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Converting cell fates: generating hematopoietic stem cells *de novo* via transcription factor reprogramming

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Abstract

Even though all paradigms of stem cell therapy and regenerative medicine emerged from the study of hematopoietic stem cells (HSCs), our inability to generate these cells *de novo* or expand them *in vitro* persists. Initial efforts to obtain these cells began with the use of embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) technologies, but these strategies have yet to yield fully functional cells. Subsequently, more recent approaches involve transcription factor (TF) overexpression to reprogram PSCs and various somatic cells. The induction of pluripotency with just four TFs by Shinya Yamanaka informs our ability to convert cell fates, and demonstrates the feasibility of utilizing terminally differentiated cells to generate cells with multilineage potential. In this review, we discuss the recent efforts undertaken using TF-based reprogramming strategies to convert several cell types into HSCs.

Keywords

hematopoietic stem cells; transcription factors; reprogramming; hematopoiesis; cell fate conversion

Introduction

All cells in the hematopoietic system originate from hematopoietic stem cells (HSCs) via a delicate balance of differentiation and self-renewal. HSCs sit at the top of a hierarchy, giving rise to multipotent progenitors (MPPs) with extensive differentiation capacity but limited self-renewal. Although we can generalize hematopoiesis with this hierarchy, the various populations of cells within stages of the hierarchy have recently been found to be quite heterogeneous.¹ Within these populations, MPPs differentiate into various lineage-restricted

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cells that constitute the hematopoietic system. The ability of these cells to repopulate the entire blood system after transplantation makes them essential for stem cell therapies to treat a wide variety of blood disorders. Even though years of research on HSCs and hematopoiesis have greatly informed regenerative medicine, we still encounter a number of problems.

Human leukocyte antigen (HLA)–matched HSCs are in great demand at least 20,000 HSC transplants are performed in the United States each year.^{2,3} Current sources of HSCs include cord blood, mobilized stem cells, and donor bone marrow. Unfortunately, these sources do not provide enough cells to meet demand. The difficulties of HLA matching in ethnic minorities and similar populations further limit the use of HSCs *ex vivo*.⁴ Furthermore, HLA-matched transplants do not fully guarantee the avoidance of graft versus host disease (GvHD) and other forms of immune rejection.⁵ Depending on the source of extracted HSCs, we encounter stringent limits in the number of functional cells usable for transplant. Using HSCs taken from umbilical cord blood (UCB) is advisable only for children owing to limited numbers of usable cells. When used in adult HLA-matched patients, we run the risk of late graft failure due to expansion of progenitors with limited self-renewal capacity.⁶ This option is further limited because UCBs are not always stored.⁷ Clearly, alternative cell sources of transplantable HSCs are necessary.

The novel technology of induced pluripotent stem cells (iPSCs) from Yamanaka and colleagues instigated the entire field of regenerative medicine. Using only four TFs, Oct3/4, Sox2, Klf4, and c-Myc, both mouse and human fibroblasts could be reprogrammed into iPSCs.^{8,9} This finding provided a framework to learn how to generate various cell types *in vitro* for clinical studies, ranging from disease models to drug discovery.¹⁰ Interest is now focused on converting cell fates without going through a pluripotent intermediate.^{11–13} Multiple studies have demonstrated our ability to directly convert fibroblasts to other cell types, such as functional/spinal motor neurons, myelinogenic oligodendrocyte progenitor cells, neural stem cells, cardiomyocytes, striated mononucleated myoblasts, multinucleated myotubes, Sertoli cells, macrophages, and hepatocyte-like cells^{14–24} (Fig. 1). In each of these tissues and these reprogramming studies, we see that a small network of TFs regulates an entire genetic program of cell identity. The differences across each network add to the complexity of cell fate, demonstrating the need to understand transcriptional regulation throughout multiple cell types.²⁵

Although it is clear that cell fate conversions can be initiated using a minimal set of TFs, reprogramming somatic cells to stem cells with the degree of multipotency that an HSC possesses has been attempted only recently.²⁶ Given the feasibility of cell fate conversions via TF overexpression, we and others are trying to establish a new paradigm for HSC study and clinical treatment. After patient cells are obtained, they can be corrected genetically, directly reprogrammed into the relevant cell type, and then used in multiple ways, such as transplants, drug discovery, and *in vitro* study that can all be used to advance stem cell medicine^{27,28} (Fig. 2).

The first attempts toward making HSCs *de novo* primarily came from studies that directly differentiated PSCs into the cell types of choice. Although the derived cells resemble HSCs

based on genetic profiles and cell surface immunophenotypes, functionality will ultimately define these cells. These cells look like HSCs, but they appear to be developmentally restricted to embryonic-like cells that are not yet competent to behave like mature HSCs that home and engraft properly.

To more closely recapitulate developmental hematopoiesis, several PSC studies have attempted to obtain HSCs through a hemogenic endothelium (HE) intermediate, the cell type theorized to give rise to HSCs.²⁹ This specialized endothelial cell emerges as a distinct lineage from the vascular endothelium and has been found to also have a specific cell surface immunophenotype that allows separation from other embryonic endothelial tissues.³⁰ HE resides in the aorta–gonad–mesonephros, one of the embryonic sites of hematopoiesis.^{31,32} At this site, these cells are thought to give rise to HSCs via a process of cell budding.^{33,34} Other features of hematopoiesis to consider include the primitive and definitive programs. It is known that primitive hematopoiesis emerges before the definitive program, and only produces erythrocytes, macrophages, and megakaryocytes for a limited time. Definitive hematopoiesis produces bona fide HSCs that emerge from HE (separate from endothelium that can generate early erythroid and myeloid progenitors) via the endothelial-to-hematopoietic transition (EHT).³⁵ Various TFs and signaling pathways are crucial for this process, such as RUNX1, SOX17, and NOTCH.³⁶ To distinguish between these two hematopoietic programs, T lymphoid potential has been used as a readout.³⁷ This readout, however, has recently been challenged as more about these two hematopoietic programs have been discovered.³⁸ In other attempts to mimic the definitive program *in vitro* using mouse PSCs, it has been shown that a hemangioblast can be generated through an HE precursor cell and will eventually undergo the primitive program.³⁹

Progress in cell reprogramming has been made through the use of small molecules. Small molecules provide many benefits, such as the opportunity to maximally optimize their use experimentally, but they can also cause various side effects by acting on the wrong signaling pathways.^{40,41} It has been demonstrated that iPSCs can be derived from somatic cells solely via small molecule compounds.⁴² This technology has also been extended to the generation of other cell types, such as neurons.^{43,45} Small molecules have also been shown to expand HSCs *ex vivo*. Studies using SR1, UM171, and VPA have all shown varying levels of success in expanding populations of HSCs.^{46,48} Cells expanded with these compounds, however, remain limited in their function.⁴⁹

To obtain highly functional HSCs *de novo*, several things must be taken into consideration. These include but are not limited to (1) selection of the appropriate TFs, (2) choice of the somatic cells to reprogram, (3) method of TF delivery and the culture conditions for the reprogramming process, and (4) the ways to mature the cells appropriately. In this review, we will focus on the various strategies that utilize TF reprogramming of both PSCs and somatic cells that bring us closer to obtaining HSCs *de novo*.

TF reprogramming PSCs

In the early attempts to obtain HSCs *in vitro*, investigators tried to directly differentiate PSCs.^{50,51} In some studies, hemogenic endothelial precursor cells were generated. These

studies attempted to mimic developmental hematopoiesis during embryogenesis *in vitro*, with varied success.⁵² Other studies tried isolated HSCs from teratomas, but functional and clinical application of these cells would be extremely difficult.^{53,54} Rather than just trying to differentiate PSCs into HSCs directly *in vitro*, others have tried to transduce PSCs or lineage-restricted cells derived from PSCs with a variety of TF cocktails to induce the hematopoietic program. For this section of the review, we will focus only on studies that utilized TF overexpression in PSCs or PSC-derived cells in their attempts to generate bona fide HSCs.

HoxB4 in mouse cells

Initial TF reprogramming strategies focused on generating HSCs *de novo* utilized the homeobox gene *HoxB4*. *HoxB4* was retrovirally introduced into mESCs, which were then co-cultured on OP9 stroma. This stroma promotes HSC survival in culture and provides important inductive signals to these cells.^{55,56} Upon transplant, it appeared that these reprogrammed cells could rescue lethally irradiated mice, but the cells only generated myeloid cells.⁵⁷ Transduction of precirculation yolk sac and ESC-derived progenitors with inducible *HoxB4* and subsequent co-culture on OP9 stroma produced cells with multilineage engraftment potential in both primary and secondary transplants. Continued expression of *HoxB4*, however, inhibited differentiation.⁵⁸ To avoid genomic integration, others tried reprogramming with transient *HoxB4* packaged in adenovirus and generated HSCs from mouse iPSCs.⁵⁹ One study found that the inclusion of *Cdx4* with *HoxB4* overexpression improved the reprogramming process.⁶⁰ These *HoxB4* studies suggested great promise in the prospect of obtaining HSCs *de novo* via TF overexpression, but they also come with various drawbacks. The transient *HoxB4* study did not determine whether the cells were capable of long-term multilineage engraftment. Mouse and human hematopoiesis do vary; what we learn in the mouse does not always apply in the human.^{61,62}

HOXB4 in human cells

HOXB4 overexpression in human ESCs demonstrated the first attempt at TF-mediated reprogramming of human cells to HSCs. HOXB4 overexpression maintained cells in an ESC state, and upon culture without cytokines they differentiated toward hematopoietic cells. Inclusion of cytokines increased the efficiency of reprogramming/differentiation. In human ESCs, enforced HOXB4 expression resulted in the development of myeloid and monocytic colonies.⁶³ Mouse ESCs by comparison generated erythroid and mixed erythroid/myeloid colonies upon *HoxB4* overexpression. To add to the discordant results seen with HOXB4, another study demonstrated that HOXB4 in human cells was unnecessary for hematopoietic development.⁶⁴ The variability in acquired hematopoietic lineages between mouse and human cells from HOXB4 overexpression calls into question the importance of HOXB4, and its reproducibility.

TF overexpression in lineage-restricted cells

Moving away from HOXB4, investigators explored other TF combinations in PSCs to determine if they could establish the hematopoietic program. One study differentiated human iPSCs into CD34⁺CD45⁺ lineage-restricted myeloid precursors and subsequently transduced them with HOXA9, ERG, and RORA. After transduction, they found that their

cells resembled progenitors capable of self-renewal and differentiation. To improve the engraftment potential of their derived cells, they added SOX4 and MYB to their TF cocktail, resulting in short-term engraftment potential generating myeloid and erythroid lineages.⁶⁵ Interestingly, this reprogramming strategy produces erythroid cells that make adult hemoglobin and can be expanded exponentially. Though these cells only possess short-term engraftment potential and do not form lymphoid cells, their single lineage production of myeloid and erythroid cells could be utilized for future studies of hematologic disease focused on those lineages.

Sox17 in mouse cells

The functions of fetal and adult HSCs differ in a variety of ways, such as their ability to proliferate and engraft.⁶⁶ Sox17 represents one major difference between these two populations of cells. Sox17 is critical in definitive fetal HSCs and dispensable in adult HSCs. Conditional KO studies demonstrated that fetal HSCs will die without Sox17, whereas adult HSCs are unaffected.⁶⁷ Sox17 overexpression in adult HSCs resulted in a restructuring of the expression profile, increasing self-renewal and multilineage engraftment potential. Sox17 overexpression in HSCs was not without problems. These cells did not possess lymphoid engraftment potential, and constitutive overexpression resulted in leukemogenesis.⁶⁸ Deletion studies in mESCs demonstrated that Sox17 is crucial for definitive hematopoiesis, but is expendable in the primitive program. Additional studies showed that Sox17 interacts with the NOTCH pathway and is required for EHT rather than for generating CD34⁺ HE.^{30,69}

SOX17 in human cells

HE development was observed after SOX17 overexpression in human PSCs as well. Although these cells formed semiadherent cell aggregates during hematopoietic culture, they gave rise to very few hematopoietic progenitors. When SOX17 overexpression was stopped, however, the cells gave rise to several hematopoietic progenitors. This demonstrates that SOX17 is important for hemogenic potential. SOX17 directly activates the transcription of several regulatory genes for vasculogenesis, hematopoiesis, and erythroid differentiation. SOX17 knockout resulted in repressed hemogenic potential.⁷⁰ This work further supports the notion that during developmental hematopoiesis HSCs travel through the HE, but this study did not examine whether these cells possess multilineage engraftment potential. This work appears promising *in vitro*, and can be further used to study developmental hematopoiesis “in a dish.”

Varying lineage acquisition with two different TF cocktails

One study found that transduction of human iPSCs with two separate TF cocktails generated HE with different lineage potentials. Using modified mRNA overexpression, gain-of-function studies revealed the two TF sets that reprogrammed fibroblasts to these lineage-restricted HE populations. GATA2 and ETV2 overexpression generated HE restricted to the production of myeloid lineage cells. GATA2 and TAL1 overexpression produced HE restricted to erythromegakaryocytic lineage potential.⁷¹ This work shows further evidence of HSCs traveling through the HE. It also demonstrates that overexpression of select factors

will confer specific lineage potential to the human iPSC-derived HE. The inability to generate cells capable of multilineage engraftment potential, however, limits their use.

TF reprogramming somatic cells

Building upon the framework established by other studies of cell fate conversion (Fig. 1), our laboratory was the first to attempt somatic cell programming into hematopoietic stem and progenitor cells (HSPCs) using non-pluripotent TFs.²⁶ Several laboratories have followed and are now trying to directly reprogram somatic cells into HSCs via TF overexpression. Most of these studies bypass a pluripotent state by not using pluripotency factors, which can generate plastic intermediates.^{72,73} Variations between each strategy, such as starting cell populations, TF cocktails, or stromal co-culture, highlight our lack of understanding this developmental process *in vitro*. We will first discuss two studies that did use pluripotency factors. A timeline of conversion studies is outlined in Figure 3 for both TF reprogramming studies with PSCs and with somatic cells.

OCT4 and induced plasticity

The first study that claimed to generate HSPCs *de novo* from human fibroblasts utilized OCT4, an essential pluripotency factor. Transduced cells were then cultured with cytokines and reprogramming media (RM) that resulted in CD45⁺ cells that gave rise to granulocytic, erythrocytic, monocytic, and megakaryotic colonies.⁷⁴ The reprogrammed cells had limited self-renewal potential and did not produce lymphoid cells. Upon transplant, engraftment could only be observed in the site of injection of the reprogrammed cells, limiting the prospects of full hematopoietic reconstitution. It is thought that the OCT4-transduced cells travel through a plastic intermediate that can be directed to differentiate into the hematopoietic lineage via RM exposure.⁷⁵ Although this study demonstrates the possibility of obtaining HSPCs from somatic cells, the process is inefficient and does not generate fully functional cells. The generation of partially pluripotent intermediates can also lead to oncogenesis, limiting the clinical use of these cells.

Lineage-restricted progenitors with SOX2-induced plasticity

Pluripotency-associated factor SOX2 (as well as miR-125b) overexpression in human fibroblasts rapidly generates CD34⁺ cells. To support the reprogramming even further, cells were placed in the mouse to utilize the *in situ* niche. These cells gave rise to only macrophages and monocyte-like progenitors.⁷⁶ Similar to the OCT4 study, the reliance on the known pluripotency factors during reprogramming causes transit through a plastic intermediate. Moreover, although the *in vivo* hematopoietic niche can provide all signals necessary for reprogramming, we cannot dissect the niche in this study and thus cannot determine the elements that support reprogramming. Furthermore, the inability to generate any hematopoietic cells other than macrophages and monocyte-like progenitors also limits future studies with this process unless they focus on these lineages.

Hemogenic induction

As previously stated, our laboratory became the first to induce a hemogenic program in somatic cells via non-pluripotency TF overexpression in mouse embryonic fibroblasts

(MEFs). We first did a large screen by transducing TFs into MEFs from our hCD34/H2BGFP mouse as a reporter for GFP⁺ cells.⁷⁷ We found that GATA2, Gfi1b, and cFos were necessary, but the addition of ETV6 was optimal for the expression of CD34 GFP⁺ cells. These cells were first generated as Prominin1⁺Sca1⁺CD34⁺CD45⁻ intermediates that possessed a global endothelial expression program that could functionally parallel embryonic HE. Continued culture produced semi-adherent hematopoietic-like cells emerging from these intermediate cells. These semi-adherent cells possess an HSC transcription program and cell surface immunophenotype. Upon placental re-aggregation culture, the cells adopted colony-forming potential *in vitro*.²⁶ A salient feature of this work is that, from somatic cells, we can instigate a developmental process using a minimal set of TFs. It is likely that the best way to obtain HSCs *de novo* will be to follow and mimic developmental hematopoiesis as closely as possible. If the hemogenic program that we induce recapitulates this process, it will be of great interest to determine if the intermediate cells described by the surface phenotype Prominin1⁺Sca1⁺CD34⁺CD45⁻ exist in known embryonic sites of hematopoiesis.⁷⁸ We are now translating these studies to human cells and have promising preliminary data. Knowing that human and mouse hematopoiesis vary in a number of ways, it will be important to determine additional signals needed for hematopoietic induction.

Blood progenitor de-differentiation and the *in vivo* niche

The laboratory of Derek Rossi took a different approach where they let the *in vivo* niche do the reprogramming by transplanting cells directly after transduction. In their studies, ectopic expression of Hlf, Runx1t1, Pbx1, Lmo2, Zfp37, and Prdm5 reprogrammed hematopoietic progenitor and effector cells back into functional HSCs. Although these cells possessed long-term multilineage and secondary transplantation potential, the process is inefficient. Inclusion of the factors Meis1 and Mycn, along with polycistronic viruses, greatly increased reprogramming efficiency. Transient expression of the TFs for 2 weeks *in vivo* was sufficient to reprogram lineage-committed progenitor cells from the BM and lineage-positive cells.⁷⁹ These studies advanced the field, but obstacles remain. Hematopoietic cells would bear the acquired or congenital mutations in hematological malignancies and preclude their use as a starting cell type. Moreover, several TFs in the cocktail are proto-oncogenes, further increasing the risk of tumorigenicity. Although the *in vivo* niche contributes to the reprogramming process, it would be impossible to dissect out the signals that play a role in hematopoietic induction and maintenance.⁸⁰ As of now, these studies have yet to be replicated with human cells.

Fetal and adult human endothelial reprogramming and the *in vitro* niche

Although it is possible to cross embryonic germ layers in the selection of the starting cell population for reprogramming experiments,⁸¹ most investigators make an effort to minimize the “epigenetic distance” that increases as you deviate from the lineage of a certain cell type.⁸² Due to the short distance between endothelial cells and hematopoietic cells, Shahin Rafii’s group selected human umbilical vein and adult dermal microvascular endothelial cells (HUVECs and hDMECs, respectively) to reprogram to HSCs. They combined conditional overexpression of the TFs FOSB, GFI1, RUNX1, and SPI1 with induction from a vascular niche. The investigators reprogrammed both endothelial cell lines into MPPs.

These MPPs possessed multilineage engraftment potential of all lineages except for T cells, and could engraft both primary and secondary recipient mice.⁸³ Their vascular niche was a monolayer of endothelial cells transduced with *E4ORF1* by adenovirus. These cells are called E4ECs and they have been shown to promote survival pathways without compromising proliferation and cell survival, while also providing signals necessary for HSPC expansion.⁸⁴ The work again demonstrates that a niche component to HSC reprogramming is crucial and must be further explored.

Reprogramming to the hemangioblast

The hemangioblast is an *in vitro* structure derived from mesodermal cells capable of generating endothelial, hematopoietic, and smooth muscle cells.⁸⁵ One study reprogrammed a variety of cell types (mouse ESCs, fetal liver cells, and fibroblasts) with Gata2, Lmo2, Mycn, Pitx2, Sox17, and Tal1 to produce expandable hemangioblasts (eHBs). Successful culture of these cells required fibroblast growth factor (FGF). Continued expression of the TFs maintained the cells in a proliferative state, and upon removal of TF overexpression these eHBs generated functional smooth muscle, endothelial, and multilineage hematopoietic cells. FGF allowed cells to expand in culture and promoted the generation of endothelial cells and leukocytes but not erythrocytes.⁸⁶ This study suffers from poor fibroblast data, making it hard to fully see feasible generation of eHBs from these somatic cells. We also do not know the multilineage engraftment capabilities of these reprogrammed cells. This work augments study of the hemangioblast as well as hematopoiesis *in vitro*.

Rapid HSC reprogramming

Rapid reprogramming of fetal and adult mouse fibroblasts into HSPCs using a TF cocktail comprising Erg, Gata2, Lmo2, Runx1c, and Scl was demonstrated by the group of George Lacaud. As in our studies these reprogrammed cells appeared to travel through an HE intermediate, and had an endothelial and hematopoietic gene expression profile. The generated progenitors formed erythroid, megakaryocytic, and myeloid cells, and could form B and T cells upon co-culture with OP9 and OP9-DL1 stroma, respectively. The use of *p53* null fibroblasts made reprogramming more efficient and more rapid, shortening the transition from 8 to 5 days.⁸⁷ It was previously shown that reprogramming somatic cells to iPSCs becomes more efficient with inactivation of *p53*⁸⁸ or with mutant *p53*.⁸⁹ Although this strategy generates hematopoietic-like cells rapidly, the loss of *p53* can lead to aberrant self-renewal and tumorigenicity of the generated cells, limiting clinical application. Functionally, these cells only possess short-term engraftment potential, necessitating further studies into this reprogramming method.

We have graphically summarized the above-discussed efforts in Figure 4 and present specific reprogramming strategies. TFs, starting cells, culture conditions, media, and cytokines, as well as the outcomes in terms of lineage production and engraftment, are compiled in Table 1.

Perspectives on the various reprogramming strategies

Obtaining HSCs *de novo* remains the “holy grail” of regenerative HSC biology because of the multiple issues associated with HSC transplant and HLA matching. Generating and studying these cells *in vitro* could also help further expand our capacity to study the nuances of hematopoiesis. Despite the attempts of several laboratories, the production of bona fide HSCs *de novo* or their expansion *ex vivo* has met with varying levels of success. Our incomplete understanding of the mechanisms by which these various cell fate conversions proceed contributes to our current inability to generate these cells.

The impact of epigenetic distance and memory^{82,90,91} complicates the selection of starting cells. Reprogramming cells more epigenetically related to HSCs undoubtedly makes the process easier, but the clinical acquisition and application of these cells must be considered. Understanding the changes to the epigenome that occur during reprogramming is essential to making this process efficient and complete.⁹²

In a perfect world, these reprogramming strategies would not use viral tools to introduce the selected TFs for overexpression. These tools, such as lentivirus, can integrate into the host cell genome and raise the risk of insertional oncogenesis.^{93,94} These concerns are tempered by the fact that several successful gene therapy studies are using this technology clinically.⁹⁵ This can also be used to our advantage, however, enabling the identification of genes involved in numerous cancers such as hepatocellular carcinoma.⁹⁶ These concerns may be overcome using modified RNAs^{97,98} or self-replicating RNA.⁹⁹ Both of these methods have been shown to efficiently reprogram fibroblasts to iPSCs.

The field of reprogramming somatic cells into HSPCs is in its infancy. With each new study, we learn more about the reprogramming process, as it appears that there are multiple ways emerging to bring the process to fruition with the eventual creation of bona fide HSCs. Many obstacles remain, the foremost being the need for expanding knowledge of the mechanisms that modify DNA and chromatin. The few years of existing efforts of hematopoietic reprogramming work towards the same goal: making an HSC *de novo*. Reaching this goal will allow us to examine hematopoiesis in more detail *in vitro*, generate patient disease models *in vitro* to study disease mechanism and novel drug discovery, and engineer platforms for gene correction and disease treatment via patient-specific HSC transplant (Fig. 2).^{81,100} The strategy that most closely follows developmental hematopoiesis may allow us to understand more about this process and how to best reprogram any cell type into HSCs. These are exciting times in the reprogramming field. We have shown that it can be done. Now we need to understand how we are doing it and make the process efficient and feasible for cell replacement therapy. The hard work has just begun.

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Figure 1. Established TF reprogramming strategies. TF reprogramming has been shown to work across a variety of cell types. This figure shows the starting and end cell populations, as well as the TF cocktails used to derive each cell type of choice. Adapted from Ref. ²⁵.

Figure 2.

Paradigm for HSPC reprogramming and clinical application. This figure displays the overall goals of HSPC reprogramming, and the most prevalent ways that this work will be applied to both research and medicine. The ideal strategy is to effectively reprogram patient cells and use them for studying hematopoiesis, drug discovery, and stem cell medicine.

Figure 3.

Timeline of attempted HSPC derivation strategies. From a variety of different mouse and human strategies, laboratories have been trying to derive functional HSPCs, with different levels of success. This timeline shows the TF overexpression strategies that attempt to generate HSPCs *de novo*.

Figure 4.

TF reprogramming strategies to obtain HSPCs. Investigators have attempted to generate HSPCs *de novo* for several years. This figure shows the multiple efforts made to obtain these cells, and the results of their reprogramming strategies based on the starting cell population and the TF cocktail. The colors of the boxed TF cocktails and arrows represent the associated strategy. An H or an M at the bottom right hand corner of each TF box denotes strategies that used human or mouse cells, respectively.

Table 1

Reprogramming strategies and materials

Reference	Kyba <i>et al.</i> , 2002 ⁸⁸	Wang <i>et al.</i> , 2005 ⁶⁰	Bowles <i>et al.</i> , 2006 ⁶³	McKinney-Freeman, <i>et al.</i> , 2008 ⁵⁷	Szabo <i>et al.</i> , 2010 ⁷⁴	Pereira <i>et al.</i> , 2013 ²⁶	Doulatov <i>et al.</i> , 2013 ⁶⁵	Riddell <i>et al.</i> , 2014 ⁷⁹	Elcheva <i>et al.</i> , 2014 ⁷¹	Sandler <i>et al.</i> , 2014 ⁸³	Pulecio <i>et al.</i> , 2014 ⁷⁶	Vereide <i>et al.</i> , 2014 ⁸⁶	Battia <i>et al.</i> , 2014 ⁸⁷
Starting cells	Mouse ESCs and yolk sac progenitors	Mouse ESCs	Human ESCs	Mouse ESCs	Human fibroblast	Mouse embryonic fibroblast	Human ESC–derived CD34 ⁺ CD45 ⁺ cells	Mouse lymphoid or myeloid progenitors and myeloid effector cells	Human ESCs and iPSCs	Human embryonic and adult endothelial cells	Human fibroblast	Mouse ESCs, fetal liver cells, and fibroblasts	Mouse fibroblast
TF combo	HoxB4	HoxB4, Cdx4	HOXB4	HoxB4	OCT4	GATA2, Gfi1b, cFos, ETV6	ERG, HOXA9, RORA, SOX4, MYB	Runx1/T1, Hlf, Lmo2, Pbx1, Zfp37, Prdm5, Mycn, Meis1	ETV2 + GATA2 or TAL1 + GATA2	FOSB, GFI1, RUNX1 SPI1	SOX2, miR-125b	Gata2, Lmo2, Mycn, Ptx2, Sox17, and Tal1	Erg, Gata2, Runx1c, Scl Lmo2
Stroma/co-culture	mESCs on MEFs	mESCs on MEFs	hESCs on MEFs	OP9	Matrigel	Gelatin, placenta reaggregation	hESCs grown on matrigel	<i>In vivo</i> niche	Matrigel	Vascular niche (E4EC cells)	–	Matrigel	OP9, OP9-DL1
Media/cytokines	SCF, VEGF, TPO, Flt3L	SCF, VEGF, TPO, Flt3L	SCF, IL3, IL6, Flt3L, G-CSF, BMP4	Flt3L, SCF, TPO, VEGF	RM (see text)	Myelocult, SCF, Flt3L, IL3, IL6	BMP4, SCF, Flt3L, G-CSF, IL3	–	SCF, TPO, bFGF	FGF2, EGF, SCF, Flt3L, TPO, IGF1, IGF2, IL3, IL6	Flt3L, TPO, SCF	FGF	SCF, IL3, G-CSF, TPO, EPO, M-CSF, IL6, IL11
CF-U	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Lineages	Erythroid myeloid lymphoid	Erythroid myeloid lymphoid	Erythroid myeloid lymphoid	Erythroid myeloid lymphoid	Erythroid myeloid megakaryocyte	Erythroid myeloid	Erythroid myeloid lymphoid (T cells <i>in vitro</i>)	All	Myeloid or erythroid + megakar-yocyte	Erythroid myeloid lymphoid (B cells only)	Erythroid myeloid	Endothelium, smooth muscle, erythroid, macrophage, megakaryocyte	Erythroid myeloid lymphoid (with p53 KO cells)
Engraft	✓	✓	N/A	✓	✓ (minimal)	X	✓ (short term)	✓	X	✓	✓	N/A	✓ (short term)
Serial transplant	✓	✓	N/A	✓	✓	X	X	✓	X	✓	X	N/A	X