007-1049.
- [137] Deng M, Lu Z, Zheng J, Wan X, Chen X, Hirayasu K, et al. A motif in LILRB2 critical for Angptl2 binding and activation. *Blood*. 2014;124:924–35. doi:10.1182/blood-2014-01-549162.
- [138] Farahbakhshian E, Verstegen MM, Visser TP, Kheradmandkia S, Geerts D, Arshad S, et al. Angiopoietin-like protein 3 promotes preservation of stemness during ex vivo expansion of murine hematopoietic stem cells. *PLoS One*. 2014;9:e105642. doi:10.1371/journal.pone.0105642.
- [139] Xiao Y, Jiang Z, Li Y, Ye W, Jia B, Zhang M, et al. ANGPTL7 regulates the expansion and repopulation of human hematopoietic stem and progenitor cells. *Haematologica*. 2015;100:585–94. doi:10.3324/haematol.2014.118612.
- [140] de Lima M, McNiece I, Robinson SN, Munsell M, Eapen M, Horowitz M, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *New Engl J Med*. 2012;367:2305–15. doi:10.1056/NEJMoa1207285.
- [141] Ko KH, Holmes T, Palladinetti P, Song E, Nordon R, O'Brien TA, et al. GSK-3 β inhibition promotes engraftment of ex vivo-expanded hematopoietic stem cells and modulates gene expression. *Stem Cells*. 2011;29:108–18. doi:10.1002/stem.551.
- [142] Huang J, Nguyen-Mccarty M, Hexner EO, Danet-Desnoyers G, Klein PS. Maintenance of hematopoietic stem cells through regulation of Wnt and mTOR pathways. *Nature Med*. 2012;18:1778–85. doi:10.1038/nm.2984.
- [143] Emmrich S, Rasche M, Schöning J, Reimer C, Keihani S, Maroz A, et al. miR-99a/100~125b tricistrons regulate hematopoietic stem and progenitor cell homeostasis by shifting the balance between TGF β and Wnt signaling. *Genes Dev*. 2014;28:858–74. doi:10.1101/gad.233791.113.
- [144] Gao Y, Yang P, Shen H, Yu H, Song X, Zhang L, et al. Small-molecule inhibitors targeting INK4 protein p18(INK4C) enhance ex vivo expansion of haematopoietic stem cells. *Nat Commun*. 2015;6:6328. doi:10.1038/ncomms7328.
- [145] Fares I, Rivest-Khan L, Cohen S, Sauvageau G. Small molecule regulation of normal and leukemic stem cells. *Curr Opin Hematol*. 2015;22(4):309–16. doi:10.1097/moh.000000000000151.
- [146] Balazs AB, Fabian AJ, Esmen CT, Mulligan RC. Endothelial protein C receptor (CD201) explicitly identifies hematopoietic stem cells in murine bone marrow. *Blood*. 2006;107(6):2317–21. doi:10.1182/blood-2005-06-2249.

- [147] Sauvageau M, Sauvageau G. Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer. *Cell Stem Cell*. 2010;7:299–313. doi: 10.1016/j.stem.2010.08.002.
- [148] Chen JY, Miyanishi M, Wang SK, Yamazaki S, Sinha R, Kao KS, et al. Hoxb5 marks long-term haematopoietic stem cells and reveals a homogenous perivascular niche. *Nature*. 2016;530:223–7. doi: 10.1038/nature16943.
- [149] Roche J, Bertrand P. Inside HDACs with more selective HDAC inhibitors. *Eur J Med Chem*. 2016;121:451–83. doi: 10.1016/j.ejmech.2016.05.047.
- [150] Elizalde C, Fernandez-Rueda J, Salcedo JM, Dorronsoro A, Ferrin I, Jakobsson E, et al. Histone deacetylase 3 modulates the expansion of human hematopoietic stem cells. *Stem Cells Dev*. 2012;21:2581–91. doi: 10.1089/scd.2011.0698.
- [151] Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nature Med*. 2010;16:232–6. doi: 10.1038/nm.2080.
- [152] de Lima M, McMannis J, Gee A, Komanduri K, Couriel D, Andersson BS, et al. Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. *Bone Marrow Transplant*. 2008;41:771–8. doi: 10.1038/sj.bmt.1705979.
- [153] Strassel C, Brouard N, Mallo L, Receveur N, Mangin P, Eckly A, et al. Aryl hydrocarbon receptor-dependent enrichment of a megakaryocytic precursor with a high potential to produce proplatelets. *Blood*. 2016;127:2231–40. doi: 10.1182/blood-2015-09-670208.