REVIEW

Blood making: learning what to put into the dish [version 1; peer review: 3 approved]

Ana G Freire¹, Jason M Butler^{1,2}

¹Center for Discovery and Innovation, Hackensack University Medical Center, Nutley, USA

²Molecular Oncology Program, Georgetown University, Washington D.C., USA

V1 First published: 23 Jan 2020, 9(F1000 Faculty Rev):38
<https://doi.org/10.12688/f1000research.21245.1>

Latest published: 23 Jan 2020, 9(F1000 Faculty Rev):38
<https://doi.org/10.12688/f1000research.21245.1>

Abstract

The generation of hematopoietic stem cells (HSCs) from pluripotent stem cell (PSC) sources is a long-standing goal that will require a comprehensive understanding of the molecular and cellular factors that determine HSC fate during embryogenesis. A precise interplay between niche components, such as the vascular, mesenchymal, primitive myeloid cells, and the nervous system provides the unique signaling milieu for the emergence of functional HSCs in the aorta-gonad-mesonephros (AGM) region. Over the last several years, the interrogation of these aspects in the embryo model and in the PSC differentiation system has provided valuable knowledge that will continue educating the design of more efficient protocols to enable the differentiation of PSCs into *bona fide*, functionally transplantable HSCs. Herein, we provide a synopsis of early hematopoietic development, with particular focus on the recent discoveries and remaining questions concerning AGM hematopoiesis. Moreover, we acknowledge the recent advances towards the generation of HSCs *in vitro* and discuss possible approaches to achieve this goal in light of the current knowledge.

Keywords

hematopoietic stem cell, AGM, hemogenic endothelium, pluripotent stem cell

Open Peer Review

Approval Status

	1	2	3
version 1 23 Jan 2020			

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

1. **Lesley M Forrester**, University of Edinburgh, Edinburgh, UK
2. **Valerie Kouskoff**, University of Manchester, Manchester, UK
3. **Igor Slukvin**, University of Wisconsin, Madison, USA

Any comments on the article can be found at the end of the article.

Corresponding author: Jason M Butler (jason.butler@hnh-cdi.org)

Author roles: Freire AG: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; Butler JM: Conceptualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Ana G. Freire is a New York Stem Cell Foundation – Druckenmiller Fellow. Jason M. Butler is a Scholar of The Leukemia and Lymphoma Society. This research was supported by The New York Stem Cell Foundation, Tri-Institutional Stem Cell Initiative (2014-004), American Society of Hematology Scholar Award, American Federation for Aging Research, National Institutes of Health (1R01CA204308, 1R01HL133021, and 1R01AG065436), and the Leukemia and Lymphoma Society Quest for Cures. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

Copyright: © 2020 Freire AG and Butler JM. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Freire AG and Butler JM. **Blood making: learning what to put into the dish [version 1; peer review: 3 approved]** F1000Research 2020, 9(F1000 Faculty Rev):38 <https://doi.org/10.12688/f1000research.21245.1>

First published: 23 Jan 2020, 9(F1000 Faculty Rev):38 <https://doi.org/10.12688/f1000research.21245.1>

Introduction

Throughout the lifespan of an organism, the supply of blood cells is guaranteed by hematopoietic stem cells (HSCs) that replenish lineage-restricted progenitors while maintaining their stem cell pool. Because these cells are capable of sustaining the entirety of the blood hierarchy, they constitute a cellular source for transfusion, immunotherapies, and transplantation. HSC transplantation constitutes a crucial therapy for hematological disorders. However, the supply for patients is insufficient owing to shortage of histocompatible donors and limited cell number. Thus, the ability to generate HSCs *in vitro* will provide access to an unlimited number of cells that are capable of long-term reconstitution of all blood cell lineages. The derivation of embryonic stem cells (ESCs) and, subsequently, induced pluripotent stem cells (iPSCs) has provided the platforms to, in theory, derive HSCs given their intrinsic property to generate derivatives of the three germ layers. Yet, despite innumerable efforts, the generation of HSCs from non-genetically manipulated mouse or human pluripotent stem cells (hPSCs) has still not been successful. A full understanding of how HSC fate is specified during embryo development is crucial, as it will provide useful cues to educate the design of *in vitro* differentiation protocols. The inefficacy in doing so thus far is related to the inherent complexity of the hematopoietic developmental process and the incomplete identification of all the critical factors that make HSC specification possible in the embryo. Comprehension of the initiating events underlying the emergence of the distinct hematopoietic waves endowed with different lineage potential as well as of the microenvironmental cues that instruct and nurture HSC emergence, maturation, and expansion is fundamental. In the following sections, we will revisit the knowledge in early hematopoietic development, with particular focus on the events occurring in the aorta-gonad-mesonephros (AGM) region, the location where HSCs are first detected in the embryo, from the origin of the HSC precursor to the molecules and niche components that guide HSC fate. Lastly, we will review recent advances in the attempt to generate long-term HSCs in the dish and the important contributions these studies provided to get closer to the Holy Grail of the hematopoietic field.

Early hematopoietic development at a glance

Blood formation is a conserved process among vertebrates that occurs in several waves, contributing different hematopoietic cell types in the yolk sac (extraembryonic) and within the embryo proper (intraembryonic). The first hematopoietic wave, called primitive hematopoiesis, emerges within the yolk sac blood islands around embryonic day (E) 7.0–7.5 in the mouse. Hematopoietic differentiation at this stage is limited to cells of the erythroid, macrophage, and megakaryocytic lineages and serves the needs of a rapidly growing embryo¹. Following the formation of primitive cells, a second wave contributes with erythro-myeloid progenitors (EMPs) as well as lymphomyeloid, B-1, and T cell-restricted progenitors before the emergence of HSCs^{2–6}. Because the first and second waves arise before the first adult-type HSCs are detected in the embryo, they are considered to be HSC-independent hematopoiesis^{1,7}. Interestingly, derivatives of the HSC-independent hematopoiesis

persist to adult life, such as the microglia in the brain and the tissue-resident macrophages found in several organs^{8,9}. The existence of long-lived functional blood cells that did not derive from an HSC challenges the classical hierarchical model of adult hematopoiesis. The hallmark of an adult HSC is its capacity of long-term, multilineage reconstitution in adult recipients in a setting of serial transplantation¹⁰. This definition set the foundation that HSC emergence begins around E10.5 in the dorsal aorta (DA) and vitelline/umbilical arteries of the mouse embryo^{11–14}. Nascent HSCs are present within intra-aortic clusters in close contact with endothelial cells (ECs) lining the floor of the DA¹⁴. In the human, intra-aortic clusters are detected around 27 days of gestation¹⁵, with HSC long-term repopulating activity being demonstrated by transplantation assay into immunodeficient mice¹⁶.

Observations made 100 years ago led to the hypothesis that blood cells emerging in the DA possess an endothelial origin^{17,18}. More recent studies have provided compelling evidence, through live imaging and lineage tracing, for the existence of a specialized vascular endothelium, the denominated hemogenic endothelium (HE), that undergoes endothelial hematopoietic transition (EHT)^{19–23}. Maturation towards HSC fate occurs through a multi-step differentiation process from hematopoietic precursors (type I and II pre-HSCs) that express genes associated with the endothelial lineage, such as *Cdh5* (or *VEcad*) and *Pecam1* (or *Cd31*) (Figure 1)^{24–28}. Later, it was discovered that HE is not restricted to the developing embryonic vasculature where HSCs emerge, existing also in the yolk sac where it serves as a precursor of primitive erythrocytes²⁹ and EMPs³⁰ as well as B⁶ and T cells⁵ prior to HSC emergence.

The emergence of HSCs in the DA is a highly dynamic and transient phenomenon that declines around E12.5 in the mouse, coinciding with the entrance of HSCs in the bloodstream and seeding of the fetal liver, which will support HSC expansion prior to migration to the fetal spleen and bone marrow^{28,31}. Additional sources where the presence of HSCs has been reported (e.g. the placenta, head, and yolk sac) were suggested to also contribute to the HSC pool in the fetal liver^{32–36}. However, the capacity of these sources to originate HSCs *de novo*, rather than promoting their expansion, remains a controversial topic which is greatly hampered by the onset of circulation around E8.25^{35,37–39}. Recently, the *de novo* generation of hematopoietic stem/progenitor cells (HSPCs) from HE existing in the bone marrow of late fetus/young adult chickens and mice has also been suggested⁴⁰.

Studies using limiting-dilution transplantation assays have estimated that the AGM contains very few HSCs (one to three) and proposed that the rapid increase in HSC number in the fetal liver could be the result of maturation of a large pool of pre-expanded pre-HSCs generated in the AGM region^{28,32}. Recent reports utilizing multicolor fate mapping in zebrafish and mouse models have suggested that the number of hematopoietic precursors emerging at the AGM, and contributing to adult hematopoiesis, is significantly higher than initially predicted^{41,42}. The functional readout of HSCs autonomously

Figure 1. Representation of the consensually accepted sequential steps of differentiation to adult-type HSCs in the AGM. HSCs derive from a precursor that descends from mesoderm and expresses endothelial genes, called hemogenic endothelium (HE). HE undergoes endothelial hematopoietic transition (EHT) and maturation to functional HSCs through intermediate HSC precursors (type I and II pre-HSCs). During this process, a precise regulation of the arterial-associated genes *Notch1* and *Sox17* and of the critical players in EHT, *Runx1* and *Gata2*, takes place. Moreover, hematopoietic markers (i.e. c-Kit, CD41, CD43, and CD45) are upregulated while genes of endothelial affiliation (i.e. VE-Cadherin and CD31) are still expressed^{24–28}. *HSCs are believed to display a similar phenotypic signature to type II pre-HSCs, but their function is demonstrated by transplantation into adult irradiated recipients. AGM, aorta-gonad-mesonephros; E, embryonic day; HSC, hematopoietic stem cell.

generated from the AGM has been facilitated by the utilization of platforms that allow *ex vivo* maturation and/or expansion, such as organ explant culture^{12,32,43}, co-aggregation culture with OP9 stromal cells^{24,44}, or co-culture with Akt-expressing AGM-derived ECs^{45,46}. It is not until E11.5 that functional AGM-derived HSCs can be robustly detected by direct transplantation into adult recipients, likely because of the achievement of a critical number of HSCs²⁴. The fact that hematopoietic precursors are able to acquire functional properties after a period of *ex vivo* co-culture with niche cells suggests that these *in vitro* platforms are able to provide the cues encountered within the embryonic environment. This anticipates that it might be possible to recapitulate *in vitro* the process of HSC generation if we recreate the complex interactions within the stem cells' niche.

AGM: the cradle of HSCs

The observation that HE located in the DA and vitelline/umbilical arteries originates HSCs highlights the importance of comprehending the combination of spatiotemporal signals and niche cells that compose the AGM region. Curiously, HE giving rise to functional HSCs is detected only within the large arteries of the embryo, whereas HE in the yolk sac is associated with both arterial and venous endothelium^{13,30,47}. This evidence, combined with the observation of direct hematopoietic cell emergence from aortic endothelium, led to the hypothesis that acquisition of arterial identity precedes HSC emergence.

The origin of the aortic HE and whether it shares a common lineage with arterial endothelium is still a topic of debate. The loss of critical regulators of arterial identity, such as SOX17 and NOTCH1^{48–50}, is detrimental for HSC generation or maintenance in the mouse embryo^{51,52}. However, a tight control of the activity of these pathways is required to ensure proper HSC specification, with downregulation being necessary during formation of the hematopoietic clusters^{53,54}. RUNX1 and GATA2 are also indispensable players during EHT (Figure 1) in the zebrafish, chick, and mouse AGM and during ESC differentiation^{23,55–60}, whose timing and duration of intervention are tightly controlled^{61,62}. Nevertheless, studies that have reported the presence of hematopoietic precursors in the subendothelial aortic layers in the mouse AGM region added complexity to our understanding of the origin of the HE^{24,63}. In addition, it has been recently shown in the chicken that the coelomic epithelium-derived mesenchyme invades through the ventral wall of the DA prior to formation of the intra-aortic clusters⁶⁴. Studies in hPSCs have identified HE and non-HE lineages during *in vitro* differentiation⁶⁵ and have suggested that the HE and the precursors of the arterial vascular endothelium represent distinct lineages deriving from hPSC-derived mesodermal progenitors⁶⁶. These observations during hPSC *in vitro* differentiation led to a proposed model of human HE development, in which HE in the subaortic mesenchyme migrates to the AGM region to integrate with the arterial ECs within the ventral wall of the DA⁶⁷. Later studies in hPSCs suggested that, after

specifying from mesodermal progenitors, HE can be specified into an arterial-type HE with lymphoid and myeloid potential *in vitro* through overexpression of *Ets1* or modulation of MAPK/ERK signaling in a process dependent on Notch signaling^{68,69}. Recently, RNA-sequencing studies in the mouse embryo revealed that the aortic HE transcriptome is very similar to that of arterial ECs of the DA^{26,70} and distinct from the yolk sac HE⁷⁰. Additionally, a recent single-cell RNA-sequencing study in human AGM reported that the HE displays an arterial gene signature⁷¹. Moreover, a study in zebrafish using a BAC *runx1* transgenic reporter showed that *runx1* expression in HE mediates silencing of arterial endothelial genes, including *dll4*, while *runx1*^{-/-} mutants maintain arterial identity⁷². This finding reconciles the previous demonstration that cells in the mouse DA that experience high levels of NOTCH1 activity through DLL4 are committed to arterial EC fate, whereas specification of HSCs depends on low levels of NOTCH1 enabled by JAGGED1⁷³. Furthermore, it suggests the aortic endothelium is the precursor of HE⁷², in line with earlier evidence provided by live imaging and lineage tracing studies in the chick, zebrafish, and mouse embryo^{19,21–23,74}.

An important observation is that the emergence of intra-aortic clusters is located at the ventral wall of the DA in the chick, zebrafish, and human AGM^{15,19,21,23}. In the mouse, although mostly ventralized, some clusters are also found along the dorsal wall^{22,75,76}. Yet repopulating activity is predominantly restricted to intra-aortic clusters localized ventrally in the DA⁷⁵. A plausible explanation might be that ventral aortic ECs, conversely to dorsal ECs, derive from splanchnopleural mesoderm, which is endowed with hematopoietic potential⁷⁷. The dorsoventral polarity of HSC function correlates with asymmetric signaling in the embryonic DA, with polarized Sonic Hedgehog (SHH) and bone morphogenetic protein 4 (BMP4) signaling

and Kit ligand (KITL) expression being reported^{78–80}. Reinforcing the transient nature of AGM hematopoiesis, a precise control of BMP activity has been shown in mouse and zebrafish in order to restrict HSC formation and allow maturation^{80–82}. In the zebrafish, fibroblast growth factor (FGF) signaling was found to play a role in modulating the BMP pathway⁸². Moreover, Tenascin C, an extracellular matrix glycoprotein that facilitates cell-to-cell interactions and cell migration, is also abundant in the mesenchymal tissue underneath the ventral wall of the human DA⁸³. A posterior study in hPSCs correlated the high production of Tenascin C in overconfluent OP9 stromal lines with the ability to induce higher hematopoietic potential⁸⁴.

Contributing to the highly dynamic nature of the AGM, a complex crosstalk between different niche components, including the recruitment of different cellular and molecular factors to the vicinity of the DA, takes place during HSC emergence (Figure 2). The subaortic mesenchyme contributes to HSC formation in the chicken embryo by inducing RUNX1 expression in the ventral aortic endothelium⁵⁸. Moreover, studies suggested a link between the developing nervous system and enhancement of HSC emergence, as shown by the inductive effect of catecholamines in the mouse⁸⁵ and neuronal serotonin in the zebrafish⁸⁶. Interestingly, from the yolk sac-derived wave, macrophages^{87,88} and neutrophils⁸⁹ contribute with local sterile inflammatory signaling that promotes HSC emergence. Evidence in the zebrafish further demonstrated that tumor necrosis factor alpha (TNF α) signaling produced by neutrophils triggers JAGGED1-mediated Notch signaling in the aortic endothelial layer, thereby promoting HSC specification⁸⁹. Furthermore, macrophages establish a close interaction with the emerging intra-aortic hematopoietic cells in zebrafish, human⁹⁰, and mouse AGM⁸⁸ and remodel the extracellular matrix, thus permitting HSC mobilization to the caudal hematopoietic

Figure 2. Representation of niche components that participate during HSC emergence in the AGM. A precise interplay between niche components, including ECs, mesenchymal cells, primitive myeloid cells, and components of the nervous system, provides the unique signaling milieu for the emergence of HSCs in the AGM region. AGM, aorta-gonad-mesonephros; EC, endothelial cell; EHT, endothelial hematopoietic transition; HSC, hematopoietic stem cell.

tissue (analogous to the mammalian fetal liver in the zebrafish)⁹⁰. Importantly, HSC repopulating activity was impaired when AGM explants were depleted of macrophages, thus indicating macrophages are crucial for the generation and/or maturation of functional HSCs in the mouse AGM⁸⁸. In addition, quail/chick orthotopic transplantations have shown that somite-derived ECs contribute distinct waves, first to build the roof of the DA and later to replace the hemogenic floor ceasing hematopoietic generation in the ventral aortic wall⁹¹. In the zebrafish, somite-derived ECs integrate the DA while contributing to HSC formation by eliciting inductive signals⁹². Though phenotypic HSC precursors are found in both subluminal and luminal layers, HSCs are localized exclusively within the endothelial aortic layer^{24,44}, strongly suggesting that the final step of maturation into functional HSCs needs instructive cues from the non-hemogenic vascular niche. Accordingly, hematopoietic precursors are able to expand and mature into functional HSCs after *in vitro* co-culture with Akt-expressing AGM-derived EC lines⁴⁵. This could be in part explained by the secretion of KITL by aortic ECs^{80,93}, a key regulator driving pre-HSC/HSC survival and maturation in the AGM region^{25,80,93}. Moreover, *Tie2-Cre::Kitl^{Δ/Δ}* mice, where *Kitl* is specifically deleted in the endothelium, showed decreased numbers of phenotypic pre-HSC II/HSCs in the AGM at E11.5, further resulting in fewer reconstituted mice upon transplantation⁹³. The instructive role of the vascular niche in adult HSC function in the bone marrow, including the supply of KITL, has been extensively demonstrated⁹⁴. The full understanding of these complex interactions and the identification of all the moving parts that take place in AGM hematopoiesis will help us design protocols that aim to derive HSCs from PSCs.

The quest for generating HSCs in the dish

Historically, hematopoietic development *in vitro* recapitulates the patterns observed in the embryo, following a step-wise differentiation toward induction and patterning of the mesoderm and commitment to the distinct hematopoietic waves⁶⁷. Although differentiation of PSCs to multiple blood cell types is feasible, the generation of *bona fide* blood stem cells is still a challenge. Strategies utilizing genetic manipulation of mouse ESC-derived hematopoietic progenitors, such as transduction with *Hoxb4*, reported long-term engraftment in irradiated adult hosts though underperformance in terms of lymphoid contribution⁹⁵. More recently, a study in non-genetically manipulated mouse ESCs has provided exciting evidence that it might be possible to obtain cells with engrafting ability within a transient time window during differentiation. However, engraftment shown in immunodeficient mice declined over time, and demonstration of repopulation in secondary recipients was not provided⁹⁶. Despite the innumerable efforts to generate HSCs from human PSCs, studies that have performed transplantation into immunodeficient mice have reported low engraftment efficiency and/or lacked proof of robust lymphoid contribution^{97–100}. Noteworthy, a study in hPSCs utilizing a differentiation protocol that enhances the expression of *HOXA* genes, shown to be downregulated in PSC-derived hematopoietic progenitors when compared to fetal liver HSPCs and cord blood CD34⁺ cells^{101,102}, was able to recapitulate an AGM-like

vessel network. Yet hematopoietic cells derived using this protocol were not capable of long-term engrafting ability after transplantation¹⁰².

Nonetheless, studies using PSCs have contributed valuable information on early cell fate determination, such as the identification of signals that repress specification into primitive hematopoiesis (i.e. inhibition of ACTIVIN-NODAL signaling and activation of WNT during early stages of differentiation)^{102–104}. Moreover, observations in this *in vitro* system suggest that an early mesoderm-derived HE specifies into distinct subsets that differ in the hematopoietic lineage potential^{68,69,105,106}. In the attempt to foster hematopoietic instruction by providing niche signals, some studies have established co-cultures with human umbilical vein endothelial cells^{97,106} or AGM-derived stromal cell lines^{98,107}. The growing knowledge on the molecular and cellular factors that instruct HSC generation and maturation will be crucial for the implementation of efficient protocols to derive HSC fate *in vitro*.

Conclusions

The ability to generate *bona fide* blood stem cells from PSCs has been intensively investigated given the impact it would have in clinical applications. The challenges are related not only to the incomplete knowledge of all the critical factors important for HSC emergence but also to the inherent complexity of the hematopoietic developmental process. In light of the understanding that erythroid-myeloid and B and T lymphoid potential is detected prior to repopulating capacity, it becomes unclear whether hematopoietic cells obtained during PSC differentiation, including T cells, represent the *in vitro* counterpart of the HSC-independent hematopoiesis occurring in the embryo. Because of the obstacles posed by direct differentiation *in vitro*, several laboratories have made efforts to achieve this goal by using the reprogramming strategy. Recent studies have reported the generation of hematopoietic cells with long-term repopulating ability and immune competence^{108,109}. Towards a safer approach, the successful generation of HSCs from reprogramming methods that do not require transgene integration will need to be tested. The generation of HSCs in the dish, by exposing PSCs to signaling cues that mimic the embryo environment, would be the safest approach. The challenge here is to identify the combination of soluble factors and inhibitors and the niche components required to drive specification of HSC fate. The recent discoveries documenting new cell types and signaling molecules that participate during AGM hematopoiesis added valuable information to the field and will help to establish *in vitro* differentiation protocols. The current knowledge supports the idea that the aortic endothelium is pivotal for HSC emergence. A specialized hemogenic endothelium, in a complex crosstalk with other cell types, undergoes EHT mediated by the interplay of several transcriptional regulators that act transiently to specify hematopoietic fate. Simultaneously, non-hemogenic ECs provide instructive cues that enable the generation of functional HSCs. Thus, to mimic the transient nature of AGM hematopoiesis, a fine tuning of the signaling milieu will have to take place to ensure progression of EHT and generation of functional HSCs. Importantly, given the evidence

that several niche components are essential for HSC emergence and maturation, the niche cells need to be included in the equation. This could be attempted either by direct co-culture or by supplementing the culture with key factors provided by the niche cells. Moreover, provided that HSC emergence is achieved, culture conditions will need to support maturation and expansion to numbers that can robustly engraft adult recipients in a transplantation setting, the only reliable readout assay to confirm the presence of functional long-term HSCs. The ability to expand long-term reconstituting HSCs from adult bone marrow or umbilical cord blood (UCB) sources *ex vivo* has also demonstrated challenges. Earlier attempts have established *in vitro* culture conditions that did not support the expansion to relevant cell numbers and/or caused adverse effects on HSPCs that resulted in improper differentiation¹¹⁰. More recently, the incorporation of the small molecules SR-1¹¹¹ and UM171¹¹² or valproic acid¹¹³ or direct co-culture with ECs¹¹⁴ to serum-free media supplemented with cytokines has shown robust levels of CD34⁺-UCB expansion with long-term repopulating ability. The *ex vivo* vascular niche platform has also been shown to support the expansion of engraftable adult bone marrow-derived CD34⁺ cells¹¹⁵. Interestingly, a protocol that enables long-term *ex vivo* culture of mouse adult bone marrow HSCs that expand robustly and maintain their functionality has

been reported recently¹¹⁶. Proven the robust generation and expansion of *bona fide* blood stem cells by direct differentiation from PSCs *in vitro*, the Holy Grail in the field will be ultimately achieved, providing not only an *in vitro* platform to model development and hematological diseases but also an unlimited source of cells for clinical applications.

Abbreviations

AGM, aorta-gonad-mesonephros; BMP4, bone morphogenetic protein 4; DA, dorsal aorta; EC, endothelial cell; EHT, endothelial hematopoietic transition; EMP, erythro-myeloid progenitor; ESC, embryonic stem cell; FGF, fibroblast growth factor; HE, hemogenic endothelium; hPSC, human pluripotent stem cell; HSC, hematopoietic stem cell; HSPC, hematopoietic stem/progenitor cell; iPSC, induced pluripotent stem cell; Kitl, Kit ligand; PSC, pluripotent stem cell; SHH, Sonic Hedgehog; TNF α , tumor necrosis factor alpha; UCB, umbilical cord blood.

Acknowledgements

The authors thank the members of the Butler Lab for useful discussions.

References

- Palis J: **Hematopoietic stem cell-independent hematopoiesis: Emergence of erythroid, megakaryocyte, and myeloid potential in the mammalian embryo.** *FEBS Lett.* 2016; **590**(22): 3965–3974.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Böiers C, Carrelha J, Lutteropp M, *et al.*: **Lymphomyeloid Contribution of an Immune-Restricted Progenitor Emerging Prior to Definitive Hematopoietic Stem Cells.** *Cell Stem Cell.* 2013; **13**(5): 535–48.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- McGrath KE, Frame JM, Fegan KH, *et al.*: **Distinct Sources of Hematopoietic Progenitors Emerge before HSCs and Provide Functional Blood Cells in the Mammalian Embryo.** *Cell Rep.* 2015; **11**(12): 1892–904.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chen MJ, Li Y, De Obaldia ME, *et al.*: **Erythroid/Myeloid Progenitors and Hematopoietic Stem Cells Originate from Distinct Populations of Endothelial Cells.** *Cell Stem Cell.* 2011; **9**(6): 541–52.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Yoshimoto M, Porayette P, Glosson NL, *et al.*: **Autonomous murine T-cell progenitor production in the extra-embryonic yolk sac before HSC emergence.** *Blood.* 2012; **119**(24): 5706–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Yoshimoto M, Montecino-Rodriguez E, Ferkowicz MJ, *et al.*: **Embryonic day 9 yolk sac and intra-embryonic hemogenic endothelium independently generate a B-1 and marginal zone progenitor lacking B-2 potential.** *Proc Natl Acad Sci U S A.* 2011; **108**(4): 1468–73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dzierzak E, Bigas A: **Blood Development: Hematopoietic Stem Cell Dependence and Independence.** *Cell Stem Cell.* 2018; **28**(5): 639–651.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Ginhoux F, Greter M, Leboeuf M, *et al.*: **Fate mapping analysis reveals that adult microglia derive from primitive macrophages.** *Science.* 2010; **330**(6005): 841–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Gomez Perdiguero E, Klapproth K, Schulz C, *et al.*: **Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors.** *Nature.* 2015; **518**(7540): 547–51.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Eaves CJ: **Hematopoietic stem cells: Concepts, definitions, and the new reality.** *Blood.* 2015; **125**(17): 2605–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Müller AM, Medvinsky A, Strouboulis J, *et al.*: **Development of hematopoietic stem cell activity in the mouse embryo.** *Immunity.* 1994; **1**(4): 291–301.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Medvinsky A, Dzierzak E: **Definitive hematopoiesis is autonomously initiated by the AGM region.** *Cell.* 1996; **86**(6): 897–906.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bruijn MF de, Speck NA, Peeters MC, *et al.*: **Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo.** *EMBO J.* 2000; **19**(11): 2465–74.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- de Bruijn MF, Ma X, Robin C, *et al.*: **Hematopoietic Stem Cells Localize to the Endothelial Cell Layer in the Midgestation Mouse Aorta.** *Immunity.* 2002; **16**(5): 673–83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Tavian M, Hallais MF, Péault B: **Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo.** *Development.* 1999; **126**(4): 793–803.
[PubMed Abstract](#)
- Ivanovs A, Rytsov S, Welch L, *et al.*: **Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region.** *J Exp Med.* 2011; **208**(12): 2417–27.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Emmel VE: **The cell clusters in the dorsal aorta of mammalian embryos.** *Am J Anat.* 1916; **19**(3): 401–421.
[Publisher Full Text](#)
- Jordan HE: **Evidence of hemogenic capacity of endothelium.** *Anat Rec.* 1916; **10**(5): 417–420.
[Publisher Full Text](#)
- Jaffredo T, Gautier R, Eichmann A, *et al.*: **Intraaortic hemopoietic cells are derived from endothelial cells during ontogeny.** *Development.* 1998; **125**(22):

- 4575–83.
[PubMed Abstract](#)
20. Eilken HM, Nishikawa SI, Schroeder T: **Continuous single-cell imaging of blood generation from haemogenic endothelium.** *Nature*. 2009; 457(7231): 896–900.
[PubMed Abstract](#) | [Publisher Full Text](#)
 21. **F** Bertrand JY, Chi NC, Santoso B, *et al.*: **Haematopoietic stem cells derive directly from aortic endothelium during development.** *Nature*. 2010; 464(7285): 108–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 22. Boisset JC, van Cappellen W, Andrieu-Soler C, *et al.*: **In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium.** *Nature*. 2010; 464(7285): 116–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
 23. **F** Kissa K, Herbomel P: **Blood stem cells emerge from aortic endothelium by a novel type of cell transition.** *Nature*. 2010; 464(7285): 112–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 24. **F** Rybtsov S, Sobiesiak M, Taoudi S, *et al.*: **Hierarchical organization and early hematopoietic specification of the developing HSC lineage in the AGM region.** *J Exp Med*. 2011; 208(6): 1305–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 25. Rybtsov S, Batsivari A, Bilotkach K, *et al.*: **Tracing the origin of the HSC hierarchy reveals an SCF-dependent, IL-3-independent CD43⁺ embryonic precursor.** *Stem Cell Reports*. 2014; 3(3): 489–501.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 26. **F** Baron CS, Kester L, Klaus A, *et al.*: **Single-cell transcriptomics reveal the dynamic of haematopoietic stem cell production in the aorta.** *Nat Commun*. 2018; 9(1): 2517.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 27. **F** Zhou F, Li X, Wang W, *et al.*: **Tracing haematopoietic stem cell formation at single-cell resolution.** *Nature*. 2016; 533(7604): 487–92.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 28. **F** Rybtsov S, Ivanovs A, Zhao S, *et al.*: **Concealed expansion of immature precursors underpins acute burst of adult HSC activity in foetal liver.** *Development*. 2016; 143(8): 1284–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 29. **F** Stefanska M, Batta K, Patel R, *et al.*: **Primitive erythrocytes are generated from hemogenic endothelial cells.** *Sci Rep*. 2017; 7(1): 6401.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 30. Frame JM, Fegan KH, Conway SJ, *et al.*: **Definitive Hematopoiesis in the Yolk Sac Emerges from Wnt-Responsive Hemogenic Endothelium Independently of Circulation and Arterial Identity.** *Stem Cells*. 2016; 34(2): 431–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 31. **F** Mahony CB, Bertrand JY: **How HSCs Colonize and Expand in the Fetal Niche of the Vertebrate Embryo: An Evolutionary Perspective.** *Front Cell Dev Biol*. 2019; 7: 34.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 32. **F** Kumaravelu P, Hook L, Morrison AM, *et al.*: **Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver.** *Development*. 2002; 129(21): 4891–9.
[PubMed Abstract](#) | [F1000 Recommendation](#)
 33. Ottersbach K, Dzierzak E: **The murine placenta contains hematopoietic stem cells within the vascular labyrinth region.** *Dev Cell*. 2005; 8(3): 377–87.
[PubMed Abstract](#) | [Publisher Full Text](#)
 34. **F** Gekas C, Dieterlen-Lièvre F, Orkin SH, *et al.*: **The placenta is a niche for hematopoietic stem cells.** *Dev Cell*. 2005; 8(3): 365–75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 35. **F** Li Z, Lan Y, He W, *et al.*: **Mouse embryonic head as a site for hematopoietic stem cell development.** *Cell Stem Cell*. 2012; 11(5): 663–75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 36. Li Z, Vink CS, Mariani SA, *et al.*: **Subregional localization and characterization of Ly6aGFP-expressing hematopoietic cells in the mouse embryonic head.** *Dev Biol*. 2016; 416(1): 34–41.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 37. **F** Ganuza M, Chabot A, Tang X, *et al.*: **Murine hematopoietic stem cell activity is derived from pre-circulation embryos but not yolk sacs.** *Nat Commun*. 2018; 9(1): 5405.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 38. **F** Samokhvalov IM, Samokhvalova NI, Nishikawa SI: **Cell tracing shows the contribution of the yolk sac to adult haematopoiesis.** *Nature*. 2007; 446(7139): 1056–61.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 39. **F** Rhodes KE, Gekas C, Wang Y, *et al.*: **The emergence of hematopoietic stem cells is initiated in the placental vasculature in the absence of circulation.** *Cell Stem Cell*. 2008; 2(3): 252–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 40. **F** Yvernogeau L, Gautier R, Petit L, *et al.*: **In vivo generation of haematopoietic stem/progenitor cells from bone marrow-derived haemogenic endothelium.** *Nat Cell Biol*. 2019; 21(11): 1334–1345.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 41. **F** Henninger J, Santos B, Hans S, *et al.*: **Clonal fate mapping quantifies the number of hematopoietic stem cells that arise during development.** *Nat Cell Biol*. 2017; 19(1): 17–27.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 42. **F** Ganuza M, Hall T, Finkelstein D, *et al.*: **Lifelong hematopoiesis is established by hundreds of precursors throughout mammalian ontogeny.** *Nat Cell Biol*. 2017; 19(10): 1153–1163.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 43. Taoudi S, Morrison AM, Inoue H, *et al.*: **Progressive divergence of definitive hematopoietic stem cells from the endothelial compartment does not depend on contact with the foetal liver.** *Development*. 2005; 132(18): 4179–91.
[PubMed Abstract](#) | [Publisher Full Text](#)
 44. **F** Taoudi S, Gonneau C, Moore K, *et al.*: **Extensive hematopoietic stem cell generation in the AGM region via maturation of VE-cadherin⁺CD45⁺ pre-definitive HSCs.** *Cell Stem Cell*. 2008; 3(1): 99–108.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 45. Hadland BK, Varnum-Finney B, Poulos MG, *et al.*: **Endothelium and NOTCH specify and amplify aorta-gonad-mesonephros-derived hematopoietic stem cells.** *J Clin Invest*. 2015; 125(5): 2032–45.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 46. **F** Li Y, Gao L, Hadland B, *et al.*: **CD27 marks murine embryonic hematopoietic stem cells and type II prehematopoietic stem cells.** *Blood*. 2017; 130(3): 372–376.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 47. Gordon-Keylock S, Sobiesiak M, Rybtsov S, *et al.*: **Mouse extraembryonic arterial vessels harbor precursors capable of maturing into definitive HSCs.** *Blood*. 2013; 122(14): 2338–45.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 48. **F** Corada M, Orsenigo F, Morini MF, *et al.*: **Sox17 is indispensable for acquisition and maintenance of arterial identity.** *Nat Commun*. 2013; 4: 2609.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 49. Lawson ND, Scheer N, Pham VN, *et al.*: **Notch signaling is required for arterial-venous differentiation during embryonic vascular development.** *Development*. 2001; 128(19): 3675–83.
[PubMed Abstract](#)
 50. Clarke RL, Yzaguirre AD, Yashiro-Ohtani Y, *et al.*: **The expression of Sox17 identifies and regulates haemogenic endothelium.** *Nat Cell Biol*. 2013; 15(5): 502–10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 51. **F** Kumano K, Chiba S, Kunisato A, *et al.*: **Notch1 but not Notch2 is essential for generating hematopoietic stem cells from endothelial cells.** *Immunity*. 2003; 18(5): 699–711.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 52. **F** Kim I, Saunders TL, Morrison SJ: **Sox17 dependence distinguishes the transcriptional regulation of fetal from adult hematopoietic stem cells.** *Cell*. 2007; 130(3): 470–83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 53. Lizama CO, Hawkins JS, Schmitt CE, *et al.*: **Repression of arterial genes in hemogenic endothelium is sufficient for haematopoietic fate acquisition.** *Nat Commun*. 2015; 6: 7739.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 54. **F** Souilhol C, Lendinez JG, Rybtsov S, *et al.*: **Developing HSCs become Notch independent by the end of maturation in the AGM region.** *Blood*. 2016; 128(12): 1567–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 55. Burns CE, Traver D, Mayhall E, *et al.*: **Hematopoietic stem cell fate is established by the Notch-Runx pathway.** *Genes Dev*. 2005; 19(19): 2331–42.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 56. Robert-Moreno A, Espinosa L, de la Pompa JL, *et al.*: **RBPJkappa-dependent Notch function regulates Gata2 and is essential for the formation of intra-embryonic hematopoietic cells.** *Development*. 2005; 132(5): 1117–26.
[PubMed Abstract](#) | [Publisher Full Text](#)
 57. de Pater E, Kaimakis P, Vink CS, *et al.*: **Gata2 is required for HSC generation and survival.** *J Exp Med*. 2013; 210(13): 2843–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 58. **F** Richard C, Drevon C, Canto PY, *et al.*: **Endothelio-mesenchymal interaction controls runx1 expression and modulates the notch pathway to initiate aortic hematopoiesis.** *Dev Cell*. 2013; 24(6): 600–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 59. **F** Kang H, Mesquita WT, Jung HS, *et al.*: **GATA2 Is Dispensable for Specification of Hemogenic Endothelium but Promotes Endothelial-to-Hematopoietic Transition.** *Stem Cell Reports*. 2018; 11(1): 197–211.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 60. **F** Lie-A-Ling M, Marinopoulou E, Lilly AJ, *et al.*: **Regulation of RUNX1 dosage**

- is crucial for efficient blood formation from hemogenic endothelium. *Development*. 2018; **145**(5): pii: dev149419.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
61. Chen MJ, Yokomizo T, Zeigler BM, *et al.*: **Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter.** *Nature*. 2009; **457**(7231): 887–91.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 62. Guiu J, Shimizu R, D'Altri T, *et al.*: **Hes repressors are essential regulators of hematopoietic stem cell development downstream of Notch signaling.** *J Exp Med*. 2013; **210**(1): 71–84.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 63. Bertrand JY, Giroux S, Golub R, *et al.*: **Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin.** *Proc Natl Acad Sci U S A*. 2005; **102**(1): 134–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 64. **F** Arraf AA, De Bruijn MFTR, Schultheiss TM: **Disruption of the aortic wall by coelomic lining-derived mesenchymal cells accompanies the onset of aortic hematopoiesis.** *Int J Dev Biol*. 2017; **61**(3–4–5): 329–335.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 65. Choi KD, Vodyanik MA, Togarrati PP, *et al.*: **Identification of the hemogenic endothelial progenitor and its direct precursor in human pluripotent stem cell differentiation cultures.** *Cell Rep*. 2012; **2**(3): 553–67.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 66. **F** Ditadi A, Sturgeon CM, Tober J, *et al.*: **Human definitive haemogenic endothelium and arterial vascular endothelium represent distinct lineages.** *Nat Cell Biol*. 2015; **17**(5): 580–91.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 67. **F** Ditadi A, Sturgeon CM, Keller G: **A view of human hematopoietic development from the Petri dish.** *Nat Rev Mol Cell Biol*. 2017; **18**(1): 56–67.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 68. **F** Park MA, Kumar A, Jung HS, *et al.*: **Activation of the Arterial Program Drives Development of Definitive Hemogenic Endothelium with Lymphoid Potential.** *Cell Rep*. 2018; **23**(8): 2467–2481.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 69. **F** Uenishi GI, Jung HS, Kumar A, *et al.*: **NOTCH signaling specifies arterial-type definitive hemogenic endothelium from human pluripotent stem cells.** *Nat Commun*. 2018; **9**(1): 1828.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 70. **F** Gao L, Tober J, Gao P, *et al.*: **RUNX1 and the endothelial origin of blood.** *Exp Hematol*. 2018; **68**: 2–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 71. **F** Zeng Y, He J, Bai Z, *et al.*: **Tracing the first hematopoietic stem cell generation in human embryo by single-cell RNA sequencing.** *Cell Res*. 2019; **29**(11): 881–894.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 72. **F** Bonkhof F, Rispoli R, Pinheiro P, *et al.*: **Blood stem cell-forming haemogenic endothelium in zebrafish derives from arterial endothelium.** *Nat Commun*. 2019; **10**(1): 3577.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 73. **F** Gama-Norton L, Ferrando E, Ruiz-Herguido C, *et al.*: **Notch signal strength controls cell fate in the haemogenic endothelium.** *Nat Commun*. 2015; **6**: 8510.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 74. **F** Zovein AC, Hofmann JJ, Lynch M, *et al.*: **Fate tracing reveals the endothelial origin of hematopoietic stem cells.** *Cell Stem Cell*. 2008; **3**(6): 625–36.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 75. Taoudi S, Medvinsky A: **Functional identification of the hematopoietic stem cell niche in the ventral domain of the embryonic dorsal aorta.** *Proc Natl Acad Sci U S A*. 2007; **104**(22): 9399–403.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 76. **F** Yokomizo T, Dzierzak E: **Three-dimensional cartography of hematopoietic clusters in the vasculature of whole mouse embryos.** *Development*. 2010; **137**(21): 3651–61.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 77. Pardanaud L, Lutton D, Prigent M, *et al.*: **Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis.** *Development*. 1996; **122**(5): 1363–71.
[PubMed Abstract](#)
 78. Marshall CJ, Kinnon C, Thrasher AJ: **Polarized expression of bone morphogenetic protein-4 in the human aorta-gonad-mesonephros region.** *Blood*. 2000; **96**(4): 1591–3.
[PubMed Abstract](#) | [Publisher Full Text](#)
 79. Wilkinson RN, Pouget C, Gering M, *et al.*: **Hedgehog and Bmp polarize hematopoietic stem cell emergence in the zebrafish dorsal aorta.** *Dev Cell*. 2009; **16**(6): 909–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 80. Souilh C, Gonneau C, Lendinez JG, *et al.*: **Inductive interactions mediated by interplay of asymmetric signalling underlie development of adult haematopoietic stem cells.** *Nat Commun*. 2016; **7**: 10784.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 81. **F** McGarvey AC, Rytsov S, Souilh C, *et al.*: **A molecular roadmap of the AGM region reveals BMPER as a novel regulator of HSC maturation.** *J Exp Med*. 2017; **214**(12): 3731–3751.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 82. Pouget C, Peterkin T, Simões FC, *et al.*: **FGF signalling restricts haematopoietic stem cell specification via modulation of the BMP pathway.** *Nat Commun*. 2014; **5**: 5588.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 83. Marshall CJ, Moore RL, Thorogood P, *et al.*: **Detailed characterization of the human aorta-gonad-mesonephros region reveals morphological polarity resembling a hematopoietic stromal layer.** *Dev Dyn*. 1999; **215**(2): 139–47.
[PubMed Abstract](#) | [Publisher Full Text](#)
 84. Uenishi G, Theisen D, Lee JH, *et al.*: **Tenascin C promotes haematoendothelial development and T lymphoid commitment from human pluripotent stem cells in chemically defined conditions.** *Stem Cell Reports*. 2014; **3**(6): 1073–84.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 85. **F** Fitch SR, Kimber GM, Wilson NK, *et al.*: **Signaling from the sympathetic nervous system regulates hematopoietic stem cell emergence during embryogenesis.** *Cell Stem Cell*. 2012; **11**(4): 554–66.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 86. Kwan W, Cortes M, Frost I, *et al.*: **The Central Nervous System Regulates Embryonic HSPC Production via Stress-Responsive Glucocorticoid Receptor Signaling.** *Cell Stem Cell*. 2016; **19**(3): 370–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 87. Li Y, Esain V, Teng L, *et al.*: **Inflammatory signaling regulates embryonic hematopoietic stem and progenitor cell production.** *Genes Dev*. 2014; **28**(23): 2597–612.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 88. **F** Mariani SA, Li Z, Rice S, *et al.*: **Pro-inflammatory Aorta-Associated Macrophages Are Involved in Embryonic Development of Hematopoietic Stem Cells.** *Immunity*. 2019; **50**(6): 1439–1452.e5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 89. **F** Espín-Palazón R, Stachura DL, Campbell CA, *et al.*: **Proinflammatory signaling regulates hematopoietic stem cell emergence.** *Cell*. 2014; **159**(5): 1070–1085.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 90. **F** Travnickova J, Tran Chau V, Julien E, *et al.*: **Primitive macrophages control HSPC mobilization and definitive hematopoiesis.** *Nat Commun*. 2015; **6**: 6227.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 91. Pouget C, Gautier R, Teillet MA, *et al.*: **Somite-derived cells replace ventral aortic hemangioblasts and provide aortic smooth muscle cells of the trunk.** *Development*. 2006; **133**(6): 1013–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
 92. **F** Nguyen PD, Hollway GE, Sonntag C, *et al.*: **Haematopoietic stem cell induction by somite-derived endothelial cells controlled by meox1.** *Nature*. 2014; **512**(7514): 314–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 93. **F** Azzoni E, Frontera V, McGrath KE, *et al.*: **Kit ligand has a critical role in mouse yolk sac and aorta-gonad-mesonephros hematopoiesis.** *EMBO Rep*. 2018; **19**(10): pii: e45477.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 94. Ramalingam P, Poulos MG, Butler JM: **Regulation of the hematopoietic stem cell lifecycle by the endothelial niche.** *Curr Opin Hematol*. 2017; **24**(4): 289–299.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 95. Lengerke C, Daley GQ: **Autologous blood cell therapies from pluripotent stem cells.** *Blood Rev*. 2010; **24**(1): 27–37.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 96. Pearson S, Cuvertino S, Fleury M, *et al.*: **In vivo repopulating activity emerges at the onset of hematopoietic specification during embryonic stem cell differentiation.** *Stem Cell Reports*. 2015; **4**(3): 431–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 97. **F** Gori JL, Butler JM, Chan YY, *et al.*: **Vascular niche promotes hematopoietic multipotent progenitor formation from pluripotent stem cells.** *J Clin Invest*. 2015; **125**(3): 1243–54.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 98. **F** Ledran MH, Krassowska A, Armstrong L, *et al.*: **Efficient hematopoietic differentiation of human embryonic stem cells on stromal cells derived from hematopoietic niches.** *Cell Stem Cell*. 2008; **3**(1): 85–98.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 99. Lu M, Kardel MD, O'Connor MD, *et al.*: **Enhanced generation of hematopoietic cells from human hepatocarcinoma cell-stimulated human embryonic and induced pluripotent stem cells.** *Exp Hematol*. 2009; **37**(8): 924–36.
[PubMed Abstract](#) | [Publisher Full Text](#)
 100. Wang L, Menendez P, Shojaei F, *et al.*: **Generation of hematopoietic repopulating cells from human embryonic stem cells independent of ectopic HOXB4 expression.** *J Exp Med*. 2005; **201**(10): 1603–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 101. **F** Dou DR, Calvanese V, Sierra MI, *et al.*: **Medial HOXA genes demarcate haematopoietic stem cell fate during human development.** *Nat Cell Biol*. 2016;

- 18(6): 595–606.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
102. Ng ES, Azzola L, Bruveris FF, *et al.*: **Differentiation of human embryonic stem cells to HOXA⁺ hemogenic vasculature that resembles the aorta-gonad-mesonephros.** *Nat Biotechnol.* 2016; **34**(11): 1168–1179.
[PubMed Abstract](#) | [Publisher Full Text](#)
 103. Sturgeon CM, Ditadi A, Awong G, *et al.*: **Wnt signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells.** *Nat Biotechnol.* 2014; **32**(6): 554–61.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 104. Kennedy M, Awong G, Sturgeon CM, *et al.*: **T lymphocyte potential marks the emergence of definitive hematopoietic progenitors in human pluripotent stem cell differentiation cultures.** *Cell Rep.* 2012; **2**(6): 1722–35.
[PubMed Abstract](#) | [Publisher Full Text](#)
 105. Garcia-Alegria E, Menegatti S, Fadlullah MZH, *et al.*: **Early Human Hemogenic Endothelium Generates Primitive and Definitive Hematopoiesis *In Vitro*.** *Stem Cell Reports.* 2018; **11**(5): 1061–1074.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 106. Rafii S, Kloss CC, Butler JM, *et al.*: **Human ESC-derived hemogenic endothelial cells undergo distinct waves of endothelial to hematopoietic transition.** *Blood.* 2013; **121**(5): 770–80.
[PubMed Abstract](#) | [Publisher Full Text](#)
 107. Gordon-Keylock SAM, Jackson M, Huang C, *et al.*: **Induction of hematopoietic differentiation of mouse embryonic stem cells by an AGM-derived stromal cell line is not further enhanced by overexpression of HOXB4.** *Stem Cells Dev.* 2010; **19**(11): 1687–98.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 108. Sugimura R, Jha DK, Han A, *et al.*: **Haematopoietic stem and progenitor cells from human pluripotent stem cells.** *Nature.* 2017; **545**(7655): 432–438.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 109. Lis R, Karrasch CC, Poulos MG, *et al.*: **Conversion of adult endothelium to immunocompetent haematopoietic stem cells.** *Nature.* 2017; **545**(7655): 439–445.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 110. Dahlberg A, Delaney C, Bernstein ID: ***Ex vivo* expansion of human hematopoietic stem and progenitor cells.** *Blood.* 2011; **117**(23): 6083–90.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 111. Boitano AE, Wang J, Romeo R, *et al.*: **Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells.** *Science.* 2010; **329**(5997): 1345–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 112. Fares I, Chagraoui J, Gareau Y, *et al.*: **Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal.** *Science.* 2014; **345**(6203): 1509–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 113. Chaurasia P, Gajzer DC, Schaniel C, *et al.*: **Epigenetic reprogramming induces the expansion of cord blood stem cells.** *J Clin Invest.* 2014; **124**(6): 2378–95.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 114. Butler JM, Gars EJ, James DJ, *et al.*: **Development of a vascular niche platform for expansion of repopulating human cord blood stem and progenitor cells.** *Blood.* 2012; **120**(6): 1344–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 115. Gori JL, Butler JM, Kunar B, *et al.*: **Endothelial Cells Promote Expansion of Long-Term Engrafting Marrow Hematopoietic Stem and Progenitor Cells in Primates.** *Stem Cells Transl Med.* 2017; **6**(3): 864–876.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 116. Wilkinson AC, Ishida R, Kikuchi M, *et al.*: **Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation.** *Nature.* 2019; **571**(7763): 117–121.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)

Open Peer Review

Current Peer Review Status:

Editorial Note on the Review Process

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

1. **Igor Slukvin**

Wisconsin National Primate Research Center, University of Wisconsin, Madison, USA

Competing Interests: No competing interests were disclosed.

2. **Valerie Kouskoff**

Developmental Hematopoiesis Group, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

Competing Interests: No competing interests were disclosed.

3. **Lesley M Forrester**

Centre for Regenerative Medicine, Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research