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Review

Making a Hematopoietic Stem Cell

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Previous attempts to either generate or expand hematopoietic stem cells (HSCs) *in vitro* have involved either *ex vivo* expansion of pre-existing patient or donor HSCs or *de novo* generation from pluripotent stem cells (PSCs), comprising both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). iPSCs alleviated ESC ethical issues but attempts to generate functional mature hematopoietic stem and progenitor cells (HSPCs) have been largely unsuccessful. New efforts focus on directly reprogramming somatic cells into definitive HSCs and HSPCs. To meet clinical needs and to advance drug discovery and stem cell therapy, alternative approaches are necessary. In this review, we synthesize the strategies used and the key findings made in recent years by those trying to make an HSC.

The Need for Patient-Specific HSPCs and Strategies to Obtain Them

Hematopoiesis (see Glossary), the process by which **hematopoietic stem cells** (HSCs) generate all the cellular elements in our blood, established the paradigm for stem cell therapy. It proceeds in a hierarchical manner anchored by self-renewing HSCs. They give rise to progenitors with limited self-renewal potential that differentiate into lineage-restricted cells, making up the immunohematopoietic system. Source material for hematopoietic transplantation is in great demand as at least 20 000 **allogeneic transplants** are performed every year [1]. Despite advances in using umbilical cord blood (UCB) and mobilized stem cells, donor material remains restricted by limited stem cells in UCB, poor mobilization, and the lack of ethnic diversity to provide sufficiently matched material [2]. Allogeneic transplants require donor and host human leukocyte antigen (HLA) matching, and can cause graft-versus-host disease (GvHD) and graft rejection [3].

To overcome the aforementioned challenges, some studies have sought to expand HSPC numbers *in vitro* through the expansion of *ex vivo* HSPCs with small molecules. Success has been reported using SR1, UM171, and valproic acid [4–6]. Although small molecules have demonstrated utility in somatic cell **reprogramming** strategies such as fibroblasts to cholinergic neurons and others, their use with hematopoietic cells is still limited [7,8]. Despite their ease of optimization experimentally, various side effects have been reported when using small molecules [9,10], and there remain limitations in both the overall function of the expanded HSPCs and who can be treated with them. For these reasons, alternative sources of transplantable allogeneic and patient-specific HSCs are required.

A paradigm shift in stem cell biology – and the beginning of the field of regenerative medicine – occurred when Yamanaka and Takahashi reprogrammed somatic cells to iPSCs using four **transcription factors** (TFs) [11,12]. Further understanding of transcriptional control in a number of different cell types [13] has expanded the use of TFs to directly change somatic cell fates without going through pluripotency [14,15]. Indeed, progress has been made in

Trends

Many reprogramming strategies attempt to derive hematopoietic stem and progenitor cells (HSPCs) *de novo* from pluripotent stem cells (PSCs) or somatic cells. Each strategy yields hematopoietic cells of varying functionality.

The *in vivo* or *in vitro* niche, cytokine supplementation, and culture media greatly influence reprogramming efficiency. Incorporating these elements into a finalized reprogramming protocol is crucial to generate *bona fide* HSCs.

Some reprogramming strategies recapitulate developmental hematopoiesis 'in a dish'. This allows us to study blood development *in vitro* as well as the pathways involved in hematologic disease.

Once perfected, HSPC reprogramming protocols will be used for hematologic disease modeling and drug discovery. Also, patient-specific HSPC transplant will circumvent the risk of graft-versus-host disease and other immunological complications.

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reprogramming fibroblasts to other cell types such as monocyte-like progenitor cells, macrophages, and angioblast-like progenitor cells, among others [16–29], but few attempts have been made at reprogramming somatic cells into a stem cell with the degree of multipotency that an HSC possesses [30]. This possibility makes the *de novo* generation of HSCs from patient-specific cells a major goal of regenerative medicine: patient cells would be harvested, genetically corrected, reprogrammed, expanded *in vitro*, and used for **autologous HSC transplant** [31,32]. Having these cells to study *in vitro* would also permit drug discovery for a range of different disorders and allow insights into the transcriptional control of hematopoiesis (Figure 1).

After decades of research, differentiating PSCs into engraftable multilineage HSCs has largely been unsuccessful [33]. Multiple studies, however, bring us much closer to such a coveted feature of regenerative stem cell biology (Table 1), which is the focus of this review. Moreover,

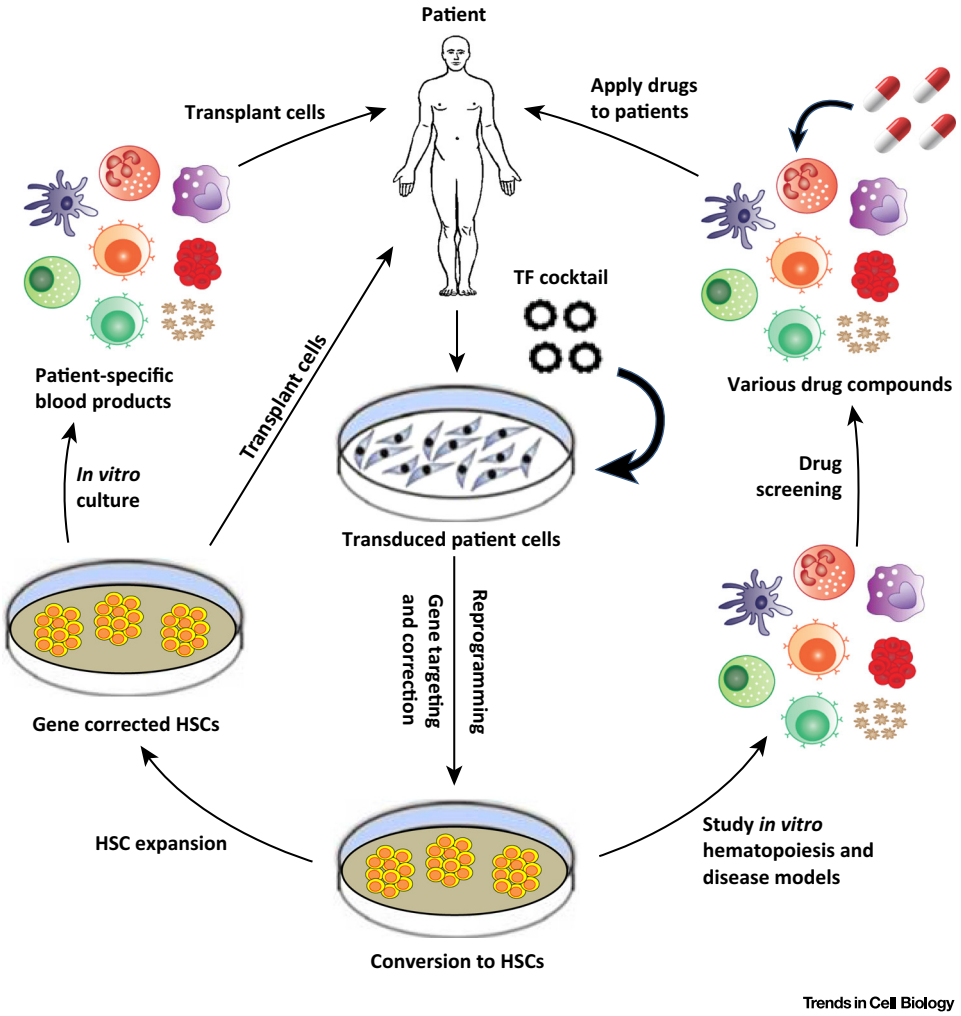

Figure 1. Patient-Specific Hematopoietic Stem and Progenitor Cell (HSPC) Derivation and Future Studies. This diagram demonstrates the general strategy of most patient-specific cell reprogramming processes and future directions. The ideal strategy is to obtain patient/donor somatic cells and reprogram to the cell type of choice, in this case hematopoietic stem cells (HSCs). These HSCs could then be used in a variety of different studies. These include but are not limited to, gene correcting the derived HSCs (or correcting the genetic defect in the obtained patient cells before reprogramming), transplantation, drug screens to identify novel therapeutics for a variety of diseases, generating patient-specific blood products and studying hematopoiesis *in vitro*.

Glossary

Allogeneic HSC transplant: transplantation of bone marrow or isolated cells from peripheral blood from a donor that is then given to a host. There are major concerns of GvHD with this form of transplant.

Autologous HSC transplant: transplantation of isolated cells (typically from UCB) belonging to the same person that requires the transplant. This type of transplant bypasses concerns related to HLA matching and GvHD. The abundance of required cells for transplant is the primary concern. Children require fewer HSCs than adults with regard to HSC transplant, thus UCB is useful primarily for children in need of transplant. Of course this is dependent upon whether the child's UCB has been banked.

Hemangioblast: unlike the HE, the hemangioblast is defined as the theorized precursor cell to endothelium, blood cells, and smooth muscle cells.

Hematopoiesis: the process by which an HSC gives rise in what is thought to be a hierarchical manner to every cell in the hematopoietic system. HSCs sit at the top of this hierarchy and give rise to progenitors that then divide into more lineage-restricted cells until they generate terminally differentiated cells such as leukocytes, macrophages, etc.

Hematopoietic stem cells (HSCs): can self-renew and differentiate down a hierarchy to form every terminally differentiated cell in the blood. A current major deficit in HSC investigative biology is our inability to culture HSCs long term, which currently hinders what we can study with them.

Hemogenic endothelium (HE): specialized endothelium theorized to give rise to HSCs via a process of cell budding. They are thought to be found in locations of embryonic definitive hematopoiesis such as the aorta-gonad-mesonephros region.

Multilineage long-term reconstitution/engraftment: the ability of a cell with self-renewing HSC potential to repopulate the hematopoietic system of an irradiated or immunocompromised host for long periods of time and give rise to all the cells in the hematopoietic system. Self-renewal can be assessed in experimental conditions by