



Concise Review: Towards the Clinical Translation of Induced Pluripotent Stem Cell-Derived Blood Cells—Ready for Take-Off

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ABSTRACT

Since their discovery in 2006, induced pluripotent stem cells (iPSCs) have opened up a world of possibilities for regenerative medicine and novel cell-based therapeutics. Now, over a decade later, robust reprogramming and expansion and differentiation protocols have been developed, and iPSC-derived cells have been used in a wide variety of small and large animal models to treat many different diseases. Furthermore, the first iPSC derivatives are on their way into clinical trials. In this line, (i) GMP-compliant generation, cultivation, and differentiation, (ii) preclinical efficacy and safety, as well as (iii) ethical and regulatory compliance of stem cell research represent important aspects that need to be evaluated for proper clinical translation of iPSCs and their derivatives. In this review article, we provide an overview of the current advances and challenges of the clinical translation of iPSC-derived blood cells and highlight the most pressing problems that have to be overcome in the next years. *STEM CELLS TRANSLATIONAL MEDICINE* 2019;8:332–339

SIGNIFICANCE STATEMENT

Given the groundbreaking discovery of induced pluripotent stem cells (iPSCs), use of these cells has been envisioned for future cell-based therapies. Nowadays, iPSCs have been differentiated toward a variety of different effector cells, and iPSC derivatives have also reached clinical translation. Although clinical transfer of iPSC-derived hematopoietic cells remains as of yet sparse, the study describes the recent advances in hematopoietic differentiation of iPSC and provides a current perspective on the GMP-compliant generation of suitable hematopoietic cells.

INTRODUCTION

Given their potential to continuously regenerate and differentiate into all cell types of an organism, pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) or induced PSCs (iPSCs) represent a highly promising source for new cell-based therapies. Following the pioneering studies by Takahashi and Yamanaka [1], murine and human iPSCs have been used to derive a multitude of therapeutically active cell types, thus laying the foundation for new treatment concepts in regenerative medicine (Fig. 1). After over a decade of research, the effectiveness and feasibility of iPSC-derivatives have been proven in small and large animal models, building the foundation for clinical application [2]. In this review article, we will give an overview of the ongoing efforts to translate iPSC-derived blood cells toward clinical use, with the main focus on hematopoietic

stem cells (HSCs) and macrophages, highlighting some of the problems that have to be overcome in the upcoming years.

PROMISES AND ETHICAL CONCERNS OF HUMAN IPSC

To broaden the clinical use of iPSC-derived cell types, current efforts focus on three cornerstones, which are dedicated to (i) GMP-compliant generation, cultivation, and differentiation of human iPSCs, (ii) preclinical efficacy and safety, as well as (iii) ethical and regulatory compliance of stem cell research. Especially, the latter has recently been considered in more detail, as human iPSCs are derived from adult-type somatic donor cells. Given the unique features of iPSCs for unlimited self-renewal and indefinite differentiation toward various cell types, these cells can be used for research purposes or

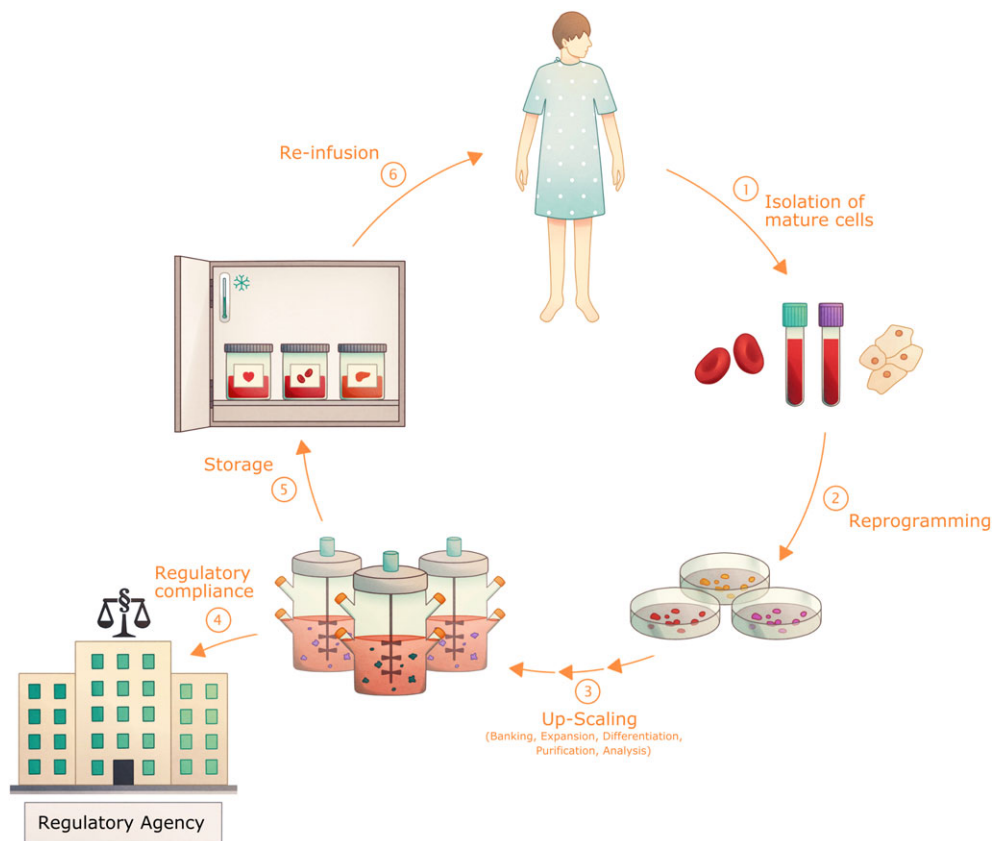


Figure 1. The clinical translation of induced pluripotent stem cell (iPSC)-derived cells. iPSCs and their derived progeny hold great potential for their use in regenerative and personalized medicine. To achieve this aim, mature cells, for example, blood cells or fibroblasts, are collected from a patient (1) and reprogrammed into iPSCs using a GMP-compliant protocol (2). After this, the cells have to go through several processes (3) including but not limited to: banking for future use and testing, expansion and differentiation in sufficient numbers via upscaling, for example, in bioreactors, purification, and functional analysis. Cells also have to go through tests by regulatory agencies regarding safety and compliance (4). Following certain cell release criteria, the cell products (5) can potentially be stored and infused back into the patient (6).

be translated into clinical practice many years after their generation. Furthermore, iPSCs contain the individual genetic fingerprint of the donor, which can be (mis)used for in depth research of the personal genetic code. Although human iPSCs can nowadays be generated within days or weeks from many different donors, the history of Henrietta Lacks should act as a memorial to follow certain national and international guidelines on stem cell research. The International Society of Stem Cell Research has also become aware of these prerequisites and has published a compendium of “Guidelines for Stem Cell Research and Clinical Translation” (see also [3]). Besides recent landmark studies on the therapeutic success of iPSC-derivatives, such guidelines are necessary to protect the rights of the cell donors (e.g., by written informed consent) and to fulfill (pre)clinical standards (e.g., by preclinical efficacy and safety studies) before an iPSC-derived cell therapeutic reaches individual patients. Given the rapid medical progress in the field of stem cell research and regenerative medicine, national stem cell societies (e.g., the German Stem Cell Network) also provide knowledge on regulatory compliance, with the aim to use the iPSC technology for disease modeling, drug discovery, and also clinical translation.

SCALABLE GENERATION AND MAINTENANCE OF iPSCs AS A PREREQUISITE FOR THE CLINICAL TRANSLATION

Since their discovery in 2006, the concept of “reprogramming” was quickly transferred from the murine to the human system [4] and then expanded toward different starting cell sources with various different reprogramming techniques [5–11] (for a more in depth overview, see [12]). The original protocol is based on introducing the four transcription factors (TFs), *OCT4*, *SOX2*, *KLF4*, and *c-Myc*, via viral vectors; although it remains the basis of iPSC generation [1], other factors and methods have also been explored. After their establishment, iPSCs were quickly compared to the “gold standard” ESCs and it was found that they are remarkably similar. However, subtle differences in transcriptomic and epigenetic profiling were discovered [13, 14]. How critical these differences are, as well as the use of different starting materials, the donor age, and reprogramming method are still debated [13, 15–17] (for a more in depth overview of the influence of donor age and of genetic variability, see [18] and [19], respectively). Furthermore, the genetic integrity and stability of iPSCs are of concern as the reprogramming process and subsequent culturing are associated with DNA damages including double strand breaks and other genomic aberrations

[20]. As such aberrations are passed down to any nucleated differentiated progeny, they could present a major safety issue. Despite these concerns, iPSCs are pushed toward clinical application because of their great therapeutic potential. However, the main difficulties still persist in GMP-compliant generation and maintenance of iPSCs, translation of cultivation and differentiation protocols toward industrial standards, and the definition of safety standards.

As the original reprogramming technique used viral vector integration, the risk of genotoxicity and nonintegrating viral and nonviral reprogramming methods have been successfully developed. For the viral reprogramming, adenovirus and sendai virus have been predominantly used [6, 21], whereas for the nonviral methods, mRNA, protein, plasmid, and transposon-based methods have been successfully employed [12]. Most of these methods, however, trade-off higher security for lower efficiency in comparison to the traditionally used lenti- or gamma-retroviral methods [22]. In hand with the nonintegrating reprogramming, also GMP-grade generation and maintenance protocols were developed [23]. This includes chemically defined and xeno-free media but also feeder-free cultivation of iPSCs. Many systems have been successfully developed so far, Wiley et al., used sendai virus-based reprogramming and culturing on recombinant human laminin [24], whereas Baghbaderani et al. used plasmid-based reprogramming and the L7 matrix from Lonza [23, 25]. Both groups demonstrate fully GMP-compliant protocols ready for clinical translation. As there is no standardized method up until this point, much work is still needed toward establishing robust and reliable protocols. The next step after the GMP-compliant reprogramming and culturing is the translation toward industrial-scale production. The aforementioned protocols work with traditional monolayer culture, but for a clinical application, up-scaling to bioreactor technology is required. Various groups have shown that cultivation of iPSCs as pluripotent aggregates in bioreactors is possible [26, 27], although the bioreactors were still inoculated with cells initially derived from feeder-based cultivation, and continuous passaging of the pluripotent aggregates remains problematic. The most important hurdle that remains is the connection of all the different parts to achieve a combined, all-in-one GMP-compliant reprogramming and maintenance protocol that can be transferred to the bioreactor and ideally be linked to various differentiation protocols. One of the few cell types for which robust and combined protocols in a bioreactor system have already been established are cardiomyocytes [28, 29], but for the majority of other cell types including the blood cells robust protocols remain sparse. Furthermore, the produced cells need to be linked to clinical use: Even though protocols for cardiomyocytes are already well established, the application of these cells in the clinic is still in the very beginning [30]. In summary, both the GMP-compliant reprogramming as well as the maintenance of iPSCs have been established. However, main challenges remain in connecting the protocols, up-scaling of the processes, and achieving standards and guidelines for all steps involved.

THE DIFFERENTIATION POTENTIAL OF HUMAN iPSCS INTO HEMATOPOIETIC STEM CELLS

Considering their three-lineage (mesoderm, ectoderm, and endoderm) differentiation potential, human iPSCs have been

shown to give rise to almost all cell types of the human organism, highlighting their attractiveness for the field of regenerative medicine. This also holds true for the hematopoietic lineage, in which the HSC gives rise to all mature blood cells in a hierarchical process. Given the high therapeutic value of HSC transplantation in clinical practice, great efforts have been put into the generation of HSCs from PSC sources. And while substantial improvements have been made in the generation of a variety of mature blood cells, the *in vitro* generation of HSCs from PSCs is still fraught with problems. The main hallmarks such as engraftment in secondary recipients and multilineage reconstitution currently remain mostly unmet. Although many protocols are published, they vary greatly in both methods used and the outcome. So far, only genetic modification of the original iPSC starting material allowed the generation of iPSC-derived functional HSCs, whereas iPSC-derived HSC-like cells from differentiation cultures employing “only” cytokine administration, small molecule-guided activation of signaling pathways, or cocultivation with endothelial/stromal cells are missing critical functionality [31, 32]. One promising approach to overcome this problem is the forced overexpression of defined TFs during the differentiation process. In the human system, Sandler et al. showed that the overexpression of *FOSB*, *GFI1*, *RUNX1*, and *SPI1* in endothelial cells together with a coculture with E4EC vascular niche cells is able to produce multipotent progenitor cells that can reconstitute primary and secondary recipients [33]. An alternative approach comes from the Daley lab, that used the inducible overexpression of the TFs *ERG*, *HOXA9*, *RORA*, *SOX4*, and *MYB* (EARS) in CD34⁺ CD45⁺ myeloid precursors derived from human PSCs (hPSCs). Following this approach, they were able to generate engraftable multilineage progenitors with myeloid and erythroid differentiation potential [34]. Of note, the additional knockdown of the epigenetic modifier and polycomb group protein *EZH1* unlocked lymphoid potential *in vitro* [35]. In addition, also the overexpression of *MLL-AF4* only has shown the generation of engraftable iPSC-derived blood cells; however, transplanted cells showed a myeloid bias and leukemic transformation at later timepoints [36]. Similarly, a screen of 26 TF candidates after hPSC differentiation in hemogenic endothelium discovered seven TFs (*ERG*, *HOXA5*, *HOXA9*, *HOXA10*, *LCOR*, and *RUNX1*) that were sufficient to generate hematopoietic stem and progenitor cells that engraft in primary and secondary recipients and generate myeloid, B and T cell lineages [37]. Here, the overexpression of these TFs is combined with a preceding morphogen-based differentiation protocol to first generate hemogenic endothelium, which is then converted into HSCs. With similar success Rafii and coworkers converted murine endothelial cells into HSCs via the transient overexpression of the TFs *Fosb*, *Gfi1*, *Runx1*, and *Spi1* and coculture with an inductive vascular niche [38]. Another approach is performed by Suzuki et al. [39] and Amabile [40], for example, who successfully generated HSCs via teratoma formation. However, this approach has clear limitations with respect to clinical translation. Even though great advances have been made, the clinical translation of *in vitro* generated transgene-free HSCs remains out of reach for the moment. This might be explained by the complex hematopoietic embryonic development, which proceeds through two distinct stages: a primitive and a definitive hematopoietic program. Whereas these programs are spatially and temporarily separated in the developing embryo, they are

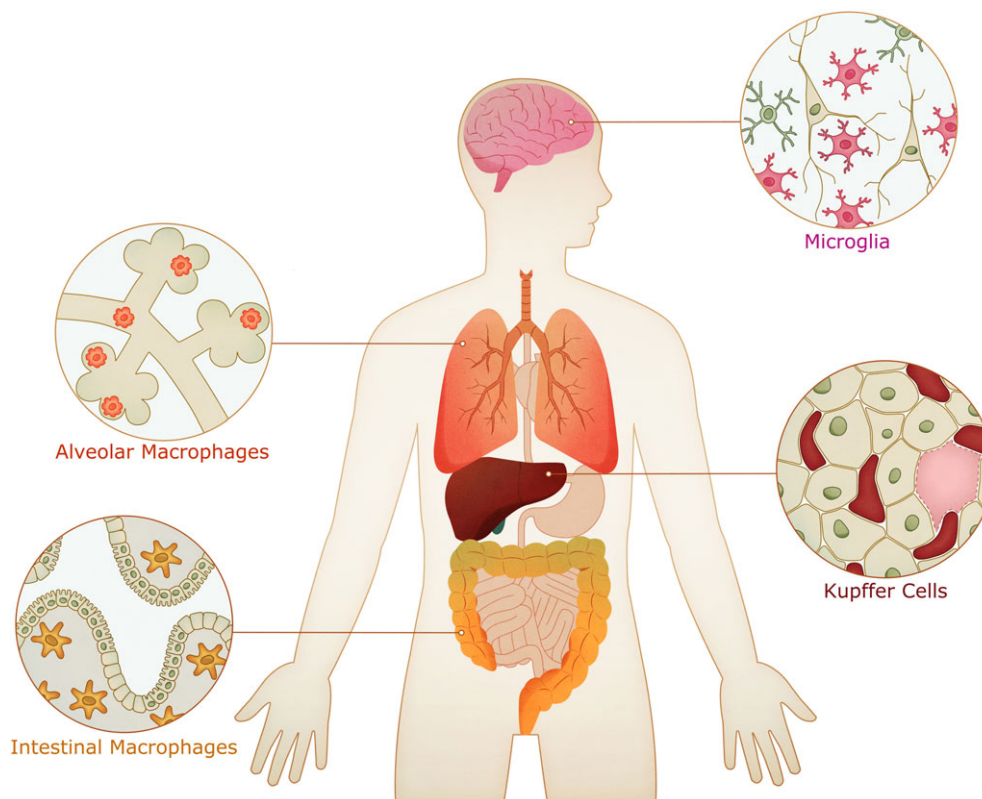


Figure 2. Localization of different macrophage subsets in different organs. Tissue macrophages play an important role in tissue homeostasis and can act as regulators in the innate immunity. Prominent examples for macrophages in different tissues are microglia in the brain, Kupffer cells in the liver, alveolar macrophages in the lung, and the intestinal macrophages. Considering the individual turnover and the ontogeny of the different macrophage subsets, generation and transplantation of induced pluripotent stem cell-derived macrophages might be a future therapeutic approach for different diseases in which tissue macrophages are impaired.

simultaneously induced during iPSC differentiation (also reviewed in [41]). Certainly, specific factors and signaling pathways are still missing to instruct the developing HSPCs to a definitive, long-term engraftable HSC. Because of these problems, many researchers have turned their attention toward the generation of further differentiated cells instead. Here, our understanding of the ontogeny of these cells *in vivo* has been the crucial guiding plan toward their *in vitro* generation.

GENERATION OF THERAPEUTICALLY ACTIVE MACROPHAGES FROM HUMAN IPSC

Macrophages have become an increasingly interesting cell type for *in vitro* generation and clinical translation, as insights into their function and ontogeny have been unveiled. Several recent publications have shown that macrophages from different organs (Fig. 2), also called tissue resident macrophages (TRMs), are of embryonic origin and originate from progenitors, which seed the different tissues before birth. Furthermore, many TRM populations have been shown to self-maintain independent of monocyte influx as, for example, the microglia in the brain, alveolar macrophages (AMs) in the lung, or the Kupffer cells in the liver (as also reviewed elsewhere [42]). Given their remarkable self-renewal and plasticity combined with their crucial role in a wide variety of diseases such as hereditary alveolar proteinosis [43] and mendelian

susceptibility to mycobacterial disease [44, 45], the *in vitro* generation of macrophages can lead to new insights into their role in pathophysiology [46, 47], while creating possible clinical applications.

The generation of human macrophages from PSCs started out with a variety of complex differentiation methods regularly including coculturing with other cells such as mouse bone marrow cells, complex cytokine cocktails, and lengthy purification steps. In 2008, however, the group of William James published a more streamlined and efficient protocol to derive macrophages from ESCs. In short, embryoid bodies (EBs) are formed and subjected to directed differentiation via M-CSF/IL-3, and the subsequently produced cells can be harvested from the supernatant [48]. The resulting cells are of high purity, transcriptionally closely related to their *in vivo* counterparts, and seem to be fully functional. Because of its simplicity and efficiency, this protocol has since become a standard for differentiating macrophages and meanwhile been further advanced to generate macrophages and granulocytes from human iPSCs [49, 50]. In addition, similar techniques have been developed to also generate microglia-like cells from human iPSCs, further demonstrating the potential to produce various macrophage subsets [51, 52]. With a well-established protocol in hand, current efforts are mainly focused on defined, GMP-compliant, and large-scale production of these cells, to push their use toward the clinical application. Here, additional steps have often been introduced to the original protocol, mainly a mesoderm priming step after EB formation, which often includes

the addition of Stem Cell Factor (SCF), Vascular Endothelial Growth Factor (VEGF), Bone morphogenetic protein 4 (BMP-4) [53]. This step seems to be made necessary when using a feeder-free cultivation of the iPSCs as either a monolayer or as aggregates. Further modifications often include single EB formation with a defined cell numbers to make the process more reproducible and a switch to media that are suitable for clinical translation such as X-Vivo, E8/E6 or mTeSR medium. With large parts of the GMP compliant and defined production of these cells established, further efforts are now concentrated on the large-scale production of these cells and establishing a link to the GMP-compliant cultivation and maintenance of iPSCs to have an all-in-one large-scale production protocol. Regarding the function of the iPSC-derived macrophages, the question remains whether they rather resemble an adult-type monocyte/macrophage or a primitive embryonic-type macrophage phenotype. Some evidence seems to be pointing toward the latter [52, 53], but how important this is for future clinical application of the cells still has to be shown. As of yet, the connection of the ontogeny and function of different macrophage subsets is poorly understood, and the question persists whether adult and embryonic macrophages have the same functionality. Furthermore, macrophages are one of the few known cell types that exist both from embryo and adult origin in the adult organism. Given the tendency of most PSC-derived cells to be more of primitive (embryonic/fetal) than definitive origin, macrophages are an interesting cell type to study the functional differences between these cells. In fact, recent studies suggest a more primitive origin of murine and human iPSC-derived macrophages [53–55]. Given the *MYB*-independent origin of murine TRMs [56], a *MYB*-independent and *RUNX1*-dependent development has been shown for human iPSC-derived macrophages [53].

First in vivo data of how these cells could be used in the clinical application came, for example, from macrophage transplantation in the context of hereditary pulmonary alveolar proteinosis (herPAP) [57, 58]. Because of the absence of AMs and therefore an empty niche, it has been shown that pulmonary macrophage transplantation of human iPSC-derived macrophages can also lead to stable long-term engraftment, adaption toward an AM phenotype, and thereby improvement of disease parameters in a humanized mouse model of herPAP [54]. Although very much similar, the concept of HSC-derived macrophage transplantation is now on its way toward clinical translation and might also be a useful application in other macrophage-related diseases and even infectious diseases.

CLINICAL TRANSLATION OF HUMAN IPSC AND FUTURE DIRECTIONS

As introduced before, human iPSCs have been differentiated into a multitude of different blood cells; however, the clinical translation of iPSC-derived cell products is still impeded. One major hurdle is our insufficient knowledge about the in vivo functionality of iPSC-derived blood cells as well as the lack of scalable differentiation protocols allowing to generate therapeutically relevant quantities of effector cells. Another potential concern relates to the contamination of the cell product with residual undifferentiated iPSCs, which can form unwanted teratomas. To circumvent this problem, improved differentiation

protocols in combination with rigorous purification strategies are currently underway. As an alternative, the addition of safety switches, which are based on suicide genes, for example, the herpes simplex virus thymidine kinase or the inducible Caspase 9 system, may be used to efficiently deplete tumor (including teratoma)-forming cells [59, 60]. Application of such a system has recently shown to eradicate approximately 95% of iPSCs, making this system an attractive safety-backup option [59]. Although current preclinical lab work relates to GMP-compliant generation, maintenance, and differentiation as well as safety concerns, one major question remaining is which iPSC line(s) should be used to generate the therapeutic cell product. First studies promised iPSC technology to provide autologous cell products and tissues for patient-specific cell-based therapies; however, this scenario is extremely labor and cost intensive, suggesting the clinical use of allogenic iPSCs. To provide suitable target cells, either genetically modified “universal” iPSCs, which, for example, expresses artificial HLA molecules [61], or the use of human iPSCs, which can be obtained from iPSC-banks, is interesting approach. Indeed, S. Yamanaka has started recently to establish a GMP-grade iPSC-bank in Japan (*CiRA's iPSC bank for regenerative medicine*), preferentially using material from HLA-homozygous donors. This approach offers the advantage that 5 to 10 allogenic donor lines would be sufficient to match 30%–50% of the Japanese population [62, 63]. Using HLA-homozygous donors greatly reduces the numbers of iPSC lines needed to cover a given population, but potential donors would have to be identified performing large screenings or using established data from HSC donors/cord blood banks. While this scenario may be applicable for Japan, more diverse populations (e.g., Brazil, United States, or India) are expected to require more iPSC lines to match the majority of their population [64, 65]. As an alternative approach, a single iPSC line, previously genetically manipulated to escape immune rejection would allow for a universal iPSC-based cell therapy approach, which can in principle be applied for all patients and thus drastically reduce the costs for cell therapy. Going this line, recent studies could already demonstrate feasibility by disrupting the beta-2 microglobulin gene-locus and introducing a single-chain HLA-E molecule into PSCs. Derivatives from these iPSCs were able to escape T cell-mediated rejection and were resistant to NK-cell lysis, highlighting their potential as allogenic alternatives [61].

Although no clinical trials employing iPSC-derived hematopoietic cells have been conducted so far, two other iPSC-derived products have been used to treat the first patients. The worldwide first clinical trial using an autologous iPSC-derived cell product has been launched in 2014 in Japan. Here, the team of Masayo Takahashi from RIKEN and Kobe City Medical Center initiated a clinical trial to treat neovascular age-related macular degeneration by transplanting iPSC-derived retinal pigment epithelial (RPE) sheets (UMIN-CTR #, UMIN000011929). To achieve this aim, the team generated a GMP-grade autologous iPSC line from patient's fibroblasts using nonintegrating episomal vectors. After vigorous safety testing of the iPSCs and the iPSC-derived cell product, the first patient has been treated in September 2014, with an intact RPE cell sheet 1 year post transplantation [2]. However, given new regulations in Japan's regenerative medicine law [66], the team of Takahashi has changed the strategy, aiming to use allogeneic donor cells instead [67, 68]. Unfortunately, one adverse event was reported for one of the patients earlier this year, which, however, was most likely

related to the surgical procedure and not the iPSC product itself [69]. Besides this pioneering trial, only one more interventional trial using allogeneic iPSC-derived mesenchymal stem cells (MSCs) is currently being conducted by the Australian Company Cynata Therapeutics. In this trial, the investigator aims to use mesenchymoangioblast-derived MSCs for the treatment of steroid-resistant acute graft-versus-host disease (ClinicalTrials.gov: NCT02923375). Currently, all eight patients treated so far have demonstrated at least a partial response, while no treatment-related serious adverse events or safety concerns have been observed [70]. The outcomes of these two trials will certainly be helpful for further clinical translation of iPSC-based therapies.

CONCLUSION

Given the pioneering work of the aforementioned clinical trials and long-standing expertise of transfusion medicine in cell-based therapies, iPSC-derived hematopoietic cells represent a promising cell type for the initiation of further iPSC-based clinical trials. Especially, the recent advances in GMP-compliant generation and differentiation of human iPSCs in combination with upscaling approaches of hematopoietic differentiation put iPSC-derived blood cells on the right path toward possible clinical translation. While various preclinical studies could already demonstrate the efficacy and safety of various iPSC-derived blood cells, more work is still needed to generate transgene-free HSCs for clinical application. Here, maybe the transplantation of macrophages into different tissues could be the forefront of clinical translation (Fig. 1). As mentioned earlier, dysfunction of macrophages

has been associated with a variety of disease entities, and for some, macrophage transplantation has been proven feasible and long lasting. Also highlighting recent efforts in the generation of other mature and immature cells, it will be a fascinating time to see when and how iPSCs will be translated into clinical application and furthermore which hematopoietic cell types will be applied first.

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AUTHOR CONTRIBUTIONS

K.H. and M.A.: manuscript writing, final approval of manuscript; N.L.: conception/design, manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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