

# The European Hematology Association Roadmap for European Hematology Research: a consensus document



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## ABSTRACT

The European Hematology Association (EHA) Roadmap for European Hematology Research highlights major achievements in diagnosis and treatment of blood disorders and identifies the greatest unmet clinical and scientific needs in those areas to enable better funded, more focused European hematology research. Initiated by the EHA, around 300 experts contributed to the consensus document, which will help European policy makers, research funders, research organizations, researchers, and patient groups make better informed decisions on hematology research. It also aims to raise public awareness of the burden of blood disorders on European society, which purely in economic terms is estimated at €23 billion per year, a level of cost that is not matched in current European hematology research funding. In recent decades, hematology research has improved our fundamental understanding of the biology of blood disorders, and has improved diagnostics and treatments, sometimes in revolutionary ways. This progress highlights the potential of focused basic research programs such as this EHA Roadmap.

The EHA Roadmap identifies nine 'sections' in hematology: normal hematopoiesis, malignant lymphoid and myeloid diseases, anemias and related diseases, platelet disorders, blood coagulation and hemostatic disorders, transfusion medicine, infections in hematology, and hematopoietic stem cell transplantation. These sections span 60 smaller groups of diseases or disorders.

The EHA Roadmap identifies priorities and needs across the field of hematology, including those to develop targeted therapies based on genomic profiling and chemical biology, to eradicate minimal residual malignant disease, and to develop cellular immunotherapies, combination treatments, gene therapies, hematopoietic stem cell treatments, and treatments that are better tolerated by elderly patients.

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## Introduction

Blood can be described as one of the human body's largest organs. It is essentially a liquid tissue containing many different types of specialized cells needed for the normal functioning of the human body. When one or more of these cell types do not perform well, a wide variety of blood disorders can result, ranging from blood cancers and coagulation and platelet disorders to very common diseases such as anemia.

Hematology is the medical discipline concerned with diagnosing and treating all of these diseases.

In the European Union (EU) alone, an estimated 80 million people are currently affected with blood disorders.

Various types of anemia affect more than 50 million children and adults in the World Health Organization's European region.<sup>1</sup> Blood cancers, some of which mainly affect young people, contribute strongly to premature cancer-related mortality and lost productivity in Europe.<sup>2</sup> Among cancers, blood cancers [leukemia, Hodgkin and non-Hodgkin lymphomas (HLs and NHLs), and multiple myeloma] together rank third after lung cancer and colorectal cancer in terms of age-adjusted mortality in the European Economic Area.<sup>3</sup>

Inherited blood diseases, such as thalassemia, sickle cell disease, and glucose-6-phosphate dehydrogenase deficiency, also affect millions of people and cause substantial morbidity and mortality. Rarer forms of congenital blood disorders represent an immense burden on those affected. Many infectious diseases affect various types of blood or blood-forming cells, causing widespread diseases such as malaria and HIV/AIDS.

In recent decades, enormous progress has been made in terms of diagnosis and treatment of these diseases. Unfortunately, many blood disorders remain incurable. Approximately 115,000 patients die each year.<sup>4</sup>

Blood disorders have immense economic consequences as well. The combined societal cost of hematologic diseases for the EU, Norway, Iceland, and Switzerland has been estimated at €23 billion per year.

At a European level, current public spending on hematology research does not match this vast medical need. Of the €6.1 billion that the European Union allocated to health research under its 7<sup>th</sup> Framework Programme (2007-2013), only 2.2% (€137 million) was granted to hematology research. That amounts to less than 0.1% of the societal cost of blood disorders in Europe over that same period.

## Milestones in hematology and the contribution from Europe

Research in hematology has fundamentally improved our understanding of the biology of hematologic diseases and resulted in many innovative discoveries. Many of these discoveries are powerful examples of how carefully designed basic research can lead to new approaches that block or interact with key pathways in diseased cells, resulting in very impressive anti-tumor effects. European hematologists have pioneered important inventions and played leading roles in developing, for example, curative approaches for patients with malignant diseases, such as lymphomas and leukemias,<sup>5,6</sup> which often affect young patients.

Key milestones included the characterization of hemoglobin (Hb),<sup>7</sup> induced pluripotent stem cells (iPSCs),<sup>8</sup> and somatic driver mutations.<sup>9</sup> The discovery of the Philadelphia chromosome and the subsequent identification of the BCR-ABL1 tyrosine kinase and its role in chronic myeloid leukemia (CML)<sup>10</sup> led to the successful development of potentially curative targeted treatment in this form of blood cancer.<sup>11</sup> This was an unprecedented rate of success and it occurred in a malignancy that previously could only be treated by allogeneic transplant in a very select number of patients. Acute promyelocytic leukemia became one of the first malignancies that could be cured without conventional chemotherapy.<sup>12</sup>

Another key development in hematology was that of a wide range of monoclonal antibodies following the original invention by Köhler and Milstein in the UK.<sup>13</sup> Humanized or fully human monoclonal antibodies are now used in hematology for both diagnostic and therapeutic purposes. The clinical breakthrough was a humanized monoclonal antibody targeting the CD20 antigen on B-cell lymphoma.<sup>14</sup> Today, monoclonal antibodies or antibody-based conjugates are used successfully in most malignant lymphomas and leukemias. They can, however, also be effective in non-malignant blood disorders such as paroxysmal nocturnal hemoglobinuria (PNH), a rare acquired clonal stem cell defect leading to increased fragility of hematopoietic cells and hemolytic anemia (HA), thrombosis, and bone marrow failure (BMF). Prognosis of patients with severe PNH used to be less than five years, but changed radically with the advent of an anti-complement monoclonal antibody that counteracts membrane fragility.<sup>15</sup> Today, PNH patients treated with this antibody have a normal life expectancy.

Severe hemophilia represents another story of unprecedented success. Patients used to be confined to wheelchairs or face the specter of death because of untreatable hemorrhage or blood-borne infections such as HIV/AIDS. Today, new recombinant substitutive therapy is completely safe and effective in long-term prophylaxis. Hematology expects to further improve in this area, with innovative factor VIII or IX molecules that have increased activity and prolonged half-life.

Gene therapy is becoming a reality for more and more blood diseases, while treatment of malignant and non-malignant hematologic diseases is impossible without blood transfusions and blood-derived medicinal products. "Haemovigilance", a European initiative that provides a surveillance registry of serious unwanted transfusion effects, is now up and running in most EU member states.

## European research policy

Governments, politicians and other policy makers carry the responsibility for making well informed decisions on regulation and funding priorities for health research and medicinal product development. The research community has a responsibility in providing policy makers with the kind of information and evidence that they need to make those informed decisions.

With respect to research funding, the authors feel that hematology was underfunded in the EU's 7<sup>th</sup> Framework Programme. The current Framework Programme (Horizon 2020) was spared major budget cuts, but raising the relative level of funding for hematology research needs to be improved.

With respect to regulation, a key issue on the table is the EU's new regulation on clinical trials on medicinal products for human use, which will come into effect in 2016. Over the past years, the number of clinical trials in Europe has decreased. These trials are key to medical research. European research groups have been instrumental in setting up multicenter clinical trials to test important new products. However, the new regulation has the potential of making future trials in Europe too expensive and too complex to carry out, especially in terms of academic research, and, therefore, may lead to a further decrease in clinical trials. A drop in the number of trials and the number of participants would harm the interests of European patients and damage Europe's knowledge infrastructure and future economy.

## The European Hematology Association Roadmap

In 2014, at its 19<sup>th</sup> Annual Congress in Milan, Italy, the European Hematology Association (EHA), Europe's largest non-profit membership organization in the field of hematology, decided to launch a Roadmap project. One of its goals was to better inform European policy makers and other stakeholders about the urgent needs and priorities of patients with blood diseases and the field of hematology. Another goal was to help the European hematology research community in harnessing resources by bringing basic researchers, clinical trial networks and patient advocates together in comprehensive study groups. A European consensus on medical and research priorities will also promote excellence and collaboration between academics and the pharmaceutical industry.

The EHA Roadmap Task Force included EHA board members and other top experts from all fields of hematology. Hundreds of hematologists, clinical trial groups, drug makers, national hematology societies, patient representatives and others were invited to provide input and advice. Many contributed to the drafting of the document and the various stages of review.

This Roadmap is the outcome of this project. It identifies the greatest unmet needs in hematology research and clinical science, describing: 1) state-of-the-art hematologic research; 2) the most urgent research priorities; and 3) the anticipated impact this research could have.

The EHA Roadmap Task Force identified nine major 'sections' in hematology: normal hematopoiesis, malignant lymphoid and myeloid diseases, anemias and related diseases, platelet disorders, blood coagulation and hemostatic disorders, transfusion medicine, infections in hematology, and hematopoietic stem cell transplantation (HSCT). For each section, the Roadmap Task Force appointed one or two editors. Together, the Roadmap Task Force and section editors drafted and reviewed a more detailed framework of 60 'subsections' of groups of diseases and conditions. Section editors selected experts from their various fields to contribute as subsection editors or authors. Each section and subsection adapted the same basic format.

Draft texts and figures were discussed by the Roadmap Task Force and section editors during three meetings between October 2014 and March 2015. Sections were then reviewed by the Roadmap Task Force, the EHA board, and a selection of experts. The final draft was sent for consultation to stakeholders such as national hematology

societies, patients' organizations, hematology trial groups, and other European organizations in, for example, overlapping disease areas. All comments were discussed and integrated before submission of the manuscript to *Haematologica*.

In all, around 300 European hematologists and top experts helped to create the Roadmap.

At the request of the EHA board, the University of Oxford simultaneously carried out a study into the societal burden and cost of blood disorders in Europe. Outcomes from their analysis also informed various parts of this Roadmap.

Some dominating topics and unmet needs can be recognized in nearly all of the nine EHA Roadmap sections. They include:

1. developing novel targeted therapies based on genomic profiling and chemical biology;
2. unleashing the power of cellular immunotherapy;
3. eradicating minimal residual disease (MRD) in hematologic malignancies;
4. creating smarter combination treatments;
5. developing better tolerated treatments for blood disorders with a special emphasis on elderly patients;
6. using gene therapy to tackle blood disorders;
7. maximizing the clinical application of hematopoietic stem cells (HSCs) for transfusion, immunomodulation, and repair.

Taken together, this EHA Roadmap highlights major past achievements in the diagnostics and treatment of blood disorders, identifies unmet clinical and scientific needs in those same areas, and will enable better funded and more focused European hematology research.

The EHA will pro-actively bring this Roadmap to the attention of all stakeholders involved in hematology, and calls upon those stakeholders to do the same.

### Acknowledgments

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## The EHA Roadmap for European Hematology Research

### Section 1. Normal hematopoiesis

#### Section editors: Jan Jacob Schuringa, Thierry Jaffredo.

Hematopoiesis, the formation of blood, is initiated in our bone marrow by hematopoietic stem cells (HSCs), first identified by Till and McCulloch in the 1960s. After cell division, these HSCs can generate progenitor cells that gradually differentiate into all the erythroid, myeloid, and lymphoid lineages that reconstitute our blood. *Via* a process termed self-renewal, they are also able to generate new stem cells to ensure a lifelong reservoir of HSCs. In the past decades, excellent *in vitro* and *in vivo* model systems have been generated that have allowed us to obtain a thorough understanding of hematopoiesis at the molecular and cell biological level. HSCs were also the first stem cells that were used in a clinical setting through bone marrow transplantation (BMT). It is, therefore, not surprising that the hematopoi-

etic system has served as a paradigm for the study of many other stem cell types as well.

We have learned much about growth factors and cytokines that regulate the fate of HSCs and their progenies. With the availability of genome-wide multiomics technologies, transcription factor (TF) networks and epigenetic landscapes of cells within the hematopoietic hierarchy are currently being characterized at a rapid pace. Step by step, we are now beginning to understand how these are interlinked and how they control the transcriptomes and proteomes of hematopoietic cells. We have learned a lot about the microenvironment within the bone marrow that keeps HSCs in their quiescent state and regulates their self-renewal. We have learned about how and where HSCs are formed during embryogenesis, and we are also beginning to better understand how HSCs age.

Fundamental translational research has been critically important in getting us where we are today. But still many questions remain. Among many others, these include the question as to how (epi)genetic aberrations cause hematologic malignancies, and how we can use these insights to develop better therapeutic strategies. It is now being realized that there is a clonal heterogeneity in many hematologic cancers, and possibly even within the normal HSC compartment. But how does this affect disease development and current treatment options? 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 us to better understand the differences between childhood and adult hematologic malignancies. Reprogramming now allows patient-specific induced pluripotent stem cells (iPSCs) to be generated, but the generation of fully functional HSCs from these is still rather challenging. Can this be improved? We live in a continuously aging society, but how does HSC aging actually affect health and disease? Within the first section, we have brought together leading scientists and clinicians in the field of hematopoiesis. They provide an overview of the current status of the field and an outlook on where future research should be directed (Figure 1). We firmly believe that combining fundamental and translational research will result in not only a better understanding of the hematopoietic system, but also in the development of better therapeutic approaches for hematologic malignancies, many of which are still difficult to treat.

### 1.1. Erythropoiesis

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#### Introduction

The major cell type in our blood is the red blood cell (RBC) or erythrocyte. RBCs transport oxygen from the lungs to other parts of the body, and from there they carry carbon dioxide back to the lungs. An adult has approxi-

mately 5 liters of blood, containing  $25 \times 10^{12}$  RBCs. Because the lifespan of an RBC is approximately 120 days, a healthy person needs to produce  $2.4 \times 10^6$  RBCs per second to maintain a constant number of RBC.<sup>16</sup> The oxygen carrier hemoglobin (Hb), composed of two  $\alpha$ -like and two  $\beta$ -like globin proteins, makes up approximately 90% of soluble protein in RBCs. RBCs and Hb form during a process called erythropoiesis, which includes the initial specification of HSCs from mesoderm during embryogenesis, the decision of these cells to self-renew or differentiate, the process of proliferation and erythroid specification, and, finally, their terminal differentiation and post-mitotic maturation. Terminally differentiating erythroid cells extrude their nucleus and shed their endoplasmic reticulum and mitochondria. The new cells enter the circulation as reticulocytes, which are still engaged in protein translation. Finally, the population of mature, biconcave RBCs with diameters of only 6-8 micrometers creates a large surface area for gas exchange, which, through RBC membrane deformability, extends from major blood vessels into the microcirculation.

Abnormally low Hb levels cause anemia. Approximately one-third of the world's population has some form of anemia, making this diverse group of disorders by far the most common clinical problem worldwide. Perturbation of erythropoiesis might be acquired and related to iron deficiency or to different systemic disorders associated with chronic inflammation (e.g. autoimmune diseases and cancers) or myelodysplasia. A multitude of different inherited anemias affect erythropoiesis by diverse mechanisms, such as thalassemias (by reduced or absent functional Hb), sickle cell disease (SCD) (by a pathological Hb variant), HAs (by defects in membrane proteins, metabolic enzymes, or pathological Hbs), Diamond Blackfan anemia (DBA) (by impaired ribosome biogenesis), Fanconi anemia (FA) (by DNA repair defects), and congenital dyserythropoietic anemia (CDA) (e.g. CDA type II by defects in protein trafficking). Polycythemia vera (PV), although not limited to erythropoiesis and also seen in myeloproliferative neoplasms (MPNs), is caused by activating JAK2 kinase mutations. The physiological and molecular mechanisms underlying these disorders are still not completely understood, while erythroid defects are also associated with many other, and often still unknown, genetic defects. Elucidation of normal erythropoiesis is, therefore, essential to develop new strategies for treating the wide variety of conditions affecting the erythroid system.

#### European research contributions

Historically, research of the hematopoietic system has driven novel biological concepts and methods, owing to the accessibility and ready purification of hematopoietic progenitor cells (HPCs) for molecular and functional analyses. Early European contributions included the Nobel Prize winning discovery of the structure of Hb<sup>7</sup> and understanding the etiology and epidemiology of inherited anemias, leading to implementation of pre-natal diagnostic programs.<sup>17</sup> Other European contributions include determining the origin of hematopoietic stem cells (HSCs), the transcriptional circuitry underlying erythropoiesis, the molecular control of differentiation *versus* apoptosis, the role of iron metabolism, and DNA sequences driving high-level expression of Hb, which are now applied in gene therapy vectors. Other discoveries, such as the roles of serotonin and transferrin receptors, also heralded significant

progress in our understanding of normal erythropoiesis. Recently, purified cells have been characterized using “omics” techniques to determine their transcriptional profiles, epigenetic programs, and responses to cell signaling. A database dedicated to erythroid disorders has been established<sup>18</sup> aiming to integrate data from fundamental and translational research with data from routine clinical care. Translational research has resulted in optimized BMT protocols, magnetic resonance imaging (MRI) monitoring of iron overload, improved iron chelation therapies, and targeted inhibition of signaling pathways mutated in (pre)leukemic conditions.<sup>19</sup>

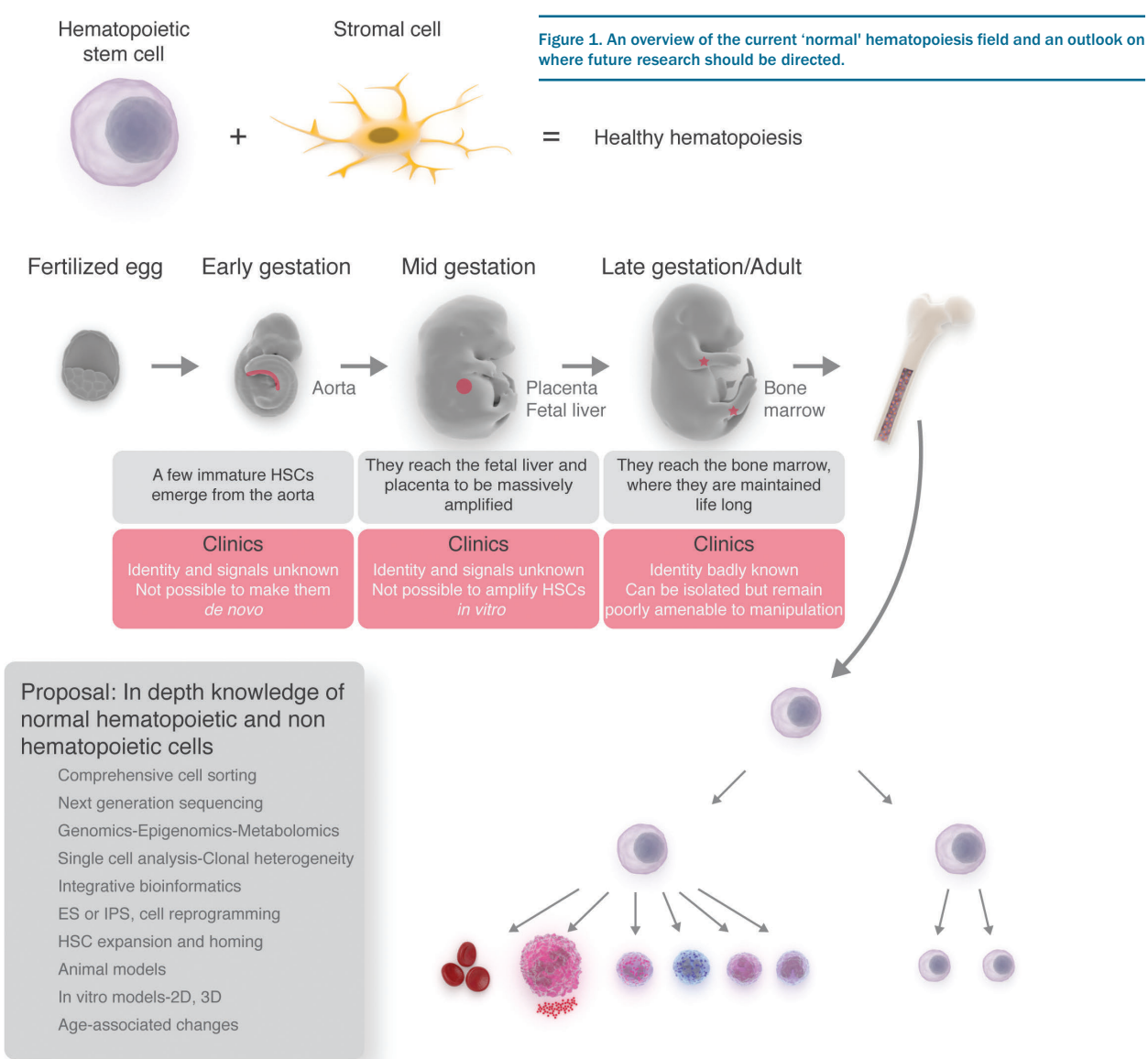
**Proposed research for the Roadmap**

Previous research has laid the foundation on which a comprehensive framework for understanding erythropoiesis can be built. Next generation sequencing (NGS) technologies have opened up exciting new avenues for qualitative and quantitative biology, with unprecedented sensitivity and specificity. For instance, mutation detec-

tion in single cells is now possible, and quantitative gene expression profiles can be generated from hundreds of individual cells in a single experiment. For the first time, this allows hierarchical relationships between cells of a single lineage to be determined and the impact of cell-cell interactions and signaling cascades on erythroid development to be unraveled. Although pioneering research will rely on the use of cellular and animal model systems, the protocols developed will be quickly translated to the study of erythropoiesis in human subjects, taking full advantage of single-cell omics analyses. Our goal is to apply this deeper understanding of erythropoiesis to improve diagnosis, prognosis, and treatment algorithms for patients with conditions affecting the erythroid system.

**Anticipated impact of the research**

Understanding the basic physiological and molecular mechanisms of normal erythropoiesis will have a direct and long-lasting impact on the medical care of patients with hereditary and acquired anemias. Firstly, improved diagno-



sis will enable clinicians to predict disease progression for individual patients much more accurately, leading to better-informed decisions on disease management by transfusion, iron chelation, cytokines or cytokine inhibitors, and splenectomy. Secondly, fundamental knowledge of normal erythropoiesis will likewise guide the development of safer and more effective curative treatments, such as those involving gene correction or gene therapy, and identification of new therapeutic targets to be exploited in public-private partnerships for the development of new treatments for erythroid disorders. Thirdly, an increased understanding of the microenvironment and cell signaling mechanisms will enable the development of clinical algorithms for management of patients with anemias. Fourthly, *in vitro* generation of fully functional human erythrocytes will ultimately bring completely defined and guaranteed disease-free human blood units to the clinic, with a major impact on transfusion medicine. Finally, the erythroid system represents a fascinating and tightly regulated process of proliferation, survival, and differentiation and has always served as a paradigm for other biological systems. New fundamental insights into erythropoiesis will, therefore, likely continue to facilitate discoveries in all fields of medicine, leading to an improved understanding of disease mechanisms and better clinical care for patients.

### 1.2. Myelopoiesis

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#### Introduction

Myeloid cells, including granulocytes, monocytes/macrophages, and dendritic cells, are key effector cells of the innate immune defense against invading microorganisms.<sup>20</sup> Myeloid cells are continuously generated from hematopoietic stem cells (HSCs) in the bone marrow through a tightly regulated process referred to as myeloid differentiation or myelopoiesis. This complex process is regulated in part by growth factors and epigenetic and transcriptional regulators that in concert orchestrate cell survival, proliferation, and, most importantly, instruction of lineage-restricted differentiation of HSCs *via* a series of hematopoietic progenitor cells (HPCs) into all types of fully mature myeloid cells. For the past two decades, a plethora of studies have demonstrated the pathognomonic link of genetic aberrations in myeloid key regulators with several hematologic disease entities, such as acute myeloid leukemias (AMLs), myeloproliferative neoplasms (MPNs), and severe congenital, as well as cyclic neutropenia. Hence, characterization of myeloid regulators and their function during steady-state hematopoiesis at both the molecular and systems level is important to understand: 1) the biology of myeloid differentiation and innate immune defense; and 2) how genetic aberrations of myeloid regulators affect normal myeloid differentiation and cause myeloid disease.

#### European research contributions

Lineage priming is an idea that was brought forward by scientists in Europe. The concept of lineage priming,

which postulates that lineage-specific transcription factors (TFs) are already present in uncommitted HSCs, is now widely accepted. European scientists have also made substantial contributions to the understanding of TF networks in hematopoiesis, as well as identification of the earliest cells in the hematopoietic hierarchy that can give rise to myeloid cells. Moreover, research groups in Germany and the Netherlands have identified mutations in the granulocyte colony-stimulating factor receptor as the cause of severe congenital neutropenia, and have shown that treatment with granulocyte colony-stimulating factor can improve survival but also lead to AML.<sup>21</sup>

Later, *HAX1* and *JAGN1* mutations were identified by a German research group as another genetic cause for severe congenital neutropenia. Other European researchers have pioneered our understanding of neutrophil differentiation and antimicrobial granule proteins of neutrophils.<sup>22</sup>

The role of the TF CEBPA as a key regulator in normal and malignant myeloid differentiation was pioneered by European research groups. Specifically, these groups applied genetic models to uncover the important role of CEBPA for granulocytic lineage commitment and differentiation, as well as the causative role for mutant CEBPA in AML, thereby dissecting the complexity of how biallelic CEBPA mutations contribute to leukemogenesis.<sup>23,24</sup>

#### Proposed research for the Roadmap

The advent of novel comprehensive omics technologies, such as RNA-seq/microarray analyses, miRNA array analysis, ChIP-seq analyses of transcriptional and epigenetic regulators, metabolomics, and finally proteomics and phosphoproteomics, allow us to define phenotypes of cellular states at the systems level. The current proposal is to apply a comprehensive omics strategy to improve our understanding of normal myeloid differentiation and innate immune defense at a systemic level and how genetic aberrations cause perturbations of normal cellular activities, resulting in myeloid disease phenotypes.

Given this, the proposal will combine omics technologies and comprehensive cell sorting to generate a state-of-the-art reference omics data set of prospectively purified human bone marrow populations representing successive stages of myeloid differentiation (i.e. HSCs, myeloid progenitors, and mature myeloid cells) in healthy subjects. The resultant data set will improve our understanding of how dynamic regulatory networks control cell fate and function during normal myeloid differentiation and immune defense. Moreover, the research community will be able to match the resultant normal reference omics data set with omics data sets of sorted bone marrow populations from patients with myeloid diseases harboring defined genetic aberrations. This strategy will allow a standardized omics data set comparison of normal and disease states, which will unravel how specific genetic aberrations in patients promote aberrant cellular activities (e.g. signaling, proliferation, metabolism, apoptosis, etc.) underlying the phenotype of specific myeloid diseases. Significantly, comprehensive data mining of the normal "reference" and patient omics data sets will allow us to identify novel diagnostic markers, as well as targets for therapeutic interventions, and ultimately improve treat-

ment and clinical outcome of patients suffering from AML, MPNs, and other myeloid diseases.

A co-ordinated European effort involving basic researchers, clinical researchers, and bioinformatic technicians is required to achieve the following aims of the proposal.

1. Establishment of a European expert group that will discuss and define a standard for cell sorting of myeloid cells, applied omics technology platforms, and the development of bioinformatic methodologies for integrated omics and clinical data analysis.
2. Establishment of core facilities/hospitals for standardized collection and biobanking of human bone marrow samples from healthy subjects for the project.
3. Establishment of core facilities/research teams for sorting of bone marrow populations according to the standard sorting strategy defined by the expert group (see point 1).
4. Establishment/identification of core facilities/research teams for omics analysis of sorted bone marrow populations according to the consensus omics standard platform defined by the expert group (see point 1).
5. Establishment of a European core bioinformatics group for concerted processing and analysis of omics data in order to generate a “reference” omics data set of normal myeloid differentiation.

Ideally, the bioinformatics group will also assist European clinicians with standardized comparison of the obtained reference omics data set and omics data sets of patients enrolled in clinical trials. In addition, the bioinformatics group will develop an open-access web-based platform allowing researchers worldwide to download and match omics data from patients with myeloid diseases for comparison with the reference omics data set.

#### *Anticipated impact of the research*

The proposed research program relies on a concerted multidisciplinary European effort to generate a comprehensive omics reference data set of myeloid differentiation. The resultant data set represents an extremely 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, the latter is important for pre-clinical and clinical research programs aiming at identifying: 1) novel diagnostic markers for improved prognostication; and 2) novel therapeutic targets for the development of more effective treatment modalities improving survival of patients with AML, MPNs, and other rare myeloid diseases.

### **1.3. Megakaryopoiesis**

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#### *Introduction*

Megakaryopoiesis is the differentiation process that leads to platelet production. This is a unique cell biology system, because platelets arise from the fragmentation

in the blood shear of their marrow precursors, namely the megakaryocytes. A large part of the platelet production is regulated by the megakaryocyte size through polyploidization. The regulation of megakaryopoiesis is dependent on a cytokine/hormone called thrombopoietin (THPO), which signals through the MPL receptor. However, THPO is not directly involved in the last differentiation steps directly responsible for platelet production. In terms of development, megakaryopoiesis is extremely close to erythropoiesis, and the regulation of megakaryopoiesis and HSCs unexpectedly share many common features concerning gene transcription and regulation by THPO with the presence of megakaryocyte-biased hematopoietic stem cells (HSCs). Megakaryopoiesis is affected by numerous acquired and hereditary disorders. Most of them target the THPO/MPL signaling or the actin and tubulin cytoskeletons, which play a central role in late stages of megakaryopoiesis.<sup>25-28</sup>

Platelet transfusion is the most common way to treat profound thrombocytopenia, but this increasing need in platelet transfusion is limited by a donor deficit, and thus, there is now a place for alternative approaches, including *ex vivo* platelet production and small molecules stimulating platelet production *in vivo*. All of these approaches require major progress to be made in basic research.

#### *European research contributions*

Researchers from Europe have played a central role in understanding the regulation of megakaryopoiesis.

1. They have been pioneers in the identification of the MPL/THPO axis and the main transcription factors (TFs) (GATA1, FLI1, TAL1, and LYL1).
2. They have largely contributed to the mechanisms of polyploidization and proplatelet formation.
3. They have developed new investigational techniques, such as 2-D and 3-D cultures, videomicroscopy, and use of shear to produce platelets.<sup>29</sup>

#### *Proposed research for the Roadmap*

The major topics that require intense research resources and efforts are listed here.

*Mechanisms of megakaryocyte commitment and differentiation from HSCs:* defining these different cellular steps in terms of transcription factors, epigenetic regulators, and growth factors involved in this cellular process will be important to: 1) increase platelet production *in vivo*; 2) develop *in vitro* techniques for somatic cell reprogramming toward the megakaryocyte lineage; and 3) increasing the megakaryocyte potential of induced pluripotent stem cells (iPSCs).

*Further characterization of the THPO/MPL functions:* MPL plays a central role in regulating megakaryopoiesis through direct signaling, as well as by clearing THPO. Studies of THPO synthesis, the precise MPL signaling pathways, including their consequences on gene regulation and MPL cell trafficking will be important for understanding the mechanisms of thrombocytopenia or thrombocytosis and for developing new molecules capable of positively or negatively regulating MPL.

*Determination of the precise mechanisms of polyploidization:*

the processes of endomitosis and its regulation by both extrinsic and intrinsic mechanisms, including ontogenesis processes, are poorly known. A complete understanding will be important for developing *in vitro* cell systems able to produce highly polyploid megakaryocytes. In addition, this topic might be relevant to understanding the processes of polyploidization in malignant tumors.

*Regulation of platelet formation:* in order to discover disease mechanisms and new therapeutic targets, it is fundamental to understand how megakaryocytes differentiate and form platelets. These mechanisms include all the bone marrow environment molecules that participate in the regulation of TFs and biochemical signaling through activation of specific receptors. New cellular techniques, including single-cell assays, should be developed, generating candidate regulators of this crucial step of platelet production. Intrinsic cellular determinants, such as the actin and tubulin cytoskeletons and their regulation, will have to be studied. It will be important to determine the precise mechanism of platelet abscission and the role of the shear. These approaches may also provide candidate molecules implicated in proplatelet formation and the migration of megakaryocytes in the marrow. The endothelium participates in the regulation of megakaryocyte function, and megakaryocytes have to remodel the basement membrane of the sinusoids in order to extend proplatelets through the vascular wall of the bone marrow sinusoids. It will be important to understand how proplatelets interact with the endothelium to reach the blood flow and be released, and how megakaryocytes and endothelial cells mutually regulate their behavior. New processes to be considered also include the role of calcium in megakaryocyte development and the importance of autophagy in megakaryocyte development within the bone marrow environment.

*Mutual regulation of megakaryocyte and bone marrow environment:* it is known that megakaryocytes are able to express and release different molecules that may regulate bone marrow homeostasis in both steady-state, post-injury conditions, and in malignant and inflammatory disorders. Alteration of these processes may lead to pathological conditions or support diseases. On this basis, it is important to understand which molecules megakaryocytes actively express and release, and their role in the bone marrow regulation.

#### *Anticipated impact of the research*

In recent decades, much progress has been made in our knowledge of megakaryopoiesis, but better understanding its regulation and the function of the increasing number of genes found to be mutated in pathology will require significant effort. The precise understanding of endomitosis and platelet formation may have important clinical consequences, particularly for developing new technologies for large platelet production *in vitro* and also new molecules capable of modifying platelet production *in vivo*. Europe has played a leading role in studies of megakaryopoiesis, but research has remained fragmented. Integrated European programs will provide the critical mass of resources and expertise needed to develop the large ambitious programs required to proceed from basic to clinical research.

#### **1.4. Lymphopoiesis**

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#### *Introduction*

The immune system constitutes the body's defense system against disease and foreign cells/micro-organisms. Immune cells are diverse, conventionally divided into innate and adaptive subsets. B and T lymphocytes are the main protagonists in adaptive immunity and are generated by a complex process referred to as lymphopoiesis, which involves numerous finely regulated steps, including the lymphoid-specific somatic rearrangement of genes encoding the immunoglobulins and T-cell receptors for antigens. Lymphocytes are generated within defined microenvironments that provide the growth factors and signals necessary for their commitment, survival, expansion, and education to self-/non-self-discrimination. A deep understanding of the steps that allow the production and amplification of lymphoid precursors and mature cells is crucial in order to obtain an efficient immune system, which is important for lymphoid-based therapies in humans.

#### *European research contributions*

European groups have a strong research record in the field of lymphopoiesis and achieved the first successful gene therapy protocols for human genetic defects affecting lymphopoiesis.<sup>30</sup> They contributed to the development of *in vitro* assays, allowing studies on T- and B-cell differentiation using bone marrow-derived stromal cell/progenitor and organotypic thymic cultures further complemented by humanized murine models in which the generation of human T and B cells could be studied.<sup>31</sup> Such tools helped identify murine and human lymphoid progenitors, as well as thymus-seeding progenitors. Moreover, lymphoid progenitor expansion conditions for cell therapy purposes have been recently described. Pioneering European studies also characterized the thymic microenvironment, crosstalks between the epithelium and developing T cells, and the role of the Notch pathway and regulators in the molecular regulation of T-cell differentiation.<sup>32</sup> Other groups focused on the early steps of B-cell development in mice and identified progenitor B-cell regulators that modulate pathological immune responses. Studies on human B-cell deficiencies emphasized species differences between mice and humans, underlining the need for the establishment of novel research strategies that help us understand species-specific peculiarities and the common features of B- and T-cell development. Several pathologies involving lymphoid deficiencies were found to be caused by defects in various steps of V(D)J recombination, implicating general and specific mechanisms of DNA repair in lymphopoiesis and telomere maintenance.<sup>33</sup> Finally, innate lymphoid cell and natural killer (NK)-cell development were described,<sup>34</sup> bringing robust cell role-players to develop new therapies.



### Proposed research for the Roadmap

Mature lymphocytes and their subsets concomitantly arise from very infrequent progenitor subsets that are not well characterized. These processes take place in complex “niches,” the settings of which are only just being unraveled. Dissecting the role of various non-hematopoietic and hematopoietic cell subsets of the bone marrow and thymus involved in lymphoid development/progenitor maintenance will favor identification of the major players in steady-state conditions and new molecular targets for anti-cancer and anti-viral immunotherapies. This knowledge will be important for modeling normal T/B/NK-cell development within their specific niches, using scaffolds as surrogate thymic and bone marrow niches to help uncover side effects and resistance mechanisms observed in chemotherapies. A precise description of the migratory pattern of developing T and B cells will be necessary to efficiently transplant newly generated, *ex vivo* lymphoid progenitors from different HSC sources, including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). Identification of drugs able to mobilize lymphoid progenitors for cell/gene therapy also needs to be investigated. Further studies are required to assess the impact of these and other drugs/small molecules on normal developmental processes, such as hematopoiesis. Such experiments will support the design of new assays for human lymphoid development. They should also stimulate drug research for acute lymphoblastic leukemias (ALLs). For realistic lymphoid-based therapies to become “routine” in the near future, one should learn from previous experiences with the successes achieved in gene therapy. These experiences emphasize the continuous need for modification of existing viral vectors to improve transduction of lymphoid progenitors, as well as the development of lymphoid-specific protocols of gene therapy.

Improved modeling of human disease and cellular therapy of immune deficiencies is also essential and is required to support the development and expansion of lymphoid cells from iPSCs or ESCs, as well as to generate lymphoid progenitors *ex vivo* from isolated hematopoietic cell populations. Identification of human HSCs biased toward T- and B-cell production will facilitate understanding of the origin of lymphoid cells and their potent expansion prior to transplant into patients.

At the molecular level, epigenetic changes and major regulatory signals (microRNA, long non-coding RNA, and chromatin modifications) need to be explored by developing robust genome-wide protocols on small cell subsets. This may help to: 1) define an “epigenetic and transcriptomic ID card” of aforementioned progenitors and B- and T-subsets in normal and pathological development; and 2) evaluate the consequences of infections, inflammations, irradiations, and hypoxia in this development. A major factor that compromises the immune system is aging, and future studies should be aimed at maintaining a fit, long-lived lymphopoietic compartment.

### Anticipated impact of the research

The primary goals of such studies are lymphoid-based therapies, the maintenance of a healthy immune system in the elderly, and the engineering of personalized treatment. The tools developed should enable clinicians to reconstitute the lymphopoiesis of patients carrying invading

pathogens and neutralize autoimmunity and response to tumor cell growth. Additional knowledge about normal lymphopoiesis will undoubtedly benefit new drug discovery for leukemia/lymphoma/myeloma therapies.

### 1.5. Hematopoietic stem cells

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#### Introduction

Hematopoietic stem cells (HSCs) were the first adult stem cells to find their way into the clinic. Indeed, each year more than 50,000 patients receive HSCs for various (benign and malignant) diseases. 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 further expand their use, or that of their more mature cellular derivatives, in the clinic. In this subsection, we will briefly discuss research topics that will need to be addressed to further expand and maximize the impact of the use of HSCs (and/or their progenies) in the clinic.

#### Proposed research for the Roadmap and anticipated impact of the research

*HSC heterogeneity and clonal diversity:* it is still not known how many stem cells contribute to blood cell development in normal individuals, nor whether all contributing HSCs in fact contribute equally.<sup>35</sup> Multiple hypotheses have been put forward over the past decades, yet we do not know how many HSCs there are in the human body, how many of these cells contribute to blood cell production, how long individual stem cells remain active, whether active stem cells can become dormant and whether this process is reversible, and to what extent active stem cells differ individually in their contribution to the various blood cell types.

*HSC aging and rejuvenation:* most patients who develop a hematologic disease (including anemia, immune senescence, lymphoma, myelodysplasia, and chronic and acute leukemias) are older, typically 65 years and over. It has become apparent that aged HSCs suffer from multiple functional defects: they show reduced self-renewal, overall produce fewer mature cells per stem cell, and are impaired in terms of generating lymphocytes. The molecular causes of these defects have not been defined, but may involve DNA damage, telomere attrition, erosion of epigenetic marks, replication stress, or loss of cell polarity, or, indeed, may result from microenvironmental perturbations.<sup>36</sup> Elucidation of the molecular cause(s) of stem cell aging will be required to assess whether, and how, it may be possible to reverse it. Reversion or prevention of stem cell aging will contribute to delaying age-related hematopoietic deficiencies.

Generation of HSCs from non-stem cells: in the post-Yamanaka era, many labs are attempting to generate *bona fide* HSCs from induced pluripotent stem cells (iPSCs), or indeed to embark on direct conversion of non-HSCs to

transplantable stem cells. Whereas these attempts initially proved to be very cumbersome, substantial progress has recently been made in this field. It has been proved possible, using an array of transcription factors (TFs), to induce hematopoietic (stem) cell activity, while the molecular mechanisms are still unknown.<sup>37</sup> The generation of functional HSCs from non-stem cells will greatly expand the clinical use of stem cells and their differentiated progenies.

*HSC expansion, transplantation, and homing:* HSCs are very rare cells, and many attempts have been made to amplify them in order to improve engraftment kinetics after transplant and to allow manipulation of these cells prior to transplantation. In the light of very significant clinical progress in the field of (hematopoietic) gene therapy, the ability to maintain, grow, and expand HSCs during gene transduction protocols becomes more important than ever. Classical protocols that used cytokines have not been successful in expanding stem cells. More recently, however, small molecule-based approaches have suggested that the massive expansion that occurs *in vivo* when few stem cells are transplanted to conditioned recipients can be recapitulated *in vitro*. Efforts to explore such expansion protocols are highly warranted and should include studies aimed at increasing the homing efficiency of transplanted stem cells to their proper niche in the bone marrow. Novel imaging tools have been developed to record, in real time, the lodging of transplanted cells to specific preferred sites.<sup>38</sup> Such tools and the insight they provide are essential for developing methods to improve homing and to identify the microenvironmental cells to which normal and leukemic cells home in transplantation settings. HSCT will benefit from *in vitro* manipulation of the graft to expand more desired cells and decrease the number of less desired cells in certain conditions (i.e. facilitate the development of “designer grafts”).

*HSC transformation, cell of origin, and pre-leukemia:* 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. It seems plausible that the epigenetic context in which a (pre)leukemic lesion first arises plays an important role in establishment and progression of the disease. Pre-leukemic lesions have been identified that appear to confer a proliferative advantage to not yet transformed primitive cells, the progenies of which then continue to accumulate additional mutations.<sup>39</sup> Cataloging these pre-leukemic events and identification of the primitive cells in which they first arise will be crucial in order to embark on approaches that allow very early detection of aberrant hematopoiesis. Understanding the value of these pre-leukemic events in the predisposition of developing a full-blown leukemia will allow us to screen mutations in elderly patients’ blood and decrease the risk of leukemia development. In addition, despite our knowledge of the mutation landscape present in leukemic stem cells (LSCs), the importance of the order in which a mutation or mutations occurred might provide us with a better understanding of the co-operative effect of different mutations. Furthermore, understanding the biology of stem cells will provide insight into how leukemic (stem) cells hijack specific biological processes exploited by stem cells to remain undifferentiated. Consequently, this insight should result in better treatment strategies.

## 1.6. Developmental aspects of hematopoiesis

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### Introduction

Throughout adult life, the hematopoietic system is a highly dynamic, self-renewing hierarchy of cells founded by robust hematopoietic stem cells (HSCs) that produce billions of mature blood cells daily. How these self-renewing HSCs in the bone marrow are first generated during embryonic life is only beginning to be understood. Already, the clinical use of umbilical cord stem cells suggests that ontogenetically early cells have a therapeutic advantage. Research into the mechanisms of hematopoietic fate determination, expansion, and homing during embryogenesis and in successive ontogenetic microenvironments will be instrumental to the production and amplification of HSCs from pluripotent stem cells (PSCs), two current issues in regenerative medicine, and this knowledge will have relevance in understanding/treating hematologic disease and childhood leukemias. Europe has been, and continues to be, the major leader in the field of developmental hematopoiesis.

Among the key challenges are:

1. HSC generation: currently limited to the embryo and occurs through the transdifferentiation of endothelial cells;
2. extrinsic microenvironmental and cell-intrinsic factors that govern the HSC generative program;
3. by understanding and harnessing the program of hematopoietic cell ontogeny, it may be possible to reprogram somatic cells and produce HSCs for regenerative therapies and leukemic treatments.

### European research contributions

European developmental biologists spearheaded the search for the embryonic origins of the adult hematopoietic system.<sup>40,41</sup> As demonstrated in chick and frog embryos, the adult blood system originates in an intraembryonic site encompassing the dorsal aorta, whereas the yolk sac contributes to transient embryonic hematopoiesis. Extensive analyses in these models and in the mouse and human intraembryonic aorta-gonad-mesonephros region clearly demonstrate that the first adult-type HSCs are generated in that region in a process called endothelial-to-hematopoietic transition. This surprising natural cell transdifferentiation event was revealed by real-time imaging of the developing aorta in mouse and zebrafish embryos. The late embryonic development of these potent HSCs has raised the question of why there are many earlier, less potent hematopoietic progenitors in the embryonic hematopoietic tissues, such as the yolk sac, placenta, and fetal liver.<sup>40,41</sup> Early hematopoietic cells (primitive erythrocytes, macrophages, etc.) support the growth of the embryo, may influence the generation of HSCs, and prepare the developing hematopoietic microenvironments to receive the cells of the adult

hematopoietic system. The unique complexity of HSCs arises through several maturational steps orchestrated by molecular regulators. Mouse deficiencies for hematopoietic transcription factors (TFs) (many of them involved in leukemic chromosomal translocations) and *in vitro* hematopoietic differentiation of embryonic stem cells (ESCs) have facilitated the identification of pivotal regulators.

#### Proposed research for the Roadmap

The key factors initiating HSC generation and the adult hematopoietic stem cell program will be found by comparing gene expression profiles obtained from embryonic endothelial/HSC precursor cells and the first HSCs generated in the aorta.<sup>42</sup> Computational methods and the comparative analysis of the transcriptomes of HSC subsets across different vertebrate models will also further define the molecular signature of HSCs through the identification of TF complexes and epigenetic regulators that each play a role in modulating the hematopoietic program during ontogeny. Mouse models and systems biology approaches are beginning to provide insight into the molecular differences between embryonic, fetal, and adult hematopoietic cells and their specific developmental microenvironments.<sup>43</sup> Considerable advances have been made regarding the cellular complexity of the bone marrow HSC niche, but very little is known about specific cellular components and the molecular signatures of the cells within the HSC supportive niches of the developing embryo.

Examination and comparison of the gene regulatory networks active in the aorta-gonad-mesonephros, placenta, and fetal liver hematopoietic supportive microenvironments will be instrumental for identifying the molecular pathways involved in specific processes, such as the emergence, amplification, and differentiation of hematopoietic progenitors and stem cells. This knowledge can then be interpreted in the context of childhood leukemias that resolve at later developmental stages. For example, Down syndrome trisomy 21 impacts fetal, neonatal, and childhood hematopoiesis, and expands HSCs and megakaryocyte-erythroid progenitors.<sup>44</sup> Acquired *GATA1* mutations in these cells lead to abnormal myelopoiesis. These initiating events are occurring *in utero* during embryonic and fetal stages when hematopoietic progenitors represent a major part of the hematopoietic system. The transient nature of these progenitors prevents their lifelong persistence and acquisition of additional mutations leading to leukemia. Understanding the cellular targets of particular leukemias during the different developmental stages and in the different microenvironmental compartments presents an important challenge for ongoing and future research. Induced pluripotent stem cells (iPSCs) from patients and animal models in which leukemia can be induced during development are among the options for such studies.

The *ex vivo* expansion of HSCs for clinical transplantation has continued to be a major challenge in the field despite many years of research. Our knowledge of the TFs and other regulators that play a role in endothelial-to-hematopoietic transition, together with the molecular programs of HSCs and their surrounding microenvironments, hold promise for unlimited production of such cells for therapeutic purposes. These data will also have an impact

on how to generate HSCs from PSCs. Yamanaka-style reprogramming may allow the *de novo* production of HSCs *via* gene transduction/factor stimulation of endothelial cells or other somatic cell types. In Europe, some success is currently being seen in the production of blood and platelet production from iPSCs.

#### Anticipated impact of the research

Taken together, by understanding all the hematopoietic cell types, progenitors, and stem cells produced in embryonic, fetal, and neonatal stages, we will begin to establish how the hematopoietic microenvironment is shaped, what mechanisms co-operate in regulating the emergence and amplification of HSCs, how this relates to changes in HSC heterogeneity during ontogeny, and how leukemia is initiated at developmentally early stages. These findings are sure to have an impact on treatment regimens, especially during postnatal periods. Moreover, once we are able to directly establish HSCs by reprogramming somatic cells from the patient, graft rejection issues will become a thing of the past and will allow all patients to receive transplants in cases of hematopoietic malignancy and failure.

#### 1.7. Mesenchymal and other stromal cells

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#### Introduction

Hemopoiesis is critically regulated by non-hematopoietic cells that are capable of controlling the production of blood and immune cells according to the demands of the organism. These stromal cells make up 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 will allow this complexity to be addressed experimentally. This will be critical in fulfilling the enormous potential of stromal cells in immune modulation, tissue regeneration, and cancer treatment.

#### European research contributions

Soon after Till and McCulloch demonstrated the existence of hematopoietic stem cells (HSCs), European researchers contributed to characterizing the hematopoietic microenvironment and extrapolating this knowledge to the clinical and technological arenas. Simultaneously with the discovery of mesenchymal stem cells (MSCs) in the bone marrow, Owen and Friedenstien dissected skeletal turnover at the cellular level and demonstrated the capacity of osteoprogenitor cells to give rise to both skeletal-forming and hematopoietic-supporting stroma. Dexter

devised protocols to maintain blood cell production in long-term culture and Schofield hypothesized that a specific niche in the bone marrow is essential to maintaining HSCs. The stem cell niche concept was later extrapolated to other organs. Remarkably, at variance from niches made of fully differentiated cells (e.g. *Drosophila* ovariole and mammalian intestine), some of the cells belonging to the niche are the immediate progenies of the MSCs, or the MSCs themselves.

#### *Proposed research for the Roadmap*

*Unraveling the physiology of mesenchymal-hematopoietic networks:* HSCs and their microenvironment probably represent the best-characterized hierarchical stem cell system in vertebrates, paving the path to understanding how other organs function. Dissecting the regulation of HSCs and their progenies by their microenvironment, and *vice versa*, will be key to optimizing the use of HSCs and therapeutically applying the emerging role of the HSC microenvironment in hematologic disorders. Ongoing research by European groups has identified MSCs *in vivo* and characterized their functions in the HSC niche. Detailed characterization of the properties of MSCs will rely on the development of markers to isolate relevant subsets of human MSCs and understand their HSC-supporting and regulatory properties at the anatomical and functional levels.<sup>48</sup>

*Understanding the role of mesenchymal elements in human disease:* future work will uncover the complexity of HSC-stroma reciprocal regulation in normal and pathological settings. Recent intravital microscopy studies showed altered physical interaction between HSCs and stroma during infection.<sup>45</sup> Mesenchymal elements have recently been experimentally implicated in the initiation, progression, and drug resistance of a variety of hematopoietic neoplasms. Osteoblastic cells are altered in chronic myeloid leukemia (CML) and effectively support leukemic stem cells (LSC) maintenance while hampering normal hematopoiesis. Parathyroid hormone stimulation of osteoblastic cells attenuates CML progression, but enhances acute myeloid leukemia (AML).<sup>46</sup> Another type of myeloproliferative neoplasms (MPN), polycythemia vera (PV), reduces nestin+ MSCs, and their rescue is associated with disease blockade.<sup>47</sup> In parallel to a concept in which (pre)malignant hematopoietic elements alter their niche to promote disease progression, emerging research is showing that primary (genetic) abnormalities in mesenchymal cells can initiate malignant transformation in hematopoietic cells.<sup>48</sup> Finally, stromal cells might promote leukemic cell survival and/or protect them from chemotherapy. A better understanding of the molecular mechanisms driving these pivotal contributions of mesenchymal cells to disease pathogenesis opens the way to not only more effective treatments, but also to novel strategies to prevent malignant evolution and the associated socio-economic burden.

*Mesenchymal cells in regeneration, immune modulation and systemic disease:* bone marrow stromal cells do not only regulate HSCs, they are also instrumental in the (re)generation of other bone marrow cell types, including bone cells. Characterizing the mechanisms by which stromal cells maintain and regulate hematopoiesis and osteogenesis will be essential for optimizing recovery following injury or bone marrow transplantation (BMT), as well as

increasing the number of patients that can benefit from cell therapies. In addition, stromal cells also have a profound impact on different immune cells, having therapeutic benefits in sepsis, autoimmune disorders, and graft-versus-host disease (GvHD). More mature stromal cells, or cells isolated from other sources (e.g. adipose tissues), also seem to display some of these immunomodulatory properties. Bone marrow stromal cells are also responsive to factors mediating diabetes, obesity, and aging, which are likely to increase in Europe in the next ten years. Understanding how the hematopoietic niches respond to these physical conditions and how these changes affect the blood cell system will be essential. Research in this area holds promise for correcting some of the debilitating effects associated with these conditions.

#### *Anticipated impact of the research*

An increase research in the hematopoietic microenvironment will, scientifically, feed into other (hierarchical) stem cell systems and, clinically, provide new ways to modulate and treat hematopoietic diseases, immune responses, and regenerative processes. Both pharmacological modulation and cellular therapy are expected to emanate from further efforts. As a result of the productive research, the European Union is already the world region with the second highest number of registered clinical trials using mesenchymal stem/stromal cells.

### **1.8. Transcriptional/epigenetic networks**

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#### *Introduction*

Maintaining a balanced output of mature hematopoietic cell lineages is critically dependent on exquisite control of cell fate choices at the stem and progenitor level within the hematopoietic hierarchy.<sup>49-53</sup> These cell fate choices are executed through the interplay of extracellular signaling pathways with the intracellular decision-making machinery. The latter is driven by networks of transcriptional 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 programs and also mediate developmental transitions when cells differentiate down a particular hematopoietic lineage.

Within these transcriptional regulatory networks, transcription factor (TF) proteins bind to specific DNA sequences, and therefore represent the primary stage of decoding regulatory information present in lineage-specific gene regulatory elements. Once bound, TF proteins recruit a number of accessory proteins with enzymatic activity, which cause either modifications to the DNA (e.g. DNA methylation) or covalent modification of histone proteins (e.g. histone methylation, acetylation, phosphorylation). These so-called epigenetic modifications in turn influence the accessibility of the DNA template for subsequent binding of further TFs, and therefore serve a critical function in the establishment of stable transcriptional programs. The epigenetic status of the chromatin

template can also influence additional aspects of transcriptional control, such as the assembly of RNA polymerase complexes on the promoter, and also the rate of transcriptional elongation. In addition, these TF-regulated networks are related to higher-order structure organization and nuclear localization of DNA elements.

#### *European research contributions*

European researchers have contributed significantly to the identification and characterization of TFs involved in normal and aberrant hematopoiesis. In addition, they have extended many of these analyses toward genome-wide mapping of normal as well as mutated TFs in primary hematopoietic cells from healthy and diseased individuals, thereby revealing multiple unanticipated functions of these proteins.

European researchers have been working 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 (e.g. Epigenome NoE, HEROIC, EPITRON, and Epigenesis). Recently, these interests specifically focused on the hematopoietic lineage with the support of BLUEPRINT, a project that set out to generate epigenomic data of more than 100 blood cell types from healthy individuals and patients suffering from blood diseases. This project has already provided 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 only be accomplished through close collaboration between experimentalists, computational biologists, statisticians, and clinicians.

#### *Proposed research for the Roadmap*

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 post-transcriptional regulation of TFs, and on how these regulate the local and global epigenetic environment, should be intensified.

Although the main TFs involved in disease development have been identified, many components that drive the functional fine-tuning of the blood cell types 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 starting point to utilize cells of the hematopoietic system in disease prevention and control.

Apart from creating additional comprehensive data sets

on 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 cell niche, environmental factors, TF binding, epigenetic alterations, and the presence of various hematologic disorders.

#### *Anticipated impact of the research*

Intensifying the research on normal blood development provides an opportunity to better understand the intrinsic molecular systems that regulate normal hematopoiesis and provide knowledge of how these mechanisms can be shaped and regulated to the benefit of the individual. It will provide a framework to which similar analysis on hematologic diseases can be compared, allowing the identification of the primary mechanisms that are disrupted and providing starting points for the development of targeted interventions. Finally, the depth to which these networks can be studied *in vitro* and *in vivo* will be of great importance to deciphering transcriptional and epigenetic networks, not only in blood cell formation, but in multiple other organ systems as well.

### **1.9. Reprogramming/induced pluripotent stem cells/embryonic stem cells**

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#### *Introduction*

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), represent a limitless source of cells for investigations ranging from developmental processes to drug discovery. Dissecting the molecular and cellular mechanisms underlying the *in vitro* differentiation of PSCs to blood progenitors has been instrumental in furthering our knowledge of the early steps of hematopoietic development. The use of human ESCs has proved particularly useful given the difficult and restricted access to human embryos. Furthermore, dissecting the *in vitro* differentiation of iPSCs derived from cells of patients with hematologic diseases is starting to provide invaluable insights into these pathological conditions. But one of the greatest promises of the stem cell field remains the *in vitro* derivation of cell populations that can be used in the clinic for therapeutic purposes. PSC-derived cells could be used to regenerate a damaged hematopoietic system or to modulate the repair of other tissues (e.g. macrophage in fibrosis) or the immune response [e.g. chimeric antigen receptor (CAR)-expressing T cells]. Finally, the fast-expanding field of direct reprogramming in which one somatic lineage is directly converted into another clinically useful cell type is gaining momentum and may complement the derivation of cell populations from PSCs.

#### *European research contributions*

In the past two decades, European researchers have contributed significantly to the field of embryonic hematopoiesis, using PSCs as a model system to decipher

the early steps of mesoderm specification and blood commitment. These efforts have led to the identification of the hemogenic endothelium as a cornerstone in the *in vitro* generation of blood progenitors.<sup>54,55</sup> Studies by several European laboratories have provided insights into the role of key transcription factors (TFs) (e.g. RUNX1, ETV2, TAL1, HOXB4, and HOXA9) and Notch signaling (e.g. DLL4 and CDCA7) in promoting hematopoiesis. Innovative studies exploring the derivation of *in vivo* engrafting blood progenitors from ESCs<sup>56</sup> and investigations on the instructive role of leukemogenic fusion genes (e.g. *MLL-AF4*) in human ESC-derived blood cells have also been undertaken in European laboratories. Furthermore, the use of disease-specific iPSCs to unravel inherited hematologic disorders, such as Fanconi anemia (FA), chronic granulomatous disease, and pyruvate kinase deficiency, provided unique clues to understanding the mechanisms underlying these diseases.

On the translational front, European teams have made seminal progress toward establishing protocols for the *in vitro* generation of platelets, macrophages, dendritic cells, and red blood cells (RBCs) and their manufacturing for use in the clinic.<sup>57</sup> Alternative methods for the derivation of hematopoietic populations are now starting to emerge with direct reprogramming as a forerunner in this fast expanding area of research. Although still in its infancy, direct reprogramming explores the instructive conversion of one somatic lineage into another mediated by the ectopic expression of TFs. Pioneering work was performed in Europe to decipher the reprogramming of committed immune cells into macrophages mediated by CEBPA.<sup>58</sup> More recently, the reprogramming of fibroblasts to hematopoietic progenitors was achieved upon ectopic expression of specific TFs.

#### Proposed research for the Roadmap

Fundamental research focusing on developmental processes of the hematopoietic specification of PSCs needs to be maintained, because this area of investigation holds the key to establishing robust and efficient protocols for the production of clinically applicable blood and immune cells. These protocols would benefit from the reproducible derivation of long-term repopulating hematopoietic stem cells (HSCs) with adult characteristics. Deriving therapeutic cells from PSCs by modulating the differentiating conditions is a priority, but other approaches should be followed in parallel. One of the new avenues to be pursued is the direct reprogramming of human non-hematopoietic cells to blood cells/progenitors for therapeutic purposes. There is still much to be understood about the fundamental aspects of somatic cell reprogramming, and funding opportunities should be provided for European groups to intensify their efforts in this groundbreaking field. Both basic and translational areas need to be explored in order to remain competitive at an international level. Another important area that European research should focus on is further developing the use of iPSCs as a model system for hematologic disease in both fundamental and applied studies. This approach represents a unique opportunity to: 1) determine the contribution of the epigenome to disease initiation and maintenance; and 2) understand the developmental impact of leukemia-specific mutations and chromosomal translocations in blood specification and cell fate decisions.

#### Anticipated impact of the research

Ultimately, harnessing the power of PSCs for regenerative medicine and the production of blood derivatives will profoundly benefit patients, families, and European society as a whole. To reach that goal, however, we will need to gain a deeper understanding of the differentiation processes leading to the generation of the desired hematopoietic subsets. We will also need to implement robust protocols for the large-scale production of desired blood products. iPSCs as model systems to understand mechanisms of disease emergence and maintenance will be instrumental in identifying and developing novel treatment options for the relevant disorders.

## The EHA Roadmap for European Hematology Research

### Section 2. Malignant lymphoid diseases

#### Section editors: Bertrand Coiffier, Gilles Salles.

Malignant lymphoid diseases represent the most frequent hematologic malignancies, with an age-adjusted estimated incidence of 24.5 per 100,000 inhabitants in Europe,<sup>59</sup> and are associated with significant mortality<sup>60</sup> and morbidity. This disease group is highly heterogeneous in terms of frequency, epidemiology, biology, genetic abnormalities, and outcome. While in a way all individual lymphoma subtypes could be seen as rare diseases, some of them are relatively common, e.g. multiple myeloma, chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphomas (DLBCLs), follicular lymphomas (FLs), and Hodgkin lymphomas (HLs). Others are less common e.g. mantle cell lymphoma (MCL), acute lymphoblastic leukemia (ALL), T-cell lymphoma, and mucosa-associated lymphoid tissue (MALT) lymphoma, or even very rare, e.g. some subsets of marginal zone lymphomas (MZLs) and HIV-associated lymphoma. After the progress made in the morphological classification of these tumors in the 1990s, the advent of large-scale genomic approaches enabled identification of multiple molecular subsets, which may further subdivide the different entities in multiple rare diseases.<sup>61-63</sup> These achievements justify the need for European-based epidemiological studies and contributions to the InterLymph consortium<sup>64</sup> to investigate the role of environmental and lifestyle factors, which, in the context of inherited genetic background, may favor the development of these malignancies.

Significant progress was also made in unraveling key biological features of these diseases, including: 1) the more precise delineation of intrinsic genetic defects in tumor cells, delineation still ongoing with NGS approaches;<sup>63,65,66</sup> 2) the growing understanding of the complex interplays between malignant cells and their microenvironment, which is especially critical in these diseases arising in lymphoid organs;<sup>67</sup> and 3) the emerging identification of constitutional genetic traits associated with an increased susceptibility to develop these malignancies.<sup>68,69</sup> Finally, several recent developments have pointed toward the existence of “lymphoid cancer stem cells,” which may represent highly desirable targets to achieve a definitive cure in these malignancies.<sup>70-72</sup> Although several European groups have already made outstanding contributions to this field, in part within large international consortia, further

achievements will only be possible if major investments can be realized. These should particularly focus on establishing new cellular and animal models (critically rare in the field of mature lymphoid malignancies) to better understand how these diseases develop and for pre-clinical assessment of new therapeutic agents.

Despite important advances in the past few years,<sup>73</sup> the survival of patients with lymphoid malignancies remains unsatisfactory. This is true for the most aggressive malignancies (e.g. ALLs, T-cell lymphomas, and some forms of DLBCL), which still are frequently fatal. In addition, the lack of cure in patients with multiple myeloma or indolent lymphoma is equally challenging. Furthermore, short- or long-term morbidities such as infertility, secondary malignancies, as well as cardiac, pulmonary, renal, or neurological dysfunction are associated with intensive treatment in HL or DLBCL. Chronic exposure to therapeutic agents such as in indolent lymphoma and CLL also represents a health burden for patients, as well as an increasingly relevant economic burden for the European Union.<sup>2,74</sup> Attention to malignancies occurring in elderly patients should also be considered in this regard given the fact that life expectancies continue to grow.

European co-operative groups have been leading clinical research in lymphoid malignancies in the past decades. Progress is being made in investigating the role of targeted agents in well-characterized molecular subsets. The number of new therapeutic agents under development in this field demands further academic research collaboration. For example, analyzing the medico-economic impacts of patient management should clarify costs and benefits of new therapeutic strategies, including those related to public health economics. These groups also need further support in their translational research activities, especially in their efforts to constitute and analyze large biobanks with high-quality clinical annotations. Efforts should also aim to eliminate the differences in outcome observed in different parts of Europe and to improve patients' survival and quality of life.

### 2.1. Hodgkin lymphoma

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#### Introduction

Classical Hodgkin lymphoma (HL) is a highly curable disease and is considered one of the most successful stories in hematology. For both localized and advanced-stage disease, more than 90% of patients are alive five years after diagnosis, and the progression-free survival (PFS) is 85%-93% for localized disease and 70%-89% for patients with advanced disease. After a first relapse, the disease remains curable in nearly half of the cases when high-dose chemotherapy with autologous stem cell transplantation (ASCT) is feasible. As HL is one of the most common

malignancies in young adults, most patients will have a very long survival. During their follow up, however, a significant proportion of patients experience serious long-term toxicities, such as second malignancies, cardiovascular diseases, and infertility. Most of these late toxicities have been related to the treatments for HL. To reduce long-term, treatment-related toxicities, optimization of the balance between the risks and benefits of the different treatment strategies is still the subject of controversy and the main goal of most clinical trials.

#### European research contributions

Two European risk models (proposed by the German Hodgkin Study Group and the European Organisation for Research and Treatment of Cancer/the Lymphoma Study Association) are commonly used and were established to separate HL into three different risk categories: early favorable, early unfavorable or intermediate, and advanced disease. Using these prognostic categories, European lymphoma groups have been major contributors in clinical trials that established ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) as a reference chemotherapy for HL. Alteration of ABVD by omitting any drug including bleomycin was also recently reported as inferior in terms of disease control.<sup>75</sup> However, as approximately 30% of patients with advanced disease relapse after ABVD, a more intensified chemotherapy, BEACOPPesc (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) was developed for these patients. It showed a better progression-free survival (PFS), although it is also associated with more toxicity.<sup>76</sup> Since the late 1980s, children and adolescents with HL have been treated with chemotherapy designed to limit cumulative doses of anthracycline, alkylating agents, and bleomycin to limit long-term toxicity. Recently, OEPA (vincristine, etoposide, prednisone, and doxorubicin) associated with COPDAC (cyclophosphamide, vincristine, prednisone, and dacarbazine) was commonly used in European studies and demonstrated global efficacy comparable to treatment with regimens containing procarbazine with, therefore, an expected lower rate of impaired fertility in both men and women.

Radiotherapy is a major contributor to the late toxicities, such as secondary cancers and cardiovascular diseases. European trials provided an important contribution to the reduction of radiotherapy for HL treatment. Indeed, several trials have shown that when given in combination with chemotherapy, the radiotherapy fields can be reduced from extended to involved. In recent years, even more restricted radiotherapy modalities, such as involved node and involved site radiotherapy, have also been proposed and evaluated in randomized clinical trials. Moreover, these trials have also demonstrated that the dose of radiotherapy can be safely reduced without compromising treatment efficacy. The dose of radiotherapy in the more favorable group of localized patients has been reduced to 20 Gy. In the vast majority of cases, radiotherapy in children and adolescents is delivered at 20 Gy and restricted to the involved site in order to limit growth abnormalities, and long-term vascular and heart toxicity. More recently, European trials in pediatric, adolescent, and adult patients have also tested the possibility of omitting radiotherapy in select patients. As it has been shown that patients with a fluorodeoxyglucose positron-emission tomography (PET)

negativity after two cycles of ABVD have an excellent outcome, it was suggested that those PET-negative patients could receive less intense therapy without any radiotherapy.<sup>77</sup> This PET-driven treatment adaptation is still restricted to clinical trials and whether this select population of patients can be safely treated without radiotherapy remains controversial and a matter of debate. Importantly, HL trials led by several European co-operative groups allowed nuclear medicine physicians to establish criteria for a good and reproducible interpretation of PET-CT scans in lymphoma patients. This 5-point scale, referred as the Deauville scale, is now commonly adopted by the international community to properly evaluate interim and end-of-treatment PET-CT scan results in the field of lymphoma.<sup>78</sup>

Apart from these clinical achievements, various European groups have a long history of research on HL pathogenesis, beginning with the identification of HL as a B-cell-derived malignancy. Most of the currently known molecular defects considered to be key alterations of HL were identified by European groups, including the deregulated NF- $\kappa$ B activity and genomic defects of components of the NF- $\kappa$ B and JAK/STAT signaling pathway.

#### *Proposed research for the Roadmap*

Research should be pursued into the etiology and epidemiology of HL with attention to genetic, immune-based and infectious (e.g. Epstein-Barr virus) determinants. Most patients with HL will ultimately be cured from their disease and experience long-term survival. We are now in a situation of trying to better identify patients who will be more readily cured from those who will need more intensified therapy (e.g. radiotherapy for localized patients and BEACOPPesc for advanced patients). This will allow us to meet the urgent need of avoiding unnecessary toxicities for the vast majority of patients who can be cured with less aggressive treatments. Special efforts towards optimizing treatment for elderly patients should also be made.

PET-CT and biomarker-driven strategies are currently being explored with the hope of individualizing treatment decisions. However, pre-treatment prognostic markers (e.g. circulating biomarkers), genomic markers, or molecular markers have to be identified to stratify patients before starting treatment or early thereafter. To develop such tools, coupling international clinical trials with the establishment of a biobank including tumor tissue and blood samples is essential. Moreover, as the pathogenesis of HL is still largely unknown, establishment of a comprehensive picture of the role of the microenvironment, genetic alterations, and molecular pathways driving pathogenesis of the disease is required. This may also lead to the identification of novel targets for treatment that could potentially cause fewer undesired side effects.

Interestingly, for the first time since the 1970s and the introduction of doxorubicin, new drugs are now becoming available in the field of HL and are suggested as treatments with a safe toxicity profile. A CD30 antibody-drug conjugate was recently approved for the treatment of relapses after high-dose chemotherapy with ASCT and for patients failing two previous lines of chemotherapy but ineligible for high-dose chemotherapy. This drug induced durable remissions and favorable long-term survival in patients

with relapsed/refractory HL.<sup>79</sup> More recently, when given as a consolidation therapy after ASCT, this drug was also shown to improve PFS of patients treated with high-dose chemotherapy for a first relapse. Finally, this drug is now being tested for both first- and second-line therapy. More recently, as pre-clinical studies suggested that Hodgkin/Reed-Sternberg cells exploit the programmed death-1 (PD-1) pathway to evade the immune system, PD-1-blocking antibodies were evaluated in ASCT and CD30 antibody-drug conjugate refractory patients and showed substantial therapeutic activity with an acceptable safety profile. The place of these new drugs and possible associations with the currently available treatment modalities need to be further refined, especially with the aim of limiting long-term toxicities.

As late toxicities and complications are a major concern in this situation, continuous and rigorous evaluation of long-term survivors also remains a major topic of interest for clinical research.

#### *Anticipated impact of the research*

As HL is a disease of the third and fourth decade of life, and one of the most common cancers in young adults, cured patients are potential long-term survivors. Reducing their risk of late toxicities, while keeping their high cure rate, is of utmost importance for increasing their individual chances of becoming active members of society in the long term. The recent successful implementation of targeted treatment strategies shows the importance and clinical relevance of unraveling the pathogenesis of HL. Moreover, knowledge about disease relapse is lacking and intensive research is needed into this. This will only be possible within large European clinical trials combined with extensive biobanking and the development of tools that allow analysis of the scarce tumor cell population characteristic of HL. In addition, these clinical trials provide a solid basis to identify, if possible, pre-treatment prognostic biomarkers to select patients who will benefit from the respective treatment regimen. Finally, the definition of the place of already-developed new drugs in our armamentarium and individualized therapy strategies is one of the main goals of the next generation of clinical trials. Special attention should be given to disseminate knowledge and innovation to vulnerable populations in Europe and elsewhere.

## **2.2. Diffuse large B-cell lymphoma and Burkitt lymphoma in adults and children**

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### *Introduction*

Diffuse large B-cell lymphoma (DLBCL) is the most common mature B-cell neoplasm, with an estimated incidence of 3.8 per 100,000. This indicates that approximately 30,000 cases occur in Europe each year, with some variations in geographic distribution.<sup>59</sup> The median age at diagnosis is 60 years. The standard method of diagnosis is



a surgical excision biopsy with morphology and immunohistochemistry study. Gene expression profiling has identified three major subtypes of DLBCL according to the cell of origin of the malignant cells: germinal center B-cell, activated B cell-like, and primary mediastinal B-cell lymphoma. A large majority of DLBCL in adolescents belongs to the germinal center B-cell subtype, and the proportion of the activated B cell-like subtype increases with age. Next generation sequencing (NGS) studies demonstrated very heterogeneous genomic alterations among these subtypes, which could be related to a variable outcome and could indicate putative targets for therapeutic interventions. The combination of the anti-CD20 antibody rituximab with chemotherapy resulted in an important improvement in survival over the past decades.<sup>73,80</sup> A proportion of 50%-90% of patients can be cured by immunochemotherapy depending on age and other clinical prognostic factors gathered in the International Prognostic Index.<sup>81</sup> As salvage treatment is often disappointing, a successful first-line treatment is the key to longer survival.

Burkitt lymphoma (BL) is an aggressive B-cell lymphoma characterized by the presence of a translocation that activates the oncogene *MYC*. The sporadic form found in Europe mostly affects children and young adults with a crude incidence of 0.22 per 100,000, accounting for 80% of B-cell lymphomas in these age groups. It is associated with immunosuppression, especially HIV infection, mostly among older patients. Intensive chemotherapy and supportive care can cure most young patients in high-income countries, but outcome is less favorable for other populations.

*MYC* translocations can also occur in lymphoma with features intermediate between DLBCL and BL. This B-cell lymphoma unclassifiable has a more aggressive behavior and potentially needs a specific therapeutic approach.

#### European research contributions

Large randomized studies conducted by European cooperative study groups have contributed to the establishment of a worldwide standard treatment. Fifteen years ago, the advantages of the combination of rituximab and standard chemotherapy CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) over chemotherapy alone were demonstrated by these study groups in young and older patients.<sup>80</sup> Further studies have proposed optimal combinations varying according to prognostic factors, the exploration of salvage treatment, the evaluation of treatment by functional imaging, and the description of biological characteristics correlated to clinical outcome in patients treated with immunochemotherapy. Efforts in children and adolescents consisted of intensive chemotherapies to increase a high-rate cure, sometimes accompanied with immediate toxicity, but with the aim of reducing long-term toxicity.

European hematologists have contributed to an international collaborative project aiming at defining the molecular definition of lymphoid malignancies. This consortium was at the origin of the identification of molecular subtypes of DLBCL and the distinction between BL and DLBCL with *MYC* translocations.<sup>82</sup> These investigators explored the genomic and transcriptional mechanisms

implicated in lymphomagenesis and identified genetic alterations modifying major cellular pathways, influencing clinical outcome of patients with DLBCL and representing possible therapeutic targets.

#### Proposed research for the Roadmap

An effort to further characterize the genomic, transcriptional, epigenetic, proteomic, and metabolomic landscape of each DLBCL subtype is a common goal in research into hematologic malignancies. Development of new cell lines and animal models representative of these subtypes are certainly needed to improve our understanding of the important biological mechanisms of the lymphoma cell, the interaction with the tumor microenvironment, and to explore the efficacy of new drugs.

The most important challenge in DLBCL is to improve survival of those patients who have refractory disease or who relapse early in the course of the lymphoma. Molecular heterogeneity is, at least in part, responsible for this outcome. If whole-exome sequencing has redefined the genetic landscape of the disease, identification and characterization of genetic alterations in this population requires a large number of samples, important clinical data, and access to extensive sequencing and analysis possibilities. This approach should overcome the inherent complexity of these alterations, the low frequency of some of them, the tumor heterogeneity, and the mechanisms of resistance.

New tools for children and adolescents will be developed to assess risk stratification, early response, and monitoring of the disease in order to tailor chemotherapy and strike a balance between acute toxicity and the risk of long-term toxicity. This will require further international collaboration, along with partnerships with adult lymphoma groups.

Early access to targeted drugs will be conditional on the ability of the European centers to collect the tumor samples and establish a viable network of platforms to exchange data, define common protocols, and share quality-control processes. A large number of new agents targeting driver mutations involved in lymphomagenesis are awaiting clinical application. The selection of patients who are likely to respond to a single agent should be based on the identification of subtypes, the exploration of pathways, or the presence of genetic abnormalities. In this view, targeting *MYC* would be a highly desirable goal in both BL and DLBCL.

Combining targeted drugs with standard first-line immunochemotherapy in order to increase its efficacy and decrease its toxicity will certainly be the major therapeutic path to be explored in the coming years. The next step could then be the advent of chemotherapy-free regimens containing a combination of targeted molecules or a combination of these molecules with monoclonal antibodies or other immune therapies. Access to platforms and development of these novel combinations will be of critical importance in elderly patients who represent the fastest-growing group and the frailest population.

New biomarkers of response and survival need to be explored. Functional imaging has been shown to be a useful tool for evaluating early response and correlating it to clinical outcome. Efforts to develop new markers of spe-

cific pathways or new evaluation modalities could help guide treatment. Circulating DNA could also be a powerful tool to help diagnosis, evaluate response to treatment, and predict relapse.

#### *Anticipated impact of the research*

All of these research directions aim at a better understanding of the biology of DLBCL and BL. Future management of patients with these diseases will have to change from empiric administration of chemotherapy to a combination of precision therapy, thus leading to more personalized treatment approaches. This change will have a major impact on increasing treatment efficacy, decreasing the rate of treatment complications, and ultimately prolonging survival. A close collaboration between patients, academic laboratories, pharmaceutical and biotech companies, and co-operative study groups must work towards ensuring this translation in adults and children. Finally, a reduction of hospital costs and optimization of treatment strategies will allow this policy to be adopted in all parts of Europe.

### **2.3. Mantle cell lymphoma**

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#### *Introduction*

Mantle cell lymphoma (MCL) represents approximately 7% of all non-Hodgkin lymphomas (NHLs) and is genetically characterized by the translocation t(11;14)(q13;q32) and the overexpression of *CCND1*. Most cases have an aggressive course and require intensive treatment. The standard approach is based on immunochemotherapy, which consists of rituximab and CHOP-like and/or high-dose, Ara-C-containing regimens followed by high-dose treatment (HDT) with autologous stem cell transplantation (ASCT). Elderly patients are usually treated with rituximab and CHOP (R-CHOP) or R-bendamustine chemotherapy, and rituximab maintenance is used for responding patients. The MCL International Prognostic Index (MIPI) can identify patients with a high risk, who still have a very poor outcome in spite of intensive treatment. Proliferation index evaluated by Ki-67 has been established as an important prognostic marker. There is growing evidence that patients who achieve minimal residual disease (MRD) negativity have a significantly better outcome than patients who remain MRD-positive.<sup>83</sup> Patients who relapsed have a very poor outcome with median overall survival of approximately 18 months. A small subset of MCL biologically characterized by IGHV hypermutation and *SOX11* negativity has a favorable outcome and usually does not require immediate treatment after diagnosis.

#### *European research contributions*

The effort in Europe has been focused on several different priorities: 1) description of a clinically relevant prognostic index with the use of some biological parameters; 2) development of treatment strategies for the young as well as the elderly population; and 3) response criteria. The German Low Grade Lymphoma Study Group established an MIPI that consists of age, lactate dehydrogenase level,

white blood cell count, and performance status.<sup>84</sup> This index has been accepted worldwide as a tool to discriminate between different outcomes. Evaluation of proliferation index by Ki-67 adds important prognostic information; the most common threshold used is 30% positive cells.<sup>84</sup> High-dose chemotherapy (HDCT) and ASCT is regarded the standard of care for young MCL patients. The addition of rituximab and high-dose Ara-C has been explored by the Nordic Lymphoma Study Group and the Lymphoma Study Association.<sup>85</sup> The European Mantle Cell Lymphoma Network showed that incorporating DHAP (dexamethasone, high-dose Ara-C, and cisplatin) led to a better outcome in these patients. Whether the improvement is due to high-dose Ara-C only or if combination high-dose Ara-C with platinum is important has not been tested; there is, however, some hint that combination (DHAP) can be better than high-dose Ara-C monotherapy.

The issue of total body irradiation (TBI) as part of HDT has not been resolved; it might well be that the incorporation of TBI results in better outcomes. Maintenance treatment after stem cell transplant has been evaluated by the Lymphoma Study Association. Although significant improvements have been achieved, young patients with a high MIPI score still have a poor outcome.<sup>85</sup> However, the majority of MCL patients are ineligible for intensive treatment with HDCT and ASCT. The treatment with R-CHOP or R-bendamustine has been accepted as standard, and the European Mantle Cell Lymphoma Network study demonstrated that rituximab maintenance for two years after R-CHOP significantly prolongs progression-free survival (PFS).<sup>86</sup> Although there is significant improvement in terms of survival, the majority of patients suffer from poor outcomes. This led to collaborative trials with new drugs including temsirolimus, lenalidomide, ibrutinib, bortezomib, and others.<sup>87</sup> It has been clearly demonstrated that outcome depends on response. Several groups have worked to define the impact of MRD negativity. Pooled data from different trials under the umbrella of the European Mantle Cell Lymphoma Network demonstrated that MRD negativity is a more important prognostic factor than classical staging.

#### *Proposed research for the Roadmap*

The general outcome of MCL patients has improved but is still worse than that of other lymphoma subtypes. The growing understanding of MCL biology and improvements in therapeutic strategies have led to a situation in which the MCL patient population is considered more heterogeneous than it was 15 years ago. Heterogeneity regarding MCL epidemiology in different parts of Europe should be investigated. On one side, there is a subgroup of MCL patients with an indolent course; these patients can be followed until treatment is required. There is, however, the need for a better biological description of this subgroup. For the other MCL patients, MIPI or Ki-67 is not yet used for treatment tailoring. All young patients outside clinical trials are treated similarly with HDCT and ASCT as standard management. There is, however, a significant survival difference according to prognostic subgroups and there is a need for individualized treatment. Patients with more aggressive MCL (blastoid variants, high Ki-67, p53 mutations or deletion, mutations in some related genes, such as *NOTCH1* or *NOTCH2*, several chromatin modifiers, such as *WHSK1*, and others) still have a very poor outcome.

On the other hand, a description of patients with good prognosis, who could be treated without HDT, is also needed. Targeted therapy at relapse seems to improve outcome, but the median survival is still only approximately two years. Due to the low incidence of MCL, prospective trials based on international collaboration are warranted. These trials should test new classes of drugs combined with established immunochemotherapy, and translational research should be included. The important issue is to collect biological samples of patients who fail the treatment in order to understand the mechanism of resistance.

Another task is to define how to use the information on MRD. Should it become a standard response criterion? Until now, this information has been used without affecting therapeutic decisions. The question of whether there is room for treatment intensification in MRD-positive patients or treatment reduction in MRD-negative patients should be answered in prospective clinical studies.

It has been accepted that post-induction treatment with rituximab improves outcome in MCL. The question that should be explored is whether targeted treatments, such as ibrutinib, lenalidomide, or others, should be used as a maintenance approach in first-line treatment.

#### *Anticipated impact of the research*

Mantle cell lymphoma is a rare lymphoma subtype with a genetically well-defined primary event and many secondary events. This disease has a very poor prognosis with some recent improvements but results are still far from satisfactory. The European collaborative effort could provide a fresh insight into the impact of different secondary genetic events in MCL and identify subgroups for individualized therapeutic approaches. The important issue is establishing tissue bank samples, not only from the time of diagnosis but also at the time of relapse. This can only be achieved through large international efforts, such as those initiated under the umbrella of the European Mantle Cell Lymphoma Network. Collaborative efforts should aim at further improvements in the outcome of this disease. Although MCL is a rare disease, it represents a paradigm for the exploration of innovative, targeted therapies.

## **2.4. Follicular lymphoma**

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#### *Introduction*

Follicular lymphoma (FL) is the second most common lymphoma, comprising approximately 20% of all non-Hodgkin lymphomas (NHLs), with an incidence in Europe of approximately 2.18 cases per 100,000 people per year. FL typically presents in middle age and in the elderly, and the median age at diagnosis is 60 years. FL arises from germinal center B cells and maintains the gene expression profile of this stage of B-cell differentiation.

Morphologically, the disease is composed of a mixture of centrocytes and centroblasts; an increased percentage of centroblasts is predictive of a poor outcome. A hallmark of the disease is the chromosomal translocation t(14;18), contributing to overexpression of the antiapoptotic protein BCL2. In addition, next generation sequencing (NGS) studies have identified several recurring mutational events targeting genes, highlighting the importance of epigenetic dysregulation in the pathogenesis of the disease and tumor microenvironment modulation through NF- $\kappa$ B and B-cell receptor signaling pathways, as well as defects in DNA repair and apoptosis, challenging the notion that t(14;18) is sufficient for tumor initiation and demonstrating the genetic heterogeneity of the disease.<sup>88</sup> Guidelines for the diagnosis of indolent lymphomas were outlined by the European Society for Medical Oncology. The majority of affected individuals exhibit a characteristic indolent disease course with multiple relapses requiring repeated courses of treatment; others develop aggressive disease and histological transformation with shortened overall survival. The disease remains incurable in most cases.

#### *European research contributions*

In the past decades, European scientists have made major contributions to the understanding of the molecular basis of the disease and the relationship between the tumor cells and their microenvironment. The conduct of large controlled randomized trials within highly organized lymphoma co-operative groups has changed the treatment of FL and improved outcome. In particular, European-led trials have paved the way to demonstrating the benefit of immunochemotherapy over chemotherapy and the advantage of maintenance anti-CD20 monoclonal antibody in first and subsequent remission, and have defined new approaches to optimize first-line treatment.<sup>89</sup>

#### *Proposed research for the Roadmap*

Despite major progress in the management of FL, the biological basis is not fully understood, and there is currently no cure. To find an effective treatment, we need to be able to determine the molecular basis of the disease so that more targeted treatment approaches can be adopted. Because there does not appear to be a single target that can be applied to all cases, a combination of novel biomarkers (based on genomic, proteomic, transcriptomic, and metabolomic analyses of biopsies) will be required. It will be important to identify those molecular events involved in early development of the disease<sup>90,91</sup> and those involved in progression and transformation.<sup>88</sup> The prognosis and clinical course of FL appears to be highly dependent on the tumor microenvironment, and immunohistochemical methods are being assessed to address this.<sup>92</sup> The validation of these techniques will require the integration of longitudinal standardized data, as well as uniform criteria for diagnosis and outcome that can be applied in the clinical setting. Better integration of basic and clinical research will also be crucial. Salient features of that integration should include the following:

1. Where possible, consent should be obtained for the procurement and storage of use of excess tissue from lymph node biopsies and normal tissue at the time of presentation and at each subsequent disease relapse for research purposes in order to investigate the molecular biology of these diseases.

2. A biobank of biopsies linked to the clinical database should be available, with protocols for standardized sampling and storage procedures adapted to genomics and functional assays (including live cells), which would form the basis of correlative and biomarker studies. Of particular importance would be the banking of longitudinal samples from patients at diagnosis and at subsequent relapses and transformation to the nature of relapsing disease.
3. A database (a co-ordinated pan-European registry) that can be accessed by all research partners, containing the biological and clinical information collected for each participating patient, should be made available.
4. Uncovering the molecular mechanisms involved in FL, especially in its early stages<sup>90-92</sup> and the processes involved in transformation, should be a common goal.<sup>88</sup> A key issue would be performing both genetic and microenvironment analyses on longitudinal and/or paired FL biopsies to obtain an integrated view on bidirectional dependency.
5. Robust biomarkers (both prognostic and predictive) should be developed, reflecting the molecular biology of the disease and the impact of the immune microenvironment for disease development and treatment outcome.<sup>92</sup>
6. Novel animal models that recapitulate disease features and allow pre-clinical investigation could also be developed. No good animal models of this disease are currently available, limiting research and drug development.
7. Academic clinical research should also address issues related to the costs and benefits of different therapeutic options and optimal strategies in the elderly population.

#### Anticipated impact of the research

The current lack of understanding of the molecular basis of the disease, the nature of the lymphoma “stem cell”, and the events involved in progression and transformation limit our ability to cure this disease. The research plans above hold the key to understanding the molecular pathogenesis of disease and identifying key targets for optimal therapeutic intervention. The characterization of the genetic, genomic, proteomic, transcriptomic, and metabolomic profile of individual patients and patient cohorts will allow the most appropriate treatment to be selected within clinical trials investigating novel targeted therapeutic agents. This will also allow identification of robust biomarkers for monitoring response to treatment in order to allow a precision therapeutic strategy to be applied to improve the survival and quality of life of patients with FL.

#### 2.5. Marginal zone lymphoma: extranodal, nodal, and splenic forms

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#### Introduction

Marginal zone lymphomas (MZLs) are a diverse group of clinic-pathological entities, comprising extranodal (also called MALT lymphoma), nodal, and splenic forms. The

ontogeny of these lymphomas is in most cases related to autoimmune disorders and chronic infections. Indeed, Sjögren syndrome, systemic lupus erythematosus, rheumatoid arthritis, and Hashimoto thyroiditis, among the former, and hepatitis C virus, *Helicobacter pylori*, and *Chlamydia psittaci*, among the latter, have been linked to MZL development. Persistent (auto)antigenic stimulation by chronic infections or autoimmune disorders leads to lymphoid proliferation, susceptible to malignant transformation; the acquisition of genetic aberrations culminates in the activation of intracellular survival pathways and clonal outgrowth due to proliferation and resistance to apoptosis. However, the interactions between cell-extrinsic (environmental factors) and cell-intrinsic (genetic, molecular, and immunological abnormalities) factors that underlie disease pathogenesis are still not completely understood.

From a clinical standpoint, MZLs are indolent disorders, often manageable with a “watch and wait” strategy, and exhibiting excellent survival when treated with conventional immunochemotherapy or radiotherapy. However, such approaches result in overtreatment of many patients affected by these indolent lymphomas. Accordingly, several investigators are exploring new active and less toxic therapies. In particular, monoclonal antibodies, immunomodulators, antibiotics, and other targeted therapies have been tested, sometimes with promising results. Nevertheless, efficacy rates are still lagging behind those achieved with conventional treatments.

#### European research contributions

In the past 20 years, European researchers have made great progress in basic, translational, and clinical research into MZL. In particular, the establishment of pathogenic links with microorganisms has boosted understanding of the mechanisms underlying MALT lymphomagenesis and advanced therapeutic concepts.<sup>95</sup> A seminal finding was that t(1;14)(p22;q32)/BCL10-IGH, t(14;18)(q32;21)/IGH-MALT1, and t(11;18)(q21;q21)/API2-MALT1, recurrently seen in MALT lymphomas, activate the nuclear factor NF- $\kappa$ B pathway. More recently, high-throughput studies have identified several genetic changes that are useful as biomarkers for disease diagnosis and refined classification (e.g. *BRAF*, *MYD88*, *NOTCH2*, and *KLF2* and *NOTCH2* mutations), and new targets to be translated into therapeutic interventions (e.g. BCR, TLR, Notch, NF- $\kappa$ B, and MAPK signaling pathways), especially in splenic MZL. Splenic MZL was also shown to display a remarkably skewed immunoglobulin gene repertoire because up to one-third of cases express clonotypic B-cell receptor immunoglobulin utilizing the *IGHV1-2\*04* gene, supporting antigen selection in splenic MZL ontogeny.

The precise diagnosis of MZL remains challenging, but real progress has been achieved. The contribution of European researchers through the Splenic Lymphoma Working Group has been important, especially for the establishment of guidelines for the diagnosis, treatment, and monitoring of splenic MZL. Their studies have been instrumental in the characterization of a broad category of variably well-defined provisional entities, involving primarily the spleen, that do not fall into any of the other distinct types of splenic B-cell neoplasms (splenic B-cell lymphoma/leukemia unclassifiable), especially splenic diffuse

red pulp lymphoma and hairy cell leukemia variant, and their precise ontogenetic relationship with splenic MZL. Furthermore, positive diagnostic markers (e.g. MNDA and FCRL4) have been established, and gene expression studies have identified a specific gene expression profile that separates nodal MZL from other lymphoma types.

The Splenic Lymphoma Working Group has also formulated standard criteria to initiate treatment and a simple but effective prognostic score for splenic MZL.<sup>94</sup> Such advancements have complemented the therapeutic progress in MZLs achieved thanks to European trials, often performed within the International Extranodal Lymphoma Study Group. An International Extranodal Lymphoma Study Group trial and a nationwide Spanish trial have established a standard of care with immunochemotherapy for extranodal MZL.<sup>95,96</sup> Moreover, the activities of drugs such as rituximab, everolimus, bortezomib, lenalidomide, and clarithromycin, among others, in MZLs have been addressed in European trials. Importantly, studies of the association between infectious agents and MZL resulted in the development of safe, cost-benefit, and effective therapeutic strategies,<sup>93</sup> as exemplified by the efficacy of antiviral therapy for hepatitis C virus-related MZL.<sup>97</sup>

#### *Proposed research for the Roadmap*

A concerted use of high-throughput platforms available in several European research centers will significantly advance our knowledge of these lymphomas. The main objectives of future studies should be the following.

1. Characterize genetic aberrations and molecular mechanisms involved in the natural history of MZL, map recurrent mutations to molecular pathways, and investigate their correlation with gene expression signature and potential oncogenic co-operation among the altered molecular pathways.
2. Unravel the ontogenetic mechanisms of splenic MZL and other lymphomas of MZ origin, through genome (e.g. whole-exome sequencing), transcriptome (e.g. RNA-seq), epigenome (e.g. DNA methylome), and immunoglobulin repertoire analysis and comparison to normal B-cell subsets from human spleen and lymph nodes.
3. Analyze multi-stage lymphomagenesis and clonal evolution, including transformation, applying deep sequencing to longitudinal samples from different phases of the disease.
4. Define functional immune profiles of disease subgroups with particular clinical and/or biological features.
5. Identify other micro-organisms that could play a pathogenic role and serve as target for more specific therapies.
6. Identify and characterize the antigens and immune pathways that drive lymphoma development, thus paving the way for tailored treatment strategies applicable to each major immunogenetic subgroup.
7. Investigate the precise mechanisms associated with chronic inflammation involved in the development and evolution of nodal MZL arising in patients with autoimmunity.
8. Address the complex interactions between the neoplastic B cells and the surrounding microenvironment,

including both cellular and humoral components.

9. Develop prospective clinical trials on risk-adapted treatments that result in improved efficacy and reduced toxicity.

#### *Anticipated impact of the research*

Advances in unraveling the molecular abnormalities and mechanisms of antigenic triggering combined with progress in genetic profiling will likely result in the identification of subjects with an increased risk of MZL and, consequently, the potential to implement prevention strategies. Knowledge of involved antigens, pathogenic mechanisms, altered molecular pathways, and the crosstalk between tumor cells and the microenvironment will promote personalized therapies, thus maximizing benefits while minimizing unnecessary toxicities and costs.

Given the recent refinement of some entities and their relative rarity, pan-European co-operation is a prerequisite for real progress. This is especially important given the emerging trend of designing clinical trials for highly select subgroups of MZL patients, which is a challenge considering the rarity of these lymphomas. Patient selection will always be based on clinical criteria, but biological and molecular parameters will be progressively incorporated as selection criteria in important trials. This is necessary for testing new compounds, as well as for guiding patient choice among the armamentarium of personalized therapies.

## **2.6. T-cell and NK-cell lymphoma**

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### *Introduction*

T-cell and natural killer (NK)-cell lymphomas are rather heterogeneous and uncommon malignancies. They represent less than 15% of all non-Hodgkin lymphomas (NHLs) worldwide but their epidemiology shows important geographic variations, partly overlapping with the prevalence of certain viral infections [e.g. Epstein-Barr virus (EBV) and human T-cell leukemia virus type 1] and linked to the heterogeneous distribution of genetic backgrounds. The current WHO classification recognizes more than 20 entities grouped according to their predominant nodal, extranodal, cutaneous, or leukemic presentation; some of them indolent, but most of them aggressive or very aggressive.<sup>98</sup> With some exceptions, such as ALK-positive anaplastic large cell lymphoma, survival is usually short. The long-term overall survival for all entities is less than 30%. Unfortunately, there is no real standard treatment for most T-cell/NK-cell lymphoma, except for NK/T-cell nasal type lymphoma where the role of L-asparaginase (alone or in combination) has been well demonstrated.<sup>99</sup> Prognostic biomarkers are not well characterized for most groups or entities. In addition, most entities lack pre-clinical models. Leading researchers in the field feel that substantial and continuous efforts should be made while appreciating the challenges represented by the rarity of these lymphomas.

### *European research contributions*

In recent years, a better understanding of most entities of the different T-cell and NK-cell proliferations has been established.<sup>100,101</sup> The different identities have become better defined, primarily through pathological classification.

Several European groups have long-lasting research activity and experience in the pathogenesis of T-cell lymphoma. In larger cohorts, genome-wide molecular profiling of available tumor material has provided new insights into the pathobiology of these diseases. This subsequently led to the identification of new markers with diagnostic, prognostic, and/or therapeutic implications. The identification of the follicular helper T-cell subset as the cell of origin of angioimmunoblastic T-cell lymphoma and a proportion of peripheral T-cell lymphoma (PTCL) represented an important step in defining markers with diagnostic and therapeutic implications. European groups described the currently known molecular signatures of most T-cell lymphomas. These findings also contributed to the discovery of several genetic aberrations and deregulated pathways, such as the involvement of mutations in epigenetic modifiers and dysregulation in important signaling pathways, including JAK-/STAT, PDGFRA, and NF- $\kappa$ B. Now there is a good chance that at least some of these pathways may serve as targets for the development of novel therapies.

New drugs such as romidepsin, pralatrexate, and belinostat have shown clinical responses in up to 30%-35% of relapsing patients but the question as to their role in daily patient management remains unanswered. Promising advances include a targeted immunoconjugate against CD30-positive T-cell lymphoma (expressed on anaplastic lymphoma, but also on some others T-cell subtypes) and small molecules against the activity of the ALK kinase (e.g. crizotinib). However, no substantial improvement has been made in defining the best first-line treatment or the role of high-dose chemotherapy and transplant for these patients. Several phase III clinical trials have been launched that evaluate therapeutic options such as transplants in first remission and the addition of new drugs to CHOP, but results are still pending.

#### *Proposed research for the Roadmap*

Insights into the molecular basis of T-cell and NK-cell lymphomas will probably help define future risk stratification, predict treatment response, and provide the basis for novel drug design. The emphasis is put on defining the best combination for first-line patients. They represent the best opportunity for finding curative treatments, as salvage treatments for these lymphomas clearly remain insufficient. Given the modest efficacy of most agents, improving outcome will likely rely on drug combinations with complementary mechanisms of action. Furthermore, we need to better identify patients who will respond to therapies, with correlative studies performed in parallel.

Despite some improvement in recent years, there is a need to characterize the genomic, transcriptional, epigenetic, and metabolomic landscape of each PTCL subtype. Development of new cell lines and animal models representative of these subtypes are also needed to explore the efficacy of new drugs, and to improve our understanding of the important biological mechanisms of the lymphoma cell and the interaction with tumor microenvironment.

The most important challenge in PTCL is to develop alternative therapies to the conventional CHOP or CHOP-like chemotherapy regimens in order to improve survival. Biologically oriented strategies with drugs targeting altered genes, pathways or surface molecules expressed in specific PTCL entities need to be developed. Despite recent whole-

exome sequencing studies, the genetic landscape and potential driver alterations remain poorly characterized. A European effort to collect clinical and biological data of PTCL patients enrolled within clinical trials or registries is critical. It should aim to perform whole-exome and whole-genome sequencing analysis on a large number of clinically well-annotated PTCL samples of each entity to identify driver alterations and novel candidates for therapy.

Early access to targeted drugs will be needed for European groups in order to collect tumor samples and establish a viable network of platforms to exchange data, define common protocols, and share quality-control processes. A number of novel compounds targeting driver mutations (e.g. demethylating agents and IDH2 inhibitors in PTCLs with mutations in epigenetic modifiers) are awaiting clinical application. The selection of patients who are likely to respond to a single agent should be based on the identification of the alterations, the exploration of pathways, or the presence of genetic abnormalities. Evaluation of targeted drug combinations should also be undertaken. Access to platforms and development of these novel combinations will be of critical importance in PTCL patients, a group of patients with a very poor outcome.

In parallel, new response and outcome biomarkers need to be explored. Functional imaging has been shown to be a useful tool for evaluating early response and correlating it to clinical outcome. Within the populations of patients collected in Europe, in view of the rarity of the diseases, efforts to develop new markers of specific pathways or new evaluation modalities could help guide treatment. Circulating DNA could also be a powerful tool to help diagnosis, evaluate response to treatment, and predict relapse.

#### *Anticipated impact of the research*

All of these projects will help describe well-defined entities with their specific genetic modifications and will enable new targets for innovative drugs to be evaluated; these could provide more efficient and personalized treatment approaches for PTCL patients and prolong their survival. PTCLs are certainly the best example of lymphomas for which there is still a need for biologically oriented novel strategies. Randomized studies will help set new standards and enable better entity-specific treatment regimens to be tested. Close collaboration between patients, academic laboratories, pharmaceutical and biotech companies, and European co-operative lymphoma study groups with a long-standing tradition of working together offers the possibility of a rapid translation of biological studies into the clinic. Finally, reduction of hospital costs and optimization of treatment strategies will allow this policy to be adopted in all parts of Europe.

### **2.7. Lymphoma and immune deficiency (including AIDS, post-transplant, and drug-induced immunodeficiency)**

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#### *Introduction*

The incidence of Hodgkin lymphoma (HL), and espe-

cially non-Hodgkin lymphoma (NHL), in patients with immune deficiencies is higher than in the normal population. Although particularly evident in patients with HIV infection, this also occurs in other situations, such as after solid organ and hematopoietic transplants, and in patients receiving immunosuppressive therapies or with autoimmune diseases. Characteristically these lymphomas have high-grade malignancy and are in advanced stage, with frequent extranodal involvement.

Since the introduction of combination antiretroviral therapy (cART), there have been a number of changes in the spectrum of cancer affecting HIV-infected individuals. Although the incidence and proportion of deaths related to non-AIDS-defining malignancies are increasing, lymphoma is still the most frequent neoplastic cause of death among HIV-infected individuals. The incidence of NHL initially fell in the cART era but has now stabilized. On the other hand, the incidence of diffuse large B-cell lymphoma (DLBCL) and primary central nervous system lymphomas has decreased, whereas that of Burkitt's lymphoma (BL) and HL has increased.

Post-transplant lymphoproliferative disorders (PTLDs) include a range of diseases ranging from benign proliferations to malignant lymphomas. Risk factors for developing PTLD include Epstein-Barr virus (EBV) infection, recipient age, transplanted organs, type of immunosuppression, and genetics. Uncontrolled proliferation of EBV-infected B cells is implicated in EBV-positive PTLD, whereas the pathogenesis of EBV-negative PTLD may be similar to that of NHL arising in the general population.

The management of lymphomas in immunosuppressed patients differs according to the cause of the immunosuppression. In HIV-infected patients, the extensive use of cART has allowed these patients to be treated with identical schedules of immunochemotherapy as those used in the general population (together with cART and adequate prophylaxis of opportunistic infections). In PTLD, the first step is the removal of immunosuppressive therapy, followed by anti-CD20 immunotherapy, moving quickly to standard immunochemotherapy schedules if response is not rapidly achieved. Sequential therapy using rituximab followed by chemotherapy has demonstrated promising results and may establish a standard of care. The remaining immunosuppression-associated lymphomas are managed like those arising in the normal population.<sup>102-106</sup>

#### *European research contributions*

Most of the achievements made through collaborative efforts in Europe have been in HIV-related lymphomas. Several national groups from European Union countries have conducted phase II trials showing similar results of the treatment of HIV-related lymphomas in the cART era. The most frequent schedules used for DLBCLs are R-CHOP, R-CDE (rituximab, cyclophosphamide, doxorubicin, and etoposide) and R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin); for BLs the most frequent schedules are R-CODOX-M/IVAC (rituximab, cyclophosphamide, vincristine, doxorubicin, and methotrexate/ifosfamide, etoposide, and cytarabine), LMB, NHL2002, Burkimab, and dose-adjusted R-EPOCH, among others. A general consensus has been reached on the improvement in out-

come with the addition of rituximab to the different chemotherapy schedules in patients not severely immunosuppressed. A new prognostic score for HIV-related lymphomas in the rituximab era (AIDS-Related Lymphoma International Prognostic Index) has been developed, combining patients from Europe and the United States included in phase II or phase III trials of immunochemotherapy. This score includes the age-adjusted International Prognostic Index (IPI), the number of extranodal sites, and the HIV-score (composed of CD4 count, viral load, and prior history of AIDS). Twenty-eight percent of patients were defined as low risk by the AIDS-Related Lymphoma International Prognostic Index and had an estimated 5-year overall survival of 78%, 52% as intermediate risk (5-year overall survival of 60%), and 20% as high risk (5-year overall survival of 50%). Another European-US study has shown that the outcome of patients with AIDS-related lymphomas has improved in the past two decades, and effective HIV-directed therapies have reduced the impact of HIV-related prognostic factors on outcome, allowing curative anti-lymphoma therapy to be used in most patients with aggressive NHL. As far as HL is concerned, the results of chemotherapy schedules used in different European countries, such as BEACOPP (Germany) and VEBEP (Italy), have been comparable to those obtained with the classical ABVD regimen (Spain and other countries). Similarly to NHL, an international effort (Europe, the US, and South America) has been made to define the prognostic factors of HL patients treated with ABVD and cART, showing the negative impact of low CD4 lymphocyte counts on overall and progression-free survival.

A comparative analysis of HIV-related lymphoma and a matched cohort of HIV-negative lymphoma patients from several European countries was conducted by the European Group for Blood and Marrow Transplantation (EBMT). Comparable survival between HIV-positive and HIV-negative NHL and HL patients undergoing autologous peripheral blood stem cell transplantation was observed, leading to the conclusion that, in the cART era, HIV-infected patients with lymphoma should be considered for autologous peripheral blood stem cell transplantation according to the same criteria adopted for HIV-negative lymphoma patients. Finally, a co-operative study from the European Group of AIDS and Tumors analyzed the autologous peripheral blood stem cell mobilization policies used in HIV-associated lymphomas, evaluated the failure rate, and identified factors influencing mobilization results.

In summary, most of the co-operative European studies on lymphomas associated with immunosuppression have been focused on therapeutic strategies and the identification of prognostic factors. Pan-European trials have also established treatment standards for the management of post-transplant B-cell lymphomas, using sequential administration of rituximab followed by rituximab-chemotherapy. However, rare post-transplant lymphoma entities remain challenging.

#### *Proposed research for the Roadmap*

##### *Basic aspects*

1. To improve the knowledge of the mechanisms of lymphomagenesis in immunosuppression-related lymphomas, especially EBV-driven lymphomagenesis and

- the relation with other viruses (e.g. other gammaherpesviruses, HIV, and hepatitis viruses).
- To evaluate the potential value of plasma load of gammaherpesviruses as a surrogate marker of residual disease in lymphomas in immunosuppressed patients.
  - To develop early biological predictors of development of lymphomas in immunosuppressed patients (e.g. EBV viral load and serum-free light chains).
  - To study the dynamics of the T-cell and natural killer (NK)-cell repertoire in immunosuppressed patients and its relation with the development of lymphomas.

#### *Clinical aspects*

The key clinical research goals are as follows.

- To develop pan-European clinical trials with new drugs, especially in the setting of relapsed/refractory patients with HL and NHL, since immunosuppressed patients (especially those who are HIV-infected) are usually excluded from current clinical trials. Given their low frequency, specific trials for these patients are required in a multicenter setting.
- To compare the strategies based on R-CHOP with those based on infusional dose-adjusted chemotherapy (e.g. dose-adjusted-R-EPOCH) in the treatment of immunosuppression-related NHL.
- To develop a joint effort to conduct specific clinical trials for the treatment of infrequent subtypes of lymphoma arising in immunosuppressed patients (e.g. plasmablastic, peripheral T-cell, and primary effusion lymphomas).
- To conduct joint trials with therapies including antiviral agents, adoptive immunotherapy (e.g. genetically modified EBV-specific cytotoxic T cells), and monoclonal antibodies targeting cytokines for the prevention and treatment of PTLD.

#### *Anticipated impact of the research*

With the extensive use of immunologically-based therapies to treat cancer and immunological diseases, the increased frequency of solid organ and stem cell transplants, and the increased life expectancy of patients with HIV infection, the number of lymphomas arising in immunosuppressed patients is expected to increase in the coming years. However, the frequency of these lymphomas is still lower than that of those involving the non-immunosuppressed population, making it essential to initiate co-operative efforts in order to improve the knowledge of the mechanisms of lymphomagenesis and to develop more effective therapies. As the first-line therapy of HL and aggressive NHL in these patients has been reasonably well standardized in Europe, efforts must mainly be focused on relapse and refractory patients, for whom promising new drugs and immunologically-based therapies are in development. Progress in the knowledge of the mechanisms of lymphoma development in these patients will contribute to improving the treatment results, and will hopefully help in the prevention of these lymphomas.

### **2.8. Chronic lymphocytic leukemia and other chronic lymphoproliferative disorders**

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#### *Introduction*

Chronic lymphocytic leukemia (CLL) is the most common leukemia among adults in Europe. The disease is characterized by a complex pathogenesis due to the interplay between genetic and microenvironmental factors and a variable clinical course, making it a paradigm for the understanding of other malignancies. Recent discoveries in biology, therapy, and the relationship between these two have led to groundbreaking advances, and underline the impact of this “translational” approach for cancer research in general.

#### *European research contributions*

Research in Europe has been at the forefront regarding CLL biology and therapy. This goes back to the defining of the Binet staging system, the discovery of pathogenic mechanisms and prognostic parameters, the development of the current standard of care, and the latest innovations regarding novel therapies and resistance mechanisms. Details of each of these are outlined in the priorities of the proposal in the following section.

#### *Proposed research for the Roadmap*

The cellular origin of CLL has been difficult to identify. The finding that the clinical behavior of CLL differs dramatically depending on the mutational load and rearrangements of their immunoglobulin genes has opened new perspectives. Although it was initially postulated that CLL with mutated IGHV corresponded to memory B cells while CLL with unmutated IGHV corresponded to naive B-lymphocytes, the current consensus is that both represent clonal expansions of antigen-experienced cells. Based on similar patterns of antigen recognition, both have been thought to derive either from marginal zone B cells or putative human B1 cells. Recent studies coupling gene expression profiling and IGHV repertoire analysis have led to the conclusion that both IGHV subtypes derive from subsets of CD5+ B lymphocytes. Surprisingly, recent data from xenotransplantation experiments and next generation sequencing (NGS) suggest that the disease may, in fact, originate within hematopoietic progenitor (CD34+) cells. Thus, although progress has been made, the precise cellular origin still needs to be elucidated.

As indicated by the importance of the immunoglobulin genes and rearrangements and the clinical success of BCR pathway inhibitors (see below), the BCR and its downstream signaling elements play a crucial role in the pathogenesis of CLL. However, as BCR pathway inhibitors cannot cure the disease and the functional pathogenic mechanisms of BCR stimulation remain elusive, further research in this area is needed. Specific topics that need to be addressed in order to allow selective therapeutic targeting of disease subsets are related to the characterization of the stimuli and interactions that activate the BCR pathway and determine their impact and the downstream signaling molecules activated by different types of BCR interactions.<sup>107</sup> Furthermore, it will be extremely important to: 1) develop rational therapeutic strategies with curative potential by combining BCR pathway inhibitors with drugs that target other essential pathways, such as apop-



tosis regulation; and 2) decipher mechanisms underlying treatment resistance.

Genomic aberrations and gene mutations have been identified as major factors determining resistance to therapy and poor survival. 17p deletion and/or *TP53* mutation remain the strongest prognostic markers in multi-variable analyses despite the improvement in treatment with immunochemotherapy (see below), and *NOTCH1* mutation may be a predictive marker indicating a decrease in benefit from the addition of CD20 antibody. Mutations of specific target structures, such as BTK, and downstream signaling molecules, such as *PLCG2*, have been identified as resistance mechanisms against targeted therapies, such as ibrutinib.<sup>108</sup> Questions remain, however; for example, those related to resistance mechanisms and their impact on treatment decisions for novel treatments such as PI3K and BCL2 inhibitors and novel antibodies. Furthermore, the outcome of some disease subgroups (e.g. 17p-CLL) still appears inferior to other subgroups, and the transformation of CLL to more aggressive lymphoma (Richter transformation) is a frequent phenomenon of unclear etiology, leading to new and urgent research questions.

The standard conventional treatment approach in CLL is now immunochemotherapy with FCR (fludarabine, cyclophosphamide, and rituximab) for young/fit patients<sup>109</sup> and chlorambucil plus anti-CD20 antibodies (rituximab, obinutuzumab or ofatumumab) for elderly/unfit patients.<sup>110</sup> Despite the dramatic improvements in efficacy with these regimens, a number of critical issues remain. The first regards when to initiate therapy in the light of novel developments; also important are the therapeutic objectives [symptom control/palliation vs. minimal residual disease (MRD) eradication/cure]. Moreover, there is a relentless pattern of relapse despite the often initially deep remissions, making cure unlikely with these regimens. Therefore, predictive factors allowing informed treatment choice (i.e. is any one treatment superior to another in a particular patient?) represent an urgent need for individualized treatment (“precision medicine”).

The improved understanding of disease biology in CLL has led to the identification of targeted therapeutic approaches against BTK (e.g. ibrutinib), PI3K (e.g. idelalisib), BCL2 (e.g. venetoclax), and CD20 (e.g. obinutuzumab and ofatumumab). These agents have provided compelling evidence not only for dramatic efficacy but also for outstanding tolerability.<sup>111</sup> Nevertheless, a number of critical questions have emerged, in particular, related to the choice and handling of these agents. With some novel agents, “benign” disease persistence (lymphocytosis) is a frequent phenomenon, whereas there is rapid disease eradication with other agents (e.g. immunochemotherapy, BCL2 antagonists), and the principles guiding treatment aims between disease control and eradication remain to be determined. New, rare adverse events (e.g. bleeding, atrial fibrillation, and colitis) have been identified in spite of the generally outstanding treatment tolerability, and the long-term (side) effects of novel treatments are unclear. Given that combination treatments have been beneficial on the one hand, whereas on the other hand the novel compounds have already shown great single agent activity, will the concept of a combination approach or a sequential

use of the novel agents lead to better long-term results? As differences are seen, on the one hand, between patients' and their disease characteristics and, on the other hand, between the specific features of each compound, will all patients be treated with the same approach, or how can subgroups be identified for the greatest individual benefit? Lastly, given the cost of indefinite treatment duration with the currently licensed novel agents, and the worldwide demand for efficacious cancer therapy, how will the issue of cost and equal access to these treatments be handled?

#### *Anticipated impact of the research*

As is often the case with breakthroughs, the answers from the biological and clinical studies mentioned above open up new questions, and it appears that the magnitude of these questions is at least as great as the advances made through a new understanding of CLL biology and treatment. Clearly, to move beyond the understanding and success witnessed already, more well-designed clinical trials are needed with the ultimate goal of cure. Of equal importance, and underlined by the development of these targeted agents in CLL, is the advance of laboratory science. Taken together, CLL can serve as a valid model system for cancer in general by highlighting the dramatic progress that can be made within a short time frame when linking biology to therapy in a truly translational approach.

### **2.9. Acute lymphoblastic leukemia**

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#### *Introduction*

Acute lymphoblastic leukemia (ALL) is a life-threatening disease if not treated immediately. ALL occurs most frequently in children under 15 years of age and accounts for 25% of pediatric cancers and less than 1% of adult cancers. ALL arises from hematopoietic stem cells (HSCs) in the bone marrow that have acquired genomic lesions that result in the survival and a proliferative capacity of immature, non-functional malignant cells at the expense of normal, functionally differentiated white blood cells. This results in an impaired immune response against pathogens, resulting in fever or infections, anemia, and decreased wound healing capacity or bleeding. The type of genomic lesions differs somewhat between children and adults; for example, *KMT2A* gene fusions are predominantly found in infants (< 1 year of age), and the *ETV6-RUNX1* gene fusion is mainly found in children, whereas the *BCR-ABL1* gene fusion is most frequently found in adults. Treatment mainly consists of combination chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT), mainly limited to high-risk categories of ALL. Due to risk-stratified treatment, more optimized treatment protocols, and improved supportive care, the 5-year event-free survival on contemporary treatment protocols is more than 80% for children and is approaching 50% for adults.<sup>112</sup> The short- and long-term side effects of therapy are considerable, however, particularly with respect to the quality of life (QoL) in at least some of the adult survivors of childhood cancer.

### European research contributions

The treatment of ALL is relatively well structured in Europe by the assembly of national and international study groups, such as the International-Berlin-Frankfurt-Munster study group for childhood ALL and the European Working Group for adult ALL (EWALL). New prognostic subtypes of ALL were identified by screening large patient cohorts, facilitating structured diagnosis and treatment of ALL. The European collaborative studies under the umbrella of EuroMRD have been key in standardizing and developing monitoring minimal residual disease (MRD) and in building risk-adapted therapies that benefit ALL patient outcomes. The prognosis of adolescents and young adults has been significantly improved by pediatric protocols. In addition, pediatric-like therapies, including the use of L-asparaginase, have significantly improved outcome for adults with ALL.

In addition to disease monitoring, major achievements have been obtained in molecularly redefining ALL. Initially, chromosomal lesions were identified, such as those affecting chromosomal copy number (e.g. high hyperdiploidy with more than 50 chromosomes, as well as good prognosis) and those leading to aberrant chromosomes [e.g. the Philadelphia chromosome t(9;22), which gives rise to the *BCR-ABL1* gene fusion and is predictive of an unfavorable outcome]. The development of the molecular toolbox, mainly driven by the deciphering of the human genome in 2001, has accelerated the oncogenomics field in the past decade. Together with their US colleagues, European researchers have significantly contributed to the molecular characterization of ALL. Gene expression profiling has identified new subtypes of B-cell precursor and T-cell lineage ALL, and deepened our knowledge of mechanisms of resistance to frequently-used chemotherapeutic drugs, such as prednisone and L-asparaginase.<sup>114</sup> The *BCR-ABL1*-like ALL subtype, which was originally identified in children, has also been identified in adults with ALL, representing a relatively large new unfavorable prognostic subtype. Genomic screens and next generation sequencing (NGS) have revealed many new fusion genes, including more than 10 *ABL1*-class and more than 10 *JAK*-class fusion genes, which result in constitutively-activated gene products that can be targeted with precision medicines, such as *ABL1* inhibitors like imatinib and *JAK* inhibitors like ruxolitinib. In addition to gene fusions, smaller genetic mutations have been identified, which often affect the survival and proliferative capacity of leukemic cells.

### Proposed research for the Roadmap

The molecular deciphering of ALL revealed the complexity of this disease; the heterogeneity among ALL patients, and between children and adults, is further complicated by the identification of mutated subclones, which can resist chemotherapy or be acquired during treatment, and which can give rise to (late) relapse.<sup>115</sup> In addition, our knowledge about the supportive (and protective) role of the bone marrow microenvironment in the progression and treatment response of leukemia is still limited. Research will improve our knowledge of the pathobiology of ALL and its interaction with the microenvironment, which presumably also plays a role in the spread of ALL to extramedullary sites, such as the central nervous system, liver, and testicles, associated

with an adverse clinical outcome. Many new genomic/molecular lesions have been identified in past years, of which a few are prognostic. It is a huge mistake to only foster research dedicated to identifying and unraveling prognostic lesions because the improvements in clinical outcome will also limit the number of new prognostic lesions that can be identified, whereas the side effects of treatment will remain considerable. Research should increasingly focus on functional studies addressing the tumor dependency of new lesions (including those found in subclones) in relevant leukemia models. Last but not least, studies need to address how new precision medicines should be combined with (reduced) up-front chemotherapy to minimize side effects without jeopardizing clinical outcome. Attention should also be paid to age-related differences in pharmacokinetics of novel and old drugs in order to individualize dosing and accelerate the implementation of new drugs in children. Support by regulatory authorities, such as the European Medicines Agency (EMA), the development of European laws to facilitate drug development in children (Pediatric Regulation, 2001/20/EC), and early access to new potential drugs for *ex vivo* (patients' leukemic cells) and *in vivo* (ALL animal models) studies are essential for accomplishing better therapies with reduced side effects.<sup>116</sup> Furthermore, harmonizing the backbone of chemotherapeutic protocols of different study groups will be highly beneficial for performing clinical trials with new drugs in rare subsets of patients and will accelerate early drug development programs together with pharmaceutical companies. Recently developed monoclonal antibodies (bispecific antibodies and immunoconjugates) in ALL need to be further evaluated and eventually integrated in the standard management of some patients, particularly in adult ALL. Early results using cellular therapy (adoptive modified T cells, such as CAR-T cells) are promising and point towards alternative innovative strategies that might be effective in ALL. Leukemias have always represented proof-of-concept cancers for solid tumors, whereby their understanding permeates to less easily accessible tumors in both adults and children. Reinforced funding for acute leukemia will, therefore, have an impact well beyond the small percentage of cancers they represent.

### Anticipated impact of the research

The treatment will change from disease-type to molecular target-type, and from risk-stratified treatment schedules to more personalized therapies with precision medicines. In addition, individualized drug dosing may prevent underdosing and hence may reduce the risk of relapse, while therapeutic drug monitoring may also prevent overdosing and associated toxic side effects. More specific therapies and new immunotherapy-based approaches are of the utmost importance, not only for improving the prognosis of high-risk patients but also for significantly reducing treatment-related morbidity for patients of all ages, and especially for long-term survivors of childhood cancer.

### 2.10. Multiple myeloma and other plasma cell neoplasms

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### Introduction

Plasma cell disorders are common hematologic malignancies; multiple myeloma, the most common of these disorders, accounts for about 2% of all neoplasms and is the second most common hematologic malignancy. The median survival of patients with myeloma is 3-7 years; however, there is significant heterogeneity depending on the characteristics of the disease, of the patient and the therapy. The prevalence of the precursor monoclonal gammopathy of undetermined significance (MGUS) increases with age, and it is estimated that approximately 3%-5% of individuals over 65 years of age may have a MGUS. The risk of developing myeloma or other lymphoproliferative disorders with underlying MGUS is approximately 1% per year and remains lifelong.

Myeloma is a disease of the elderly with a median age at diagnosis of around 65-70 years. In the past two decades, there has been a major increase in the number of patients over 75 years of age who are diagnosed and receive therapy for myeloma, and this has been largely attributed to the major demographic changes that have occurred in Europe. Thus, more patients of advanced age will require therapy, presenting a major challenge in terms of management and health care costs. Despite the recent improvements in the survival of patients with myeloma, there is a significant minority of patients who have a very poor prognosis even with the use of the most intensive therapies, including transplantation. This group includes patients who are refractory to both proteasome inhibitors and immunomodulatory drugs, as well as patients with high-risk cytogenetics [del17p, t(4;14), and add1q], plasma cell leukemia, extramedullary relapses, or myeloma of the central nervous system. New therapies and innovative strategies are urgently needed for the treatment of these patients.

The recent advances made with the introduction of new drugs are also a challenge for the health care system due to the high cost of these therapies. Furthermore, these therapies are moving forward to front-line/early applications, they are combined very intensively, and many of them are given continuously until disease progression, resulting in substantial additional costs. Therefore, there must be a prudent allocation of health care resources in order to provide the best therapy for patients within a sustainable health care system.<sup>117-121</sup>

### European research contributions

The European Myeloma Network (EMN) was established in 2003 by integrating 27 research institutions and 14 trial groups with the intent of supporting development of novel diagnostics and therapies for multiple myeloma. Now, the EMN is a legal entity and has initiated co-operative clinical trials and laboratory research in different research fields in plasma cell dyscrasias. The close relationship between these research areas facilitated the exchange of information and experience, and has created a spirit of co-operation within the European area, pro-

moting clinical and laboratory research in myeloma and related disorders.

Different groups have initiated research programs and projects within the EMN and most of the researchers in Europe have been involved. As a result, several clinical trials have been conducted in myeloma, both multicenter phase III and phase I and II. The results of these clinical studies have framed the contemporary management of the disease in Europe. Importantly, through the collaborative network of the EMN it has been possible to conduct large clinical trials in rare diseases, such as Waldenström macroglobulinemia and light chain systemic amyloidosis, which represent major advances in the field. Through the EMN, a network of laboratories is working on several projects and participates in European Framework Programmes. As a result, the EMN has regularly published recommendations and guidelines on the management and other aspects of myeloma and related disorders, and these provide guidance to European and other physicians who care for myeloma patients, thus advancing the quality of care of patients with plasma cell malignancies.

### Proposed research for the Roadmap

Genetic studies have revealed the complex nature of myeloma and other plasma cell disorders. Large-scale genetic studies will provide new insights into the pathogenesis of plasma cell malignancies but, importantly, will uncover mechanisms of development, resistance, and relapse. Biobanking will be crucial for collecting sufficient high-quality samples. Genetic studies require the development of additional tools for interpretation and application of the results of the genetic mapping. The EMN has set up a network for biobanking, and for providing guidance and facilities.

Asymptomatic myeloma and MGUS are models for progression of the disease, and the integration of advanced genomics will provide the required knowledge of the evolution of plasma cell malignancies. Myeloma is characterized by inherent genomic instability and evolution of clonal disease has been shown for different pathways. Studies on disease evolution and genetic instability, integrating next generation technology and the detailed and prospective evaluation of the genetic evolution of the disease, will provide a framework for understanding mechanisms of resistance and escape.

The role of the microenvironment is crucial in the development and evolution of the disease, and genetic and functional studies that will address the role of other cells in the microenvironment of the plasma cell can delineate the pathobiology of myeloma and provide new rational targets for therapy. Although mechanisms of resistance are crucial in order to build new therapies and combinations of existing drugs, it is also important to develop technologies and *in vitro/ex vivo* systems that can provide reasonable sensitivity and specificity and predictive tools for responses to various therapies in order to avoid toxicity and reduce health care costs. In particular, valid and reproducible animal models are needed in multiple myeloma. These require advanced technologies in order to become widely available and usable.

In view of the deep responses that are now attained by a

significant fraction of patients with myeloma, the prognostic importance and the characteristics of minimal residual disease (MRD) are issues of intensive investigation. MRD may be considered a surrogate for cure or survival in clinical trial settings, but the different aspects of MRD need to be thoroughly and prospectively assessed. Technologies that involve multicolor flow cytometry, next generation sequencing (NGS), and single cell analysis will provide the data needed to build new regimens and modify therapeutic targets. In this setting, it will not be sufficient to monitor the disease by the traditional serum and urine electrophoresis, and new markers of disease for both diagnostic and monitoring procedures need to be developed and validated.

Imaging of the disease is another area of intensive research, which may provide crucial information on the extent, prognosis, and response of the disease. New technologies (PET/CT, PET/MRI, DWI-MRI) and advanced imaging software can identify disease foci with high sensitivity. Over the next few years, these advances will change the landscape of disease imaging and it is expected that evaluation of response will imply imaging criteria.

Survival outcome (usually progression-free survival and, less often, overall survival) is the main end point of most clinical studies that aim to improve disease outcome. However, the availability of new therapies with different toxicity profiles and the changing demographics of the disease require an appraisal of quality of life (QoL) as a critical end point of clinical studies. Redefining outcome based not only on metrics of survival end points, but also on QoL, is an area of intense study, with major social and economic impact, which will become important for the choice of therapy, as well as for the approval and financial compensation of new drugs. Furthermore, well-designed clinical trials, including more investigator-initiated efforts, are needed. Appropriate treatment options need to be harmonized within corporate groups to explore and answer questions such as: 1) when should treatment be initiated? 2) for how long should a regimen be given? 3) is cure rather than long-term disease control attainable, and in which patient cohort? and (4) what exactly benefits ultra-high-risk patients (i.e. patients with poor cytogenetics, RISS-3, plasma cell leukemia or extramedullary disease)?

#### *Anticipated impact of the research*

The intensification of research in the field of plasma cell dyscrasias and related disorders is expected to improve outcome, according to all end points. This has been proved in the past decades where a major survival improvement has changed the landscape of these diseases as a result of new therapies, advanced technologies, and improved criteria for definition, diagnosis, and treatment initiation. These improvements reflect the major advances in our understanding of the disease biology, which have led to the improvement of therapies in terms not only of the availability of new drugs, but also by improving treatment strategies and delivery of therapy. It is also crucial that several aspects of this research result in the development and establishment of new technologies in genetics, single cell analysis, *ex vivo* predictive systems, and imaging. In addition, a better understanding of the disease and patients' needs will allow a rational allocation of health care resources, with significant social impact.

## The EHA Roadmap for European Hematology Research

### Section 3. Malignant myeloid disease

#### Section editor: Hartmut Döhner.

The malignant myeloid diseases that are discussed in this section are disorders of hematopoietic progenitor cells (HPCs) characterized by varying degrees of cell maturation defects and/or uncontrolled proliferation. These disorders commonly affect older patients and will, therefore, constitute an increasing burden for caregivers in an aging society.

In recent years, European scientists have made major contributions to the understanding of the molecular basis of these disorders. Key disease-associated gene mutations have been discovered in myelodysplastic syndromes (MDSs), acute myeloid leukemias (AMLs), and myeloproliferative neoplasms (MPNs).<sup>9,122,123</sup>

The conduct of large controlled randomized trials with highly organized leukemia co-operative groups, in conjunction with the implementation of correlative science programs on well-annotated patient samples, has been one of the great assets of the European hematology community. The introduction of 1<sup>st</sup>- and 2<sup>nd</sup>-generation ABL1 TKIs in chronic myeloid leukemia (CML), characterized by the *BCR-ABL1* gene fusion, has been the paradigm for the successful development of precision medicine in cancer. New strategies are now being studied that aim at curing this previously fatal disease. The discovery of the *JAK2* mutation has been instrumental in rapidly bringing the first *JAK1/JAK2* inhibitor into the clinic for the treatment of patients with MPNs.<sup>124,125</sup> European investigators have also played a leading role in the development of hypomethylating agents for the treatment of MDS and AML.<sup>126,127</sup> European co-operative groups have shown that around 95% of patients with acute promyelocytic leukemia can be cured by a chemotherapy-free combination therapy with the vitamin A derivative all-trans-retinoic acid and arsenic trioxide.<sup>128</sup> For the great majority of patients with MDS and AML, however, progress has been very modest, and a high unmet medical need remains.

The European research groups, under the umbrella of the European Hematology Association (EHA) and the European LeukemiaNet (ELN),<sup>129</sup> and in collaboration with international investigators, have been instrumental in providing fundamental information to the scientific community by publishing recommendations and guidelines for clinical and laboratory practice of all the malignant myeloid diseases discussed here.<sup>130-133</sup>

Nevertheless, major challenges remain, as outlined in the following subsections. To further advance precision medicine for these disorders, a complete understanding of the disease biology will be needed. With the advent of novel technologies, comprehensive analyses of the genomes, epigenomes, transcriptomes, and metabolomes of these heterogeneous disorders will become possible. Robust bioinformatics tools need to be developed that are capable of processing such complex data and that can be applied on a clinical scale. Due to the demographic devel-

opment in Western countries, particular attention should be paid to the study of older patients in order to improve their outcome and, importantly, also their quality of life (QOL).

A joint effort of clinicians and scientists, research consortia, and leukemia co-operative groups on an international level, in close collaboration with the biotech and pharmaceutical industry, will be essential for more rapid scientific achievements.

### 3.1. Myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms

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#### Introduction

Myelodysplasia is a term used to describe morphological abnormalities in one or more of the major myeloid cell lineages of hematopoiesis and is a typical feature of myelodysplastic syndromes (MDSs). MDSs are clonal disorders of hematopoiesis with a propensity to evolve into acute myeloid leukemia (AML), caused by somatic mutations that occur in hematopoietic stem cells (HSCs). These disorders include primary conditions as well as secondary and therapy-related forms. Primary MDSs occur mainly in older people as a result of stem cell aging, and their crude incidence rate is 4 per 100,000 people per year, indicating that more than 30,000 new cases are expected in Europe each year. Myelodysplasia is also found in other myeloid malignancies, in particular in the so-called myelodysplastic/myeloproliferative neoplasms (MPNs), which include chronic myelomonocytic leukemia and atypical chronic myeloid leukemia (CML). MDS and MDS/MPNs show marked clinical heterogeneity, ranging from conditions with an indolent clinical course and a near-normal standardized mortality ratio to entities with very poor prognosis. A risk-adapted treatment strategy is, therefore, mandatory for these disorders. Several treatments have been proposed for MDS, but only a few have met evidence-based criteria of efficacy. At present, the only treatment with a potentially curative effect is allogeneic hematopoietic stem cell transplantation (HSCT), but less than 20% of all MDS patients are eligible for such treatment and have a donor. Azacitidine can prolong survival in patients with high-risk MDS, while erythropoiesis-stimulating agents and lenalidomide improve anemia in patients with low-risk MDS and the MDS associated with deletion 5q, respectively. Red cell transfusion remains the mainstay of therapy for many patients with MDS.

#### European research contributions

In the past few years, the genetic basis of MDS has been revealed by means of massive parallel DNA sequencing, and seminal studies have been performed in Europe.<sup>134,135</sup> Approximately 90% of MDS patients carry one or more oncogenic mutations, and two-thirds of them are found in individuals with a normal karyotype. Driver mutations have been identified in genes involved in RNA splicing, DNA methylation, chromatin modification, transcription regulation, DNA repair, and signal transduction. Only six genes are consistently mutated in 10% or more MDS

patients, while a long tail of more than 50 genes are mutated less frequently. Seminal contributions have also been made in pediatric hematology, for example, in elucidating the genetic predisposition to juvenile myelomonocytic leukemia.<sup>136</sup> European hematologists have provided pivotal contributions to developing effective treatments for MDS, including erythropoietin and azacitidine.<sup>126,137</sup> Recommendations for treatment of the individual patient with MDS have also been developed.<sup>130</sup>

#### Proposed research for the Roadmap

Myeloid malignancies appear to be propagated by rare self-renewing mutant HSCs. However, the cellular and molecular mechanisms that regulate development, propagation, and therapy resistance of these myelodysplastic stem cells remain unknown. Studies are needed to: 1) delineate the stem and progenitor cell hierarchy in order to identify the cancer-propagating cells in MDS and MDS/MPN patients; 2) characterize the cellular and molecular mechanisms underlying disease development, progression, and therapy resistance; and 3) identify therapeutic targets suitable for efficient elimination of the MDS-propagating cells.

In order to decipher the genetic complexity of MDSs, prospective studies of comprehensive mutational profiling of acquired gene mutations should be performed in large patient populations, ideally within clinical trials. The combined analysis of the genome and transcriptome may identify the impact of recurrent molecular abnormalities on gene expression. Particular focus should be given to spliceosome mutations, which occur in about half of all patients with MDS and are highly specific for this myeloid malignancy, suggesting an important role in disease pathogenesis.

Gender and age significantly influence prognosis of MDS patients; in particular, age is an independent adverse prognostic factor. One or more comorbidities are found in more than half the patients at the time of diagnosis, and they have a significant impact on survival. Studies that analyze the relationships between genotypes, gender, age, and comorbidities are needed. The findings of these studies should be used to develop prognostic/predictive models.

Outcome improvements in MDS patients remain modest. Identifying drugs that may further improve survival of patients with high-risk MDS and drugs that may inhibit ineffective erythropoiesis and improve anemia in those with low-risk MDS represents a priority. The importance of spliceosome and epigenetic mutations in MDS suggests that novel drugs targeting these pathways should be specifically investigated. Patient inclusion in clinical trials should be encouraged. The relationship between the genetic basis of MDS and the outcome of allogeneic HSCT should be explored, and more effective transplantation procedures should be developed.

#### Anticipated impact of the research

The current lack of understanding of the molecular mechanisms that regulate MDS stem cell development and their escape from therapeutic targeting is limiting our ability to efficiently eliminate the cells required for MDS propagation. The research lines described above have the potential to decipher these mechanisms.

The current diagnostic approach to MDS and MDS/MPNs includes a complete blood count, peripheral blood and bone marrow morphology, and cytogenetics. Mutational profiling of peripheral blood has the potential to dramatically improve our approach to the diagnosis of myeloid malignancies, leading to a clinically relevant molecular classification of these disorders.

The characterization of genomic and transcriptomic profiles of each individual patient with MDS or MDS/MPNs will allow the most appropriate treatment to be selected, patients to be enrolled in *ad hoc* clinical trials investigating new targeted therapeutic agents, and molecular biomarkers for monitoring response to treatment to be identified. This will eventually lead to precision medicine strategies.

### 3.2. Acute myeloid leukemia

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#### Introduction

Acute myeloid leukemia (AML) is a clonal disorder arising from hematopoietic progenitor cells (HPCs) characterized by defects in their maturation program and by uncontrolled proliferation.<sup>138</sup> AML is the most common form of acute leukemia with an estimated incidence of 3 per 100,000 individuals, resulting in 15,000 newly diagnosed patients each year in Europe. AML most commonly affects the elderly population, males more commonly than females, with a median age that has reached 70 years. The incidence of the disease will rapidly rise due to the proportional increase of the aging population. A further increase is expected from the rising incidence of therapy-related AML (i.e. myeloid neoplasms occurring in cancer survivors after successful treatment of a primary cancer). A particularly significant unmet medical need lies in the management of older patients with AML. Whereas in younger patients cure rates of 40%-50% can now be achieved, the outcome of older patients has remained very poor, in particular for those patients who are considered unsuitable for intensive chemotherapy. The backbone of treatment for AML, the combination of an anthracycline and cytarabine, has not changed in decades. This demonstrates the urgent need for the development of new agents, the mechanisms of action of which are based on a better understanding of the disease biology. Using novel genomics technologies, such as next generation sequencing (NGS) techniques, major progress has been made in deciphering the heterogeneity of the disease; however, translating this knowledge into clinical practice is lagging behind.

#### European research contributions

In Europe there is a long-standing tradition of controlled randomized trials within well-organized leukemia co-operative groups and this has largely contributed to the scientific achievements of European hematology. The

European LeukemiaNet (ELN) has facilitated the internationally embraced ELN recommendations on the diagnosis and management of AML as well as the consensus statement on allogeneic hematopoietic cell transplantation (HCT).<sup>131,139</sup> European hematologists have played a leading role in the improved management of a particular form of AML, acute promyelocytic leukemia, by showing superiority of the chemotherapy-free combination of the vitamin A derivative all-trans-retinoic acid and arsenic trioxide over conventional treatment, with cure rates of approximately 95%, a first highly successful step toward precision medicine in AML.<sup>128</sup>

European investigators have made major contributions to our understanding of the molecular basis of AML, as exemplified by the discovery of the mutation in the nucleophosmin 1 gene (*NPM1*), one of the most important biomarkers currently used in the clinic.<sup>140</sup> The identification of new biomarkers was paralleled by the introduction of the concept of minimal residual disease (MRD) detection either by quantitative polymerase chain reaction (PCR) or by flow cytometry, which is now implemented in many treatment algorithms. Recently, investigators have shown that clonal hematopoiesis with somatic mutations previously implicated in hematologic cancer (*DNMT3A*, *ASXL1*, and *TET2*) is increasingly common as people age, and it is associated with an increased risk of hematologic cancer. The data from this study are instrumental for the further understanding of the biology of AML in the aging population, one of the main challenges that we are now facing.<sup>141</sup>

#### Proposed research for the Roadmap

Concerted efforts from basic, translational, and clinical hematologists will be required to make major advances in the forthcoming years.

One important prerequisite to advance the field is the further understanding of the disease pathogenesis. This includes the identification and characterization of pre-leukemic cells and leukemic stem cells (LSCs), the analysis of the clonal architecture of genomic lesions, and their clonal evolution during the disease course, as well as the analysis of primary and acquired resistance mechanisms. To capture the entire complexity of leukemia biology, it will be instrumental to also analyze the transcriptional and epigenetic landscape of the leukemic cells. Integrated analysis of these complex omics data sets will require the parallel development of appropriate bioinformatics tools. These studies should be performed on well-annotated biosamples from patients treated in controlled clinical trials.

A particular focus should be on the study of older patients, who in the past have been largely under-represented in both clinical trials and correlative science studies. Instruments need to be developed to better define patients who are considered fit for intensive therapy *versus* those who *a priori* should be considered for investigational treatment.

A large number of new drugs targeting leukemic drivers or a multitude of deregulated pathways are awaiting clinical application. Careful pre-treatment selection by extensive molecular profiling will pave the way to a successful

outcome. Examples for targeting of defined subgroups are: AML with *IDH1/IDH2* mutations (using small molecule IDH inhibitors), AML with *FLT3* mutations [tyrosine kinase inhibitors (TKIs)], AML with *KMT2A* rearrangement (DOT1L or CDK6 inhibitors), and AML with *DNMT3A* mutations (hypomethylating agents).

Another promising route of investigation is offered by the new avenues of immunotherapy, beyond the further development of the concept of allogeneic hematopoietic cell transplantation that will remain a mainstay in the management of AML patients. New immunotherapy approaches, such as vaccination, CAR T cells, natural killer (NK) cells, bispecific T-cell engagers, novel monoclonal antibodies, and immunoconjugates, hold great promise for treatment of bulk disease or for targeting residual LSCs.

Finally, harmonization and standardization of complex diagnostic procedures, such as gene panel diagnostics and monitoring of MRD, need to be realized on an international level, because results from these diagnostic tests are expected to have a major impact on informing patient management.

#### Anticipated impact of the research

The program aims at further understanding the complex molecular heterogeneity of the disease. Deciphering this enormous complexity will be essential for the development of personalized approaches to AML treatment.

Given the current knowledge of the clonal architecture of the disease, no single drug is expected to cure it; rather, the combination of established therapies with novel agents that target disease-associated molecular lesions will be needed. Special attention must also be paid to the better management of older patients, given the more unfavorable biology and the still dismal outcome of the disease in these patients.

Dissecting the molecular trajectories of the disease using well-annotated biosamples from patients treated in innovative clinical trials will be instrumental to achieve these goals. These research programs are expected to make a major contribution to improving outcome in patients with this fatal disease.

### 3.3. Chronic myeloid leukemia

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#### Introduction

Chronic myeloid leukemia (CML) is a malignant neoplastic disease of the hematopoietic stem cells (HSC). CML is typically linked with the Philadelphia chromosome, a shortened chromosome 22 as the result of a reciprocal translocation of chromosomes 9 and 22 leading to fusion of *BCR* and *ABL1* genes. CML constitutes approximately 15% of all leukemia and occurs with an incidence of approximately 1.2 per 100,000 people. CML was almost always fatal until 15 years ago, but the excellent

results of BCR-ABL1 TKI treatment are raising the expectation that a considerable proportion of patients will achieve a treatment-free remission. The use of interferon (IFN)  $\alpha$  in parallel with or after tyrosine kinase inhibitor (TKI) therapy is associated with the induction of an immune response against the leukemic clone with further improvement of the remission rate. An essential part of the management of CML patients is rigorous use of cytogenetic and molecular follow up with standardized methods to regularly assess the residual disease status.<sup>132</sup> Prevalence of patients with CML treated with TKIs is expected to increase by approximately 10% per year so that CML is a challenge for health care systems worldwide. With average treatment costs in Europe of between €40,000 and €70,000 per patient per year, the challenge is how to maximize patient benefit with an affordable allocation of resources.

#### European research contributions

European co-operative CML study groups were established 30-40 years ago and have continued to contribute to the optimization of management. The impact of interferon (IFN) has been investigated in a series of large studies. IFN as an immunomodulatory agent has activity in CML and has resulted in sustained cytogenetic remissions in an important minority of patients. Meta-analyses of conflicting studies revealed new prognostic factors for IFN response. In 1998, the EUROscore was presented to better discriminate patients with a favorable, intermediate, and unfavorable outcome. The place of stem cell transplantation in disease management had been gradually evolving, having been displaced as first-line treatment by 2002, and then moving to a 3<sup>rd</sup>-line option after the licensing of the 2<sup>nd</sup>-generation TKI in 2006. From 2001 on, European investigators have participated in the clinical development of five TKIs. National and supranational [the European LeukemiaNet (ELN)] networks of European CML investigators and clinicians have provided fundamental knowledge for clinical practice. National and multinational studies with imatinib, IFN  $\alpha$ , Ara-C, nilotinib, and dasatinib have contributed to understanding the biology of TKI response, the impact and potential problems of combination therapies, and base-line and time-dependent prognostic factors. As a result of a study of the ELN involving more than 2000 patients, a new score predicting the chance of a complete cytogenetic response on imatinib therapy has been presented.<sup>142</sup> The predictive value of early molecular response, deep molecular response, and the velocity of response has been established in Europe.<sup>143</sup> ELN expert recommendations for CML management were published in 2006, 2009, and 2013, and have become a key reference for CML treatment worldwide.<sup>132</sup>

In basic and translational research, European investigators significantly contributed to the understanding of the mechanisms of TKI resistance and how to prevent and overcome it. Molecular monitoring of CML has been developed, optimized, and standardized in Europe, allowing accurate quantification of residual disease in a dynamic range of six orders of magnitude.<sup>144</sup> Such contributions permitted the successful attempt to discontinue treatment after deep molecular response.<sup>145</sup> Currently, the mechanisms that allow persistence of *BCR-ABL1*-positive stem cells are a major focus of research. Other research directions are the origin of CML, the clonal molecular evolu-

tion, and the characterization of the *BCR-ABL1*-negative hematopoiesis. Still, challenges remain, in particular in those patients who develop resistance mechanisms and eventually fail current treatment options.

#### *Proposed research for the Roadmap*

Tyrosine kinase inhibitors have substantially improved survival of CML patients. There is reasonable expectancy to cure the disease in a significant proportion of patients. The main objective is to integrate the leading European national trial groups in CML to form a co-operative network for advancements in CML-related research and health care. A clinical trials platform was created to promote the performance of clinical trials with new drugs and/or treatment strategies. Standardization of diagnostic and therapeutic procedures allows outcome to be compared across Europe. The formation of an exemplary “European platform to cure CML” is proposed to consolidate and lead international efforts to improve CML therapy with harmonized methods of molecular monitoring, definition of prognostic factors, and assessment of quality of life (QOL), as well as to reveal the biology of CML stem cells in order to induce immune response or to target specific features. We aim to improve: 1) the rate of deep molecular response; and 2) the rate of patients in durable remission after stopping TKIs. New induction therapies, combination with immunotherapies or stem cell active drugs, and new approaches of stem cell transplantation after treatment failure are methods to improve treatment responses. Patients in durable deep molecular remission after withdrawal of TKIs are considered cured of the disease. The complexity of CML blast crisis pathophysiology, the failure of TKIs to eradicate CML at the stem cell level, and the observation of molecularly defined *BCR-ABL1*-negative clones demand further research despite major improvements in the standard of care for CML. A better understanding of the events governing LSC behavior might lead to the biological cure of CML and effective treatment of blast crisis. Translational studies will contribute to early outcome prediction and treatment surveillance. Biostatisticians and patient advocacy groups co-operate with the study groups and with a European clinical trials platform that will support the co-ordination of the studies.

#### *Anticipated impact of the research*

In-depth molecular and cellular characterization of CML patients will facilitate personalized medicine with regard to diagnosis, prognostication, and therapeutic decisions. Overall, this will have a major impact on lowering the disease burden, reducing the rate of complications, and, prospectively, prolonging survival. The cost of novel technologies and treatments might be balanced by their more specific application as well as a favorable impact on patients' QoL and the lessening of the burden for caregivers; this will translate to a more general favorable impact for society by reducing use of resources and improving individual work capabilities. Standardization of diagnostics and therapeutic procedures will further strengthen integration. The resultant comparability of outcome will facilitate recognition of interstudy differences and rare subtypes. Recommendations, network meetings, training courses, and exchange of researchers will spread excellence and raise standards of research and patient care across Europe. The CML network and its activities provide the critical mass for added value and

European leadership. The impact on the future management of CML patients is expected to be considerable, because a rational advanced treatment design will likely lead to higher remission rates, longer survival, and a higher proportion of patients in whom treatment can be permanently discontinued as an indicator of cure.

#### **3.4. Myeloproliferative neoplasms**

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#### *Introduction*

The chronic Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) are disorders of hematopoietic stem cells (HSCs) and include polycythemia vera (PV), essential thrombocythemia (ET), and primary and post-PV/post-ET myelofibrosis. These are chronic disorders usually affecting individuals in middle to advanced age; the estimated overall incidence in Europe is 1-5 people per 100,000 a year. Life expectancy is close to normal in ET and modestly reduced in PV, whereas in primary myelofibrosis median survival is 5-6 years. There is no reliable estimate of the prevalence of MPNs; however, the prevalence is likely rising due to earlier diagnosis and prolonged survival. MPN patients suffer from major cardiovascular events and, less commonly, hemorrhages. Patients, and particularly those with myelofibrosis, may present with disabling constitutional symptoms, including marked cachexia, and suffer the consequences of abnormal cell proliferation with massive splenomegaly, hepatomegaly, and foci of extramedullary hematopoiesis. MPNs have an intrinsic tendency to transform to acute leukemia.

#### *European research contributions*

European researchers have a long-standing record of achievements in this field regarding both basic/translational and clinical research. European researchers have discovered the *JAK2V617F* mutation, *JAK2* exon 12 mutations, and *CALR* mutations that constitute major diagnostic criteria in the up-dated WHO classification.<sup>146-148</sup> The characterization of the key pathogenetic role of activated JAK/STAT signaling has been instrumental to directing pharmaceutical research that culminated in the approval of the first JAK1 and JAK2 inhibitor, ruxolitinib. The pivotal COMFORT-II study in myelofibrosis patients has been co-ordinated and performed in Europe;<sup>124</sup> the international pivotal RESPONSE study in PV has been co-ordinated and largely carried out in Europe as well.<sup>125</sup> European researchers first discovered genetic haplotypes predisposing to MPNs and highlighted the prognostic impact of subclonal mutations in primary myelofibrosis. National and supranational [European LeukemiaNet (ELN)] networks of European investigators and clinicians specifically focusing on MPNs have produced fundamental knowledge for clinical practice, including the drawing up of clinical risk scores, definitions of drug intolerance and resistance, treatment response criteria, and clinical end points for novel drug studies. Major phase III studies that established standards of care for MPN patients were performed in Europe, such as: 1) the ECLAP study, which demonstrated the safety and



efficacy of low-dose aspirin for thrombosis prophylaxis in PV; 2) the PT-1 and the ANAHYDRET study, which compared hydroxyurea *versus* anagrelide for high-risk ET patients; and 3) the CYTO-PV trial, which established the optimal hematocrit target in PV. European researchers have produced the largest prospective experience of stem cell transplantation in myelofibrosis.

#### *Proposed research for the Roadmap*

In spite of the above achievements in molecular characterization of MPNs, additional genomic research is needed to identify novel phenotype driver mutation(s) in the approximately 20% of patients with ET and primary myelofibrosis who still lack a molecular marker; reaching this goal will certainly facilitate earlier and more accurate diagnosis. However, a more ambitious goal is the discovery of the initiating mutation for MPNs, because the currently known mutations are certainly required for disease manifestation but are not essential for its development. Disease progression to either secondary myelofibrosis or acute leukemia is a particularly important aspect that is poorly understood and has not been studied in adequate depth, notwithstanding its eventual occurrence in more than 25% of patients with ET and PV whose conditions are regarded as more indolent. A better understanding of the molecular framework of MPNs would be important for improving our ability to subcategorize patients according to their risk of disease progression and dying. This would also permit appropriate therapies to be better selected. For example, stem cell transplantation may be a curative option, but it carries considerable risk; conversely, the recently developed JAK2 inhibitors must be credited with an incredible efficacy for symptomatic disease management, possibly resulting in prolongation of survival, but are unable to cure the disease. Thus, research is also needed to better delineate the cell-intrinsic abnormalities that determine and/or accompany these diseases; such insights might enable development of novel and more efficacious therapeutic strategies. Lastly, better animal models are required as an integral part of proposed research plans in order to facilitate a full understanding of the functional consequences of mutations and to test novel therapies. Overall, these studies might be greatly facilitated by supporting and reinforcing the existing networks of European scientists and clinicians in order to share carefully annotated patient samples and take advantage of existing technological platforms and expertise.

#### *Anticipated impact of the research*

Detailed molecular and cellular characterization of MPN patients would facilitate personalized medicine in the context of diagnosis, prognostication, and therapeutic decisions. Overall, this would have a major impact on lowering the disease burden, reducing the rate of complications, and, prospectively, prolonging survival. The cost of novel technologies and treatments might be balanced by their more specific application as well as a favorable impact on patients' quality of life and the lessening of the burden for caregivers; this would translate into a favorable outcome for society in general by reducing use of resources and improving individual work capabilities. Scientific achievements frequently result in patent development, which, by strengthening the relationships between European academia and the industry, facilitates public and private investments.

## The EHA Roadmap for European Hematology Research

### Section 4. Anemias and related diseases

#### Section editor: Achille Iolascon.

Anemia affects 1.6 billion people worldwide<sup>1</sup> and has a huge direct impact on human health and economic well-being, as well as being associated with worse prognosis and higher treatment costs because of the numerous comorbid diseases. Global anemia prevalence is approximately 47% in children under the age of five years, 42% in pregnant women, and 30% in non-pregnant fertile women.<sup>1</sup> The consequences of morbidity associated with chronic anemia extend to loss of productivity due to impaired work capacity, cognitive impairment, increased susceptibility to infection, and in the elderly, a huge contribution to comorbidities, which places a substantial economic burden on health care systems.<sup>149</sup> Nevertheless, anemia frequently goes unrecognized and untreated, causing high direct and indirect costs both to the individual and at a national level.

The globalization of migration flows in recent decades has increased the multicultural diversity of our societies. According to the Organisation for Economic Co-operation and Development, the percentage of foreign-born populations within the European Union in 2008 ranged from 4% in Finland to 37% in Luxembourg, with an overall average of 8%.<sup>150</sup>

Health care services in these countries have to deal with increasingly culturally diverse populations. Due to the movement of immigrants in Europe, there is a new epidemiology of acquired and inherited anemias. It is important to know the exact distribution of the different forms of anemia in each country in order to plan healthcare interventions. To carry this out, it appears mandatory to have European guidelines for diagnosis and establish a common and more sustainable therapeutic approach.

Moreover, clinical trials on new drugs and therapeutic procedures could ameliorate the quality of treatment of patients affected with these diseases, that are mainly inherited, enhance their quality of life, and extend their life expectancy.

Co-ordinated efforts should be made to develop strategies for prevention of acquired and inherited anemias in individuals at risk in Europe. Detailed epidemiological studies in all countries, especially in Western Europe, are a prerequisite for the implementation of effective prevention programs. For a correct diagnosis, it is mandatory to share a common diagnostic flowchart for each of the main forms of anemias.

Thus, new tools urgently need to be developed to reliably diagnose anemias and this fits well with the needs of personalized medicine. We expect that the development of such diagnostic tools will improve timely diagnosis throughout Europe, especially in those countries where it is difficult to gain access to "classical" diagnostic tests.

In the past 15 years, hematology research has made a big leap forward. The emergence of sophisticated genetic

and molecular tools [e.g. next generation sequencing (NGS) techniques] have allowed spectacular progress to be made in our understanding of the structure and function of the blood cells in health and disease. Our general aim will be to solve several hematologic problems using these new approaches.<sup>151,152</sup>

Research on rare iron-related genetic diseases that are informative biological models may contribute to a further understanding of iron metabolism. Precise diagnosis of these diseases might help avoid unnecessary and costly diagnostic tests and possibly harmful treatments.

#### 4.1. New technologies for anemia and related disorders

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##### Introduction

The production of the oxygen carrier red blood cells (RBCs) is called erythropoiesis, a process that begins with pluripotent hematopoietic stem cells (HSCs) and terminates with the production of RBCs. Anemia, defined as a decreased amount of hemoglobin (Hb), can result from blood loss as well as from decreased synthesis of Hb, decreased RBC production, or increased RBC destruction. Examples include hemolytic anemias (HAs), anemia of inflammation, chronic kidney disease (CKD), some forms of myelodysplastic syndromes (MDS), and bone marrow failure (BMF). In Europe, it is estimated that each year there are 8 new cases of MDS per every 100,000 people, 13% of people present clinical or biochemical evidence of CKD, and anemia of inflammation affects approximately 50% of patients with chronic inflammatory disease. Clinical management includes administration of anti-inflammatory molecules in anemia of inflammation, erythropoiesis-stimulating agents and iron in CKD, and blood transfusions, administration of hematopoietic growth factors, low-intensity chemotherapy, and bone marrow transplantation (BMT) in MDS. Hb disorders, such as  $\beta$ -thalassemia,  $\alpha$ -thalassemia, and sickle cell anemia (SCA), are monogenic disorders characterized by reduced or altered synthesis of the  $\beta$ - or  $\alpha$ -globin chain, components of the oxygen carrier Hb. Causes of morbidities and mortality associated with hemoglobinopathies are extramedullary hematopoiesis, iron overload, thrombosis, pain, bone defects, and liver and heart failure. Hemoglobinopathies represent the most frequent disorder worldwide, with at least 300,000 children born with these disorders every year. Clinical management has been focusing on supportive therapy, such as blood transfusions, iron chelation, and management of pain. BMT has also been utilized as a definitive curative option, although it is not without risks. Additional anemias are due to dietary limitations, such as folate, vitamin B12, and iron deficiency, while others are due to infections or exposure to toxic agents.

##### European research contributions

Advances have been made in our understanding of molecules and pathways that could be targeted to improve the anemia or the secondary manifestations associated with

defective erythropoiesis, such as splenomegaly, bone abnormalities, iron overload, and pain. B-cell chronic lymphocytic leukemia (CLL)/lymphoma 11A (BCL11A) and Krüppel-like factor 1 (KLF1), which modulate the production of fetal hemoglobin, are important modifiers of clinical severity in  $\beta$ -thalassemia and SCA.<sup>153</sup> JAK2 and growth differentiation factor 11 (GDF11), modulators of erythropoiesis, have been negatively associated with anemia in  $\beta$ -thalassemia, SCA, and MDS.<sup>154</sup> Cell adhesion molecules, such as E-, L-, and P-selectin, are being investigated for their potential role in hemolysis, inflammation, pain, and thrombosis in SCA.<sup>155</sup> Hepcidin (HAMP), the main regulator of iron absorption, has been associated with increased iron absorption in  $\beta$ -thalassemia and iron-restricted anemia in anemia of inflammation.<sup>154</sup> Molecules that control dietary iron absorption in the gut and HAMP production in the liver, such as hypoxia-inducible factor 2 $\alpha$ , divalent metal transporter 1, duodenal cytochrome B, ferroportin, transferrin, erythroferrone, and type II transmembrane serine protease, have also been investigated for their contribution to iron overload and anemia in  $\beta$ -thalassemia.<sup>154</sup> Despite huge progress, however, there is still no radical treatment for  $\beta$ -thalassemia and SCA, besides allogeneic HSCT, which has limitations such as donor histocompatibility. Gene therapy and gene editing approaches have been introduced as realistic alternatives to treat  $\beta$ -thalassemia and SCA. The technology for viral-mediated gene addition of the  $\beta$ -globin chain gene reached the clinical trial stage with promising results in two ongoing clinical trials in the United States.<sup>154</sup> This technology allows insertion of the curative gene by random integration in the genome. However, this might be associated with genotoxicity (i.e. undesirable gene disruption or oncogene activation). Gene editing technologies are focusing on the correction of mutations in the  $\beta$ -globin gene or in modifying genes that modulate fetal hemoglobin expression.<sup>156</sup> The latter technology could be intrinsically safer, because it does not require random integration of a curative transgene.

##### Proposed research for the Roadmap

All of these new potential targets and technologies may translate into clinical treatments with a positive impact on the management of these disorders. For instance, new agents that target GDF11 are now in phase I clinical trials for hemoglobinopathies and MDS.<sup>154</sup> Drugs that limit iron absorption and improve anemia are showing very promising results in pre-clinical studies.<sup>154</sup> As some of these drugs might target additional molecules, however, negative side effects following long-term treatments have not yet been excluded. Gene therapy trials for the cure of  $\beta$ -thalassemia show very promising results, but long-term effects are unknown. In Europe, there has been significant progress in generating appropriate gene therapy vectors, but this needs to be further supported in order to reach the clinic. Gene editing requires additional studies before it can be

**Table 1. Key priorities in a European Anemia Research Roadmap.**

- Epidemiology of anemias in Europe
- Common flowcharts for diagnosis
- Pathogenesis studies of rare inherited anemias to have new therapeutic targets
- Enhancement of clinical trials for new drugs
- Use of new technologies for a personalized diagnosis and therapy

translated into mainstream clinical therapy.<sup>156</sup> Genetic variability will likely influence the efficacy of these new therapeutics or therapies. For this reason, we recommend the use of next generation (omics) technologies to assess the role of genetic makeup of the patients, modifiers, and environment. This will help clinicians develop precision medicine (i.e. prevention and treatment strategies that take individual variability into account, leading to personalized treatment for each patient).<sup>157</sup> Therefore, as many of these approaches are still under characterization or in an early phase of development, we propose investing in these new lines of investigations and technologies in order to validate their potential and transform them into effective and safe ways of treatment (Figure 2).

#### Anticipated impact of the research

The clinical cost and socio-economic impact of these disorders is tremendous. Children affected by hemoglobinopathies require life-long transfusion for survival but, eventually, much morbidity develops, leading to a decreased life span. Anemia of inflammation, CKD, and MDS predominantly impact the adult population, representing a growing and pressing issue in Europe. Therefore, efforts to develop new scientific discoveries and therapeutics can have a major impact on the growing and aging population in Europe at many different levels. This new source of information and potential novel technologies might have a profound impact, not only on these disorders, but also on many other diseases that require management of RBC production, such as in cancer therapies or following BMT. Moreover, many additional incurable disorders could benefit from the development of gene therapy and gene editing technologies developed for  $\beta$ -thalassemia and SCA.

#### 4.2. Iron-deficiency anemia

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#### Introduction

Iron-deficiency anemia (IDA) is the most common form of anemia worldwide, affecting almost 1 billion people. There are strong differences in IDA prevalence between developing and high-income countries, and even among European countries.<sup>158</sup> Individuals at risk are those with increased iron needs, such as pre-school children, adolescents, and young (especially pregnant) women. Pathological causes of IDA, such as malabsorption and chronic blood loss, are common and may be associated with cancer, especially in the elderly. IDA poses a major burden to society: it has been reported to cause cognitive defects in children, increased morbidity and mortality in pregnancy, reduced physical performance in workers, and is a common comorbidity in the elderly. Genetic causes of IDA are extremely rare, often remaining undiagnosed, though relevant in children. Also challenging is the diagnosis of IDA in the context of anemia of chronic disease (ACD), a condition frequently found in the elderly. Diet fortification is an effective preventive modality of IDA, although there are concerns

that iron may worsen infections, as shown in children living in malaria-endemic areas.<sup>159</sup> Treatment of IDA is apparently simple, because several (oral and intravenous) iron preparations are available.

#### European research contributions

European researchers have contributed identifying hepcidin, the key liver hormone regulating systemic iron homeostasis, defining its role in genetic iron-related disorders and clarifying how high hepcidin induces iron sequestration and iron-deficient erythropoiesis in ACD.<sup>160</sup> Our advanced understanding of iron metabolism has allowed the recognition of rare genetic iron-related anemias that are challenging to diagnose. The e-rare JTC 2009 HMA-IRON project has helped raise awareness of these novel entities in Europe. Orphanet and the European Network for Rare and Congenital Anaemias (ENERCA) ([www.enerca.org](http://www.enerca.org)), a European network of expert centers, offer online tools useful for the diagnosis of these rare anemias. From genetic iron-refractory IDA, characterized by high hepcidin levels and iron refractoriness, the lesson is that hepcidin levels should be low/undetectable to allow oral (and pharmacological) iron absorption and that, when needed, intravenous iron should be preferentially employed in the presence of inflammation with high hepcidin. However, analytically and clinically validated hepcidin tests are available in only a few European centers.



Figure 2. A roadmap for research into new technologies in anemias and related disorders.

The results of clinical trials comparing the efficacy of novel intravenous preparations with oral iron drugs are available or being processed. Intravenous preparations appear safe even at high doses (up to 1 g) administered in a single infusion. However, experience is limited, and criteria for using oral or intravenous iron are only partially defined. Long-term effects and cost-effectiveness also need to be evaluated.

#### Proposed research for the Roadmap

Co-ordinated efforts should be made to develop strategies for the following.

1. Prevention and treatment of IDA in individuals at risk in Europe. From the available data, Eastern European countries show a higher IDA prevalence than countries in Western Europe.<sup>158</sup> Detailed epidemiological studies in all countries, especially Western countries, are a prerequisite for the implementation of effective prevention programs.
2. A better understanding of the impact of iron-deficiency on physical performance and cognitive and physical development in children, even independently from anemia; understanding is now based only on epidemiological evidence, also necessitating molecular and biological studies. A flowchart should be developed and shared for differential diagnosis of IDA, IDA in ACD, and ACD, and for therapeutic criteria.<sup>161</sup>
3. Clinical trials focused on the new intravenous iron preparations should be designed to compare their efficacy and side effects. There is a need for evidence-based strategies for accurate and timely diagnosis and optimal treatment of genetic iron-related anemias.
4. Clarify the possible genetic propensity to develop iron deficiency and the relationship of iron with erythropoiesis efficiency. We have just started to understand how erythropoiesis adapts to iron deficiency. It is unclear why and how iron deficiency induces microcytosis and through which mechanisms it increases platelet production in severe cases. Iron deficiency is a positive modifier of ineffective erythropoiesis in pre-clinical murine models of  $\beta$ -thalassemia,<sup>162</sup> but the mechanisms have not been explored and should be verified in patients.
5. The iron-related changes in the composition of gut microbiota, with a prevalence of pathogens over the beneficial lactobacilli, is an emerging problem in developing countries that needs to be further explored considering the relevance of microbiota for human health and the safety of oral iron supplementation in developing countries.

#### Anticipated impact of the research

To correct IDA is simple and usually inexpensive. The underlying cause in some cases is more relevant than anemia itself. Society will benefit from programs aimed at controlling iron and Hb levels in all age groups. Research on rare genetic iron-related diseases that are informative biological models may contribute to further understanding iron metabolism and its regulation. Their precise diagnosis might lead to avoiding unnecessary and costly diagnostic tests and possibly harmful treatments.

Elucidating the role of iron in ineffective and effective erythropoiesis would benefit patients with  $\beta$ -thalassemia and other inherited anemias and may have implications

for acquired disorders of erythropoiesis in myeloproliferative disorders [e.g. polycythemia vera (PV)] and low-risk MDSs. The results of clinical trials may indicate when and how intravenous iron should be safely used. Targeted therapies are the results of increased knowledge. The discovery of hepcidin is fostering its therapeutic manipulation as a novel approach to control iron levels. Trials with hepcidin antagonists that aim at making the sequestered iron in inflammation available for erythropoiesis are ongoing in European centers and beyond. Epidemiological surveys, biological studies, and clinical observations suggest an important role for iron in common disorders, including heart failure, obesity, CKD, diabetes, and metabolic syndrome. Studies triggered by IDA have the potential to help establish the optimal levels of iron according to age and sex in these disorders.

#### 4.3. Dyserythropoietic and hyporegenerative anemias

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##### Introduction

Congenital dyserythropoietic anemias (CDAs) and Diamond Blackfan anemia (DBA) are rare hereditary anemias caused by abnormal erythropoiesis that is ineffective in the former and hypo/aregenerative in the latter. The prevalence of the CDAs in Europe varies according to European region and is 0.1-3 cases per million live births, while that of DBA is 4-7 cases per million live births.<sup>163</sup>

The CDAs are characterized by moderate to severe anemia, distinct morphological features in bone marrow late erythroblasts, and development of secondary iron overload. The morphological classification initially proposed by Heimpel and Wendt (CDA I, II, and III) is still valid in clinical practice and is now supported by the identification of different genes mutated in each type.<sup>164</sup> CDA I is caused mainly by mutations in *CDAN1*, but also in *C15orf41*. CDA II is a result of mutations in *SEC23B* and CDA III of *KIF23*. However, there are families that fulfill the general definition of the CDAs but do not conform to any of the three classical types (CDA variants). Moreover, mutations in erythroid-specific TFs genes *GATA1* and *KLF1* have been described in few such patients. The protein encoded by *KIF23* is a mitotic kinesin crucial for cytokinesis; however, the possible role of the proteins encoded by *CDAN1*, *C15orf41*, and *SEC23B* in erythropoiesis is still unknown.

Children with DBA classically present with severe macrocytic anemia in the first year of life. The bone marrow discloses a paucity of erythroid precursors. Approximately 30% of DBA patients also have physical anomalies (e.g. craniofacial, thumb, and cardiac malformations). The risk of solid tumors, myelodysplastic syndromes (MDS), or leukemia is elevated in DBA and was calculated to be 20% by the age of 46 years.<sup>165</sup> Following the first year of life, the anemia is currently treated with corticosteroids. Infants in the first year of life or patients who do not respond to steroids or require high doses with unacceptable toxicities receive chronic red blood cell (RBC) transfu-

sions.<sup>166</sup> These patients often develop substantial iron overload and require careful monitoring to detect this, as well as iron chelation therapy. Stem cell transplantation is an alternative to chronic transfusions. DBA is the first ribosomopathy described resulting in haploinsufficiency of 16 genes encoding ribosomal proteins, *RPS19*, *RPL5*, *RPS10*, *RPL11*, *RPL35A*, *RPS26*, *RPS24*, *RPS17*, *RPS7*, *RPL26*, *RPS27*, *RPS28*, *RPS29*, *RPL15*, *RPL27*, and *TRS2*. Mutations in ribosomal genes account for 60%-70% of DBA cases. The effect of decreased ribosomal activity *in vivo* and in a tissue-specific manner is unknown; p53 activation has been observed in bone marrow from DBA patients, after depletion of ribosomal proteins. Recently, it has been shown that rare mutations in the *GATA1* gene can cause DBA. Subsequently, an elegant study suggested that impaired translation of GATA1 mRNA (as a consequence of ribosomal protein haploinsufficiency) is an important factor in mediating the erythroid defect observed in DBA.

#### European research contributions

Almost all the work carried out on CDAs, including the description of the clinical picture and identification of the genes involved, was done in Europe. Heimpel made an important contribution to the field, becoming the first to diagnose the disorder and to describe its morphological and clinical features. Iolascon did much of the work on CDA II, defining the molecular genetics and the genotype-phenotype correlation of this disease. The genes mutated were mainly described in Europe. Although the major gene mutated in CDA I (*CDAN1*) was identified in Israel, the second gene causing CDA I was described in the UK. The CDA II gene identification was an Italian-German collaboration; that of the CDA III gene was performed in Sweden.

The main DBA patient registries are kept in France, Germany, Italy, the UK, and the US. Israel also keeps a registry of bone marrow failure (BMF) syndromes that includes Diamond Blackfan anemia (DBA). Collaboration among European groups led to the identification of the first DBA gene, *RPS19*. Subsequently, many collaborative papers explored several aspects of genotype-phenotype correlation. A group from Lund has developed many cellular and mice models of DBA, and is involved in gene therapy and new drug research for the anemia.

Educational activities train young doctors and researchers in their understanding of these disorders, and patients have organized themselves into national organizations throughout Europe.

#### Proposed research for the Roadmap

We suggest programs to improve diagnosis and optimal clinical care for patients with these rare disorders, as well as basic research programs to better understand the role of the proteins encoded by mutated genes in erythropoiesis (Figure 3).

1. Improve CDA and DBA European registries by harmonization and collaboration among the existing national registries to create a unique European database.
2. Improve molecular diagnosis and identification of potential new genes by using next generation sequencing (NGS) methods. The first step in this proposal is to employ targeted NGS with a panel of known genes mutated in CDAs, DBA, and other rare anemias. If no

mutation is identified by targeted NGS, a whole-exome sequence will be performed to identify new causative genes.

3. Perform functional studies to define the role of proteins, encoded by known and any new genes in erythropoiesis. The proteins will be studied in relevant human erythroid progenitors grown in liquid medium CD34<sup>+</sup> cells and also using patients' induced pluripotent stem cells (iPSCs).
4. Investigate the role of new drugs that modulate erythropoiesis (anti-JAK2 and TGF- $\beta$  ligand modifiers) using *in vitro* models of erythropoiesis. Anti-JAK2 has the potential to decrease ineffective erythropoiesis and thus ameliorate anemia in CDAs. TGF- $\beta$  ligand modifiers correct anemia by promoting late-stage erythropoiesis and have recently been shown to decrease transfusion requirements and serum ferritin level with favorable safety profile in patients with  $\beta$ -thalassemia.<sup>167</sup> Because of the similarity in pathogenesis between thalassemia and CDAs, their study in CDAs is warranted.
5. Implement studies for CDAs/DBA. Gene therapy has also been proved to cure diseases that affect hematopoietic cells, such as severe combined immunodeficiency.

#### Anticipated impact of the research

As CDAs/DBA are rare disorders, there is often misdiagnosis or a delay in diagnosis which may result in years of inappropriate therapy, including iron preparations. Patient registries and cutting-edge molecular technology will contribute to accurate diagnosis and optimal therapy for most patients. Defining the role of the proteins encoded by mutated genes in CDAs and DBA in erythropoiesis may eventually be exploited therapeutically. Gene therapy

#### Dyserythropoietic and hyporegenerative anemias road map

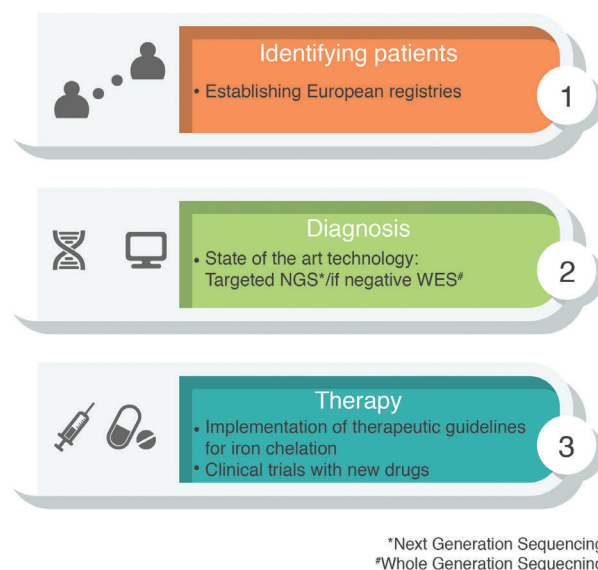


Figure 3. A roadmap for research into dyserythropoietic and hyporegenerative anemias.

approaches and new drugs that modulate erythropoiesis have the potential to ameliorate the anemia and iron overload, and thus improve patients' quality of life and survival.

#### 4.4. Hemolytic anemias, including membrane and enzyme defects

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##### Introduction

Hemolytic anemias (HAs) are a heterogeneous group of hereditary and acquired disorders. Among hereditary forms, the most common are defects of the red cell membrane and enzymopathies that disturb red cell metabolism. Except for glucose-6-phosphate dehydrogenase deficiency, which affects more than 400 million people, the most frequent congenital hemolytic disease in Europe is hereditary spherocytosis, a cytoskeletal defect with a prevalence of 1-5 cases per 10,000 individuals. Other hereditary HAs are rare or extremely rare (Figure 4).

Hereditary HAs are characterized by anemias of variable degree, from fully compensated hemolysis to severe and transfusion-dependent anemia. Other manifestations of clinical significance include jaundice, splenomegaly, and iron overload. Hydrops fetalis has been reported in rare cases. In some enzymopathies (those involving genes with ubiquitous expression) and in rare conditions, non-hematologic symptoms, such as neurological/neuromuscular impairment, may also be present. Because the pathophysiology of around 30% of hereditary HAs is poorly understood, these disorders represent a substantial and heterogeneous group of diseases that still lack easy-to-apply tools for diagnosis, clinical management, and patient stratification. Moreover, epidemiological data in Europe are generally still incomplete, and the estimated prevalence of some defects varies widely among countries. This is likely due to the limited and incomplete availability of diagnostic tools.<sup>163</sup>

Although the general diagnosis of anemia is part of daily clinical practice, the differential diagnosis of HAs is often difficult, requiring specialized analyses available in only a few expert EU centers. In addition, the conclusive diagnosis is often delayed, thus increasing the overall costs of the health care systems and causing considerable degrees of distress for patients and their families.

It has been calculated that the cost of diagnosis for one of these anemias is between €850 and €2500; that can triplicate or even quadruplicate if a conclusive diagnosis is not reached.

Although the total number of affected individuals is substantial, the rarity and heterogeneity of HAs have resulted in limited interest from the pharmaceutical industry.

##### European research contributions

In past 20 years, inherited HAs have been the object of

intensive research by internationally recognized EU groups, aimed at elucidating the molecular bases of these diseases, as is the case for hereditary stomatocytosis and enzymopathies.<sup>168,169</sup>

A great step forward in the classification of these rare defects, as well as in the identification of expert centers for diagnosis, has been made in the past ten years by ENERCA. ENERCA is an EU project currently in its fourth phase (e-ENERCA). An important outcome is the ENERCA White Book containing the recommendations and the definition of the criteria that Centers of Expertise and local centers have to fulfill as health care providers.<sup>170</sup>

Due to the concerted efforts of several EU groups, a new edition of diagnostic guidelines for hereditary spherocytosis is about to be published.<sup>171</sup> However, no specific guidelines are currently available for the rarer HAs.

##### Proposed research for the Roadmap

*Create an EU network and registry for rare HAs:* through the work carried out by ENERCA, it has been possible over the past years to map EU expert centers for the referral of cases with RBC cytoskeletal membrane disorders, hereditary stomatocytosis, and RBC enzymopathies. This experience has shown that an officially recognized European Reference Network (ERN-RA), combining different areas of expertise and dedicated specialists, is needed to better define these disorders and share common diagnostic and therapeutic flowcharts. The creation of European registries for these rare disorders will also be of great help to increase knowledge about their prevalence and to collect a greater number of patients; this will improve clinical diagnosis, allow a better definition of complications, and facilitate possible therapeutic trials.

*Understand the pathophysiological mechanisms and identify new genes to develop new diagnostic tools:* despite detailed, exhaustive hematologic and molecular investigations, approximately 10%-15% of HA patients remain undiagnosed. Moreover, the wide heterogeneity of their phenotypic expression has made it difficult in the past to develop easy-to-apply molecular diagnostic tools. The advent of next generation sequencing (NGS) technologies make these new approaches useful tools to investigate the genetic basis of undiagnosed cases and to identify new nosological entities. Moreover, the reduction in cost of these technologies may allow the development of NGS-based diagnostic tools (i.e. by creation of a panel of known genes) and their market development.

*Develop new therapeutic approaches (e.g. new drugs and gene therapy models):* whereas new drugs and therapeutic approaches recently became available for acquired HA, no specific or curative treatments are available for congenital HAs except for hematopoietic stem cell transplantation (HSCT). Because most of these defects are monogenic, gene therapy may represent a therapeutic option. In this respect, a promising approach concerns the use of gamma-retroviral vectors that has proved effective in correcting the disease in a pyruvate kinase-deficient mouse model as recently developed by EU groups. A therapeutic and clinically applicable lentiviral vector has recently received the orphan drug designation for the treatment of pyruvate kinase deficiency by the European Commission

(EU/3/14/1130). Finally, investigation of new drugs that could increase specific enzymatic activity and/or activate isoenzymes could pave the way for attractive therapeutic approaches to RBC enzyme disorders.

#### Anticipated impact of the research

A correct diagnosis will have a major impact on patients' quality of life (QoL) and survival, especially by the early detection of complications such as iron overload, and will allow for appropriate genetic counseling of patients and their families. A more timely diagnosis will also result in a significant reduction of the overall costs of the health care systems.

The increased knowledge of pathophysiology of these disorders and the identification of new nosological entities will be of great help in improving the diagnosis and will offer the basis for the development of new therapeutic approaches for HAs.

Finally, the creation of EU registries for rare HAs will improve awareness of these rare disorders and their prevalence.

#### 4.5. Congenital bone marrow failure, aplastic anemias, and paroxysmal nocturnal hemoglobinuria

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#### Introduction

Bone marrow failure (BMF) syndromes are a heterogeneous group of diseases characterized by a quantitative deficiency in one or more blood cell lineages. Inherited BMFs include different entities, such as Fanconi anemia (FA) (which is due to impaired DNA repair and cytokine hypersensitivity),<sup>172,173</sup> dyskeratosis congenita, Diamond Blackfan anemia (DBA), and Shwachman-Diamond syndrome (all associated with impaired ribosomal or telomere function). Inherited BMFs are rare disorders, the most common of which is FA (1-3 per 500,000 newborns).<sup>172</sup> Indeed, the majority of BMFs are acquired forms, mostly idiopathic; the most typical form, idiopathic aplastic ane-

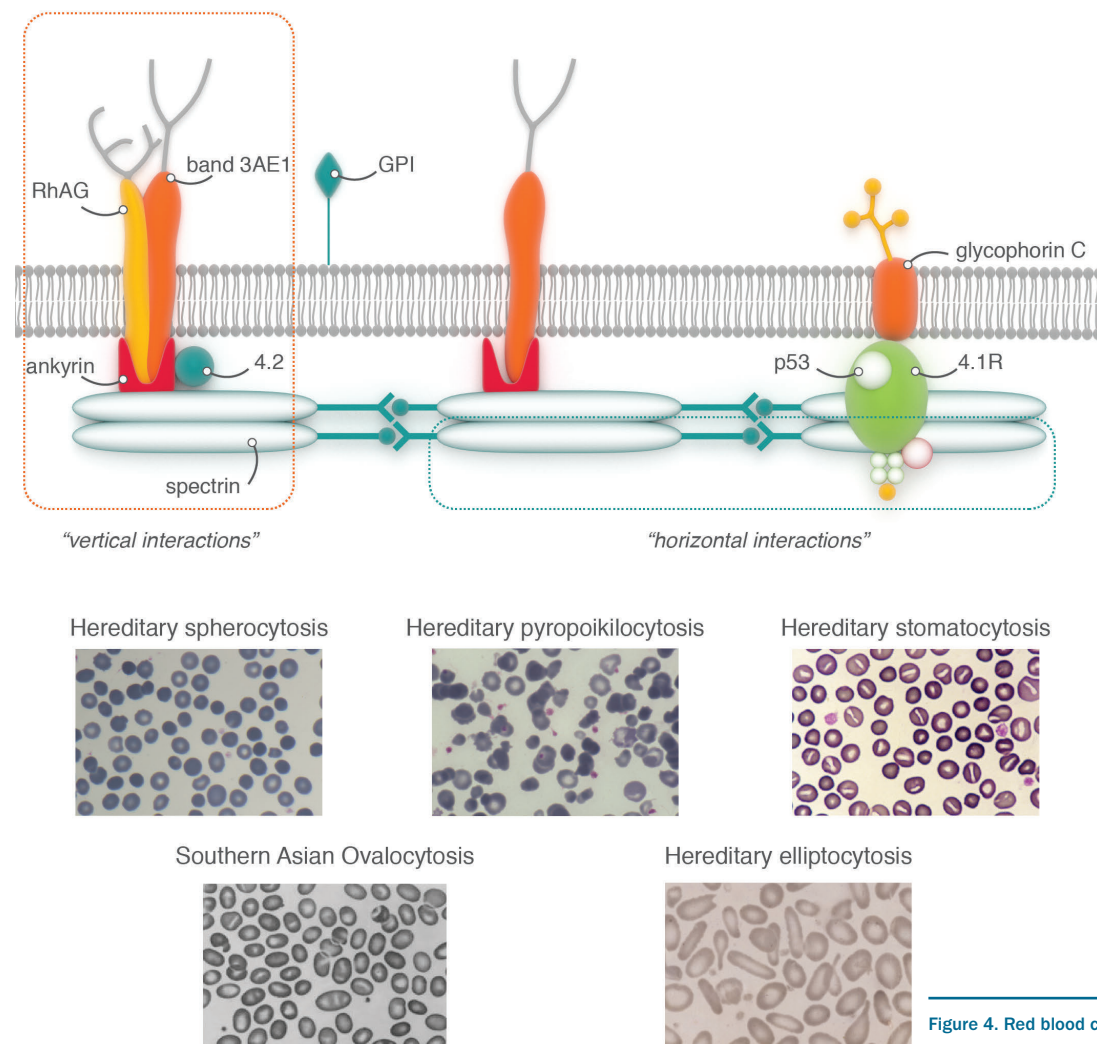


Figure 4. Red blood cell membrane disorders.

mia (IAA), has an incidence of 1-5 people per million in Western countries. Another less common acquired BMF is paroxysmal nocturnal hemoglobinuria (PNH); here the underlying bone marrow disorder is associated with the expansion of an abnormal, non-malignant, blood cell population that is deficient in the expression of glycosylphosphatidylinositol-linked proteins due to a somatic *PIGA* mutation. As well as BMF, which is seen in only a proportion of PNH patients, the clinical phenotype of PNH is characterized by anemia due to complement-mediated intravascular hemolysis secondary to the lack of the glycosylphosphatidylinositol-linked complement regulators CD55 and CD59, along with an increased frequency of major thromboembolic events.

#### European research contributions

The improved understanding of all BMFs has led to better patient management in Europe; the Working Party for Severe Aplastic Anemia (WPSAA) of the EBMT has contributed greatly to improved clinical outcome in this field. Milestones, where European hematologists have taken the lead, include the first observations on the use of antithymocyte globulin (ATG) as treatment for IAA and the first use of hematopoietic stem cell transplantation (HSCT) in patients with FA. There have also been improvements in diagnostic strategies in BMF, mostly in the differential diagnosis of inherited versus acquired forms of BMF. (The former are often cryptic and may appear even in adolescents or adults.) In terms of treatment, the most relevant improvements include: 1) intensive immunosuppressive treatment (mainly for acquired IAA); 2) anticomplement treatment (for hemolytic forms of PNH); 3) HSCT, for inherited BMFs, IAA, and, more rarely, PNH. These improvements are particularly relevant given that they were achieved in the setting of rare disorders where there are innate difficulties in making progress. The database of the WPSAA of the European Group for Blood and Marrow Transplant (EBMT) contains data on more than 11,000 patients with different subtypes of BMFs, thereby providing a unique opportunity for investigating many different critical aspects of these diseases. The WPSAA of the EBMT continues to run a multinational database to collect data from all European BMFs; the aim is to combine this retrospective work with prospective studies to address further improvements in the complex treatment of these disorders.

#### Proposed research for the Roadmap

Patients suffering from BMFs continue to represent a challenge for the medical community because of their poor prognosis when the underlying disease is not controlled. Additional efforts are needed to offer the most appropriate treatment to all European patients and improve current standards of care. The WPSAA of the EBMT is dedicated to this goal through different research lines.

1. *Improvement of non-transplant treatment for acquired IAA:* current immunosuppressive treatment for acquired IAA is based on the combination of horse ATG and cyclosporine A. The recent withdrawal of the horse ATG preparation from the European market has had detrimental effects on outcome in European IAA patients, as demonstrated in several studies,<sup>174,175</sup> leading the WPSAA to highlight the need for this ATG preparation for IAA patients.<sup>175,176</sup> In addition, ongoing efforts are investigating the benefit in randomized,

controlled trials of the addition of newer agents (e.g. the THPO-mimetic agent eltrombopag) on the “scaffold” of standard immunosuppression (which may be different according to the severity of IAA).

2. *Improvements in HSCT for inherited and acquired forms of BMF:* HSCT remains a key treatment option for all BMF patients, with the current indication depending on the phase/severity of the disease and on the availability of alternative treatments. Possible improvements in HSCT procedures will exploit different (and possibly combined) strategies:
  - a) identification of improved HSCT protocols (including pre-transplant conditioning and pre-, peri-, and post-transplant immunosuppression) to reduce post-transplant mortality and morbidity (e.g. comparing different immunosuppressive regimens);
  - b) development of novel HSCT protocols in the setting of unrelated donor HSCT, aiming to neutralize the detrimental effect of a non-related donor, such that unrelated HSCT could be used earlier in the treatment algorithm of IAA;
  - c) investigation of HSCT from alternative donors, such as human leukocyte antigen (HLA)-haploidentical donors and cord blood units, aiming to offer a HSCT option to all candidate patients;
  - d) identification of novel HSCT procedures tailored to specific conditions (e.g. for PNH or for FA and other inherited BMFs)
3. *Observational studies on PNH:* the treatment of PNH has been revolutionized in the past decade by the introduction of the anticomplement agent eculizumab. The WPSAA is currently looking at the actual role (and most appropriate procedures) of HSCT in PNH in the eculizumab era, as well as evaluating possible unmet medical needs that may still remain during this treatment.
4. *Observational studies on the natural history of BMFs:* these studies aim to improve the diagnosis, classification, and definition of response categories of both acquired and inherited BMFs, with the goal of identifying burning clinical questions to be investigated by specific investigations.

#### Anticipated impact of the research

The management of BMF represents an urgent medical need not only for individual patients but also for society as a whole. Many affected patients will become unproductive and require lifelong, expensive treatments. In the past four decades, BMFs have changed from inevitably fatal diseases into curable ones, with an overall survival rate approaching 70% at ten years. These outcome data can still be improved. It is only through the design and execution of stringent and well-focused studies that the scientific community will learn how to deliver better treatment options to these patients. Furthermore, improvement of the management of BMF will lead to a better use of increasingly restricted resources, which will also have a positive impact on society, also from a financial point of view.

#### 4.6. *Thalassemia and congenital hemoglobinopathies*

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### Introduction

The thalassemia syndromes are a heterogeneous group of inherited hemolytic anemias (HAs) characterized by reduced or absent production of one or more of the globin chains of hemoglobin (Hb). This leads to imbalanced globin chain synthesis that is the hallmark of thalassemia syndromes. In hemoglobinopathies, globin chain synthesis is usually balanced but one chain is abnormal: HbS, HbC, and HbE are the most common and relevant.

The thalassemias and hemoglobinopathies are the most common single gene disorders in the world population, with estimated carrier number of more than 270 million and an annual birth rate of more than 300,000. They are most frequent in southern Asian, Middle Eastern, and Mediterranean countries, and North and Central Africa. As a result of migration, however, these conditions are found all over Europe.<sup>177</sup>

### European research contributions

The natural history of these diseases has changed significantly in Europe during the past decades due to three main reasons:

1. carrier screening and prenatal diagnosis;
2. advances in diagnosis and conventional treatment;
3. HSCT.

*Carrier screenings and prenatal diagnosis:* a couple identified at risk for a severe form of thalassemia or hemoglobinopathy can be offered prenatal diagnosis to avoid the birth of an affected child. Prenatal diagnosis of thalassemia was introduced in Europe in the late 1970s, initially performed through globin chain synthesis on cord blood, and then in the 1980s by DNA analysis. Since then, the birth rate of children with thalassemia in Cyprus and Italy has dropped almost to zero. However, the wide variability of the phenotype of many mutation combinations demands great experience and counseling. Prenatal diagnosis may be difficult for religious and cultural reasons in recent migrants.<sup>178</sup>

*Improvement of conventional treatment:* for thalassemias, the improved understanding of the pathophysiology and the availability of safe and high-quality blood in Europe has allowed an optimal suppression of ineffective erythropoiesis by appropriate transfusion therapy. Iron chelation has had a major impact on morbidity and mortality in Europe. The standard chelation therapy for more than 40 years was deferoxamine, given by continuous subcutaneous infusion 5-7 days per week. The long-term efficacy of deferoxamine has been extensively documented in large cohorts of patients. Unfortunately, long-term compliance with daily subcutaneous infusions is a serious limiting factor. This has led to identifying safe, effective oral iron chelators. At present, two oral iron chelators are available: deferiprone and deferasirox. Deferiprone was registered in Europe and only recently in the United States and Canada. Deferiprone may be more effective than deferoxamine in protecting the heart from the accumulation of

iron. Combined deferoxamine/deferiprone therapy is used in high-risk patients, such as those with heart iron or cardiac dysfunction. The more recent oral iron chelator, deferasirox, has been shown to be effective and safe in removing excess iron from different organs, including the heart. While the availability of oral iron chelators has improved patients' compliance, the introduction of non-invasive techniques to quantify tissue iron, especially MRI T2\* to measure myocardial iron, has significantly contributed to optimizing and intensifying iron chelation, reducing cardiac morbidity and mortality.<sup>179</sup>

*HSCT:* allogeneic HSCT in thalassemia syndromes has been increasingly successful during the past three decades, mainly in  $\beta$ -thalassemia major. Predictors of transplant outcome established by the Pesaro group categorized patients into three risk classes. The probability of thalassemia-free survival for patients under 17 years of age who receive the allograft from an HLA-identical relative is above 85% in class 1 or 2 patients and is much lower in young patients in class 3. The progressive adjustment of conditioning therapy in class 3 patients and in adults (over 17 years of age) has also significantly improved outcome in this class. HSCT from unrelated donors has a higher risk of acute and chronic graft-versus-host disease (GvHD), particularly in thalassemia. A recent study from Eurocord reported no mortality and better outcome in 33 patients with class 1 and 2 thalassemia who received cord blood HSCTs from HLA-identical siblings, suggesting that related cord blood HSCT is a safe procedure for thalassemia patients. The European experience in bone marrow transplantation (BMT) in thalassemia represents a milestone for thalassemia treatment in the world. HSCT in sickle cell disease (SCD) has also been developed in Europe, and recent recommendations have been proposed.<sup>180</sup>

### Proposed research for the Roadmap

*Boost transplantation options:* HSCT may be considered a definitive treatment for the major Hb disorders; however,

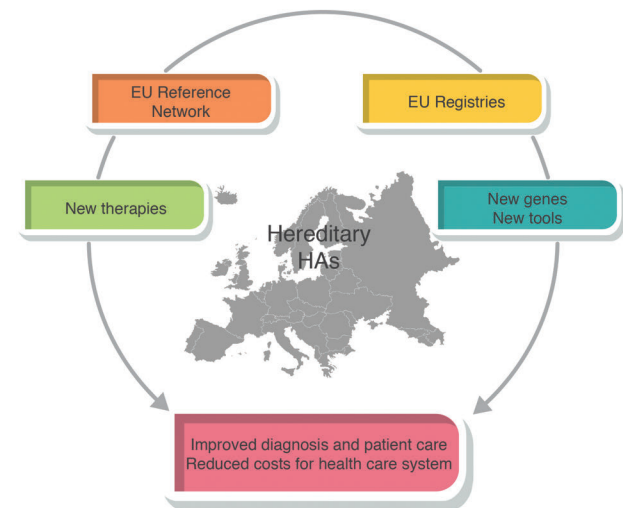


Figure 5. Proposed priorities for European research on hereditary hemolytic anemias.

most patients with thalassemia lack a compatible sibling donor and thus there is interest in using alternative donors. In this context, other approaches require further development:

1. matched unrelated donor;
2. matched unrelated cord blood;
3. mismatched related or haploidentical donors.

**Pharmacological intervention:** although there is currently no definitive treatment (with the exception of HSCT), the potential of correcting the globin chain imbalance through pharmacological intervention is an approach that holds tremendous promise and could lead to widespread therapeutic options for patients. This includes identification of new and potent fetal hemoglobin inducers or new molecules that may potentially modulate the ineffective erythropoiesis, such as agents that block the activity of certain TGF- $\beta$ -family cytokines, as well as JAK2 kinase inhibitors.<sup>181</sup>

**Gene therapy:** gene therapy is the major investment for the future for the cure of thalassemias and SCD.

**Quality of life (QoL):** more research work should be undertaken to investigate the impact of good social and psychiatric support in improving the QoL of this group of patients. Most thalassemia and sickle patients in Europe are immigrants who, in addition to their lifelong disease, are exposed to extra stress when they move to a new country with regulations and a social and cultural life that are different from that of their country of origin.

#### **Anticipated impact of the research**

During the past three decades, the improvement in diagnosis and management has enabled patients to live normal lives but has increased the economic and social burden. The cure of thalassemias and hemoglobinopathies represents an urgent medical need, not only for individual patients, but also for society as a whole.

#### **4.7. Iron overload disorders**

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#### **Introduction**

Iron overload represents a major health problem worldwide. Excess iron accumulates in vital organs of the human body and increases the risk for liver disease (cirrhosis or cancer), heart failure, diabetes mellitus, metabolic syndrome, osteoarthritis, and hypogonadism, and in some cases it causes premature death. Iron overload can be a consequence of inherited diseases, such as hereditary hemochromatosis, the most frequent genetic disorder in the Caucasian population (carrier frequency of 1:8). Similarly, patients with “iron-loading anemias” (e.g. with  $\alpha$ -thalassemia) present with elevated iron levels. Iron accumulates dramatically in patients that require regular blood transfusions. Furthermore, mild to moderate elevation of

tissue iron levels exacerbates the pathologies of common acquired diseases, such as chronic liver disease, diabetes, atherosclerosis, and cardiovascular disease. Iron misdistribution in the brain hallmarks the main neurodegenerative disorders (i.e. Alzheimer and Parkinson diseases).

#### **European research contributions**

Research into mechanisms that cause iron overload was fueled by the discovery of mutations in the *HFE* gene as the cause of hereditary hemochromatosis. Subsequently, European researchers identified more aggressive subtypes of hereditary hemochromatosis,<sup>182</sup> as well as novel disease entities characterized by iron accumulation (e.g. ferroportin disease).<sup>183</sup> The discovery by European researchers of the iron-regulatory hormone hepcidin and its target receptor ferroportin improved our understanding of how iron overload develops in hereditary hemochromatosis and provided new insights into mechanisms that underlie iron accumulation in blood diseases caused by insufficient or malfunctioning red blood cells (RBCs) [e.g. myelodysplastic disease syndromes (MDS) and thalassemias].<sup>180</sup> These anemic patients frequently require blood transfusions, which exacerbates iron overload. (One unit of RBCs contains 200-250 mg iron.) Iron overload causes oxidative stress, and up till now, use of phlebotomy and chelation therapies has been common to prevent iron toxicity. European research groups established disease models for iron overload disorders to identify mechanisms that control iron balance.<sup>184</sup> These important research findings not only gave an insight into the classical iron-related disorders, but also significantly improved our knowledge of how iron accumulates and contributes to the pathologies of acquired diseases, such as chronic liver disease, heart failure, and diabetes mellitus. Importantly, basic research into iron metabolism disorders was successfully translated into novel therapeutic opportunities. Together with European biotech companies, novel therapies were defined that are currently being tested in clinical trials.<sup>185</sup> Educational activities train young doctors and researchers in their understanding of iron-related disorders throughout Europe. Patients have organized themselves in the European Federation of Associations of Patients with Haemochromatosis.

#### **Proposed research for the Roadmap**

The past decades provided us with important insights into cellular and systemic iron metabolism that have improved understanding of the pathophysiology of iron overload disorders. Despite that, fundamental questions remain unanswered.

1. An improved understanding of the etiology and pathogenic mechanisms of iron overload in inherited disorders. We need to identify:
  - a) the signals sent from the erythroid compartment to regulate systemic iron homeostasis and how these signals are controlled and sensed by different organs;
  - b) how iron traffics inside cells;
  - c) how iron causes toxicity;
  - d) how different organs handle iron or heme that is released during hemolysis.
2. An improved understanding of clinical implications of iron overload in inherited disorders. We need to understand how iron influences the early stages of erythropoiesis and how iron overload damages ery-

thropoiesis in conditions such as  $\beta$ -thalassemia, as well as how the kidneys handle iron, which undergoes glomerular filtration and reabsorption with important physiological and potentially therapeutic implications. Similarly, the nervous system poses an enormous challenge. Despite the fact that patients with high plasma and systemic iron levels are protected from iron accumulation in the brain, local alterations of iron metabolism contribute to neurodegeneration. We need to devise strategies to prevent or ameliorate its course. We still do not fully understand the role that iron overload plays in inflamed or infected tissues and how this affects the immune system. The link between mild to moderate iron overload and type 2 diabetes or other highly prevalent insulin-resistant conditions (e.g. the metabolic syndrome) is increasingly recognized, but the molecular mechanisms underlying these associations are largely unknown.

3. An unanswered question remains as to how iron metabolism differs in the fetal, neonatal, and infant stages and how this compares with adulthood. Furthermore, there are uncertainties regarding when chelation therapy should be started and what the safe levels of body iron burden are. An improved understanding of basic cell biology principles of iron metabolism will allow further exploration of disease states and will help devise new therapeutic concepts.
4. Novel diagnostic means to identify patients with iron overload at risk of clinical progression and development of comorbidities. We need to identify modifier genes that affect the pathological course of iron overload disorders and that can be applied as diagnostic parameters. Epidemiological and prospective cohort studies are required to substantiate our knowledge of how perturbations of iron homeostasis are linked to disease progression and development of comorbid complications in other acquired disorders, such as cancer and cardiovascular, liver, kidney, or bone disease.
5. Clinical trials to evaluate the potential of targeted therapies in reducing systemic iron levels in widespread diseases, including atherosclerosis, diabetes, chronic liver disease, and neurodegenerative disorders. Integrated pre-clinical and clinical approaches are needed to continue the translation of novel targeted approaches that were designed based on the impressive progress in unraveling the regulation of hepcidin and ferroportin expression, in addition to commonly applied therapies, such as phlebotomy and iron chelation.

#### Anticipated impact of the research

Continued research into iron metabolism will discover novel iron-related genes and regulatory mechanisms that maintain iron homeostasis. This will improve our understanding of the etiology, pathogenic mechanisms, and clinical implications of iron overload in inherited disorders, as well as in those diseases where iron accumulates secondary to primary disease pathology (e.g. iron-loading anemias, dysmetabolic iron overload syndrome, atherosclerosis, and chronic liver disease). This is expected to have a major impact on the treatment of hereditary and acquired iron overload disorders. The identification of “iron signatures” in iron overload diseases will be an important diagnostic means of identifying patients with iron overload at

risk of clinical progression and development of comorbidities or to differentiate true iron overload from other disorders characterized by high hyperferritinemia. Clinical trials will evaluate the potential of targeted therapies in reducing systemic iron levels in widespread diseases, including atherosclerosis, diabetes, chronic liver disease, and neurodegenerative disorders.

#### 4.8. Anemia in the elderly

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##### Introduction

Anemia is associated with an increased risk of adverse outcome in older adults, including hospitalization, impaired cognitive capacities, diminished quality of life (QoL), frailty, and higher mortality. Analyses have revealed a prevalence of anemia (WHO definition: Hb <130 g/L for men; <120 g/L for women) of 12% in adults over 65 years of age living in the community, 40% in those admitted to the hospital, and 47% in nursing home residents (Table 2). Based on an overall proportion of 17% in the general population,<sup>186,187</sup> approximately 15 million elderly citizens (over 65 years of age) in the European Union are affected by anemia. Hence, anemia is a frequent condition in the elderly population, exceeding 40% in those aged 85 years or over.

Anemia has been associated with increased morbidity, mortality, and hospital stays. Despite this clinical importance, anemia in the elderly is often neglected, and evidence-based guidelines for diagnostic workups and individualized therapeutic algorithms are lacking.

Causes of anemia in the elderly include nutritional deficiency in approximately one-third (primarily iron deficiency), while one-third have chronic inflammation (ACD) or chronic kidney disease (CKD). While ACD is caused by cytokine- and hepcidin-mediated processes,<sup>188</sup> the underlying mechanisms of anemia in approximately 30% of cases remain unexplained.<sup>189</sup> Moreover, decreased testosterone levels have been considered as an underlying mechanism.

**Table 2.** Late-life anemia is frequent in the elderly. Numbers based on a cohort of 19,758 university hospital inpatients and outpatients.<sup>203</sup>

Age group (years)	Anemia prevalence*	
	Men	Women
64-69	18%	13%
70-74	21%	16%
75-79	25%	20%
80-84	34%	25%
85-89	40%	29%
>89	47%	33%
All	23.4%	19.3%

\*As defined by WHO (Hb <12 g/dL in women and <13 g/dL in men).

### European research contributions

The rational therapy of anemia is hampered by the difficulty of dissecting the underlying pathological mechanisms and the lack of evidence-based guidelines. Whereas a \$16 million grant supports studies of unexplained anemia in the United States (Partnership for Anemia: Clinical and Translational Trials in the Elderly; [www.agingportfolio.org/projects/project/5U01AG034661-02](http://www.agingportfolio.org/projects/project/5U01AG034661-02)), very few European programs have addressed issues of anemia in the elderly.

A network for the recognition, epidemiological surveillance, and medical education of rare anemias was established in Europe (ENERCA). The aim is to offer an improved public health service in rare anemias.

Based on clinical studies, several drugs have so far achieved approval by the European Medicines Agency, including different iron formulations and erythropoiesis-stimulating agents. Studies on promising drugs, including drugs directed at hepcidin,<sup>190</sup> TGF- $\beta$  superfamily ligand traps, and novel oral iron supplementations, are ongoing.

### Proposed research for the Roadmap

We propose to study the epidemiology of anemia in the elderly and to raise awareness among health care providers. The most essential objective is to develop and perform evidence-based clinical treatment strategies and health care interventions, which are based on a refined pathological algorithm. The innovative combination of new anti-inflammatory drugs and novel iron formulations will improve health care interventions in end-of-life anemia.

Objectives of the research agenda include the following.

1. Study Hb concentrations as well as the epidemiology and prognostic impact of anemia in distinct cohorts of elderly patients in select geographical regions throughout Europe. Structured evaluation of comorbidities and functional capacities will allow the estimation of the prevalence and relative contribution of different chronic diseases and frailty on Hb concentrations.
2. Raise awareness of the clinical relevance of a structured workup, diagnosis, and treatment. A European competence network on "anemia in the elderly" encompassing relevant stakeholders, such as physicians, researchers, health care providers, regulatory institutions, and patient groups, for the dissemination and utilization of up-to-date, evidence-based recommendations will be established. The network will actively facilitate the diffusion of evidence-based guidelines among patient organizations and European anemia study groups.
3. Develop a new pathological classification of anemia, particularly addressing unexplained anemia, in different patient groups. This will be based on parameters such as hepcidin, erythroferrone, cytokine, and hormone levels in combination with established hematologic parameters such as serum ferritin, reticulocyte count, Hb concentration, and soluble transferrin receptors.
4. Infer individualized algorithms based on these refined laboratory analyses that will assist differential diagnosis and rational treatment for the principal therapeutic interventions in anemia:
  - a) supplementation of oral or IV iron;
  - b) erythropoiesis-stimulating agents;
  - c) antihepcidin strategies; and

- d) new drugs, such as TGF- $\beta$  superfamily ligand traps.
5. Exclude congenital disorders of erythropoiesis, including thalassemias and congenital dyserythropoietic anemias (CDAs), because inherited anemias might represent an underlying cause of anemia.
6. Evaluate the effect of these tailored health care interventions on Hb levels, survival, and other health-related outcomes. Patient-reported outcomes, including scores to assess fatigue and quality of life (QoL) and objective functional parameters (e.g. gait speed), will be included and analyzed.

### Anticipated impact of the research

It is anticipated that a refined definition of the causes and pathological classification of anemias in the elderly will widen our understanding and achieve the following goals.

1. Develop and distribute new health care interventions in anemia:
  - a) develop and validate simple and evidence-based guidelines for diagnosis and treatment of anemia in the elderly, with particular consideration of gender aspects;
  - b) improve therapeutic outcome by clinical studies;
  - c) reduce morbidity and mortality related to anemia in a group of vulnerable patients through tailored treatment.
2. Develop an algorithm for intervention in cases that are likely to succeed.
  - a) exclude inherited anemias;
  - b) identify early patients in whom specific strategies are ineffective or even harmful;
  - c) develop a predictive model to classify patient populations with high likelihood of treatment response.
3. Assess and improve clinical outcome of anemia by generation of new core outcome sets:
  - a) raise awareness of novel therapeutic options for anemia;
  - b) generate clinical evidence even in non-fit elderly patients displaying comorbidities;
  - c) comprehensively address patients' needs and support treatment algorithms by using parameters that are relevant to the patient and society, including patient-reported outcomes, QoL, and functional capacities;
  - d) generate new and innovative core outcome sets and add them to classical parameters;
  - e) form the basis for cost-effective use of diagnostic and health care resources for diagnosis and treatment.
4. European competitiveness in the field includes the following:
  - a) generate data to form the basis for a rational design of clinical trials to apply for European Medicines Agency registration and marketing of novel therapeutic substances;
  - b) generate a European competence network on "anemia in the elderly."

### 4.9. Sickle cell disease

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### Introduction

Sickle cell disease (SCD) is a rare hereditary red cell disorder caused by a point mutation in the  $\beta$ -globin gene that results in the synthesis of pathological hemoglobin S (HbS). The term SCD is used to indicate different genotypes that cause the characteristic clinical syndromes (SS, SC,  $\beta$ S).

SCD is a chronic and disabling disorder, that is still associated with a high mortality. In the past two decades, due to immigration fluxes from areas endemic for SCD, SCD has spread throughout the European Union (EU). The prevalence of SCD newborns and SCD carriers in the EU is approximately 1-5 in 10,000 and one in 150, respectively. Epidemiological predictions suggest an increasing global burden of SCD between 2010 and 2050, making SCD an emerging public health problem with limited therapeutic options. In fact, the years lived with disability for hemoglobinopathies and SCD is estimated to be 10,197, which is a dramatic observation, because the years lived with disability for cardiovascular disorders is 21,985. In the past two decades, the survival of children with SCD has significantly improved due to the introduction of hydroxycarbamide therapy and the comprehensive care provided through pediatric age to adulthood. However, the shape of survival curves for SCD has not been affected by these therapeutic approaches and has been shifted to the second and third decade of life, showing a mortality rate that remains high for young adult SCD patients.<sup>191-195</sup>

### European research contributions

Historically, SCD was first described by James Herrick in 1910. The identification of the molecular defect and the characterization of the peculiar biochemical properties of pathological HbS that polymerize when deoxygenated have definitely opened a new era of research in the SCD field. Studies on red cell membrane physiology in SCD have allowed the identification of the key membrane ion systems involved in the generation of dense dehydrated red cells, which play an important role in the pathogenesis of acute and chronic clinical manifestation of SCD. Increasing fetal Hb levels was explored as a therapeutic strategy to delay HbS polymerization. This resulted in the introduction of hydroxycarbamide treatment for SCD patients with improvement of patients' survival and ameliorations of clinical outcome, such as pain crisis rate and chronic complications with some impact also on their quality of life (QoL). European research contributions have included: 1) the long-term evaluation of hydroxycarbamide in treatment of pediatric and adult patients with SCD; 2) the development of comprehensive sickle cell centers for multidisciplinary care of SCD patients; and 3) the development of hematopoietic stem cell transplantation (HSCT) programs for pediatric patients with severe forms of SCD. In addition, EU research has contributed to the development of the first transgenic mouse model for SCD, which has improved knowledge of the disease, starting from identification of new mechanisms of disease to testing new therapeutic targets in SCD. Furthermore, the availability of animal models for SCD has allowed the European research community to open new high-risk and high-innovative therapeutic approaches to SCD through the development of different strategies for gene therapy in the disease. This has recently been moved from benchside to bedside in an ongoing trial in patients with various

hemoglobinopathies and also SCD. The European scientific community has also contributed to the definition of the biocomplexity of SCD, which involved chronic inflammation, vasculopathy, oxidative stress (i.e. free heme), and cytokine storm, as well as to the clinical definition and characterization of severe chronic organ complications related to SCD, such as pulmonary hypertension and cerebrovascular disease. In conclusion, European research has made an important contribution to state-of-the-art advances in the field of SCD and to a widening of knowledge of the disease within the international scientific community.

### Proposed research for the Roadmap

Although significant progress has been made in our knowledge of SCD, treatment strategies remain unsatisfactory for both acute and chronic clinical complications. Thus, future research horizons in the disease should face up to that SCD biocomplexity which makes it a multiorgan disease.

The recent development of high-throughput techniques for both molecular and protein analysis, integrated with a rigorous phenotype characterization of the large EU SCD cohort of patients, will allow us to identify new biomarkers of disease severity to generate subgroups of patients to be driven to personalized medicine (secondary outcomes: SCD EU registry, biobanking).

These will also allow us to select new targets for future development of new therapeutic options. The presence of both *in vitro* and *in vivo* animal models for SCD within the EU research teams will ensure the exploitation of new therapeutic approaches will lead to clinical trials in the near future. These might involve SCD vasculopathy, chronic hemolysis, free heme, or novel fetal hemoglobin inducers.

In addition, the novel imaging techniques developed for functional studies will help the scientific community learn more about the disease and the management of SCD with the generation of new diagnostic approaches to organ damage to facilitate early treatment. This will have an impact on the natural history of SCD and improve the survival of adult patients.

### Anticipated impact of the research

Future European research programs on SCD will widen our knowledge of SCD and will deliver: 1) new therapeutic molecules for clinical management of SCD; 2) new profiling of disease severity for personalized medicine; 3) optimization of SCD patient care; and 4) clinical trials addressing basic aspects of clinical care.

These will positively affect: 1) patient health outcome and QoL; 2) national and European health systems by reducing hospitalization lengths and care costs; and 3) national and European welfare spending due to the reduction in disabilities of SCD patients and in the level of sick leave from work.

In addition, the novel identified therapeutic molecules would have an impact on the management of SCD in other high-income countries, such as the United States, as well as in low- and middle-income countries, such as

Africa and India, where SCD is a significant health problem. These will also stimulate public-private partnerships for orphan drug development largely based on innovation, generating new opportunities in the context of international competition.

## The EHA Roadmap for European Hematology Research

### Section 5. Platelet disorders

#### Section editor: Carlo Balduini.

Knowledge about platelet disorders has greatly expanded in recent years, and this topic has become one of the most complex in the field of hematology. Many new diseases have been identified, and a better understanding of old diseases revealed their complexity in terms of etiology, pathogenesis, and clinical features.<sup>196-200</sup> Moreover, we are now recognizing that the peculiar genetic background of each patient may modulate the clinical expression of both acquired and inherited platelet disorders,<sup>201</sup> while even slight differences in the mutations affecting one gene may be the origin of quite different clinical pictures in subjects with inherited thrombocytopenias (ITs).<sup>202</sup> Finally, the therapeutic armamentarium has been enriched with new, targeted drugs interfering specifically with the pathogenic mechanisms of diseases and promising to modify their natural history.<sup>199,203-205</sup> Thus, platelet disorders have truly entered the era of personalized medicine, and European researchers have played a key role in achieving this goal.

Despite the remarkable advances made, the increase in knowledge has not gone forward at the same rate for the different diseases. For instance, the number of well-defined inherited forms of thrombocytopenia has increased from 3 to more than 20 in the past 15 years,<sup>196</sup> while that of inherited disorders of platelet function have changed little, and the majority of affected subjects still remain without a definite diagnosis because the nature of their illness has not yet been identified.<sup>206</sup> Also, some forms of acquired platelet disorders (APDs), in spite of their high prevalence, remain poorly defined; this is the case of, for example, platelet dysfunction in chronic liver and kidney diseases, for which we do not have a clear understanding of clinical relevance, standardized diagnostic methods, or validated therapeutic approaches. Drug-induced platelet dysfunction is even more important considering the impact it has on general health, especially with the increasing number of subjects receiving antiplatelet drugs for the prevention of thrombosis. In addition, for patients receiving drugs that interfere with platelet function, we have no evidence-based guidelines to help them deal with bleeding or hemostatic challenges. Another undefined issue of great clinical relevance is the differentiation between primary and secondary immune thrombocytopenia (ITP) and other forms of acquired thrombocytopenia, especially those associated with infection. Finally, new curative approaches for ITP based on restoring the immune dysregulation are required. Major outcomes of new therapeutic studies should be rooted in bleeding assessment and quality of life (QoL) more than on platelet count.<sup>207</sup> Given the great expertise being applied to platelet studies, stimulating European

research on these neglected topics is expected to lead quickly to a better management of these conditions, to the benefit of patients and the health care system.

Another major problem in the field of platelet disorders is the gap between what is done in everyday clinical practice and what should be done. Many diseases are rare or exceedingly rare, and awareness of these forms is not widespread in the medical community. As a consequence, affected patients often receive misdiagnoses and inappropriate treatments.<sup>208</sup> Moreover, when the right diagnosis is suspected, its confirmation requires tests that are available in only a few specialized laboratories.<sup>209</sup>

The resulting diagnostic delay due to logistic difficulties can even put the lives of patients at risk because, as in thrombotic microangiopathies, a very early therapeutic intervention maximizes the chances of survival.<sup>210,211</sup> Thus, creating a network of centers for the diagnosis of specific platelet disorders is expected to have a strong impact on the quality of care for affected subjects. Moreover, centralizing diagnosis may facilitate the creation of registries and conduct of collaborative clinical trials, which are essential for widening our knowledge and improving treatment of rare diseases (Figure 5).

#### 5.1. Congenital platelet disorders: number and function

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#### Introduction

Knowledge in the field of inherited thrombocytopenias (ITs) and inherited platelet function disorders (IPFDs) has greatly improved in the past 15 years. More than 20 new genes responsible for ITs and IPFDs have been identified, leading to the definition of novel nosographic entities and better characterization of some disease phenotypes. This has also provided novel information that widens our understanding of human platelet production and function. Despite impressive advances, many gaps still have to be filled. In a considerable proportion of patients, a definite diagnosis still cannot be made because their ITs and IPFDs are still unknown. For example, nearly 50% of patients with ITs are affected by disorders that have not yet been identified. Moreover, the clinical features of some disorders that have had their genetic defects identified remain poorly characterized, as the information currently available comes from studies conducted in select single families or small series, thus hampering a general representation of clinical features of patients and preventing a data-driven clinical management. Differential diagnosis of ITs and IPFDs is currently based on pre-genetic laboratory assays that are usually complex and only available in a few centers; in addition, the diagnostic significance of laboratory findings is often uncertain. Therapy of ITs and IPFDs also needs to be improved. For most patients, no evidence-based protocols are available for treatment of bleeding or management of the bleeding risk associated with hemostatic challenges. Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for a few, very severe forms, and alternative treatments are needed for patients with these forms for whom HSCT is not possible. Finally,

validated alternative options to platelet transfusions for the treatment of major bleedings or prophylaxis of hemostatic challenges are needed.

#### European research contributions

Researchers from Europe identified 15 novel genes responsible for ITs and seven genes causing IPFDs.

Through the construction of patient databases, European groups have provided a thorough definition of the mutation spectrum, clinical features, and genotype/phenotype correlations of some disorders, including *MYH9*-related disease, Bernard-Soulier syndrome, Glanzmann thrombasthenia, Wiskott-Aldrich syndrome/X-linked thrombocytopenia, and thrombocytopenia 2. European researchers have provided effective tools to standardize the diagnosis of both ITs and IPFDs; European networks have developed methodologies that combine phenotyping of IPFDs and application of next generation sequencing (NGS) to unravel the genetic complexity of these diseases.<sup>196,212,213</sup> By using *in vitro* models of megakaryopoiesis or animal models, European investigators identified the pathogenesis of several ITs and IPFDs, including congenital amegakaryocytic thrombocytopenia, disorders deriving from *FLI1* or *RUNX1* haploinsufficiency, and thrombocytopenia 2.<sup>214,215</sup> Groups from Europe contributed to the improvement of HSCT in Wiskott-Aldrich syndrome or congenital amegakaryocytic thrombocytopenia, performed the first clinical trials of gene therapy for Wiskott-Aldrich syndrome, and successfully tested a drug for increasing platelet production in one form of IT.

#### Proposed research for the Roadmap

*Identify new genes responsible for ITs and IPFDs:* the first step toward this goal is the recruitment of large series of patients with ITs and IPFDs of unknown genetic origin through international co-operative efforts to collect biological samples and clinical data of patients. Application of NGS approaches (whole-exome or -genome or RNA sequencing) appears the most powerful tool for identifying candidate genes. The demonstration of pathogenicity of genetic variations requires functional studies that preferably use *in vitro* models of human platelet biogenesis or, alternatively, animal models (although these do not always reproduce the phenotypes observed in IT/IPFD patients).

Establish national and international registries of patients with known genetic defects: registries should be aimed at two main objectives.

1. To define the clinical consequences of the mutations responsible for ITs or IPFDs by the systematic investigation of large series of consecutive patients. This concerns not only the disorders that will be defined by the recognition of new causative genes, but also some ITs and IPFDs with known molecular defects but a yet poorly characterized clinical picture.
2. To define evidence-based protocols for the management of bleeding risk.

Optimize *in vitro* models of human platelet biogenesis (megakaryopoiesis and platelet formation) and platelet function for pathogenetic investigations and pre-clinical studies of novel therapies. We have identified three priorities.

1. To optimize *in vitro* models of platelet biogenesis obtained by peripheral blood of IT and IPFD patients. Patient-derived models are those that more closely reproduce human diseases. Because marrow sampling is often not feasible for ethical reasons, improved *in vitro* models obtained by circulating progenitors or using induced pluripotent stem cells (iPSCs) derived from peripheral blood should be prioritized. The establishment of a patient-derived European bank of iPSCs would provide an unlimited source of cells for pathogenetic studies.
2. To develop models of platelet biogenesis that reproduce as close as possible the bone marrow microenvironments: the endosteal niche that regulates megakaryocyte differentiation/maturation and the vascular niche where platelets are released into flowing blood.
3. To improve models that reproduce the conditions of human circulation to investigate platelet function defects.

*Validate single-step sequencing approaches for diagnosis of ITs and IPFDs:* the single-step sequencing of all known IT/IPFD genes (or whole-genome sequencing) as the first diagnostic approach for patients with ITs or IPFDs may prove more effective and cheaper than the complex and time-consuming sequence of traditional assays currently in use.

*Identify and/or develop novel therapeutic options:* these include the following.

1. Defining the role of THPO mimetics and other drugs that stimulate megakaryopoiesis with different mechanism in the treatment of ITs.
2. Promoting studies of gene therapy for the most severe forms of IT and IPFD.

#### Anticipated impact of the research

Identifying new genes responsible for ITs and IPFDs will increase the number of patients for whom it is possible to make a molecular diagnosis and will lead to the identification of new key players in platelet production and function. Validating single-step sequencing of all the causative genes as the first-line diagnostic approach for IT and IPFD patients will make it easier and more effective to reach a molecular diagnosis. The definition of the clinical phenotypes deriving from the different mutations is the basis for providing patients with a personalized, genotype-driven prognostic assessment, and, therefore, to set the appropriate follow up, choose the best treatments, and offer correct genetic counseling. Optimizing *in vitro* models of platelet biogenesis and function will provide powerful tools to validate the pathogenicity of the genetic variations identified by NGS, identify novel therapeutic approaches, and test them in pre-clinical studies.

#### 5.2. Acquired non-immune thrombocytopenia and acquired disorders of platelet function

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## Introduction

The understanding of the regulation of platelet function and number has increased enormously during the past decade. However, most of the advancements come from the study of inherited platelet disorders or immune thrombocytopenia (ITP). In spite of this, qualitative and quantitative APDs, different from ITP, are very common but relatively little-studied. Several systemic disorders of large epidemiological impact, such as chronic kidney and liver disease, are associated with APD and a bleeding diathesis. Despite the large epidemiological burden, the clinical relevance of APD is still unclear, and no consensus is available on its assessment and treatment. Moreover, many drugs and foods transiently modify platelet function and may provoke, especially when taken before surgery, increased bleeding. Finally, nonimmune thrombocytopenia (NITP) is very frequent in acutely ill patients, especially in association with infection, but not well understood due to its multifactorial pathogenesis, difficult differentiation from ITP, heterogeneity of involved pathogens, comorbidities, and the potential confounder represented by antimicrobial drugs.<sup>216</sup> Differentiation from ITP and other forms of acquired thrombocytopenia is a crucial issue because treatment is completely different.

## European research contributions

European researchers have strongly contributed to characterizing APD in chronic liver and kidney disease.<sup>217-219</sup> European investigators clarified, in particular, the role of altered platelet formation *versus* platelet destruction in thrombocytopenia and of *in vivo* platelet activation, platelet “exhaustion”, altered nitric oxide signaling, and enhanced nitric oxide formation in platelet dysfunction. Similarly, the role of uremic toxins and enhanced generation of nitric oxide in uremic platelet dysfunction have been shown by European investigators.<sup>218</sup> The bleeding risk associated with drug-induced platelet dysfunction in patients undergoing surgery and its management has been the object of position statements from European experts.<sup>219</sup> Finally, several studies have documented the strong and independent negative prognostic value of thrombocytopenia in critically ill patients.<sup>220</sup> Mechanisms causing pathogen-induced thrombocytopenias, including decreased platelet production by megakaryocyte invasion and/or enhanced platelet consumption by direct interaction with platelet receptors or by antiplatelet antibodies or immune complexes, have been described by European researchers.<sup>220</sup> European researchers have made an important contribution towards identifying the role of platelet toll-like receptors (TLRs) in the response to infection.<sup>220</sup> TLR expression enables activated platelets to bind and kill bacteria during sepsis and stimulate neutrophils to extrude their DNA-forming neutrophil extracellular traps that capture bacteria and perpetuate platelet activation, promoting thromboinflammatory processes or disseminated intravascular coagulation.<sup>220</sup>

## Proposed research for the Roadmap

*Clarification of the clinical relevance of APD in chronic liver and kidney disease:* uncertainty remains about the clinical relevance of APD in the hemostatic abnormalities of these chronic conditions. A large international, collaborative, prospective study on the prognostic value of a clinical bleeding risk score, previously employed in congenital hemostatic disorders and ITP, may clarify the clinical rele-

vance of the mild mucocutaneous bleeding diathesis typical of liver or kidney failure patients. Moreover, studies employing new and sensitive techniques for the assessment of platelet/vessel wall interactions and correlating the results with clinical bleeding may conclusively unravel the significance of APD in chronic liver and kidney disease. In addition, a rational diagnostic algorithm for the identification of clinically relevant APD needs to be generated. Finally, the best management of impaired platelet function and number needs to be established by prospective, European collaborative studies. Another issue to be developed is the role of platelets in liver regeneration and fibrosis.

*Drug-induced platelet dysfunction in patients undergoing surgery:* clinical relevance and treatment. The relevance of drug-induced platelet dysfunction in patients undergoing surgery and of its management may initially be evaluated by a large European retrospective survey assessing the relationship between pre-surgical drug intake and surgical bleeding. Previous experience from the EHA-SWG shows that surveys, although based retrospectively on clinical records, may provide clinically relevant information when the database obtained is sufficiently large. The survey will analyze different drug treatments and various types of surgery, classified as major or minor and by organs/systems. Another subject of future research is the possibility of guiding surgery and minimizing prophylactic platelet transfusions by pre-operative platelet function testing with point-of-care devices; appropriately designed, collaborative prospective studies will clarify this important issue.

*Infection-associated thrombocytopenia (IATP): mechanisms, diagnosis, and treatment.* Identification of the mechanism(s) leading to IATP (decreased platelet production, enhanced platelet consumption, or a combination of both), its diagnosis, and treatment are the crucial objectives of future research. The questions to be addressed are whether the dynamics of IATPs differ from other NITPs; whether platelet count profiles differ depending on the invading pathogen; if the immature platelet fraction is a reliable index of thrombopoietic activity; what the bleeding risk is depending on the degree of thrombocytopenia (using the ISTH score); if THPO receptor agonists are safe and may minimize prophylactic platelet transfusions in IATP; what the frequency is of antiplatelet antibodies/immune complexes in IATPs; what immune mediators are expressed by platelets during infection (cytokines, CD40, CD154, TLR, and P-selectin); what the expression profile is of platelet-, monocyte-, and endothelial cell-derived microparticles and their receptors; and what the platelet-induced inflammatory responses are that can be protective or detrimental to the host. To answer these questions, an IATP registry and prospective multicenter studies complemented by murine sepsis models will have to be established.

*The differentiation between ITP and NITP, clinical and laboratory diagnosis:* the rapid differentiation between ITP and acquired NITP is a crucial task of future research as the clinical management of the two conditions is very different. Better and more rapid tests for the confirmation of ITP are urgently required. In the area of pregnancy, a collaborative network for thrombocytopenia should be established to better document the natural history in mothers and fetuses.



### Anticipated impact of the research

The clarification of the clinical meaning of APD in disorders of large epidemiological impact will improve clinical knowledge in the complex hemostatic impairment of these diseases and provide a guide to their diagnosis and management. Moreover, understanding of the impact of drug-induced platelet dysfunction on surgical bleeding and its possible prediction by laboratory testing may greatly reduce surgical and cardiovascular morbidity and mortality. In addition, the proposed research will provide further insights into the mechanisms leading to thrombocytopenias associated with infections and improve their management. Finally, the differentiation between ITP and NITP will allow more appropriate and rapid treatment.

### 5.3. Primary and secondary immune thrombocytopenia and fetal neonatal alloimmune thrombocytopenia

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#### Introduction

Primary immune thrombocytopenia (ITP) is a bleeding disorder of unknown etiology characterized by low platelet count, immune-mediated platelet destruction, and reduced platelet production. As with many other autoimmune disorders, it is thought that environmental and genetic factors may incite the autoimmune response against some platelet and megakaryocyte membrane glycoproteins. Immune dysregulation manifests with a complex pathophysiological mechanism, including peculiar features of antigen-presenting cells and B and T cells. ITP occurs in all age groups, with half of patients older than 60 years of age and 20% older than 75. Data for estimation of frequency of this disorder are primarily from Europe and include an incidence of 1.9 to 6.4 per 100,000 children per year and 3.3 per 100,000 adults per year. In children, a minority of patients has severe bleeding and persistence of symptomatic thrombocytopenia, which is not predictable at an early stage of the disease. In adults, comorbidity and co-medication affect the bleeding phenotype, and there is an increasing number of patients (currently approx. 20%) with secondary forms of ITP, which have still not been well studied. Severe hemorrhages and fatal outcome increase with age and may become a cause of death in approximately 5%-10% of refractory cases. It has been realized that primary ITP is not a clinical-pathological entity, but rather has a heterogeneous background. Secondary ITP occurs in patients with malignancy, systemic autoimmune disease, chronic viral infection, primary immune deficiency, or drugs.

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) due to fetomaternal alloimmunization to paternal human platelet antigens (HPAs) is a rare disorder (incidence one in 1000 live births), but causes severe thrombocytopenia and a high bleeding risk in fetuses and neonates. Intracranial hemorrhage occurs in approximately one per 10,000 fetuses and neonates. Screening of all pregnant women for HPA alloimmunization has been suggested, because the risk of complications is very high and effective

intervention is available. The rarity of the disorder, and the fact that alloantibodies are not identified in many cases, makes FNAIT difficult to study except through large transnational collaborative groups.

#### European research contributions

For both ITP and FNAIT, European researchers are playing a key role in epidemiology and clinical research. There has been a significant increase in knowledge of the natural history of ITP based on registries. It has been recognized that research activity should be co-ordinated for an effective use of resources. These international collaborative activities have resulted in the development of practice guidelines; standardizing of definitions, terminology, and outcome criteria; therapeutic outcome measures, such as assessing bleeding; and co-ordination of research activities.<sup>221</sup> New innovative therapies have been successfully introduced and have questioned current therapeutic strategies and stimulated expert discussions.<sup>222,223</sup>

Despite the relative rarity of FNAIT, several important advances have been made in the past ten years. Firstly, new diagnostic methods, including fetal genotyping on maternal blood samples and antibody detection using aptamers or recombinant peptides, have been developed and are gradually being introduced into clinical practice. Secondly, improvements in the management of pregnant women with a positive history of FNAIT have resulted from systematic studies of the use of antenatal immunomodulation with IVIG in place of invasive treatment (*in utero* platelet transfusion).<sup>224,225</sup> Finally, large screening studies have shown the potential benefit of testing in combination with planned delivery and prophylactic perinatal platelet transfusion.

#### Proposed research for the Roadmap

The pathophysiological mechanisms of ITP and FNAIT are still not well understood and need to be studied in more detail in order to precisely diagnose the disease at an early stage, identify predictors of severe bleeding, and find novel treatments. In both ITP and FNAIT, the functional effects of putative or known auto- and alloantibodies on megakaryopoiesis and thrombopoiesis should be studied, as having a better understanding of this may help guide new treatment protocols with existing drugs as well as develop novel therapies.

*Pathophysiology of ITP:* new findings of immune responses in autoimmune disorders include the identification of the role of antigen-presenting cells, T and B cells, and their interactions. Regulatory T cells maintaining self-tolerance are involved in modulating ITP pathogenesis. Study of the increased platelet mass due to various therapies, and its role in presenting platelet autoantigens to T cells and potential effects on activating and suppressing regulatory T cells, may elucidate novel pathomechanisms. Improving the knowledge of the pathogenesis of ITP is an essential starting point for identifying effective diagnostic tests for this form of thrombocytopenia, a very important goal that has not yet been reached.

*Pathophysiology and management of FNAIT:* the assessment of platelet function in thrombocytopenic individuals is challenging and may be important for predicting bleeding risk. Thus, the development of functional assays for

diagnostic and management applications is needed. The application of high-throughput genomics to the identification of causal antibodies and/or antigens should be investigated. Effective therapies for antenatal and post-natal management should be developed and assessed. Some promising avenues include the development of modified HPA-1a recombinant antibodies and monoclonal antibodies to induce neonatal Fc receptor blockade. Studies in animal models and clinical trials are a priority for further development and testing of these strategies.

*Clinical proposals in children and adults with ITP:* a diagnostic algorithm in children and adults with chronic primary ITP should be developed to adequately diagnose patients at acceptable costs. Clinical research activity in all age groups is required to assess treatment end point alternatives or additional to the platelet count, such as bleeding and health-related quality of life (QoL), and to develop innovative treatments. Criteria for defining both the bleeding and the thrombotic risk, which seems to be increased in some cases, are required to develop personalized treatment strategies in all age groups of patients, particularly in elderly patients.

*New treatments of patients with ITP:* the introduction of innovative treatments, such as THPO receptor agonists, may change current therapeutic strategies, reducing the rate of splenectomies and reserving this intervention to the few patients who do not achieve a good QoL with medical treatments. The occasional observation that THPO receptor agonists may induce durable responses in a small subgroup of adult patients that have been weaned from therapy may reflect a tolerance-like activity of these drugs and should be studied in animal models. New curative approaches of ITP based on restoring the immune dysregulation are required. Outcomes of new therapeutic interventions should be rooted in bleeding assessment and QoL more than on platelet count measurements.

#### *Anticipated impact of the research*

The systematic study of immune response mechanisms in health, autoimmune (ITP), and alloimmune (FNAIT) disorders will provide more insights into a highly complex system and may identify, not only new diagnostic tools with the potential to define patient prognosis (mild and moderate disease or more severe forms with life-threatening bleedings), but also better and more personalized therapeutic approaches.

#### **5.4. Heparin-induced thrombocytopenia and other drug-dependent immune thrombocytopenias**

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##### *Introduction*

Drug-induced immune thrombocytopenias (DITPs) result from drug-dependent antibodies destroying platelets in the presence of drugs. DITPs are a major safe-

ty concern for patients and also for approval of new drugs. DITPs are: 1) life-threatening and require rapid recognition to allow appropriate measures to be taken to avoid harm for the patient; 2) iatrogenic adverse effects, with medico-legal implications; 3) relevant for drug approval, with a major economic impact on the development of new compounds. As drug-induced immune reactions are infrequent, they are typically recognized at a late stage of clinical development, or even only after approval. When they then cause the drug to be withdrawn from the market, several hundreds of millions of euros have often already been spent; and 4) DITPs are models to understand mechanisms causing the human immune system to attack self-proteins.

DITPs are based on different mechanisms: 1) the drug may alter the immune system that produces autoantibodies against several tissues; or 2) the drug or its metabolite(s) binds to platelets and thereby induces the formation of antibodies that cause platelet destruction. The most frequent DITP is heparin-induced thrombocytopenia (HIT).<sup>226</sup> HIT is currently the underlying cause of more than 95% of all confirmed DITPs. Whereas most DITPs increase the risk for bleeding, HIT is prothrombotic and affects 0.5% of intensive care patients and approximately 1%-3% of cardiac surgery patients. By conservative assumption, the incidence of HIT is 1:10,000 in-hospital patients, making DITP a substantial health issue in Europe.

Diagnosis of DITPs is based on clinical criteria followed by laboratory confirmation of drug-dependent antibodies.

*Clinical criteria for non-HIT DITPs:* clinical criteria for non-HIT DITPs are the exposure to the candidate drug started approximately 1-2 weeks before the onset of thrombocytopenia and recovery from thrombocytopenia after discontinuing the candidate drug.

*Clinical criteria for HIT (e.g. 4Ts score):* clinical criteria for HIT include: 1) decrease in platelet count by more than 50% from the highest platelet count; 2) decrease in platelet count between days 5 and 10 after start of heparin; 3) often associated with new thromboembolic complications; and 4) no other obvious cause of thrombocytopenia.

These clinical criteria for DITPs are not very specific, and diagnosis requires confirmation of drug-dependent antibodies by laboratory tests. The currently available tests, however, have major limitations.

*Laboratory tests for non-HIT DITPs:* laboratory tests for non-HIT DITPs show low sensitivity (but high specificity), are restricted to specialized laboratories, and are poorly standardized.<sup>227</sup> In contrast, the widely available HIT laboratory tests<sup>228</sup> are easy to perform, show high sensitivity but a low predictive value, and have unsatisfactory specificity, whereas the much more specific functional assays are technically demanding and not widely available.

Open issues include the following.

1. There is a strong need for sensitive but also specific screening tests.
2. Access to appropriate testing is needed throughout Europe.

3. A better understanding of the pathogenesis to develop preventive measures is needed.
4. Current treatments of HIT and its clinical sequelae are a major cost burden for hospitals.

#### European research contributions

Several European groups made major contributions to the pathogenesis of DITPs<sup>229</sup> and developed test systems and treatment recommendations. Access of physicians and patients to appropriate laboratory testing is well developed in some European countries (e.g. France, Germany, Austria, Switzerland, the Netherlands, and the UK).

#### Proposed research for the Roadmap

*A better understanding of the pathogenesis of DITPs:* understanding the pathogenesis of DITPs is important not only for drug development but also for gaining new insights into pathological states of the immune system.

1. With regard to drug development, it is of major relevance that several polyanionic drugs, including DNA/RNA-based<sup>230</sup> and polysaccharide-based drugs, can induce a HIT-like syndrome. *In vitro* assays able to predict potential immunogenicity of polyanionic compounds based on their interaction pattern with PF4 are needed for the development of polyanion-based drugs, such as RNA aptamers and antisense drugs, and the rapidly developing field of carbohydrate engineering, which allows screening of new drugs for their immunogenicity during pre-clinical development.
2. The immune response to PF4/polyanion complexes is so frequent that this immune reaction can be studied systematically in humans. This may be instrumental to better understanding which factors lead the immune system to develop antibodies against endogenous proteins other than PF4.
3. Recent data make it highly likely that PF4 is a protein involved in pathogen host defense. It acts as a danger signal for the immune system. Further understanding of these mechanisms could optimize antibacterial and antiviral treatments and even identify new strategies for anti-tumor treatment.
4. For the other drug-dependent thrombocytopenias, first evidence suggests that the immune reaction is caused by binding of the drug to the hypervariable region of a pre-formed IgG antibody, which thereby gains high affinity to an endogenous protein. A better understanding of this mechanism may become very important for understanding other autoimmune disorders.

*Improvement in diagnostic methods of DITPs:* widely applicable assays for drug-dependent antibodies are needed. Especially for HIT, an easy-to-apply assay with a high positive predictive value is one of the main needs in most laboratories. Establishing networks in Europe providing rapid access to diagnostic assays for DITPs with locally available screening tests and a rapid turnaround time, followed by confirmatory tests with high specificity, will be a solution for a currently unmet need.

#### Anticipated impact of the research

Adverse immune reactions are the biggest threat for the development of new biotherapeutic drugs. Understanding

the underlying mechanisms will not only improve patient safety but will also strengthen the European biopharmaceutical industry. All DITPs are caused by antibodies reacting with endogenous (self) cells. Identifying the underlying mechanisms at the molecular level will help to understand mechanisms of autoimmunity.

#### 5.5. Thrombotic thrombocytopenic purpura and other thrombotic microangiopathies

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#### Introduction

Thrombotic thrombocytopenic purpura (TTP) is a form of thrombotic microangiopathy (TMA) characterized by microangiopathic hemolytic anemia (HA), thrombocytopenia, and organ failure of variable severity generated by microvascular aggregation of platelets causing ischemia in the brain, heart, kidneys, and other organs. TTP is distinct from other TMAs such as the hemolytic uremic syndrome (HUS), in which renal involvement due to fibrin-rich thrombi is the prominent feature, or the HELLP syndrome in pregnant women. TMAs are very rare: the incidence of TTP is between 6 and 10 per million of the population, and for atypical HUS (aHUS), approximately 0.5 per million of the population.

Thrombotic thrombocytopenic purpura is usually idiopathic, although it can also occur in association with HIV infection, connective tissue disease, pregnancy, or cancer. TTP is most frequently an acquired disorder, but rarely it derives from inherited defects in *ADAMTS13*, which may manifest itself not only in childhood but also in adult life, sometimes in association with pregnancy.

Early recognition and treatment of TMAs is essential, because they are most often fatal when left untreated. Moreover, an early differentiation among the different TMAs is crucial given the availability of targeted therapies for each form.

#### European research contributions

In the past 15 years, advances have delineated the molecular mechanisms of most of the TMA syndromes, including TTP, aHUS, and the HELLP syndrome, providing evidence that they are caused by distinct molecular defects.<sup>231</sup> In particular, complement dysfunction and immune-mediated *ADAMTS13* deficiency are responsible for aHUS and TTP, respectively. The novel concepts and disease mechanisms identified in the laboratory were rapidly and successfully transferred into the clinic for the benefit of patients, and recent studies reporting on the use of monoclonal antibodies in the management of TTP and HUS provided convincing examples of translational medicine. Indeed, the B-cell depleting monoclonal antibody rituximab successfully treated refractory or relapsing acquired TTP.<sup>232</sup> The results of two international studies involving multiple European teams clearly indicated that the complement blocker eculizumab represents a breakthrough in the management of aHUS by preventing the evolution to end-stage renal disease and allowing dialyzed patients to have a successful kidney transplant.<sup>233</sup> Therefore, the rapid distinction between TTP and aHUS at the time of diagnosis is now mandatory.

From the research point of view, TTP represents a relevant model to better understand the interrelations between microbes, other environmental influences, the immune system, and the endothelium within a still uncharted specific genetic background. In this regard, three European groups reported independently that HLAs DRB1\*11 and DQB1\*03 were both susceptibility alleles for acquired TTP and confirmed the protective role of DRB1\*04.<sup>234</sup> Future large-scale studies should lead to the identification of additional genetic risk factors associated with acquired idiopathic TTP and in other forms of TMA.

The ability to increase our knowledge and experience in the field of TMAs was challenged in the past by the low incidence of these diseases and their clinical heterogeneity. Several national groups have recently set up large registries that include hundreds of patients with various forms of TTP, however, and these reports have shed light on the epidemiology, clinical presentation, prognosis, and long-term outcome of the disease.<sup>235</sup> This provides evidence that collaborations at the national and international level remain key to the continued advancement of the knowledge and treatment of rare diseases. Collaborative works have progressively led to the proposal of consensual treatment modalities and the definitions of treatment responses based on large series of patients. Though arbitrary and based only on clinical experience, these definitions are progressively and advantageously shared by different groups and may foster a common language that can allow fruitful meta-analyses in the future.

#### *Proposed research for the Roadmap*

1. Raise awareness of TMAs through multidisciplinary educational programs for general medicine physicians, emergency department physicians, and all other specialists possibly involved in the management of TTP. Create a network of laboratories to facilitate patients' access to specific tests (ADAMTS13 measurement and genetics and complement genetics) to prove the nature of their diseases and personalize treatment.
2. Identify tools for quickly distinguishing TTP from HUS and other TMAs in order to use early targeted therapies for each form of TMA.
3. Develop an international registry for TTP to identify:
  - a) the genetic risk factors that, interacting with environmental factors, are responsible for the onset of TTP;
  - b) relevant early prognostic factors to adapt treatment to the severity of the disease;
  - c) parameters that identify patients at risk of relapse (e.g. decreasing ADAMTS13 levels in remission).
4. Organize international clinical trials to:
  - a) identify the efficacy of early introduction of targeted therapies (e.g. B-cell depleting therapies for TTP and complement blockers for HUS);
  - b) test the effect of innovative, promising compounds (e.g. recombinant ADAMTS13 and blockers of the von Willebrand factor-glycoprotein Ib/IX pathway);
  - c) verify the efficacy of B-cell depleting therapies in the prevention of TTP relapses.

#### *Anticipated impact of the research*

The improvement of knowledge of TTP and other TMAs at a multidisciplinary level will increase their diag-

nosis in emergency situations, and undoubtedly improve patients' prognosis. The development of clinical trials at the international level (mandatory to achieve significant patient numbers given the rareness of TTP) will allow rapid evaluation of new strategies in the early management of TTP, and for the prevention of relapses. The understanding of genetic factors involved in the occurrence of autoimmune TTP, as well as the interaction between genetic and environmental factors will increase our knowledge about the initiation of the disease and, again, better prevention of its occurrence.

The proposed initiatives are needed to improve the knowledge of clinicians about this disease and related TMAs that require a diagnosis and an adapted treatment in emergency situations. Moreover, through collaboration with industry, Europe's role in the improvement of knowledge of TTP and its management will become more important, at a time when the field of rare diseases is becoming a major goal.

## The EHA Roadmap for European Hematology Research

### Section 6. Blood coagulation and hemostatic disorders

#### Section editor: Sabine Eichinger.

Thrombotic and bleeding disorders are a global disease burden with considerable morbidity and a high mortality. Estimates for the European Union (EU) arrived at a death toll of 500,000 venous thrombosis (VT)-related deaths per year.<sup>236</sup> About one in 300 people is affected by an inherited bleeding disorder. Uncontrolled bleeding is a major cause of death not only among these patients but also among those with an acquired bleeding disorder, including liver disease and severe trauma.

Europe has a long-standing tradition in basic, translational, and clinical science in practically all areas of blood coagulation and hemostatic disorders.<sup>237</sup> The identification of coagulation factors, their interplay within the clotting system, and the role of platelets in hemostasis are all seminal discoveries made in Europe.<sup>238-242</sup> Owing to the dedication and scientific curiosity of physicians in many European countries, the clinical aspects and pathophysiological features of genetic and acquired coagulation disorders have been described for the first time. Based on this knowledge, coagulation assays were developed and the foundation for standardized nomenclature in thrombosis and hemostasis was laid. Drugs that saved or improved the lives of millions of people worldwide, such as antithrombotics or procoagulants, have been developed by researchers based in Europe. Notably, these research activities are not clustered in certain parts of the continent but are ongoing across the whole of Europe, from the far north to the south, from east to west.

In the past decades, Europe has experienced exciting developments and dramatic breakthroughs that offer a myriad of possibilities to continue along the road of enlightenment in order to unravel still hidden secrets of thrombosis and hemostasis, thereby further improving the management of the diseases. Moreover, we saw changes and will see even more so in the upcoming years throughout the continent due to an expanding EU, an aging population, increasingly self-determined patients, intra- and interconti-

mental migration, and a globalization that facilitates collaboration with research institutions also outside Europe. These developments pose substantial challenges to researchers, physicians, and health authorities alike, but at the same time pave the way for exploring novel tools and approaches in research and treatment options. Increasing demands of regulatory authorities and decreasing financial resources also weigh heavy on the shoulders of the academic research community in particular.<sup>243</sup> The burden can be alleviated at least to some extent by defining needs and major goals, focusing on main research questions, streamlining resources, combining efforts, and intensifying collaborations.

Scientists and physicians working in the field of blood coagulation and hemostatic disorders are privileged by the fact that bleeding and thrombotic disorders are of utmost relevance in almost all medical disciplines, which *per se* fosters collaboration and provides a fruitful and inspiring atmosphere for research.

The following chapters give an overview of the developments and multi-disciplinary aspects of hemostasis and thrombosis. Although hematologists concern themselves with clinical, consultative, and research aspects of arterial thrombosis, including acute coronary syndromes and cerebral infarction, we will focus here on venous thromboembolic diseases and bleeding disorders. We describe major needs and cutting-edge questions in basic and clinical research, and provide a view not only of the future but also beyond.

### 6.1. Genomics in hemostasis

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#### Introduction

Inherited disorders of the hemostatic system can be divided into those that increase the risk of bleeding and those that increase the risk of thrombosis. Common inherited bleeding disorders include the hemophilias and von Willebrand disease, but a multitude of rare inherited bleeding disorders involving blood coagulation factors or platelets also amount to a significant disease burden. Today, we have good evidence that the severity of the bleeding phenotype can be modified by multiple common genetic variations, such as the ABO blood group and the activation status of platelets, as well as other confounders.

Severe inherited disorders that lead to thrombosis are quite rare and mostly limited to homozygosity or compound heterozygosity for loss of function mutations in genes encoding natural anticoagulants. Lifetime risk of thrombosis is influenced by inherited risk factors, such as factor V Leiden; PT20210A; genetic variations in platelet glycoproteins, such as P-selectin; and also the ABO blood group.

#### European research contributions

The field of human genetics in Europe has a proud his-

tory of leadership in the study of inherited hemostatic disorders. Technological breakthroughs were always quickly incorporated into European laboratories and clinics. For example, in the early days of recombinant DNA technology, restriction fragment length polymorphism-based diagnosis was immediately implemented for genetic diagnosis and counseling in hemophilia A and B.<sup>244</sup> Common risk factors for VT were among the first risk factors for complex diseases to be characterized in detail and applied for risk stratification, and most of the research in this area was led by European research groups.<sup>245</sup>

#### Proposed research for the Roadmap

**Bleeding:** in hemophilia A, genetic imprinting studies and sequencing of the entire *F8* gene have shown that, in rare cases, mutations in intronic regions of the *F8* gene can cause a severe hemophilia phenotype. Furthermore, there are preliminary data to suggest that the severity of hemophilia is influenced by the number of circulating activated platelets present in the circulation. An answer to this important question can be obtained only if many hemophilia centers collaborate more actively. In addition, new knowledge has been obtained regarding the epigenetic modification and imprinting of the *F8* gene in females. However, it is currently unclear whether certain mutations (e.g. the intron 22 inversion) are associated with skewed imprinting. To clarify this, large numbers of patients with and without the mutation will have to be tested again, underlining the need for collaboration.

The diagnosis and study of rare bleeding disorders, including those involving blood platelets, will greatly benefit from the introduction of next generation sequencing (NGS) technology.<sup>246</sup> To take full advantage of this, a stable and sustainable database of genetic variation in hemostatic genes is required. There is also a need for standardization of clinical reporting of genetic test results for hemostatic disorders to have long-term stability of reference sequences to avoid confusion about “versioning” of DNA sequences, and the locus reference genomic sequences aim to achieve this.

**VT:** high-throughput genotyping technologies in the framework of genome-wide association studies have led to the identification of at least eight new loci associated with the risk of first VT.<sup>247</sup> However, the currently known genetic factors explain only approximately 5% of VT heritability.<sup>248</sup> An important question is how to discover the missing heritability. The following research strategies can be used to achieve this.

1. Increase sample sizes by pooling large collections of comparable data. Importantly, the number of variants discovered is strongly correlated with sample size. Thus, increasing the sample size will increase the number of discovered variants.
2. Risk factors for first and recurrent VT may not be identical. Identifying genetic risk factors specific for recurrence is important as it could influence treatment decision, in particular duration of anticoagulant treatment after a first VT event. To date, no genome-wide association study has been published on recurrent VT and there is a need for a collaborative effort to reach a sufficient sample size to approach this question.
3. Missing heritability for VT can be attributed to rare

variants (minor allele frequency less than 0.5%). Such variants are not sufficiently captured by current genome-wide association genotyping arrays. The best method for the detection of rare single nucleotide polymorphisms is sequencing (ideally whole-genome or alternatively exome) using NGS technology. These approaches should be scaled up both in families with a strong history of VT and in unselected individuals.

4. Missing heritability is not necessarily explained by simple Mendelian genetics. Epigenetic processes that influence gene expression may be highly important. Investigating these processes might provide new insights into the molecular mechanisms underlying VT, as recently suggested for other human diseases. For this purpose, specific high-throughput technologies are becoming available to quantify non-coding RNA expression and methylation profiles from cells, tissues, and blood.

The second goal for VT is to translate the genetic discoveries into useful clinical applications that lower the burden of thrombotic disease. This process should primarily focus on the prevention of secondary thrombosis after a first event. Clinical trials need to be designed that stratify patients with a first event based on genetic and non-genetic risk factors. Patients with a high risk should be treated differently from patients with a low or intermediate risk, and the effect of such triaging on the number of thrombotic and bleeding events should be recorded. This precision medicine approach should lead to guidelines for thrombosis prevention that are tailored to the individual characteristics of the patient. Research groups in Europe have a strong track record and the infrastructure to carry out such trials.

#### *Anticipated impact of the research*

With the advent of genome-wide association studies and NGS technologies, the introduction of genomic analysis in the clinical care of patients with inherited bleeding and thrombotic disorders is rapidly progressing, and there is a need to achieve the safe and appropriate introduction of this technology in clinical care delivery. In parallel, standardized and harmonized tools to collect clinical data should be developed, adopting an ontological approach, in order to attribute the right clinical significance to the specific genetic abnormality. This should lead to a lower disease burden and improved public health.

The discovery of the missing heritability in VT may point to genes that operate outside the canonical coagulation system. This may yield drug candidates that decrease thrombotic risk without increasing bleeding risk, which would revolutionize treatment and prevention.

## **6.2. Novel mechanisms for coagulation activation**

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### *Introduction*

Activation of the plasmatic coagulation cascade generates the key enzyme thrombin that is pivotal for converting the soluble plasma protein fibrinogen to fibrin and the activation of blood platelets. Together, these form

hemostatic plugs to seal sites of vascular injury. Thrombin is essential not only for hemostasis (the prevention of bleeding) but also for thrombosis (the formation of blood clots in vessels). Since the first description of the waterfall model of co-factor-amplified, consecutive proteolytic activation of coagulant protease zymogens 50 years ago, a wealth of biochemical data has delineated the details of blood clotting *in vitro*. Coagulation initiates through the cellular enzyme-co-factor complex of coagulation factor VIIa and tissue factor or factor XIIa that is activated in the context of contact with exogenous or endogenous polyanions, including DNA, RNA, and polyphosphates (Figure 6). The main routes of coagulation initiation have been refined by description of amplification loops connecting these pathways (e.g. the direct activation of the anti-hemophilic factor IX by tissue factor–factor VIIa or the feedback activation of factor XI by thrombin). Thrombin generation is tightly controlled by plasma serine protease inhibitors that prevent intravascular clotting, and thus thrombosis. Important information on the physiological regulation of coagulation initiation has been further uncovered in patients with thrombophilia and validated by *in vivo* studies in model organisms. Key roles are played by the vascular antithrombotic mechanisms provided by the anticoagulant thrombomodulin-protein C-protein S pathway and plasma- or platelet-derived tissue factor pathway inhibitor. Although the principles of initiation and regulation of coagulation are well laid out, much remains to be learned about the fine-tuning of these responses to vascular injury and the implications for normal hemostasis and pathological thrombosis.

### *European research contributions*

European scientists were major contributors to recent conceptual advances in our understanding of molecular connections between innate host defense mechanisms and coagulation, and the relevance of these interactions to thromboembolic disease. The factor XII-dependent contact pathway has experienced a flurry of research activities after the realization that factor XII and factor IX deficiencies, while causing minimal hemostatic impairments, confer resistance to vascular thrombosis in a variety of animal models.<sup>249</sup> Complementary research has identified pathophysiologically relevant activators of this pathway, including DNA and RNA released in the context of cell damage and polyphosphates derived from platelets and microbial pathogens.<sup>250</sup> Initial clinical proof-of-concept studies emphasize the feasibility of therapeutic intervention in the contact pathway for antithrombotic benefit without impairment of hemostasis during surgery.

In recent years, complex interactions of the coagulation cascade have been uncovered, not only with platelets but also with multiple intra- and extravascular cell types. These studies led to the realization that coagulation enzymes and their cognate receptors mediate crucial cell signaling events in angiogenesis, inflammation, and immunity.<sup>251</sup> Conversely, effector mechanisms of innate immunity couple coagulation with inflammation. Activation of immune cells by injury signals are known to trigger a variety of acute and chronic inflammatory diseases, but the same cellular signaling mechanisms are increasingly recognized as directly responsible for the generation of procoagulant tissue factor-bearing thrombin-

flammatory microparticles. In addition, the complement system serving host defense is connected through multiple reciprocal effects with the coagulation cascade. Complement activation not only enhances coagulation by perturbing membrane integrity, exposure of negatively charged phospholipids, and direct activation of coagulation factors, but also through thiol-disulfide exchange-dependent and protein disulfide isomerase-dependent activation of tissue factor on monocytic cells.<sup>252</sup> These redox and innate immune reactions are emerging as important regulators of both coagulation and platelet activation, and should remain a priority for future studies.

A key function of the coagulation system is to generate fibrin that serves as a transitional barrier at sites of injury. Fibrin formation also serves to wall off and protect the organism from invading pathogens. Novel surveillance functions of leukocytes have been defined that promote similar protective functions within the vascular bed. In these processes, termed immuno-thrombosis,<sup>253</sup> neutrophil-derived proteases degrade tissue factor pathway inhibitor leading to localized tissue factor-dependent coagulation and fibrin formation. Alternatively, neutrophils can respond under certain conditions with coordinated chromatin remodeling and expulsion of nuclear DNA that entrap intravascular micro-organisms. The formed neutrophil extracellular traps also serve as a matrix for platelet deposition and both extrinsic and intrinsic coagulation activation. These primarily host-protective mechanisms are now recognized as crucial contributors to the development of thrombosis, in particular in the venous system. Important research into the multicellular interactions in low- and high-flow vascular beds remains to be pursued to understand the unique causes of coagulation initiation in the venous and arterial system.

#### Proposed research for the Roadmap

Coagulation research has evolved novel technologies and *in vivo* and translational approaches to move from simple test-tube research to a detailed understanding of coagulation initiation in specific vascular and extravascular locations. This productive path should be continued with a special focus on understanding the common and discriminating regulatory mechanisms of coagulation activation in thrombosis *versus* hemostasis. The specific research areas with a high likelihood of return in resolving these fundamentally important questions are as follows.

1. Triggers, modulators, and regulators of intrinsic and extrinsic coagulation activation in the venous and arterial circulation.
2. Contributions of the coagulation system to the complex multicellular interactions of the blood and the vascular endothelium under physiological and pathological conditions, including cancer.
3. Thromboinflammatory circuits contributing to vascular dysfunction, thrombosis, or hematologic and immunological disorders.
4. Environmental, metabolic, age, and gender effects on the reciprocal interactions of the hematopoietic and the coagulation systems.

#### Anticipated impact of the research

These areas of research are highly significant for the rapidly evolving landscape of antithrombotic therapy. The availability of diverse target selective anticoagulants has already changed the practice of hematology, and future advances in anticoagulant therapy are on the horizon. The proposed research areas will have an impact on these developments by defining new interactions and pathogenic roles of the coagulation system in hemato-oncological

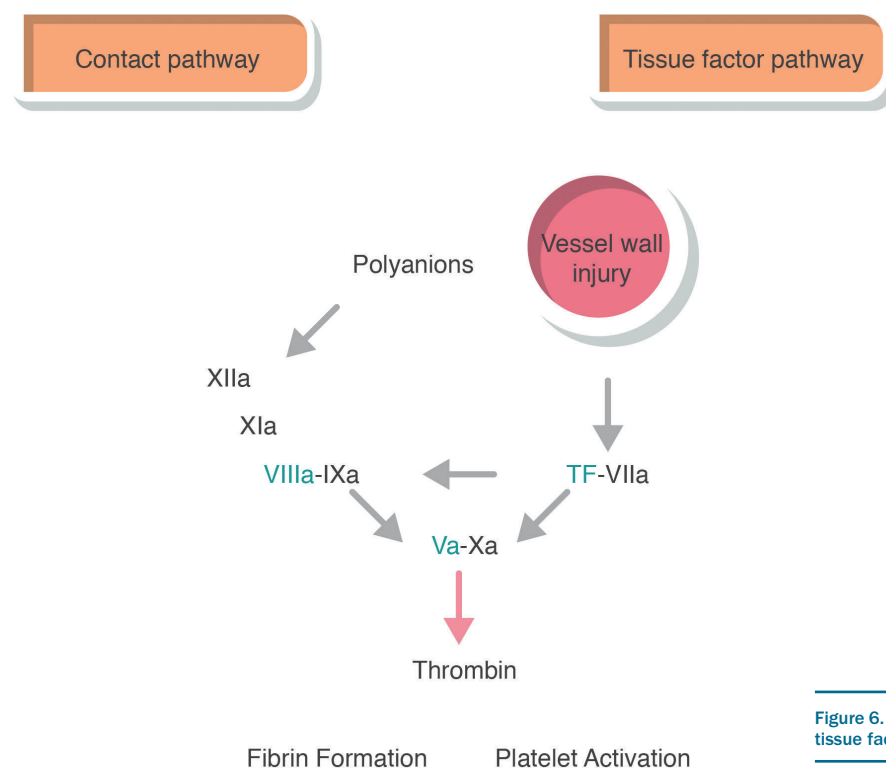


Figure 6. Synergistic activation of coagulation by contact and tissue factor pathways.

diseases and vascular medicine. New insights into coagulation-supported pathomechanisms can be used to tailor and individualize antithrombotic therapy with new anti-coagulants and may indicate additional areas for therapeutic interventions with antithrombotic drugs in more complex thromboinflammatory diseases.

### 6.3. Venous thromboembolism

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#### Introduction

Venous thromboembolism (VTE), a syndrome consisting of deep vein thrombosis and/or pulmonary embolism (PE), occurs in one to 2 per thousand people per year. VTE is one of the leading causes of death in Europe. It is estimated that in France, Germany, Italy, Spain, Sweden, and the UK together, VTE occurs in more than 750,000 people per year and that VTE-related death affects more than 370,000 individuals annually in these countries.<sup>256</sup> Millions of VTE events or deaths per annum are expected in the European Union. Most importantly, VTE is one of the best examples of a preventable disease as antithrombotic treatment is highly effective in both primary and secondary prophylaxis. It is therefore of major importance to improve the diagnostic and therapeutic strategies in order to reduce the number of people affected by this frequent and potentially fatal disease.

#### European research contributions

European scientists were responsible for many important breakthroughs in both basic and clinical thrombosis research. This subsection addresses only a few of them.

The recognition of important pathomechanisms leading to VT or PE has an essentially European origin. The German pathologist Rudolf Virchow the etiology of VTE was already clarified back in 1865, postulating that thrombi occurring within the veins, particular in those of the extremities, become dislodged and migrate to the pulmonary vasculature. A few years later, the French internist Armand Trousseau described for the first time the association between cancer and vessel wall inflammation due to blood clots, which are recurrent and appear in different locations over time. In modern thrombosis research, European scientists have greatly capitalized on advances in genetics to identify important determinants of VT, such as the factor V Leiden mutation and the G20210A mutation in the prothrombin gene,<sup>254-256</sup> which represent the two most frequent congenital risk factors of VT known today.

A tremendous amount of pioneering work in the field of antithrombotic treatment has been carried out in Europe. Sir John Vane discovered the mechanism by which acetylsalicylic acid (aspirin) inhibits platelet function, thereby paving the way for large clinical trials that the effectiveness of aspirin in reducing the incidence of both arterial and venous thrombosis on firm ground. As for the treatment of acute PE, the first (and for ethical reasons last) placebo-controlled trial was carried out in England to show that patients greatly benefit from anticoagulant treatment with heparin in terms of both morbidity and mortality. Only recently, European researchers were the masterminds behind the development of the novel anticoagulant drugs rivaroxaban and dabigatran, which selec-

tively inhibit distinct coagulation factors (factor Xa or thrombin), thereby protecting patients with VTE from recurrence to the same extent as the vitamin K antagonists but at a lower risk of (major) bleeding and without the need of regular coagulation monitoring.

#### Proposed research for the Roadmap

Venous thromboembolism is a multicausal disease, and its development can be explained by the interaction of various genetic and environmental risk factors. It is the main task of future thrombosis research to find strategies for identifying individuals/patients at risk for a first/recurrent thrombotic event on the basis of their risk factor profile, to delineate methods of prevention and prove that such a personalized medicine approach is beneficial. To accomplish this ambitious task, large clinical studies are required that enable differentiation between individuals at a high or low risk for thrombosis. In these studies, clinical and biochemical characteristics have to be complemented with genetic determinants of VTE. It is important to recognize that the development of VTE is to a great extent genetically determined. It is very doubtful that the presence or absence of a few, so far unknown, strong risk factors determines the thrombosis risk. It is much more likely that, according to the common variant–common disease hypothesis, VTE development is driven by common variants in many genes, which occur with a high frequency in the general population, with each variant at each gene exerting a small additive or multiplicative effect on the disease phenotype.<sup>257</sup>

Specifically, future research on VTE should do the following.

1. Explore the incidence of VTE in well-defined populations of patients or so far unaffected individuals by the use of prospective observational studies with large numbers of individuals included.
2. Identify single genes, networks of genes, and signaling pathways responsible for VTE development using genome-wide linkage association studies.
3. Investigate the relationship between VTE occurrence and hemostatic system activity by measuring (molecular) markers of platelet and coagulation activation.
4. Enable the construction of prediction tools that are capable of differentiating between high- and low-risk individuals on the basis of clinical, genetic, and molecular evidence. This should be achieved in all groups of patients with VTE including those with VTE provoked by surgery, pregnancy or trauma and also in patients with cancer.
5. Improve our knowledge on the association between hematologic malignant and non-malignant diseases and the risk of VTE.

Upon completion of these studies, findings have to be validated in separate studies, and eventually management studies will be required to prove that such a personalized risk stratification strategy is helpful.

#### Anticipated impact of the research

The large number of people suffering from VTE is a serious challenge to European health care systems now and will continue to be so in the years to come. The research proposed in this document will provide indications for



prevention in patients as well as in those up till now healthy individuals. It is a step-by-step plan that firstly consists of the definition of populations that might benefit, and subsequently of the estimation of risk based on clinical, molecular, and genetic features, eventually allowing a personalized prevention and treatment strategy. Only a concerted action that brings together basic scientists and clinically orientated researchers will eventually be successful at achieving such an ambitious goal.

#### 6.4. Venous thrombosis in aging populations

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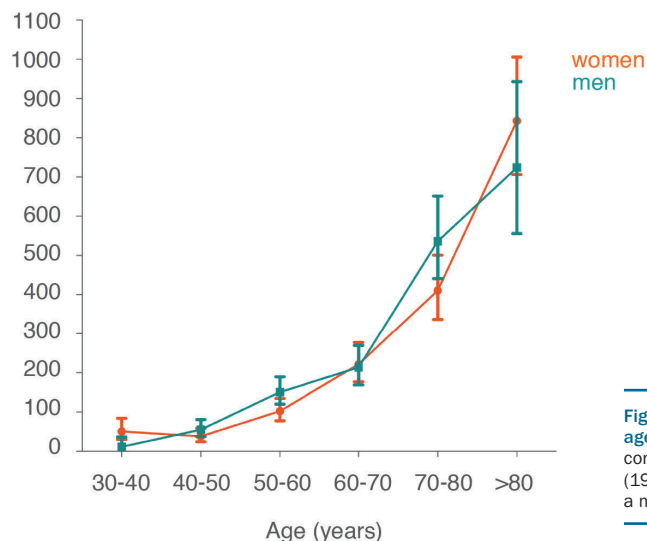
##### Introduction

Advancing age is associated with a shift of the hemostatic balance in a prothrombotic direction. The incidence of VT [e.g. deep vein thrombosis and pulmonary embolism (PE)] increases sharply with age. The overall incidence of first symptomatic VT is one to 2 per 1000 person-years in the general population, increasing from one per 10,000 person-years in the age group 25 to 30 years to 5-8 per 1000 person-years in those above 75 years of age<sup>258</sup> (Figure 7). Therefore, the risk of VT is 50- to 80-fold higher in the older population, leading to a high attributable risk for age-related factors. In the Scandinavian Thrombosis and Cancer study, a merged cohort including individual data from three large population-based cohorts in Scandinavia (the Tromsø Study, the HUNT Study, and the Danish Diet, Cancer and Health study), approximately 45% out of a total of 2444 subjects with a first VT were over 70 years of age. Based on these results, age accounted for 78% of the VT events occurring in this age group (attributable risk), whereas previous estimations of the population attributable risk were approximately 90%, indicating that 90% of total incidence of VTE could be explained by age-related factors.<sup>259</sup>

##### European research contributions

Despite a striking increase in the incidence of VT with age, few studies have addressed etiology and prevention strategies in the elderly. Some studies have reported age-related changes of the hemostatic system, such as increased plasma concentrations of procoagulant coagulation factors (fibrinogen, factor V, factor VII, factor VIII, factor IX, and von Willebrand factor) and inhibitors of the fibrinolytic system (PAI-1 and TAFI),<sup>260</sup> but none have investigated the interactive effect between age and these hemostatic factors on VT risk. A major proportion of the VT events (45%-60%) occur during or shortly after hospitalizations. However, the thrombotic risk associated with hospitalization has not been studied in the elderly. Because the incidence of hospitalization doubles with age, the population attributable risk is expected to be highest in the elderly (10% in the young and approximately 40% in the elderly).<sup>259</sup> Short-term bed rest in subjects over 65 years of age was associated with a 6-fold higher VT risk than in subjects without bed rest,<sup>261</sup> suggesting that a strategy to either avoid bed rest or offer prophylaxis will substantially affect the VT incidence in elderly. The overall risk of VT in cancer patients is 5- to 7-fold higher than in individuals without cancer, and cancer-related VTs account for 20%-25% of all VTs in the general population. Due to the high incidence of cancer in the elderly, it has been estimated that the population attributable risk for VT in cancer patients is 15% in the young and 35% in the elderly. Recently, Blix *et al.* calculated age-specific population attributable risks of VT due to cancer based on incidences of cancer and VT in the Tromsø Study and found a smaller actual difference than postulated between the young (<50 years, population attributable risk 14%) and the elderly (>70 years, population attributable risk 18%).<sup>262</sup> These findings show that malignancy does not explain a substantial proportion of the VT events in the elderly, and, most importantly, that studies focusing on risk factors in the elderly are urgently needed, because extrapolations do not suffice. A limited number of studies have investigated the impact of age-specific risk factor on VT risk, and prelimi-

Incidence rate per 100,000 person-years



**Figure 7. Venous thrombosis (VT) incidence increases with age.** Incidence rates per 100,000 person-years with 95% confidence intervals. Data are derived from the Tromsø Study (1994–2012), in which 26,853 individuals were followed for a median of 17.7 years, and a total of 710 first VTs occurred.

nary data have shown that thickening of the venous valves occurs with age, which may contribute to the high incidence of thrombosis in the elderly.

#### *Proposed research for the Roadmap*

The key question is: why does the incidence of VT increase with age? We believe that the answer is multifaceted and involves at least three fundamental aspects: 1) aging may be associated with an increased prevalence of conventional risk factors (e.g. immobility, malignancy, comorbidity, hemostatic factors, and genetic factors); 2) VT risk may be conferred by age-specific risk factors (e.g. reduced muscle strength, endothelial dysfunction, venous insufficiency, and frailty); 3) synergistic effects with age may occur for established risk factors, meaning that the impact of a risk factor on VT may differ between young, middle-aged, and elderly individuals.

The following issues need to be addressed.

1. Investigate the prevalence of established risk factors (e.g. immobility, malignancy, comorbidity, surgery, hemostatic factors, and genetic factors) in the elderly and their effect on the thrombotic risk in different age groups.
2. Identify novel risk factors of VT in the elderly by applying state-of-the-art technologies in imaging, biochemistry, genomics, genome-wide methylation patterns, and proteomics.
3. Investigate joint effects between age and thrombotic risk factors on VT risk.
4. Develop risk prediction models for VT in high-risk situations (e.g. malignancy, hospitalizations for acute medical conditions, and surgery) specifically for the elderly.
5. Identify age-specific risk factors (e.g. muscle strength, endothelial dysfunction, venous insufficiency, and hemodynamic changes) and elucidate underlying mechanisms for VT risk.

These issues should be addressed in observational studies with validated information on exposures, effect modifiers, and end points, and with no upper age limit. Risk prediction models should be developed and validated in prospective cohort studies.

#### *Anticipated impact of the research*

The total suffering and economic burden caused by VT is tremendous, particularly among the elderly. VT is associated with short- and long-term complications, such as fatal or non-fatal recurrence (affecting 30% of the patients within 10 years), post-thrombotic syndrome (affecting 25%-50% of VT patients), and pulmonary hypertension, all of which severely impair mobility and quality of life (QoL). Extrapolated data from six EU countries estimated that a total of 680,000 deep vein thrombosis events, 430,000 PE events, 610,000 post-thrombotic events, and 540,000 VT-related deaths occur in the EU each year.

As the European population is becoming older, the incidence of VT is expected to increase. Therefore, efforts are urgently needed to identify inherited and acquired risk factors and age-specific factors, and reveal their interactions with advanced age, on risk of VT.

Targeted prevention strategies are needed to reduce the incidence of VT in the elderly in order to reduce personal suffering and the health burden in Europe, and promote healthy aging.

### **6.5. Bleeding disorders**

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#### *Introduction*

Inherited abnormalities of coagulation factors or platelets lead to lifelong bleeding. Hemophilia A and B due to deficiency or dysfunction of factor VIII and factor IX account for the majority of the severe disorders. Von Willebrand disease is mostly a milder but much more prevalent disorder of primary hemostasis. The remaining defects, such as factor II, V, VII, X, XI, and XIII, are very rare, with a prevalence of around one in 1 million. There are 106,000 people with an inherited bleeding disorder in Europe according to the World Federation of Hemophilia Survey of November 2014. Mild deficiencies may be asymptomatic until suitable challenges, such as surgery or trauma, and may go undiagnosed until adult life.

Due to their low prevalence, current knowledge of the genetic, laboratory, and clinical characteristics of these disorders remains limited, making their diagnosis and management difficult. Their treatment involves replacement of the missing factor with plasma-derived or recombinant concentrates. Their management has recently improved with the development of new extended half-life drugs. Gene therapy is also emerging as a possible future treatment with recent reports of success in hemophilia B. The development of antibodies against the replaced factor (inhibitors) is a major issue, affecting up to 30% of patients with severe hemophilia.

The rare coagulation disorders present significant difficulties in management. Affected individuals suffer increased morbidity and mortality as a result of recurrent, often spontaneous, bleeding, and there are wide variations in the availability and quality of specialized health care delivery in Europe.<sup>263-267</sup>

#### *European research contributions*

In the past decade, European researchers have made major contributions to the field, with some large collaborative projects funded by the European Commission. The PedNet registry of European pediatricians has contributed to studies on severe hemophilia in terms of genotype, phenotype, role of prophylaxis, and risk of inhibitor development by endogenous and exogenous factors. Large collaborative efforts of UK and French researchers have also investigated inhibitor development. The Malmö International Brother Study identified genetic determinants of inhibitor development. The European Haemophilia Network project established the hemophilia center standards required for delivery of care, and the European Haemophilia Safety Surveillance project prospectively monitors the safety of hemophilia treatments across Europe.

The European Network of Rare Bleeding Disorders identified the critical levels in clotting factor deficiency leading to clinical problems and has established prospective studies to determine the natural history of the disorders. The multicenter European study Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand Disease documented that von Willebrand factor mutations in type 1 disease are common and established the use of bleeding scores in the diagnosis of the disease.

#### *Proposed research for the Roadmap*

We propose the following topics for future research in the area.

1. The use of new technologies, such as next generation DNA sequencing, to clarify the genetic determinants involved in the complex multifactorial process of inhibitor development in hemophilia.
2. The harmonization of standards for collecting and sharing data on the development of inhibitors in patients previously untreated and previously treated in Europe for both standard and novel engineered products (e.g. the longer-acting factor concentrates).
3. The establishment of an optimal data collection system through a European network to obtain more accurate data on safety and efficacy of novel bioengineered coagulation factors in the post-marketing investigation period.
4. The monitoring of the safety and efficacy of gene therapy clinical trials and ensuring collaboration in the consistent long-term collection and sharing of these data at a European level.
5. The collection of data on the prevalence and management of the most frequent age-related hemophilic comorbidities, in particular cardiovascular disease.
6. The prospective collection of data on the natural history of the very rare bleeding disorders, which can only be carried out by multinational, worldwide collaboration.
7. The investigation of the ascertainment, diagnosis, clinical implications, and natural history of mild bleeding disorders.
8. The improvement of bleeding risk prediction in subgroups such as fragile and elderly patients.
9. The collaboration between diagnostic manufacturers and clinical services to improve the quality, sensitivity, and economic value of laboratory assays.

#### *Anticipated impact of the research*

Developing appropriate European networks based on real collaboration and appropriate data sharing and agreed methodologies will significantly enhance the power of the data collected, minimize bias, and prevent wastage of resources due to the current models of individual working. The aim is to improve the health and quality of lives of European citizens with these rare inherited bleeding disorders through improved awareness, diagnosis, and knowledge of the clinical manifestations and sequelae of these disorders.

### **6.6. Women, hemostasis, and thrombosis**

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#### *Introduction*

Inherited bleeding disorders are lifelong conditions that remain largely undiagnosed in women and result in significant morbidity and impaired quality of life (QoL). A World Federation of Hemophilia global survey in 2013 showed that the majority of patients with von Willebrand disease, the most common bleeding disorder reported by 107 countries, are women in their reproductive years.<sup>268</sup> Affected women suffer significant reproductive health problems, with heavy bleeding during menstruation, ovulation, and postpartum. There is an increased risk of pregnancy loss in women with severe deficiency of certain coagulation factors. Affected newborns are at an increased risk of intracranial hemorrhage during birth. The incidence of intracranial hemorrhage is unknown, however, without consensus on the optimal mode of delivery.

Venous thromboembolism (VTE) is rare in women of childbearing age, but pulmonary embolism (PE) is a leading cause of maternal mortality. The optimal diagnostic approach for PE during pregnancy and postpartum has not been established, and lung imaging carries risks, including those of radiation exposure for both the mother and baby. Low molecular weight heparin is the standard treatment of VTE in pregnancy and postpartum, although the evidence for dosing regimens is limited. Thrombophilias, both acquired and heritable, increase the risk of VTE in relation to pregnancy and hormonal intake; increase the risk of recurrent pregnancy loss and placenta-mediated complications, such as pre-eclampsia, placental abruption, and fetal growth restriction, and may be associated with recurrent implantation failure in assisted conception.

#### *European research contributions*

Research has highlighted increased menstrual and gynecological morbidity in women with bleeding disorders. Heavy menstrual bleeding is now recognized as a predictor for bleeding disorders in women. A systematic review of literature showed a 13% prevalence of von Willebrand disease in women with menorrhagia, with a higher prevalence of 18% in the European population.<sup>269</sup>

Analysis of cell-free fetal DNA in maternal plasma has been established as a non-invasive prenatal diagnosis method for assessment of fetal sex in pregnant carriers of hemophilia and can be used to detect fetal genotypes for hemophilia mutations in male fetuses.<sup>270</sup> The role of multidisciplinary management of pregnancy has also been established for safe delivery, with regional block for pain relief and anesthesia during labor and reduced risk of postpartum hemorrhage. Postpartum hemorrhage remains a leading cause of maternal mortality and morbidity in Europe. Hypofibrinogenemia has been shown to be a good marker for progression to severe postpartum hemorrhage.<sup>271</sup> However, how to assess the fibrinogen level and when to administer fibrinogen remains controversial.

The risks of thrombosis and pregnancy morbidity associated with heritable thrombophilias have been established. VTE risk assessment scores have been proposed to guide VTE thromboprophylaxis and for exclusion of PE during pregnancy and postpartum. Low-dose aspirin plus

heparin have been demonstrated to lead to a significant increase in live births in women with antiphospholipid syndrome-related recurrent miscarriage. The TIPPS trial did not show benefit with antenatal low molecular weight heparin in women with heritable thrombophilia; however, the power calculations were based on an aggregate of adverse outcomes rather than individual obstetric complications.<sup>272</sup> The role of heparin in women undergoing assisted conception is unclear.

#### Proposed research for the Roadmap

##### Bleeding disorders

1. Studies in women with heavy menstrual bleeding on assessing underlying bleeding disorders, the predictive value for the menstrual pictorial blood assessment chart and bleeding assessment tool, and to validate the benefits of incorporating these tools into routine clinical practice.
2. Studies to assess gynecological problems and treatment options in women with bleeding disorders; development and validation of a disease-specific QoL tool, to develop optimal care services.
3. Assessment of the role of local hemostatic mechanisms within the endometrium as an underlying cause for gynecological pathologies, such as heavy menstrual bleeding and endometriosis, and also in implantation and early placental development, in particular in women with recurrent early pregnancy loss and recurrent implantation failure following *in vitro* fertilization.
4. Refinement of non-invasive prenatal diagnostic techniques for the prenatal diagnosis of severe and common mutations, such as intron 22 inversion; qualitative research to explore feelings that influences decision making about reproductive choices.
5. Pooling of European prospective data to establish the risk of intracranial hemorrhage with various modes of delivery, and the impact of neonatal intracranial hemorrhage on neurocognitive development.
6. For women with rare bleeding disorders, multicenter studies and/or a web-based international registry to provide more evidence for management.
7. Assessment of real-time hemostatic monitoring by point-of-care testing in postpartum hemorrhage, to guide a tailored approach to management of coagulopathy and administration of blood components/products.

##### Thrombotic disorders

1. Prospective studies to establish the optimal strategies for management of VTE (stratification of risk factors and clinical prediction rules for suspected VTE, the potential use of higher D-dimer cut-off values, and appropriate low molecular weight heparin dosing) in pregnancy and postpartum.
2. Prospective studies to investigate the pathogenesis, diagnostic validity, management implications, and long-term outcome in women with thrombotic and obstetric complications, associated with antiphospholipid antibodies.
3. Research to define the optimal management of pregnancy in women with heritable thrombophilia and other thrombotic states, including hemoglobinopathies, MPNs, and thrombotic microangiopathies.
4. Assessment of combining cardiovascular measure-

ments with circulating biomarkers for early prediction of pre-eclampsia; development of diagnostic algorithms; definition of mechanisms for improved outcome with aspirin or other agents; definition of the role of complement.

5. Prospective studies on the effects of heparin in assisted conception.

#### Anticipated impact of the research

Research will enable improved diagnosis and an evidence-based approach for minimizing obstetric and gynecological morbidity. This will improve quality of life (QoL) and, in turn, educational achievement and productivity at work.

Definition of the diagnosis of mild bleeding disorders will enable assessment as to whether this improves reproductive health outcome and/or has a health economic benefit. Development of non-invasive prenatal diagnosis for hemophilia and information on the risk of intracranial hemorrhage during birth improve obstetric management, avoiding unnecessary intervention and medicalization of delivery. Development of strategies for the prevention and diagnosis of VTE and obstetric morbidity will reduce maternal and fetal/neonatal morbidity and mortality. A further understanding of the pathogenesis of pregnancy morbidity associated with thrombotic states and predictive biomarkers will inform optimal management. Appropriate management of obstetric antiphospholipid syndrome, as well as other obstetric morbidity associated with thrombotic states, will reduce long-term disability in the offspring, along with its health care and economic implications. Improvements in the understanding of mechanisms responsible for pre-eclampsia will achieve the long-term goal of curing this condition.

## The EHA Roadmap for European Hematology Research

### Section 7. Transfusion medicine

#### Section editor: Anneke Brand.

Transfusion therapy started a century ago with the aim of rescuing soldiers with large blood loss. Since then, transfusions reduced maternal mortality and enabled major surgery, leukemia treatment, and transplantation. Despite a lack of studies on when and how much a patient needs, transfusions became increasingly used. Recent studies lowering transfusion thresholds, conducted in surgical and intensive care patients, mostly favor a restrictive policy.<sup>273</sup> However, for most indications, the balance between benefit and harm of transfusion is still unknown.<sup>274,275</sup> Although in rich countries transfusions are safe, alertness for emerging infections remains, and for under-resourced countries and immunocompromised patients transfusion-transmitted infections still pose a considerable risk. Besides transfusion-transmitted infections, harm caused by transfusion results from transfusion-induced alloimmunization and transfusion-related immunomodulation, causing (transient) immune suppression.<sup>276</sup>

Transfusion represents a huge (economic) market, albeit with a wide range of use in Europe. Reported by 32 of 47

(70%) member states, on average 37 units of red blood cells (RBCs) are transfused per 1000 inhabitants (range 8–126 units), identifying three countries with a possibly insufficient blood supply (<20 units/1000). One-third of platelet transfusions are supplied by apheresis, and 8.5 L plasma/1000 inhabitants (range 0–49 L) is fractionated into medicinal products. In about half of the countries, all RBC and platelet transfusions are leukocyte-reduced. All countries have legally binding national regulations and 90% have a hemovigilance registry.<sup>277</sup> Future blood supply needs are expected to reflect an increase in patients needing blood components because of age-related morbidity coinciding with less accrual of young donors and donors from ethnic minorities.

Although for approximately half of the patients transfusion is a single lifetime event, patients with hereditary anemias from Europe's former colonies and residents in Mediterranean and eastern Europe need lifelong RBC support, leading to iron overload.

Overarching research proposals include the following (Figure 8).

1. Evidence-based indications of virtually all (individual, pooled/cellular, or plasma-derived) blood components demand new research methodology for clinical trials, as well as (bio)markers for indication and monitoring of effects.<sup>278,279</sup> Improved disease-specific IVIG products require human research on the mechanisms of immunomodulation and optimal dose and timing of administration. Hemovigilance would include surveillance of thrombotic complications of blood products.
2. Technology is available to match donors and recipients for almost any antigen avoiding alloimmunization, but scarcity of matched donors makes this impossible for all transfusions.<sup>280,281</sup> An immunovigilance registry of allo-(RBC, HLA, and HPA) immunized patients will assist selection of eligible patients (patient groups) for pre-emptive matching.<sup>282</sup> Such a registry can also stimulate collaborative studies that aim to reverse antibody production<sup>283</sup> and explore new treatment for severe complications of incompatible transfusions, transplants, or unborn children.<sup>284</sup>
3. For (emerging) transfusion-transmitted infections, the best future solution must be sought. Additional tests for immune-compromised patients and pathogen-reduction methods that optimally preserve intended (also red) cell functions should be compared. In the long term, *ex vivo* culture of transfusion-transmitted infection-safe (and antigens defined) blood cells lie ahead.<sup>285</sup> These three approaches need triage for feasibility, costs, and patient selection.
4. Good donor management, not solely considering them as resource material, must safeguard donors of blood, cells, tissues, and organs.<sup>286</sup> Achievements of transfusion medicine (non-remunerated donors, good manufacturing practice, traceability, and hemovigilance) can support quality management of new cellular products for immunotherapy and repair treatment.<sup>287</sup>

### 7.1. Conventional blood products: indications and usage

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### Introduction

The most commonly transfused blood component remains red blood cells (RBCs), followed by platelets and plasma. Granulocyte transfusions are still considered an experimental product. Bleeding with shock and anemia is an undisputed indication for RBC transfusion. However, RBCs are commonly used to correct anemia in patients without bleeding to improve the oxygen-carrying capacity, for instance in critically unwell patients and bone marrow failure (BMF) disorders. Although the evidence base for RBC transfusion practice is incomplete, randomized studies in surgery and intensive care settings consistently support a more restrictive use of RBCs, with no evidence of benefit (and arguably harm by increasing post-operative infections and organ failure) for maintaining patients at higher Hb thresholds (liberal strategy).<sup>288</sup> The past ten years have also seen an increasing scrutiny of RBC usage in medical patients (accounting in the UK for approx. two-thirds of all RBC transfusions) compared with surgical indications. The degree to which the optimal Hb transfusion "trigger" should be modified for patients with specific risk factors (e.g. coronary disease, radiotherapy, and chemotherapy) remains unclear, and clinical dilemmas are clearly shown when considering the appropriate RBC transfusion threshold in the extremes: elderly, and premature neonates.

The evidence for a prophylactic platelet transfusion threshold of  $10 \times 10^9$  /L instead of higher triggers has come from randomized controlled trials in hematology patients with chemotherapy-associated thrombocytopenia.<sup>289</sup> More recent studies even questioned the effectiveness of prophylactic platelet transfusions in all patients with (onco) hematologic diseases. There is a lack of evidence to guide use of platelet transfusions to cover invasive/surgical procedures in patients with platelet dysfunction and/or thrombocytopenia, and guidelines for these indications outside hematologic settings remain largely based only on expert opinion.

Fresh frozen plasma contains pro- and anticoagulant factors and other proteins. Most guidelines describe the use of laboratory coagulation tests to guide administration of plasma, but there are uncertainties about the value of standard coagulation tests in predicting clinical bleeding risk and a lack of evidence of benefit for the prophylactic administration of fresh frozen plasma in non-bleeding patients.

### European research contributions

Major steps toward questioning the evidence base for use of blood were established in Europe, beginning with descriptive data by the Sanguis Study Group, which in 1994 reported large differences between hospitals and clinical teams in the use of RBC transfusions (and other blood products) for the same surgical procedures with no clear clinical explanation. Many subsequent activities (e.g. the European Society of Anaesthesiology Clinical Trial Network, established in 2010) have facilitated clinical research in anesthesia and intensive care. An ongoing study termed the European Transfusion Practice and

Outcome Survey has collected transfusion and outcome data from large numbers of patient transfusion episodes from many centers across Europe. According to reports from the European Blood Alliance, although average RBC use per 1000 inhabitants in Europe is substantially lower than in the US, large variations in usage rates are still reported between European countries, and audits indicate greater than 15% inappropriate use.

Several leading clinical trials have been conducted in Europe for platelets, red cells, and granulocyte and fresh frozen plasma transfusions,<sup>289-292</sup> but given the broad ranges of clinical setting where transfusions occur, many research gaps remain.

#### *Proposed research for the Roadmap*

*Clinical studies on appropriate use/linking to ongoing studies and patient input:* the aforementioned European Transfusion Practice and Outcome Survey on blood usage for surgical interventions in Europe still has no counterpart for (onco) hematologic diseases. There is an opportunity to explore current hospital databases to characterize blood use in (onco) hematology and hemoglobinopathy, to generate key base-line information on blood usage practice. Incorporation of data collection for transfusion usage and bleeding outcome in hemato-oncology trials should be encouraged. Policies to enhance patient engagement, for instance in transfusion-dependent myelodysplasia, are required. This could be achieved by introduction of patient-reported outcomes related to physical activity, well-being, and quality of life (QoL) as part of post-transfusion follow up.

Research is warranted to identify (predictive) risk factors for severe thrombocytopenic bleeding. A European registry is required that reports bleeding after commonly applied interventions in hematology (e.g. lumbar puncture and organ biopsies), in relation to platelet count, coagulation profile, and use of platelet transfusions and/or plasma/coagulation factors. Studies on transfusion management for pre-term infants and elderly patients undergoing cancer treatment are essentially lacking. Emphasis should also be given to medical and economic considerations of different approaches to transfusion or alternatives, including hematopoiesis-stimulating agents, iron, and prohemostatic drugs. Indications and patterns of use of granulocyte transfusions are unknown.

*Better biomarkers identifying transfusion needs and results:* the hemoglobin (Hb) concentration is still used to define the need for red cell transfusions, calculate the dose of RBCs required, and monitor the response to transfusion or alternative treatment. However, Hb is a surrogate marker, and research should address better-targeted measures of oxygen requirements that can identify specific patient needs. Similarly, to identify patients with a high bleeding risk requiring prophylactic platelet or plasma transfusions, the safety of alternatives (near-patient point-of-care tests) and the value of biomarkers for endothelial damage preceding bleeding should be explored.

*Physician behavior and education:* many interventions are undertaken to change transfusion practice based on suspected wrong practice or guidelines, but there are uncertainties about their effectiveness and durability. There is a need to define the determinants of transfusion behavior to deliver

optimal transfusion practice. Electronic blood ordering and better information technology support for prescribers may be of value. Prevention of hospital-acquired anemia is one key tenet of patient blood management, defined as a patient-centered, evidence-based approach of good clinical transfusion practice. Patients may lose significant volumes of blood for laboratory evaluation. In pre-term infants, this is even the major cause of transfusions. Non-invasive assays or micro-testing would help minimize the need for transfusions.

#### *Anticipated impact of the research*

Blood components are biological products and a costly resource. The uptake of patient blood management, which includes consideration of transfusion alternatives, remains highly variable. Studies in surgical and intensive care patients generally support a restrictive use of blood components. There are hardly any studies in (onco) hematologic diseases. The proposed research will contribute to patient blood management for hematologic patients.

### **7.2. Plasma-derived and recombinant human plasma proteins**

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#### *Introduction*

Plasma products are produced by (non-profit) blood establishments or by the pharmaceutical industry. Plasma is recovered from whole blood donations or collected by (sometimes remunerated) plasmapheresis. Plasma is used as single-donor fresh frozen plasma units (quarantine, methyl-ene-blue-treated) or as pooled plasma products requiring a pathogen-reduction treatment. Pooled plasma and fractionated plasma products (e.g. factor VIII, albumin, prothrombin concentrate, immunoglobulins, von Willebrand factor, and fibrinogen), as well as factors produced by recombinant techniques (e.g. factor VIIa, factor VIII, and factor IX), are pharmaceutical products. The huge variation in usage of various plasma-derived and recombinant products per 1000 inhabitants in the European member states is not explained by differences in plasma-product-dependent diseases. According to a 2010 report by the International Plasma Fractionation Association 2010, high-dose IVIG usage in European member states is, on average, 36.5 g per 1000 inhabitants (range 1.2–97 g), compared with an average of 120 g per 1000 US inhabitants.

Indications for recombinant and plasma-derived coagulation factors comprise substitution in massive bleeding and prevention of bleeding in patients with congenital or acquired coagulation factor deficiencies. Patients with primary or acquired hypogammaglobulinemia are substituted with immunoglobulins. Anti-RhD immunoglobulin is applied for immunoprophylaxis of hemolytic disease of the newborn. IVIG is used for many (auto-)immune-mediated disorders and to neutralize alloantibodies. IVIG exerts multiple immunoregulatory mechanisms, such as anti-idiotypic activity, inhibition of activation of B cells and antigen-presenting cells such as dendritic cells and macrophages, enhancement of IgG catabolism, and reciprocal regulation of regulatory T cells and pathogenic Th17

and Th1 cells. The precise mechanisms underlying its beneficial effects in various diseases are still largely unknown. Many indications for IVIG are not evidence-based, and off-label use is frequent.

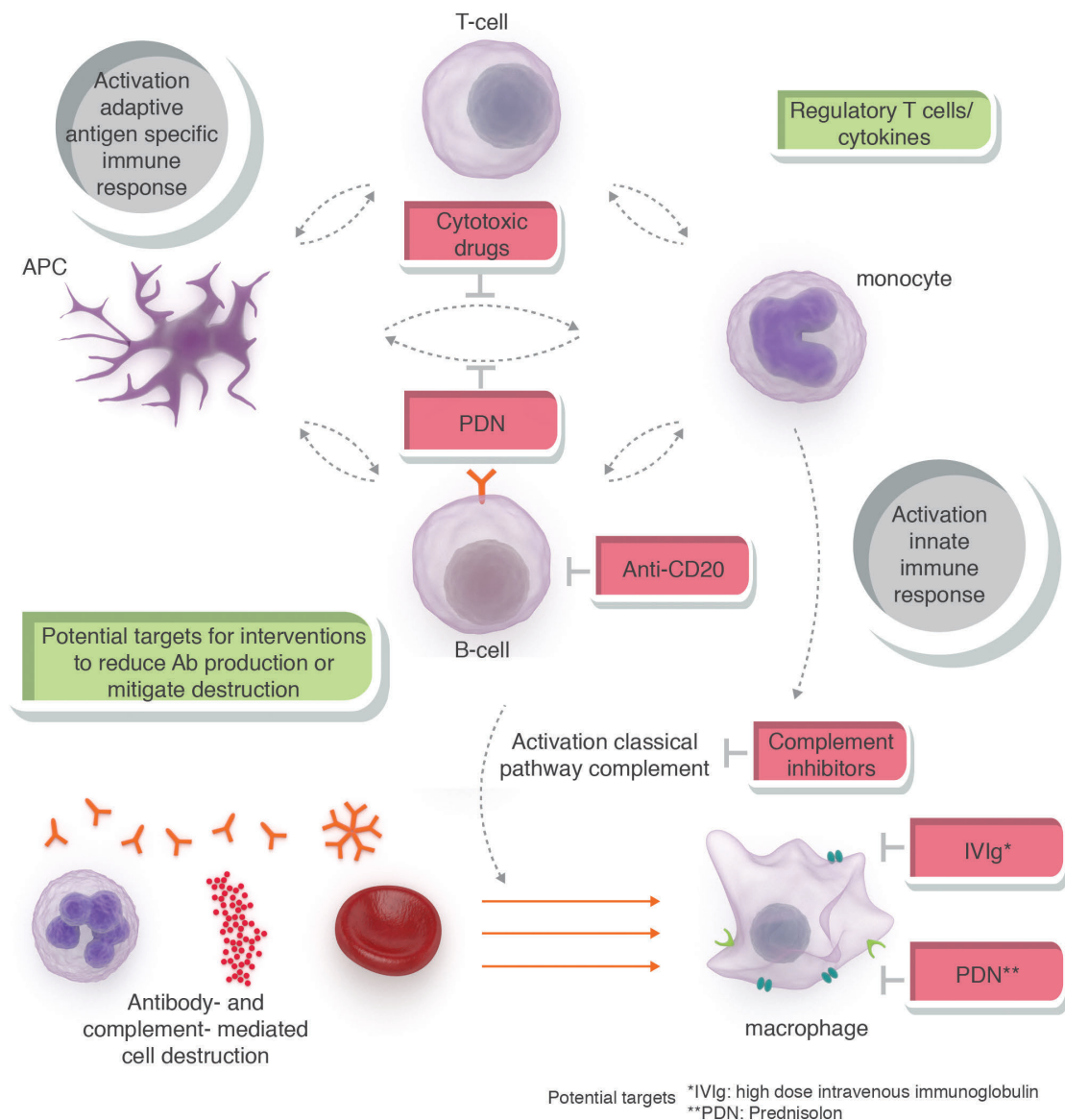
*European research contributions*

The past decades have seen enormous progress in the development of therapeutic coagulation factors, greatly improving the treatment of bleeding disorders.

Improvement in the very nature of the recombinant products has been constant, starting, in the case of procoagulant factor VIII, from albumin-stabilized products to 3rd-generation products that are devoid of contact with mammal proteins in both the production and purification

phases. Recent efforts aspire to generating products with longer half-lives, to improve patients' quality of life (QoL), or to fully human products to ensure normal glycosylation and sulfation, aiming to reduce alloimmunization and neutralizing antibodies.

It was a European discovery by Imbach in 1981 that high-dose IVIG increased the platelet count in an autoimmune thrombocytopenia patient, and since then a large number of immune-mediated diseases are treated with IVIG. European researchers and physicians have made a considerable contribution toward understanding the mechanisms of action of IVIG and conducted randomized clinical trials with IVIG contributing to the evidence base for (new) indications.



**Figure 8. Activation of the adaptive antigen-specific immune response.** Potential targets for interventions to reduce Ab production or mitigate destruction. Potential interventions include high-dose IVIG and PDN (prednisolone).

**Proposed research for the Roadmap**

*Immunogenicity of coagulation factors and gene therapy:* replacement therapy with procoagulant products is complicated by the occurrence of neutralizing antidrug antibodies. Particularly in patients with hemophilia A, the prevalence of these antibodies may reach 30% and pose a major clinical concern. Several strategies are being investigated to reduce the immunogenicity of therapeutic factor VIII. These include alteration of factor VIII moieties implicated in its processing/recognition by immune effectors, control of the inflammatory status of the patients at the time of replacement therapy and induction of active immune tolerance, for example, upon oral or transplacental transfer of factor VIII,<sup>293</sup> or using cell therapy for tolerance induction.

Pre-clinical studies with recombinant adeno-associated viral vectors encoding variant human factor VIII<sup>294</sup> are encouraging and may lead to a successful gene therapy strategy for hemophilia A similar to that achieved with hemophilia B.

*Prediction and treatment of massive bleeding:* evidence-based assays to predict bleeding severity and monitor optimal substitution (bedside point-of-care and coagulation factor levels) are required. European consortia should continue to investigate the optimal mix of coagulation factor suppletion and drug treatment in massive bleeding of various origins (e.g. military and civil casualties, major surgery, postpartum hemorrhage, and congenital or acquired bleeding disorders) (see Section 6). Addressing these questions should proceed independently and free of any influence from producers.

*Thrombotic complications:* for both antifibrinolytic treatment and coagulation factor substitution, the window of opportunity may be small, depending on the underlying condition, and late treatment may enhance thrombotic complications. Also, possible contamination of coagulation factors in IVIG products carries a risk of thrombotic events. A step has recently been made to remove coagulation factor impurities in IVIG. Besides hyperviscosity, thrombogenic activity of some products may play an additional role. Thrombotic complications should be monitored in plasma product surveillance systems, preferably European-wide. National hemovigilance registrations, which are in place in many European member states, can possibly facilitate this.

*Innovation of IVIG products and identification of biomarkers:* the same IVIG products are used for substitution of immunodeficiency conditions and for immunomodulation in immune-mediated diseases. For substitution, research should aim for the conception of highly concentrated immunoglobulin products to reduce the volume of injection by subcutaneous route. For immunomodulation studies, identification of biomarkers to predict IVIG responders and appropriate dose are warranted. Although controversies regarding dependence of Fc-sialylation toward anti-inflammatory functions of IVIG exist,<sup>295</sup> the necessity of CD209 for the IVIG-mediated expansion of regulatory T cells is established in both humans and mice.<sup>296,297</sup> Sialylation-mediated structural modifications can be mimicked by specific amino acid modifications at position 241 (F→A) of the CH2 domain.<sup>297</sup> This variant

IgG Fc molecule demonstrated anti-inflammatory effects similar to IVIG. Therefore, sialylation is not mandatory for anti-inflammatory effects of IVIG. The eventual clinical application of these innovative IVIG products needs to be further explored.

**Anticipated impact of the research**

In well-resourced countries with access to products to combat bleeding after trauma, delivery, or surgery, validated assays to predict bleeding risk and monitor treatment can improve survival. Despite its complex clinical environment, European groups treating these patients and adjustments to (inter)national reporting systems will help to arrive at more evidence-based use of available treatments. Reducing the incidence of immune response to coagulant factors, such as anti-factor VIII, would reduce the cost associated with patients' management.

As IVIG is the driving force for plasma collection and represents a huge budget, appropriate dosage, treatment window, cellular and molecular mechanisms, and randomized clinical trials to confirm off-label usage may ultimately save costs. Similarly, clinical and basic research can ultimately lead to the conception of IVIG similars or IVIG-derived therapeutic molecules for treating specific pathological conditions.

**7.3. Hemapheresis**

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**Introduction**

Hemapheresis encompasses a method of obtaining one or more blood components by using a special blood separation device processing whole blood, removing the component of interest, and returning the residual components of the blood to the donor/patient during or at the end of the process. Apheresis can be divided into apheresis to collect blood components from donors/patients and therapeutic apheresis. Component apheresis involves collection of plasma, red blood cells (RBCs), and platelets from blood donors for the purpose of direct cellular therapy or further modification. Both patients and donors donate peripheral hematopoietic stem cells (HSCs) and other mononuclear cells. Therapeutic apheresis is performed to remove large numbers of cells or plasma from patients for specific disease treatment and is applied for more than 75 different, mostly immune-mediated, rare diseases, varying from macular degeneration to sickle cell crisis.<sup>298</sup>

In 2007, the American Society for Apheresis started an initiative to classify the applied indications for therapeutic hemapheresis according to evidence base, and these recommendations are regularly up-dated. The latest update of 2013<sup>299</sup> recommends hemapheresis for 25 diseases either as a first-line lifesaving treatment or a valid adjuvant to other therapies. Still, there are more than 50 benefit-of-doubt indications. Hemapheresis serves a multitude of medical disciplines (neurology, nephrology, dermatology, ophthalmology, dermatology, cardiology, and



hematology), requiring joint studies and multidisciplinary recommendations. Besides the most pivotal plasma-exchange indication (TTP), for hematology, the collection of cells for autologous and allogeneic use play an increasing dominant role. Of the more than 20,000 hematopoietic stem cell transplants (HSCTs) performed worldwide each year, more than 70% are obtained by blood stem cell apheresis.

#### European research contributions

The European Society for Hemapheresis collaborates with the national apheresis societies to establish standardized education and certification programs for specialized nurses for apheresis. There is also co-operation in contributing to evidence-based medicine indications (e.g. systemic lupus erythematosus, as well apheresis treatment in inflammatory bowel disease). The evolving use of blood stem cell, lymphocyte, and monocyte/dendritic cell collections resulted in dedicated working parties within the European Group for Blood and Marrow Transplantation (EBMT) and the International Society for Hematotherapy and Graft Engineering. Two Italian scientific societies for hematology and hemapheresis (SIDEM and GITMO) produced best practice recommendations for stem cell mobilization in children and adults.<sup>299</sup>

Extracorporeal photopheresis is a treatment in which blood mononuclear cells collected by apheresis are incubated with methoxsalen and exposed to UV light, and subsequently reinfused to the patient. Extracorporeal photopheresis is by UK and Italian scientific/clinical consensus groups recommended as 2<sup>nd</sup>-line treatment for graft-versus-host disease (GvHD) after allogeneic stem cell transplantation and for scleroderma and other autoimmune diseases.<sup>300</sup> Because of the high costs, however, many patients cannot be treated with extracorporeal photopheresis.

Stem cell donations by healthy donors pose special medico-ethical problems. Surveys in Europe and the US reveal that the care for the family donor differs from the strict guidelines from the World Marrow Donor Association aiming at optimal donor safety for unrelated donors. Collections of mononuclear cells and subsequently *ex vivo* preparation of the collected leukocytes for cellular therapies is currently being explored as a novel potent anticancer treatment or antiviral vaccination therapy. This requires a further commitment and poses a burden for the volunteer donor.

#### Proposed research for the Roadmap

*Therapeutic apheresis:* there remains an ongoing need to contribute to evidence base indications for therapeutic apheresis. Given the orphan character of diseases this requires broad collaboration with all involved institutes in Europe and the establishment of European databases in conjunction with the scientific societies of various medical disciplines.

*Stem cell donation by autologous, related, and unrelated donors:* for stem cell donation, the establishment of a global standardized system for related donor care comparable to unrelated volunteer donors is in progress. This initiative is a joint effort of the World Marrow Donor Association and EBMT, resulting from the establishment

of an EBMT board committee on donor follow up (2012) with one of the goals to set up a donor follow-up registry for all EBMT related and unrelated stem cell donors and to provide the possibility for systematically collecting adverse events and longer follow up for family donors. The safety of new stem cell mobilization drugs (e.g. biosimilars and CXCR4 agonists) requires European collaboration and uniform medico-ethical procedures.<sup>301</sup>

*Plasma and platelet donation from unrelated donors:* a donor-related aspect is whether the repeated exposure to citrate (binding calcium) and protein removal is harmful and poses risks for osteoporosis and hypogammaglobulinemia in the long term, an important issue for recommendation on plasma- or apheresis-derived platelet products.<sup>302</sup>

#### Anticipated impact of the research

Hemapheresis represents a unique medical (supportive) specialty for patient treatment and depends highly on industry-driven technology and drug development to obtain optimal efficacy. Moreover, a huge part of the worldwide allogeneic hematopoietic transplants, as well as the European blood supply of plasma and platelet products, depends on apheresis. Although short-term adverse effects of patient as well as donor apheresis are minimal, long-term effects of many procedures applied in healthy (volunteer, non-remunerated) donors are less well studied. The proposed research subjects can provide the European community with information about the risk and safety issues for donors, and promote convenient devices and drugs for their voluntary donations.

#### 7.4. Immunological transfusion complications: alloimmunization/transfusion-related immunomodulation/ hemovigilance

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#### Introduction

Currently, donor counseling, sensitive laboratory tests, good manufacturing practice, and hemovigilance have substantially increased transfusion safety with respect to transfusion-transmitted infections. However, blood transfusions also bear immunological risks, yet lack a surveillance system. Although alloantibodies can result from pregnancy, most are induced by transfusions. Red blood cell (RBC) antibodies can cause (sub)acute hemolytic transfusion reactions and hemolytic disease of the fetus/newborn. Leukocyte/HLA antibodies can cause transfusion-related acute lung injury and limit the chance of finding a compatible (organ/stem cell) graft. HLA and platelet antibodies destroy transfused blood platelets needed for leukemia treatment and stem cell transplantation. Many European countries apply universal leukoreduced transfusions, which reduces HLA immunization, but HLA antibodies still hamper optimal platelet transfusion and transplantation treatment. Except (Rh-K) matching of RBC transfusions for (pre)fertile women and for

patients with hemoglobinopathies, applied by some European countries, measures to avoid antibody formation are not yet possible. Although some countries have a national registry for RBC/HPA-immunized pregnant women, the overall prevalence of alloimmunization across Europe is unknown. However, immunization has a huge economic impact in the need to provide safety nets for immunized patients needing blood or transplants, or who are pregnant.

Besides antigen-specific immunization, clinical studies suggest transfusion-related immunomodulation, an ill-defined complication also affecting the innate (not antigen-specific) recipient immune system, transiently impairs resistance against infections, enhancing organ damage and resulting in unknown consequences for cancer immunosurveillance.

Research on the mechanism of alloimmunity has mostly focused on animal models, even though pregnancies and blood transfusions in humans provide unique models for systematic studies of *in vivo* reactions of the human immune system.

#### European research contributions

In the past decade, European research groups have advanced our knowledge of the genetics, polymorphism, expression, function, and pathophysiology of the many blood groups expressed on RBCs, leukocytes, and platelets. Molecular genotyping of most antigens is now possible for donors and patients, and even fetal polymorphic antigens can be determined in maternal blood during pregnancy. Demonstration of the absence of (fetal) RhDpos DNA in Rh-Dneg gravidas avoids unnecessary immunoprophylaxis with human anti-D immunoglobulin and saves money.<sup>303</sup> A European platform performed preparations for implementation of “RBC bloodmatch on a chip.”<sup>304</sup> For stem cell and organ transplantation and donor selection for platelet transfusions, provided electronic matching programs can select HLA-compatible donors from large international registries. HLA-net aims at networking researchers in bone marrow transplantation (BMT), epidemiology, and population genetics to improve the molecular characterization of genetic HLA diversity of human populations with an impact on both public health and fundamental research (Allele Frequency Net Database). A European subgroup of the Allele Frequency Net Database (EUROSTAM/HLA-net) is setting up an HLA allele frequency database to enable renal transplantation in highly sensitized patients on the basis of acceptable HLA mismatches.<sup>305</sup> The European platelet immunology working party contributed insight in fetal alloimmune thrombocytopenia.<sup>306</sup> Unraveling the minor histocompatibility antigens aims at dissecting the T-cell immunity causing graft-versus-host disease (GvHD) and graft-versus-tumor effect (see Section 9).

#### Proposed research for the Roadmap

Four broad research goals are proposed.

**Epidemiology:** the current EU hemovigilance registries could be extended with immunovigilance. Registration of alloantibodies is important to define which phenotypes we should focus on for blood and stem cell donor recruitment (e.g. ethnic minorities), for composition of high-

throughput RBC, HLA, and HPA typing platforms of donors, recipients, pregnant females, and fetuses. Immunovigilance would also serve as post-marketing surveillance of pathogen-reduced products to exclude immunogenic neoantigen formation. A registry of transfusion-dependent RBC diseases in Europe can identify the incidence and stimulate collaborative treatment studies for hyperhemolysis, a life-threatening transfusion complication.

**Prevention:** prevention of alloimmunization requires better matching and the detection of potentially dangerous memory responses. Currently, about 300 RBC antigens are genetically and/or molecularly defined. Approximately 40 more residual (orphan) groups need to be unraveled, while new RBC groups are still discovered through unexpected antibodies. It is important to know their genetic backgrounds for inclusion in platforms for high-throughput donor and patient typing. The polymorphism of the HLA system with more than 10,000 identified alleles complicates relevant clinical matching. Every HLA allele poses a unique combination of antigenic epitopes, but many epitopes are shared with other HLA alleles.<sup>307</sup> Consequently, the immune response to a foreign HLA antigen can be explained by a restricted number of epitopes. New strategies should focus on matching for the relevant immunogenic epitopes between donor and recipient rather than matching for the HLA alleles. The complex pathophysiology of high and low responder individuals to alloantigens needs studies of genetic and environmental factors for T- and B-cell activation, memory, and antibody persistence. Instead of studies in mice, pregnancy and transfusions in humans can enhance insight.

**Reversal of immunity:** alloimmunization cannot be completely abolished. To reverse antibody production and memory immune cells is extremely complex. For this purpose, the effect of selected drugs/immune cells shall be explored on the behavior of B/plasma cells, regulatory mechanisms at the T-cell level, antibody affinity development, and the role of (post-pregnancy) chimerism.

**Mitigating severe alloimmune complications:** antibodies can cause severe (lethal) complications, such as hyperhemolysis after transfusion, severe bleeding and lack of compatible platelet donors, graft rejection, and fetal morbidity and mortality. New drugs mitigating complement activation and other sequels (cytokine storm) of antigen-antibody reactions may save lives and safeguard transplanted organs.

To reach these goals, translational research programs including bioinformatics analyzing big-data output<sup>304-307</sup> should be combined with basic studies using the unique situation of the highly ethical exchange of alloantigens by blood transfusions.

#### Anticipated impact of the research

For patients suffering from a wide range of (onco) hematologic diseases, transfusions, stem cell transplantation, and immunotherapy can be indispensable. Alloimmunization hampers optimal access and increases costs related to these treatments. The proposed research will result in the reduction of immunization and of dam-

age caused by immune incompatibility, and can reduce the current high cost of care for alloimmunized patients.

### 7.5. Blood-borne infections and hematologic patients: lines of research

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#### Introduction

The donation of organs, tissues, and cells as well as the pooling of large numbers of donor plasma units for protein fractionating bears the risk of transmission of pathogens to recipients. For hepatitis B, hepatitis C, HIV, and variant Creutzfeldt-Jakob disease, donor counseling, testing, product processing, and traceability of donors and recipients are successfully applied to limit the risk of transmission by transfusion and transplantation. However, the increase in global travel, intensive animal farming, and climate change may cause a quick spread of emerging bacterial, viral (e.g. West Nile virus, chikungunya virus, and dengue virus), and parasitic infections.

#### European research contributions

Safeguarding blood products and other substances of human origin (SoHO), intended for human treatment, is regulated in the European Union by several directives, launched from 2004 to 2006 (2004/23/EC, 2006/17/EC, and 2006/86/EC). The European Commission leads the regulatory activities, co-ordinates the work of national competent authorities for SoHO, and runs Rapid Alert platforms monitoring serious adverse reactions and events caused by SoHO therapies and traceability from donor to patient and *vice versa*. The European Network of Competent Authorities for Tissues and Cells participates in the vigilance and surveillance of SoHO. For the blood supply, the European Blood Alliance emerging infectious disease monitoring group ensured quick monthly information with recommendations for donor/donation exclusion, in particular related to traveling.

In 2005, the European Centre for Disease Prevention and Control was established in Stockholm. Its mission is to identify, assess, and divulge information about the current and emerging threats to human health posed by infectious diseases. The center communicates with Coordinating Competent Bodies in each member state to strengthen and develop continent-wide disease surveillance and early warning systems. In the field of SoHO safety, the center provides member states and the European Commission with the best advice available concerning infectious risks of donations and human use of SoHO. The center also develops risk assessments and tools related to infectious safety of SoHO and provides weekly maps of areas affected by West Nile virus infection, to support blood banks and pharmaceutical production plants to take preventive measures and help with validation of infectious disease tests.

#### Proposed research for the Roadmap

In the Western world, the risk that blood transfusions and blood-derived products transmit blood-borne infections to patients is very small. For example, the introduction of sensitive nucleic acid amplification testing for HIV1/2, hepatitis B virus, and hepatitis C virus reduced

transmission of these agents to one residual infection in 1-10 million transfusions. Nevertheless this risk is not zero, and for hematologic patients the risk is higher than for many other categories of recipients. Besides prolonged immune impairment in transplant recipients, it has been shown that patients treated with chemotherapy for hematologic cancer may also lose pre-existing immunity.<sup>308</sup> It is obvious that the ongoing research in pathogen inactivation and pathogen removal is of great importance for blood recipients that are especially vulnerable to blood-transmitted infections (see Subsection 7.6; pathogen inactivation and removal). An important topic, especially relevant for blood disorders, is the question of whether blood and blood-derived products for immunosuppressed hematologic patients should be safer than for other categories of patients. At least three elements of this question are candidates for applied research.

1. Does severe (iatrogenic) immunodeficiency warrant additional safety measures for blood transfusions and blood-derived products [e.g. pooled plasma and hematopoietic stem cells (HSCs)] in hematologic patients, and if so, which agents and which safety measures must be considered and what are the triggers for their implementation? At the same time, it seems desirable to apply this question on a more practical level to at least two specific infectious agents.
2. In parts of the Western world, currently there is a high incidence of silent hepatitis E virus genotype 3 infection among blood donors. Transmission *via* blood and blood-derived products has been demonstrated. Hepatitis E virus is a non-enveloped virus and is not sufficiently inactivated by the current solvent-detergent procedures used for pathogen reduction (PR) of pooled plasma products, which are in large quantities administered to patients with thrombotic thrombocytopenic purpura (TTP). In immunosuppressed patients, hepatitis E virus genotype 3 may cause silent, chronic infection that rapidly leads to cirrhosis.<sup>309</sup> Chronic hepatitis E is often interpreted as a drug-induced liver injury or a sign of graft-*versus*-host disease (GvHD), which may lead to an increase in immunosuppressive treatment and thus may worsen the viral infection. From a technical point of view, donor screening for hepatitis E virus genotype 3 RNA is possible, but so far it has not been implemented.
3. Parvovirus B19 (B19V) infection has a seasonal and cyclic nature: each year in springtime blood donors may harbor acute, asymptomatic B19V infection. In addition, every four years, there is a marked increase of B19V infections. In immunosuppressed patients, exposure to B19V may cause severe and chronic anemia, which can be controlled only by repeated blood transfusions or monthly administration of IVIG. To prevent B19V infection of vulnerable patients, some blood transfusion services (e.g. in the Netherlands) provide "B19V-safe" blood for specific categories of recipients. It is desirable to evaluate this policy in the EU member states.

#### Anticipated impact of the research

With the implementation of serological and molecular testing, at least in high-income countries, transfusion-transmitted infections of a limited number of specific pathogens (HIV, hepatitis B virus, hepatitis C virus, and

human T-cell leukemia virus) have become extremely rare. Full control of infectious disease transmission has not yet been achieved because many pathogens are not included in test protocols and new agents continue to emerge. The immune status of recipients has a considerable impact on the outcome of transfusion-transmitted infections. This particularly affects hematologic patients who received anti-lymphocytic agents or underwent stem cell transplantation. The results of the proposed research can be used to better assess patient risks, enhance the cost-effectiveness of treatments, and increase the safety of blood and blood-derived products for hemato-oncology patients across Europe.

### 7.6. Pathogen reduction of blood components

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#### Introduction

Nowadays, thanks to the measures adopted to increase the safety of transfusions (including optimized donor selection programs and mandatory screening tests), the residual risk of suffering a transfusion-related pathogen infection is extremely low, especially with regard to viral infections. Transfusion medicine research has, therefore, moved toward the development of methods intended to reduce the risks posed by bacterial contamination, particularly in platelet components. Transfusion of bacteria-contaminated platelets can cause a septic reaction in the recipients (1:20,000 to 1:50,000). The fatality rate is expected to be around 10%; however, transfusion-associated bacterial infections may be underestimated due to the fact that platelet transfusions are frequently administered to patients suffering from hemato-oncology diseases where the use of antibiotics-based therapies may mask the symptoms of a septic transfusion reaction. To prevent platelet transfusion-related bacterial sepsis, several countries have implemented bacterial detection, but unfortunately, available bacterial screening methods (even the more sensitive ones) are not able to completely eliminate the cases of this infectious complication of transfusion. Moreover, one should not forget the issue of novel emerging pathogens, including not only undiscovered strains of bacteria, but also viruses with genomic mutations susceptible to immune escape mechanisms or known parasites/viruses that are continuously on the rise in non-endemic regions in view of globalization and climate changes (e.g. *Plasmodium* spp., *Babesia* spp., *Trypanosoma cruzi* and HIV, as well as dengue, West Nile, and chikungunya viruses).<sup>310,311</sup> The arduous search for the zero-risk strategy to reach a completely safe blood transfusion will probably never end, although a complicated and intense debate about the implementation of pathogen reduction (PR) technologies continues. This is due to the paradox that innovation directly related to the safety of blood products is most frequently associated with impairment of a product's intrinsic quality. Thus, scientists have to find the equilibrium between safety and clinical efficiency. This observation means that we urgently need basic as well as clinical research. Although in some European countries PR technologies for plasma and platelets fulfill the overall criteria of acceptability, there are currently no

acceptable PR methodologies for whole blood and red blood cells (RBCs).

#### European research contributions

The variety of emerging pathogens and the costs of development of new counseling and detection assays were (besides more ideological purposes for under-resourced countries) driving forces to develop techniques for PR of cellular blood components, often in close collaboration between research centers and pharmaceutical companies. For platelets, three PR techniques have been developed and approved in Europe.<sup>311</sup> One is based on the addition of amotosalen and illumination with UVA light; a second combines the addition of riboflavin (vitamin B2) and illumination with 265 to 370 nm UV light; and the third applies only UVC (below 280 nm) under loose strong agitation. However, in France only A-L (Intercept) has been approved (by the National Agency for the Safety of Medicines and Health Products, in 2003). The first two methodologies are approved for clinical application or in phase III studies, while Theraflex UV is currently in phase I-II.<sup>311</sup> In Europe, there is a wide range of use of PR-treated platelets for routine transfusion. Published hemovigilance data predominantly concern the A-L (Intercept) method with all reports confirming both the safety and efficacy of A-L-treated platelets in a huge number of platelet transfusions.<sup>312</sup> If on the one hand, PR technologies are designed to irreversibly disrupt nucleic acids of pathogens, on the other, they also cause collateral reactive oxidation-related damage to platelet proteins, thus enhancing the platelet storage lesion. Comparison of such damage by different PR techniques has been shown by a consortium represented by four (Switzerland, Italy, France, Germany) European research groups, along with a Canadian group.<sup>313</sup> Studying platelet proteome changes revealed that each PR technique affects different protein pathways relevant for essential platelet functions.<sup>313</sup> A great challenge is posed by PR of RBCs for which UV light cannot be applied. Currently, the S-303-based technology (Cerus Corporation, Concord, USA) is the pre-eminent system for PR of whole blood and RBCs.<sup>314</sup>

#### Proposed research for the Roadmap

The following are important issues to be examined: 1) to what extent PR of platelets affect the potential to prevent/treat bleeding; 2) immunogenicity of PR-treated cellular blood products; 3) clinical comparison of blood products treated by different PR techniques; 4) PR of RBCs; and 5) assessment of cost-effectiveness of PR interventions.

#### Anticipated impact of the research

The proposed research will contribute to improving the quality of established commercial technologies for PR in platelets and plasma through the co-operation of academic expert centers in proteomic analysis and cellular biology. Moreover, experience gained in the studies performed in platelets will facilitate the development of novel technologies for PR in RBCs and whole blood. The achievement of a unique procedure carried out on whole blood will contribute to reducing the high costs of current PR procedures. Besides this important economical benefit, the development of a global PR reduction technology for whole blood will significantly reduce the frequency and severity of pathogen transmission with blood transfusions.

### 7.7. Toward large-scale production of blood cells for transfusion purposes

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#### Introduction

Blood transfusions in Europe depend on the recurrent, non-remunerated contribution of blood by donors. Part of the donor population is genetically typed. In addition, banks store typed erythrocyte units in liquid nitrogen, available for patients with alloantibodies having no other option. It is, however, logistically impossible to provide all patients that require regular blood transfusions with matched blood products.

A solution to this problem would be the availability of cultured red blood cells (cRBCs) and cultured platelets (cPLTs) that are perfectly matched for all blood groups. When successful, this will be a major clinical breakthrough providing transfusion-dependent patients with a non-immunizing safe transfusion product, likely diminishing iron overload in transfusion-dependent patients.<sup>315-319</sup>

#### European research contributions

Over the past decades, several major steps towards cultured human blood cells were established in Europe, paving the way to major advances in our understanding of the mechanisms that regulate erythropoiesis/megakaryopoiesis. The first key development for the *ex vivo* production of erythroid cells was made at the end of the last century when liquid culture systems replaced semisolid culture, allowing the expansion and differentiation of pure erythroid cultures in distinct medium conditions. This enabled large-scale culture of cRBC from hematopoietic stem cells (HSCs) of adult or umbilical cord blood origin, which was achieved with different cocktails of cytokines and additives. Improved culture conditions yielded high numbers of fully mature cRBCs and cPLTs, and the prospect of culturing blood cells for transfusion purposes became closer to a reality. Industrial production of both cRBCs/cPLTs has been tackled by simplification of protocols. Procedures without stroma or xenogenic components, using pharmaceutical-grade reagents, have been developed, allowing the production of cRBCs/cPLTs for transfusion from various stem cell sources. The first proof of concept (autologous mini-transfusion with cRBC from adult CD34<sup>+</sup> cells) in a healthy volunteer was performed in Paris.

Erythropoiesis and megakaryopoiesis should ideally be initiated from an unlimited source, such as induced pluripotent stem cells (iPSCs). The big advantage of iPSCs is that they can be selected for their phenotype of interest, or manipulated to generate specific phenotypes. Several EU teams can now produce cRBCs/cPLTs from various

sources (HSCs, human ESCs, iPSCs), but not yet at sufficient numbers or quality. An alternative may reside in immortalized cell lines, for which a Japanese team provided proof of concept by production of cRBCs and cPLTs.

The major remaining challenge for *in vitro* blood production is the affordable cost. The development of bioreactor conditions for cost-effective production at a therapeutic scale, however, is still at an early phase. The production of cRBCs/cPLTs is optimized in standard liquid bioreactors under good manufacturing practice conditions to generate enough cells for mini-transfusions for further clinical trials. In addition, 3-D bioengineered scaffolds are being tested for more efficient stem cell expansion and cost reduction. This has brought our EU consortium to a lab-to-market stage. For cPLTs in particular, 3-D culture is required, which requires the development of flow devices that are based on microfluidics and engineered biomaterials mimicking the bone marrow environment, such as silk and hydrogels.

#### Proposed research for the Roadmap

The next step is to optimize the production of cRBCs/cPLTs at the required scale for safe patient transfusion. EU blood supply centers and scientists must cooperate to: 1) fulfill regulatory compliance of cRBCs/cPLTs as new advanced therapy medicinal products (ATMPs), to define the release criteria and set functional standards for cRBCs/cPLTs; 2) prepare cRBCs/cPLTs for clinical studies; and 3) optimize culture conditions to reduce costs for the large scale required for clinical use. The key objectives for the next few years can be summarized in three steps.

1. *Achieving regulatory compliance*: the objective is to define standards for quality and safety regulations and release criteria that comply with the good manufacturing practice requirements for the production of ATMPs. These standards should be used by regulatory committees throughout Europe that have to decide on the use of cRBCs/cPLTs in clinical trials. European teams need to organize consensus approach and progress meetings with stakeholders that include experts in transfusion technology, immunohematology, clinical transfusion science, hematologists, representatives of patient organizations, and representatives of donor councils throughout the European research area.
2. *Establishing proof of principle by large-scale transfusion of cRBCs/cPLTs*: proof of principle by large-scale transfusion of cRBCs/cPLTs should be established by transfusion studies in healthy volunteers, giving insight into: 1) the stability and functionality of cRBCs/cPLTs after administration; and 2) the risk of immunization against neoantigens. The aim of the clinical trials will be to validate the release criteria set *in vitro* for their suitability to predict cellular function *in vivo*.
3. *Therapeutic-scale production at reasonable cost*: the aim is to generate cRBCs/cPLTs more efficiently without compromising the functional parameters and thresholds that define a safe cRBC/cPLT product. Modified culture conditions should result in a more efficient, automated, and economical process. There is a need to devise optimal bioreactor conditions to culture cells at high density in a low-cost medium. Immortalized

cells derived from universal donors that generate suitable, high-quality transfusion units for (almost) all patients, without adverse transfusion reactions, have to be designed. Efficient expansion and maturation of these cells may be supported by small molecular compounds. Chemical libraries can be tested to find substances that control or enhance expansion and maturation of erythroid/megakaryocytic cells, and to determine expression of appropriate types of Hbs for cRBCs.

#### *Anticipated impact of the research*

cRBCs and cPLTs derived *in vitro* with rare or near universal phenotypes would revolutionize blood transfusion practices for future safe blood transfusions.

Development of culture conditions to produce pharmaceutical quantities of blood involves a considerable amount of knowledge, innovation, and technology generation. To this end, EU teams should collaborate closely with industrial partners. In addition to the transfusion market, RBCs may be manipulated for drug delivery, promising a further revolution in blood transfusion practices.

## The EHA Roadmap for European Hematology Research

### Section 8. Infections in hematology

#### Section editor: Catherine Cordonnier.

Parallel to great progress made in the treatment of hematologic malignancies in the past 20 years, the research carried out by the hematology community has led to equally valuable progress in the incidence, causes, presentation, and mortality of infectious complications. These changes have usually been poorly anticipated, and sometimes not identified before several trials have assessed a new drug. Since tyrosine kinase inhibitors (TKIs) have been made available for chronic myeloid leukemia (CML), the indication for allogeneic hematopoietic stem cell transplantation (HSCT) has decreased considerably in this disease, and although TKIs impact on immunity, infection is now extremely rare in this setting before acute transformation of the disease. On the other hand, new monoclonal antibodies such as rituximab, which is highly efficient in many CD20-positive lymphoid malignant disorders, have created a risk of prolonged B-cell deficiency, increasing a natural risk of infection due to encapsulated pathogens and impairing the response to vaccines. In general, few new therapeutic approaches have been devoid of adverse infectious risks: the older age of HSCT candidates increases the risk of many infections after transplant; alemtuzumab treatment increases the risk for cytomegalovirus (CMV) infection and disease; eculizumab, a humanized anti-C5 monoclonal antibody used for treatment of paroxysmal nocturnal hemoglobinuria (PNH), results in susceptibility to meningococcal infection, requiring vaccination. However, many unmet needs remain and will continue to remain as long as new therapies are being developed. Understanding the mechanism of predisposition to certain pathogens is of crucial importance to develop strategies, as are exhaustive epidemiological data each time a

new treatment or strategy is assessed in hematology. This should be done not only in the classical high-risk patient populations such as neutropenic patients or HSCT recipients, but also in patients with lympho- or myeloproliferative chronic disorders who are increasingly being managed as outpatients and deserve specific considerations in terms of control of infection.

Europe has been highly active in the field. The Infectious Diseases Group of the European Organisation for Research and Treatment of Cancer has been one of the very first in the world to prospectively study febrile neutropenia.<sup>320</sup> Twenty-five years ago, the European Group for Blood and Marrow Transplantation (EBMT) created a dedicated working party for infectious complications after HSCT. The EBMT was able to show that death from infections significantly declined over time since the very early times of transplant, although it remains a major concern.<sup>321</sup> The European Conference on Infections in Leukemia was initiated ten years ago to share practices and expert opinions, elaborate guidelines for the management of infectious complications in hematology patients, and define new areas of research. More recently, the European Society of Clinical Microbiology and Infectious Diseases created a group for immunocompromised patient populations. Pharmaceutical companies have also been very active in developing new anti-infective drugs for high-risk patients. However, the evolution of bacterial resistance worldwide, more aggressive therapies for some conditions, and an older age limit for performing HSCT mean these advances are associated with a greater risk of patients dying from infection, often before benefiting from the treatment of their underlying disease. This is especially the case in acute leukemia or high-risk lymphoma patients, allogeneic HSCT recipients, and primary immunodeficient children. Several issues concern all of these populations, and targeted studies should be encouraged.

1. We need to better anticipate the infectious risk and adopt a more preventative approach on an individual basis. This implies good epidemiological data in hematology patients, better identification of specific infectious risks, and the development of scoring systems<sup>322</sup> to better target prophylaxis, which, for many reasons, including toxicity, resistance, and cost, cannot be universal.
2. We need new classes of antibacterials to overcome the inevitable increase of multi-drug resistance, which is a worldwide phenomenon and may soon constrain us to give up curative treatment in hematologic diseases. In parallel, we should explore how to limit the administration of the available antimicrobials as far as possible each time they are needed. To reach this goal, antimicrobial stewardship in the hematology ward is crucial, and yet more difficult than with other patient populations.<sup>323</sup> This requires studies and resources.
3. In order to challenge the empirical anti-infectious approach widely used in many hematology patients, we need to develop sensitive, direct or indirect markers of bacterial, fungal, and viral infection and assess their clinical value in prospective trials. The main goal should be to restrict the administration of anti-infectives as much as possible, on the basis of infection markers.

4. Faced with the real lack of new antibacterials, we need to develop alternative strategies to prevent and treat infection. To this end, vaccination, monoclonal antibodies, and targeted cellular therapy should be properly assessed in well-designed prospective and controlled trials. Except after HSCT, where it has been possible to produce evidenced-based guidelines, vaccination has been poorly explored in hematology patients so that clinical practice is heterogeneous. Prospective trials on vaccination in specific diseases should be encouraged, taking into account the timing of the therapeutic program of the underlying disease.<sup>324</sup>

Although the role of the pharmaceutical industry is of the utmost importance to produce new tests and new drugs and support large, well-designed trials, more academic involvement is essential for translational research, epidemiology, and prospective randomized trials within the field.

The unmet needs of infectious complications in hematology patients are mainly observed in four different settings.

1. HSCT recipients.
2. Neutropenic patients.
3. Non-neutropenic patients with acquired immune deficiencies.
4. Primary immune deficiencies.

We also think that exploring genetic predisposition to infections in these patients will greatly help anticipate and manage the risks.

### 8.1. Infections in HSCT recipients

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#### Introduction

Infections have been major obstacles to the success of allogeneic stem cell transplantation from the beginning, more than 40 years ago. The immunosuppression needed for successful transplantation, including the conditioning regimen, prevention of, and treatment of graft-versus-host disease (GvHD), all contribute to these risks. Transplant strategies are continuously evolving with the introduction of new conditioning regimens, an increased utilization of alternative donors (e.g. haploidentical donors), and single and double cord blood grafts. Many of these new techniques will affect the speed and degree of immune reconstitution and be related to the risk of infections. Haploidentical transplantation is performed in an increasing number of patients. However, the strategies used to perform allogeneic transplantations vary between centers. Strategies are likely to affect the immune reconstitution in different ways, and knowledge of the risks of specific infection in short, intermediate, and long-term perspectives are still limited.

Patient populations are changing, especially with more elderly patients undergoing allogeneic transplantation. Elderly patients are more vulnerable to infections such as

pneumococci and influenza, and are also likely to have comorbidities that influence the risk for infections.

Viral infections have been recognized as important for outcome, especially in allogeneic stem cell transplant recipients. Despite many advances in the field, these infections are still associated with morbidity and mortality. Several new antiviral drugs, as well as cytomegalovirus (CMV) vaccines are in late clinical development. Techniques allowing monitoring of CMV-specific immune responses in the individual patient are also being developed and might be valuable in the management algorithm. A challenge for the next few years will be to implement these new agents and techniques in the clinical management of stem cell transplant recipients, taking into consideration efficacy, toxicity, and cost aspects. New drugs are likely to be more expensive than the currently available agents, but might have significant advantages in terms of toxicity. In addition, vaccines and specific T-cell therapies are under development, and strategies for their implementation need to be developed addressing efficacy, safety, and costs.<sup>325</sup>

The importance of human herpesvirus 6 has been discussed for at least two decades, but the diagnostics and management of these infections have been controversial. Recent studies suggest that human herpesvirus 6 is an important pathogen mainly causing complications in the central nervous system in patients with poor or delayed T-cell reconstitution, such as cord blood transplant recipients.<sup>326</sup> For the moment, the available antiviral drugs are not very effective in controlling this virus.

A changing epidemiology of viral infections poses new challenges. Respiratory viruses such as respiratory syncytial virus and influenza are well recognized as important pathogens especially after allogeneic stem cell transplantation. During the past decade, at least 10 new respiratory viruses have been described, of which several have the potential to become relevant pathogens.

Previously known viruses can change their epidemiological pattern and appear as important pathogens. This is illustrated by the recent emergence of severe infections caused by enterovirus D68 in outbreaks in both North America and Europe.<sup>327</sup> Recent experience also includes the emergence of the West Nile virus, outbreaks of chikungunya virus, and the expanding areas of the world where patients are at risk of dengue virus infections. A recently recognized potentially important pathogen is the hepatitis E virus that has been associated with chronic hepatitis and possibly the rapid development of cirrhosis in small patient series.<sup>328</sup> Several of these viruses can also be transmitted from stem cell donors, possibly requiring testing for new viruses.

Vaccines are important for preventing infections in the general population. There are major gaps in our knowledge of how best to utilize vaccines in stem cell transplant.<sup>329</sup>

#### European research contributions

European centers and collaborative groups, such as the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation (EBMT) and the

European Organisation for Research and Treatment of Cancer working groups, have been active in the field of infectious diseases in transplant recipients. Topics of particular interest for European investigators include the management of viral infections due to CMV, Epstein-Barr virus (EBV), respiratory syncytial virus, and polyomavirus (BK). Other topics include the development of vaccination strategies for HSCT recipients, randomized studies of antibacterial agents, and diagnosis and management of fungal infections. Many of these studies have changed practice, not only in Europe.

#### *Proposed research for the Roadmap*

1. Immune reconstitution and infections with special emphasis on new transplant strategies.
  - a) studies are needed in patients undergoing haploidentical transplantation;
  - b) studies addressing the specific needs of elderly patients must also be developed.
2. Herpesviruses.
  - a) CMV: strategies are needed for introduction of new prophylactic, monitoring, and therapeutic strategies in patient management;
  - b) Human herpesvirus 6: new strategies must be developed through proper controlled clinical trials.
3. Respiratory viruses - new viruses.
  - a) Careful surveillance and rapid recognition of respiratory viruses by sensitive and specific diagnostic techniques are necessary for the development of infection control and management strategies. New antiviral drugs against respiratory syncytial virus, parainfluenza viruses, and influenza are undergoing clinical tests, and well-designed studies in the HSCT patient population are important.
4. T-cell therapy for infections.
  - a) Approaches aiming to improve the immune reconstitution with a minimal risk of uncontrollable GvHD, such as the use of suicide gene-expressing T cells, should be encouraged;
  - b) Multi-specific T cells with activity against several infections are interesting options, especially for prevention of viral infections;
  - c) T cells against aspergillosis might become important, because the development of new antifungals remains limited;
  - d) CMV-specific T cells are commercially available and need to be tested in larger trials.
5. Vaccines.
 

The following topics need to be addressed:

  - a) whether different vaccine schedules should be used in patients having undergone different transplant procedures needs to be examined;
  - b) new vaccines, such as inactivated varicella-zoster vaccines, CMV vaccines, and vaccines against human papillomaviruses, should be evaluated in well-designed studies.

#### *Anticipated impact of the research*

In past decades, major improvements have been achieved in infectious disease management in allogeneic HSCT recipients. Despite these advances, infections remain important causes of non-relapse mortality. New infections can quickly become severe threats, as shown by the 2009 influenza pandemic. Therefore, the impact of the proposed projects will be in two areas: to further

reduce the morbidity and mortality of infections, and to develop management strategies that quickly meet new challenges.

## **8.2. Infections in neutropenic patients**

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#### *Introduction*

Infections are the leading cause of death in patients with hematologic malignancies undergoing myelosuppressive chemotherapy. The majority of infections are caused by bacteria, but invasive fungal or viral infections also occur frequently.

#### *European research contributions*

A variety of European scientific associations and groups have played a leading role in advancing the diagnosis, prevention, and treatment of infectious diseases in patients with hematologic malignancies. In particular, Europeans have pioneered research in the field of invasive fungal diseases. Most of the diagnostic tools (e.g. galactomannan antigen and the detection of *Aspergillus* nucleic acid by PCR) have been developed and validated in Europe, leading to the globally adapted definitions of invasive fungal diseases by the European Organisation for Research and Treatment of Cancer-Mycoses Study Group in 2008 ([www.eortc.org](http://www.eortc.org)) and the European Aspergillus PCR Initiative ([www.eapcri.eu](http://www.eapcri.eu)) has developed a standard for detecting *Aspergillus* nucleic acid. Moreover, European clinical trials of antifungal treatment and prophylaxis have resulted in an overall survival benefit. Beyond this, European groups have led the field in clinical studies on antibiotic treatment and thus paved the way for internationally recognized clinical guidelines on antimicrobial strategies in patients with hematologic malignancies.

#### *Proposed research for the Roadmap*

Despite this progress, there are medical and scientific needs that remain unmet in the field of infections in neutropenic patients. The most pressing topics include: 1) the need for antimicrobial stewardship in the face of emerging multi-drug-resistant bacteria given the lack of new antimicrobial agents; 2) the challenge of community-acquired respiratory viruses; 3) improvements in control of infection; and 4) establishing the role of the microbiome.

#### *Proposed research topics:*

*Antimicrobial stewardship in prophylaxis and therapy:* the current standard of care for neutropenic patients is a pre-defined form of antimicrobial prophylaxis and empiric antimicrobial therapy as soon as fever occurs. This has been a lifesaving approach for many years, but several issues remain: 1) improving the initial response to antimicrobial therapy; 2) establishing the minimum duration of systemic antimicrobial therapy after defervescence, as current approaches often result in unnecessarily prolonged use of antibiotics; and 3) in the era of multidrug-resistant pathogens,<sup>330</sup> the current recommendations for



antimicrobial prophylaxis and treatment, even after establishing an etiology, need to be reevaluated. Thus, we propose research regarding the following topics.

1. Use of therapeutic drug monitoring to personalize dosing and improve response to prophylaxis and initial empirical therapy.
2. Prospective comparison of the duration of antibiotic treatment, including in de-escalation (step-down) strategies, with or without guidance by inflammatory markers such as C-reactive protein, procalcitonin, interleukin-6, and markers of gut function (e.g. citrulline).
3. Prospective evaluation of different antimicrobial prophylaxis regimens utilizing commonly used fluoroquinolones, as well as "older" drugs such as cotrimoxazole, with prospective monitoring of inflammatory markers and citrulline.
4. Prospective evaluation of a systematic approach based on clinical and laboratory findings to guide pre-emptive compared with empiric antimicrobial therapy including antifungal therapy.
5. Clinical trials of novel antibacterial agents with new targets and mechanisms of action in neutropenic patients.

*Community respiratory viruses (CRV):* a series of outbreaks have brought CRVs to the attention of clinicians and scientists.<sup>331</sup> CRVs are regarded as an uncommon cause of fever during neutropenia, because they tend to occur seasonally and are difficult to diagnose, but they have not yet been studied systematically. However, the increasing number of reports of fatal CRV infections has illustrated the need for a better understanding of CRV epidemiology. Further research is warranted, and we suggest the following topics for clinical studies.

1. Studies on epidemiology, seasonality, and clinical relevance of CRVs in neutropenic patients using modern diagnostic methods.
2. Prospective evaluation of infection control measures, especially in asymptomatic patients shedding the virus.
3. Clinical development of novel antiviral agents with activity against relevant CRVs.

*Efficacy of infection control measures:* infection control measures are adopted routinely to reduce the risk of hospital-acquired infections. These measures include protective isolation, use of gloves and gowns, rigorous disinfection measures, reduction of inhaled potentially infective particles by air filtration and masks worn by patients leaving their rooms or wards, and low-microbial hospital diets. However, there is no evidence of benefit from these measures in terms of reducing infection-related morbidity and mortality.<sup>332</sup> In the light of an increasing threat from multi-drug-resistant bacteria and fungi among hematologic patients, a comprehensive review is long overdue. What is needed is a series of prospective, randomized clinical studies focused on the use of the following.

1. Low-microbial hospital diet.
2. High-efficiency air particle filtration.
3. Disposable gowns, gloves, and masks for caregivers and visitors.

4. Well-fitting masks for patients outside their treatment rooms.

*Microbiome:* in recent years, a rapidly evolving insight into the essential role of the intestinal and skin microbiome for the quality of immune responses in normal and immunocompromised individuals has been attained. This has been shown to have a significant impact on the clinical course of allogeneic stem cell transplant recipients, but potentially also on the general interaction between host and tumor cells.<sup>333,334</sup> There is a need to better understand the pathogenesis and background of fever and infections in patients undergoing myelosuppressive chemotherapy, for distinguishing febrile immune reactions from infections due to microbial pathogens, and for designing more specific ("targeted") antimicrobial or anti-inflammatory treatment in this severely immunocompromised patient cohort. Clinical and translational studies are needed in neutropenic patients to explore the role of the microbiome in the following.

1. The intestinal tract.
2. The oral cavity and teeth.
3. The respiratory system.
4. The skin.

#### *Anticipated impact of the research*

These projects will result in the following.

1. A significant improvement of survival rates of neutropenic patients.
2. An improvement of the quality of life of neutropenic patients through better prophylaxis of infectious events, deletion of unnecessary procedures, shortening of antimicrobial therapy duration, and subsequent reduction of collateral damages of antibacterials.
3. Reduction of unnecessary antimicrobial prophylaxis and therapy leading to reduced pathogen and commensal resistance rates.
4. Evidence-based update of the guidelines on the rational use of infection control measures for severely immunocompromised patients.
5. A fundamental revision of the current approach to fever and infections in neutropenic patients.

### **8.3. Infections in non-neutropenic patients other than hematopoietic stem cell transplantation recipients**

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#### *Introduction*

There is a growing population of patients with hematologic malignancies who receive chemotherapeutic agents that do not cause neutropenia but result in other types of severe immunodeficiencies, such as T-cell defects and hypogammaglobulinemia. These patients are at risk of developing potentially life-threatening infections, which are different from those commonly seen during neutropenia, due to differences in the underlying immunodeficiency. There is little reliable and systematic information on infections in non-neutropenic hematologic patients, even though they significantly outnumber those with neutropenia.<sup>335</sup>

Studies on infectious complications in patients with

hematologic malignancies are paramount for determining the efficacy of chemotherapeutic agents. Indeed, even a drug with a 100% curative effect of the underlying hematologic disease cannot be used if it results in a 90% rate of severe infections, which cannot be prevented or treated. Thus, the lack of advances in infectious diseases (e.g. the shortage of efficacious antibiotics against multidrug-resistant gram-negative bacteria) may result in unacceptably high mortality, hampering advances in chemotherapy.<sup>336</sup>

Most of the infections developing in subjects undergoing different chemotherapeutic treatments are not unexpected, and they could be predicted and prevented if timely studies on the infectious complications were to be carried out. The monoclonal antibody eculizumab is a good example; it predisposes patients to meningococcal infection, and this could be foreseen based purely on the knowledge of its mechanism of action. Eculizumab mimics the C5 complement deficit, which can occur as an inherited immunodeficiency, and it is associated with repeated or relapsing meningococcal infections. Secondly, if registration trials were properly designed, the true rate of specific infections could have been established before the drug was marketed and proper preventive measures could be designed. Another example is alemtuzumab: here, no regular monitoring of cytomegalovirus (CMV) reactivation was performed in the first efficacy studies. Dedicated studies and appropriate recommendations on CMV management in this setting could only be made several years later. Last but not least, post-marketing monitoring of infectious complications would benefit from a standardized and dedicated approach so that real-life incidence of infectious complications can be properly assessed.

Preventing infectious complications has always been the most appealing approach, yet the benefit of prophylaxis must be carefully weighed against its short- and long-term side effects, such as drug toxicity and a change in epidemiology or resistance patterns. The latter is of particular importance in our era of multidrug-resistant bacteria, yet it has rarely been evaluated in trials aimed at short-term benefits.<sup>337</sup> Antibiotics are the only drugs in which improper use does not reflect on the patient being treated, but rather on other patients and generations of future patients.

In addition, there is a global understanding that hospital stay is associated with several risks and substantial costs, such as colonization and infection with nosocomial pathogens, including resistant bacteria or *Clostridium difficile*. Along with a reduced risk of health care-associated infections, the undeniable benefits of outpatient therapy are better quality of life (QoL) for patients and reduced costs.<sup>338</sup> Although some neutropenic patients are also cared for in their home environment, outpatient management is particularly suitable for non-neutropenic populations. In order to perform secure treatment in outpatient settings, all management procedures, such as diagnostic methods, infection control measures, and treatment, should be adapted to the outpatient condition. Therefore, rapid diagnosis, possibly based on a point-of-care approach, should be developed; oral or once-daily intravenous therapy should be preferred.

#### European research contributions

Multidisciplinary efforts, including efforts from infec-

tious disease specialists, hematologists, microbiologists, and infection control specialists, are needed in the field of infectious complications in hematology patients, and several studies have been successfully carried out in Europe for more than 40 years.<sup>339</sup> Scientific organizations and societies, such as the Infectious Diseases Group of the European Organisation for Research and Treatment of Cancer, the European Conference on Infections in Leukemia, the European Group for Blood and Marrow Transplantation (EBMT), the Immunocompromised Host Society, the European Conference on Infections in Leukemia and the European Society of Clinical Microbiology and Infectious Diseases, have significantly contributed with studies and guidelines on the management of infectious diseases in patients with hematologic malignancies.

#### Proposed research for the Roadmap

Several initiatives at different levels, ranging from basic science to large epidemiological studies, should be undertaken to comprehensively address the problem of infections in non-neutropenic populations. Major areas of interest requiring research resources and efforts include the following.

1. Background and basic science studies aiming at understanding the intimate mechanism of action and the potential proinfectious effect of novel molecules or biological agents, with certain infectious problems possibly anticipated even before clinical trials.
2. Inclusion in clinical trials of new agents of an *ad hoc* section for the detection of infectious complications, designed and reviewed by experts in the field of infectious diseases.
3. Setting up pre- and post-marketing registers of infectious complications in patients receiving chemotherapy, in order to establish long-term safety of new products and to react immediately when unexpected problems arise, which would also allow full assessment of the risk profile of novel therapies.
4. Studies on the need for prophylaxis of bacterial, viral (including hepatitis B), and fungal (including *Pneumocystosis pneumonia*) infections, its efficacy, cost-effectiveness, and short- and long-term benefits and risks.
5. Studies on the role of vaccination for preventable diseases (e.g. influenza and pneumococcus), and implementation of new efforts for new approaches.
6. Tuned-up revaccination strategies.
7. Studies in the non-neutropenic population on the performance of diagnostic assays based on antigen or DNA detection for bacterial and fungal diseases, and further development of immunological diagnosis by means of monitoring cellular immune responses to specific pathogens (e.g. ELISPOT).
8. Novel rapid diagnostic methods, to be used especially in the outpatient setting (e.g. point-of-care tests and molecular diagnostics).
9. Studies on effective and convenient outpatient therapy of infections, including oral antibiotics and antibiotics with prolonged half-lives.

#### Anticipated impact of the research

Implementing the studies and initiatives described above, we should be able to obtain a more complete and

thorough approach to infectious complications in non-neutropenic patients. Thus, mortality and morbidity can be reduced, providing full benefit of novel therapies by minimizing the drawback of unexpected or uncontrolled infections. The individualization of the management of infectious risk and the development of outpatient infection control protocols and tools for diagnosis and treatment of infections should result in better quality of life and reduced cost.

#### 8.4. Infections in primary immune deficiencies

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##### Introduction

Although individually rare disorders, there are now more than 200 genetic primary immunodeficiencies (PIDs) described, with 1:2500 people having one PID or another. The lifetime burden of infection in this group is high and infections can be unusual and difficult to treat. The risk is increased by immunosuppression associated with hematopoietic stem cell transplantation (HSCT).

Early diagnostics and effective treatment for bacterial, viral, and fungal infection is important, as is surveillance within the bone marrow transplantation (BMT) unit setting.

The research is relevant for various patient populations.

1. Severe combined immunodeficiencies, including conditions such as X-linked severe combined immunodeficiency, adenosine deaminase deficiency, and Omenn syndrome.
2. Combined immunodeficiency syndromes, including conditions such as Wiskott-Aldrich syndrome, T-cell activation defects, MHC class II deficiency, and CD40 ligand deficiency.
3. Antibody deficiencies, including X-linked agammaglobulinemia, common variable immunodeficiency, and selective IgA/specific antibody deficiencies.
4. Diseases of immune dysregulation, including hemophagocytic lymphohistiocytosis and X-linked lymphoproliferative syndrome.
5. Congenital defects of phagocyte number, function, or both, including disorders such as chronic granulomatous disorder.
6. Defects in innate immunity, including conditions such as NEMO deficiency and complement deficiencies.

##### European research contributions

More than 200 PIDs have now been genetically defined and infectious complications and prognosis are more predictable. Viral PCR tests enable much more rapid accurate diagnosis. Furthermore, new antiviral and antifungal drugs improve outcome. Clearer guidelines for the management of febrile neutropenia and research networks for studying infections in the immunocompromised have been developed through learned societies such as the European Society for Paediatric Infectious Diseases and the European Society of Clinical Microbiology and Infectious Diseases.

##### Proposed research for the Roadmap

*Viral infections:* improved rapid methods for detection of viruses are now in clinical use. PCR methodology allows an accurate identification and quantification of viral load, as well as better planning of treatment strategies and monitoring of therapeutic effects; however, some caveats remain.

1. There is a need for better treatments; current approaches are associated with significant toxicities for the patient (e.g. foscarnet and cidofovir) and potentially also for the staff (e.g. nebulized ribavirin). The prolonged treatment required confers a risk of emerging resistance. Little is known about efficacy and toxicity of combination therapy in these complex cases. Particular viral treatment challenges include Epstein-Barr virus (EBV), adenovirus, and cytomegalovirus (CMV), where cellular (virus-specific cytotoxic lymphocytes) and antibody (monoclonal antibody, e.g. respiratory syncytial virus-specific palivizumab) therapies seem to be of limited effectiveness and not always easy to arrange and administer.
2. Improved prevention and treatment of respiratory infections are needed, especially for rhinovirus, respiratory syncytial virus, parainfluenza, influenza, and adenovirus.<sup>340</sup>
3. Human papillomaviruses cause severe massive skin warts in specific immunodeficient patients, and better therapeutic options are needed.
4. New live attenuated vaccines, such as the rotavirus vaccine, given prior to PID diagnosis, confer new challenges. Ideally, PIDs should be diagnosed prior to first vaccinations.

*Central line-associated bloodstream infections:* this remains a significant issue in oncology and chronic diseases. The morbidity and mortality of central line-associated bloodstream infections mean that all children with fevers and central lines undergo blood culturing and empirical broad-spectrum antibiotic treatment, affecting antimicrobial resistance in a high-risk population.<sup>341</sup>

1. There remains a need for more timely diagnostics and better prevention of central line-associated bloodstream infections, including adjuncts or alternatives to antibiotic use (e.g. biofilm inhibitors).
2. A better antimicrobial stewardship is required to preserve antibiotics for now and the future in an era of increased antimicrobial resistance.

*Gastrointestinal infections:* PID patients are affected by many bacterial and viral gut pathogens with an ongoing impact on nutrition and fluid management, a significant cause of morbidity and mortality.

1. Improved diagnostics and treatments for *Cryptosporidium* spp. are required.<sup>342</sup>
2. Improved treatment modalities for enteroviruses in the context of X-linked agammaglobulinemia, where paralysis and death can result from ongoing infection, are required.
3. A better understanding of the human gut microbiome and its impact on the immune system is needed.
4. Improved diagnostics to distinguish gut graft-versus-host disease (GvHD) from infectious enteritis requiring distinct management are needed.

*Nosocomial infections:* PID patients who require prolonged or multiple hospital admissions are at a very high risk of nosocomial infections. These potentially increase the length of hospital stay, having not only clinical but also economic implications on health services.

1. Better estimation of the prevalence of nosocomial infections (e.g. norovirus and sapovirus) is needed. The morbidity and mortality associated with these infections require effective preventative strategies and new therapeutic interventions.
2. For those patients requiring ventilation, ventilator-associated pneumonia is an important cause of morbidity. Better anti-infective and anti-inflammatory treatments are needed here.

*Infections associated with biologics:* biological agents, particularly monoclonal antibodies to proinflammatory cytokines, are being widely used, increasing the risk of infections in a high-risk population:<sup>343</sup>

1. More information regarding specific infectious risks of this family of agents would be useful to allow appropriate prophylaxis to be instituted.
2. More information regarding how this family of agents affects immune response to vaccination is warranted. While aiming to prevent vaccine strain infections, whenever possible, appropriate protection with vaccination is needed. Timing of vaccination is likely to be important and should be considered prior to commencing immune-modulating therapy.

*Fungal diagnostic markers and treatment monitoring:* morbidity and mortality due to invasive fungal infections are increasing in PID patients, especially those who are on immunosuppression.<sup>344</sup> *Candida* spp. and *Aspergillus* spp. diagnostics are currently limited to culture and histopathology, with serum markers such as galactomannan showing limited reliability.

1. Surveillance throughout HSCT could be improved with better rapid diagnostic tests to allow more timely management and reduced mortality.
2. Call for standardized diagnostic tests to be established to allow universal diagnostic criteria to be set and commercial assays or protocols to set up services.
3. Large interindividual variabilities of antifungal drug levels are observed in children. Pharmacokinetic/pharmacodynamic data are limited in this group and are warranted for prompt informed adjustment of dosing, allowing optimal treatment with these potentially lethal infections.
4. Optimal duration of antifungal treatment is ill defined, as is subsequent secondary prophylaxis.

*Surveillance of infections post HSCT:* PID patients preparing for HSCT are screened for blood, respiratory, and gut pathogens. Although reasonably controlled pre-HSCT, these pathogens can cause significant problems as the child progresses through HSCT with the conditioning and immunosuppressive regimens. A further understanding of control mechanisms and preventative strategies are needed for CMV, EBV, adenovirus, and cryptosporidium in the PID patient leading up to HSCT.

#### *Anticipated impact of the research*

The impact of these proposed research areas will have a huge benefit on the management of PID patients. Their vulnerability to both common and opportunistic pathogens leaves them at a high risk even before they go through the HSCT process. We have a limited repertoire of prophylactic antimicrobials, immunoglobulins, and treatment modalities for these patients. Even those who are appropriate for and successfully transplanted are highly susceptible at various points. Therefore, in order to minimize the impact of their underlying diseases and HSCT, improved diagnostics, therapeutics, and monitoring are desperately needed.

### **8.5. Genetic predisposition factors for infection in hematology and hematopoietic stem cell transplantation patients**

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#### *Introduction*

Infectious complications represent a major challenge in patients undergoing intensive chemotherapy for the treatment of hemato-oncological diseases and/or hematopoietic stem cell transplantation (HSCT). The most common pathogens are gram-positive and gram-negative bacteria (usually causing bloodstream infections during the course of neutropenia); fungi such as *Candida* or *Aspergillus* species (causing invasive infections during the course of neutropenia or after HSCT); and viruses such as cytomegalovirus (CMV) (that can be reactivated and/or cause disease after HSCT). Although specific risk factors, such as patient age, comorbid conditions, and type and duration of immunosuppression, have been identified, it is still difficult to accurately predict which patient will develop which infection, and at which time. Because most infections are severe, prophylactic and empirical drugs are increasingly administered, exposing patients to potentially unnecessary side effects and the development of resistance.<sup>345</sup> It is becoming clear that an individualized risk assessment may dramatically improve the appropriateness of antimicrobial use in such immunocompromised patients.

Over the past 15 years, a number of studies have evaluated whether genetic factors influence susceptibility to infections in hematologic patients. These studies have been made possible through several concomitant events, including the availability of the full human genome in the early 2000s; the development of new, rapid, and inexpensive genotyping techniques; and major discoveries in the field of innate immunity. At the molecular level, the detection of pathogens is mediated by pattern recognition receptors located on the surface or within immune cells. Pattern recognition receptors detect specific molecular patterns from microorganisms and mediate immune response and cytokine production. Therefore, polymorphisms in gene-encoding cytokines and pattern recognition receptors, such as TLRs and C-type lectin receptors (e.g. CLEC7A), were extensively studied.

However, such studies were often limited by several factors, including problematic design, lack of precise definitions for infections, inappropriate control selection, small sample size, increasing use of prophylactic regimen, failure to account for important covariates, and/or lack of correction for multiple testing.<sup>345</sup> Another important limitation has been the lack of a definite role for some of the reported genes and/or their polymorphism(s) in the immune response mechanisms against the offending pathogen. So far, existing data have not supported the use of such polymorphisms for individual risk stratification in clinical practice. However, recent associations have been reported that are more promising, because they have been replicated in different studies and/or different populations at risk and/or are supported by strong functional evidence.

#### European research contributions

Most genetic association studies in hemato-oncological patients focused on susceptibility to invasive fungal infections (n=30). Only a limited number of studies analyzed the risk of developing bacterial (n=8) and viral infections (n=4). Among studies reporting associations supported by replication and/or convincing functional evidence, all were performed on susceptibility to invasive fungal infections in relatively large cohorts of Caucasian patients undergoing HSCT,<sup>346-348</sup> mostly in European centers.<sup>347,348</sup>

In a study of 702 HSCT recipients (336 included in a discovery and 366 in a replication cohort), rs4986790 and rs4986791 single nucleotide polymorphisms in *TLR4* were associated with an increased risk for the development of invasive aspergillosis. The relevant polymorphisms were found in the donor, not the recipient, a specific feature that was observed in subsequent studies of HSCT patients. Although *TLR4* is a detector for O-linked mannan, an important component of the fungal cell wall, the exact mechanism by which the polymorphisms influenced fungal pathogenesis has remained controversial. In another study of 205 HSCT patients, a stop codon polymorphism in *CLEC7A* (Y238X, found either in the donor and/or the recipient) was associated with susceptibility to invasive aspergillosis.<sup>347</sup> *CLEC7A* is the detector of  $\beta$ -glucan, a key component of the fungal cell wall. The Y238X polymorphism leads to *CLEC7A* deficiency and was also associated with different forms of *Candida* infections.<sup>349</sup> Although promising, associations with relatively infrequent polymorphisms, such as those in *TLR4* (minor allele frequency ~0.05) and *CLEC7A* (minor allele frequency ~0.08), may be difficult to replicate and implement as risk predictors in clinical practice.

A more frequent polymorphism in donor pentraxin 3 (*PTX3*, rs3816527, minor allele frequency ~0.43) was associated with susceptibility to invasive aspergillosis in a study of 268 HSCT recipients. The investigators replicated the association in an independent cohort of 330 HSCT patients. Furthermore, a similar association was observed in a cohort of 1101 solid organ transplant recipients.<sup>350</sup> *PTX3* is a pattern recognition receptor that can directly bind to *Aspergillus* conidia, thereby acting as an opsonizing factor for complement activation and phagocytosis, or can interact with *CLEC7A* or *TLR4* to enhance immune recognition. *PTX3* variants were associated with reduced *PTX3* production in neutrophils with defective phagocyt-

ic activities and reduced *Aspergillus* clearance. Altogether, these features currently make *PTX3* rs3816527 the most promising marker for invasive fungal infections in hemato-oncological patients.<sup>345</sup>

#### Proposed research for the Roadmap

*Implement prospective cohorts of hemato-oncological patients:* efficient exploration of host genetics in susceptibility to infections among hemato-oncological patients needs data from a much larger number of patients than most studies have been able to collect so far. This could be obtained by implementing a large multicenter cohort with efficient structures for prospective data collection (e.g. electronic case report forms, use of standard definitions for relevant phenotypes, and systematic quality checking) and biobanking (e.g. centralized storage and processing and genotyping) in accordance with existing regulations.

*Improving the quality of genetic association studies:* there is an urgent need to improve the quality and reliability of genetic association studies. Future proposals need to follow stringent criteria for cases and control enrollment (e.g. standard definition of cases, controls exposed to the same risk as cases, and period of observation and/or use of time-dependent analyses), study design (e.g. complete publication of polymorphisms tested, rationale for gene/polymorphism selection, and replication for relevant associations), genotyping (e.g. standardization and quality controls), and statistics (e.g. power calculation, multiple testing correction, and multivariate analyses accounting for all factors influencing the phenotype).

#### Anticipated impact of the research

Well-conducted studies based on large, multicenter cohorts are needed to further establish the role of host genetics on susceptibility to infections in high-risk hemato-oncological patients. Such factors may contribute to improved infectious risk stratification and individualized prevention strategies.

## The EHA Roadmap for European Hematology Research

### Section 9. Hematopoietic stem cell transplantation and other cell-based therapies

#### Section editor: Willem Fibbe.

More than 35,000 hematopoietic stem cell transplantations (HSCTs) were performed in Europe in 2011. Of these, 60% were autologous and 40% were allogeneic transplants. It is worthy of note that allogeneic HSCT remains the single potentially curative option for many patients with hematologic malignancies, despite recent progress with chemotherapeutic treatment modalities. Allogeneic stem cell transplantation largely relies on the principle that the immune system has the power to cure hematologic tumors, provided that tolerance is achieved for the immune cells that mediate these activities. With the development of the reduced intensity conditioning regimens aiming primarily at tolerance induction rather than at eradicating disease, allogeneic stem cell transplantation can now be applied in elderly patients and in patients with significant comorbidities. Issues that remain to be solved, and that require a better understanding,

include graft-versus-host disease (GvHD), promotion of immune reconstitution, and mechanisms underlying the graft-versus-tumor effect. In particular, following reduced intensity conditioning, the transplant procedure itself may not be sufficient to eradicate disease. Therefore, additional steps are required that include post-transplant immunotherapy, such as donor lymphocyte infusions or other forms of cellular immunotherapy. In this regard, significant scientific progress has been made in the past few years with the development of novel approaches in this setting. These include the targeting of extracellular antigens through chimeric antigen receptor (CAR) T cells, T-cell therapy with gene-modified T-cell receptors, and strategies aiming at amplification of *in vivo* immune responses. Through these “checkpoint-blocking agents”, potentially curative immune responses become apparent by blocking inhibitory signals to immune cells. The identification of antigens underlying these responses will be of crucial importance to further develop disease- or patient-specific forms of immunotherapy. The disadvantage of checkpoint blockers *via* inducing autoimmunity underscores the need for further research into this promising new approach.

Expansion of hematopoietic stem and progenitor cells may aim at solving the problem of hematopoietic and immune reconstitution that is observed particularly following cord blood transplantation. A further understanding of the hematopoietic niche in regulating HSC expansion and differentiation is required to develop novel expansion technologies. This will also be important for the further development of genetically repaired hematopoietic stem cells (HSCs) through the use of artificial nucleases. Through these novel technologies, it may be possible to edit the genome of patients with congenital hematologic abnormalities, thereby bypassing the need for allogeneic stem cell transplantation (ASCT). A requirement to make this technology successful will be amplifying the corrected HSCs to the numbers that are required for functional engraftment. This will enable a novel form of ASCT.

These new therapeutic strategies require substantial manipulation of cells, including expansion, differentiation, and gene transfer.

Cells that have undergone this more than “minimal manipulation” are regarded as advanced therapy medicinal products (ATMPs) and are regulated as drugs. As such, approval is required of a national regulatory and ethical committee before clinical studies can be undertaken. In addition, ATMPs have to be produced under good manufacturing practice conditions and require an Investigational Medicinal Product Dossier and an Investigator’s Brochure. There is a clear need to make the regulatory procedure less complicated in order to allow an easier and more rapid development of these novel therapeutics.

### 9.1. Allogeneic stem cell transplantation

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#### Introduction

*Prevention and control of graft-versus-host disease (GvHD):*

during the past decade, our understanding of the pathophysiology of acute GvHD has greatly improved. Much has been learned from pre-clinical models and less from correlations with clinical observations or therapeutic interventions. Little progress has been made since the mid 1980s, and GvHD still develops in approximately 40%-60% of recipients. Thus, there is a deep need to develop newer approaches to mitigate and effectively treat GvHD, which may facilitate the wider use of allogeneic hematopoietic stem cell transplantation (HSCT).

Promoting immune reconstitution without increasing GvHD is still the main challenge, especially after umbilical cord blood or T-cell depleted haploidentical HSCT, which are associated with prolonged immunodeficiency.

While a slow T-cell reconstitution is regarded as primarily responsible for deleterious infections, GvHD, and relapse, the importance of innate immune cells for disease and infection control is currently being re-evaluated. Relapse following allogeneic HSCT has remained unchanged throughout the past three decades, as recently reviewed in the second workshop convened by the National Institutes of Health.<sup>351</sup>

#### European research contributions

Clinical trials mainly conducted in the European Union (EU) demonstrated that intensifying GvHD prophylaxis by T-cell depletion with antithymocyte globulin prevents GvHD. Up to 60% of patients who develop GvHD will have inadequate responses to corticosteroids, however, portending a dismal prognosis. Trials conducted have demonstrated that high doses are not more efficient than standard dose steroids, and that adding any new drug to steroids improves outcome.

#### Proposed research for the Roadmap

Considering the increased utilization of hematopoietic cell transplantation, the morbidity and mortality associated with GvHD, and the limitations inherent to contemporary therapies, novel approaches are urgently needed. Two main priorities have been identified.

1. Developing translational research at the European level on human GvHD to study pathophysiology and develop biomarkers to assess disease severity.
2. Favoring the development of clinical trials with ancillary studies based on strong biological background, leading to rapid development to phase III trials through “pick-the-winner” phase II trials.

A variety of approaches have been explored pre-clinically and clinically, including cytokines, keratinocyte growth factor, growth hormone, cytotoxic lymphocytes, and mesenchymal stromal cells (MSC) or blockade of sex hormones.

Dissecting GvHD from graft-versus-leukemia has been the aim of several decades of unsuccessful pre-clinical and clinical studies. A recent study<sup>352</sup> suggests that the combined use of regulatory and conventional T cells prior to selected CD34<sup>+</sup> cell transplants may result in a surprisingly low rate of relapse, without increasing GvHD. In other words, we now have several studies suggesting that leukemia relapse can be diminished either by pre-transplant, peri-transplant, or post-transplant interventions. We also have new cellular tools.

Prospective trials with a control arm are badly needed to prove this is the case.

Strategies to prevent or treatment post-HSCT relapse<sup>351</sup> include the following.

*Prophylaxis pre-transplant:* several agents can be given before HSCT in the attempt of reducing relapse; pre-HSCT azacytidine, other chemotherapy, and bispecific antibodies have been used in phase II trials. In particular, one study has reported interesting results in refractory or relapsed patients with acute myeloid leukemia (AML): a short course chemotherapy, given prior to the conditioning regimen, produced survival in excess of 50%, but only if patients had received one to two courses of induction therapy.<sup>353</sup> If patients had received more than two courses of chemotherapy, results were very poor.<sup>353</sup> Therefore, AML patients with primary induction failure should proceed to transplant immediately after the second course of failed chemotherapy, rather than attempting sequential intensive additional chemotherapy.

The role of pre-transplant azacytidine in patients with refractory anemia with excess blasts is controversial; unfortunately there has been no prospective randomized trial, and a large retrospective study by the French transplant group has failed to show any advantage of pre-transplant azacytidine on post-transplant relapse or survival.<sup>354</sup>

Prospective randomized trials are necessary to assess whether a short course of chemotherapy the week prior to conditioning in AML or azacytidine in refractory anemia with excess blast patients will reduce the risk of post-transplant relapse.

*Prophylaxis post transplant:* prophylactic tyrosine kinase inhibitors (TKIs) have been tested prospectively post HSCT against controls and have been shown to reduce the risk of relapse in patients with Ph<sup>+</sup> acute lymphoblastic leukemia (ALL). Recent data would suggest that prophylactic post-transplant panobinostat or azacytidine may be beneficial in patients with AML; these data warrant confirmation through controlled trials.

*Preemptive treatment of molecular relapse:* markers for minimal residual disease (MRD) are required to implement pre-emptive treatment: in acute myeloblastic leukemia, treatment driven by WT1-based MRD, with donor lymphocyte infusions, has limited success. The use of bispecific antibodies and cellular therapy with CAR T cells<sup>355</sup> may produce significantly better results and should be tested prospectively.

*Treatment of hematologic relapse:* once hematologic relapse has occurred, outcome depends on the pace of the underlying disease; in chronic disorders, long-term survival is still possible.<sup>351</sup> In patients with acute leukemia, the major predictor of outcome is the interval between the first transplant and relapse:<sup>351</sup> patients relapsing within six months are at a very high risk of early death, whereas delayed relapses can be successfully treated in a proportion of cases with a second transplant or chemotherapy and donor lymphocyte infusions.<sup>351</sup> The use of bispecific antibodies and CAR T cells may offer better results,<sup>355</sup> and trials are under way to assess optimal protocols.

#### *Anticipated impact of the research*

HSCT is increasingly performed in Europe (15,000 per year). Recent progress now allows transplantation in older patients (over 60 years of age). Prediction and improved treatment of early immunological complications remain an unmet medical need.

Progresses in managing infectious complications have contributed to improved survival rates during the past ten years. Further improvement should incorporate recent knowledge about innate lymphoid cells and immune response to cancer to improve long-term outcome.

### **9.2. Immune-based therapies for hematologic malignancies**

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#### *Introduction*

Targeted therapy in patients with hematologic malignancies aims at generating new agents, such as monoclonal antibodies, that are less toxic than conventional chemo- or radiotherapy. Monoclonal antibodies are effective in a number of hematologic malignancies. Most of the currently identified targets for monoclonal antibodies are also expressed on non-malignant cells. The ability to genetically engineer structure and function of these antibodies has significantly improved their effectiveness.

T lymphocytes recognize antigens through a unique antigen-specific T-cell receptor (TCR), promoting the elimination of a given target and amplifying the attack through recruitment of other components of the immune response. T cells can target antigens derived from both intracellular and extracellular proteins, including peptides encoded by mutated genes. T lymphocytes can actively distribute themselves within tissues and in the tumor environment and have the potential for *in vivo* expansion and self-maintenance, as they can establish a memory compartment.

Donor lymphocyte infusion is used to treat relapsed or residual tumor cells following allogeneic stem cell transplantation. In addition, adoptive transfer of antigen-specific cytotoxic T lymphocytes and CD4<sup>+</sup> T-helper cells has been used to treat viral or fungal infections.

Immunotherapy based on a personalized dendritic cell cancer vaccine represents an innovative approach for hematologic malignancies. In some cancer entities, dendritic cell/peptide vaccines have demonstrated clinical benefit in phase III trials. A clear shortcoming of cancer vaccines is the difficult standardization of the antigenic material. Therefore, most investigators favor mixing relevant immunogenic peptides to be used directly as peptide vaccines or loaded onto dendritic cells as professional antigen-presenting cells.

Checkpoint blockade is a form of releasing the brakes on tumor-specific T cells, allowing them to persist and expand to attack malignant cells. Cancers can develop in part as a consequence of cancer-induced immunosuppression. In many individuals, immunosuppression is mediated by CTLA4 and PD-1, two immunomodulatory receptors

expressed on T cells. Monoclonal antibody-based therapies targeting CTLA4 or PD-1 have shown significant clinical effects in patients with hematologic malignancies, such as Hodgkin lymphoma (HL).

Recent advances have allowed genetic modifications of T cells to provide robust, personalized lymphocytes that target specific tumor-associated antigens. Gene transfer in human T lymphocytes can be accomplished by several means. Here, long-term culture is required, significantly compromising their capacity to survive long term *in vivo*. Gene delivery can also be achieved through retroviral vectors. These vectors can be manufactured on a large-scale producing stable integration into the genome of the T cell and its progeny. Adverse consequences due to insertional mutagenesis in T cells have not yet been reported. Lentiviral vectors have also been used to engineer T cells. These vectors are particularly attractive when less differentiated T-lymphocyte subsets are targeted, as they have the unique ability to infect T cells even upon minimal activation, a property lacking in retroviral vectors. Novel nonviral systems (Sleeping Beauty and *PiggyBac*) allow larger fragments of DNA to be inserted than viral vectors permit.

T cells redirected to specific surface antigens on malignant cells by engineered CARs are also emerging as powerful therapies for hematologic malignancies. More than 10 clinical trials using CAR T cells for treatment of hematologic malignancies have been reported. Dramatic responses were observed, especially in patients with B-lineage ALL, even in high-risk patients. Similar to T-cell engaging antibodies, clinical use of CAR T cells especially in patients with advanced disease was associated with significant but reversible toxicity.

#### European research contributions

New bispecific antibodies also have the properties of selective antigen specificity and T-cell activation. Very high response rates and long-lasting remissions, especially after the application of the CD3/CD19-directed bispecific antibody blinatumomab, indicate that in some patients either the tumor is eradicated during T-cell activation and expansion or, more likely, a memory T-cell response directed against the tumor cells was induced or expanded. The development of bispecific antibodies for hematologic malignancies was mainly done in Europe.<sup>356,357</sup> Also new targets for bispecific antibodies will be tested in pilot trials in Europe, including multiple myeloma and AML.

Technologies of gene modification of T cells have been intensively developed in Europe.<sup>358</sup> However, Europe is lacking behind the large number of clinical trials initiated in the US. Currently, European patients are queuing up to receive treatment with CAR T cells for hematologic malignancies in US centers due to the lack of clinical trials in Europe. European biotech companies are also moving to the US for a much more rapid transfer of CAR T approaches into the clinic. T-cell therapy has been developed for clinical use in Europe, as have novel strategies of cell selection.<sup>359,360</sup> TCR gene transfer has been developed by groups in Europe, and pilot trials are ongoing.

#### Proposed research for the Roadmap

The following questions and research priorities have been identified.

1. Which antigens expressed by both malignant and non-malignant cells can be safely targeted?
2. Do all tumor cells express similar tumor-specific antigen(s)?
3. Can the tumor be eradicated if only some tumor cells are susceptible to immune-mediated targeting?
4. Are there tumor stem/initiating cell-specific antigens?
5. Can stroma-specific antigens be used to target the tumor?
6. How are long-lasting remissions following treatment with bispecific antibodies induced?
7. How can resistance be overcome, especially target loss variants?
8. How can the neurotoxicity of the constructs be explained?
9. How can the significant cytokine release syndrome following treatment with bispecific antibodies be reduced, especially in patients with more advanced disease?
10. How can specificity by combinatorial approaches targeting several tumor antigens on the tumor cell be increased?
11. Are trispecific antibodies a better strategy?

Other topics include strategies to: reduce the side effects of CAR T-cell therapy; improve long-term persistence of CAR T cells; improve efficacy of CAR T cells by combination with other T-cell activation modifiers; overcome resistance to CAR T cells, especially target antigen loss on tumor cells; and develop combinatorial approaches to improve. Efficacy and tolerability of CAR T-cell approaches tackling tumors other than the CD19<sup>+</sup> B-cell malignancies will have to be shown.

In addition, the best and most specific T-cell epitopes and most suitable T-cell subsets for adoptive T-cell therapy must be identified, and strategies to improve *in vivo* expansion must be developed. We also need to maximize the surface expression of the introduced TCR, optimize the translation of the introduced TCR  $\alpha$  and  $\beta$  chain, identify high avidity T cells, and increase TCR affinity to promote *in vivo* persistence.

To optimize induction of tumor-specific T cells and response to vaccination, we need better adjuvants to improve the mode of antigen delivery. However, little is known about the identity of tumor antigens that represent the targets of activated T cells. Currently, genomic and bioinformatic approaches are applied to identify tumor-specific mutant proteins following anti-PD-1 and/or anti-CTLA4 therapy. In addition, efficacy of checkpoint blockade is dependent on a sufficiently broad T-cell repertoire. Therefore, strategies to enhance T-cell repertoire could enhance strategies involving checkpoint blockade.

#### 9.3. The hematopoietic stem cell niche: regulation of hematopoietic stem cell function and significance for hematopoietic stem cell expansion

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### Introduction

Europe has a long-lasting track record of research focusing on the hematopoietic stem cell (HSC) niche. In fact, in 1978, Manchester-based Ray Schofield was the first to postulate that HSC function critically depends on their occupancy of specific niches located within the bone marrow. Since then, numerous cell types comprising the HSC bone marrow niche have been identified, and some of the molecular signals that they transmit to HSCs have been discovered. These signals trigger the cell-intrinsic molecular mechanisms known to regulate HSC fate. Here we provide a short summary of these studies, and discuss the new challenges and questions faced by the HSC niche field. A better understanding of the HSC niche is critical to develop both novel therapies for and preventative strategies against hematologic disease. Furthermore, delineating the molecular pathways by which the niche regulates HSC fate decision may contribute to solving the unmet clinical need for HSC *ex vivo* expansion and enhanced *in vivo* reconstitution activity for regenerative therapies.

### European research contributions

Both functional studies and direct observation of HSCs *in vivo* and *ex vivo* have been critical in developing our knowledge of the HSC niche and have highlighted how several stromal and hematopoietic cells must co-operate to ensure that the necessary number of hematopoietic cells are produced every day. European researchers have both contributed to and led these studies since their earliest days. Osteoblastic cells were the first to be identified as a component of the HSC niche and subsequently several perivascular mesenchymal cells, including nestin<sup>+</sup> progenitor cells, PDGFR/NG2/LepR-expressing cells, Schwann cells, neuronal terminations, adipocytes, and, within the hematopoietic lineages, macrophages, regulatory T cells, and megakaryocytes, have all been shown to produce molecular signals directing HSC quiescence and proliferation, self-renewal, and differentiation. These signals act through direct cell-cell interactions, such as Notch-Delta and integrin signaling; short-range gradients, such as Wnt, CXCL12, Ang1, and SCF; and long-range signals, such as cytokines and hormones. More recently, the chemical and physical composition of the bone marrow microenvironment has begun to be studied, including factors such as oxygen levels and matrix stiffness.

### Proposed research for the Roadmap

Given the complexity of the bone marrow microenvironment, the abundance of most niche components, and the rarity of HSCs themselves, a central question within the field is whether unique and rare combinations of niche cells are required to ensure correct stem cell function or whether the bone marrow space is, in fact, more fluid than postulated and stem cells are free to migrate between multiple niches to undergo different fates. To this end, intravital microscopy has shown that infection-exposed, highly engrafting HSCs are migratory and interact with larger niches than stem cells at steady state.<sup>45</sup> Although there is a clear appreciation that bone marrow physiology alters with age, most dramatically seen in the age-associated accumulation of adipocytes, we are only just beginning to understand how this affects the HSC-supporting function of the niche.<sup>361,362</sup>

Clearly this is a phenomenon that is likely to contribute

to the decreased efficacy of bone marrow transplantation (BMT) as a therapeutic modality in elderly individuals and is, therefore, an area that requires further attention. Interestingly, not only stem but also progenitor cell function is influenced by their microenvironment, and progenitor niches need to receive further attention as their manipulation is likely to lead to better short- and long-term recovery of BMT patients.

Moreover, the bone marrow microenvironment is deeply affected by the development of hematologic malignancies,<sup>47</sup> and it is, therefore, critical to understand the impact of both leukemia and chemotherapy on HSC niches, and how to restore the bone marrow microenvironment to improve outcome for therapeutic BMT. In addition, to prevent the development of hematologic malignancies, it is critical to study and understand their initial steps, when the niche conditions that support expansion of specific, often mutant, clones of HSCs are established.

A better understanding of the relationship between hematopoietic stem and progenitor cells and the bone marrow microenvironment is needed not only to improve current BMT-based therapies, but also to enable us to culture or even expand HSCs for therapeutic purposes. Currently, as few as 5% of banked cord blood samples contain a sufficient amount of stem cells to be deemed usable for transplantation. Achieving efficient *in vitro* expansion of HSCs has long been a Holy Grail of the experimental hematology community, as this would both increase the size of the stem cell pool transplanted into each patient and, therefore, ensure more rapid recovery, while dramatically expanding the pool of usable cord bloods, increasing by orders of magnitude the number of successful haplotype matches. In order to achieve this aim, and potentially revolutionize HSCT by opening up novel sources of donor cells, significant further research needs to be performed in order to ascertain how HSC-supportive signals provided by the niche can be provided artificially *in vitro*.

Cytokines have so far been the main factors used to improve HSC culture conditions. Co-culture systems, including stroma and hematopoietic cells in two or three dimensions, are currently being developed and will likely provide much insight into what molecular, chemical, and physical factors need to be combined to achieve efficient, clinical-grade, feeder-free expansion of HSCs. When not only freshly isolated but also genome-edited HSCs are expanded and transplanted, the patient population that will benefit from these studies will be even greater.

### Anticipated impact of the research

The past 30 years have seen many exciting discoveries highlighting the dazzling complexity of the HSC niche and providing some clues to its plasticity in response to stress, de-regulation when hematologic disease occurs, and critical factors that may be combined to achieve HSC expansion for therapeutic purposes. However, our knowledge about the cellular and molecular components of the hematopoietic niches and spatial organization and dynamics remain extremely rudimentary, and strong research efforts are required to enable potential future therapeutic use. The clinical need for better niche regeneration and larger availability of HSCs remains critically

unmet, and further *in vivo* and *in vitro* studies relying on genetically engineered mouse strains, xenograft models, clinical samples, and refined culture conditions are critical to start making sense of the complexity of the HSC niches. Prolonged observation of stem cells *in vivo* and *ex vivo*<sup>363</sup> will allow an understanding of how self-renewal is achieved at the single cell level and how pre-malignant clones develop into full disease, so that not only improved therapies but also effective preventive strategies will be developed.

This research will affect all patients with hematologic disease, as well as all of those requiring BMT for other reasons, and ultimately the population as a whole by reducing the burden of these diseases and, most importantly, their incidence.

#### 9.4. Human pluripotent stem cells: source of hematopoietic stem cells and hematopoietic progenitor cells

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##### Introduction

The present availability of human pluripotent stem cells (PSCs) (see subsection 1.9) raises promise for a universal resource for cell-based therapies in regenerative medicine. Rapid progress has been made in generating human PSCs amenable for clinical applications, culminating in reprogramming of adult somatic cells to autologous human PSCs that can be indefinitely expanded *in vitro*. However, how to differentiate human PSCs to specific lineages efficiently and how to select for cells that will function normally upon transplantation in adults remain major challenges. The hematopoietic lineage has been particularly refractory, and derivation of adult-type hematopoietic stem cells (HSCs) has not yet been possible, even though various other blood lineages can be obtained.<sup>364</sup> If HSCs could be generated from a patient's own human PSCs, genetic engineering could easily be used to correct genetic defects prior to differentiation into transplantable HSCs, which would overcome some caveats of conventional hematopoietic stem cell transplantation (HSCT) therapies.

Skin fibroblasts were the first human somatic cells to be reprogrammed into human induced pluripotent stem cells (iPSCs), but blood cells are increasingly used because of the availability of banked (cord and peripheral) blood samples covering a range of diseases, ages, genders, and ethnic backgrounds. Complementing human iPSC generation are attempts at "direct reprogramming" using sets of lineage-specific transfer factors (TFs) that act as master regulators and convert cells to a new differentiation state without intermediate pluripotency. Generating human iPSCs from mature B cells requires silencing of lineage-specific factors, such as PAX5, so that direct reprogramming into blood cells where this would not be necessary may, therefore, be quite feasible.<sup>365</sup>

The first steps in directed differentiation of human PSCs are guided by morphogens (e.g. Wnt, TGF- $\beta$ , activin, and BMP) important in development. Human PSCs progress through a primitive-streak-like stage before forming the three germ layers, endoderm, ectoderm, and mesoderm,

which is the layer giving rise to (hemogenic) endothelial cells and blood. While early embryonic stages are quite faithfully mimicked in culture, patterning of the mesoderm requires anatomical structure of the embryo, embryonic cell-to-cell interactions, expression of patterning genes (*Cdx/Hox*), certain cell non-autonomous effects, and exposure to physical stimuli (flow) that occur at specific stages of development. Human PSC derivatives thus remain immature, and to date, all human PSC-derived HSCs have been deficient in their developmental potential and ability to self-renew and engraft upon transplantation in mice, even though animal studies have shown that this is possible in the context of a developing embryo. PSCs do have the innate ability to differentiate into fully functional, definitive HSCs. Normal HSC development during embryogenesis occurs in several distinct temporal/spatial waves, each characterized by its own set of HPCs. Only those produced from the latter, or "definitive", wave give rise to mature, functional HSCs, but during *in vitro* differentiation, it is believed that the HSC-like cells produced are from the more primitive waves. These give rise to HSC-like cells biased toward myeloid lineages at the expense of lymphoid potential. These are unable to self-renew in culture and lack long-term engraftment capacity. The key to overcoming this HSC bottleneck will be understanding: 1) the ontogeny of human HSCs; 2) how they progress at the molecular level to become mature, adult HSCs; and 3) intrinsic and extrinsic factors that govern HSC behavior and function.

##### European research contributions

Several prominent European hematologists are using genomic approaches (e.g. gene expression and microRNA profiles and TF and histone ChIP data) to examine the molecular mechanisms that regulate cell fate decisions (e.g. self-renewal *versus* differentiation and quiescence *versus* proliferation and apoptosis) by HSCs or HPCs and their more differentiated progenies. This focus on genetic modification of stem cells especially using gamma-retroviral and lentiviral vectors has brought Europe to the global forefront of genetically modified stem cell therapy for treatment of various types of severe combined immunodeficiencies. This has been in part through EU FP7-funded projects that included PERSIST, EuroStemCell, and NOV-EXPAND, among others. This momentum is poised to combine HSC expansion with gene editing approaches that will move the field forward toward innovative therapies in the coming decade.

##### Proposed research for the Roadmap

An exciting new development in the gene therapy field is the use of designer nucleases, most recently CRISPR/Cas9-based, that are proving exceptionally efficient in engineering the genome.<sup>366</sup> Recent work has established the potential for site-specific gene editing to repair disease mutations in the human genome by targeting the integration of a corrective cDNA into the *IL2RG* locus of HSCs from a severe combined immunodeficiency-X1 patient.<sup>367</sup> For homologous recombination-based gene editing approaches to be successful, HSCs need to proliferate and template DNA harboring the corrective sequences needs to be efficiently introduced into the target cells. This can be readily achieved in human iPSCs so that these cells are prime targets for gene editing techniques. As gene editing can also be done in primary HSCs,

however, it is crucial to find conditions that allow HSCs to proliferate without loss of phenotype. Therefore, expansion protocols (see subsection 9.1) are directly relevant for the gene repair described here. Niche signals, expansion, directed differentiation toward induced HSCs, and genetic modification of HSCs and their progenies are related research topics that need an integrated approach for optimal exploitation in the clinical context.

#### **Anticipated impact of the research**

A major bottleneck to curing genetic diseases originating from mutations in HSCs is that even though efficient methods for gene targeting are now available, HSCs from neither adult nor human PSC sources can be expanded sufficiently for this to become routine practice. The combined projects in subsections 9.1 and 9.2 seek to address this and bring safe gene therapy to the clinic *via* HSCs.

### **9.5. Advanced therapy medicinal products and other cell therapies**

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#### **Introduction**

Advanced therapy medicinal products (ATMPs) comprise somatic cell therapy medicines, gene therapy medicines, and tissue-engineered medicines or combinations. The first class of ATMPs includes mesenchymal stem cells (MSCs). These are adult, fibroblast-like multipotent cells characterized by their ability to differentiate into tissues of mesodermal origin, including adipocytes, chondrocytes, and osteocytes. MSCs have been initially isolated from bone marrow, but can also be expanded from a variety of other tissues including periosteum, muscle connective tissue, adipose tissue, umbilical cord (blood), amniotic fluid, and placenta. Isolation relies on their ability to adhere to plastic surfaces and they are able to expand significantly by consecutive passaging *in vitro*. The lack of uniform criteria to define MSCs has prevented efforts to compare results from different experimental and clinical studies, and therefore the International Society for Cellular Therapy formulated minimal criteria for defining MSCs.

Mesenchymal stem cells have also been shown to possess broad immunoregulatory abilities, which can influence both adaptive and innate immune response *in vitro* and *in vivo*. The mechanisms through which MSCs exert these functions, either through cell-to-cell contact or by secretion of soluble factors, are still not completely understood. Recent findings indicate that MSCs are able to actively interact with cells of the innate immune system through which they may display both anti-inflammatory and proinflammatory effects. This ability to adopt a different phenotype in response to sensing an inflammatory environment is not yet captured in assays that are currently used to characterize these cells. The putative role of stromal cells in maintaining tissue homeostasis serves as the basis for their application in disorders resulting from auto- or alloimmune responses, including graft-*versus*-host disease (GvHD) and autoimmune disorders. These potential anti-inflammatory properties of MSCs are distinct from their stem cell characteristics.

Cell-based gene therapy is an innovative therapeutic approach based on the possibility of permanently introducing a gene of interest in the genome of human cells, thus conferring a new function to the cells and their progenies. The first clinical trials of gene therapy, performed in the 1990s with gamma-retroviral vectors, have produced promising clinical results. The recent introduction of innovative gene transfer vectors, such as self-inactivating lentiviral vectors, have further increased the efficacy and safety profile of gene therapy. Recently, the introduction of artificial nucleases, molecules able to mediate DNA strand breaks in selected genomic regions, allow not only a gene of interest to be added in human cells but also a cellular gene and thus a cellular function to be precisely substituted, thus offering new hope for the cure of several diseases.<sup>368-372</sup>

#### **European research contributions**

Whereas the development of cellular therapies in the US is primarily driven by biotech, the role of academic developments in Europe is more prominent. A broad range of phase I and II studies have been performed.

Following the first report on a pediatric patient suffering from grade IV refractory acute GvHD of the liver who was rescued by the infusion of bone marrow-derived MSCs, the cells have been studied in the context of hematopoietic stem cell transplantation (HSCT), as well as immunoregulating and regenerative therapies. These studies confirm the safety of MSC therapy and indicate significant response rates in steroid-resistant acute GvHD, solid organ transplantation, Crohn disease (fistulas), and other autoimmune disorders.

Cell-based gene therapy is currently being tested in clinical trials for the cure of genetic and acquired diseases. In recent years, patients affected by genetic diseases, such as immunodeficiencies, metabolic diseases, and thalassemias, have been enrolled in phase I-II clinical trials based on the infusion of autologous HSCs genetically modified with viral vectors, to express the correct gene and restore cellular functions. Initial results have been highly promising.

Gene therapy has been also recently applied to treat patients affected by hematologic malignancies. In this setting, T lymphocytes have been modified by viral vectors to express genes selected to increase the safety and efficacy of cancer immunotherapy. Suicide genes have been successfully introduced in allogeneic lymphocytes, to promote anti-tumor responses and control side effects. Molecules conferring cancer specificity, such as TCRs and CARs, have been introduced in patients' T cells, to mediate tumor regression. In initial phase I-II clinical trials with CAR-redirectioned T cells, striking complete remissions have been observed in patients affected by B-cell malignancies.

#### **Proposed research for the Roadmap**

The clinical efficacy for the treatment of allo- (e.g. acute GvHD and solid organ transplantation) and autoimmune (e.g. Crohn disease and multiple sclerosis) disorders is currently being studied in several randomized phase II and III studies. In addition to demonstrating improved outcome, it will be crucial to gain further insight into the biology of response. Advanced immunological monitoring that will

take into account the inflammatory status in the host at the time of treatment in conjunction with extensive analysis of the products will be crucial for the development of a signature that is associated with outcome. This will be crucial for the development of next generation MSC products with enhanced therapeutic efficacy.

Several hurdles need to be cleared before ATMP therapy can be fully exploited in Europe. Multicenter phase III studies across Europe, required to validate promising approaches tested in phase I-II studies, must be carried forward together with many national regulatory agencies, some of which interpret the European guidelines on ATMPs in different ways. This complex scenario largely explains why and how the successful clinical trials with CAR T cells have been performed mainly in the US. The scientific and medical community, and most importantly, patients and patients' associations, urgently need the use of cell-based gene therapy to be promoted in Europe, and this can be achieved only through harmonization, simplification of the procedures required for trial approval, and dedicated funding.

Finally, the full exploitation of T-cell-based cancer gene therapy to additional cancer types requires a co-ordinated and multidisciplinary effort to speed up the generation of new cancer-specific receptors, able to target a wide range of cancer subtypes. This important aim, that can now be reached, thanks to the most innovative technologies currently available, can be achieved only by multidisciplinary teams and dedicated funding.

#### *Anticipated impact of the research*

The development of a biomarker signature will allow the design of MSC products with enhanced therapeutic efficacy that can be applied in a variety of auto- and alloimmune disorders.

#### **Appendix**

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