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REVIEW ARTICLE

Human-induced pluripotent stem cell-derived blood products: state of the art and future directions

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***In vitro* cultured blood cells for transfusion purposes provide a safe alternative to donor blood, particularly for patients who require recurrent transfusions, and can be used as carriers of therapeutic molecules. *In vitro* derivation of hematopoietic cell types from human-induced pluripotent stem cells (iPSCs) allows for a constant, well-defined production pipeline for such advanced therapeutic and medicinal products. Application of selected iPSC-derived hematopoietic stem cells and hematopoietic effector cells in transplantation/transfusions would avoid the risk of alloimmunization and blood-borne diseases, as well as enable the production of enhanced blood cells expressing molecules that enforce blood cell function or endow novel therapeutic properties. Here, we discuss the state of the art approaches to produce erythroid, megakaryoid and myeloid cells from iPSCs and the biological and technical hurdles that we need to overcome prior to therapeutic application.**

Keywords: blood product; differentiation; erythrocyte; erythroid; granulocyte; iPSC; large-scale production; macrophage; megakaryocyte; platelet

iPSCs: from research to large-scale GMP-grade iPSC products

The first human-induced pluripotent stem cell (iPSC) lines were generated in 2007 by Takahashi *et al.* [1] using retro-virus transcription factor (TF) delivery. Subsequent to this breakthrough, iPSC generation and culturing systems greatly improved with respect to delivery of the transgenes (footprint-free), reprogramming conditions (feeder-free, xeno-free), and the large-scale production and differentiation of iPSCs, leading toward clinical use [2–5]. The transition from research-grade to good manufacturing practice (GMP)-grade iPSC derivation and cultivation has taken place in recent years. The technical know-how is available, although the costs involved with respect to producing a large number of GMP-iPSCs has decelerated this process substantially [6,7]. Lately, the aim of autologous patient-specific iPSCs has shifted toward universal iPSCs, which could provide products for larger patient cohorts [8–10]. In the case of *in vitro* blood products, universal blood group-based iPSCs, via selective removal of blood groups or HLA-ABC deficient platelets and myeloid cells, may potentially serve a broad range of patients who can benefit from these novel cellular therapies [11].

Abbreviations
AGM, aorta-gonad-mesonephros; CB, cord blood; CSF, colony-stimuating factor; DOX, doxycycline; EB, embryoid body; EBL, erythroblast; EMP, erythro-myeloid progenitor; ESC, embryonic stem cell; GMP, good manufacturing practice; Hb, hemoglobin; HiDEP, human iPS cell-derived erythroid progenitor; HSC, hematopoietic stem cell; IL, interleukin; imMKCL, immortalized MK cell line; iPSC, induced pluripotent stem cell; MK, megakaryocyte; MPB, mobilized peripheral blood; MΦ, macrophage; PBMC, peripheral blood mononuclear cell; RBC, red blood cell; SCF, stem cell factor; SR-1, stemregenin 1; TF, transcription factor; TPO, thrombopoietin.

iPSC differentiation toward the hematopoietic lineage: distinct waves during ontogeny

Induced pluripotent stem cell and embryonic stem cells (ESCs) are pluripotent stem cells that correspond to cells within the inner cell mass of a blastocyst-stage embryo. The differentiation of these cells toward the hematopoietic lineage mimics blood development from this early embryonic stage. There are three hematopoietic waves during human development, which are spatio-temporally separated: (i) yolk sac-primitive; (ii) yolk sac-definitive with erythro-myeloid progenitors (EMPs), which later migrate to the fetal liver; and (iii) aorta-gonad-mesonephros (AGM)-definitive, producing the first hematopoietic stem cells (HSCs). AGM-HSCs first migrate to the fetal liver, before populating the bone marrow, and supply life-long hematopoiesis [12,13]. The first wave produces primitive red blood cells (RBCs), megakaryocytes (MKs) and macrophages (MΦs) from the hemangioblasts in the yolk sac [14]. Primitive RBCs are larger in size compared to definitive RBCs, enucleate late in circulation and express embryonic globins, whereas primitive MKs have low polyploidization-, low production of enlarged platelet- and low coagulation-potential [15,16]. MΦs that are generated in this first wave are tissue-resident and persist until adulthood [17,18]. The second wave of hematopoiesis arises from EMPs, originating from hemogenic endothelium in the yolk sac [19]. EMPs can produce erythroid, megakaryoid and myeloid cells, but have no long-term engraftment potential [20]. When blood circulation starts, EMPs migrate toward the fetal liver, where they give rise to their progeny. EMP-RBCs do enucleate and express predominantly fetal hemoglobin (Hb). EMP-MKs become polyploid, albeit to a lesser extent compared to adult MKs, and produce less platelets [21]. These first waves are dependent on PU1 transcriptional regulation, whereas MYB, a TF indispensable for HSC-dependent erythropoiesis, is not essential at this stage [18,22]. EMP-MΦs are tissue-resident, and persist throughout adult life via localized self-renewal capability [23]. HSC-RBCs enucleate and their preliminary globin content gradually changes from fetal to adult type. HSC-MKs become highly polyploid up to 128N and produce large amounts of platelets (> 1000).

Hematopoietic *in vitro* differentiation of human iPSCs, considering the current technical knowledge, presumably corresponds mainly to yolk sac primitive/definitive and migrated fetal liver EMP hematopoiesis. Differentiation of iPSCs is commonly initiated by the formation of a 3D-like aggregate structure [embryoid body (EB), ESsac], which is assumed to allow cell–cell contact and spatio-temporal changes that are important factors in cell-type specification. However, monolayer (2D) iPSC differentiations are also able to give rise to all three germ layers and the subsequent structures contain 3D properties. The multitude of slightly different protocols in iPSC differentiation, however, makes it difficult to conclude whether 2D or 3D differentiations give rise to distinct differentiation trajectories or not. Presently, *in vitro* hematopoietic differentiation of iPSCs generally involves the induction of mesoderm using a combination of vascular endothelial growth factor, activin, WNT, basic fibroblast growth factor and bone morphogenetic protein 4. Subsequent differentiations are generally supplemented with hematopoietic growth factors and cytokines, which have been shown to induce hematopoietic differentiation from cord or adult $CD34^+$ HSPC, such as stem cell factor (SCF), interleukin (IL)-3, IL-6, thrombopoietin (TPO) and fms-like tyrosine kinase 3, although the protocols differ slightly in their timing, growth factor combination and concentration.

A major aim of the field is to establish protocols that efficiently generate HSCs *in vitro* with long-term engraftment potential. The capacity of iPSC to yield HSCs would benefit both the direct application in HSC transplantation and improvement of the functionality/potency of produced RBCs, MKs and myeloid cell types.

In this review, we summarize the technical improvements that have taken place in the past 5 years, such that clinically relevant, functional cells of the erythroid/megakaryoid/myeloid lineages are produced using human iPSCs. We also discuss the characteristics and clinical relevance of the end products currently achievable within each of the three lineages.

Erythropoiesis

Spatio-temporally produced RBCs during development have similar roles (oxygen transporter and CO_2 clearance); however, some of their characteristics are site-specific, such as their specific globin subunit composition within Hb, oxygen-binding capacity and enucleation potential. It is important to understand how the different waves of erythropoiesis that occur through development are initiated during iPSC differentiation. The Hb content of the RBCs is generally used to define their developmental stage. Hb contains two α- and two β- globin polypeptides, each bound to an iron-containing heme molecule. Yolk sac primitive RBCs start to express HbZ (embryonic; ζ2ε2), followed by Hbe (embryonic; α2ε2). EMP-RBCs predominantly express HbF (fetal; α2γ2), with varying amounts of Hbe and

HbA (adult), which might relate to its yolk sac or its later progressed existence in fetal liver. HSC-RBCs in the fetal liver express HbF but gradually switch to HbA (HbA1: α2β2 and HbA2: α2δ2) upon migration of hematopoiesis to the bone marrow. EMP-RBCs and AGM-RBCs, at a certain point of ontogeny, both express a high amount of HbF, which renders them indistinguishable based on globin content. Therefore, Hb content alone defining between EMP- vs. AGM-origin at this specific stage is not adequate or possible, such that other discriminatory markers are necessary, but are not yet described in humans. Besides the globin subtypes, other parameters are also site-specific. Primitive erythrocytes enter the circulation as nucleated cells, are much larger than their definitive counterparts and primitive erythroblasts (EBLs) expand with less extent compared to HSC-EBLs. Definitive EBLs have gained the ability to expand in large numbers, and enucleate before entering the circulation. Whether iPSC-derived erythropoiesis follows the course of *in vivo* developmental processes or the transcriptional program is disordered compared to the normal ontogeny is not well known and may also depend on the differentiation protocol. With the current technical knowledge, the derived iPSC-RBCs presumably correspond mainly to yolk sac primitive/definitive and fetal liver EMP-derived erythropoiesis, with low expansion and enucleation potential, with debatable clinical relevance. The field is still seraching for the factor(s) allowing the generation of iPSC-derived erythroid cells via HSCs that have the ability to efficiently enucleate *in vitro*. Different strategies have been utilized aiming to overcome this barrier, leading to the large-scale production of functional *in vitro* RBCs. Here, we focus on the most recent improvements of the iPSC-erythroid field.

Advances in differentiation approaches

Gene editing-free differentiation

In 2015, Dorn *et al*. applied a EB-based method previously reported by in 2010 Lapillone *et al*. [24,25]. The erythroid differentiation potential of five iPSC lines showed ~20–28% enucleated iPSC-RBCs (based on cytospin counts), improving the earlier result of 10% (the method of enucleation calculation was not shown). The iPSC-RBCs were expressing predominantly HbF (relative to the other globin types), some degree of embryonic globins and, in contrast to the earlier report (0% β-globin), 2–7% HbA [24,25]. Their enucleation rate was relatively higher than the previously published methods using iPSCs, which might be a consequence of line to line variations and/or culture condition differences. The way of measuring and calculating the enucleation rate might also result in variance between reports, thus making comparisons between protocols from different laboratories rather difficult. The enucleation potential is generally reported based on reticulocyte count/cytospin or immunocytochemistry slides or based on fluorescence-activated cell sorting assays using DNA staining. For enucleation calculation, some studies used total reticulocyte count, whereas others normalized this to the seeded iPSC number/iPSC-EBL number.

The ectopic expression of lineage-specific key TFs in iPSC is often used to promote lineage-specific differentiation. In the past 5 years, only a few novel methods have been published without the overexpression of certain TFs. Olivier *et al*. [26] introduced an EB-based protocol with the use of small molecules. Inhibition of glycogen synthase kinase 3 by inhibitor VIII, or inhibition of cAMP by 3-isobutyl-1-methylxanthine at early stages of differentiation, resulted in higher cellular yield, as well as improved cell quality and hematopoietic-specific gene expression. We reported a simplified monolayer protocol based on growth factor supplementation, which can give rise to erythroid/megakaryoid/granulocytic cells [27]. Both systems are GMP-compatible, moving toward possible clinical application. These protocols also increased the yield compared to earlier published methods and, most importantly, reached 90–100% erythroid cell purity without any pre-selection. However, irrespective of the methods, the mature RBCs, expressing primarily HbF, showed no improvement in respect of the development stage accompanied by low enucleation.

Other studies have attempted to selectively isolate cells with distinct developmental characteristics during iPSC to erythroid differentiation, aiming to understand the process. In 2016, Fujita *et al*. further developed the ESsac-based [28] system and used CD34 and/or CD235 to distinguish primitive erythroid progenitors ($CD34^-$ or $CD235^+$) from definitive erythroid progenitors ($CD34^+$ or $CD235^-$) derived from ESC/iPSCs [29]. Elevated numbers of $CD45^+$, β-globin- expressing cells, concomitant with lower ε-globin expression, indicated a definitive population that arose during erythroid differentiation of pre-selected cell fractions. The enucleation potential between the two cell fraction was not shown [29]. In 2014 and 2017, Carpenter *et al*. established a feeder-based protocol and suggested a slightly different marker combination to distinguish between definitive progenitors ($CD45^+/CD235^-$) and primitive erythroid cells ($CD31^+/CD235^-$) [30,31]. Differentiation of definitive progeny showed a maturation toward a more definitive erythroid program

comparable to cord blood (CB)-erythroid cells with respect to globin expression [30,31]. However, reticulocyte counts from pre-selected definitive progenitors (~10%) did not differ from the current enucleation ability of iPSC-derived RBCs [32]. To follow definitive erythroid development on the basis of β-globin expression and at a single cell level, a β-globin reporter (GFP^+) iPSC system has been established and combined with single cell RNA sequencing [33]. GFP^- and GFP^+ expressing cells were compared using single cell RNA sequencing following erythroid differentiation, where the GFP^- population predominantly expressed ε- and γ- globins, in contrast to the GFP^+ cells, which expressed lower ε-, elevated γ- globin and some degree of β-globin. Despite the β-globin RNA expression, only ~1% of cells exceeded the GFP detection threshold, suggesting that the cells inefficiently translate the transcripts into protein. The single-cell analysis also revealed that one cell can express all three globin types to different degrees, instead of a heterogeneous population containing cells from distinct waves. The data tend to support the hypothesis that iPSC-erythroid cells correspond to a definitive EMP-derived cell population, with a gradient of the degree of erythroid maturation. Taken together, these studies indicate that iPSC-erythroid cells are able to perform the γ to β globin switch *in vitro*. In addition, they suggest a more confident correspondence of iPSC erythroid differentiation with *in vivo* ontogeny. The enucleation process and potential of human EMP-derived RBCs *in vivo* are largely unknown; therefore, it is difficult to determine whether the low enucleation rate using the above-mentioned system is a result of a technical or biological inefficiency. This further denotes the paramount importance of identifying discriminatory markers between primitive/definitive, EMP/AGM, embryonic/fetal/adult erythropoiesis.

Forward programming/overexpression/knockdown

Comparison of the transcriptome and proteome of iPSC-derived erythroid cells with definitive erythroid cells [cultured from CB and/or mobilized peripheral blood (MPB)/peripheral blood mononuclear cell (PBMC)] revealed the differential expression of various core erythroid genes and proteins. These included major erythroid transcription regulators that are also known to influence globin switching, including a reduced expression of BCL11A, KLF1, SOX6, c-KIT and cMYB [34–38]. In addition, ARID3A, specific to primitive erythropoiesis, was observed to be poorly expressed in adult but strongly expressed in iPSC-erythroid cells. Furthermore, aberrant expression of genes involved in protein degradation, autophagy (VCPIP1, TRIM58) and cell-cycle regulation (PITX1) was seen in iPSC-erythroid cells [38,39]. Most of the proteins involved in the enucleation process occurring via cytoskeleton remodelling, such as TUBB2A, MAP1LC3B, CTNNA1 and MARCKS, were present in iPSC-erythroid cells, although at lower levels compared to adult-erythroid cells, which may explain their impaired enucleation [37]. These studies led to experiments aiming to compensate for the differentially expressing genes/TFs during iPSC-erythroid differentiation using overexpression/forward programming and knockdown approaches.

Although most studies reflect the terminal differentiation potential of erythroid cells, the expansion potential of iPSC-derived EBLs is also significantly lower compared to definitive EBLs, which is currently a limiting factor with respect to establishing a sufficient yield in a cost-effective manner. The expansion potential of human definitive EBLs is dependent on erythropoietin and SCF (c-KIT ligand) and can be extended using glucocorticoid agonists, allowing sufficient numbers for transfusion purposes (although currently at a significant cost) [40,41]. c-KIT overexpression aiming to complement the lack of expression in iPSC-erythroid cells was found to have a positive effect on proliferation compared to the control, but still remained lower compared to definitive erythroid cells [38]. c-KIT expression in definitive erythroid cells is largely dependent on cMYB, for which the expression is also low in iPSC-derived erythroid cells [42]. Instead of ectopic expression of specific proteins that may functionally complement iPSC erythropoiesis (e.g. c-KIT), a better strategy may be to express specific transcriptional regulators that drive major erythroid processes, such as cMYB.

Direct interference in globin subunit expression by overexpressing specific globin regulators has been reported. For example, BCL11A-L overexpression increased HbA1 levels, with a concomitant reduction in HbF, although Hbe expression remained similar [34]. BCL11A-XL and/or KLF1 overexpression also increased the level of β-globin expression in human iPS cell-derived erythroid progenitor (HiDEPs) [43]. Simultaneous with β-globin elevation, the embryonic (ε and ζ) globins were repressed in HiDEPs, suggesting that the same approach might have a beneficial effect with respect to progressing iPSCs to a more definitive erythroid state in terms of globins. However, it should be noted that HiDEPs already express a low level of β-globin in contrast to iPSC-erythroid cells, which may indicate that HiDEPs are developmentally more mature compared to iPSC-erythroid cells [36]. Yang *et al.* [35]

reported a lower overall cell number but better enucleation rate when the expression of KLF1 was induced in iPSCs following hematopoietic commitment. However, in contrast, a more embryonic program was observed in iPSC-erythroid cells upon KLF1 activation, with no alteration in BCL11A expression [35,36]. It was concluded that the interaction between KLF1 and its target genes is a complex process and also that the overexpression of multiple TFs is likely required to enhance a more definitive erythroid program. It should also be noted that strategies aiming to increase HbA at the dispense of HbF or embryonic globins by interfering with globin regulators may not progress the cells to a more definitive/adult program but, instead, increase adult globin expression in an embryonic/fetal state cells. Thus, efforts should not be concentrated on globin switching but rather on driving iPSC-hematopoiesis further with respect to development.

Recently, KLF1 induction was shown to drive iPSC-derived MΦs into an erythroblastic island phenotype [35]. iPSC-erythroid differentiation combined with iPSC-MΦs increased the reticulocyte count from ~4% to 5% and ~6% using KLF1-induced iPSC-MΦs, indicating a slight positive effect of erythroblastic island MΦs in the process, although this does not ultimately solve the iPSC-erythroid differentiation defect [44].

Rouzbeh *et al.* [45] investigated the enucleation process of ESC-erythroid cells (using H1 ESC) and observed that 20 days of EB culture (+25 days terminal differentiation) resulted in 55% enucleated RBCs, whereas 9 days of EB culture resulted in no enucleated cells. Notably, knockdown of miR-30a, showed elevated enucleation to 51% ± 10% in the 9 days of EB culture using H1 ESC and 10% with H9 ESC. Because iPSCs adopt a near ESC phenotype, their differentiation has implications for iPSC performance; however, iPSC-erythroid differentiation normally underperforms compared to that of ESCs; therefore, the EB culture length- and miR-30a knockdown effect remained to be tested for iPSCs. In 2019, Trakarnsanga *et al.* [46] investigated vimentin expression during iPSC-erythroid differentiation and found its expression to stay constant, in contrast to adult erythropoiesis, where vimentin declined during late terminal differentiation and turned off at the time of enucleation. Knocking down vimentin when the majority of cells resembled basophilic EBLs resulted in cell death, highlighting the complexity of the vimentin-related process, which requires further study [46].

Functionality/applicability

As described above, the erythroid differentiation of iPSC follows the waves of ontogeny but currently arrests at a certain time point of development. Enucleation rates are low in accordance with primitive erythropoiesis, even when Hb subtypes suggest definitive erythropoiesis. The question remained as to whether the currently achievable iPSC-RBCs are functionally similar or comparable to adult RBCs to allow eventual use in the clinic. iPSC-derived reticulocytes show lobular phenotypes comparable to definitive-reticulocyte populations, as well as normal contractile actin rings (observed by F-actin staining with confocal microscopy). However, the iPSC-derived cells are fragile and rarely exhibit a biconcave shape (~1 : 10–20, using scanning electron microscopy) [24,37].

iPSC-RBCs contain a mixture of Hbe, HbF and HbA, which might influence normal oxygen transport post-transfusion. Hbe and HbF binds oxygen with greater affinity compared to HbA, which has been proposed to provide the developing embryo with efficient oxygen as a result of enhanced competitive binding during pregnancy. In agreement with the globin expression profiles, iPSC-RBCs exhibit a higher O_2 affinity than adult RBCs. However, they have a similar oxygen-binding capacity compared to CB-RBCs, which contain fetal Hb such as iPSC-RBCs in contrast to adult RBCs [47]. A naturally occurring mutation in a healthy human cohort living with increased HbF or with hereditary persistence of Hb indicates the biological compatibility of HbF-RBCs with normal life [48]. Therefore, partial HbF expression would not be a problem. Indeed, it is considered as a potential therapy for patients with sickle cell disease, as well as for β-thalassemia patients.

The *in vivo* functionality of iPSC-erythroid cells was also investigated by injecting nucleated cells into NOD/SCID-mice. The low number of iPSC-RBCs in the mouse blood (0.3–0.7%, detectable for 4 days) did not allow enucleation to be analysed [47]. However, this study [47,49], as well as work by Doulatov *et al.* [47,49] showed that the predominantly HbF, Hbe-low expressing iPSC-erythroid cells are able to silence embryonic globins and perform the γ- to β- globin switch *in vivo*. These results reported by Doulatov *et al.* [47,49] were obtained using $CD235^+$ human erythroid cells, which were isolated from mouse blood following retro-orbital vein injection of iPSC-erythroid cells. However, Doulatov *et al.* [47,49] analysed $CD235^+/CD71^+$ human erythroid cells isolated from the mouse bone marrow following iPSC-hematopoietic progenitor injection into the femur [47,49]. Furthermore, in later work, Sugimura *et al.* [50], were able to isolate enucleated iPSC-RBCs, after injecting ERG, HOXA5, HOXA9, HOXA10, LCOR, RUNX1 and SPI1 overexpressing nucleated iPSC-hematopoietic

progenitors into NSG mice [50]. These *in vivo* experiments confirmed the relevance of iPSCs as a potential RBC source and suggest technical rather than biological inefficiencies in the process that need to be overcome. Furthermore, the *in vivo* assays indicate that certain factor(s) within the niche are lacking in the *in vitro* cultivation methods. In addition, these tests showed that iPSC-derived erythroid cells have the potential for post-transfusion enucleation. It is arguable whether a nucleated transfusion product is safe and might be feasible in the future because MΦs are likely able to remove the nuclei. Although the human body may have this capacity, it is highly unlikely to occur when needed in a sudden burst immediately after transfusion. In addition, it appears to be risky to rely on post-transfusion enucleation, especially when using a highly carcinogenic origin such as iPSC, making it unlikely to be accepted by regulatory authorities as therapy.

One transfusion unit contains $1–2 \times 10^{12}$ RBCs, with this number being achievable *in vitro* using definitive sources (CB, MPB, PBMC) because their enucleation potential is ~90% [51]. Currently, the cell number/surface ratio is a limiting factor when using these cells, which will require bioreactor settings instead of static cultures. Bioreactors might lead to shear stress for the cells, and this needs to be investigated more elaborately in RBCs to enable the design of RBC-specific bioreactors. The low enucleation potential and instability, as often observed in iPSCs-RBCs, would further elaborate the technical limitations of up-scaling, indicating the need for improvement of iPSC-erythroid differentiation before its actual clinical application can even be considered.

Megakaryopoiesis

Megakaryopoiesis is the process occurring within the bone marrow that leads to the production of platelets. It is crucially dependent on the growth factor TPO and its receptor c-MPL, exemplified by the observation that its knockout in mice results in dramatically reduced platelet counts (> 90%) [52]. During megakaryopoiesis, megakaryoid cells increase their size and become polyploid, at the same time as producing alpha and dense granules that contain the proteins involved in clot formation. Terminally differentiated MKs form cytoplasmic extensions, called pro-platelets, which are shed into the bloodstream, and further mature into platelets. Currently, platelet transfusions still depend on the voluntary donation of blood, although this demand is expected to exceed the supply in the future; thus, alternative sources are needed [53]. To enable *in vitro* platelet production for transfusion products, the processes need to be improved to drive expansion, differentiation and pro-platelet formation in megakaryoid cells at a molecular level [11,27,54–60]. During ontogeny, there are multiple waves of megakaryopoiesis, each harbouring different characteristics, such as cell size, surface marker expression, polyploidization and platelet formation capacity. For example, besides being smaller in size, the polyploidization of primitive/fetal megakaryoid cells is also lower compared to adult MKs [21]. Surprisingly, this does not overtly affect the functionality of the resulting platelets. Currently, the biggest technical challenges of *in vitro* megakaryopoiesis are the expansion of progenitors and the shedding of platelets. Although MKs successfully generate pro-platelet-like extensions *in vitro*, the number of platelet-like particles is relatively modest compared to the thousands of platelets shed by MKs in the bone marrow. In addition, the shedding of platelet surface markers (CD42, GPVI) by proteolysis *in vivo* is related to platelet function and age, and influences platelet clearance [61]. This proteolysis already occurs during platelet production *in vitro* and should always be circumvented throughout this process.

Advances in differentiation approaches

iPSC to MK differentiation

The embryoid body-based method is a commonly applied initiation step in iPSC-MK differentiation. However, in contrast, other systems, such as micro carrier culture or 2D differentiation have shown a superior MK yield of between ~30 and 6900 MK/iPSC [11,27,62]. These systems improved the iPSC-MK output substantially, even compared to $CD34^+$ cultures yielding 10–20 MK/$CD34^+$ cells [27]. Besides the method of differentiation, the composition of the differentiation mix (cytokines, small molecules) can also influence the efficiency of megakaryopoiesis. For example, platelet stimulation by TPO or agonist to c-MPL (TPO receptor) are used in the clinic for some diseases to improve platelet counts (thrombocytopenias, myelodysplastic syndromes) [63,64]. Interestingly, specific agonist binding to c-MPL elicits slightly different downstream signalling. For example, incubation with the c-MPL agonist TA-316 increased self-renewal and maturation of iPSC-MKs by at least two-fold and with a higher platelet yield compared to recombinant human TPO [57]. This specific agonist leads to an iPSC-MK-biased differentiation by upregulating vascular endothelial growth factor-A and basic fibroblast

growth factor, allowing the possibility of regulating fms-like tyrosine kinase 1 and IL-3 signalling in an autocrine-paracrine-based manner [65,66]. Another way of improving megakaryopoiesis is by the utilization of factors that directly alter gene expression known to be important for specific megakaryoid stages. For example, iBET151, which is a bromodomain and extra-terminal motif protein (BET) inhibitor, inhibits the interaction of bromodomain containing TFs with acetylated histones [11]. This compound blocks cMYC and increases GATA1 expression, which are crucial for megakaryoid maturation, leading to a two- to four-fold increase of MKs and decreased $CD14^+$ myeloid cell commitment. Although the efficiency of iPSC-MK differentiation has been improved, these approaches still fall short with respect to providing mature MKs in sufficient numbers for clinical applications.

Forward programming

Long-term production/expansion potential combined with cryobank iPSC-MK progenitors might further increase the obtainability of clinically relevant cell numbers. Exogenous expression of GATA1, FLI1 and TAL1 in iPSCs from the first day of differentiation enables continuous megakaryoblast production for up to 134 days, thereby greatly improving the expansion potential [55], albeit with decreased viability from day 90 onward. These long-term cultures yield 2×10^5 MK/iPSC, with only the addition of TPO and SCF from day 10 onward. Another combination of factors that was used to augment megakaryoid yield from iPSCs comprised doxycycline (DOX) inducible cMYC, BMI1 and BCL-XL [54,67]. DOX-on stimulated the proliferation of megakaryoblast and generated immortalized MK cell lines (imMKCLs), whereas DOX-off within these imMKCL cultures induced the terminal differentiation of MKs. Using these imMKCLs (DOX-on), a 10 000 fold increase in cell number is reached within 15–20 days in a bioreactor setting and this expansion could be extended further for over 70 days. These forward reprogramming approaches enable large-scale MK production, which is crucial for the subsequent *in vitro* thrombopoiesis. However, these strategies involve integrative lentiviral mediated ectopic expression of TFs or transcriptional regulators. Thus, rigorous validation will be needed to ensure the absence of nucleated material before their application in clinical setting can be approved. It would be of interest to understand these transcriptional regulator pathways in their entirety so that they can be activated without the need for ectopic expression. This also has to involve an improvement of the knowledge of the niches that support megakaryopoiesis within the different hematopoietic tissues.

iPSC-MK maturation and thrombopoiesis

In vivo, besides in the bone marrow, MKs have been found to reside and shed platelets within the lungs [68,69]. This might open the possibility of transfusing mature MKs, which would become entrapped inside the lung, where they would shed their platelets, thus nullify the need to generate mature platelets prior to transfusion. Initial transfusion experiments in mice indicate the feasibility of this approach; however, capillary obstruction within the lungs remains a serious risk upon MK injection [69]. In addition, transfused MKs were able to produce ~15 platelets per cell in clodronate-treated mice. To achieve a similar amount of produced platelets compared to a thrombocyte transfusion unit, 6.7×10^9 MKs would need to be injected [69]. The extruded nucleus of these transfused MKs after platelet formation could, however, harbour a potential risk of autoimmune diseases. Additionally, the MK numbers required for this approach are obtained so far only by the overexpression of cMYC, BMI1 and BCL-XL. The introduction of genetically modified nucleated cell into a recipient, however, would further elaborate the potential risk that must be taken into account when considering such methods for therapy application.

Proper MK maturation *in vitro* is essential for an efficient thrombopoiesis. Different cell-surface markers correspond to MK maturation, among which are CD41a and CD42. CD41a is also expressed on hematopoietic progenitor cells and should therefore always be combined with CD42 expression to show megakaryoid commitment. Although high percentages of CD41a are obtained by multiple groups (50–99%), only the inducible cMYC, BMI1 and BCL-XL imMKCLs model achieved almost complete CD42b positivity (98–99.8%; others 30–60%) [11,27,54,55,57–59,62]. Alongside this surface marker combination, polyploidization is another hallmark of MK maturation. Polyploidization rates vary between the *in vivo* MKs that arise from distinct development waves of hematopoiesis [70]. iPSC-MKs exhibit a broad range and degree of polyploidization. Within $CD41a^+$ cells, 38%-55% are > 4N, whereas, based on CD42b, this percentage has a broader spread of between 6% and 51% [55,57–60,62]. This makes it difficult to assess whether this variation in polyploidization is caused by biological (ontogeny) or technical (differentiation method) factors.

Ultimately, mature MKs need to shed (pro)platelets, which is even more challenging during *in vitro* megakaryopoiesis. Because platelets express the same surface markers as MKs, their discrimination has to be carried out based on size. However, distinguishing between cell debris and the actual pro-platelets is rather difficult [54,55,71]. Intracellular biomarkers such as calcium indicators (e.g. calcein) can be used to demonstrate the integrity of the pro-platelet cell membrane. Using this assay, Moreau *et al.* [55] reported 32% of viable platelet-like particles obtained by iPSC differentiation, in contrast to 40% with CB-CD34$^+$-derived pro-platelets. This type of platelet integrity assessment should become a standard in iPSC-derived platelets research/production, and *in vitro* generated platelet concentrates should demonstrate recovery rates comparable to current transfusion standards (~67%) [72].

Currently, the maximum yield of iPSC differentiation is 0–5 pro-platelets/MK [11,55], which is dramatically less compared to *in vivo* definitive-megakaryopoiesis (> 1000/MK) but remarkably close to the *ex vivo* 3–5 pro-platelets production of CD34-MKs [11,55,71]. The addition of small molecules such as the antagonist of the aryl-hydrocarbon receptor, stemregenin 1 (SR-1), can further increase the static production of pro-platelets to ~10/MK in PBMC-CD34$^+$ cultures [71]. This clearly indicates that factors are still lacking from the *in vitro* systems to enable an *in vivo* level of platelet production. One of these factors is presumably shear stress, which leads to increased thrombocyte production [73]. Pro-platelet production can be even increased further with a collagen-based scaffold in a flow bioreactor system to 29 platelets/MK [56]. The combination of turbulence, shear and SR-1 still allowed a reasonable increase in platelet shedding, up to ~70–80 pro-platelets/MK [54]. With these improvements, for the first time, an iPSC-derived thrombocyte transfusion unit (~1×10^{11}) was produced in an 8L bioreactor (three different iPSC clones, range ~$0.5–1.3 \times 10^{11}$). In these turbulence cultures, the enhanced release of IGFBP2, macrophage migration inhibitory factor and nardilysin was reported (using imMKCLs) to positively affect platelet shedding [54]. IGFBP2 and macrophage migration inhibitory factor promote extracellular matrix formation and thereby support cell clustering and pro-platelet elongation, whereas nardilysin mediates the shedding of pro-platelets. The addition of these factors in shear conditions, with low/absent turbulence, improved platelet production [54]. Platelets were stained for annexin V (a marker of apoptosis, staining phosphatidylserine) to identify viable bioreactor produced pro-platelets. Viable platelets comprised 85–95% of the total yield, although static conditions yielded only ~40%, which is in a range similar to that reported previously using calcein staining [54,55].

Functionality/applicability

It remains to be determined whether this relatively large amount of cellular debris causes detrimental immunological or other cellular responses after transfusion; thus, efforts have already focused on purifying the platelets from these *in vitro* cultures. Harvesting of pro-platelets, either by the collection of supernatant or by a hollow fibre module, is needed to generate concentrated transfusion units [54]. Isolated pro-platelets exhibit an ultrastructure similar to that of circulating human platelets (2–3 μm), although being slightly larger in size (2.38–8 μm), with alpha and dense granules [11,54,55]. Combined with these morphological platelet characteristics, key phenotypic surface receptors CD41a/CD42b should also be expressed. Both of these receptors are normally present on the iPSC-derived pro-platelets (70–97%) [11,54]. Importantly, CD42b shedding from the membrane, which is a marker of activation, can be inhibited during *in vitro* platelet generation with selective inhibition of ADAM17 (KP-457) at 37 °C, thereby retaining its high expression [74]. These steps are necessary to generate and store functional *in vitro* cultured platelets.

In vitro spreading, aggregation, clot retraction and activation assays allow the assessment of iPSC pro-platelets effector function. Besides the *in vitro* characteristics, *in vivo* functionality of *in vitro* iPSC pro-platelets also needs to be assessed. Accordingly, pro-platelets were injected into NOD/SCID or NOG mouse. As a result of the rapid depletion (phagocytosis) of human platelets in mice by splenic or liver MΦs [75], these transfusion models were performed in clodronate-treated mice to deplete the mouse MΦs. The circulating iPSC-derived pro-platelets showed a peak in platelet numbers 2 h post-injection, which diminished strongly within the first 24 h, after which platelets were no longer detected [11,54,76]. Interestingly, after transfusion of large-sized iPSC-derived pro-platelets (8 μm), an increase in platelet numbers combined with a size reduction was observed, suggesting that *in vitro* pro-platelets further mature *in vivo* [54]. Importantly, it was also reported that these iPSC-derived pro-platelets actively participated in clot formation *in vivo,* which was comparable to donor-derived platelets [11,54,55]. Taken together, these functional assays confirm that the *in vitro* generation of functional platelets from iPSCs is feasible for clinical application using currently available state of the art techniques.

Further improvement in the platelet yield to *in vivo* standards (> 1000 platelets/MK) with respect to *in vitro* storage and *in vivo* survival is critical, which would greatly contribute to the establishment of these procedures in a cost-effective manner. Standardization of protocols and functional test is required and will help the field to compare and combine the best aspects of each approach. In 2018, the Japanese government approved the start of a clinical trial using imMKCL-platelet concentrates to treat aplastic anaemia patients [77]. Patients will be transfused three times, with a gradually increased amount of platelets, up to a full transfusion unit. The trial will supply data on the post-transfusion survival and function of the *in vitro*-derived platelets, as well as on the safety of such treatments, and hopefully pave the way for their broader application. With the current model systems, additional large-scale compound screening can be realized to test their effects on pro-platelet formation [78]. Lysates of MKs could also be used directly as a source of growth factors for application in regenerative therapies [62]. Furthermore, strategies investigated by Zhang *et al*. [79], in a study that passively packaged functional plasma proteins into iPSC-MK/platelets, could help to increase the potency of *in vitro* platelets by supplying plasma proteins that are not generated *in vitro* but are essential for platelet function. In addition, (passive) loading of specific drugs into *in vitro* platelets, such as doxorubicin, could broaden their therapeutic potential [80].

Myelopoiesis

Myeloid cells are a broad group of specialized cells that include monocytes, MΦs and granulocytes. These cell types all have immune-regulatory functions. However, presently, iPSC-derived myeloid cells are most commonly utilized as disease-models and for drug testing (e.g. Gaucher disease, Alzheimer, Mendelian) and, to a lesser extent, for cellular therapy purposes [81–84]. The interesting origin of tissue-resident MΦs such as microglia, kupffer cells and mammary gland MΦs has been (genetically) tracked to early yolk sac and fetal myelopoiesis [85]. In addition, specific tissue-resident MΦs display self-renewal independent of AGM-HSC bone marrow-derived monocytes [23,86]. The implication of these data for cellular therapy is that such embryonically derived MΦs can be generated by culture systems that recapitulate embryonic development, such as iPSCs. Multiple myeloid differentiation methods have been developed and reported to generate myeloid cells, which are reviewed here.

Advances in differentiation approaches

Myeloid cell differentiation toward monocytes/MΦs has been performed by multiple groups, using both EB or monolayer approaches, with or without the use of a feeder-layer [87–91]. By contrast to iPSC-MK/erythroid differentiation, no overexpression or forward programming strategies are reported to progress iPSC-MΦ differentiation. MΦs can be broadly polarized toward a pro-inflammatory M1 or tissue-supporting M2 type, using granulocyte macrophage-colony-stimulating factor (CSF)/interferon-y or IL-4/IL-10/macrophage-CSF, respectively. Most iPSC differentiations have the capability to produce MΦs for up to 4–5 months [89]. Myeloid cell yield calculations are not normalized to iPSC input but, generally, ~0.5–1 × 10^6 monocytes per well per week of a six-well plate can be achieved [89]. Ackermann *et al*. [92] introduced the first iPSC-MΦ bioreactor utilization. Using a 250-mL reactor, a stable production of ~1–3 × 10^7 MΦ per week could be reached and maintained for up to 3 months. Recently, Cao *et al*. [93] introduced a 2D differentiation method that resulted in a total monocyte yield of ~1.5–2 × 10^7 (~39 monocyte/input iPSC) after 15 days of differentiation, which could be further differentiated to MΦs in 6 days. Alternatively, a combination of the above methods with cryobanking of iPSC-monocytes would be beneficial for reaching clinically relevant numbers of MΦ production by simultaneous differentiation. MΦs also have the potential to be further differentiated toward specific tissue-resident cells such as microglia (using IL34), which reside in the neural compartment. These cells could be potentially used to deliver gene therapy across the blood–brain barrier in neuropathologies. The yield of these microglia cultures, is relatively low, at ~0.5–2 cells per well per week per iPSC input, although there are differences between the yield calculations [94–96]. Because these specific microglia cells are formed only during the first two waves of hematopoietic development, iPSC differentiation also provides a unique system to study their biology, besides their potential use in cellular therapy products [94,95,97].

Induced pluripotent stem cell to granulocyte differentiation has been investigated less intensively compared to the other myeloid cell types. Multiple groups have reported on methods that are EB- or monolayer-based [27,89,98]. EB-based differentiation in a six-well plate can yield up to 0.5 × 10^6 granulocytes per well per week [89]. Our group developed a monolayer granulocyte differentiation that yields ~2 × 10^4 cells/iPSC (~2.5 × 10^6 per 6-cm dish) [27]. Besides the differences in the cell number calculations, the technology (2D vs.

3D) itself does not allow a direct comparison between the two systems. Slight adjustments in the combination of growth factors may allow a better specification for specific myeloid lineages from these differentiation methods (e.g. IL-5 for eosinophils, IL-3 for basophils or granulocyte-CSF for neutrophils) [99].

Functionality/applicability

Induced pluripotent stem cell-derived MΦs are morphologically and ultrastructurally similar to PBMC-derived MΦs (M2). In addition, they have the ability to phagocytose and to produce multiple cytokines (IL-6, IL-1β, tumor necrosus factor α etc.) [89,93]. Within the clinic, iPSC-derived MΦs could be applied in combination with chemotherapy not only to deliver drugs, providing local cytokine production, but also to combat opportunistic multidrug-resistant bacterial infection [100]; for example, during infection with antibiotic-resistant bacteria, such as the *Pseudomonas aeruginosa*. This bacteria is listed by the World Health Organization as one of the three most critical pathogens that urgently require novel treatment options [92,101]. Ackermann *et al*. [92] showed the potential of iPSC-MΦs with respect to clearing *P. aeruginosa* in a pulmonary infection model. In this model, iPSC-MΦ transfusion prevented the onset of acute infection or rescued mice with an established infection. It was reported that these iPSC-MΦs were also exclusively engrafted in the lung, which suggests that they are retained in the targeted organ. Translating this type of intervention into human application would require 1×10^{10} iPSC-MΦs in the case of a patient weighing 60 kg. This is amount of cells that could be achieved using a 40–60-L cell culture production in their small scale bioreactor system (250 mL) [92]. Another potential application of iPSC-MΦs could be in cancer treatment, using genetically engineered cells that overexpress interferon β, which negatively influences angiogenesis in tumors [102,103]. Tissue-specific iPSC-microglia cells could be exploited as delivery vehicles for protein, DNA or mRNA, thereby enabling gene transfer at specific sites through the blood–brain barrier [104].

Induced pluripotent stem cell-generated granulocytes are capable of reactive oxygen species production upon treatment with 4β-phorbol l2-myristate 13-acetate and are able to phagocytose fluorescence-activated cell sorting beads [27,89]. Furthermore, these cells have the potential to migrate toward an IL-8 gradient, albeit this is somewhat attenuated (~50% less) compared to peripheral blood-granulocytes [89]. Lachmann *et al*. [89] also reported iPSC-neutrophil extracellular trap capability comparable to peripheral blood-neutrophils. (iPSC)-Granulocyte transfusion is a promising option for use following HSC transplantation or after chemotherapy aiming to overcome the initial state of immunodeficiency that protects or combats infections [105]. Although this is a promising application, up to this point, the donor-derived granulocyte transfusions did not demonstrate a clear therapeutic benefit [106–108].

Conclusions and perspectives

Donor-derived blood transfusions are the most common form of cellular therapy. Approximately 112 million units of blood are collected worldwide per year and are either transfused directly or processed further for medicinal products, indicating an unmet need for supplementation. *In vitro* produced blood products are an attractive source with respect to overcoming alloimmunization and preventing the transfer of blood-borne diseases, which remains a risk with donor-derived transfusion products. CB- or peripheral blood- stem cells are a commonly used source of *in vitro* blood derivatives for research and for the recent rapidly developing experiments promoting clinical application. However, such sources are still somewhat limited with respect to their donor dependency, which is problematic, especially in rare blood group phenotypes, or in both personalized and universal medicine approaches. Thus, a standardized, immortal source such as iPSCs would provide an alternative solution. Furthermore, they can be also utilized for other purposes, such as local cytokine production, genome editing, gene therapy and, together with large-scale production, as drug delivery vesicles. Focusing on the non-lymphoid blood cells, such as the erythroid, megakaryoid and myeloid lineages derived from iPSCs, considerable improvements have been made. Although iPSC-derived blood products are promising and a few iPSC-blood derivatives are entering clinical trials (e.g. T-cells/platelets), there are still various hurdles to overcome for most of the blood lineages. Progression is somewhat limited by the different protocols and procedures currently employed within different laboratories, making comparisons difficult. Platelet production from iPSC-MKs can currently yield a full thrombocyte transfusion unit (first clinical trial is in progress), although the yield could still be improved further to *in vivo* quantities. Up-scale experiments of iPSC-MΦs suggest the feasibility of large-scale production; however, this remains to be tested. Notably, iPSC-MΦs offer more broad application possibilities compared to MKs or RBCs as a result of their specific and specialized role within

tissues. Technical improvements to culture-specific MΦs may be needed, however, which could involve complex niche-related environments because most monocytic-derived MΦs mature within their respective tissues.

Currently, iPSC-RBCs have the most challenges to overcome, both biological and technical. Their embryonic/fetal Hb expression, together with limited enucleation and reticulocyte yield and stability, requires more basic research before clinical translation is feasible. A more broad understanding of the underlying processes that drive iPSC-hematopoietic differentiation to developmentally more mature (adult type) erythrocytes would be a significant improvement.

To move all of these approaches toward the clinic, as discussed above, GMP facilities using GMP-grade materials are needed. This will undoubtedly further increase the costs of any product derived from iPSCs. Such GMP-grade production is momentary unaffordable for many research teams/clinics, hampering clinical translation. The gap between research and clinic should be bridged with the support of the (pharmaceutical) industry (e.g. bioreactors, recombinant growth factors production). In addition, clear guidelines regarding the use of iPSC-derived products have to be formulated to enable transparent legislation. This would ultimately lead to more clinical trials and the translation of iPSC-derived cellular products to the clinic. In conclusion, blood products derived from iPSCs have the potential to support future donor-derived medical activities, whereas novel application incentives can greatly broaden their applicability.

References

1 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K and Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872.

2 Wang Y, Chou BK, Dowey S, He C, Gerecht S and Cheng L (2013) Scalable expansion of human induced pluripotent stem cells in the defined xeno-free E8 medium under adherent and suspension culture conditions. *Stem Cell Res* **11**, 1103–1116.

3 Kropp C, Kempf H, Halloin C, Robles-Diaz D, Franke A, Scheper T, Kinast K, Knorpp T, Joos TO, Haverich A *et al.* (2016) Impact of feeding strategies on the scalable expansion of human pluripotent stem cells in single-use stirred tank bioreactors. *Stem Cells Transl Med* **5**, 1289–1301.

4 Badenes SM, Fernandes TG, Cordeiro CS, Boucher S, Kuninger D, Vemuri MC, Diogo MM and Cabral JM (2016) Defined essential 8 medium and vitronectin efficiently support scalable xeno-free expansion of human induced pluripotent stem cells in stirred microcarrier culture systems. *PLoS One* **11**, e0151264.

5 Sullivan S, Stacey GN, Akazawa C, Aoyama N, Baptista R, Bedford P, Bennaceur Griscelli A, Chandra A, Elwood N, Girard M *et al.* (2018) Quality control guidelines for clinical-grade human induced pluripotent stem cell lines. *Regen Med* **13**, 859–866.

6 Haase A, Glienke W, Engels L, Göhring G, Esser R, Arseniev L and Martin U (2019) GMP-compatible manufacturing of three iPS cell lines from human peripheral blood. *Stem Cell Res* **35**, 101394.

7 Baghbaderani BA, Tian X, Neo BH, Burkall A, Dimezzo T, Sierra G, Zeng X, Warren K, Kovarcik DP, Fellner T *et al.* (2015) cGMP-manufactured human induced pluripotent stem cells are available for pre-clinical and clinical applications. *Stem Cell Rep* **5**, 647–659.

8 Mattapally S, Pawlik KM, Fast VG, Zumaquero E, Lund FE, Randall TD, Townes TM and Zhang J (2018) Human leukocyte antigen class I and II knockout human induced pluripotent stem cell-derived cells: universal donor for cell therapy. *J Am Heart Assoc* **7**, e010239.

9 Jang Y, Choi J, Park N, Kang J, Kim M, Kim Y and Ju JH (2019) Development of immunocompatible pluripotent stem cells via CRISPR-based human leukocyte antigen engineering. *Experiment Mol Med* **51**, 3.

10 Lee S, Huh JY, Turner DM, Lee S, Robinson J, Stein JE, Shim SH, Hong CP, Kang MS, Nakagawa M *et al.* (2018) Repurposing the cord blood bank for haplobanking of HLA-homozygous iPSCs and their usefulness to multiple populations. *Stem Cells* **36**, 1552–1566.

11 Feng Q, Shabrani N, Thon JN, Huo H, Thiel A, Machlus KR, Kim K, Brooks J, Li F, Luo C *et al.* (2014) Scalable generation of universal platelets from human induced pluripotent stem cells. *Stem Cell Rep* **3**, 817–831.

12 de Bruijn MF, Speck NA, Peeters MC and Dzierzak E (2000) Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. *EMBO J* **19**, 2465–2474.

13 de Bruijn MF, Ma X, Robin C, Ottersbach K, Sanchez MJ and Dzierzak E (2002) Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. *Immunity* **16**, 673–683.

14 Palis J (2016) Hematopoietic stem cell-independent hematopoiesis: emergence of erythroid, megakaryocyte, and myeloid potential in the mammalian embryo. *FEBS Lett* **590**, 3965–3974.

15 Kingsley PD, Malik J, Emerson RL, Bushnell TP, McGrath KE, Bloedorn LA, Bulger M and Palis J (2006) "Maturational" globin switching in primary primitive erythroid cells. *Blood* **107**, 1665–1672.

16 Tober J, Koniski A, McGrath KE, Vemishetti R, Emerson R, de Mesy-Bentley KK, Waugh R and Palis J (2007) The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. *Blood* **109**, 1433–1441.
17 Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, Beaudin AE, Lum J, Low I, Forsberg EC *et al.* (2015) C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* **42**, 665–678.
18 Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, Garner H, Trouillet C, de Bruijn MF, Geissmann F *et al.* (2015) Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* **518**, 547–551.
19 McGrath KE, Frame JM, Fegan KH, Bowen JR, Conway SJ, Catherman SC, Kingsley PD, Koniski AD and Palis J (2015) Distinct sources of hematopoietic progenitors emerge before HSCs and provide functional blood cells in the mammalian embryo. *Cell Rep* **11**, 1892–1904.
20 Muller AM, Medvinsky A, Strouboulis J, Grosveld F and Dzierzak E (1994) Development of hematopoietic stem cell activity in the mouse embryo. *Immunity* **1**, 291–301.
21 de Alarcon PA and Graeve JL (1996) Analysis of megakaryocyte ploidy in fetal bone marrow biopsies using a new adaptation of the feulgen technique to measure DNA content and estimate megakaryocyte ploidy from biopsy specimens. *Pediatr Res* **39**, 166–170.
22 Chen MJ, Yokomizo T, Zeigler BM, Dzierzak E and Speck NA (2009) Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature* **457**, 887–891.
23 Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, Brija T, Gautier EL, Ivanov S, Satpathy AT *et al.* (2014) Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* **40**, 91–104.
24 Dorn I, Klich K, Arauzo-Bravo MJ, Radstaak M, Santourlidis S, Ghanjati F, Radke TF, Psathaki OE, Hargus G, Kramer J *et al.* (2015) Erythroid differentiation of human induced pluripotent stem cells is independent of donor cell type of origin. *Haematologica* **100**, 32–41.
25 Lapillonne H, Kobari L, Mazurier C, Tropel P, Giarratana MC, Zanella-Cleon I, Kiger L, Wattenhofer-Donzé M, Puccio H and Hebert N *et al.* (2010) Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine. *Haematologica* **95**, 1651–1659.
26 Olivier EN, Marenah L, McCahill A, Condie A, Cowan S and Mountford JC (2016) High-efficiency serum-free feeder-free erythroid differentiation of human pluripotent stem cells using small molecules. *Stem Cells Transl Med* **5**, 1394–1405.
27 Hansen M, Varga E, Aarts C, Wust T, Kuijpers T, von Lindern M and van den Akker E (2018) Efficient production of erythroid, megakaryocytic and myeloid cells, using single cell-derived iPSC colony differentiation. *Stem Cell Res* **29**, 232–244.
28 Takayama N, Nishikii H, Usui J, Tsukui H, Sawaguchi A, Hiroyama T, Eto K and Nakauchi H (2008) Generation of functional platelets from human embryonic stem cells *in vitro* via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors. *Blood* **111**, 5298–5306.
29 Fujita A, Uchida N, Haro-Mora JJ, Winkler T and Tisdale J (2016) beta-globin-expressing definitive erythroid progenitor cells generated from embryonic and induced pluripotent stem cell-derived sacs. *Stem Cells* **34**, 1541–1552.
30 Razaq MA, Taylor S, Roberts DJ and Carpenter L (2017) A molecular roadmap of definitive erythropoiesis from human induced pluripotent stem cells. *Br J Haematol* **176**, 971–983.
31 Yang CT, French A, Goh PA, Pagnamenta A, Mettananda S, Taylor J, Knight S, Nathwani A, Roberts DJ, Watt SM *et al.* (2014) Human induced pluripotent stem cell derived erythroblasts can undergo definitive erythropoiesis and co-express gamma and beta globins. *Br J Haematol* **166**, 435–448.
32 Zhang Y, Wang C, Wang L, Shen B, Guan X, Tian J, Ren Z, Ding X, Ma Y, Dai W *et al.* (2017) Large-scale *ex vivo* generation of human red blood cells from cord blood CD34(+) cells. *Stem Cells Transl Med* **6**, 1698–1709.
33 Vanuytsel K, Matte T, Leung A, Naing ZH, Morrison T, Chui DHK, Steinberg MH and Murphy GJ (2018) Induced pluripotent stem cell-based mapping of beta-globin expression throughout human erythropoietic development. *Blood Adv* **2**, 1998–2011.
34 Ochi K, Takayama N, Hirose S, Nakahata T, Nakauchi H and Eto K (2014) Multicolor staining of globin subtypes reveals impaired globin switching during erythropoiesis in human pluripotent stem cells. *Stem Cells Transl Med* **3**, 792–800.
35 Yang CT, Ma R, Axton RA, Jackson M, Taylor AH, Fidanza A, Marenah L, Frayne J, Mountford JC and Forrester LM (2017) Activation of KLF1 enhances the differentiation and maturation of red blood cells from human pluripotent stem cells. *Stem Cells* **35**, 886–897.
36 Trakarnsanga K, Wilson MC, Lau W, Singleton BK, Parsons SF, Sakuntanaga P, Kurita R, Nakamura Y, Anstee DJ and Frayne J (2014) Induction of adult levels of beta-globin in human erythroid cells that intrinsically express embryonic or fetal globin by

transduction with KLF1 and BCL11A-XL. *Haematologica* **99**, 1677–1685.
37 Trakarnsanga K, Wilson MC, Griffiths RE, Toye AM, Carpenter L, Heesom KJ, Parsons SF, Anstee DJ and Frayne J (2014) Qualitative and quantitative comparison of the proteome of erythroid cells differentiated from human iPSCs and adult erythroid cells by multiplex TMT labelling and nanoLC-MS/MS. *PLoS One* **9**, e100874.
38 Merryweather-Clarke AT, Tipping AJ, Lamikanra AA, Fa R, Abu-Jamous B, Tsang HP, Carpenter L, Robson KJ, Nandi AK and Roberts DJ (2016) Distinct gene expression program dynamics during erythropoiesis from human induced pluripotent stem cells compared with adult and cord blood progenitors. *BMC Genom* **17**, 817.
39 Kingsley PD, Greenfest-Allen E, Frame JM, Bushnell TP, Malik J, McGrath KE, Stoeckert CJ and Palis J (2013) Ontogeny of erythroid gene expression. *Blood* **121**, e5–e13.
40 van den Akker E, Satchwell TJ, Pellegrin S, Daniels G and Toye AM (2010) The majority of the *in vitro* erythroid expansion potential resides in CD34(-) cells, outweighing the contribution of CD34(+) cells and significantly increasing the erythroblast yield from peripheral blood samples. *Haematologica* **95**, 1594–1598.
41 Leberbauer C, Boulmé F, Unfried G, Huber J, Beug H and Müllner EW (2005) Different steroids co-regulate long-term expansion versus terminal differentiation in primary human erythroid progenitors. *Blood* **105**, 85–94.
42 Vegiopoulos A, Garcia P, Emambokus N and Frampton J (2006) Coordination of erythropoiesis by the transcription factor c-Myb. *Blood* **107**, 4703–4710.
43 Kurita R, Suda N, Sudo K, Miharada K, Hiroyama T, Miyoshi H, Tani K and Nakamura Y (2013) Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated red blood cells. *PLoS One* **8**, e59890.
44 Lopez-Yrigoyen M, Yang C-T, Fidanza A, Cassetta L, Taylor AH, McCahill A, Sellink E, von Lindern M, van den Akker E, Mountford JC *et al*. (2019) Genetic programming of macrophages generates an *in vitro* model for the human erythroid island niche. *Nat Commun* **10**, 881.
45 Rouzbeh S, Kobari L, Cambot M, Mazurier C, Hebert N, Faussat AM, Durand C, Douay L and Lapillonne H (2015) Molecular signature of erythroblast enucleation in human embryonic stem cells. *Stem Cells* **33**, 2431–2441.
46 Trakarnsanga K, Ferguson D, Daniels DE, Griffiths RE, Wilson MC, Mordue KE, Gartner A, Andrienko TN, Calvert A and Condie A (2019) Vimentin expression is retained in erythroid cells differentiated from human iPSC and ESC and indicates dysregulation in these cells early in differentiation. *Stem Cell Res Ther* **10**, 130.
47 Kobari L, Yates F, Oudrhiri N, Francina A, Kiger L, Mazurier C, Rouzbeh S, El-Nemer W, Hebert N, Giarratana MC *et al*. (2012) Human induced pluripotent stem cells can reach complete terminal maturation: *in vivo* and *in vitro* evidence in the erythropoietic differentiation model. *Haematologica* **97**, 1795–1803.
48 Borg J, Papadopoulos P, Georgitsi M, Gutiérrez L, Grech G, Fanis P, Phylactides M, Verkerk AJ, van der Spek PJ, Scerri CA *et al*. ((2010) Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. *Nat Genet* **42**, 801–805.
49 Doulatov S, Vo LT, Chou SS, Kim PG, Arora N, Li H, Hadland BK, Bernstein ID, Collins JJ, Zon LI *et al*. (2013) Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage-restricted precursors. *Cell Stem Cell* **13**, 459–470.
50 Sugimura R, Jha DK, Han A, Soria-Valles C, da Rocha EL, Lu YF, Goettel JA, Serrao E, Rowe RG, Malleshaiah M *et al*. (2017) Haematopoietic stem and progenitor cells from human pluripotent stem cells. *Nature* **545**, 432–438.
51 Heideveld E, Burger P, Thiel-Valkhof M, Sellink E, Heshusius S, Visser A, von Lindern M and van den Akker E (2018) The Role of Macrophages in Human Erythropoiesis, University of Amsterdam.
52 Murone M, Carpenter DA and de Sauvage FJ (1998) Hematopoietic deficiencies in c-mpl and TPO knockout mice. *Stem Cells* **16**, 1–6.
53 O'Brien KL, Mohammed M and Uhl L (2018) Management of a hospital transfusion service during a nationwide blood product shortage. *Arch Pathol Lab Med* **142**, 779–781.
54 Ito Y, Nakamura S, Sugimoto N, Shigemori T, Kato Y, Ohno M, Sakuma S, Ito K, Kumon H, Hirose H *et al*. (2018) Turbulence activates platelet biogenesis to enable clinical scale *ex vivo* production. *Cell* **174**, 636–648. e618
55 Moreau T, Evans AL, Vasquez L, Tijssen MR, Yan Y, Trotter MW, Howard D, Colzani M, Arumugam M, Wu WH *et al*. (2016) Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. *Nat Commun* **7**, 11208.
56 Shepherd JH, Howard D, Waller AK, Foster HR, Mueller A, Moreau T, Evans AL, Arumugam M, Bouët Chalon G, Vriend E *et al*. (2018) Structurally graduated collagen scaffolds applied to the *ex vivo* generation of platelets from human pluripotent stem cell-derived megakaryocytes: enhancing production and purity. *Biomaterials* **182**, 135–144.

57 Aihara A, Koike T, Abe N, Nakamura S, Sawaguchi A, Nakamura T, Sugimoto N, Nakauchi H, Nishino T, Eto K *et al.* (2017) Novel TPO receptor agonist TA-316 contributes to platelet biogenesis from human iPS cells. *Blood Adv* **1**, 468–476.
58 Eicke D, Baigger A, Schulze K, Latham SL, Halloin C, Zweigerdt R, Guzman CA, Blasczyk R and Figueiredo C (2018) Large-scale production of megakaryocytes in microcarrier-supported stirred suspension bioreactors. *Sci Rep* **8**, 10146.
59 Borger AK, Eicke D, Wolf C, Gras C, Aufderbeck S, Schulze K, Engels L, Eiz-Vesper B, Schambach A, Guzman CA *et al.* (2016) Generation of HLA-Universal iPSC-derived megakaryocytes and platelets for survival under refractoriness conditions. *Mol Med* **22**, 274–285.
60 Liu Y, Wang Y, Gao Y, Forbes JA, Qayyum R, Becker L, Cheng L and Wang ZZ (2015) Efficient generation of megakaryocytes from human induced pluripotent stem cells using food and drug administration-approved pharmacological reagents. *Stem Cells Transl Med* **4**, 309–319.
61 Gardiner EE and Andrews RK (2014) Platelet receptor expression and shedding: glycoprotein Ib-IX-V and glycoprotein VI. *Transfus Med Rev* **28**, 56–60.
62 Baigger A, Eicke D, Yuzefovych Y, Pogozhykh D, Blasczyk R and Figueiredo C (2018) Characterization of induced pluripotent stem cell-derived megakaryocyte lysates for potential regenerative applications. *J Cell Mol Med* **22**, 4545–4549.
63 Brierley CK and Steensma DP (2015) Thrombopoiesis-stimulating agents and myelodysplastic syndromes. *Br J Haematol* **169**, 309–323.
64 Rodeghiero F, Pecci A and Balduini CL (2018) Thrombopoietin receptor agonists in hereditary thrombocytopenias. *J Thromb Haemost* **16**, 1700–1710.
65 Bruno E, Cooper RJ, Wilson EL, Gabrilove JL and Hoffman R (1993) Basic fibroblast growth factor promotes the proliferation of human megakaryocyte progenitor cells. *Blood* **82**, 430–435.
66 Casella I, Feccia T, Chelucci C, Samoggia P, Castelli G, Guerriero R, Parolini I, Petrucci E, Pelosi E, Morsilli O *et al.* (2003) Autocrine-paracrine VEGF loops potentiate the maturation of megakaryocytic precursors through Flt1 receptor. *Blood* **101**, 1316–1323.
67 Hirose S, Takayama N, Nakamura S, Nagasawa K, Ochi K, Hirata S, Yamazaki S, Yamaguchi T, Otsu M, Sano S *et al.* (2013) Immortalization of erythroblasts by c-MYC and BCL-XL enables large-scale erythrocyte production from human pluripotent stem cells. *Stem Cell Rep* **1**, 499–508.
68 Lefrancais E, Ortiz-Muñoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, Thornton EE, Headley MB, David T, Coughlin SR *et al.* (2017) The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature* **544**, 105–109.
69 Wang Y, Hayes V, Jarocha D, Sim X, Harper DC, Fuentes R, Sullivan SK, Gadue P, Chou ST, Torok-Storb BJ *et al.* (2015) Comparative analysis of human *ex vivo*-generated platelets vs megakaryocyte-generated platelets in mice: a cautionary tale. *Blood* **125**, 3627–3636.
70 Elagib KE, Brock AT and Goldfarb AN (2018) Megakaryocyte ontogeny: clinical and molecular significance. *Exp Hematol* **61**, 1–9.
71 Strassel C, Brouard N, Mallo L, Receveur N, Mangin P, Eckly A, Bieche I, Tarte K, Gachet C and Lanza F (2016) Aryl hydrocarbon receptor-dependent enrichment of a megakaryocytic precursor with a high potential to produce proplatelets. *Blood* **127**, 2231–2240.
72 Slichter SJ, Corson J, Jones MK, Christoffel T, Pellham E, Bailey SL and Bolgiano D (2014) Exploratory studies of extended storage of apheresis platelets in a platelet additive solution (PAS). *Blood* **123**, 271–280.
73 Jiang J, Woulfe DS and Papoutsakis ET (2014) Shear enhances thrombopoiesis and formation of microparticles that induce megakaryocytic differentiation of stem cells. *Blood* **124**, 2094–2103.
74 Hirata S, Murata T, Suzuki D, Nakamura S, Jono-Ohnishi R, Hirose H, Sawaguchi A, Nishimura S, Sugimoto N and Eto K (2017) Selective Inhibition of ADAM17 Efficiently Mediates Glycoprotein Ibalpha Retention During *Ex Vivo* Generation of Human Induced Pluripotent Stem Cell-Derived Platelets. *Stem Cells Transl Med* **6**, 720–730.
75 Hu Z and Yang YG (2012) Full reconstitution of human platelets in humanized mice after macrophage depletion. *Blood* **120**, 1713–1716.
76 Moreau P, Leblond V, Bourquelot P, Facon T, Huynh A, Caillot D, Hermine O, Attal M, Hamidou M, Nedellec G *et al.* (1998) Prognostic factors for survival and response after high-dose therapy and autologous stem cell transplantation in systemic AL amyloidosis: a report on 21 patients. *Br J Haematol* **101**, 766–769.
77 NEWS K (2018) Japan Approves Blood Transfusions Including Platelets from iPS Cells. https://english.kyodonews.net/news/2018/09/78505251bf3f-japan-approves-blood-transfusions-including-platelets-from-ips-cells.html.
78 Seo H, Chen SJ, Hashimoto K, Endo H, Nishi Y, Ohta A, Yamamoto T, Hotta A, Sawaguchi A, Hayashi H *et al.* (2018) A beta1-tubulin-based megakaryocyte maturation reporter system identifies novel drugs that promote platelet production. *Blood Adv* **2**, 2262–2272.
79 Zhang N and Newman PJ (2019) Packaging functionally important plasma proteins into the alpha-

granules of human-induced pluripotent stem cell-derived megakaryocytes. *J Tissue Eng Regen Med* **13**, 244–252.
80 Xu P, Zuo H, Chen Wang R, Ahmed A, Hu Y and Ouyang J (2017) Doxorubicin-loaded platelets as a smart drug delivery system: an improved therapy for lymphoma. *Sci Rep* **7**, 42632.
81 Sgambato JA, Park TS, Miller D, Panicker LM, Sidransky E, Lun Y, Awad O, Bentzen SM, Zambidis ET and Feldman RA (2015) Gaucher disease-induced pluripotent stem cells display decreased erythroid potential and Aberrant Myelopoiesis. *Stem Cells Transl Med* **4**, 878–886.
82 Aflaki E, Borger DK, Moaven N, Stubblefield BK, Rogers SA, Patnaik S, Schoenen FJ, Westbroek W, Zheng W, Sullivan P *et al*. (2016) A new glucocerebrosidase chaperone reduces alpha-synuclein and glycolipid levels in iPSC-derived dopaminergic neurons from patients with Gaucher disease and parkinsonism. *J Neurosci* **36**, 7441–7452.
83 Takamatsu K, Ikeda T, Haruta M, Matsumura K, Ogi Y, Nakagata N, Uchino M, Ando Y, Nishimura Y, Senju S *et al*. (2014) Degradation of amyloid beta by human induced pluripotent stem cell-derived macrophages expressing Neprilysin-2. *Stem Cell Res* **13**, 442–453.
84 Zhang H, Xue C, Shah R, Bermingham K, Hinkle CC, Li W, Rodrigues A, Tabita-Martinez J, Millar JS, Cuchel M *et al*. (2015) Functional analysis and transcriptomic profiling of iPSC-derived macrophages and their application in modeling Mendelian disease. *Circ Res* **117**, 17–28.
85 Gomez Perdiguero E, Schulz C and Geissmann F (2013) Development and homeostasis of "resident" myeloid cells: the case of the microglia. *Glia* **61**, 112–120.
86 Jappinen N, Félix I, Lokka E, Tyystjärvi S, Pynttäri A, Lahtela T, Gerke H, Elima K, Rantakari P, Salmi M *et al*. (2019) Fetal-derived macrophages dominate in adult mammary glands. *Nat Commun* **10**, 281.
87 Duan F, Huang R, Zhang F, Zhu Y, Wang L, Chen X, Bai L, Guo W, Chang SC, Hu X *et al*. (2018) Biphasic modulation of insulin signaling enables highly efficient hematopoietic differentiation from human pluripotent stem cells. *Stem Cell Res Ther* **9**, 205.
88 Yeung ATY, Hale C, Lee AH, Gill EE, Bushell W, Parry-Smith D, Goulding D, Pickard D, Roumeliotis T, Choudhary J *et al*. (2017) Exploiting induced pluripotent stem cell-derived macrophages to unravel host factors influencing Chlamydia trachomatis pathogenesis. *Nat Commun* **8**, 15013.
89 Lachmann N, Ackermann M, Frenzel E, Liebhaber S, Brennig S, Happle C, Hoffmann D, Klimenkova O, Lüttge D, Buchegger T *et al*. (2015) Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies. *Stem Cell Rep* **4**, 282–296.
90 Mukherjee C, Hale C and Mukhopadhyay S (2018) A simple multistep protocol for differentiating human induced pluripotent stem cells into functional macrophages. *Methods Mol Biol* **1784**, 13–28.
91 Brault J, Goutagny E, Telugu N, Shao K, Baquié M, Satre V, Coutton C, Grunwald D, Brion JP, Barlogis V *et al*. (2014) Optimized generation of functional neutrophils and macrophages from patient-specific induced pluripotent stem cells: *ex vivo* models of X(0)-linked, AR22(0)- and AR47(0)- chronic granulomatous diseases. *BioRes Open Access* **3**, 311–326.
92 Ackermann M, Kempf H, Hetzel M, Hesse C, Hashtchin AR, Brinkert K, Schott JW, Haake K, Kühnel MP, Glage S *et al*. (2018) Bioreactor-based mass production of human iPSC-derived macrophages enables immunotherapies against bacterial airway infections. *Nat Commun* **9**, 5088.
93 Cao X, Yakala GK, van den Hil FE, Cochrane A, Mummery CL and Orlova VV (2019) Differentiation and functional comparison of monocytes and macrophages from hiPSCs with peripheral blood derivatives. *Stem Cell Rep* **12**, 1282–1297.
94 Douvaras P, Sun B, Wang M, Kruglikov I, Lallos G, Zimmer M, Terrenoire C, Zhang B, Gandy S, Schadt E *et al*. (2017) Directed differentiation of human pluripotent stem cells to microglia. *Stem Cell Rep* **8**, 1516–1524.
95 Muffat J, Li Y, Yuan B, Mitalipova M, Omer A, Corcoran S, Bakiasi G, Tsai LH, Aubourg P, Ransohoff RM *et al*. (2016) Efficient derivation of microglia-like cells from human pluripotent stem cells. *Nat Med* **22**, 1358–1367.
96 Abud EM, Ramirez RN, Martinez ES, Healy LM, Nguyen CHH, Newman SA, Yeromin AV, Scarfone VM, Marsh SE, Fimbres C *et al*. (2017) iPSC-derived human microglia-like cells to study neurological diseases. *Neuron* **94, 278–293**, e279.
97 Haenseler W, Sansom SN, Buchrieser J, Newey SE, Moore CS, Nicholls FJ, Chintawar S, Schnell C, Antel JP, Allen ND *et al*. (2017) A highly efficient human pluripotent stem cell microglia model displays a neuronal-co-culture-specific expression profile and inflammatory response. *Stem Cell Rep* **8**, 1727–1742.
98 Nayak RC, Trump LR, Aronow BJ, Myers K, Mehta P, Kalfa T, Wellendorf AM, Valencia CA, Paddison PJ, Horwitz MS *et al*. (2015) Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem cells. *J Clin Investig* **125**, 3103–3116.
99 Sweeney CL, Teng R, Wang H, Merling RK, Lee J, Choi U, Koontz S, Wright DG, Malech HL *et al*. (2016) Molecular analysis of neutrophil differentiation from human induced pluripotent stem cells delineates

the kinetics of key regulators of hematopoiesis. *Stem Cells* **34**, 1513–1526.
100 Lee S, Kivimae S, Dolor A and Szoka FC (2016) Macrophage-based cell therapies: the long and winding road. *J Control Release* **240**, 527–540.
101 Willyard C (2017) The drug-resistant bacteria that pose the greatest health threats. *Nature* **543**, 15.
102 Jablonska J, Leschner S, Westphal K, Lienenklaus S and Weiss S (2010) Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. *J Clin Investig* **120**, 1151–1164.
103 Koba C, Haruta M, Matsunaga Y, Matsumura K, Haga E, Sasaki Y, Ikeda T, Takamatsu K, Nishimura Y and Senju S (2013) Therapeutic effect of human iPS-cell-derived myeloid cells expressing IFN-beta against peritoneally disseminated cancer in xenograft models. *PLoS One* **8**, e67567.
104 Polazzi E and Monti B (2010) Microglia and neuroprotection: from *in vitro* studies to therapeutic applications. *Prog Neurobiol* **92**, 293–315.
105 Dale DC and Price TH (2009) Granulocyte transfusion therapy: a new era? *Curr Opin Hematol* **16**, 1–2.
106 Price TH, Boeckh M, Harrison RW, McCullough J, Ness PM, Strauss RG, Nichols WG, Hamza TH, Cushing MM, King KE *et al.* (2015) Efficacy of transfusion with granulocytes from G-CSF/dexamethasone-treated donors in neutropenic patients with infection. *Blood* **126**, 2153–2161.
107 Pham HP, Rogoza K, Stotler B, Duffy D, Parker-Jones S, Ginzburg Y, Bhatia M, Cairo M and Schwartz J (2012) Granulocyte transfusion therapy in pediatric patients after hematopoietic stem cell transplantation: a 5-year single tertiary care center experience. *J Pediatr Hematol Oncol* **34**, e332–e336.
108 Estcourt LJ, Stanworth SJ, Hopewell S, Doree C, Trivella M and Massey E (2016) Granulocyte transfusions for treating infections in people with neutropenia or neutrophil dysfunction. *Cochrane Database Syst Rev* **4**, CD005339.