PCR,[28] Nuclease-Assisted Mutation Enrichment[29]).

The last decades have witnessed significant progress in elucidating the origin, characteristics and potential applications for the analysis of ccfDNA. Given its ability to assess dynamic and comprehensive tumor processes, ccfDNA, as a source for ctDNA, holds great promise for the implementation of personalized and dynamic cancer therapies. In addition, the low detection limit and ease of sampling may critically improve post-treatment monitoring compared to surveillance imaging. Resolving the remaining technical and practical challenges will allow ccfDNA to prove its clinical utility and revise cancer patient care.

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Hematopoietic stem cells meet induced pluripotent stem cells technology

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Hematopoietic stem cells (HSCs), the source of the entire blood cells repertoire, represent the first stem cells identified in an adult tissue, the bone marrow, and the first stem cells used as a therapy in humans, through bone marrow transplantation.[1] Although this therapeutic approach is well established and used to treat a variety of hematological conditions, including leukemia, several aspects limit its application. A lack of matched donors poses a serious barrier, especially for patients from ethnic minority backgrounds. Finding the perfect match between donor and recipient, and obtaining a large number of HSCs, represent two of the unmet major clinical challenges.

Development in a parallel area of stem cells research, induced pluripotent stem cells (iPS) technology, might provide new avenues to circumvent the limitations posed by the scarce number of HSCs available for transplantation. A decade ago the team led by Nobel prize winner Shinya

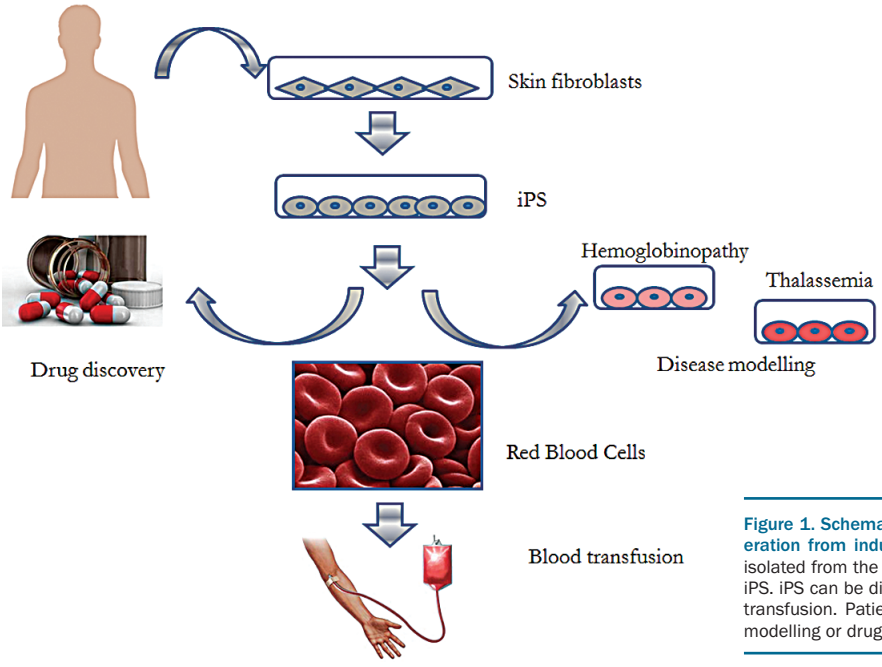

Figure 1. Schematic representation of Red Blood Cells (RBCs) generation from induced pluripotent stem cells (iPS). Fibroblasts are isolated from the skin of the patient and reprogrammed *in vitro* into iPS. iPS can be differentiated *in vitro* in RBCs and be used for blood transfusion. Patient-specific iPS can be powerful tools for disease modelling or drug discovery processes.

Yamanaka authored a breakthrough study describing the generation of pluripotent stem cells from adult cells.[2,3] Since then, many scientists have tried to develop hematopoietic stem cells and blood cells from adult cells *via* iPS.

Szabo *et al.* showed that expression of the reprogramming gene and transcription factor *OCT4* could induce the generation of HSCs from adult fibroblasts.[4] With an analogous approach, Pereira *et al.* reprogrammed mouse fibroblasts into HSCs by introducing a set of transcription factors, including *Gata2*, *Gfi 1b*, *Fos* and *Etv6*.[5] One of the major obstacles to the reprogramming is the epigenetic code that sets a strong barrier between the differentiation potential of an adult cell and a stem cell. Based on this consideration, Doulatov *et al.* decided to attempt the reprogramming of human committed myeloid cells into HSCs, identifying 13 essential transcription factors.[6] Although these studies showed that the reprogramming of a variety of cells into HSCs is achievable, none of them were able to achieve long-term peripheral blood reconstitution *in vivo* and to establish the full repertoire of HSCs through serial transplantations. These are hallmarks of the ability of the cells to differentiate and self-renew and represent the gold standards for stem cells. In a more recent study, Riddell and colleagues reprogrammed differentiated somatic blood cells, pro-B cells, by using a cocktail of transcription factors, namely *Hlf*, *Lmo2*, *Pbx1*, *Prdm5*, *Runx1t1*, and *Zfp37*.[7]

Of course the cocktail used by Riddell and colleagues includes potent leukemic oncogenes that limit the immediate clinical use of HSCs generated by this application. Non-integrating methods and controllable expression systems of the transcription factors are currently under investigation for the clinical translation of these products.

The first clinical trial with iPS derived cells started over two years ago in Japan, where a patient affected by macular degeneration of the retina was injected with iPS derived cells.[8] Although the treatment proved safe and effective, last September the trial was halted because of mutations found in the cells prepared for a second patient, and due to new laws regulating regenerative medicine products in Japan.[9]

However the scientific community strongly believes that the iPS technology holds better promise than embryonic stem cells (ESC) for clinical translation. Currently there is no open source of human ESC (hESC) in the UK[10] and hESC products are not patentable in Europe;[11,12] moreover, hESC research has been restricted due to ethical consideration regarding the use of human embryos.[13] Furthermore, no good manufacturing practices (GMPs) grade hESC lines of O Rh negative blood type are available, a critical aspect in order to develop widely transfusable red blood cells. Novosang is a project born from a consortium between British academic groups and blood services planning to launch the first human clinical trial using red blood cells produced by iPS in 2017.[14]

Stem cell research is a very hot topic that has led to hope and hype, with exaggerated predictions and claims regarding a potential clinical translation of these products. Considering the steep progress made in iPS technology in less than ten years we can optimistically expect progress for clinically proofed induced hematopoietic stem cells (iHSCs). Moreover, iHSCs hold the promise of becoming potent tools for modelling diseases *in vitro* and for drug discovery (Figure 1). These are two particularly important aspects in the hematology field, where the lack of appropriate humanized models and ethical concerns over the use of xenotransplantation and *in vivo* toxicity studies limit both the understanding of the biology of the disease and

the screening of new drugs.

The marriage between the HSC and iPS technology therefore represents a very promising and interesting area of stem cell research.

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