Gustav Steinhoff *Editor*

Regenerative Medicine - from Protocol to Patient

5. Regenerative Therapies II

Third Edition

Springer

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Editor
Gustav Steinhoff
Department of Cardiac Surgery
and Reference and Translation Center
of Cardiac Stem Cell Therapy (RTC),
Medical Faculty
University of Rostock
Rostock, Mecklenburg-Vorpomm, Germany

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Foreword: Regenerative Medicine: From Protocol to Patient

Third Edition

The vision to unravel and develop biological healing mechanisms based on evolving molecular and cellular technologies has led to a worldwide scientific endeavour to establish *Regenerative Medicine*. This field is involving interdisciplinary basic and (pre)clinical research and development on the repair, replacement, regrowth or regeneration of cells, tissues or organs in congenital or acquired disease. Stem cell science and regenerative biology is prompting the most fascinating and controversial medical development of the twenty-first century. It can be envisaged that this development will establish completely new molecular and cellular techniques for medical diagnosis and therapy. An early rush of scientific development was set up more than one hundred years ago by the physiology of blood regeneration (Hall and Eubanks, 1896) and successful vascular surgical techniques for organ transplantation (Carrel and Guthrie, 1905). However, the clinical realization of allogenic blood transfusion lasted until the discovery of the blood group antigens (Landsteiner and Levine, 1928) and successful routine allogenic organ and bone marrow transplantation even until the end of the last century.

Similar to the field of allogenic cell and organ transplantation it seems that *Regenerative Medicine* again condenses mankind's visions, hopes and fears regarding medicine: hopes of eternal life and effective treatment of incurable disease as well as fears of misuse of technology and uncontrolled modifications of life are polarizing the scientific field. The development and public acceptance of new ethical and regulatory guidelines is a necessary process to support the further clinical development. Nevertheless, the vision of a new medicine using the regenerative power of biology to treat disease and restructure the organism is setting the aim for scientific, technological and medical development. Viewing the great expectations to restructure and regenerate tissue, organs or organisms, the current attempts of scientist and physicians are still in an early phase of development.

The field of *Regenerative Medicine* has developed rapidly over the last 20 years with the advent of molecular and cellular techniques. This collection of volumes on

Regenerative Medicine: From Protocol to Patient aims to explain the scientific knowledge and emerging technology as well as the clinical application in different organ systems and diseases. The international leading experts from four continents describe the latest scientific and clinical knowledge of the field of *Regenerative Medicine*. The process of translating science of *laboratory protocols into therapies* is explained in sections on basic science, technology development, and clinical translation including regulatory, ethical and industrial issues.

This collection is organized into five volumes: *(1) Biology of Tissue Regeneration, (2) Stem Cell Science and Technology, (3) Tissue Engineering, Biomaterials and Nanotechnology, (4) Regenerative Therapies I, and (5) Regenerative Therapies II.*

Biology of Tissue Regeneration (Volume 1) focuses on regenerative biology with chapters on extracellular matrix, asymmetric stem cell division, stem cell niche regulation, (epi)genetics, immune signalling, and regenerative biology in organ systems and model species as axolotl or zebrafish.

Stem Cell Science and Technology (Volume 2) provides an overview as classification of stem cells and describes techniques for their derivation, programming and culture. Basic properties of differentiation states as well as function in human organism are illustrated, and areas of stem cell pathologies in cancer and therapeutic applications for these cells are discussed with emphasis on their possible use in *Regenerative Medicine*.

Tissue Engineering, Biomaterials and Nanotechnology (Volume 3) focuses the development of technologies, which enable an efficient transfer of therapeutic genes and drugs exclusively to target cells and potential bioactive materials for clinical use. Principles of tissue engineering, vector technology, multifunctionalized nanoparticles and nanostructured biomaterials are described with regard to the technological development of new clinical cell technology. Imaging and targeting technologies as well as biological aspects of tissue and organ engineering are depicted.

Regenerative Therapies I (Volume 4) gives a survey on history of Regenerative Medicine and clinical translation including regulation, ethics, and preclinical development. Clinical state-of-the art, disease-specific approaches of new therapies, application technology, clinical achievements, and limitations are described for the central nervous system, head and respiratory system. *Regenerative Therapies II (Volume 5)* contains state-of-the-art knowledge and clinical translation of *Regenerative Medicine* in the cardiovascular, visceral and musculoskeletal systems.

These volumes aim to provide the student, the researcher, the health-care professional, the physician and the patient a complete survey on the current scientific basis, therapeutical protocols, clinical translation and practised therapies in *Regenerative Medicine*. On behalf of the sincere commitment of the international experts, we hope to increase your knowledge understanding, interest and support by reading the book.

After the successful introduction in 2011 with 41 chapters, this work has been actualized and extended for the third edition with into five volumes containing 60 chapters.

Department of Cardiac Surgery and Reference and Translation Center of Cardiac Stem Cell Therapy (RTC), Medical Faculty
University of Rostock
Rostock, Mecklenburg-Vorpomm, Germany
gustav.steinhoff@med.uni-rostock.de
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Gustav Steinhoff

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Contributors

W.K. Aicher Department of Urology, University Hospital of Tuebingen, Tuebingen, Germany

B. Amend Department of Urology, University Hospital of Tuebingen, Tuebingen, Germany

Takayuki Asahara Department of Regenerative Medicine Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan

Oliver Bachmann Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Augustinus Bader Stem Cell Biology and Cell Technology, Medical Faculty, Biotechnological-Biomedical Center (BBZ), Leipzig University, Leipzig, Germany

Arner Berner Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia,

Department of Trauma Surgery, University of Regensburg, Regensburg, Germany

Tobias Cantz Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Translational Hepatology and Stem Cell Biology, REBIRTH Cluster of Excellence, Hannover Medical School, Hannover, Germany

Hendrikus J. Duckers Julius Research Institute, University Utrecht, Utrecht, The Netherlands

Georg N. Duda Julius Wolff institute and Centre for Musculoskeletal Surgery, Berlin-Brandenburg Centre for Regenerative Therapy, Charité-Universitätsmedizin Berlin, Berlin, Germany

Joshua O. Eniwumide Julius Wolff institute and Centre for Musculoskeletal Surgery, Berlin-Brandenburg Centre for Regenerative Therapy, Charité-Universitätsmedizin Berlin, Berlin, Germany

Mathias Freund Department of Internal Medicine III, University of Rostock, Rostock, Germany

Christina Irene Günter Clinic for Plastic and Hand Surgery, University Hospital Rechts der Isar, Technische Universität München, Munich, Germany

Hermann Haller Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany

Dietmar W. Hutmacher Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Masaaki Ii Division of Research Animal Laboratory and Translational Medicine, Osaka Medical College, Takatsuki, Osaka, Japan

Elmar Jäckel Department of Gastoenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Atsuhiko Kawamoto Unit of Regenerative Medicine, Institute of Biomedical Research and Innovation/Translational Research Informatics Center, Foundation for Biomedical Research and Innovation, Chuo-ku, Kobe, Japan

Carsten Keil Department of Gastoenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Evi Lippens Julius Wolff institute and Centre for Musculoskeletal Surgery, Berlin-Brandenburg Centre for Regenerative Therapy, Charité-Universitätsmedizin Berlin, Berlin, Germany

Hans-Günther Machens Clinic for Plastic and Hand Surgery, University Hospital Rechts der Isar, Technische Universität München, Munich, Germany

Michael P. Manns Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Haruchika Masuda Department of Regenerative Medicine Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan

Thomas Mittlmeier Department of Trauma and Reconstructive Surgery, University of Rostock, Rostock, Germany

Julia Nesteruk Reference and Translation Center for Cardiac Stem Cell Therapy (RTC), Department of Cardiac Surgery, University Rostock, Rostock, Germany

Ulrich Nöth Department of Orthopedic and Trauma Surgery, Ev. Waldkrankenhaus Spandau, Berlin, Germany

Michael Ott Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Centre for Experimental and Clinical Infection Research, a joint venture between the Hannover Medical School (MHH) and the Helmholtz Centre for Infection Research (HZI), TWINCORE, Hannover, Germany

Johannes C. Reichert Department of Orthopedic and Trauma Surgery, Ev. Waldkrankenhaus Spandau, Berlin, Germany

Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Michael Schmitt Cellular Immunotherapy, University Clinic Heidelberg, Heidelberg, Germany

Roland Schmitt Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany

Amar Deep Sharma Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Junior Research Group "miRNA in Liver Regeneration", REBIRTH Cluster of Excellence, Hannover Medical School, Hannover, Germany

Michael Sittinger Tissue Engineering Laboratory and Berlin-Brandenburg Center for Regenerative Therapies, Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Sajoscha Sorrentino Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany

Gustav Steinhoff Department of Cardiac Surgery and Reference and Translation Center of Cardiac Stem Cell Therapy (RTC), Medical Faculty, University Rostock, Rostock, Mecklenburg-Vorpomm, Germany

Arnulf Stenzl Department of Urology, University Hospital of Tuebingen, Tuebingen, Germany

Ioannis Stratos Department of Trauma and Reconstructive Surgery, University of Rostock, Rostock, Germany

Bodo E. Strauer Department of Internal Medicine, Division of Cardiology, Pneumolody and Angiology, Heinrich - Heine - University, Dusseldorf, Germany

Lei Wang Cellular Immunotherapy, University Clinic Heidelberg, Heidelberg, Germany

Maria Patapia Zafiriou Institute of Pharmacology and Toxicology, Heart Research Center Goettingen, University Medical Center Goettingen, Georg-August-University Goettingen, Goettingen, Germany

DZHK (German Center for Cardiovascular Research), Göttingen, Germany

Laura C. Zelarayán Institute of Pharmacology and Toxicology, Heart Research Center Goettingen, University Medical Center Goettingen, Georg-August-University Goettingen, Goettingen, Germany

DZHK (German Center for Cardiovascular Research), Göttingen, Germany

Wolfram-Hubertus Zimmermann Institute of Pharmacology and Toxicology, Heart Research Center Goettingen, University Medical Center Goettingen, Georg-August-University Goettingen, Goettingen, Germany

DZHK (German Center for Cardiovascular Research), Göttingen, Germany

Chapter 1
Blood

Michael Schmitt, Lei Wang, and Mathias Freund

Abstract The transplantation of stem cells from the bone marrow, peripheral blood or cord blood has become a clinical procedure since the 1980s and is now annually performed in 10,000 of patients in the autologous and allogeneic setting world-wide. Refinement of human leukocyte antigen typing as well as recent advances in immunosuppression, anti-infective prophylaxis and therapy as well in supportive care have much improved the outcome of patients with leukemia and lymphoma, aplastic anemia, as well as hereditary diseases of the hematopoietic system. This is still an experimental therapy and patient subgroups that profit most from hematopoietic stem cell transplantation need to be defined. Consideration and classification of co-morbidity indices as well as cytogenetic risk factors are pivotal for making decisions on transplantation modalities. Modern conditioning regimens allow balancing of allo-effects against malignant cells versus normal tissue even in elderly patients. Recent innovations in cellular therapy combine allogeneic stem cell transplantation with genetically engineered or specifically selected T cells and potentially natural killer (NK) cells. Depletion of regulatory T cells and vaccination after allogeneic stem cell transplantation constitute further approaches to improve the long-term outcome of transplanted patients.

Keywords Autologous and allogeneic hematopoeitic stem cell transplantation (HSCT) • Donor lymphocyte infusion (DLI) • Chimeric antigen receptor (CAR) • T cell receptor (TCR) • T cell therapy

1.1 Blood: A Long Way from Replacement to Regeneration

Have you ever asked yourself why the red lights at the crossing are red? It is the color of blood that warns for danger. Blood has ever fascinated man. It is the topic of myths and rites. The shedding of blood initiates deeply rooted fears.

M. Schmitt (✉) • L. Wang
Cellular Immunotherapy, University Clinic Heidelberg, Heidelberg, Germany
e-mail: Michael.Schmitt@med.uni-heidelberg.de

M. Freund
Department of Internal Medicine III, University of Rostock, Rostock, Germany
e-mail: mathias.freund@onkologie-rostock.de

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The first documented attempt to replace this essence of life and to cure deadly disease has been performed in Paris on the 15th of June 1667 by Jean-Baptiste Denis. Blood from a lamb was transfused to a 15 year old boy who survived. More than 300 years later Landsteiner discovered the A, B, O blood group system. He received the Noble Prize for his research in 1930. He laid the fundament for the first successful blood transfusion 1907 in New York by Reuben. 1915 it became possible to conserve blood for transfusion by the addition of citrate. Blood group testing was refined with the discovery of the N, M, and P system and finally in 1939 by the discovery of the Rh system. Blood transfusions increasingly became an essential treatment on the battle fields of the Second World War and later in the Korean War.

As spontaneous recovery from blood loss is evident in healthy subjects and as there are blood diseases in which lifelong blood replacement would be needed the need to advance from replacement to regeneration was obvious. An era of intensive research on bone marrow and its stem cells began in the 1950s.

It was discovered that the infusion of spleen cells promoted blood regeneration and led to survival of supralethal total body irradiation (Barnes and Loutin 1953; Ford et al. 1956; Nowell et al. 1956; Vos et al. 1956). However in 1957 first attempts of clinical bone marrow transplantation in man were unsuccessful in the majority of cases. The reasons were allograft failure and progressive disease (Thomas et al. 1957). Two years later Thomas reported the first successful allogeneic bone marrow transplants from identical twins in two patients with acute lymphoblastic leukemia.

It is remarkable that this program had been started by E.D. Thomas in 1955. It was years before stem cell assays were developed by Donald Metcalf (Bradley and Metcalf 1966) and Leo Sachs (Ginsburg and Sachs 1963). The theoretical and experimental basis of stem cell transplantation had been left behind by clinical application.

Although many human blood stem cell transplantations were carried out between 1958 and 1968, the outcome had not been encouraging. Out of 203 patients transplanted in these times, 125 experienced graft failure, 49 developed lethal graft-versus-host disease (GvHD), and only 11 achieved long-term engraftment. Only three patients were alive when Bortin reported these results in 1970 (Bortin 1970). Many researchers left the field and some voices declared hematopoietic stem cell transplantation as dead.

Those who were not discouraged returned to the laboratory and animal models. After progress in the understanding of the HLA-system and the development of GvHD prophylaxis by immunosuppression transplantation went back to the clinics. The Seattle group realized that patients with far advanced malignant disease and poor general status had a dismal outcome in contrast too patients in the earlier stages (Thomas et al. 1975). It was an enormous adventure at that time to transform this finding into a clinical consequence: to recommend the dangerous procedure to patients in remission, to patients with non malignant or low malignant but long-term dismal disease, and children.

This courage of the early clinical researches and the following developments in high resolution HLA typing, worldwide donor programs, the improvement of the

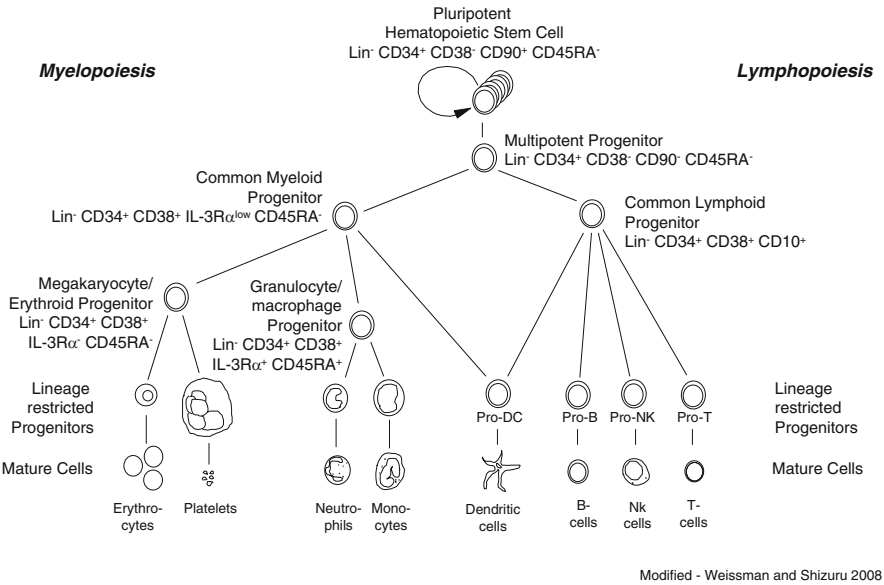

Fig. 1.1 Pedigree of the normal hematopoiesis. Through the influence of cytokines and growth factors like interleukin 3 (*IL-3*), stem cells differentiate into mature cells of the peripheral blood. Partially differentiated progenitor cells are characterized by surface markers, so called "clusters of differentiation" (*CD*) as indicated

conditioning regimen and supportive care have established hematopoietic stem cell transplantation as the first true regenerative therapy. We can learn from that development for other areas of regenerative medicine.

Yet the field of hematopoietic stem cell transplantation is still dynamic. New indications emerge as the procedure is improved. Other indications vanish as their conservative treatment advances. It is the venue of this chapter to give an insight into the actual status of this treatment option.

1.2 Hematopoietic Stem Cells

1.2.1 Basic Properties

Hematopoietic stem cells have the capacity to self-renew and to differentiate in all mature blood lineages (Fig. 1.1). They have been first identified in the mid 1950s by their capability to rescue lethally irradiated mice by reconstituting the entire repertoire of hematopoietic cells. Hematopoietic stem cells are scarce with a frequency of 1:10,000–1,000,000 bone marrow cells. Without stress the majority of stem cells rest in a quiescent state while only a small fraction enters the cell cycle and proliferates to give rise to differentiated progenitors. During infections, acute bleeding, or chemotherapy a large fraction of the stem cells may proliferate.

The regenerative capacity of the stem cells has its evidence in the fact that despite the short lived nature of blood cells a continuous supply of these cells is given even in very long living persons without clinical signs of insufficiency. The self renewal potential of the hematopoietic stem cells is associated with the activity of telomerase. The telomeres at the end of the chromosomes shorten during cell division. This process is reduced by telomerase, a reverse transcriptase which synthesizes new telomeric DNA (Morrison et al. 1996). Telomere shortening is associated with cell cycle arrest, replicative senescence and chromosomal instability. It might be an inhibitory mechanism against the evolution of malignant cell clones.

Despite the activity of telomerase in hematopoietic stem cells, their replication capacity is limited. Serial stem cell transplantations in mice can be done with minimal stem cell numbers for five to seven times until hematopoietic insufficiency occurs (Harrison and Astle 1982). On the other hand it should be noted that transplantation is a severe stress for stem cells. The regenerative potential of stem cells under normal conditions is enormous. It has been concluded from these mouse experiments that hematopoietic stem cells should be able to function normally through at least 15–50 life spans. Therefore hematopoietic insufficiency should not be expected in even very old subjects.

1.2.2 Characterization of Hematopoietic Stem Cells

The study of hematopoietic stem cells is difficult because of their low frequency in the bone marrow. Specific markers or tests for a definitive identification of stem cells are lacking. So in most instances methods have to be combined for the characterisation of stem cells.

1.2.2.1 Surface Markers

In mice hematopoietic stem cells are fairly well characterized by surface markers. A single murine bone marrow cell which is CD34−/lo, CD117+ Sca1+ (stem cell antigen) and negative for lineage-specific antigens is capable of self renewal and multilineage differentiation when transplanted into a recipient mouse (Ema et al. 2000). In humans the phenotypic properties of hematopoietic stem cells are far less well defined.

1.2.2.2 Stem Cells and the Concept of “Niches”

There exists a more than 30 year old concept that the number and behavior of hematopoietic stem cells (HSCs) is regulated by physically discrete locations within the bone marrow for which the French term “niches” was coined. Despite the fact that the precise identities of the niche cells are not yet well defined and controversial, there is an increasing body of evidence that HSCs are retained within the niches by specific adhesion molecules and chemokine gradients (Papayannopoulou and

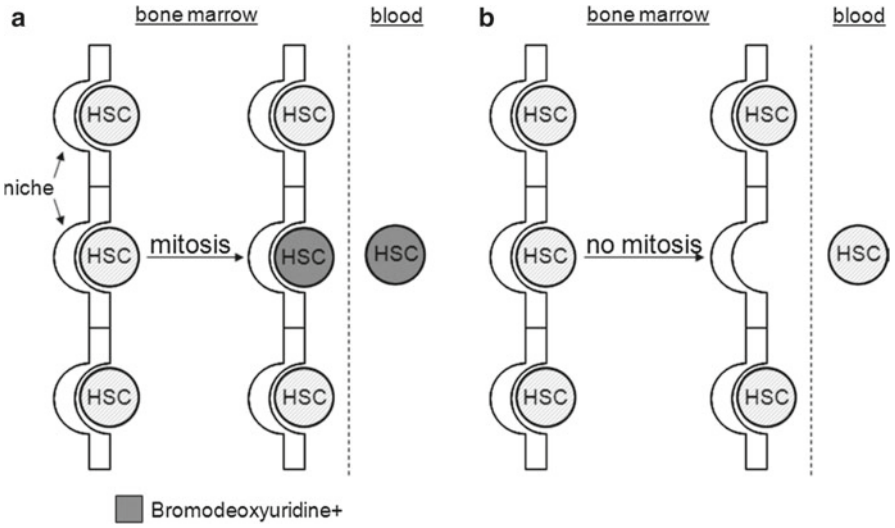

Fig. 1.2 HSC egress is either division dependent or independent (from Bhattacharya et al. 2009). HSCs can either undergo an extrinsically asymmetric division, in which one daughter cell is positioned away from a supportive niche and can thus intravasate to the blood (**a**) or can exit the supportive niche in the absence of cellular division (**b**). In the former model, all HSCs in the blood would be expected to have incorporated BrdU (*gray shaded cells*) after an appropriate feeding period, while the latter model would predict similar low BrdU incorporation rates between bone marrow and blood HSCs

Scadden 2008). By these interactions, HSCs can be assured that they receive appropriate supportive signals that allow them to retain their stem cell identity. In contrast to this concept, there are data suggesting that recipient bone marrow can be readily displaced by transplanted marrow in an efficient and linear dose-dependent manner, even in the absence of conditioning (Colvin et al. 2004). These authors have described a model where HSCs do not reside locked into fixed positions in the bone marrow, but instead they would receive their regulatory signals through limiting quantities of freely diffusible factors.

Work by Irvin Weissman and co-workers (Bhattacharya et al. 2009); (Czechowicz et al. 2007) clearly demonstrated that a certain degree of HSC replacement occurs even in the absence of conditioning. Recent studies could demonstrate that egress of HSCs can be stimulated pharmacologically through administration of plerixafor (AMD3100), an inhibitor of CXCR4. This resulted in the clearance of niches from HSCs. As HSCs and progenitors have been demonstrated to circulate under physiological conditions, a steady-state HSC egress from niches might also allow the engraftment of donor HSCs. One to five percent of HSCs in the murine model enter into the circulating pool every day.

Weissman's group fed their mice with bromodeoxyuridine and found out that HSCs in the circulating pool incorporate the dye at the same rate as bone marrow HSCs (Fig. 1.2). This suggests that HSCs egress from the bone marrow to the blood without cell division and can leave behind them vacant HSC niches (Bhattacharya et al. 2009).

Fig. 1.3 Stem cell donor during leukapheresis. A leukapheresis device (Spectra Optia™) is put into a circuit between the left and the right cubital veins. It separates white blood cells containing stem cells by a density gradient method

1.2.3 Stem Cell Sources

Initially, bone marrow was obtained from healthy HLA-matched sibling donors of the patients. Donors were subjected to intubation and anesthesia. In a prostate position bone marrow was aspirated from the upper posterior iliac crest and transferred into a transfusion bag. After stem cell counting and microbiological and virological evaluation of the bone marrow blood preparation the stem cell containing bone marrow is transfused into the patients by the way of a central venous catheter.

Bone marrow aspiration under sterile conditions in the operation theater is costly and cumbersome. Moreover, stem cells mobilized from the bone marrow niches into the peripheral blood by subcutaneous administration of granulocyte-colony stimulation factor (G-CSF) or plerixafor to the donor will result in a 1 week earlier engraftment when compared with bone marrow. Therefore most of the stem cell preparations given nowadays to adult patients are peripheral blood stem cells collected by leukapheresis (Fig. 1.3). Through magnetic cell separation using anti-CD34 monoclonal antibodies labeled with magnetic beads (Fig. 1.4) a highly purified fraction of CD34+ stem cells can be prepared. Only for younger patients with e.g. aplastic anemia (see below), bone marrow stem cells are preferred due to the better reconstitution of the bone marrow with all its components (Schrezenmeier et al. 2007).

A novel source for hematopoietic stem cell is cord blood. Its application has been initiated by Eliane Gluckman at the Hôpital Saint Louis, Sorbonne VII Paris

Fig. 1.4 Purification of stem cells through a magnetic cell separation device (CliniMACS® by Miltenyi, Bergisch Gladbach/Teterow, Germany). Cells (in the *red* bag above the device) are labeled by monoclonal antibodies (*moAbs*) against the stem cell marker CD34. These moAbs are coupled with magnetic beads. The beads stick to a magnet during the first run of a buffer (the bag with a clear solution on the right side) though the column. When the column is detached from the magnet, a second eluate containing the marker-positive (stem) cells can be obtained

(Czechowicz et al. 2007) and has been practiced in more than 4,000 patients in Japan (Kodera 2008). Hitherto, the transplantation of cord blood derived stem cells is restricted by the number of stem cells from this source when put into relation with the average body weight of a European patient undergoing HSCT. Application of several cord blood preparations as "dual" or even "triple" cord blood transplantation gets into practice (Arachchillage et al. 2010). While the extended time of engraftment make the patient even more prone to opportunistic infections, cord blood stem cells do obviously not have to be HLA-identical Mismatches cause less GvHD in the cord blood setting than in the setting of PBSCT or bone marrow transplantation (Barker et al. 2010).

1.2.4 Stem Cell Doses for Transplantation

Different sources of stem cells like bone marrow, peripheral blood and cord blood can yield different amounts of stem cells with various pros and cons (Table 1.1):

For autologous transplantation one would like to administer 2×10^6/kg body weight (BW) of the recipient. In the case of allogeneic HSCT the desirable CD34+

Table 1.1 Stem cell sources and stem cell doses

Source	Pro's/con's	CD34+ stem cell count
Bone marrow	Aspiration requires general anesthesia	Median of 2.8×10^6/kg body weight (BW)
Peripheral blood	Easy collection, but G-CSF side effects	Median of 7.0×10^6/kg BW
Cord blood	Easy, immediately available, partial HLA mismatches acceptable	Median of 0.2×10^6/kg BW

stem cell count would be at least $>2.5 \times 10^6$/kg BW, better $>5.0 \times 10^6$/kg BW for peripheral blood stem cells, and at least $>1.0 \times 10^6$/kg BW, better $>2.5 \times 10^6$/kg BW for bone marrow blood stem cells.

1.3 Principles of Hematopoietic Transplantation for Regeneration in Blood Diseases

Hematopoietic stem cell transplantation can be performed in two principally different situations: (1) Stem cells can be harvested in a patient with malignant disease and can be used to induce and/or accelerate hematopoietic regeneration after myelosuppressive or myeloablative treatment procedures. (2) Stem cells from a healthy volunteer donor can be transplanted for hematopoietic recovery of patients with non-malignant and malignant blood disorders.

1.3.1 Autologous Transplantation

The rationale in the autologous setting is to deliver as intensive cytotoxic treatment to the patient as possible. The basis of this concept is the finding that in a certain dose range of irradiation or cytotoxic treatment, the effect on the tumor increases in a steep linear relationship (Fig. 1.5). However this dose range is not equal in all tumors and with increasing doses there is increasing damage to hematopoiesis and organs. As organ damage may occur later in some agents an autologous transplantation of hematopoietic stem cells might open a therapeutic window for dose intensification.

This concept (Fig. 1.6) has been proven most convincingly in lymphomas, Hodgkin's disease and multiple myeloma. Attempts to apply high dose chemotherapy in other diseases as sarcomas or some other solid tumors have not been as successful, probably due to the fact that chemotherapies active in these diseases are very toxic to the organs so that the window opened by autologous hematopoietic transplantation is small or non-existing. Although autologous transplantation is given even in disseminated hematologic disease as the acute leukemias, this treatment principle is not convincing in these entities as potentially tumor stem cells are also re-infused with the graft. Purification of grafts by chemical of immunologic

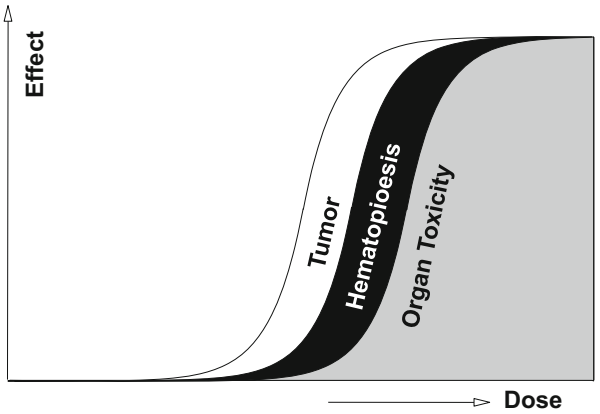

Fig. 1.5 Dose of cytotoxic treatment and toxic effects on tumor, hematopoiesis and organs. The higher the dose, the myelotoxicity or even organ toxicity

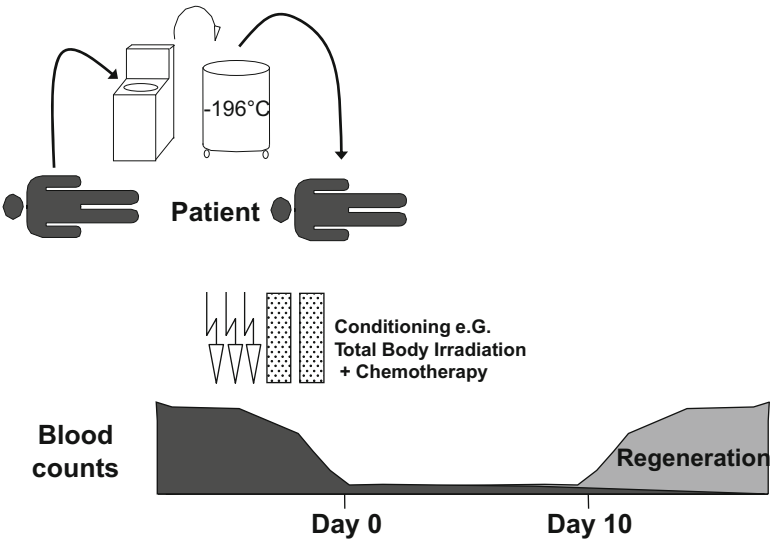

Fig. 1.6 Schema of autologous transplantation. Stem cells are collected from a hematological patient by leukapheresis after mobilization with e.g. cyclophosphamide and granulocyte-colony stimulating factor (*G-CSF*). Stem cells are stored in liquid nitrogen. The patient undergoes a conditioning regimen with e.g. total body irradiation (*TBI*) and cyclophosphamide. Thereafter the autologous stem cells are given back to the patient. The blood counts drop under a conditioning regimen of TBI and chemotherapy. Regeneration of hematopoiesis starts by day 10 after transplantation

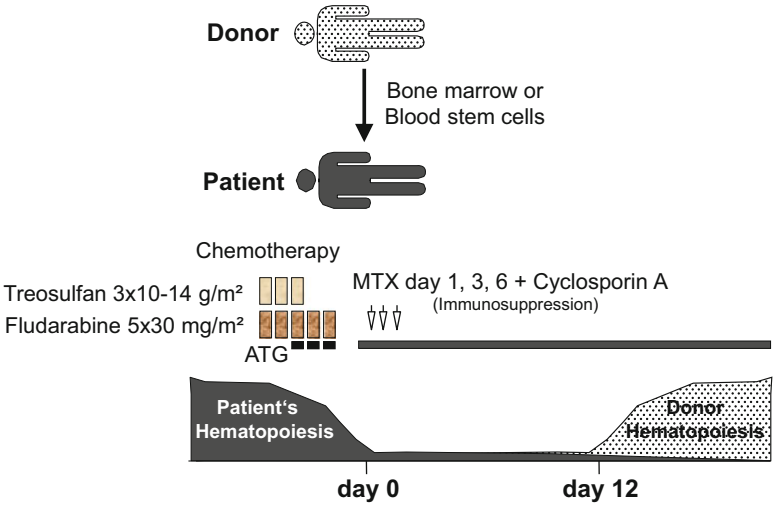

Fig. 1.7 Schema of allogeneic transplantation with the treosulfan-fludarabine conditioning regimen. After a conditioning with a treosulfane and fludarabine containing chemotherapy regimen the patient obtains stem cells from the bone marrow or peripheral blood of a healthy donor. To avoid graft rejection immunosuppression with e.g. methotrexate (*MTX*) or cyclosporine A is administered. As early as by day 12 reconstitution of the hematopoietic system is effective

methods have not yielded better results and are hampered by side effect e.g. immunosuppression by T-cell depletion in CD34+ selected grafts.

1.3.2 Allogeneic Transplantation

The basic concept of allogeneic transplantation is to replace malignant or deficient hematopoiesis by tansplantation from another individual (Fig. 1.7). Consequently allogeneic transplantation has been first applied in irradiation injuries and aplastic anemia.

As immunocompetent cells are normally transferred with the graft and as minor immunologic disparities might lead to a graft-versus-host reaction concomitant immunosuppressive prophylaxis has to be given in the allogeneic setting. However in contrast to organ transplantation the immunosuppression can be omitted in most patients after immunologic reconstitution.

Allogeneic transplantation is widely applied in children and adult patients with genetic diseases, immunodeficiency syndromes, aplastic anemia, leukemias and lymphomas.

1.4 Diagnostics and Indications of "*blood*" Regenerative Therapies

Histocompatibility is a basic prerequisite for allogeneic transplantation. Future concepts of allogeneic transplantation might overcome this principle to some extent. Another very important limitation for the application of allogeneic transplantation is the presence of comorbidities of the patients which become more prevalent with increasing age. A third overwhelming important factor for the outcome after autologous and allogeneic transplantation is the disease status and risk group. The latter is discussed later.

1.4.1 HLA Compatibility

The human leukocyte antigen (HLA) system is the human equivalent of the major histocompatibility complex. T cell recognition of peptide epitopes derived from functional or structural proteins is essential for the engraftment or rejection of the transplant and directly linked to GVHD and GVL reactions. Two classes of HLA molecules can be distinguished: HLA-A,-B and -Cw are class I molecules with three alpha subunits and a beta-2 microglobulin molecule, while class II molecules such as HLA-DR,-DQ and –DP are composed of two alpha and two beta subunits (see Fig. 1.8) (Klein and Sato 2000a, b). In their groove, HLA-ABC molecules present 9–11 amino acid residues long peptides to the T cell receptor of CD8+ T lymphocytes while class II HLA-D molecules present 15–20mer peptides to CD4+ T lymphocytes (Rammensee et al. 1993).

Minor histocompatibility antigens (miHAgs) such as HA-1 (den Haan et al. 1998) can both elict GVHD (Mutis et al. 1999) but can also contribute to the recognition of leukemic blast i.e. to the GVL (Goulmy et al. 1996) effect. Moreover natural killer cells, their surface molecules and killer cell inhibitory (KIR) molecules contribute GVHD and GVL, and are of pivotal importance for allogeneic stem cell transplantations in the haploidentical setting (Moretta et al. 2009).

1.5 Standardized Treatment, Technologies

1.5.1 Autologous Hematopoietic Stem Cell Transplantation (HSCT)

1.5.1.1 Conditioning Regimens

Stem cell transplantation requires always the preparation of the bone marrow compartment in particular but also the whole patient to receive the graft. The term "conditioning" has been coined for this central process of the stem cell transplantation

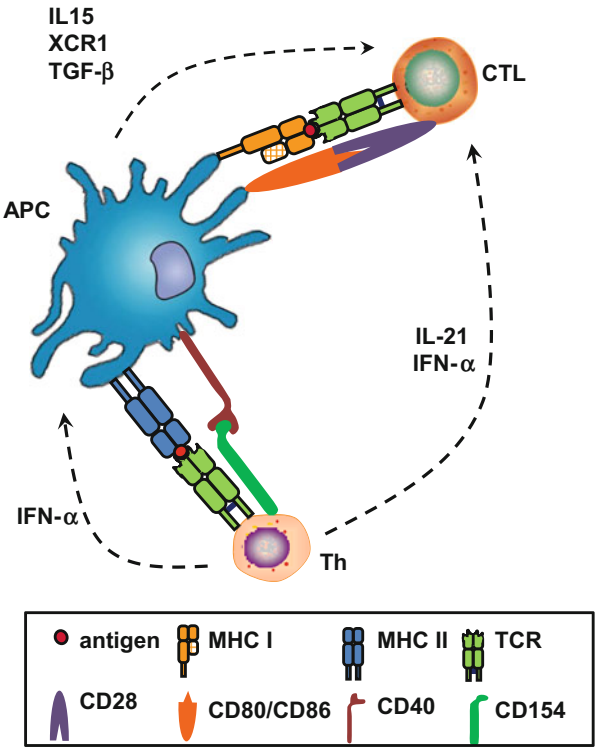

Fig. 1.8 Recognition of antigen epitopes by CD4+ and CD8+ T lymphocytes. Peptides derived from antigens are presented on HLA-molecules class I and II. A second signal is effective by binding of costimulatory molecules with their ligands. Interaction of T helper cells and cytotoxic T cells occurs through interleukines. (*CTL* CD8+ cytotoxic T lymphocytes, *Th* CD4+ T helper cells, *APC* antigen presenting cell, *TCR* T cell receptor, *IL* Interleukin, *DC* clusters of differentiation, *CD28, CD40, CD80, CD86, CD154* Co-Stimulatory molecules, *HLA* human leukocytes antigen, *TGF β* Transforming growth factor beta, *XCR* chemokine (C motif) receptor 1, *IFN-α* Interferon-alpha, *IL-15/-21* Interleukin-15/ -21)

which constitutes an integral part of the HSCT. However, there is an ongoing debate on how to bring the patient in the best condition possible to accept the graft.

The "creation of space" has been postulated as a major goal of conditioning. The original concept was that immature progenitor cells occupy circumscriptive bone marrow niches to gain the necessary support from feeder cells of the stroma for their proliferation and maturation. To this end the patient's stem cells have to be eradicated to provide the donor stem cells with access to these important niches so that engraftment might occur. The concept of reduced intensity conditioning (see below) with a fading in-fading out phenomena of donor stem cells versus host stem cells has at least to some extent relativated this concept. One might not necessarily have to eradicate the complete bone marrow to give a graft a realistic chance to engraft successfully. However, this depends on the underlying disease and the dynamic of the malignant clone.

In general the antitumor activity of the conditioning regimen is also needed to further reduce the tumor burden before transplantation. This is particularly true in autologous transplantation where no graft-versus-tumor effect is present.

For autologous HSCT standard conditioning regimens are in common practice according to the disease entity. For patients with multiple myeloma, 200 mg/m^2 melphalan has shown the best survival. For patients older than 60 years this dose might be reduced to 140 mg/m^2. Lymphoma patients (Hodgkin's disease (HD) and Non-Hodgkin-Lymphoma (NHL)) obtain most commonly a combination of carmustine (bis-chloronitrosourea; BCNU), etoposide, cytarabine and melphalan (Mills et al. 1995). Purging of transplants for lymphoma patients constitutes an interesting approach; however it can be associated to hypoglobulinemia or secondary malignancies such as MDS or AML (Gyan et al. 2009). For patients with acute myeloid leukemia (AML), autologous HSCT has become rather rare, even more in Europe than in the US. The reasons are multiplex. In the last decade there has been no major progress in that field. Some centers report higher survival of patients receiving a purged transplant. But this has never been proven in a randomized trial. Most importantly, only half of the patients allocated to autologous HSCT for AML reach the transplantation because of relapse of the disease or a poor graft. For acute lymphoblastic leukemia the picture is even clearer. Several studies showed no difference for the comparison of chemotherapy versus autologous HSCT or even a significantly inferior outcome for auto-HSCT (Goldstone et al. 2008).

1.5.1.2 Mobilization

A standard protocol for the mobilization of autologous stem cells requires cyclophosphamide 1.5 g/m^2 on day 1 followed by 10 μg/kg BW/day G-CSF on days 2–12. Stem cells can be collected on days 10–12 when the WBC count reaches 8 G/L post nadir. In the case of poor mobilizers plerixafor (AMD3100) might be given in concert with G-CSF: on day 4 give additionally plerixafor at a dose of 160–240 μg/kg BW i.v. or i.m. 6–12 h before the intended harvest.

1.5.2 Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

1.5.2.1 Conditioning Regimens

The favorable results of allogeneic stem cell transplantation depend not only on chemotherapy and irradiation, but also on the allo-effect which is introduced by the graft from a family donor or unrelated donor. Therefore conditioning regimes for allogeneic HSCT must include immunosuppression to prevent a host-versus-graft reaction. Transplanted donor cells might be immediately attacked by immune cells of the host. Natural killer (NK) cells, T lymphocytes as well as dendritic cells (DCs)

are involved in the complex interplay. There is a particular need for immunosuppression in the case of increasing human leukocyte antigen (HLA) disparities. The risk for graft rejection is also increased through pre-sensitization against minor histocompatibility antigens (miHAgs), e.g. through multiple blood product transfusions into the host preceding the allogeneic HSCT.

From the historical perspective, HSCT has been understood as a potential cure for patients irradiated through atomic bomb explosions or nuclear accidents. Therefore, total body irradiation (TBI; 12 Gray [Gy]) was tested first as conditioning method. TBI was efficient in eliminating the hematopoietic system, but TBI alone could not eradicate the leukemic clone. Only by adding cyclophosphamide (Cy) to TBI in patients at early stage disease, the first successful allogeneic HSCTs could be performed in the 1970s. In further studies TBI was replaced by the "radiomimetic" busulfan (Bu), i.e. Bu/Cy. Other alkylating drugs like melphalan (Mel) or carmustine (BCNU), as well as "leukemia-specific" drugs like cytarabine (ARA-C), etoposide (ETO) and 6-thioguanine (6-TG) followed in a conditioning regimen termed BACT. As for TBI/Cy and Bu/Cy there are several differences in toxicities (more venous occlusive disease [VOD], more permanent alopecia with Bu/Cy), but both regimens are comparable in terms of long-term survival of the patients with the exception of ALL, where TBI/Cy is more effective.

With regard to the reduction of transplantation-related mortality (TRM) and the quality of life (QoL) the concept of "reduced intensity conditioning (RIC)" was born. In preclinical experiments the requirements for a stable engraftment were evaluated, and subsequently low and even lowest dose TBI (2–4 Gy) were used as well as conditioning regimens with fludarabine (Flu), Bu, Mel and Cy. RIC concepts became particularly interesting in the context of donor lymphocyte infusions (DLIs) which where inaugurated at the begin of the 1990s as a tool to bring patients with myeloid disease back into remission.

A revolutionary development in recent conditioning regimens is the postponement of cyclophosphamide after transplantation of the graft. Allo-HSCT has two major limitations. The first relates to the procedure's toxicity, including conditioning regimen toxicity, graft-versus-host disease (GVHD), and infection. The second limitation is the lack of histocompatible donors. A human leukocyte antigen (HLA)-matched sibling or unrelated donor cannot be identified expeditiously for up to 40 % of patients. In general HSCT from partially HLA-mismatched donors, or HLA-haploidentical relatives has been complicated by unacceptably high incidence of graft rejection, severe GVHD, and non-relapse mortality. Recently, Luznik et al. have developed a method to selectively deplete allo-reactive cells in vivo by administering high doses of cyclophosphamide in a narrow window after transplantation (Fig. 1.9). Using high-dose, post-transplantation cyclophosphamide, crossing the HLA barrier in allo-HSCT is now feasible and donors can be found for nearly all patients (Luznik et al. 2012; Kanakry et al. 2014).

In haploidentical hematopoietic stem cell transplants, post-transplant cyclophosphamide together with standard prophylaxis reduces the incidence of GVHD to acceptable rates without the need for T cell depletion. In matched related and unrelated donor settings, cyclophosphamide alone or in combination with solely

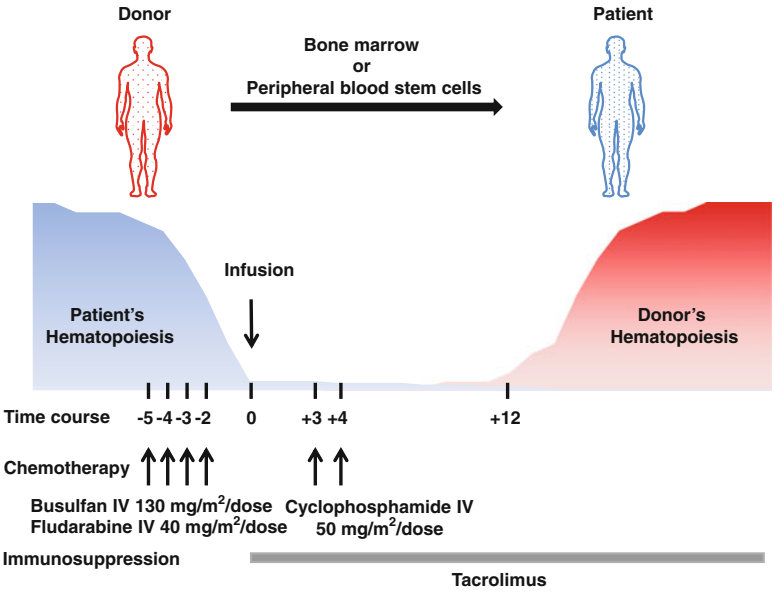

Fig. 1.9 Transplantation platform. The treatment schema is shown. Busulfan dosing was adjusted based on measured pharmacokinetics. Only Tacrolimus was administered as graft-versus-host disease prophylaxis after post-transplantation cyclophosphamide on days +3 and +4

Tacrolimus has produced encouraging results. Interestingly, only a low incidence of chronic GVHD has been observed (Al-Homsi et al. 2015).

1.5.2.2 Graft-Versus-Host Disease (GvHD)

Graft-versus-host disease (GvHD) constitutes one of the most serious complications after allogeneic stem cell transplantation. The underlying pathomechanism is the recognition of host-specific proteins by T cells of the donor which were transferred together with the donor stem cells or maturated thereof. Moreover dendritic cells of the host are involved in this complex interaction of the immune system depicted schematically in Fig. 1.10.

Clinical manifestations of GvHD can be detected on skin, liver, and GI tract. A macula-papular rash can develop on the upper part of chest and back as well as a palmo-plantar exanthema. It might involve the entire integument and might lead to desquamation or even the development of bullae. Cholestatic hepatopathy with classical jaundice, increased serum levels of bilirubin as well as of cholestatic enzyme (whereas transaminases rather show no specific changes) are signs of GvDH of the liver. This needs to be differentiated from the veno-oclusive disease (VOD) which occurs rather early after allogeneic HSCT even before immunoreconstitution and subsequently the basis for a GvHD occurs. VOD is characterized by liver pain, ascites, impaired flow of intrahepatic veins and elevated serum levels of bilirubin and cytokeratin 17.