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Editorial

THE EVE OF A HISTORIC BREAKTHROUGH IN THE REALM OF HUMAN PLURIPOTENT STEM CELL-DERIVED HAEMATOPOIETIC STEM CELLS

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Haematopoietic stem cells (HSCs) are the most critical cell type in the human blood system, capable of differentiating into all types of blood and immune cells, thereby fully rebuilding the haematopoietic and immune systems. Haematopoietic system transplantation represents one of the earliest successful approaches in stem cell transplantation, effectively used to treat immunodeficiency, autoimmune diseases, and advanced cancers (including haematologic malignancies and solid tumours) [1–3]. However, the limited source of bone marrow donors poses a significant challenge, and bone marrow transplantation can also cause considerable harm to donors. The scarcity of donor cells and the difficulties associated with human leukocyte antigen/major histocompatibility complex (HLA/MHC) matching greatly restrict the application of this treatment [4].

Cord blood and mobilised peripheral blood contain HSCs that can be utilized for transplantation therapy; however, their content is relatively low, and the quantity of HSCs collected from a single individual is often insufficient for transplantation [5,6].

The establishment of two types of human pluripotent stem cells (hPSCs), namely human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), offers a potentially unlimited source of HSCs [7,8]. Theoretically, these cells can be differentiated into HSCs and functional blood and immune cells. Nevertheless, it is technically easier to obtain the latter two types of cells, as achieving and maintaining the cellular status of HSCs through haematopoietic differentiation from hPSCs *in vitro* is exceedingly challenging. While hiPSCs share many cellular characteristics and functions with hESCs, hiPSCs circumvent the ethical implications associated with human embryos, as they can be derived from reprogramming most types of terminally differentiated cells. This provides a convenient and abundant cell source while minimizing the risk of immune compatibility conflicts after HSC transplantation, making them the most promising source of HSCs and other stem cells [9,10]. As the inventor of hiPSCs, Prof. Yamanaka established the hiPSC bank at Kyoto University in 2008. Since then, numerous similar hiPSC banks have been set up worldwide to collect diverse hiPSC lines with various genetic backgrounds, addressing the cell therapy needs of patients [11].

Moreover, hiPSC-derived blood and immune cells have been successfully generated in *in vitro* differentiation systems, with notable progress in clinical applications. Currently, despite challenges related to quantity and quality, certain types of blood cells, such as red blood cells (RBCs) and platelets, can be produced at scale from hiPSCs using these methods [12,13]. Notably, platelets have shown significant advancements in clinical trials [13], alongside various immune cell types, including neutrophils, T cells, natural killer (NK) cells, and dendritic cells (DCs) [14–17]. Century Therapeutics Inc. is currently testing two projects in clinical trials: CNTY-101 for autoimmune diseases (NCT06255028) and for B-cell malignancies (NCT05336409). Fate Therapeutics Inc. also has three projects in Phase 1 trials, including FT819 for systemic lupus erythematosus (NCT04629729), FT825 for solid tumours (NCT06241456), and FT522 for B-cell lymphoma (NCT05950334). However, efforts over the years to differentiate hPSCs into HSCs *in vitro* have not yielded substan-

tial progress, with seldom preclinical studies achieving significant results, making this a challenging frontier in stem cell biology [18].

It is important to acknowledge that some progress has been made in hPSC differentiation into HSCs in recent years. Research indicates that transplantable HSCs were generated by injecting hPSCs into immunodeficient mice; however, this method poses a risk of tumour formation *in vivo*, rendering it unsafe for clinical application [19,20]. Sugimura *et al*. [21] developed a novel approach that induced hPSCs into HSC precursor cells through overexpression of transcription factors such as *RUNX1*, demonstrating the capability to generate functional HSCs *in vitro* with multipotent haematopoietic capacity in mice. Nonetheless, the overexpression of multiple tumour-related factors and transgenic manipulations limit clinical applications, and the inherent tumorigenic potential of HSC precursor cells raises safety concerns [21]. Overall, while advances have been made, research on hPSC differentiation into HSCs remains exploratory, facing challenges of low efficiency, incompleteness, and instability. This highlights a critical need for a deeper understanding of the regulatory mechanisms involved in HSC generation [22].

Since 2023, significant progress has emerged in the generation of hiPSC-derived HSCs. A study published in *Cell Stem Cell* by the team of Guyonneau-Harmand and Jaffredo reported the discovery of a non-transgenic and non-embryonic feeder layer culture system that, following optimisation, can induce hPSCs to generate HSCs with continuous transplantation capabilities, fully reconstructing the haematopoietic system *in vitro* [23]. Their method is simple and elegant, requiring no special reagents or techniques, and the results are promising, aligning well with clinical application requirements. Although replicable results from other laboratories are still pending, the simplicity of their differentiation method enhances the potential for broader adoption.

A similar breakthrough was recently reported in *Nature Biotechnology* by Elefanty's group [24]. They successfully differentiated hiPSCs to form embryoid bodies (EBs) in a defined culture medium and patterned mesoderm in a Homeobox A (HOXA) configuration. Following this, they specified hemogenic endothelium using bone morphogenetic protein 4 (BMP4) and vascular endothelial growth factor (VEGF), ultimately achieving differentiation into CD34+ blood cells through an efficient endothelial-to-haematopoietic transition. The HSC-like cells derived from four independent hiPSC lines were transplanted into immunodeficient recipient mice, demonstrating a close resemblance to functionally defined HSCs in 25–50 % of cases. These significant advancements achieved through traditional methods offer a glimmer of hope for ultimately resolving the functional differentiation of hPSCs into HSCs *in vitro*. The anticipated success of this approach is noteworthy, as the EB-based method may closely mimic actual embryonic development [25]. Importantly, they utilized a feeder-free *in vitro* differentiation system, suggesting that further breakthroughs in such systems could improve the quantity and quality of hPSC-derived HSCs. Utilizing nanomaterials or an extracellular matrix (ECM) to replicate the natural haematopoietic microenvironment (characterized by optimal stiffness, low oxygen levels, and porous conditions) could prove beneficial. There have already been successful attempts in this area within *in vitro* differentiation systems [26,27], indicating that important work still lies ahead.

In comparison to cell therapies that utilize functional cells, whether sourced from donation or derived from stem or progenitor cells (hPSCs or adult stem/progenitor cells), therapies involving hPSC-derived HSCs or other adult stem cells offer unparalleled advantages. hPSC-derived adult stem cells, including HSCs, have the potential to rebuild corresponding organs and tissues *in vivo*, thoroughly cure genetic diseases or cancers while restoring their functions simultaneously. In contrast, functional cell infusions, such as RBC and platelet transfusions, typically provide only temporary alleviation of disease conditions and necessitate long-term or even lifelong repeats of treatment, creating a substantial economic burden and the potential for treatment tolerance [28]. From both an economic and a patient welfare perspective, the transplantation of hPSC-derived HSCs represents a far superior choice.

It is well recognized that the production and clinical application of hPSC-derived HSCs present significant technical challenges. However, these cells offer a powerful means for curing genetic diseases, cancers, and immune disorders, generating profound and immeasurable social and economic value, which motivates us to overcome these technological obstacles. After three decades of diligent research and effort, we are now witnessing the dawn of breakthroughs that herald potential clinical applications in the future. Thus, it is essential to remain dedicated to achieving final success in this critical area of research.

List of Abbreviations

HSCs, haematopoietic stem cells; hPSCs, human pluripotent stem cells; hiPSCs, human induced pluripotent stem cells; hESCs, human embryonic stem cells; HLA, human leukocyte antigen; MHC, major histocompatibility complex; NK cells, natural killer cells; DCs, dendritic cells; BMP4, morphogenetic protein 4; VEGF, vascular endothelial growth factor; EBs, embryoid bodies; ECM, extracellular matrix; RBCs, red blood cells; HOXA, Homeobox A.

Availability of Data and Materials

Not applicable.

Author Contributions

PYW and BC contributed to the design of this work. PYW and BC contributed to the interpretation of data. BC drafted the work. PYW revised critically for important intellectual content. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. PYW is serving the Editorial Board members of this journal. We declare that PYW had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to KZ.

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