The EHA Research Roadmap: Normal Hematopoiesis

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Thierry Jaffredo[1], Alessandra Balduini[2], Anna Bigas[3,4,5], Rosa Bernardi[6], Dominique Bonnet[7], Bruno Canque[8], Pierre Charbord[1], Anna Cumano[9], Ruud Delwel[10], Charles Durand[1], Willem Fibbe[11], Lesley Forrester[12], Lucia de Franceschi[13], Cedric Ghevaert[14], Bjørn Gjertsen[15,16], Berthold Gottgens[17], Thomas Graf[18], Olaf Heidenreich[19], Olivier Hermine[20], Douglas Higgs[21], Marina Kleanthous[22], Hannes Klump[23], Valerie Kouskoff[24], Daniela Krause[25], George Lacaud[26], Cristina Lo Celso[27], Joost H.A. Martens[28], Simón Méndez-Ferrer[29], Pablo Menendez[5,30,31,32,33], Robert Oostendorp[34], Sjaak Philipsen[35], Bo Porse[36,37,38], Marc Raaijmakers[10], Catherine Robin[39,40], Henk Stunnenberg[19], Kim Theilgaard-Mönch[36,37,38,41], Ivo Touw[10], William Vainchenker[42] Joan-Lluis Vives Corrons[43], Laurent Yvernogeau[1], Jan Jacob Schuringa[44]

Correspondence: Thierry Jaffredo (thierry.jaffredo@upmc.fr) or Jan Jacob Shringa (j.j.schuringa@umcg.nl).

In 2016, the European Hematology Association (EHA) published the EHA Roadmap for European Hematology Research[1] aiming to highlight achievements in the diagnostics and treatment of blood disorders, and to better inform European policy makers and other stakeholders about the urgent clinical and scientific needs and priorities in the field of hematology. Each section was coordinated by 1–2 section editors who were leading international experts in the field. In the 5 years that have followed, advances in the field of hematology have been plentiful. As such, EHA is pleased to present an updated Research Roadmap, now including 11 sections, each of which will be published separately. The updated EHA Research Roadmap identifies the most urgent priorities in hematology research and clinical science, therefore supporting a more informed, focused, and ideally a more funded future for European hematology research. The 11 EHA Research Roadmap sections include Normal Hematopoiesis; Malignant Lymphoid Diseases; Malignant Myeloid Diseases; Anemias and Related Diseases; Platelet Disorders; Blood Coagulation and Hemostatic Disorders; Transfusion Medicine; Infections in Hematology; Hematopoietic Stem Cell Transplantation; CAR-T and Other Cell-based Immune Therapies; and Gene Therapy.

[1]*Sorbonne Université, Institut de Biologie Paris Seine, Laboratoire de Biologie du Développement/UMR7622, Paris, France*
[2]*Department of Molecular Medicine, University of Pavia, Italy*
[3]*Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain*
[4]*Josep Carreras Leukemia Research Institute (IJC), Barcelona, Spain*
[5]*Centro de Investigación Biomedica en Red-Oncología (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain*
[6]*IRCCS San Raffaele Scientific Institute, Milan, Italy*
[7]*The Francis Crick Institute, London, United Kingdom*
[8]*INSERM U976, Universite de Paris, Ecole Pratique des Hautes Etudes/PSL Research University, Institut de Recherche Saint Louis, France*
[9]*Unité Lymphopoïèse, Département d'Immunologie, INSERM U1223, Institut Pasteur, Cellule Pasteur, Université de Paris, France*
[10]*Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands*
[11]*Leiden University Medical Center, The Netherlands*
[12]*Centre for Regenerative Medicine, Institute for Regeneration and Repair, University of Edinburgh, Scotland*
[13]*Università degli Studi di Verona, Italy*
[14]*University of Cambridge, United Kingdom*
[15]*Department of Medicine, Hematology Section, Haukeland University Hospital, Bergen, Norway*
[16]*Department of Clinical Science, Centre for Cancer Biomarkers CCBIO, University of Bergen, Norway*
[17]*Wellcome - MRC Cambridge Stem Cell Institute and Department of Haematology, University of Cambridge, United Kingdom*
[18]*Center for Genomic Regulation, Barcelona Institute for Science and Technology and Universitat Pompeu Fabra, Barcelona, Spain*
[19]*Prinses Máxima Centrum voor kinderoncologie, Utecht, The Netherlands*
[20]*Department of Hematology and Laboratory of Physiopathology and Treatment of Blood Disorders, Hôpital Necker, Imagine institute, University of Paris, France*
[21]*MRC Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom*
[22]*The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*
[23]*Institute for Transfusion Medicine, University Hospital Essen, Germany*
[24]*University of Manchester, United Kingdom*
[25]*Goethe University Frankfurt and Georg-Speyer-Haus, Frankfurt am Main, Germany*
[26]*Cancer Research UK Manchester Institute, The University of Manchester, United Kingdom*
[27]*Imperial College London, United Kingdom*
[28]*Department of Molecular Biology, RIMLS, Radboud University, Nijmegen, The Netherlands*
[29]*University of Cambridge and NHSBT, Cambridge, United Kingdom*
[30]*Josep Carreras Leukemia Research Institute, Barcelona, Spain*
[31]*RICORS-RETAV, Instituto de Salud Carlos III, Madrid, Spain*
[32]*Department of Biomedicine, School of Medicine, Universitat de Barcelona, Barcelona, Spain*
[33]*Institució Catalana de Recerca i Estudis Avancats (ICREA), Barcelona, Spain*
[34]*Department of Internal Medicine III, Technical University of Munich, School of Medicine, Germany*
[35]*Department of Cell Biology, Erasmus University Medical Center Rotterdam, The Netherlands*
[36]*The Finsen Laboratory, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Denmark*
[37]*Biotech Research and Innovation Center (BRIC), University of Copenhagen, Denmark*
[38]*Novo Nordisk Foundation Center for Stem Cell Biology, DanStem, Faculty of Health Sciences, University of Copenhagen, Denmark*
[39]*Hubrecht Institute-KNAW and University Medical Center Utrecht, The Netherlands*
[40]*Regenerative medicine center, University Medical Center Utrecht, The Netherlands*
[41]*Department of Hematology, Rigshospitalet/National University Hospital, University of Copenhagen, Denmark*
[42]*Gustave Roussy, Villejuif, France*
[43]*Red Blood Cell and Hematopoietic Disorders Research Unit, Institute for Leukaemia Research Josep Carreras, Badalona, Barcelona*
[44]*Department of Experimental Hematology, University Medical Center Groningen, The Netherlands*

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Fundamental as well as translational research has been critically important to get us where we are today in the field of hematopoiesis studies. Still, many questions remain. Among many others, these include: How do (epi)genetic aberrations cause hematologic malignancies, and how can we use these insights to develop better therapeutic strategies? It is now being realized that clonal heterogeneity exists in many hematologic cancers and possibly even within the normal hematopoietic stem cell (HSC) compartment, but how does this affect disease development and current treatment options remains largely unknown. In contrast to adult life, HSCs are rapidly expanding during embryogenesis, so can we unravel those mechanisms and apply them to in vitro HSC expansion protocols for clinical use? A thorough understanding of embryonic versus adult hematopoiesis might also help to better understand differences between childhood and adult hematologic malignancies. Reprogramming now allows the generation of patient-specific iPSCs, but the generation of fully functional HSCs from these cells is still rather challenging, can we improve this? We live in a continuously aging society, but how does HSC aging actually affect health and disease? Within this section, we provide an overview of the current status of the field and an outlook on where future research should be headed (Fig. 1). We firmly believe that combining fundamental and translational research will result in not only a better understanding of the hematopoietic system but also the development of better therapeutic approaches for hematologic malignancies, many of which are still difficult to treat.

Erythropoiesis

The major cell type in blood is the red blood cell (RBC). RBCs transport oxygen from the lungs to other parts of the body. RBCs are formed during a process called erythropoiesis. This includes the initial specification of HSCs during embryogenesis and the decision of HSCs to contribute to the erythroid lineage.[2]

About a third of the world's population has some form of anemia. Acquired anemias are often related to iron deficiency or to systemic disorders such as chronic inflammation. Inherited anemias affect erythropoiesis by diverse mechanisms, such as insufficient hemoglobin (Hb) production (thalassemias), pathological Hb variants (sickle cell disease), defects in membrane proteins or metabolic enzymes (hemolytic anemias), impaired ribosome biogenesis (Diamond Blackfan anemia), and DNA repair defects (Fanconi anemia). Activating JAK2 kinase mutations cause excess RBC production (polycytemia vera). The physiological and molecular mechanisms underlying RBC disorders are still incompletely understood, hampering the development of new treatments.

Historically, research of hematopoiesis has driven novel biological concepts, owing to the accessibility of hematopoietic cells for molecular and functional analyses. Early European contributions include the Nobel Prize-winning discovery of the structure of Hb[3] and understanding the epidemiology of inherited anemias, leading to implementation of prenatal diagnostic programs.[4] Additional European contributions include

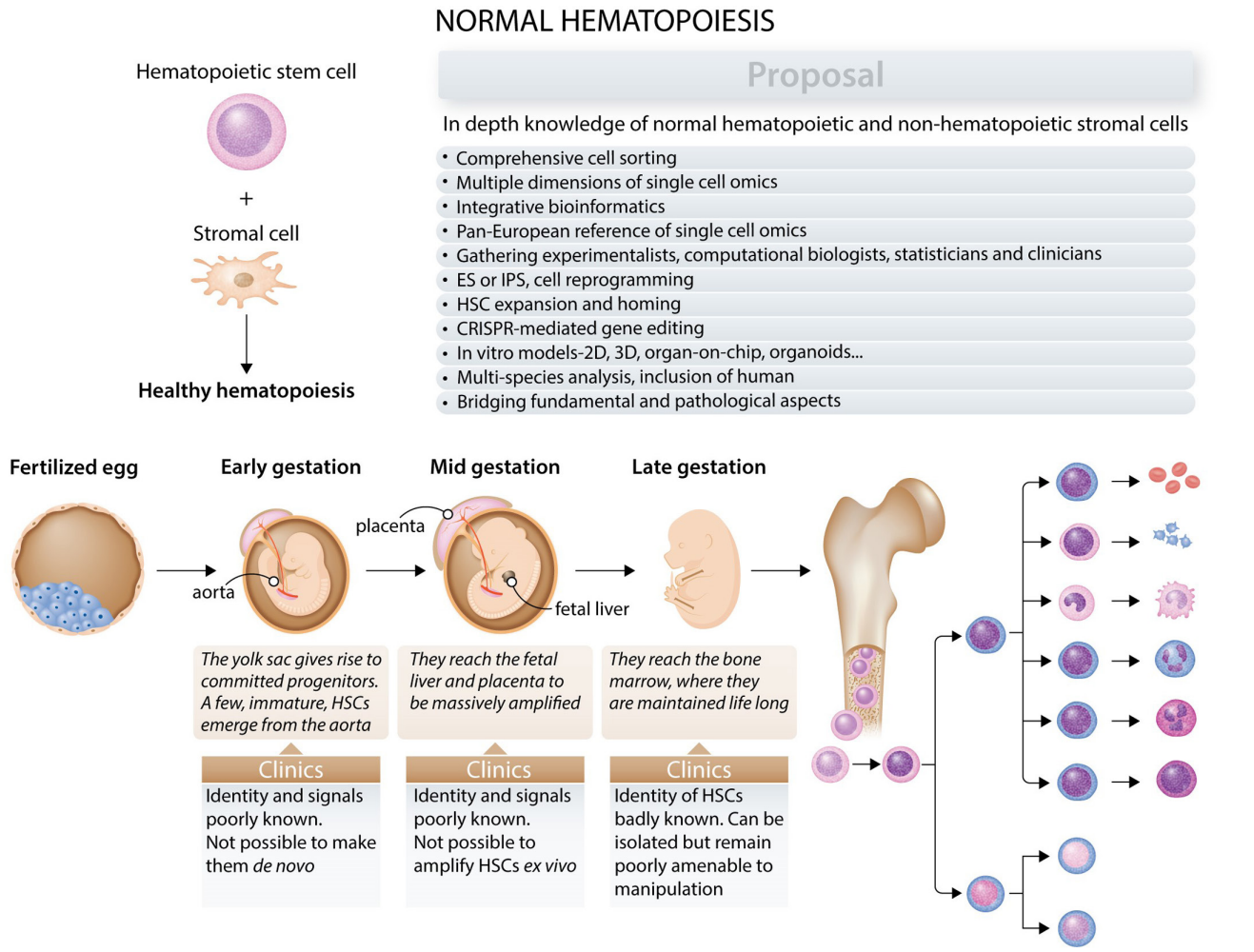

Figure 1. Overview of the current field and an outlook on where future research should be headed.

determining the origin of HSCs, the transcriptional circuitry underlying erythropoiesis, the role of iron metabolism, and DNA sequences driving Hb expression which are now applied in gene therapy. A database dedicated to erythroid disorders has been established.[5] Translational research has resulted in optimized stem cell transplantation protocols, magnetic resonance imaging for monitoring of iron overload, improved iron chelation therapies, and targeted inhibition of signaling pathways under (pre)leukemic conditions.

Previous research has laid the foundation from which understanding erythropoiesis will be extended. For instance, single cell analysis allows hierarchical relationships between cells to be determined and the impact of cell–cell interactions and signaling cascades to be unraveled. The protocols developed will be quickly translated to clinical applications, as demonstrated by CRISPR-mediated gene editing.[6] The goal is to apply this deep understanding of erythropoiesis to improve diagnosis, prognosis, and treatment of patients with RBC conditions.

The fundamental understanding of erythropoiesis will have direct impact on medical care of patients with RBC conditions. First, improved diagnosis will lead clinicians to better-informed decisions on disease management. Second, novel curative treatments will be developed, such as those involving gene correction/addition and drugs targeting specific signaling pathways. Third, in vitro generation of RBCs will bring guaranteed disease-free transfusion units to the clinic. Finally, fundamental insights in erythropoiesis will continue to facilitate discoveries in other fields of medicine, leading to better clinical care.

Myelopoiesis

The body´s immediate defense against invading microorganisms is orchestrated by myeloid effector cells of the innate immune system including granulocytes, monocytes/macrophages, dendritic cells, and rare mast cells. These cells are generally short-lived, and require life-long replenishment by HSCs and their progeny, which gradually transit along their differentiation trajectories and ultimately become highly specialized myeloid effector cells. This complex process, referred to as myelopoiesis, is tightly regulated by growth factors, epigenetic and transcriptional regulators, which coordinate early lineage-specification, cell cycle exit, and terminal differentiation. Consequently, genetic aberrations of key regulators of myeloid differentiation programs have been causally linked to the majority of myeloid diseases such as acute myeloid leukemias (AMLs), myeloid dysplastic syndromes and proliferative neoplasms (MDSs/MPNs), and severe congenital as well as cyclic neutropenia. Hence, in the context of disease, functional characterization of myeloid regulators during steady-state hematopoiesis and infections at both the molecular and system levels is pivotal to understand (1) the biology of myeloid effector cells and their role in innate immune defense and (2) how genetic aberrations affect normal myelopoiesis and cause myeloid disease.

For more than 2 decades, European scientists have contributed substantially to the understanding of myelopoeisis as highlighted in the 2016 EHA roadmap for European Hematology Research.[1] Since then, the European research community has continued this endeavor with a particular focus on myeloid regulators and networks in health and disease, and the of characterization of novel myeloid differentiation trajectories in the hematopoietic hierarchy. The latter is exemplified by a selection of recent key European achievements: (1) Paul et al[7] conducted a seminal single-cell transcriptomics study highlighting that commitment toward specific myeloid lineages is a very early event during hematopoiesis, which is not linked to transitional stages of differentiation via progenitors with multi- and bi-lineage potential, as described in the "classic" models of hematopoiesis. (2) European research consortia have pioneered the mapping of mutational landscapes and aberrant regulatory networks of several myeloid diseases, leading to the identification of novel myeloid regulators, and the development of knowledge bank tools allowing for prognostication and guidance of therapeutic stratification of patients with myeloid diseases.[8,9] (3) Transcriptomics studies comparing identical normal and malignant myeloid progenitors have mapped gene expression signatures of disease states in patients, which reflect aberrant signaling pathway activities that contribute to dissect disease biology, and are both prognostic relevant and druggable by novel therapeutic modalities.[10–12]

The advent of novel comprehensive omics technologies such as single-cell RNA-seq, single-cell ATAC-seq, ChIP-seq analyses of transcriptional and epigenetic regulators, and more recently single-cell proteomics allows to map the phenotypic cellular states of the myeloid differentiation landscape in health and disease, at a hitherto unprecedent resolution.

We propose a coordinated European effort that will develop and apply standards for comprehensive single cell omics analyses and establish a Pan-European "reference" single cell library of myeloid cells in health and disease. Ultimately, this single-cell "reference" library will improve our understanding of normal myeloid differentiation (and hematopoiesis in general) and innate immune defense at the system level but also how genetic aberrations of myeloid regulators cause perturbations of normal cellular states can lead to myeloid disease, and how these states can be targeted by novel therapeutic approaches and modalities.

A coordinated European effort involving basic and clinical researchers including bioinformaticians is required to achieve the following key aims of the proposed research program:

1. Establishment of a European expert group that will discuss, establish, and continuously update standard operating procedures (SOPs) for single-cell omics analyses of bone marrow and blood cells in general and myeloid cells in particular. These SOPs should include (a) general processing of normal bone marrow and blood cells, (b) single-cells omics protocols and technology platforms, and (c) the development of bioinformatics methodologies for integrated single cells omics and clinical data analysis.
2. Implementation of designated European core facilities/hospitals that will implement standardized collection and biobanking of human bone marrow and blood samples from healthy subjects and patients for single cell omics analyses.
3. Implementation of specialized European core facilities/research teams for standardized single-cell omics analysis of bone marrow and blood cells according to the single-cell omics SOPs defined by the expert group.
4. Implementation of a European core computational consortium for concerted standardized processing and analysis of single cell omics data to generate a "reference" single-cell omics library of normal and genetically perturbated cells from healthy subjects and patients with myeloid disease. Importantly, the computational consortium should develop open-access web-based platforms allowing researchers and clinicians worldwide to download and match single-cell omics data with their own data including those from patients with myeloid diseases enrolled in clinical trials.

The proposed research program conducted by a multidisciplinary concerted European effort will generate a comprehensive single-cell omics reference library of myeloid differentiation in health and disease. The resultant single cells library represents a most powerful tool for the research community, as it can be used in part as a reference of how expression and activity of genes, proteins, or signaling pathways change during normal myeloid differentiation and are perturbed by genetic aberrations in myeloid diseases. Significantly, this will have substantial impact on complementary European clinical research programs and trials aiming at identifying (1) novel diagnostic markers for improved prognostication and

(2) therapeutic targets for effective personalized combination therapies of patients with AML, MDS, MPN, and other rare myeloid diseases.

Megakaryopoiesis

Megakaryopoiesis is the differentiation process that leads to platelet production. This is a unique cell biology system because platelets arise from the fragmentation of their marrow precursors, namely the megakaryocytes (MKs), in the blood shear. Platelet production is both regulated by the MK size through polyploidization and number. The regulation of megakaryopoiesis is dependent on a cytokine/hormone called thrombopoietin (TPO), which signals through the thrombopoietin receptor MPL. Megakaryopoiesis is affected by numerous acquired and hereditary disorders that involve transcription factors, TPO/MPL signaling and the actin and tubulin cytoskeletons. The classical treatments of thrombocytopenia include TPO mimetics and platelet transfusion from donors but novel approaches are required.

European researchers have played a central role in the understanding of the regulation of megakaryopoiesis both at the cellular and transcription level and have developed cutting edge cell/molecular biology technologies.

Key advances have been achieved among the last 5 years by the European researchers.

1. Two different pathways of MK commitment have been identified, one directly from a MK-biased HSC playing a major role in MK stress, and the second by a hierarchical pathway through an MK-Erythroid progenitor.[13,14] The presence of megakaryopoiesis in the lung that may lead to a platelet production by atypical hypoploid megakaryocytes expressing an hybrid phenotype between classical MKs and dendritic cells was also identified.[15,16]
2. Proplatelet formation is not the unique mechanism to produce platelets as they may be produced by MK rupture in stress conditions and membrane budding in both basal and stress conditions.[17]
3. Characterization of 2 physic parameters involved in platelet shedding; shear stress[18] and turbulence[19] that is associated with the secretion of at least 3 mediators increasing platelet production. An approach combining immortalized MK cell lines derived from iPSC and these new parameters has allowed the in vitro production for transfusion purpose.[19] In parallel, the development of 3D scaffolds has allowed an increase in production of functional platelets from iPSC and hematopoietic progenitors.[20,21]

The major topics that require intense research resources and efforts include:

1. Mechanisms of MK commitment and differentiation from HSC. The new techniques on single cells allowing to study transcriptome, chromatin accessibility and occupancy, as well as DNA methylation associated with cell biology may allow to understand the mechanisms of MK/platelet bias and commitment. It may also permit to precisely understand the stress megakaryopoiesis. Furthermore, it will be possible to precisely determine the ontogeny of lung MKs and the MK heterogeneity.
2. Regulation of platelet formation. There is evidence that at least three different processes (proplatelet formation, MK budding, and MK rupture) are involved in platelet formation. How these different mechanisms are regulated, and how they contribute to the basal and stress platelet production still remains to be determined. These approaches may also provide candidate molecules implicated in the final regulation of MK differentiation and which may permit to increase platelet production.
3. Mutual regulation of MKs and bone marrow environment. There is evidence that MKs are able to express and release different molecules that may regulate bone marrow homeostasis in both, steady-state and pathological conditions. Furthermore, MKs are implicated in the immune and inflammatory responses. On this basis, it is important to identify the molecules actively expressed by MKs, the release of which is implicated in the bone marrow and immune regulation.

The precise understanding of the molecular mechanism of MK differentiation as well as of platelet production may have important clinical consequences in: understanding numerous disorders affecting MK lineage; developing new technologies for large platelet production in vitro; and the identification of new molecules capable to modify platelet production in vivo.

Lymphopoiesis

Lymphoid lineage development is by far the most complex differentiation process within the hematopoietic system. Following gradual acquisition of the lymphoid identity, lymphoid progenitors undergo subsequent diversification into T, B, and natural killer/innate lymphoid cells (NK/ILC) lineage-specified precursors. While NK/ILC rapidly acquires cytotoxic or cytokine producing effector functions, differentiation of the T and B precursors continues with somatic V(D)J recombination of T cell receptor (TCR) or Ig loci and subsequent elimination of auto-reactive clones, resulting in mature T and B cells that leave the thymus and the bone marrow, respectively. In this section, we will focus on the initial steps of lymphoid differentiation preceding TCR/Ig rearrangement, a field in which European groups have a strong research record.[22–25]

Future studies in the field should lead to a better integration of the synchronic and diachronic time scales of lymphoid development.

At the synchronic differentiation level, recent technological advances in vivo fate-mapping genetic models and multiomics approaches at single-cell resolution have largely confirmed earlier studies based on analyses of phenotypic/function relationships. However, despite recent progresses, there are still major gaps in our knowledge of the molecular machinery driving lymphoid development trajectories. It remains difficult to accommodate microenvironment-driven *instructive* differentiation schemes with the Waddington-based cell-autonomous models of lineage-commitment. A limitation of current models of lymphoid development is that they do not take into account the link between cell proliferation, that is, between symmetric or asymmetric cell division, and lineage diversification at the origin of increasing heterogeneity across cell generations. These issues are all the more important that they are beginning to be addressed in myeloid development studies.[26,27]

Another conceptual consideration for development of synthetic models of lymphoid differentiation is to accommodate phenotype/function and transcriptional approaches with recently developed chromatin conformation analyses that allow for dissection of enhancer/promoter interactions at the base-pair resolution.[28] Human lymphopoiesis is far less well characterized than in the mouse. Earlier studies led to identification of two $CD7^+$ or $CD10^+$ lymphoid-restricted subsets which are now referred multilymphoid progenitors, but the developmental status of these populations remains uncertain. Their relationships with recently described $CD127^{-/+}$ early lymphoid progenitors also need to be clarified.[22] In coming years, lymphoid development studies in humans should focus on the establishment of clonal scale maps of developmental trajectories. A special attention should also be given to human–mouse cross-species approaches that will permit to distinguish species-specific from evolutionary conserved regulatory mechanisms.

At the diachronic development level, future models of lymphoid development will need to integrate ontogeny-related changes in lymphocyte production patterns in order to determine whether ontogeny-related changes in lymphocyte production patterns are based on independent progenitors and/or if are accompanied by concomitant changes in lymphoid architecture.

Progress in the field will be essential to understand the differences in the age incidence of B versus T cell leukemias helping to devise therapeutic strategies to reconstitute lymphoid cell subsets in disease and aging.

Hematopoietic stem cells

HSCs were the first adult stem cells to find their way into the clinic. Therefore, HSCs are frequently regarded as a role model in adult stem cell biology. Notwithstanding their very successful and beneficial clinical applicability, many aspects of basic HSC biology remain unresolved, precluding additional rational approaches to even further expand them, or their more mature cellular derivatives' usage in the clinic. Here, we briefly summarize the most recent advances in the field of stem cell research in the last five years and proposed new roadmaps research topics that will need to be addressed to further expand and maximize the impact of the use of HSCs in the clinic.

HSC hierarchy and contribution of HSC

The contribution of stem and multipotent progenitor cells to steady-state blood maintenance has been recently challenged. Similarly, a number of recent studies obtained from single-cell analysis have also questioned the routes by which lineage differentiation occurs.[29] These evolving views of hematopoiesis have broad implications for our understanding of adult stem cell function. Nevertheless, most of these data have been obtained in mice and few in human.[30] Furthermore, most of the new data relied heavily on scRNAseq analysis, which have limitations on identifying closely related cell types. Thus, further studies are needed to define HSC heterogeneity and their cell fate decision both at steady state and under stress. Another aspect that will need to be addressed is to if/how the BM niche influences of the activity of HSC subsets and their differentiation.

HSC aging and rejuvenation

The effects of aging on HSCs have started to be defined.[31] Changes in the composition of the HSC pool as well as loss of cell polarity can be due to intrinsic properties, extrinsic microenvironmental perturbations and accumulation of mutations. Reversion or prevention of stem cell aging should help delay age-related hematopoietic deficiencies.

Generation of editing HSC for therapy

The discovery of the (CRISPR)-Cas9 nuclease (Cas9/sgRNA) systems in 2012, has revolutionised gene therapies for HSCs. The number of genome-editing strategies in HSCs that could offer therapeutic potential for blood diseases and immune system have dramatically increased. The HSC-based genome-editing field is primed to enter clinical trials in Europe in the coming years.[32]

HSC expansion, transplantation, and homing

A number of small molecules-based approaches and/or refined cultured conditions have suggested that ex vivo expansion of HSCs could be achievable.[33] Still, efforts to increase the homing of the transplanted (expanded or not) HSCs and better defined stem-cell niche properties and location is still warranted.

HSC transformation, cell of origin, and preleukemia

It has become clear that the identity of the hematopoietic stem or progenitor cell in which a leukemic event first arises plays a crucial role in the biology of the disease. Preleukemic lesions have been identified in healthy aged individuals. Understanding what influences the expansion of these preleukemic clones, overtime, and why certain individuals are more likely to develop a full-blown leukemia will allow us to decrease the risk of leukemia development. Additionally, understanding how leukemic (stem) cells outcompete the normal HSC overtime and altered the BM microenvironment might help us device new treatment strategies.[34]

Developmental hematopoiesis

Throughout adult life, the hematopoietic system is a highly dynamic hierarchy of cells founded by robust self-renewing and multipotent HSCs that produce billions of mature blood cells daily. The use of HSCs for cell and gene therapies is continuously growing, although with the limitation of obtaining a source of HLA-compatible cells. During the last 5 years, significant progress has been made by the European researchers regarding a better identification of the different hematopoietic cell populations and the microenvironments or niches where HSCs emerge (ie, in the embryonic aorta) and expand (ie, in the fetal liver) to form the pool of adult HSCs, as well as the molecular orchestration of HSC emergence and expansion processes. This precise fundamental knowledge is salient since it will make the foundation of future translational research. However, despite these advances, the generation of HSCs from pluripotent stem cells or using direct cell reprogramming remains elusive. This is likely because the intrinsic and extrinsic cues regulating HSC specification, expansion, and homing as it occurs physiologically during embryogenesis and in successive ontogenic microenvironments are not fully understood. This knowledge is, however, indispensable if one intends to understand/treat hematologic diseases and childhood leukemias.

Developmental hematopoiesis is characterized by the following rules:

1. HSC generation is currently limited to the embryo and occurs through the transdifferentiation of endothelial cells endowed with an hemogenic potential.
2. Extrinsic cues and cell-intrinsic cues, yet to be fully defined, govern the hematopoietic generative program.

In this subsection, we will summarize the advances made over the past 5 years and propose new issues that need to be addressed to create and amplify HSCs for therapeutic purposes.

Hemogenic endothelium commitment

How endothelial cells acquire a hemogenic potential is a key question. Interestingly, both the yolk sac and the embryo carry hemogenic endothelium but only the one from the embryo is capable of generating HSCs.[35] An in depth knowledge of the intrinsic transcriptional program(s) operating to specify hemogenic endothelium able to give rise to HSCs is required, preferably at the single cell level. This is required since hematopoietic production in vitro from the hemogenic endothelium is limited to progenitors. Recently, hemogenic endothelium generating HSCs has also been described in the forming bone marrow.[36]

The comparison between hemogenic endothelial cells specified in different anatomical sites and at different time points of development will certainly shed light on this cell commitment, which is still elusive. For a comprehensive view, several species including human should be studied and compared since the cellular and to many extents the molecular control of hematopoietic stem cell production in the aorta is strongly conserved between species. This allows to functionally test specific genes or signaling pathways.

Gene regulatory networks operating to drive hemogenic endothelium to HSCs

Based on previous studies mostly conducted in bulk populations, some genes, mostly transcription factors, have been identified and used to transduce cells to produce HSCs.[37] This production however remains extremely low and cannot be used for therapeutic applications. Despite the proof of concept, the efficiency and robustness in HSC generation of these studies is extremely low. Little is known about the gene cascades regulating the fine-tuned progression steps leading to HSC production. Transcriptomic data either in bulk and/or at the single cell level have recently started to be generated from different types of samples but the number of cells collected is small in the embryo and reliable systems of ex vivo differentiation of HSCs are still lacking, for example, pluripotent stem cell differentiation (see chapter "Reprogramming/induced pluripotent stem cells/embryonic stem cells"), gastruloids, aorta reconstruction/on a chip. In particular, gastruloids should give exciting insights into how extrinsic environmental signals instruct hemogenic endothelium and trigger the endothelial to hematopoietic transition to produce HSCs.

Dynamics of the hematopoietic waves in the different embryonic sites

Despite active research on the main hematopoietic sites in the embryo, that is, yolk sac, dorsal aorta, placenta, and fetal liver,[38–41] the precise hematopoietic cell types and the hematopoietic hierarchies present in these different locations are not fully identified. More importantly, most of these data have been obtained using the mouse embryo model as the mammalian embryo reference, while very few studies focused on the human embryo due to the lack of tissues. In a dynamic point of view, innovative barcoding systems are needed to identify the different stem and progenitor cell compartments and their routes of differentiation. Combined approaches generating bulk and single cell RNA-Seq approaches are also necessary to precisely identify these cell types and establish their close relationships.

Embryonic niches

In addition to establishing the intrinsic programs driving hemogenic endothelium specification, HSCs, and progenitor cell differentiation, the microenvironments should also be strongly scrutinized. Little is known about the first niches harboring/specifying hemogenic endothelium in the yolk sac and the embryo. Recently, 2 articles using spatial transcriptomics revealed the molecular complexity of the aortic hematopoietic microenvironment.[42,43] Of note, the fetal liver—and the placenta in the case of the mouse embryo—are 2 organs orchestrating HSC amplification that have hardly been studied regarding this point.

The embryonic origins of leukemia

Even though many leukemic events occur after birth, several hematological diseases were shown to take place during pregnancy and are accounting for 30% of childhood and adolescent cancers.[44] Understanding the initial mutation events triggering leukemia and modelling the initiation and progression of the disease will be of prime importance for the future. Access to human samples and manipulation of human pluripotent stem cells will certainly be a key issue. Bringing together people from developmental hematopoiesis and from childhood leukemia will help in the success.

Taken together, progress into these major axes should significantly enhance our understanding of the hematopoietic hierarchy, how the hematopoietic microenvironment is shaped, which mechanisms co-operate in regulating the commitment of hemogenic endothelial cells, how HSCs emerge and amplify, how this relates to changes in HSC heterogeneity during ontogeny, and how leukemia is initiated at early developmental stages. One of the strongest challenges will be to collect and integrate the multiple dimensions of omics applied to the hematopoietic cells and to combine them with similar if not identical approaches applied to the niches. These dimensions span from genomics, population or single-cell transcriptomics, spatial transcriptomics, single-cell epigenomics, and metabolomics. This unprecedented effort should gather biologists, computational biologists and, if required, clinicians to produce a comprehensive view of HSC production and hematopoietic development in health and disease. In depth knowledge of these should quickly pave the way toward ex vivo generation of HSCs for therapeutic purposes.

Mesenchymal stromal cells

Hemopoiesis is critically regulated by nonhematopoietic cells that support the production of blood and immune cells according to the organismal demands. These stromal cells compose the so-called hematopoietic microenvironment. It is becoming increasingly clear that different stromal populations regulate distinct subsets of hematopoietic cells, and vice versa. The complexity of these networks is further increased by the recognition of heterogeneity among bone marrow stem cells and their progenies. Recent technological developments (most notably single-cell studies) have allowed to describe candidate subpopulations. However, functional studies will be critical in the near future for fulfilling the enormous potential of stromal cells in immune modulation, tissue regeneration, and cancer treatment.

Understanding the specific functions of MSC subsets

HSCs and their microenvironment represent probably the best-characterized hierarchical stem cell system in vertebrates, paving the path to understanding how other organs function. Recent European research has revealed the cellular and spatial organization of different bone marrow MSC subsets.[45] Computational methods have been developed to infer candidate gene networks in HSC-supporting MSCs[46] and potential ligand-receptor interactions.[47] Future studies will be essential to reveal the specific functions and potential of MSC subpopulations.

Understanding the role of MSCs in hematological malignancies

Recent European research has demonstrated a key role for MSCs in the initiation, progression, and drug resistance of a variety of hematological neoplasms through paracrine or cell-contact-dependent mechanisms.[48] Extracellular vesicles from lymphoma B cells render MSCs protumorigenic.[49] Notch2

signaling on MSCs triggers Wnt-dependent survival of chronic lymphocytic leukemia.[50] Particularly, Wnt5A from MSCs is required for the engraftment of normal and leukemic HSCs.[51] MSCs remodel the extracellular matrix to support the progression of B-cell acute lymphoblastic leukemia (ALL),[52] support the growth of myeloproliferative neoplasms[53] or differentiate into myofibroblasts in myelofibrosis.[54] Finally, mitochondrial transfer from MSCs protects ALL and AML cells from oxidative stress induced by chemotherapy.[55,56] Therefore, elucidating and targeting niche-dependent mechanisms of tumorigenesis and resistance will be key to eradicate cancer.

MSCs in hematopoietic regeneration

Over the past 5 years, European research has shown that MSCs enhance HSC mobilization for subsequent apheresis from peripheral blood and transplantation (HSCT).[57] Targeting MSCs could help mitigate the damage to normal hematopoiesis by aging, AML, infection or iron overload in β-thalassemia.[58–61] Additionally, MSC coinfusion could increase the efficiency of HSCT[62] and minimize the risks of graft failure in gene therapy applications associated with low conditioning regimens and infusion of limited numbers of gene-edited HSCs.[63]

MSCs in tissue regeneration, immune modulation, and systemic disease

MSCs have a profound impact on different immune cells, having therapeutic benefits in sepsis, autoimmune disorders or graft-versus-host disease, and may influence the outcome of immunotherapies. Additionally, research on MSCs could influence the outcome and treatment of steadily increasing systemic conditions, such as diabetes, obesity, and aging.

Increasing research in the hematopoietic microenvironment and particularly MSCs will, scientifically, feed into other stem cell systems and, clinically, provide new ways to modulate and treat hematopoietic diseases, immune responses, and regenerative processes. As a result of this productive research, Europe continues to be the world region with the second highest number of registered clinical trials using MSCs.

Transcriptional/epigenetic networks

Maintaining a balanced output of hematopoietic cell lineages is critically dependent on cell fate choices at the stem and progenitor level within the hematopoietic hierarchy. These choices are executed at the single-cell level through the interplay of extracellular signaling pathways with the intracellular decision-making machinery. The latter is driven by networks of transcriptional and epigenetic regulators that interact in a combinatorial fashion and can form larger protein complexes with different functions dependent on composition and cellular context. These establish cell type-specific transcriptional and epigenetic programs and mediate developmental transitions when cells differentiate down a particular hematopoietic lineage.

European researchers have contributed significantly to the identification and characterization of individual transcription factors (TFs), TF networks and epigenomic changes involved in normal and aberrant hematopoieisis. In addition, they have extended many of these analyses toward mutated TFs in primary hematopoietic (single) cells from healthy and diseased individuals, thereby revealing multiple unanticipated functions of these proteins.[25,41,64–66]

European researchers have been acting at the forefront of epigenetic research, a prime research interest of the European Union, which has supported a multitude of projects on this subject in the past decade (eg, Epigenome NoE, HEROIC, EPITRON, Epigenesys, BLUEPRINT, Human Cell Atlas). These projects revealed many new insights into the interplay of TFs with chromatin to establish epigenetic patterns that define cell type functionality, but also led to the realization that much can still be learned about how blood cell types develop and how we could modulate their activities to prevent or counteract disease. This will require a concerted effort to better understand regulatory networks in both normal hematopoiesis and disease and will be accomplished only through close collaboration between experimentalists, computational biologists, statisticians, and clinicians.

Mutations in transcriptional and epigenetic regulators are some of the most common mutations in hematologic malignancies. Given that these proteins function as regulatory network components, it will be important to gain an understanding of the malignant state as a perturbation of wider regulatory networks. Research on the concerted actions as well as posttranscriptional regulation of TFs and on how these regulate the local and global transcriptomic and epigenetic environment should be intensified.

Although main TFs involved in disease development have been identified, many components that drive the functional fine-tuning of blood cell types and individual cells within are still not known. These are likely controlled by the niche in which the cells reside, the availability of metabolites-both endogenous (amino acids, sugars, and vitamins) and exogenous (drugs, food additives, and toxins)-and other environmental factors. In addition, it has become clear that not only the adaptive immune system confers memory potential, but that also cells of the innate immune system can be trained and are functionally dependent on past and present behavior, offering another entry point to utilize cells of the hematopoietic system in disease prevention and control.

Apart from creating additional comprehensive data sets on single-cell genomics, epigenomics, and metabolomics, integrative analysis of data is mandatory. Computational, conditional dependency models need to be established, for example by creating Bayesian networks, to formulate the relationship between the individual cell's niche, environmental factors, TF binding, epigenetic alterations, and the presence of various hematologic disorders.

Reprogramming/induced pluripotent stem cells/embryonic stem cells

Pluripotent stem cells (PSCs) including embryonic stem cells (ESCs) and induced PSCs (iPSCs), represent a limitless source of cells for investigations ranging from developmental processes to drug discovery. One of the most significant promises of the stem cell field is the in vitro derivation of cell populations usable in the clinic for therapeutic purposes. A comprehensive understanding of the molecular and cellular processes leading to blood cell emergence and maintenance is of paramount importance to fulfil this promise.

Over the last 5 years, European researchers have made seminal contributions to several interconnected areas in this research field. Studies into embryonic hematopoiesis using murine and human PSCs have further defined, sometimes even redefined, our understanding of hemogenic potential, cellular intermediates, signalling pathways, and molecular players implicated in blood cell emergence. High throughput sequencing has led to a wealth of data at both, population and single-cell levels.[37,67] These data revealed the importance of cell cycle regulation as a driver of differentiation, demonstrated the generation of hematopoietic stem cells-like cells from differentiated human PSCs, and highlighted gene regulatory network dynamics driving blood cell emergence. Together, these studies have helped

uncover the blueprint of blood cell generation revealing how similar PSC-derived cells are to their in vivo counterparts.

Reprogramming and forward programming have remained at the forefront of European investigation. Experimental protocols have been successfully devised for the generation of blood cell populations, including megakaryocytes and erythroblasts, both through direct reprogramming of fibroblasts and forward programming of PSCs. Dendritic cells were obtained through somatic reprogramming, while hematoendothelial progenitors were generated through human PSC forward programming. Ground-breaking studies have explored the molecular mechanisms of reprogramming and their associated changes in chromatin organization and epigenetic landscape.[68,69]

Many groups across Europe have demonstrated the tremendous potential of in vitro generated cell populations in cell replacement therapies. Macrophages, granulocytes, megakaryocytes, and erythroblasts were produced using various approaches ranging from forward programming of PSCs, optimization and standardization of differentiation protocols, to bioreactor-based mass production.[70] Pioneer studies have established the efficacy of these in vitro-derived cell populations using preclinical disease models. For example, transplanted PSC-derived macrophages were shown to reduce liver fibrosis, improve pulmonary alveolar proteinosis, or rescue mice from Pseudomonas aeruginosa-mediated acute infections.[71]

Over the last 5 years, impressive developments were made on the front of in vitro derivation of cell populations for therapeutic purposes. Translating these progresses into successful therapies will be the next significant challenge, from producing good manufacturing practices-compatible cell populations to establishing safety protocols and setting up clinical trials. The fundamental research on hematopoietic specification using PSCs led to important breakthroughs over the last 5 years, but the generation of in vitro derived hematopoietic stem cells remains elusive. However, this scientific endeavour is still worth pursuing, given the remarkable potential clinical implications.

Summary box: Main research & policy priorities

1. Further insight is needed into the mechanisms by which (epi)genetic alterations cause hematological malignancies.
2. A thorough understanding of embryonic versus adult hematopoiesis will help to better understand differences between childhood and adult hematologic malignancies.
3. Further improvement into the generation of patient-specific iPSCs and the generation of fully functional HSCs thereof is warranted.
4. In-depth understanding of normal and malignant hematopoiesis via fundamental research is essential to translate findings to the clinic and develop more effective therapies for hematological malignancies.

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