Stem Cell Biology in Health and Disease

Thomas Dittmar · Kurt S. Zänker
Editors

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Editors
Dr. Thomas Dittmar
Witten/Herdecke University
Institute of Immunology
Stockumer Str. 10
58448 Witten
Germany
e-mail: thomas.dittmar@uni-wh.de

Prof. Dr. Kurt S. Zänker
Witten/Herdecke University
Institute of Immunology
Stockumer Str. 10
58448 Witten
Germany
e-mail: ksz@uni-wh.de

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Preface

Within the last decade there has been a dramatic increase in the understanding and application of biological principles within stem cell therapies, which has made it necessary to produce a book which intends to summarize much of the body of knowledge concerning *Stem Cell Biology in Health and Disease*. Although some of the treatments have been suggested for many years, knowledge and technology have now progressed sufficiently to allow us to test many of the different concepts with human embryonic, induced pluripotent, organ-specific and resident, cancer and mesenchymal stem cells in animal models and clinical settings – alone or in combination with other therapies in cardiovascular and neurodegenerative diseases, in diabetes and against cancer.

Studies on stem cells have been hampered in the past by the ethical and biological difficulties in preparing sufficient cell numbers in a reasonable characterized and pure form. In stem cell research we are now on the threshold of a revolution; a revolution that will have major ramification for human medicine. Giant strides in our understanding of stem cell biology and the elements that control the biological behavior of the different traits of stem cells have made it possible to intervene directly with regenerative life processes and to open a novel chapter in the fight against cancer.

Chapter 1 shortly summarizes the historical hall marks of stem cell research in biology; Chapter 2 describes the hematopoietic stem and progenitor cells in clinical use; Chapter 3 describes the protocols to expand hematopoietic stem cells ex vivo; Chapter 4 highlights one important feature of hematopoietic stem/progenitor cells, namely cell migration; Chapter 5 opens the books on properties of mesenchymal stem cells for cancer cell therapy; Chapter 6 reviews intensively alternative embryonic stem cell sources to solve both ethical concerns and the allogeneic nature of human embryonic stem cells for therapeutic use; Chapters 7 and 8 describe the role of stem cell therapy in Multiple Sclerosis and Parkinson's Disease; Chapters 9, 10 and 11 introduce novel perspectives on cancer stem cells stimulating a provocative discussion of the complexity of cancer origin, and their niches of existence either in a tumor mass or in chronically inflamed microenvironment, e.g. inflamed periodontium (Chapter 12); Chapters 13 and 14 directly address hematopoietic and solid cancer stem cells and Chapter 15 embarks on a novel role of the diversity of cancer stem cells in tumor relapse and metastases formation. Chapter 16 describes

new therapeutic approaches to eliminate cancer stem cells and Chapter 17 puts the focus on a molecular target family in cancer stem/progenitor cells - the ATP-binding cassette membrane transporters - which are promising therapeutic entities. Multiple key references are provided by the authors at the end of each chapter, and the reader is encouraged to consult these sources as well, because due to the limited space of a monograph the technical details cannot be presented in a survey of this type.

Again, we would like to thank all distinguished authors for their valuable contributions to provide with this book a robust ground for the avalanche of discoveries that will deluge the field of stem cell research in the years to come.

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Thomas Dittmar
Kurt S. Zänker

Contents

Contributors

Wolfgang H. Arnold Institute of Anatomy, Faculty of Dental Medicine, Witten/Herdecke University, Alfred-Herrhausenstr. 50, 58448 Witten, Germany, wolfa@uni-wh.de

Bruce C. Baguley Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand, b.baguley@auckland.ac.nz

R.A. Barker Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge, CB2 2PY, UK; Department of Neurology, Addenbrookes Hospital, Cambridge, CB2 2QQ, UK; Edith Cowan University, Perth, Australia, rab46@hermes.cam.ac.uk

Surinder K. Batra Department of Biochemistry and Molecular Biology, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, Nebraska 68198-5870, USA, sbatra@unmc.edu

Sebastian Becher Department of Periodontology, Faculty of Dental Medicine, Witten/Herdecke University, Alfred-Herrhausenstr. 50, 58448 Witten, Germany, basti becher@yahoo.com

Jan Cerny Department of Medicine and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01536, USA, Jan.cerny@umassmed.edu

C. Cobaleda Departamento de Fisiología y Farmacología, Edificio Departamental, Universidad de Salamanca, Campus Unamuno s/n, 37007-Salamanca, Spain, ccobalhz@usal.es

Aous Dannan Department of Periodontology, Faculty of Dental Medicine, Witten/Herdecke University, Alfred-Herrhausenstr. 50, 58448 Witten, Germany, aousdannan@yahoo.com

Thomas Dittmar Institute of Immunology, Faculty of Medicine, Witten/Herdecke University, Stockumer Str. 10, 58448 Witten, Germany, thomas.dittmar@uni-wh.de

Ian D. Duncan Department of Medical Sciences, University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706, USA, duncani@svm.vetmed.wisc.eduq

Xueli Fan Department of Medicine and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01536, USA, Xueli.fan@umassmed.edu

Graeme J. Finlay Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand, g.finlay@auckland.az.nz

Georg Gassmann Department of Periodontology, Faculty of Dental Medicine, Witten/Herdecke University, Alfred-Herrhausenstr. 50, 58448 Witten, Germany, georg.gassmann@uni-wh.de

R. Laguna Goya Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge, CB2 2PY, UK, rl342@cam.ac.uk

Wolf-Dieter Grimm Department of Periodontology, Faculty of Dental Medicine, Witten/Herdecke University, Alfred-Herrhausenstr. 50, 58448 Witten, Germany, wolfg@uni-wh.de

Jürgen Hescheler Institute for Neurophysiology, Center for Physiology and Pathophysiology, Medical Center; Center for Molecular Medicine Cologne, University of Cologne, 50931 Cologne, Germany, j.Hescheler@uni-koeln.de

JeanMarie Houghton Department of Medicine and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01536, USA, JeanMarie.Houghton@umassmed.edu

R. Jiménez Departamento de Fisiología y Farmacología, Edificio Departamental, Universidad de Salamanca, Campus Unamuno s/n, 37007-Salamanca, Spain, rajim@usal.es

Benjamin Kasenda Department of Hematology and Oncology, University of Freiburg Medical Center, D-79106 Freiburg, Germany, benjamin.kasenda@googlemail.com

Susannah H. Kassmer Department of Laboratory Medicine, Yale Stem Cell Center, Yale University, New Haven, CT, USA, susannah.kassmer@yale.edu

Kathrin Krause Institute of Immunology, Witten/Herdecke University, 58448 Witten, Germany, thomas.dittmar@uni-wh.de

Eric C. Larsen Department of Medical Sciences, University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706, USA, larsene@svm.vetmed.wisc.edu

Hanchen Li Department of Medicine and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01536, USA, Hanchen.li@umassmed.edu

Narges Zare Mehrjardi Institute for Neurophysiology, Center for Physiology and Pathophysiology, Medical Center, University of Cologne, 50931 Cologne, Germany; Department of Stem Cells, Royan Institute, Tehran, Iran, nzaremeh@uni-koeln.de

Murielle Mimeault Department of Biochemistry and Molecular Biology, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, Nebraska 68198-5870, USA, mmimeault@unmc.edu

Markus Müschen Leukemia Research Program, Childrens Hospital Los Angeles; Leukemia and Lymphoma Program, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90027, mmuschen@chla.usc.edu

Melia G. Nafus Department of Biomedical Sciences, Cornell University, Ithaca, New York, 14853, USA, mgn@cornell.edu

Christa Nagler Institute of Immunology, Witten/Herdecke University, 58448 Witten, Germany, christa.nagler@uni-wh.de

Bernd Niggemann Institute of Immunology, Witten/Herdecke University, 58448 Witten, Germany, niggemann@uni-wh.de

Alexander Yu. Nikitin Department of Biomedical Sciences, Cornell University, Ithaca, New York, 14853, USA, an58@cornell.edu

M. Pérez-Caro OncoStem Pharma, Salamanca, Spain, mpc@oncosp.com

J. Pérez-Losada Departamento de Medicina, Facultad de Medicina, Universidad de Salamanca, Campus Unamuno s/n, 37007-Salamanca, Spain, jperezlosada@usal.es

Stathis Philippou Institute of Pathology, Faculty of Medicine, Ruhr University Bochum, 44801 Bochum, Germany, Stathis.Philippou@rub.de

Sandra Pommey Department of Medicine, Immunology Research Centre, St. Vincent's Hospital, University of Melbourne, Melbourne, Victoria, Australia, Sandra.pommey@svhm.org.au

Michael Punzel Institute of Transplantation Diagnostic and Cellular Therapeutics, Universitätsklinikum Düsseldorf, 40225 Düsseldorf, Germany, mpunzel@itz.uni-duesseldorf.de

Georg Reith Institute of Immunology, Witten/Herdecke University, 58448 Witten, Germany, Georg.Reith@gmx.de

A. Sagrera OncoStem Pharma, Salamanca, Spain, asa@oncosp.com

I. Sánchez-García Experimental Therapeutics and Translational Oncology Program, Instituto de Biología Molecular y Celular del Cáncer, CSIC/Universidad de Salamanca, Campus Unamuno s/n, 37007-Salamanca, Spain, isg@usal.es

Tomo Šarić Institute for Neurophysiology, Center for Physiology and Pathophysiology, Medical Center; Center for Molecular Medicine Cologne, University of Cologne, 50931 Cologne, Germany, tomo.saric@uni-koeln.de

Sarah Schwitalla Second Department of Medicine, Klinikum rechts der Isar, Technical University of Munich, 81675 Munich, Germany, sschwitalla@googlemail.com

Jeanette Seidel Medizinische Klinik II m. S. Hämatologie/Onkologie, Charité Campus Mitte, 10117 Berlin, Germany, jeanette.seidel@charite.de

John Stagg Cancer Immunology Program, Sir Donald and Lady Trescowthick Laboratories, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia, John.Stagg@petermac.org

Calin Stoicov Department of Medicine and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01536, USA, Calin.stoicov@umassmed.edu

James E. Trosko Department of Pediatrics/Human Development, College of Human Medicine, Michigan State University, East Lansing, Michigan 48824, MI, USA, James.Trosko@hy.msu.edu

Gabor Varga Department of Oral Biology, Semmelweis University, Budapest, Hungary, varga-g@net.sote.hu

Yaming Wei Guangzhou Institute of Clinical Medicine, Guanzhou Municipal First People's Hospital, Guangzhou Medical College, Guangzhou, China, weiyaming@yahoo.com

Xin Ye Institute of Clinical Blood Transfusion, Guangzhou Blood Center, Guangzhou, China, huang_yexin@yahoo.com.cn

Kurt S. Zänker Institute of Immunology, Witten/Herdecke University, 58448 Witten, Germany, ksz@uni-wh.de

Chapter 1
Introduction

Thomas Dittmar and Kurt S. Zänker

Within the past years our knowledge about stem cell biology in health and disease has changed dramatically. What rather sounded like Science Fiction 10–15 years ago, namely that e.g., stem cells from bone marrow or from adipose tissue can be used for regenerative medical approaches, or that it is possible to create donor specific stem cells (so-called induced pluripotent stem cells (iPS cells), exhibiting embryonic stem cell (ESC) properties) simply by transducing 2–4 transcription factors, has now become reality. Likewise, the knowledge that cancer tissues are hierarchically organized like normal tissues, namely comprising of a small amount of tumorigenic cancer stem cells (CSCs) and a huge mass of non-tumorigenic cancer cells will play a crucial role in the development of novel anti-cancer strategies.

It is remarkable what has been achieved in the field of regenerative medicine within the past 10–15 years. In summary, this is an exciting story of what is possible in stem cell-based regeneration strategies, but it is also a story about a long and stony way with lots of unknown pitfalls.

In 1999/2000 first data have been published demonstrating that bone marrow-derived stem cells (BMDCs) can develop into hepatocytes [1, 2]. These original studies, being performed in rodents, were the first hints that stem cells of the bone marrow do not only give rise to cells of the blood lineage, but can also differentiate into cells of a different germ layer, a phenomenon, which has been referred to as "transdifferentiation" [3]. Till then (and to date), BMDCs were/are commonly used for bone marrow reconstitution after high-dose chemotherapy of patients with malignant hematopoietic disorders, such as multiple myeloma [4] or acute leukemias [5], or solid tumors [6].

The finding that BMDCs, and later on other types of adult stem cells, e.g., adipose-derived stem cells (ASCs) or neural stem cells (NSCs), are capable to transdifferentiate into various tissues, thereby restoring tissue integrity [7], offered perspectives for novel therapeutical approaches to heal various severe diseases, such as heart attack, liver cirrhosis, and neuronal degenerative disorders (stroke,

T. Dittmar (✉)
Institute of Immunology, Faculty of Medicine, Witten/Herdecke University, Stockumer Str. 10, 58448, Witten, Germany
e-mail: thomas.dittmar@uni-wh.de

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Parkinson Disease, etc.). Among adult stem cells, particularly BMDCs and ASCs raised (and still raise) great expectations for stem cell-based tissue regeneration strategies. Both stem cell types are easily accessible (BMDCs from bone marrow via aspiration or apheresis from mobilized donors, ASCs from liposuction) and possess an enhanced transdifferentiation capacity as verified by a plethora of excellent animal studies (for review see [7–9]). BMDCs can give rise to liver, skeletal muscle, gastric mucosa, and small intestinal epithelial cells [7]. The differentiation potential of ASCs includes adipocytes, cardiomyocytes, chondrocytes, endothelial cells, myocytes, neuronal-like cells, and osteoblasts [8]. However, there are some concerns about the overall pluripotency of adult stem cells. In contrast to ESCs and iPS cells, it is not possible to transdifferentiate adult stem cells functionally in certain tissues, like cardiomyocytes and dopaminergic neurons, in-vitro. In addition to that, even in vivo studies presented inconsistent data concerning the transdifferentiation capacity of adult stem cells. For instance, in 2001, Orlic and colleagues reported that transplanted adult bone marrow cells repaired myocardial infarcts in mice [10]. Examination of the infracted region after a period of 9 days following transplantation demonstrated that newly formed myocardium, comprising of proliferating myocytes and vascular structures, occupied about 68% of the infracted region [10]. Moreover, the functional competence of the left repaired ventricle was improved for several hemodynamic parameters [11] suggesting that efficient myocardial repair by application of BMDCs is conceivable. Only one year later, in 2002, Strauer et al. already reported about the repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans [12]. After standard therapy for acute myocardial infarction (AMI), 10 patients were transplanted with autologous BMDCs via a balloon catheter placed into the infarct-related artery during balloon dilation [12]. After 3 months of follow-up, patients of the cell therapy group showed a significantly decreased infarct region, a significantly increased infarction wall movement velocity, and a significant improvement in stroke volume index, left ventricular end-systolic volume and contractility [12].

At a first glance, these data might tell a successful “form bench to bedside” story. However, in 2004, two independent studies demonstrated that BMDCs do not undergo transdifferentiation into cardiomyocytes in myocardical infarcts [13, 14]. Murry and colleagues showed that only 1–3 cells per 100,000 cardiomyocytes were of bone marrow origin [14], which is in clear contrast to 68% as reported by Orlic et al. [10]. Likewise, data of Balsam and colleagues provided evidence that BMDCs rather adopted mature hematopoietic fates in ischemic myocardium than to transdifferentiate into cardiomyocytes [13]. Balsam and colleagues speculated that there may be differences in their anesthetic and/or surgical technique and that these may resulted in a different outcome [13], whereas Murry and colleagues assumed subtle differences in the protocols, e.g., differences in trace components in the stem cell preparation or different assays used to detect cardiomyogenic differentiation, which might explain the discrepant results [14]. In a long-term study Meyer and colleagues were able to show that a single dose of intracoronary bone marrow-derived HSPCs did not provide long-term benefit on left ventricular systolic function after acute

myocardial infarction (AMI) as compared with a randomized control group [15]. Similar results were reported recently by Choi and colleagues demonstrating a lack of additional benefit of intracoronary transplantation of autologous peripheral blood stem cells in AMI patients [16]. However, both studies reported that after 6 months the left ventricular ejection fraction was significantly improved in the cell therapy group [15, 16], which may point to a stem cell specific effect.

Further disadvantages of most adult stem cells are (i) that they do not remain in a stem cell state under in vitro conditions and (ii) that they can only expanded for limited passages. Both disadvantages omit long-term cultures of adult stem cells, which is in contrast to ESCs and iPS cells that could be cultivated nearly unlimited. For instance, bone marrow-derived hematopoietic stem/progenitor cells (HSPCs) can be cultured for 5–7 days without a significant decrease of CD34/CD133 expression. Longer cultivation periods is associated with a decrease of these two HSPC marker molecules indicating induction of differentiation. To delay the autologous differentiation capacity of HSPCs, e.g., for ex vivo expansion approaches optimized culture medias have been developed, which mostly vary in the choice of supplemented cytokines. Using optimized culture conditions it is possible to expand HSPCs ex vivo without a noteworthy level of differentiation. On the other hand, these optimized culture condition might have different effects on the expanded cells. We have recently demonstrated that the stromal cell-derived factor-1α (SDF-1α) induced migratory activity of cultivated murine HSPCs strongly depended on the used cytokine combinations [17]. For instance, cultivation of murine HSPCs in the presence of stem cell factor, thrombopoietin and Interleukin-11 yielded in the third highest expansion rate of all tested cytokines and cytokine combinations [17]. However, analysis of the migratory behavior revealed that these cells did not react to SDF-1α stimulation with an increased locomotory activity [17], which could be a severe side-effect if such cells would be used for HSPC transplantation for bone marrow reconstitution.

In contrast to adult stem cells, ESCs remain in their stem cell state in vitro and can be propagated nearly unlimited. Moreover, these cells possess an unlimited differentiation capacity in vitro and in vivo. However, human ESCs are still a subject to controversial and ethical discussions since isolation of human ESCs prerequisites the destruction of a human embryo (or the killing of a putative human life). Another disadvantage of ESCs is that they could not be administered directly in degenerated tissues while this would result in teratoma formation (which nicely illustrates their unrestricted differentiation capacity). Thus, these cells could only be implanted after in vitro pre-differentiation. However, pre-differentiated ESCs exhibit an overall lesser survival rate when removed from culture and being transplanted. Ultimately, transplantation of pre-differentiated ESCs prerequisites immunosuppression of the patients to avoid the risk of graft rejection, which, however, is associated with other risks and concerns. The latter problem could be overcome by generating "patient/custom-made embryonic cell lines", so-called therapeutic cloning. Even if this technique would be feasible one day the other two problems (ethical debate and risk of tumor formation) would remain.

Within the past two to three years a novel embryonic stem cell-like type has emerged, so-called iPS cells. These cells can be generated by viral transfection of two or four transcription factors into adult stem cells or adult somatic cells, respectively [18, 19], which ultimately leads to a redirection of this cell types towards and embryonic-like, undifferentiated state. In fact, induced pluripotent stem cells possess several ESC characteristics, such as morphology, proliferation, gene expression, telomerase activity, epigenetic status, and the capacity of unrestricted differentiation. Like ESCs, the latter property is associated with teratoma formation in-vivo if iPS cells are transplanted undifferentiated. However, even if iPS cells will be pre-differentiated prior implantation, they might bear potentially tumorigenic risks since these cells were generated by using the proto-oncogene c-myc and viral vectors, which integrate randomly into the host genome. Whether human iPS cells, either generated without the use of c-myc [18, 20] or without viral integration [21] using plasmids, will find their way into clinical use has to be elucidated in future studies. Nonetheless, the benefit of such cells would be that they behave like ESCs, thus being capable to differentiate into various tissues, and "patient/custom-made iPS cells" can be generated, which supersedes immunosuppression.

A severe side-effect of most, if not all, stem cells is their potential tumor-initiation capacity. It is well recognized that ESCs and iPS cells induce teratomas in-vivo if implanted in a undifferentiated state. Pre-differentiation of both ESCs and iPS cells could minimize this risk, whereby iPS cells might still bear potentially tumorigenic risks if such cells were generated by the use of the proto-oncogene c-myc and viral vectors, which integrate randomly into the host genome. With prolonged passage for >4 months, human ASCs have been observed to undergo malignant transformation, which was correlated with karyotypic abnormalities, tumor formation in immunodeficient mice [22], and epithelial-mesenchymal transition [23]. Nearly 4 years ago, Houghton and colleagues demonstrated that gastric cancer originates from BMDCs, which have been recruited and transformed malignantly by chronically inflamed gastric mucosa tissue [24]. In addition to gastric cancer there is compelling evidence that also other epithelial cancers, such as benign and malignant tumors of the skin, Kaposis sarcoma, and Barretts' adenocarcinoma of the esophagus might originate from BMDCs (for review see [25]).

The inherent tumorigenic capacity of stem cells points to another type of stem cells, which has gained much of attention within the last decade: cancer stem cells (CSCs) (for review see [26]). CSCs have been described as a rare population of cancer cells exhibiting stem cell properties such as self-renewing, differentiation, tissue reconstitution, and multiple drug resistance. Because of their tumor initiation capacity and resistance against cytotoxic drugs and radiation CSCs [27–29] have not only been linked to primary tumor formation, but also to metastases and cancer relapses. The knowledge that a tumor is organized hierarchically like normal tissue, namely comprising of a small number of stem cells, which give rise to differentiated cells, thereby maintaining tissue integrity and organ function, is of crucial interest for our understanding how to treat cancer in future times. The dilemma of current cancer therapies (conventional chemotherapy, radiation therapy, hormonal therapy, humanized monoclonal antibodies, and/or inhibitors) is that although most cancer

patients respond to therapy, only few are definitely cured [30]; a matter, which applies to both solid tumors as well as hematological disorders. This phenomenon, which has been entitled as “the paradox of response and survival in cancer therapeutics” [30] has been compared to “cutting a dandelion off at ground level” [30, 31]. Current cancer therapies are designed to target highly proliferating tumor cells and determination of tumor shrinking concomitant with mean disease free survival of patients are commonly used as read-outs for the efficacy of the appropriate therapy. While such strategies eliminate the visible portion of the tumor, namely the tumor mass, they mostly fail to eliminate the unseen root of cancer, namely CSCs. Thus, elimination of the unseen root of cancer, CSCs, would mean to have a chance to cure disease. However, there is increasing evidence that both metastases and cancer relapses might be initiated by specific CSCs, referred to as metastatic CSCs (mCSCs) [32] and recurrence CSCs (rCSCs) [33]. Quite recently, Hermann and colleagues identified a specifically metastatic CSC subpopulation in pancreatic cancer [34], whereas Shafee et al. demonstrated that the cisplatin resistance of murine mammary CSCs was associated with genetic aberrations in the platinum resistant cells [35]. These findings suggest that different cancer stage specific CSCs exist, which might play a role in the development of anti-CSC strategies. Is it possible to eliminate distinct CSC subtypes with a single anti-CSC strategy or demand distinct CSC subtypes distinct anti-CSC strategies? The answer to this question can not be given yet since only a handful of data exist for mCSCs and rCSCs so far.

In summary, it is remarkable what has been achieved in only 10–15 years in the field of stem cell biology in health and disease. Even if still some problems, being associated with stem cell-based regeneration strategies (e.g., choice of the stem cell type (adult stem cells, ESCs, or iPS cells), how to apply them (by injection, by infusion etc.), exist, we know from several animal studies that stem cell-based regeneration strategies are feasible and that it will be only a matter of time when such approaches will become reality in humans. Likewise, the knowledge that CSCs exist has changed our understanding of the disease cancer and will help us to develop novel anti-cancer strategies. There is a growing list of CSC specific target molecules/pathways, which might be used for selective CSC elimination or which could be used to drive CSCs from their stem cell state into a more differentiated state, thereby making these cells susceptible to conventional cancer therapy. So, we the scientists, physicians, and patients should be optimistic what the future will bring in the field of stem cell biology in health and disease.

We are glad that so many internationally recognized experts accepted our invitation to contribute to this exciting book. We sincerely thank them all for their interest in this important topic and that they, despite other duties and responsibilities, found the possibility to present excellent and comprehensive overviews of the most important recent findings in their field of scientific engagement within this topic. We would also like to thank Cristina Aves dos Santos, Sara Huisman, and Peter Butler from Springer Publishers for their kind assistance and excellent collaboration on this project, as well as for giving the opportunity to realize this book project.

We hope that this book may encourage new scientific approaches within the field of stem cell biology in health and disease as well as closer interdisciplinary collaborations on this fascinating and important issue in the future.

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Part I
Bone Marrow-Derived Stem Cells

Chapter 2
Hematopoietic Stem and Progenitor Cells in Clinical Use – Transplantation and Mobilization

Michael Punzel

Abstract It took exactly 100 years from the original discovery of the blood formation within the bone marrow until the first successful clinical bone marrow transplantation has been performed. Today, the transplantation of hematopoietic stem cells from various sources, such as bone marrow, mobilized stem cells as well as umbilical cord blood has become a routine procedure, reaching currently more than 10,000 transplantations per year in the allogeneic setting and over 40,000 autologous transplantations. Although, the number of transplantations is increasing every year, the field is constantly changing in terms of conditioning procedures and clinical indications. In addition, the increase in the availability of multiple graft sources for allogeneic transplantation, such as related or unrelated living donors versus frozen umbilical cord blood as well as the choice between mobilized peripheral blood versus steady state bone marrow is challenging not only for transplant physicians but also for the donors.

This chapter provides an overview about the history of stem cell transplantation, current procedures and future developments in terms of donor selection and graft choices for hematopoietic stem cell transplantation.

Keywords Hematopoietic stem/Progenitor cells · Bone marrow · Peripheral blood stem cells (PBSC) · Umbilical cord blood (UCB) · Stem cell transplantation · Stem cell mobilization · G-CSF · AMD3100 · Graft-versus-host-disease (GVHD) · CD34

Contents

M. Punzel (✉)
Institute of Transplantation Diagnostic and Cellular Therapeutics, Universitätsklinikum Düsseldorf, 40225 Düsseldorf, Germany
e-mail: mpunzel@itz.uni-duesseldorf.de

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2.1 Historical Aspects

The origin of blood formation within the bone marrow was discovered in 1868 independently by Ernst Neumann [1] and by Giulio Bizzozero [2]. The German hematologist Arthur Pappenheim postulated in 1898 a monophyletic basophil mononuclear precursor for all blood cells, followed by the "common stem cell" concept of Alexander Maximow which suggested a common stem cell among the small blood lymphocytes [3, 4].

Although the interest in this field had been present since these initial observations, research efforts took another step after the first atomic bomb explosions in the wake of world war II in attempts to prevent the lethal effects of irradiation. One of the most important discoveries at that time was the observation that marrow failure and subsequent lethality of photon beam irradiation in mice could be reduced by shielding the spleen and femur with lead [5]. In the following years experimental evidence from animal experiments in rodents could demonstrate that intravenous infusion of bone marrow protected them from lethal irradiation [6]. Although there was a long controversy about the origin of the protective effects of marrow infusions, in the mid-1950s it was well accepted that not humoral factors but transplantable hematopoietic stem cells are responsible for marrow protection [7, 8].

In 1957 the pioneer of clinical stem cell transplantation, E. Donnall Thomas, published results on infusing unrelated bone marrow into six patients. Although all patients died and only one of them had transient engraftment, this particular report is considered as the seminal paper of modern hematopoietic stem cell transplantation. Thomas and colleagues showed for the first time that human bone marrow could be collected in significant quantities and could be administered safely after cryopreservation [9]. Two years later, Thomas′s team performed the first successful bone marrow transplantation in a 3-year-old girl with leukemia using marrow donated from her identical twin. The girl did well for six months until her leukemia relapsed [10].

At this time it became evident, that alloreactivity is one of the most crucial factors for this therapeutic concept in two ways: On one hand the alloreactivity is directed against the tumor cells and protects the patient from relapse but on the other hand it caused fatal graft-versus-host disease (GVHD) if no identical twin has served as bone marrow donor. Doubts were raised if the "allogeneic barrier" could ever be passed since it turned out that the graft-versus-host (GVH) reaction in man was much more violent compared to inbred rodents [8]. The fatalities of allogeneic marrow infusions in the clinic setting caused most investigators to abandon such studies in the 1960s.

However, under the impetus of accumulating knowledge of the human histocompatibility system, researchers laid the foundation for modern bone marrow transplantation. The Seattle group around ED Thomas developed matching strategies for bone marrow transplantations in dog experiments and related their results to the human leukocyte antigen (HLA)-system [11, 12].

Important knowledge to the field was added by Till and McCulloch in a series of experiments, which are generally considered as the beginning of the modern area of hematopoietic stem cell biology. Starting in 1961 the group demonstrated clonogenic colony formation of all hematopoietic lineages in the spleen (colony-forming-unit-spleen; CFU-S) in lethally irradiated mice after transplantation with bone marrow cells from healthy donor animals [13–15]. Thus, for the first time evidence was provided for the dose dependent, clonal repopulation, differentiation and self-renewing capacity of hematopoietic stem cells.

The area of modern clinical bone marrow transplantation began in November 1968 when Robert Good from the University of Minnesota, USA carried out the first marrow transplantation in a 5-month-old boy with hereditary immunodeficiency that had killed 11 male members of his extended family with marrow from his 8-year-old matched sister [16]. Only 4 months later the Seattle group performed the first successful adult bone marrow transplantation in a patient with advanced leukemia using bone marrow from an HLA-matched sibling [17].

In the early years bone marrow transplantation was still restricted to patients with end-stage or refractory disease status and most patients were in poor condition at the time of transplantation, which resulted in a high proportion of deaths related to this therapy. Due to the myeloablative conditioning regimen that consisted of chemotherapy and total body irradiation various efforts had been made in the 1970s to decrease this transplant related mortality. On the one hand, a continuous improvement in the supportive therapy of blood cell substitution, antifungal, antimicrobiotic and antiviral chemoprophylaxis as well as nutritional supportive care could be achieved. On the other hand, the introduction of effective immunosuppressive agents in the GVHD-prophylaxis regimen, i.e. methotrexate and cyclosporine A improved the outcome of transplantation continuously [18, 19].

Important observations on the road to common practice for stem cell transplantation were published in the mid 1970s by the Seattle group: (i) Patients that were in better clinical condition at the time point of transplantation had a better long-term survival than those in poor condition, (ii) 75% of patients with advanced hematological disease relapsed after HSC-Tx, and (iii) the general proof of significant disease-free long-term survival in the first large cohort of patients with leukemia/lymphoma and aplastic anemia after failure of conventional therapy was encouraging to the field [17, 20]. Consequently, the number of patients referred for bone marrow transplantation at earlier stage of disease and in good clinical condition improved the field of allogeneic stem cell transplantation to full recognition as a clinical routine procedure in hematologic malignancies. As all subsequent studies confirmed the success of this treatment, E. Donnall Thomas received the Nobel Price for his pioneering work in clinical bone marrow transplantation in 1990.

2.2 Stem Cell Donors

While the number of transplants involving related donors increased continuously and proved to be successful, only 25–35% of patients had a matched sibling donor available. Further advances in histocompatibility typing technologies made it possible to include unrelated donors. In the beginning, serological matching for HLA-A, HLA-B and HLA-DR-loci and a non-reactive mixed lymphocyte culture (MLC) was required for donor selection and proved to be feasible in early clinical studies [21, 22]. In 1974 the initiative of recruitment of unrelated volunteers willing to donate bone marrow for anybody was started by Shirley Nolan in the United Kingdom in the search for bone marrow donors for her son, Anthony [23]. The Anthony Nolan Trust was the first active donor registry in the world. Today in almost every developed country registries with HLA-typed volunteers have been established, which have raised the chance for patients to find a suitable unrelated donor. Per November 2008 more than 12.5 [24] million donors have been registered world wide, of those more than 25% are registered in Germany [25]. This corresponds to more than 10% of all Germans between the age of 18–60 who have volunteered for a possible bone marrow donation.

In Germany there are currently 29 national and local donor registries [25]. Since one third of all transplants worldwide requires a graft from a foreign country, searching all the national and local registries in the world step by step separately is virtually impossible and only at considerable expense and time [26]. Thus, several platforms and networks have been established to provide an easy accessible listing of all donors nationwide as well as worldwide. Beginning in 1988 the Bone Marrow Donors Worldwide (BMDW) database has been summarizing the data of most registries in the world [27]. The World Marrow Donor Association (WMDA) has defined policies and procedures for international data exchanges [26].

Since more than 95% of all unrelated transplants are facilitated through the pool of complete HLA-typed donors, it was of great importance that the number of donors which have been typed for HLA-A, -B and -DR increased up to 9.6 million. This relates to approximately 75% of all available donors worldwide [27]. However, due to the diversity of HLA-allele and haplotype frequencies in human populations, the vast majority of patients that can be provided with a full matched donor belong to the Northern European (Caucasian) ethnicity only. Therefore, many efforts have been undertaken to establish ethnic minority programs within most of the registries, i.e. within the largest single registry worldwide, the National Marrow Donor Program (NMDP) in the USA. This has resulted in a significant increase of donor availability especially for the Afro-American population within the NMDP [28].

Currently, the optimal choice for an unrelated donor is a full allele-match for HLA-class I (HLA-A, -B, -C) as well as two matched gene loci of HLA-class II (HLA-DRB1, -DQB1). This requires expensive high resolution DNA-typing. Challenges in terms of transplantation outcome still remain in undetected variations of the human major histocompatibility complex (MHC) as well as in non-genetic factors such as the disease status of the patient.

During the 1980s, umbilical cord blood, which is collected from the umbilical cord and placenta of healthy newborns, has emerged as an alternative clinical source for hematopoietic stem cell transplantation. Elaine Gluckman performed in 1988 in Paris the first successful clinical transplantation in a six-year old boy suffering from Fanconi-anemia using umbilical cord blood (UCB) from a sibling [29]. In 1992, the first public UCB-bank was established in the New York Blood Center followed by institutions in many countries. In 1993, the first unrelated UCB-transplantation was performed at Duke University in the USA. Today, there are more than 330,000 UCB-units stored and available through the BMDW database and it is estimated that more than 14,000 unrelated UCB-transplantations have been performed so far [27, 30].

There are major differences between stem cell transplantations using grafts from adult donors or alternatively from UCB. UCB-transplants require fewer nucleated cells/kg body weight ($>2.5\times10^7$/kg) than bone marrow grafts ($>2\times10^8$/kg) and only 3 HLA-loci (HLA-A, -B, -DRB1) are relevant for transplantation at allelic level. Due to the lower alloreactivity of cord blood derived immune cells grafts with a limited HLA-disparity (1–2 allele mismatches) are suitable for transplantation [31, 32]. Over the last years it became evident that the nucleated cell dose, which correlates directly with the number of hematopoietic stem and progenitor cells in the UCB-transplant, is of higher priority than a full HLA-match [31–33]. This is significant since in the early years of UCB-banking many UCB-grafts were stored with only limited cell numbers [34, 35]. For this reason UCB-transplantations had been performed almost exclusively in children until the end of the last century [34, 35].

To overcome these limitations and to provide sufficient cell doses for adult patients novel graft selection strategies are under investigation. One attempt is the simultaneous transplantation of two UCB-units if the cell number of one single cord is insufficient, called “double cord blood” transplantation. Both of the two UCB-units must be matched to each other as well as to the patient appropriately, at least with 5/6 relevant alleles [36]. Another strategy has been the use of purified haploidentical stem and progenitor cells in conjunction with one UCB-unit. The haploidentical stem cells provide rapid engraftment and serve temporarily as “bridging cell unit” until the UCB engrafts and finally rejects the haploidentical cells from the patient’s relative [37, 38]. Based on these encouraging results and the increasing availability of suitable UCB-units in the BMDW-database, UCB-transplantation will become a valid alternative in the field of adult stem cell transplantation also for adults [31, 35, 39, 40].

2.3 Stem Cell Mobilization and Autologous Transplantation

Encouraged by the rapid clinical development in the field of allogeneic bone marrow transplantation along with the feasibility of harvesting, processing, cryopreserving and reapplication of bone marrow cells, the concept of high dose chemotherapy

with subsequent autologous transplantation has been proven safe and feasible for lymphohematologic malignancies as well as certain immune disorders [40]. Unlike allogeneic transplantations high dose chemo- and radiation therapy with autologous stem cell support can be performed in elderly patients as well without the significant mortality of transplantation related complications. Between 2002 and 2006 sixty-two percent of all autograft recipients were older than 50 [41].

Since 1962 it has been known that peripheral blood leukocytes fully reconstituted lethally irradiated mice of the same genetic strain [42]. In humans, hematopoietic stem cells in the peripheral blood were reported in the early 1970s [43, 44]. An increase in the amount of human hematopoietic stem cells in the peripheral blood was observed after chemotherapy for the first time in 1976 [45]. The amount of hematopoietic stem and progenitor cells in the peripheral blood was determined by the number of colonies that could be generated in semisolid methylcellulose cultures. These colonies have been defined as Colony-Forming Units (CFUs) at different stages of maturation. The numbers of CFUs for granulocytes and macrophages (CFU-GM), CFUs for erythroid colonies (CFU-E) as well as the number of CFUs for more primitive CFU-GEMM (mixed colonies for granulocytes, erythroid cells and monocytes/macrophages) directly relates to the amount of vital stem and progenitor cells with repopulating capacity in the peripheral blood [43, 44, 46–48]. Thus, such colony assays are still in place as quality control measurement of cryopreserved stem cells. Finally, the technical development of cell separators made it possible to collect clinically relevant amounts of stem cells from the peripheral blood [49]. The disadvantage of time delay inherent in the methylcellulose assays lead to the application of immunophenotyping for stem and progenitor cell determination. One of the most important discoveries in the field was the establishment of the CD34-membrane glycoprotein as a surrogate marker for the clinical enumeration of human stem and progenitor cells for transplantation [50, 51].

Initial mobilization regimens and proof of principle for the feasibility of autologous transplantations were pioneered in 1979 by Goldman and colleagues in 6 patients with myeloproliferative disorders [52]. The first successful clinical transplantation after myeloablative radiochemotherapy with large numbers of chemotherapy mobilized peripheral blood stem cells (PBSC) being transplanted was performed in 1985 in Heidelberg, Germany. The rapid hematopoietic reconstitution within 9 days suggested an advantage over bone marrow and paved the way for the preferred use of mobilized PBSC as stem cell source today [53]. To et al. established the modern chemotherapy based mobilization regimen in the autologous transplantation setting as single infusion of cyclophosphamide (4 g/m^2) that is still the gold standard, despite minor modifications [54–56].

The discovery and clinical development of human hematopoietic growth factors such as Granulocyte-colony stimulating-factor (G-CSF) and Granulocyte-Macrophage colony-stimulating factor (GM-CSF) allowed the collection of larger amounts of hematopoietic stem cells compared to chemotherapy alone [57]. Since the mobilizing effect of G-CSF was better than GM-CSF the latter did not make it to a widespread clinical use. The addition of G-CSF to chemotherapy based mobilization regimens led to the favorable use of mobilized PBSC as autologous grafts [58].

Today, the use of mobilized peripheral blood accounts for 90% of all autotransplants in children and for more than 95% in adults [41]. As minimal required cell dose, 1–2×10^6 $CD34^+$ cells per kg body weight have been established without any clinical benefit using $CD34^+$ cell doses $>8\times10^6$/kg [55, 59].

Clinical indications and frequencies of high dose therapies with autologous stem cell transplantation have changed over the past decade. The number of autologous transplantations that is performed annually had risen from approximately 5,000 in early 1990 to almost 40,000 in 1999 worldwide [41]. This was mainly due to the introduction of high dose chemotherapy in solid tumors, such as malignant melanoma, small cell lung cancer, colon cancer and in particular breast cancer. The initial enthusiasm about preliminary results turned into disappointment after the first randomized studies did not show any significant survival differences compared to conventional treatment. The latter data together with the disclosure of scientific misconduct in one of the breast cancer trials [60] has virtually abandoned autologous transplantations in the treatment of most non-hematologic malignancies. However, high dose therapy in other diseases, such as multiple myeloma or systemic amyloidosis has emerged as preferred treatment modality and thus, the number of autologous transplantations is on the increase again since 2002. Today, multiple myeloma is the most common indication for high dose therapy and autologous transplantation with a 3-year survival probability of 68% [41]. Similar results could be obtained for relapsed diffuse large cell B-cell lymphoma (DLBCL) with a 3-year survival probability of 61% in chemosensitive disease as well as for relapsed or aggressive follicular lymphoma (FL) with a 3-year survival probability of 73% in chemosensitive disease [41].

Several major studies have shown the advantages of mobilized peripheral blood over bone marrow as stem cell source for autologous transplantation [61–63]. Patients that have received autologous mobilized PBSC-transplantations showed a more rapid granulocyte and platelet recovery, enhanced immune reconstitution and subsequently a reduced transplant related morbidity [64–66].

2.4 Allogeneic Transplantation

The emergence of mobilized PBSC as preferred autologous stem cell source has sparked the use of G-CSF in healthy donors to obtain allogeneic PBSC-grafts with similar advantages as has been shown for the autologous setting [67, 68]. Studies that compared G-CSF-mobilized PBSC with bone marrow as graft source in related allogeneic HLA-identical transplantations demonstrated similar results for hematopoietic recovery as observed in the autologous setting: more rapid engraftment, less infectious complications and a lower transplantation related mortality were advantages of the PBSC-group [69–71]. Except one study, the rate of acute GVHD was not different in both graft sources but chronic GVHD was more frequent in patients that received PBSC-transplants [70–72]. Subsequently, the use of mobilized PBSC as preferred graft source for allogeneic transplantation has

increased markedly in the last decade. With the exception of pediatric transplantation procedures, mobilized PBSC has been the most common source of allogeneic grafts from 2002 to 2006 in patients older than 20 years, with the use of PBSC twice as much as bone marrow [41]. The number of allogeneic grafts collected in Germany went over 3,000 in 2006, more than in any other country of the world. The data show that already 80% of these grafts were collected from mobilized PBSC and the numbers are rising [25].

Despite the preferred use of mobilized peripheral stem cells in allogeneic transplantations controversies still exist about long-term outcome from both adult graft sources [73].

Since most studies have demonstrated an increased risk of chronic GVHD in mobilized PBSC-transplantation, it is not yet clear whether this will result in higher late mortality or in a decrease of the relapse rate due to a prolonged graft versus malignancy effect. A meta-analysis of several randomized trials that compared the outcome of PBSC versus marrow as graft source in full matched sibling transplantations showed significant improvement in disease-free survival at 5 years (54–47%) which was associated with increased chronic GVHD (51–35%) and decreased relapse rate (24–32%) in favor of PBSC-grafts [74]. However, a recent study that had the longest follow up for matched sibling transplants so far could not confirm the improved 5-year disease-free survival from the metaanalysis after 6 years, despite confirmation of the increased chronic GVHD incidence [75]. Since the patient cohorts in both analysis were different, the advantage of mobilized PBSC in matched sibling transplants remains unclear. The first comprehensive analysis that compared bone marrow transplantations with mobilized PBSC allografts in matched sibling transplantations in the pediatric setting demonstrated a significant increased mortality of PBSC-transplants clearly attributed to the higher incidence of GVHD in the PBSC-group [76].

First data on long-term follow up in unrelated donor transplantations demonstrated an expected higher incidence in extensive chronic GVHD in the PBSC group (85 vs. 59%, $p<0.01$) compared to bone marrow grafts [77–79]. The differences in GVHD, however, did not relate to any differences, neither in disease-free and overall survival, nor in relapse rates [79]. Depending on the underlying disease, it has been shown that in contrast to acute myeloid leukemia (AML), the use of unrelated mobilized PBSC as graft source in acute lymphatic leukemia (ALL) is associated with an increased transplant-related mortality (TRM) [80]. Additional data analysis, long-term follow up of the studies and the first prospective randomized clinical trial that compares unrelated bone marrow transplantation versus mobilized PBSC may finally solve these questions.

The gold standard of stem cell mobilization is currently the subcutaneous application of 10 μg G-CSF per day per kg body weight for 4–5 days followed by the apheresis collection. Although there is great practical experience in the use of G-CSF, the biological effects of mobilization have not been fully understood. G-CSF binds to its receptor, which is present on almost all cells of the myeloid lineage; from very few receptors on the most primitive progenitors in the bone marrow up to high density expression on neutrophil granulocytes in the peripheral blood [81, 82]. Upon

G-CSF stimulation in the bone marrow, certain proteases, released from neutrophil granulocytes, such as the neutrophil elastase (NE) and cathepsin G (CG) as well as metalloproteinases, such as Matrix Metalloproteinase-9 (MMP-9) will be released to cleave adhesion molecules that are important for hematopoietic stem cell trafficking and mobilization. In particular, the disruption of Very Late Antigen-4 (VLA-4) with its receptor Vascular-adhesion molecule-1 (VCAM-1) as well as effects on stroma cell derived factor-1 (SDF-1) and its chemokine-receptor-4 (CXCR-4) may play an important role in the release of primitive stem and progenitor cells from the microenvironment and their trafficking into the blood stream. Other molecules that have been shown to be involved in mobilization and trafficking of human stem and progenitor cells are CD44 and L-selectin [83–85].

Due to the widespread use of G-CSF with more than 10,000 allogeneic donors that receive G-CSF for clinical mobilization every year, safety issues have to be taken in consideration for healthy individuals [85, 86]. Few reports have shown that G-CSF can affect the genomic stability in hematopoiesis of healthy individuals, however long-term consequences remain largely speculative [87, 88]. Acute leukemias have been observed in siblings, stimulated with G-CSF [89, 90] while major concerns of long-term consequences of G-CSF application, i.e. in children had been already present [91]. Since there is no evidence that G-CSF causes any long-term effects in normal donors, the reported cases of leukemia in matched siblings may have occurred by chance – due to the fact of a generally higher risk of leukemia in first degree relatives. Thus, long-term follow up of all healthy donors that have undergone G-CSF mobilization is necessary.

About 4% of healthy individuals do not mobilize sufficient numbers of $CD34^+$ stem and progenitor cells into the peripheral blood [92]. The reasons why this small cohort of "poor mobilizers" fail to release the $CD34^+$ cells from the bone marrow into the blood remains unclear. Donor age >38 years, low baseline levels of $CD34^+$ cells and single daily application instead of two applications per day were identified as predictors for poor mobilization [93–95]. In autologous patients that receive chemotherapy (cyclophosphamide) followed by G-CSF the mobilization failure rate is much higher and depends on previous chemotherapy, i.e. the cumulative dose of alkylating agents [92].

Recently, the better understanding of mechanisms in stem cell mobilization led to the discovery and subsequent clinical development of new mobilizing agents. The diversity and large number of hematopoietic growth factors, chemokines and cytotoxic agents that induces the release of hematopoietic stem and progenitor cells into the peripheral blood is somewhat surprising. Besides the clinically approved G-CSF and GM-CSF several cytokines, such as interleukin-3, interleukin-8, recombinant human growth hormone and stem cell factor, had been tested but did not make it to clinical use [84].

The introduction of a pegylated G-CSF molecule (Pegfilgrastim) with prolonged half-life into clinical use resulted in a more convenient single dose application but did not change the poor mobilization responses in some patients [96]. Clinical trials are currently underway to determine the efficacy of Pegfilgrastim as mobilizing agent in patients for autologous transplantations as well as in healthy donors.

A specific CXCR-4 antagonist, called AMD3100, that reversibly inhibits the binding of SDF-1 to its receptor, is probably the most promising mobilizing agent of a new kind that has successfully passed clinical phase III studies and is expected to get clinical approval in Europe by 2009 [97–100]. Most importantly, in combination with G-CSF this drug allowed the mobilization of sufficient numbers of $CD34^+$ cells into peripheral blood in poor mobilizers that previously failed G-CSF-mobilization [101, 102]. A single dose of AMD3100 causes a rapid and significant release of $CD34^+$ cells from the bone marrow within 1 h. The number of peripheral progenitors peaks after 9 h and declines to baseline levels within 24 h, which allows stem cell harvest on the same day of application [84, 98, 103]. Although a single injection of AMD3100 results in a lower yield of $CD34^+$ cells, it acts synergistically with G-CSF [101, 102].

Recently, two reports have demonstrated that a clinical grade antibody (natalizumab) approved to treat multiple sclerosis, was able to release clinically significant amounts of $CD34^+$ stem and progenitor cells into peripheral blood by blocking VLA-4 [104, 105].

2.5 Outlook

The increased availability of registered unrelated stem cell donors as well as suitable umbilical cord blood units has remarkably improved the outcome of allogeneic stem cell transplantations over the recent years and opens the perspective to choose from several available graft sources according to the specific conditions of each individual patient. This also includes the use of related haploidentical donors in various clinical settings. These donors are only partially HLA-matched relatives of the patients that are usually immediately available for transplantation workup. Based on initial results that have shown the feasibility of this approach despite the risk of graft failure and severe GVHD, modern concepts of haploidentical transplantations have incorporated reduced intensity conditioning (RIC) in the transplant procedure combined with high dose enrichment of $CD34^+$ stem and progenitor cells [106–113]. Instead of purification of $CD34^+$ cells by positive selection, the depletion of selected lymphocytes that leaves monocytes, Natural Killer cells (NK-cells) and/or T-cell-subsets within the graft, has opened the perspective of targeted allogeneic immunotherapy by choosing stem cell donors that exhibit specific graft versus tumor/leukemia alloreactivity in the NK-cell repertoire but does not show significant graft versus host reactivity [112, 114–119].

The clinical introduction of AMD3100 and other possible mobilizing agents could change the field in many ways due to their different biological properties compared to G-CSF. Chemokine-receptor inhibitors release primitive hematopoietic cells into the blood stream that have differential cell cycle properties than G-CSF mobilized cells. The immunomodulatory effects of AMD3100 in the hematopoietic system differing from those observed after G-CSF treatment, the significant increase of circulating endothelial and angiogenic progenitor cells in the peripheral blood as

well as additional (still unknown) properties open the exciting perspective of novel therapeutic approaches using mobilized peripheral stem cells.

In addition, the release of stem cells from the niche by chemokine receptor inhibitors or antibodies against certain adhesion molecules, such as VLA-4, may lead to novel approaches to treat hematologic malignancies by releasing leukemic stem and progenitor cells from the niche into the peripheral blood that results in cell cycle entry and subsequently enhanced susceptibility to chemotherapy.

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