Establishing a Hematopoietic Stem Cell Transplantation Unit

A Practical Guide

Éliane Gluckman
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Preface

Hematopoietic stem cell transplantation (HSCT) is a lifesaving, costly, and complex procedure. More than one million transplants have been performed worldwide. Results are dependent on the center infrastructure, staff expertise, quality of transplant outcomes reporting, and training programs.

In order to help new centers in developed and low-income countries to set up transplant units, this book aims to provide simplified practical guidelines which could be implemented in most centers and countries.

For this purpose, we have invited a group of internationally established experts in their corresponding HSCT fields to share their knowledge and present, as clearly as possible, the steps toward the successful establishment of an HSCT program.

Hematopoietic stem cell transplantation has been investigated for more than 60 years. Among the pioneers, George Mathé, Edward Donnall Thomas, and Mortimer Bortin were the first to demonstrate the feasibility of HSCT in humans and to describe the immunological complications represented by rejection and graft-versus-host disease (GVH-D). Jean Dausset and Jon Van Rood were the first to describe the role of human leucocyte antigen (HLA) in transplantation. They established the first rules of donor-recipient selection according to HLA identity, first in selecting siblings, then by establishing large registries of unrelated donors. George Santos and Rainer Storb were the first to describe the conditioning regimen for HSCT, including the combination of cyclophosphamide associated with total body irradiation or busulfan, which is still the gold-standard conditioning regimen. More recently, reduced-toxicity and non-myeloablative conditioning regimens have been developed in order to decrease transplant-related complications. Since this early time, progress has been made with steady improvements in transplantation procedure outcomes. Major advances have been achieved due to better donor selection, using high-resolution HLA typing; the development of unrelated donor registries and cord blood banks; and the use of family mismatched transplants.

Improvements in pretransplant conditioning, GVH-D prevention, and anti-infectious agents have had a major impact on decreasing transplant-related mortality, particularly in the past decade.

Proper patient selection, disease identification, and stratification of candidates for transplantation is also a major, and constantly evolving field with progress in the molecular diagnosis of malignancies and testing for minimal residual disease. Prevention of relapse in malignant diseases is a major concern, but new cell therapy and genetic engineering methods are promising and rapidly evolving therapeutic technologies that will, it is hoped, reduce this risk in the future.

The objective of this book is to help new HSCT centers to start transplant units in developed or low-income countries and to provide guidelines for existing centers to upgrade their practices or implement new policies and procedures, as well as therapies, according to current international standards and regulations.

Requirements for developing an HSCT program include the definition of infrastructure facilities; the availability of blood transfusion services and radiology, microbiology, pathology, and laboratory facilities for hematology, molecular biology, immunology, and HLA typing; and the presence of pharmacy facilities with access to chemotherapy and anti-infective agents.

Staff availability and training are also extremely important, including adequately qualified physicians, nurses, laboratory technicians, and data managers.

Education, training, and collaboration are also essential to attract staff members and increase national and international collaborations.

Before implementing a transplant unit project, it is fundamental to have a proper estimate of the needed number of autologous and/or allogeneic transplants to be performed per year, and to determine whether the projected transplant volume would be sufficient to build adequate experience in transplantation and justify the initiation of such project. Next, it is important to establish a network of physicians and healthcare professionals to refer patients and ascertain adequate post-transplant care. Active communication and perhaps twinning with other established centers must be sought for advice and program development. Also, the evaluation of resources is essential for building the program; a new center should determine in advance the priorities for patient selection, type of transplant, and patient follow-up. Plans for expanding the transplant program and increasing the number of transplants per year should, likewise, be determined in advance. For the long-term sustainability of the program, commitment is also of the utmost importance, not only by the head of the transplant program, but also by all the medical and non-medical personnel, administration, competent authorities, and payers. Getting started and containing costs while maintaining high-quality standards is the main challenge for developing a new transplant unit.

Using the information provided here, as a backbone, will help centers in discussions with their local hospitals, university authorities, governments, and legislators and this will help centers to adapt their programs according to the specific population needs and resources available. Consequently, this will increase the number of patients with access to transplantation in developed as well as low-income countries. With the basic information provided by this book, we hope that each center will be able to establish their priorities and develop a strategic plan for transplantation in their community according to local needs, regulatory and ethical laws, and resources.

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Abbreviations

APBMT	Asia-Pacific Blood and Marrow Transplantation Group
ASBMT	American Society for Blood and Marrow Transplantation
ASHI	American Society for Histocompatibility and Immunogenetics
AUP	Area under the peak
BMT	Bone marrow transplant
CD	Cluster of differentiation antigen
CDC	Complement-dependent cytotoxicity
CE	Conformité Européenne
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
Ct	Cycle threshold
CT	Computed tomography
CVC	Central venous catheter
CWD	Common and well-documented HLA alleles
ddPCR	Droplet digital polymerase chain reaction
DSA	Donor-specific antibodies
DTT	Dithiothreitol
EBMT	European Society for Blood and Marrow Transplantation
EDTA	Ethylenediaminetetraacetic acid
EFI	European Federation for Immunogenetics
EPT	External proficiency testing
ET	Education and training
GVH-D	Graft-versus-host disease
GVT	Graft-versus-tumor
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigens
HSCT	Hematopoietic stem cell transplantation
HSV	Herpes simplex virus
HTLV-1	Human T-cell lymphoma virus type 1
H&I	Histocompatibility and immunogenetics
ICU	Intensive care unit

Ig	Immunoglobulin
Indel	Insertion-deletion
IPA	Isopropanol
IV	Intravenous
JACIE	Joint Accreditation Committee International Society for Cellular Therapy (ISCT) European Group for Blood and Marrow Transplantation (EBMT)
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
NGO	Non-governmental organization
NGS	Next-generation sequencing
NMD	Non-malignant disorders
NMDP	National Marrow Donor Program
PCD	Plasma cell disorders
PCR	Polymerase chain reaction
PRA	Panel reactive antibodies
RDT	Rapid diagnostic test
qPCR	Quantitative polymerase chain reaction
SBT	Sequence-based typing
SC	Subcutaneous
SNP	Single-nucleotide polymorphisms
SOP	Standard operating procedure
SSO	Sequence-specific oligonucleotide
SSP	Sequence-specific priming
STR	Short tandem repeats
TBI	Total body irradiation
TDM	Therapeutic drug monitoring
VNTR	Variable number tandem repeats
WBMT	Worldwide Network for Blood and Marrow Transplantation
WHO	World Health Organization
WMDA	World Marrow Donor Association

Chapter 1
Global Perspectives on Hematopoietic Stem Cell Transplants (HSCTs)

Alois Gratwohl

Introduction

Intuitively, and ideally, patients, donors, and transplant physicians should know the requirements for, and the potential risks and benefits of hematopoietic stem cell transplant (HSCT) before they agree in a joint decision to proceed with a transplant procedure. Is there a reasonable chance for long-term survival with an acceptable quality of life compared with other therapeutic options? Has everything been undertaken to minimize the risks for the donor, whose benefit is solely altruistic? Is sufficient infrastructure available for the complex HSCT procedure, and will the costs be covered? As obvious as these questions are, answering them requires a great deal of information [1, 2].

This idea is not new. Data collection has been the key element in the development of the field since the very beginning. The first comprehensive survey, in 1970, carried out by Bortin, the founder of the International Bone Marrow Transplant Registry, the later Center for International Blood and Marrow Transplant Research (CIBMTR; www.cibmtr.org) represents a prime example [3]. Only 3 of the 203 patients in this worldwide series were alive at the time of the report; but all 3 had received bone marrow from their human leucocyte antigen (HLA)–identical sibling donors. Despite the poor outcome, the data did provide proof of concept that the infusion of bone marrow cells could restore hematopoiesis after so-called supralethal total body irradiation. This finding fostered further research, and created the hope that HSCT could potentially yield a tool to save life after radiation exposure in the event of a nuclear war. The major impact of HLA matching of donors and recipients gave clinicians a tool to successfully treat severe hematological disorders and to replace missing recipient hematopoiesis by using donor type bone marrow cells

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[1, 4]. HSCT procedures continued, and Bortin's study initiated systematic data collection by organizations such as the CIBMTR, the European Society for Blood and Marrow Transplantation (EBMT; www.ebmt.org), and the Asia-Pacific Blood and Marrow Transplantation Group (APBMT; www.apbmt.org).

What has changed is the complexity of the process. It no longer suffices to show that "it can be done." HSCT should provide, for each individual patient, a better outcome than any non-transplant treatment strategy, in terms of overall survival, quality of life, and costs [5]. Before the advent of targeted therapies, just five patient and donor criteria, as in the EBMT risk score, were considered sufficient for deciding in favor or against a transplant procedure [6]. Since the advent of these therapies, the number of single disease-, patient-, donor-, and transplant technique-associated risk factors has multiplied, paralleled by a similar increase in non-transplant treatment options. Insight into potential risk and benefit factors will grow rapidly in the era of precision medicine and big data. In addition, center-specific microeconomic and country-specific macroeconomic factors that add to or decrease risk have to be integrated. The decision to proceed with HSCT or to rely on a non-transplant strategy might soon need to be delegated to computer algorithms [6–11]. Awareness of these complexities is even more important for a new transplant team, ready to embark on HSCT. They are not isolated, but whether they like it or not, they are part of a global network. This outline might help such teams to better understand the mechanisms behind decision-making, and help them to select their specific program in order to fulfill the goal of HSCT.

WHO, Worldwide Network for Blood and Marrow Transplantation (WBMT), and Activity Surveys

The World Health Organization (WHO; www.who.org) has always followed the field of transplantation, for various reasons. In recent years, it recognized organ transplantation in general as a valuable treatment option, but saw concerns with organ trafficking and potential donor abuse [12, 13]. Therefore, WHO released, in 2010, its "guiding principles for cell, organ and tissue transplants" [14]. The guidelines were adopted by the World Health Assembly; hence they are valid for all 190 WHO member states. Among these principles, data collection and data analysis of organ procurement and transplant procedures have been declared as integral parts of the therapy. Member states share the responsibility of providing and organizing data streams; so do all transplant organizations and transplant teams.

Within this framework of oversight on transplant numbers and collection of organs from donors, the WBMT (www.wbmt.org) has been recognized as a non-governmental organization in formal collaboration with the WHO. The WBMT has taken up the task of obtaining the minimal required information on global numbers of HSCT procedures. It is preparing to extend this data collection to cellular therapies in general and eventually to all "medical products of human origin" [15].

To accomplish its task, the WBMT can rely on the activity survey system of the EBMT [16]. In the early years of HSCT, processes for data collection of transplant numbers and outcomes were simultaneous. With time and with increasing numbers of HSCTs, the complexity of the data collection process and the need for quality requirements became evident. Data on transplant numbers became as important as data on transplant outcomes. However, accounting for the former, transplant activity, has to be timely. Reporting of outcome, in contrast, requires time. Several years might be needed to assess the impact of a novel technology correctly. Some teams might preferentially report patients with poor outcomes; an early death requires less time for data management. Other teams might, intentionally or not, omit patients with poor outcomes. Evidently, data reporting has to be coordinated. Furthermore, reporting has to be consistent. A double cord transplant for one patient might arrive in five bags on two separate days. Administration might require two customs declarations, five quality control reports, and several bills for one transplant and one patient. Obviously, scientific data and administrative data have to match, and some regulations are mandatory [2, 17, 18].

In this setting, the EBMT introduced, in 1990, the "EBMT activity survey" as a quality control tool [19, 20]. Since then, all EBMT members have been asked to report, in January, the numbers of transplants in the preceding year by main disease, donor type, and stem cell source. Teams were also asked to keep a unique patient number (UPN) file for each patient with HSCT. The patients' outcomes could be reported later on to the EBMT disease-specific data file. In a simple audit system, teams were audited regularly by an external team to verify numbers in the activity survey and to compare numbers in the survey with the reports on outcome. The system proved to be very valuable as a quality control instrument, and was integrated into the JACIE (joint initiative of the EBMT and the International Society for Cellular Therapy [ISCT]) quality management system (www.jacie.org); it has been maintained since, and has been adopted by the WBMT.

Global WBMT Data Collection System

The WBMT activity survey data collection system is based on the EBMT activity survey sheet [16]. Information is collected annually from each of the 190 WHO member states, according to their WHO regional offices [21], regarding numbers of patients with a first HSCT, classified by main disease, donor type, and stem cell source. Data flow is channeled from country-specific national organizations, where they exist, through the EBMT (www.ebmt.org) in Europe, the CIBMTR (www.cibmtr.org) for the United States, the Canadian Blood and Marrow Transplantation Group (CGBMT; www.cgbmt.org) for Canada, the Latin American Blood and Marrow Transplantation Group (www.labmt.net) for Latin America, the Asia-Pacific Blood and Marrow Transplantation Group (APBMT; www.apbmt.org) for Southeast Asia, the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR; www.abmtrr.org) for the Western Pacific region, the Eastern Mediterranean Blood

and Marrow Transplantation Group (EMBMT; www.embmt.org) for the Eastern Mediterranean region, and the African Blood and Marrow Transplant Group (AFBMT; www.afbmt.org) for Africa. Unrelated donor and cord blood information is supplemented with data from the World Marrow Donor Association (WMDA; www.wmda.info) and from Bone Marrow Donors Worldwide (BMDW; www.bmdw.org).

HSCT Activity in the Global Context

The WBMT global overview of HSCT activity is still in part fragmentary, incomplete, and lagging in time. The availability of 2012 data only in 2016 is still too slow [5, 22–24]. In addition, there are no comprehensive data yet on the outcomes of HSCT on a global level. However, several WBMT reports do provide a comprehensive overview of the achievements and deficiencies of HSCT; they illustrate the tremendous diversity and the uneven global distribution of the procedure. It took more than 50 years from the first published attempt in 1957 to arrive at one million transplants in 2012 (Fig. 1.1). Substantial numbers of HSCTs have been performed only in the past two decades. And, hidden by the figures, there were several substantial "bumps" in the curves between 1957 and 2012 [25].

In 2012, close to 70,000 HSCTs were performed worldwide in 79 of the 190 WHO member states (Fig. 1.2) [22–24]. Autologous HSCTs were done in 76 of the states, allogeneic HSCTs in 72, unrelated donor HSCTs in 56, and cord blood HSCTs in 46 (Fig. 1.3). No HSCTs were performed in very small countries

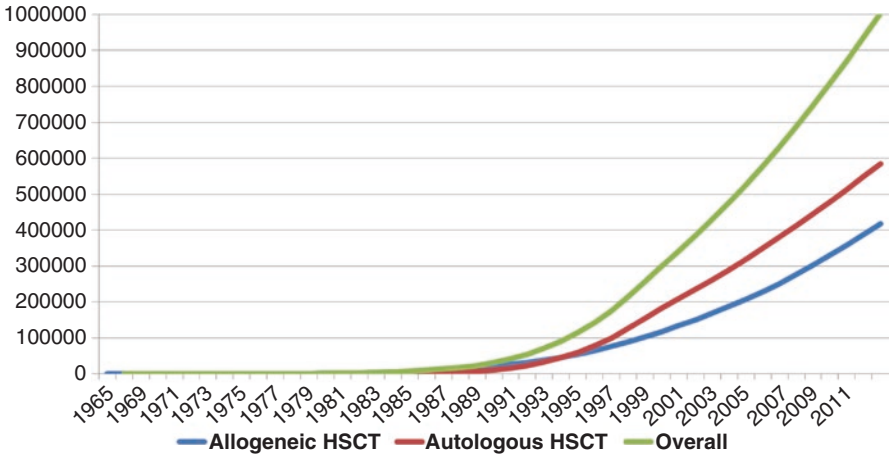

Fig. 1.1 Global evolution of hematopoietic stem cell transplant (HSCT) over time. The figure depicts the evolution of allogeneic (*blue line*), autologous (*red line*), and combined numbers (*green*) of HSCTs from the first published report in 1957 to the one millionth transplant in 2012. Reprinted with permission [5]. The figure shows the relative differences in the global use of allogeneic compared with autologous HSCT in earlier and more recent times. It cannot visualize the several ups and downs in individual disease or donor type categories [25]

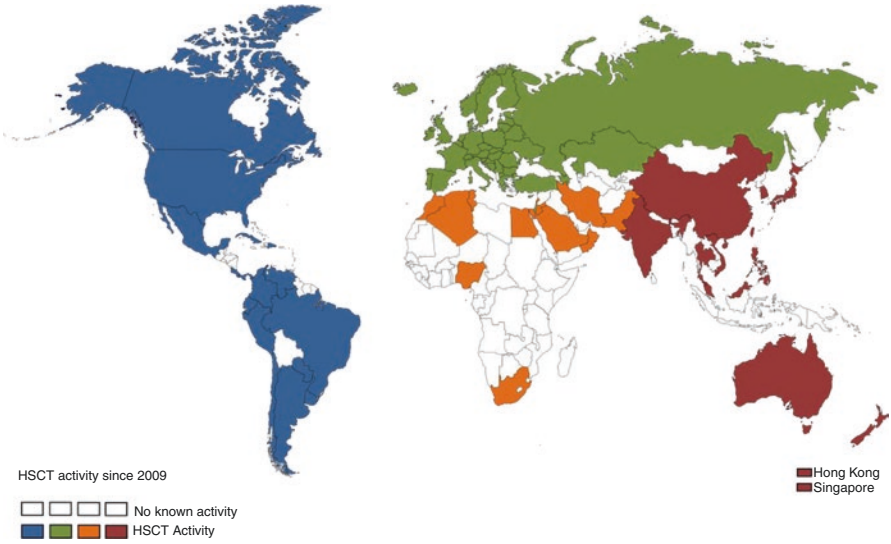

Fig. 1.2 Global HSCT activity. The figure depicts countries known to perform autologous and/or allogeneic HSCTs for hematological disorders in 2012. Colors indicate regions as defined by the World Health Organization (WHO) regional offices: *blue* (http://www.who.int/classifications/network/ro/en/) , *green* Europe, *red* Southeast Asia/Western Pacific, and *brown* Eastern Mediterranean/Africa. Data kindly provided by Helen Baldomero, WBMT activity survey office, Basel

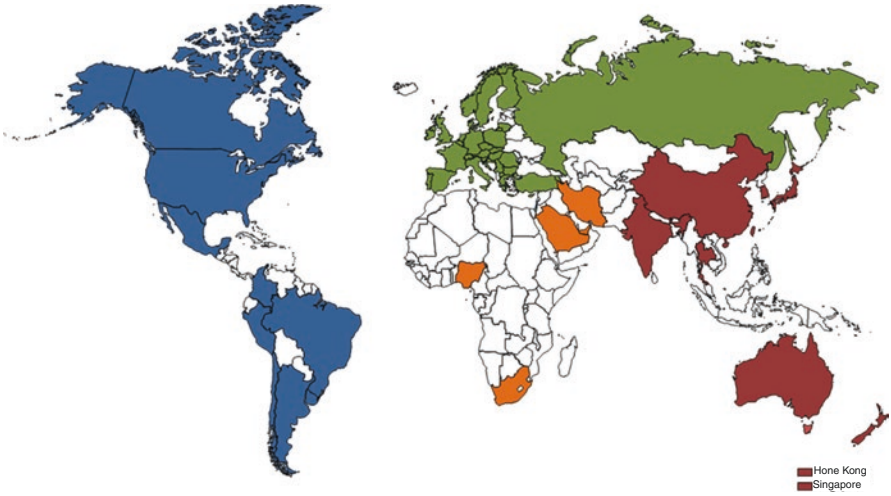

Fig. 1.3 Unrelated donor infrastructures. The figure depicts countries with an unrelated donor registry and/or a cord blood bank in 2016. Colors indicate regions as defined by the WHO regional offices: *blue* America, *green* Europe, *red* Southeast Asia/Western Pacific, and *brown* Eastern Mediterranean/Africa. Data kindly provided by Helen Baldomero, WBMT activity survey office, Basel

(countries with a population of less than 300,000 inhabitants or with a surface area of less than 700 km^2) or in very poor countries (those with a gross national income per capita of less than 1260 US$). Numbers of transplants and transplant rates varied widely. Unsurprisingly, these figures were strongly associated with macroeconomic factors and influenced by infrastructure. More transplants were performed in countries with higher gross national income per capita and in countries with a well-functioning large unrelated donor registry (Fig. 1.4). Transplant rates (number of HSCTs per ten million inhabitants) for all HSCTs (allogeneic and autologous combined) ranged from less than 1 to more than 1000 (median ≈ 250). The corresponding transplant rates for allogeneic HSCTs ranged from less than 1 to more than 500 (median ≈ 100) and for autologous HSCTs from less than 1 to more than 600 (median ≈ 160). Data showed a wide variation in how transplants were distributed within countries. Numbers of transplant centers ranged from 1 to 381 centers per country, corresponding to team densities (number of HSCT teams per ten million inhabitants) of less than 1 to more than 30 teams per ten million inhabitants, resulting in absolute numbers of HSCTs performed by individual teams ranging from 1 to more than 300. The main

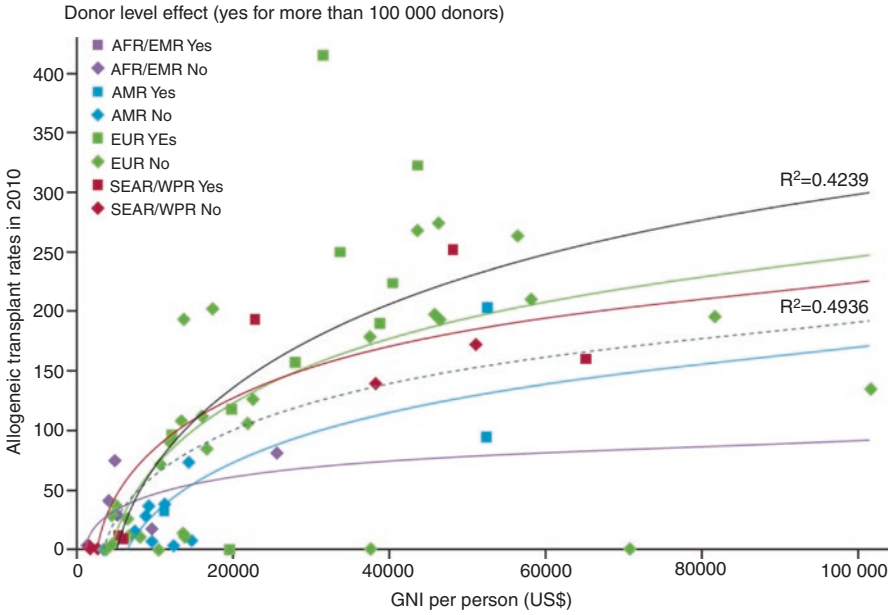

Fig. 1.4 Country-specific macroeconomic factors and transplant rates. The figure depicts the transplant rates in participating countries in 2010 according to the gross national income per capita (GNI/cap) and the presence or absence of an unrelated donor registry with more than 100,000 registered donors categorized by WHO regional office distribution. *AFR/EMR* African/Eastern Mediterranean office, *AMR* Pan-American office, *EUR* European office, *SEAR/WPR* Southeast Asian/Western Pacific region office. *Black lines* represent trend lines of countries with (*unbroken line*) more than 50,000 registered unrelated donors and with (*broken line*) no registry or fewer than 50,000 donors. Reprinted with permission [5]

indications are limited (Fig. 1.5). Allogeneic HSCTs are primarily used for patients with leukemia (≈70%), lymphomas (≈15%), and non-malignant hematological disorders (≈15%), with autologous HSCTs primarily used for lymphomas (≈85%), solid tumors (≈10%), and acute myeloid leukemia (AML; ≈3%).

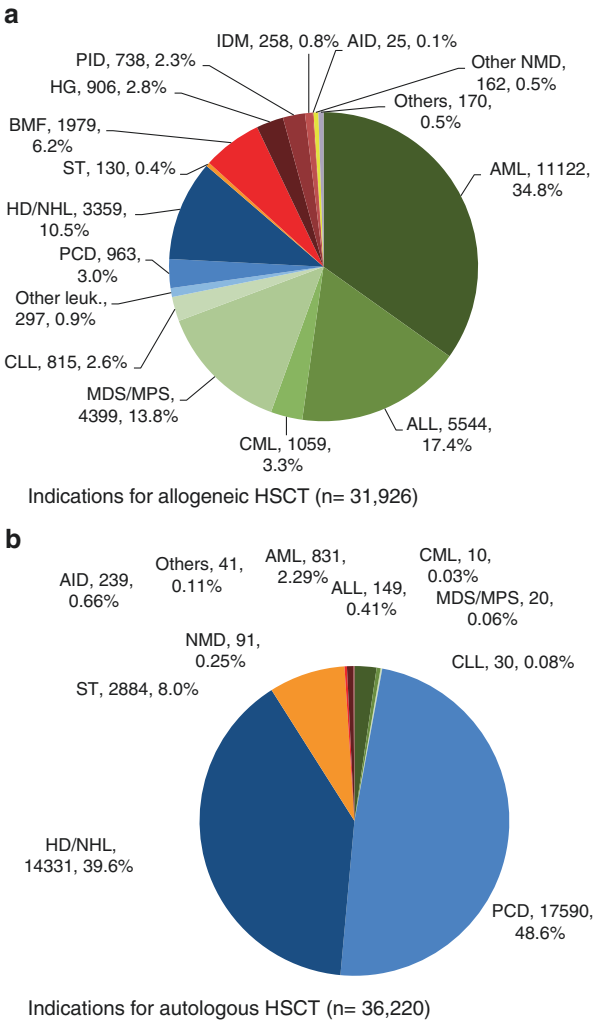

Fig. 1.5 Main indications for HSCT worldwide in 2012. The figure illustrates the proportions of main indications for allogeneic (*top*) and autologous (*bottom*) HSCT. Figure adapted from [23]. *AML* acute myeloid leukemia, *ALL* acute lymphoid leukemia, *CML* chronic myeloid leukemia, *CLL* chronic lymphocytic leukemia, *MDS/MPS* myelodysplastic/myeloproliferative neoplasias, *AID* autoimmune disorders, *PCD* plasma cell disorders, *NHL/HD* non-Hodgkin's/Hodgkin's lymphoma, *PID* primary immunodeficiencies, *NMD* non-malignant disorders, *IDM* inherited disorders of metabolism. (**a**) Indications for allogeneic HSCT (n = 31.926). (**b**) Indications for autologous HSCT (n = 36.220)

This simply covers only part of the reality. There are wide variations between and within WHO regional offices and between and within countries, and also wide variations regarding the selection of donor type, main disease indications, and use of stem cell source. On the global level, fewer allogeneic than autologous HSCTs are performed (47%; ratio of allogeneic to autologous HSCTs 0.88; Table 1.1; Fig. 1.1). This ratio also holds true for both Americas and Europe. In contrast, more allogeneic than autologous HSCTs were performed in some parts of the world, specifically within the Southeast Asia/Western Pacific regions (ratio 1.66) and WHO Eastern Mediterranean Regional Office (EMRO)/Africa regions (ratio 1.35). The use of autologous or allogeneic also HSCT varied within regions. Of note, no country within Europe showed more allogeneic than autologous HSCTs (Table 1.2). The selection of donor type for allogeneic HSCT varied as widely as the type of HSCT between and within regions. The proportion of unrelated donor transplants in the WHO regions ranged from less than 10% to more than 50%; in single countries it varied between less than 1% and more than 80% (median ≈ one-third). In the EMRO/African region the majority of allogeneic transplants were from sibling donors (≈90%), with an emphasis in indications on non-malignant disorders. In the Southeast Asia/Western Pacific region only about one-third of all allogeneic donors were identical siblings, only slightly less than the number of haploidentical donors (≈20%). The latter are of specific interest and showed increasing trends in recent years in all regions [23, 26].

Table 1.1 Diversity in use of donor type

	Ratio allo	% Allo	% Sibling	% Unrel	% Haplo
Africa/EMRO	1.35	0.58	0.87	0.08	0.04
(http://www.who.int/classifications/network/ro/en/)	0.78	0.44	0.39	0.54	0.07
Southeast Asia/ Western Pacific	1.66	0.62	0.35	0.48	0.18
Europe	0.70	0.41	0.36	0.56	0.07
Global	0.88	0.47	0.39	0.51	0.10

Data derived from [23]
Allo allogeneic, *Unrel* unrelated, *Haplo* haplodentical, *EMRO* Eastern Mediterranean Regional Office

Table 1.2 Diversity of disease selection and choice of stem cell source within Europe

	Ratios				
	Allo/auto	% Auto AML	% Allo MM	solid tumor auto	BM SAA
Mean	0.56	0.07	0.03	0.08	0.08
Min	0.00	0.00	0.00	0.00	0.00
Max	0.92	0.28	0.13	0.17	0.91

Data derived from [26] and based on 30 participating European countries. Numbers presented are means, minimum and maximum proportions
AML acute myeloid leukemia, *Auto* autologous, *MM* multiple myeloma, *BM* bone marrow, *SAA* severe aplastic anemia

A wide variation of practice is observed in use of transplant techniques for the different disease categories when Europe only is looked at (Table 1.2). Some countries limit their HSCT activity to autologous HSCT, with no country preferring allogeneic over autologous HSCT. The proportion of autologous HSCTs for AML varies from 0 to 27%, the proportion of allogeneic HSCTs for multiple myeloma from 0 to 13%, and the proportion of solid tumors among the autologous HSCTs from 0 to 17%. Variations in the choice of stem cell source are even higher. In Europe, the use of bone marrow as a stem cell source for aplastic anemia, the preferred choice, varies from 0 to 91% (Table 1.2) [27].

Consequences

These few selected numbers and figures just reflect current practice. They are neither wrong nor right, and some numbers might not fit with current recommendations [28, 29]. But today, these numbers imply some consequences. They have to be seen in the context that center- and country- specific economic factors have a direct impact on transplant outcome [10]. Nonrelapse mortality (NRM) and Relapse incidence (RI) decrease systematically with the increasing disease-specific experience of transplant centers, leading to improved overall survival in centers with longer experience and higher patient volume. Furthermore, outcome is better in countries with more resources and better infrastructure. These findings are independent of the well-known disease, patient, and donor risk factors [6, 7]. New centers embarking on a new transplant program have to be aware of this reality (Table 1.3). Just doing a few transplants is conceptually wrong today. Transplant directors should have a clear vision, sufficient resources, and available staff, including staff for quality and data management; their center should be integrated into a disease-specific pre- and post-transplant patient path. The center needs to have sufficient support from their institution to be able to achieve a reasonable number of transplants rapidly in order to build up experience within a reasonable time frame. They should arrive at

Table 1.3 Key questions for any new transplant team in view of global data

Unmet need	Is there a sufficiently large patient population that could profit from HSCT and cannot be served by existing transplant teams within their own or a neighboring country?
Network	Is there an informed disease-specific network of physicians for referring and for ascertaining post-transplant care?
Resources	Are there sufficient infrastructure and personnel resources for ascertaining pre-transplant evaluation, donor search, transplant procedure, after-care, quality management, data collection, and teaching?
Commitment	Is there adequate staff and sufficient support from administration, competent authorities, and payers to reach a reasonable number of HSCTs within a reasonable time frame?

HSCT hematopoietic stem cell transplant

performing a minimum of 20 HSCTs per year within 3 years. According to their country-specific characteristics they should decide to either select specific disease categories or split the task with neighboring centers.

The numbers presented in global activity surveys have consequences for established transplant centers, as well as for transplant organizations and for competent authorities, in several aspects. It is hard to find scientific reasons for some practice variations between centers and countries that range from 0 to 100%. Practice is rather driven by individual expectations and habits (25). If evidence is lacking, professional organizations are challenged to provide the evidence with appropriate studies. Patients, donors, and payers might no longer accept individual transplant policies outside quality control systems. If evidence is available, professional organizations should act in order to implement best practice. They should keep the lead and not leave decision-making to non-professionals.

In conclusion, global data are probably the most essential tool for new transplant teams. Such data will provide the framework for justifying their activity and guiding their patient selection, as well as serving for quality control.

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Chapter 2
General Principles of HSCT

Dietger Niederwieser

Introduction

In 1957 Edward Donnall Thomas reported, for the first time, a radical new approach to treat cancer by radiation and chemotherapy followed by the intravenous infusion of bone marrow [1]. Although the first six patients all died, a change of blood group provided evidence of transient engraftment. Even so, another decade of laboratory and clinical investigations was needed to achieve the first real success [2]. Since then, many new discoveries in basic science, immunology, and pharmacology, together with intensive interdisciplinary cooperation and evaluations of registry data have contributed to making stem cell transplantation a successful treatment worldwide [3, 4]. Today 70,000 hematopoietic stem cell transplantations (HSCTs) are performed annually around the world; a registry of more than 28 million unrelated donors and a dense network of scientific societies are in place to make the treatment accessible to a large number of patients [5]. The main principle of replacing a diseased marrow with a healthy hematopoietic system has remained valid throughout the 60 years since the first transplantations, while a range of modifications and new insights have constantly improved the approach. These days, the complexity and the multidisciplinarity of HSCT call for a team of experienced physicians and nurses. The correct indication and stage of the disease, and the optimal pairing of recipient and donor, are essential for a successful HSCT. Patients and transplant physicians should know the requirements for, and the potential risks and benefits of HSCT, but also the alternative therapeutic options. Informed consent is required for performing such a complex and costly procedure.

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During the first decade of HSCT (1957–1968), only 3 patients with immunodeficiency survived, out of a total of 203 transplanted patients with aplastic anemia, hematological malignancies, and immunodeficiency [2]. Two major problems were identified as pivotal for the success of changing a hematopoietic system: graft failure (graft rejection) and graft-versus-host disease (GVH-D). Both complications were addressed in animal models in the 1960s before HSCT became successful [6–8].

Engraftment

Two main factors were discovered to be essential for engraftment in animals, and later in humans: sufficient pretreatment of the recipient ("create space for the new marrow") and appropriate donor selection. By the mid-1960s, the research team of E. Donnall Thomas found that some dogs given "sufficient" irradiation followed by transplantation of grafts matched with rudimentary canine histocompatibility methods survived. After human leucocyte typing techniques were developed, Thomas decided to restart human HSCT. With the use of sufficient pre-treatment (referred to as the "conditioning regimen") and a human leucocyte antigen (HLA)–matched sibling donor, a few patients survived, even some with therapy-resistant disease and some with severe aplastic anemia [9–11]. The conditioning regimen optimized during the first decades of HSCT is effectively still in use today. Instead of 1000 cGy, six fractions of 200 cGy (total 1200 cGy) total body irradiation (TBI) followed or preceded by 2 × 60 mg/kg cyclophosphamide (Cy/TBI) is considered the gold standard. Subsequently, chemotherapy-only conditioning regimens using busulfan in combination with cyclophosphamide (Bu/Cy) were developed as an alternative to radiation [12]. During the 1980s, there were numerous attempts to increase the antitumor activity, based on the dose-response effect, by increasing the density and/or dose of conditioning. However, the higher toxicity and mortality of the increased conditioning cancelled out the positive effects, and no real improvement in outcome was achieved. For these reasons, the two conditioning regimens described above remain the standard for HSCT and are considered to provide a reasonable balance between antitumor effect and excessive toxicity.

The second important determinant for engraftment is appropriate donor selection. In contrast to solid-organ transplantation, stringent matching of donor and recipient turned out to be a prerequisite for successful hematopoietic engraftment from the start. It is therefore not surprising that the first successful HSCT was performed between identical twins, an ideal tissue-matching situation. Later on, the realization that recipient immune reactions against donor cells were responsible for rejection of the graft led to donor-recipient matching and to successful HSCT. At that time, matching was restricted to serological class I major histocompatibility complex (MHC) antigen determination and to a negative mixed lymphocyte culture for MHC class II, but was sufficient to grant identity at the antigen and allele level

in siblings. Finally, the number of nucleated cells in the bone marrow graft was found to be decisive for the speed and extent of engraftment [11]. Injections of a few milliliters of bone marrow into the bone marrow cavity itself were reported long before 1957, but these were nowhere near sufficient to establish a stable state of surviving donor cells in the recipient, known as chimerism. The use of more than 2×10^8 bone marrow nucleated cells/kg patient body weight infused into the bloodstream, and not necessarily injected into the bone marrow cavity, was usually sufficient to obtain engraftment. This was another important discovery of HSCT, as was the tendency of donor T-cells in the graft to reduce the rejection rate.

Graft-Versus-Host Disease (GVH-D)

A wasting syndrome, described very early on in mouse transplant models, was recognized to be caused by the reaction of donor immune cells against host tissues [6–8]. This condition, called GVH-D, affects skin, gut, or liver, leading to varying degrees of impairment in the recipient and even to their death; the condition remains a major complication in human transplant situations despite extensive tissue matching. The introduction of methotrexate, as prophylaxis, significantly decreased GVH-D mortality, and the subsequent availability of cyclosporine further reduced the risk and severity of GVH-D. The donor T-cell reaction was found to be directed primarily against recipient keratinocytes, bile duct cells, and gut cells, with cytokines being major mediators and modulators. The targets recognized by donor T-cells on the surfaces of the recipient cells were called minor-histocompatibility antigens (MiHAs), in contrast to the previously identified major histocompatibility antigens (MHAs), but despite being suspected to be immunogenic peptides of intracellular processed proteins, they remained chemically unidentified for many years. The work of Els Goulmy finally characterized one of the MiHAs, known as HA-1 [13]. MiHAs were found to be MHC-restricted and presented in the MHC groove to donor T-cells. Cytokines modulate this reaction by controlling the expression of MHC antigens and MiHAs on GVH-D target cells [14, 15]. Keratinocytes have been shown to be susceptible to donor T-cells only upon activation by interferon (IFN)-gamma, which explains the association between infections and GVH-D observed clinically [16]. In the late 1970s, investigators observed that GVH-D was associated not only with a detrimental but also with a beneficial effect on relapse rate [17]. The so-called graft-versus-tumor (GVT) effect was substantiated by discovering MiHAs on the surfaces of tumor cells. Importantly, a GVT effect was also described without clinically significant GVH-D, confirming that there can be a reaction to tumor-specific antigens. These observations inspired scientists to look in more detail for a dissociation between GVH-D and GVT reaction.

The removal of donor T-cells from the graft (T-cell depletion) is the most effective prophylaxis for GVH-D. However, the reduction in GVH-D comes at the cost of an increase in both the rejection rate and the relapse rate.

After recognizing and dealing with the two major barriers (rejection and GVH-D), the practice of HSCT increased steadily from the early 1970s and spread around the world as the only curative treatment for many previously incurable diseases. Today, rejection rates are very low, and GVH-D, as a cause of transplant-related mortality (TRM), has decreased substantially, although it remains the most frequent cause of death. Overall, TRM and morbidity have decreased continuously up to the present day, largely due to continual improvements in supportive care.

Supportive Care

Destroying the hematopoietic system by the use of a conditioning regimen leads to an aplasia that endures for the 2–3 weeks required for the graft to establish a new self-sustaining hematopoiesis. In order to avoid life-threatening complications, hemoglobin and platelet levels are maintained above a critical threshold by transfusions. Over the same period, patients are kept in isolation rooms to prevent bacterial infections. Fungal infections remain a threatening complication and are of clinical importance in prolonged aplasia. Antifungal prophylaxis is therefore an established part of modern treatment regimens. Antiviral therapy has turned out to be more challenging, with the administration of immunoglobulins offering the only option for many years. These days, antiviral medications are available to avoid both Herpes infection and cytomegalovirus (CMV) reactivation. Medications for both these conditions have had a high impact in improving survival and reducing transplant-related morbidity and mortality after HSCT.

Recurrence of the Disease

Relapse of the underlying disease remains the major complication following HSCT. Relapse incidence can be improved considerably by transplanting early on and in disease remission, but overall no real improvement in relapse rates has been observed over the years. Efforts to reduce disease recurrence should therefore be on the agenda. Reduction of disease recurrence may be achieved, e.g., by the quantification of minimal residual disease (MRD) using molecular markers and by tailoring immunosuppression accordingly in the early stages following HSCT. Another approach might be to reduce the molecular tumor load prior to HSCT. Trials using epigenetic therapy after HSCT, with the aim of reducing the relapse risk, are currently underway. The infusion of donor lymphocytes has made a major contribution to reducing relapse rates, but unfortunately this applies only to immunogenic tumors.

Donor Lymphocyte Infusion (DLI)

In the late 1980s, the GVT effect was successfully used to treat post-HSCT relapses without chemotherapy. Kolb et al. described long-lasting remissions in patients with relapsed chronic myeloid leukemia (CML) after HSCT [18]. Today DLIs are given after T-cell depletion to accelerate immune reconstitution, and/or to prevent or treat relapse. For the prevention or treatment of relapse, early application of DLI and the immunogenicity and kinetics of the disease are important determinants of success.

Important Developments in HSCT

While the conditioning regimen was originally considered to be the main anti-tumor mechanism in HSCT, with the graft being required to rescue the patient, the GVT effect was later recognized to be an additional antileukemic tool. Its role in HSCT remained underestimated for many years. Today we know that both the conditioning regimen and the GVT effect contribute to tumor cell reduction and to the eradication of the malignant stem cells. Limitations to this curative treatment include the availability of matched donors and the toxicity/mortality of the procedure, which, for many years, restricted the use of HSCT to younger patients. Multiple efforts to make the treatment accessible to patients without a (family) donor and to older patients followed.

Autologous HSCT

Separation of the high-dose chemo/radiotherapy effect from the allogeneic effect has been attempted both in patients with hematological malignancies and in those with solid tumors, with the aim of reducing TRM. High-dose chemo/radiotherapy was used to kill the tumor; the patient with the irreversibly damaged bone marrow was then rescued using their own cryopreserved autologous marrow (autologous HSCT). This concept became so popular that the number of autologous HSCTs even exceeded that of allogeneic HSCTs [5]. The treatment was indeed characterized by low mortality, but unfortunately also by a very high relapse rate, thought to be due either to the reinfusion of malignant cells, or to the lack of the allogeneic GVT effect. Patients who achieve bone marrow remission, mainly those with lymphoma or myeloma, currently provide the most frequent indication for this treatment, while the use of autologous HSCT for other indications (e.g., breast cancer) has decreased considerably. In order to reduce relapse incidence, sequential (“tandem”) autologous HSCT has been carried out, especially in multiple myeloma, and it has been shown to be superior to single transplants. Chemotherapy responsive pediatric solid tumors are a frequent indication for autologous HSCT, although the HSCT activity in both pediatric and adult leukemia is currently decreasing worldwide.

T-Cell Depletion

The removal of T-cells from the graft provides a potential way of reducing the mortality of GVH-D. E-rosette separation was used initially, but was subsequently replaced by immunomagnetic selection (negative $CD3^+$ or positive $CD34^+$ selection) [19]. Unfortunately, ex-vivo T-cell depletion was associated with higher rejection and relapse rates and slower T-cell reconstitution. Overall results were therefore not superior to conventional matched related HSCT. The frequency of ex-vivo T-cell-depleted HSCT is limited in matched related and unrelated HSCT, but the approach is frequently and successfully used in haploidentical related stem cell transplantation to avoid GVH-D (alternative stem cell transplantation). In-vivo T-cell depletion using antithymocyte globulin (ATG) or alemtuzumab as part of the conditioning regimen is considered standard in unrelated HSCT.

Unrelated HSCT

In order to make HSCT accessible to patients for whom no related donor is available (approximately one-third of patients with siblings), unrelated donors were first used, in the early 1980s. Initially the outcome was clearly inferior to outcomes with related donors. However, with the availability of molecular typing, results improved markedly and they are now indistinguishable from those of related HSCT. Unrelated HSCT has, in the meantime, become more frequent than related HSCT, not only because of the improved outcome, but also because of the availability of more than 28 million potential donors in donor registries (https://www.wmda.info/). This voluntary humanitarian movement, which is spectacular and unprecedented in history, currently provides up to 80% of donors for patients around the world who lack a suitable related donor. Many donors are already molecular typed for 12 out of 12 antigens/alleles and are quickly available on request.

Source of Stem Cells

For many years bone marrow was used as the only source of stem cells (bone marrow transplantation). With the discovery of the presence of sufficient stem cells in the blood following chemotherapy and mobilization with granulocyte colony-stimulating factors, peripheral blood stem cells were first used as a source in autologous HSCT. The avoidance of anesthesia, the possibility of collecting a larger number of stem cells, and faster hematopoietic engraftment provided the main incentives for the use of peripheral blood stem cells instead of bone marrow in autologous HSCT. The switch from bone marrow to peripheral blood stem cells was slower in allogeneic HSCT, mostly because of an increase in GVH-D incidence in

some randomized trials. Today, it is generally accepted that, in hematological malignancies, GVH-D incidence may be higher with peripheral blood stem cells, but this is probably balanced by a decreased relapse rate. These days, peripheral blood stem cells provide the predominant source of stem cells for allogeneic HSCT. In non-malignant diseases, and in children, bone marrow remains the primary source [20]. Similarly, cord blood has been used in patients who lack HLA-matched donors. Thus, the original term "bone marrow transplantation" has been largely replaced by the more accurate "hematopoietic stem cell transplantation", specifying the source (peripheral blood, bone marrow, or cord blood).

Efforts to Reduce Morbidity and Mortality

Reduced-Intensity Conditioning (RIC)

Allogeneic HSCT was confined from the beginning to younger patients without comorbidities. In order to provide curative treatments for elderly patients, concepts emerging in the 1990s investigated strategies to reduce TRM. These efforts relied on a reduced or minimal conditioning regimen sufficient to establish chimerism and to have the subsequent GVT effect. Diverse reduced or minimal conditioning regimens with different degrees of myelosuppression and immunosuppression were introduced [21]. Two major models were identified, both of which resulted in 100% donor chimerism: one concept relied on reduced conditioning followed by the GVT effect (RIC-HSCT) and the second concept was based exclusively on the GVT reaction (non-myeloablative conditioning [NMA]-HSCT). RIC conditioning regimens include busulfan 8/fludarabine/antithymocyte globulin (ATG), melphalan 180/fludarabine, and thiotepa/cyclophosphamide and are less myelosuppressive than conventional HSCT, but still enable HSCT in selected elderly patients, with resultant low rates of morbidity and mortality. Conditioning regimens aiming at immunosuppression rather than myelosuppression are referred to as NMA-HSCT. These protocols are based either on chemotherapy (e.g., cyclophosphamide or melphalan 140) or low-dose TBI in combination with fludarabine, and result in little or no aplasia. Decreasing radiation dose and varying immunosuppression (cyclosporine and mycophenolate mofetil in a dog model were used to define protocols leading to stable hematopoietic engraftment. The pros and cons of the two methods are minimal toxicities with higher relapse rates on the one hand (NMA-HSCT) and increased toxicities with lower relapse rates on the other (RIC). As a consequence, RIC-HSCT is more commonly used in selected patients up to age 70 years with minimal comorbidities, while NMA-HSCT is given to all patients aged 60–75 years (without selection or age effect), including those with major comorbidities, which is a major risk factor for HSCT. The increasing population of older patients has the highest incidence of malignant diseases and is the most susceptible to the morbidity and mortality of cytotoxic treatment. Currently, HSCT is considered the only curative treatment in older patients with a variety of hematological malignancies, and this population accounts for about 40% of all allogeneic HSCT today.

Autologous Followed by Allogeneic HSCT (Auto–Allo)

An interesting approach has been studied in detail in patients with multiple myeloma, where an autologous HSCT aiming at reducing the tumor burden can be followed by an allogeneic HSCT with NMA conditioning. Such protocols lead to a considerable reduction in mortality in matched related combinations compared with conventional allogeneic HSCT and are superior to auto alone or tandem auto-auto [22].

HSCT in Patients with Refractory or Relapsed Disease

It is generally accepted that patients should be in remission of their disease at the time of HSCT. Unfortunately, not all patients are responsive to induction therapy, either during the initial treatment or in the relapse situation. For these patients, protocols have been developed to, firstly, decrease the tumor burden by chemotherapy and then to carry out HSCT with minimal tumor burden. The most common protocol of this type is called fludarabine, high-dose cytarabine and amsacrine (FLAMSA) followed by 4-Gy TBI, high-dose cyclophosphamide, antithymocyte globulin and HSCT it is able to induce long-term remission in some patients with refractory disease or those with persistent relapse of their disease [23].

Alternative Donor Transplants

Related and unrelated HSCTs are important curative therapies for many hematological and non-hematological diseases. Although access to unrelated donors has markedly increased the availability of matched donors, a significant proportion of patients still lack a suitable donor. Depending on the ethnic diversity of histocompatibility antigens, about 20–40% of patients in Europe and up to 80% of patients in other regions do not have a suitable donor and need alternative donor transplants to cure their hematological diseases, bone marrow failures, or genetic inborn errors. Two concepts have substantially increased the chances of performing HSCT in patients without a matched related or unrelated donor, thus increasing the curative potential for many patients: the use of haploidentical related HSCT (parents or sibling) and cord blood HSCT. A third concept, post-cyclophosphamide HSCT, has been developed recently and is currently being tested in clinical trials. Alternative donor HSCTs are usually associated with an increased frequency of infections and other complications and should be restricted to centers with extensive experience in the field of allogeneic HSCT.

Haploidentical HSCT

The removal of the effector cells of GVH-D from the graft has made haploidentical HSCT a reality. Such HSCTs were performed primarily in children following in -vitro T-cell depletion to <5000 T-cells/μL kg body weight and using high-dose

$CD34^+$ cells [24]. Granulocyte engraftment is very fast, but engraftment of T-cells is delayed, particularly in non-pediatric patients. It is not unusual to see delayed T-cell reconstitution (up to 3 months) after HSCT, which makes patients susceptible to viral infections. Approaches to hasten recovery have been identified and are still under investigation. One possibility is to use T-cell depletion instead of positive CD34+ selection. Protocols adding natural killer (NK) cells to CD34+ cells are also being investigated and selective αβ-T-cell depletion is being tested in clinical studies. Finally, T-cell add-backs, using cells transduced with suicide genes is also an interesting approach. More recently, unmanipulated bone marrow or peripheral blood has been used with cyclophosphamide administration post-transplant.

Post-Cyclophosphamide Transplants

An interesting approach for alternative donor HSCT has been developed by the Baltimore group [25]. By removing activated (mainly allo-reactive) T-cells a few days after HSCT, mismatched donors can be used without the need to remove T-cells ex vivo. A cytotoxic drug such as cyclophosphamide, which kills activated T-lymphocytes but preserves memory T-lymphocytes and hematopoietic stem cells, is given on days 4 and 5 after HSCT. Clinical studies are currently being performed to investigate the outcome and especially the long-term effect on relapse incidence.

Cord Blood

The use of umbilical cord blood as a stem cell source was pioneered by Eliane Gluckman [26]. The major advantages of the method include the rapid availability of frozen grafts and the acceptance of up to two antigen mismatches. On the downside, the number of stem cells is limited, restricting the use of cord blood transplants largely to children. Further disadvantages include a higher rejection rate, slow engraftment, and the expense of establishing a cord blood bank. However, the method has been constantly improved to enable its use in adults (e.g., by combining two grafts for HSCT) and today it is becoming an important alternative to haploidentical HSCT.

HSCT for Non-malignant Diseases

Non-malignant diseases such as severe aplastic anemia (SAA) and inborn errors are standard indications for HSCT. In contrast to the treatment of malignant disease, the major aim here is to establish a functioning hematopoietic system without GVHD. For this indication, bone marrow is preferred to peripheral stem cells and the duration of prophylactic immunosuppression should be prolonged as much as possible. A typical conditioning regimen for SAA is cyclophosphamide 4 × 50 mg/kg

for 4 days; fludarabine with ATG also plays a potentially important role. The frequency of SAA differs markedly between world regions, as do the frequencies of thalassemia and sickle cell anemia. Such disorders may well become frequent indications for HSCT in the future.

Treatment of Rejections After HSCT

As described above, graft rejection following HSCT is now relatively rare. However, the sensitization of patients using transfusions from related donors before HSCT is of continuing relevance, as is the development of antibodies to HLA antigens due to multiple transfusions. Such situations have become very rare even in patients with SAA, because the potential of multiple transfusions to cause subsequent rejection is well known, and the problem can usually be avoided. For the same reason, leucocyte-deprived platelets prior to HSCT and packed red cells are used for transfusion. If HLA-antibodies against donor cells are detected, plasmapheresis and rituximab may be used. Because of the potential for complications, such transplants should be performed only at experienced centers.

Treatment of Relapses After HSCT for Malignant Diseases

Although a relapse after HSCT used to be incurable, relapses can now be treated successfully with a second or even a third HSCT, using RIC or NMA-HSCT. Usually another donor is used, but successful second transplants involving the original donor have also been reported [27].

Indications for Transplant

The indication for HSCT is mainly determined by three different factors: the risk of treating the underlying disease with conventional therapies, the risk of HSCT, and the relapse incidence after HSCT. As an example, CML was a frequent indication before the availability of tyrosine kinase inhibitors (TKIs). These days, HSCT for chronic phase CML is restricted to those patients not responding to or intolerant of TKI therapy. Other diseases, such as the acute leukemias, are now the most frequent indications for HSCT. However, even within the acute leukemias, indications for transplants will depend upon the relapse rate associated with the particular leukemia type [28]. Subtypes with relapse rates higher than the TRM are considered today to be indications for HSCT. In the future, the determination of MRD might provide an increasingly accurate marker of indications for HSCT. Despite changes

in indications, the frequency of HSCT is still overall increasing annually, by 5–20%, with acute myeloid leukemias (AMLs) in complete response (CR) ≥ 1 currently providing the most frequent indication for HSCT. Irreversible bone marrow failures are considered to be indications for HSCT, as are inborn errors such as thalassemia and sickle cell anemia. Major indications for autologous HSCT are diseases with minimal or no bone marrow involvement, such as lymphoma, multiple myelomas, and solid tumors. Attempts to apply autologous HSCT in patients with bone marrow involvement such as chronic phase CML or in patients with acute leukemia with or without purging are currently not popular because they have not achieved the desired results. Although TRM was very low in these cases, disease recurrence posed a major problem. In the context of establishing an HSCT unit, the question of indication depends primarily on the type of patients the unit has. Depending on the financial situation, the curative potential of the treatment can be of considerable importance. Autologous HSCTs are usually performed more frequently in countries with high or intermediate income.

Conclusions

The primary idea of exchanging a hematopoietic system has become feasible only after extensive experimental work, multidisciplinary discoveries, and the cooperation of scientific societies during the past few decades. Today, HSCT has advanced, now being an established curative treatment for more than 70,000 patients per year with hematological and non-hematological diseases worldwide. Experience and understanding of the general principles of HSCT remain of fundamental importance for every important step in HSCT, and at the end for a successful HSCT. It is of utmost importance to start such programs with trained physicians and dedicated teams who are able to understand and treat the complications of HSCT. The registration of activities and twinning with other HSCT centers using the technologies available today might help in establishing an HSCT unit. The program should not start with more sophisticated activities, but should grow continuously according to the patient population and the resources available. Constant activity is mandatory to maintain the training of personnel, and a quality system should make the best use of the financial resources.

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Chapter 3
The HSCT Program Structure: Minimal Requirements

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Introduction

Hematopoietic stem cell transplantation (HSCT) is a complex procedure that requires multiple components that need to be in place in order for the procedure to be done successfully and safely for patients and donors. The establishment of HSCT programs with centralized and dedicated patient care has evolved over time, and despite the different models, the program requirements are similar. According to a Worldwide Network for Blood and Marrow Transplantation (WBMT) study, the number of transplant recipients surpassed one million in 2012; these procedures were done at 1516 transplant centers worldwide [1]. Although most of the transplants so far have been performed at centers in the United States, European countries, and Japan, growth is also seen now in other regions of the world, particularly in the Asia-Pacific region and Latin America [2]. Despite this growth, there are significant gaps in the availability of and access to transplantation services in several regions of the world [3, 4]. The establishment of transplant programs in regions of need can catalyze HSCT activity, expand access to patients in need of this treatment, and influence the development of other ancillary services that, by themselves, have additional downstream effects towards overall improvement of care for other patients. For example, through the development of a transplant program, there are investments in an intensive care unit, increased access to blood products, and upgrades in microbiology and radiological services, all of which have a beneficial impact on multiple other areas of medicine. Although the importance of the

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development of specialized services such as HSCT is recognized, in certain regions the flow of resources is never constant and there are many more urgent competing problems in the delivery of medical care that affect greater numbers of the population. In these situations of scarce resources, it is important to identify the minimal requirements for a functioning HSCT program that is capable of appropriately delivering safe care. Understanding these requirements might assist in the planning for the development of new HSCT programs and in optimizing resource utilization. For established programs that are expanding, needs increase and additional components are required. Thus, understanding of all the components of a transplant program in relation to whether it is new or expanding; whether the focus is on children, adults, or all patients; and whether the type of transplant is autologous, allogeneic or both, can help to streamline the planning and optimal delivery of care.

The need for a dedicated centralized and multidisciplinary HSCT program stems from the nature of the HSCT procedure and the potential association with life-threatening complications. Accreditation bodies have been created in order to improve the quality of care and to standardize and optimize the activities of three primary functions within a program: the clinical program, the cell collection, and the cell processing services. International HSCT accreditation bodies include the Foundation for the Accreditation of Cellular Therapy (FACT) [5] and the Joint Accreditation for the International Society of Cellular Therapy and European Society for Blood and Marrow Transplantation (JACIE) [6]. These bodies have established joint sets of standards that span all the activities related to HSCT and cellular therapies. These standards form a blueprint for quality management of a transplant program, and meeting these standards through the accreditation process is one of the components necessary for an established transplant program. Though program accreditation cannot be considered a minimal requirement for starting a program, the standards are a helpful reference to what ultimately needs to be in place; accreditation also requires a minimum number of transplants to be performed in order to test whether all components set in place are working optimally. Thus, if accreditation is an option and it is available in the region, it should be considered a goal of the program.

This chapter will review the different components required for the function of a transplant program in relation to size and transplant type, and will focus mainly on the minimal requirements for establishing a new transplant program. The objective of this chapter is to provide a general overview of all components that should be considered. Details on accreditation requirements will not be discussed, but can be found from those organizations dedicated to accreditation [5].

Transplant Program Structure

Multiple components must be in place for the routine use of HSCT for patient treatment. Collaborative services between clinical practice and the laboratory, multidisciplinary professionals, and ancillary services are the hallmark of a good transplant program. The main domains of a transplant program include: infrastructure, ancillary

laboratory services including blood banking, ancillary clinical services, core personnel in the transplant team, and quality management. Each of these categories has different components that may vary according to the level of development of the transplant program. Components needed for starting new transplant programs are different from those needed for established programs planning to expand.

The focus of the transplant program, i.e., whether the program is focused mainly on autologous HSCT, allogeneic HSCT, or both, also determines different sets of requirements. The focus depends on several factors, from the current experience and skills of the center prior to initiating the program, to the population and the prevalence of diseases in the region that the center is serving. For example, in regions with a higher prevalence of nonmalignant diseases, including hemoglobinopathies and severe aplastic anemia that are being treated by hematologists, initiating a program with a primary focus on allogeneic HSCT makes sense [7–10]. Thus, the differences between autologous and allogeneic HSCT are important to highlight.

Autologous transplants are mainly used for the treatment of hematologic malignancies, with the exception of a small subset of patients with autoimmune diseases [3, 11]. The goal of autologous HSCT is mainly disease control and the extension of disease-free intervals, and it is rarely curative. The complication rates s observed after an autologous HSCT are similar to the complication rates of other treatments for malignancies; for example, induction chemotherapy for acute myeloid leukemia.

Conversely, allogeneic HSCTs have a curative impact on both malignant and nonmalignant diseases [3, 11]. Allogeneic HSCT has a significantly higher risk of morbidity and mortality compared with autologous HSCT and as a result, allogeneic HSCT requires a number of unique components in order to maximize its safety and efficacy.

The decision to start with an autologous vs. an allogeneic HSCT program depends on the center – both in terms of the expertise of the personnel, particularly the transplant team leader, and in terms of the infrastructure and support facilities available at the center. Figure 3.1 outlines two models of a transplant program expansion. Panel A outlines the start of an autologous HSCT program before an allogeneic HSCT program. The rationale is that the autologous HSCT program could assist as a stepping stone for the development of more complicated allogeneic

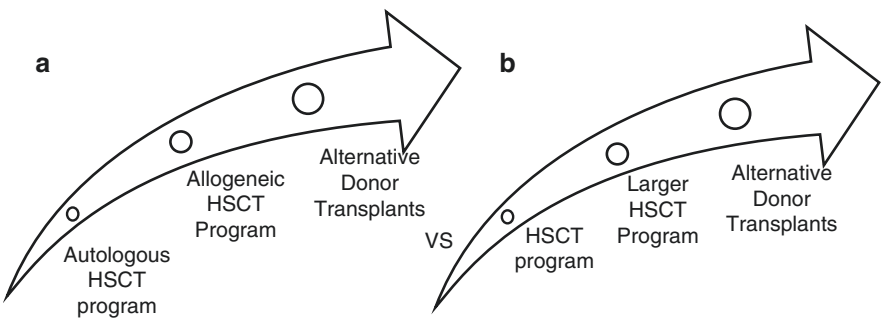

Fig. 3.1 Models for expansion of a hematopoietic stem cell transplant program

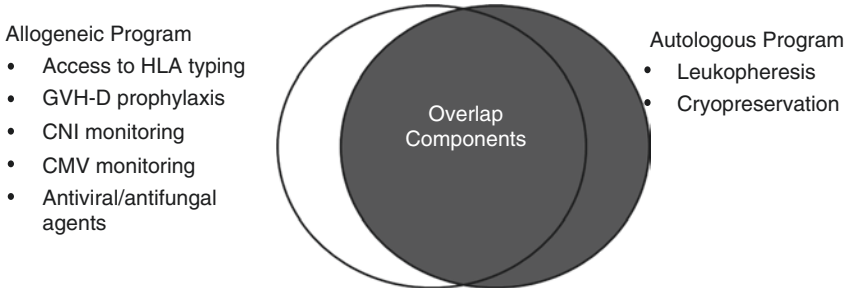

Fig. 3.2 Non-overlapping minimal requirements for autologous and allogeneic hematopoietic stem cell transplantation (HSCT) programs

transplants. This sequence of expansion allows a center to follow ("experience") a learning curve, to make sure all systems are in place and working optimally before implementing allogeneic transplants. Panel B shows an approach for the development of both types of transplants at once. This approach is possible when the lead transplant physician has adequate expertise in allogeneic transplants and the service is being established in areas with a high prevalence of nonmalignant diseases where the initial investments are focused on curative approaches. This approach is feasible, as most of the requirements for the functioning of an allogeneic transplant program would indeed be sufficient for an autologous program also, except for the need for graft cryopreservation (Fig. 3.2).

Minimal Requirements for Starting HSCT Programs

The urge to start performing transplants has many roots, such as the need to expand treatments already available for certain diseases based on their prevalence in the population, or an interest in having specialized services. However, HSCT cannot be safely performed without a programmatic approach, and establishing these programs is expensive. Thus, referencing a minimal set of requirements might assist in establishing a new program by having these elements in place and using resources optimally. Table 3.1 outlines the minimal requirements according to categories, which are expanded below.

Infrastructure

Before the idea of establishing an HSCT program can be considered, there is a fundamental need for institutional support. Assigning physical space that can be dedicated for transplants, investing in ancillary services, and hiring and training personnel are all necessary elements to be considered by the institution's leadership. The physical requirements should be a minimal number of single-bed rooms for inpatient

Table 3.1 Minimal requirements for a hematopoietic stem cell transplant program

Domain	Requirements for transplant center development
Infrastructure	
Infrastructure	Institution support
	Tertiary care center
	Dedicated patient rooms
	Access to intensive care and emergency services
	Cell processing laboratory
Ancillary laboratory services	
Blood banking	Availability of red blood and platelets for transfusion
	Availability of leucocyte-reduced and irradiated blood products[a]
	ABO typing
	Apheresis service[b]
HLA	Access to HLA typing laboratory[c]
Laboratory	Cell counter and chemistry laboratory
	Calcineurin inhibitor serum levels[c]
Microbiology	Basic bacterial and fungal cultures
	CMV detection (antigenemia or PCR)[c]
	Serology for CMV, hepatitis, HIV, HSV, syphilis, and HTLV1
Ancillary clinical services	
Pharmacy	Access to chemotherapy agents used in the conditioning regimen
	Access to antiemetics
	Access to medication used for treatment and prevention of GVH-D**
	Access to broadspectrum antibiotics
	Access to antiviral and antifungal medication for prophylaxis and treatment
Radiology	Standard X-ray and CT scanner or MRI
Other ancillary studies	Placement of central line access
Personnel	
Staff	Medical director BMT-trained hematologist/oncologist or immunologist with >6 months of training in a BMT unit
	Nursing staff with hematology/oncology experience or trained in handling chemotherapy and infection control
	Pharmacist with experience in handling chemotherapy
Quality and data management	
Quality management	Available clinical protocols or guidelines for standardization of practices[d]
	Strategy for data collection and understanding transplant outcomes

[a]Blood products should be, at a minimum, leucocyte-reduced; irradiated products are also important but not critical

[b]Apheresis services are critical for an autologous program, but not for an allogeneic program, as bone marrow harvest is an alternative

[c]Outlines specific elements for an allogeneic program

[d]Examples here include a common approach for the treatment of neutropenic fever, approach to patients with fever, antibiotic prophylaxis, and transfusion practices, among others

care, located in an area serviced by nurses who are familiar with the care of patients who are undergoing intensive chemotherapy and have prolonged cytopenias. It is desirable that at least some of the nurses receive training in a transplant unit elsewhere. There is no minimal requirement for number of rooms for the program and no absolute requirement for high-efficiency particle air (HEPA) filtration or laminar flow units, although HEPA filtration units or rooms for patients who are immunocompromised should be considered for any starting program, as these elements are important and preferred for decreasing the likelihood of bacterial and fungal infections. Over time, the requirements for performing transplants have evolved from laminar flow units with positive pressure and HEPA filtration, with the mandatory use of personal protective equipment, to less restrictive practices. The practices currently vary [12], and many centers perform allogeneic transplants in non-HEPA filtered rooms or even in an outpatient setting [12–14]. Nosocomial infections are a common problem; they are even more challenging in transplant recipients, in whom there is a high probability of the infections being life-threatening. Several considerations are required to minimize this risk; HEPA filtration has been shown to decrease the incidence of fungal infections, and to minimize exposure to fungal spores during building construction or remodeling [15–17]. Additional universal approaches for isolation and hand washing are also extremely important. Local environmental conditions are also a consideration when deciding on this aspect of the infrastructure. For example, open environments with high concentrations of fungal spores, and contaminated ventilation duct systems or water supplies could all increase the rate of nosocomial infection. Steps toward evaluating the environment and investing in means to reduce infectious complications must be considered a priority.

Transplant recipients have a risk of developing life-threatening complications, which makes it important to have HSCT programs only in tertiary care centers. Access to emergency room and intensive care services is an element of tertiary care that is needed to serve the HSCT program. Intensive care services include access to inotropic agents, hemodialysis or other renal replacement therapies, mechanical ventilation, and access to pulmonary critical care professionals. Combined units, where rooms can serve for both standard and critical care, are an alternative model. Another essential component of the infrastructure is a laboratory responsible for cell-processing activities, including but not limited to: cell counts, product evaluation, minimal manipulation, cellular product release oversight, cryopreservation, and maintenance of cellular product catalogs. Minimal requirements for a cellular processing laboratory are reviewed elsewhere [18]. Alternative models that include shared services with an affiliated blood bank could be considered.

Ancillary Laboratory Services

HSCT programs are dependent on basic ancillary laboratory services that span from blood banking to the routine clinical laboratory testing that is done for day-to-day clinical decisions, including reliable cell counts and biochemical assessments of

electrolytes and renal and hepatic functions. Access to ABO typing and blood for the transfusion of platelets and red blood cells is an absolute requirement; these blood products must be cocyte-reduced and irradiated to avoid the risk of transfusion-associated graft-versus-host disease (GVH-D)-like reactions when infused in patients who are severely immunosuppressed, as are transplant recipients. It is important that blood products are available from blood banks that meet minimal standards according to international blood bank societies, such as the American Association for Blood Banking (AABB) or equivalent. In areas with scarce resources, another important reference for the quality of blood products is the African Society for leuBlood Transfusion (AfSBT), which, in collaboration with international groups, has developed a stepwise approach for the preparation and availability of blood products [19]. According to the AfSBT implementation of standards, blood products meeting steps 1 and 2 could be considered to be within minimal requirements. For the rapid availability of blood, blood banking at the same facility as the HSCT is preferred, but this not imperative.

In the setting of allogeneic HSCT, access to HLA testing for the search and verification of anti-human leucocyte antigen (HLA) antibodies is a minimal requirement. HLA testing and verification of antibodies can be part of blood banking services and, like blood banking, can be performed in reference laboratories. It is important that the laboratory responsible for HLA typing follows the guidelines of the American Society of Histocompatibility and Immunogenetics for standard testing and reporting of results.

In the setting of autologous HSCT, the cell processing laboratory has some additional requirements that involve cryopreservation of the cell product, according to the timing of the collection and the infusion. This ideally requires access to a controlled-rate freezing system for optimal stem cell viability. However, this requirement has been debated recently, given the costs and constraints of these cryopreservation systems. Some centers have demonstrated approaches that avoid cryopreservation by shortening the period from collection to infusion [20]. This approach could be considered as an alternative, although for certain indications, like multiple myeloma, a second autologous HSCT is performed as salvage, and this cannot be performed without appropriate long-term cryopreservation.

Microbiology is another critical laboratory component that must be available; this includes basic bacterial and fungal cultures for blood, urine, and cerebrospinal and other body fluids. In the setting of allogeneic HSCT, viral monitoring and testing, mainly for cytomegalovirus (CMV) and Epstein Barr virus (EBV), must be available as well, and with sufficiently fast turnaround results to allow implementation of treatment. Depending on the prevalence of other viral infections in certain regions, access to testing for other viruses, e.g., hepatitis, will also determine the scope of this requirement. Pre-transplant testing for assessing patient eligibility must include serology for previous exposure to CMV, EBV, hepatitis B and C, human immunodeficiency virus (HIV), and human T-cell lymphoma virus type 1 (HTLV-1), in addition to syphilis. The same serology must be available for donor testing for related donors.

Additional specialized laboratory tests for allogeneic HSCT include the monitoring of drug levels. The most common approach for the prevention of GVH-D is the

use of calcineurin inhibitors (cyclosporine or tacrolimus). Monitoring of drug levels is critical for the routine clinical care of transplant recipients, as the levels must be within the therapeutic range in order to minimize toxicities and to avoid breakthrough of GVH-D-related signs and symptoms. As for the other laboratory tests, the availability of testing and fast turnaround of drug level results are imperative to maximize clinical utility.

Ancillary Clinical Services

Clinical pharmacy has become a critical component of the minimal requirements for clinical services. Polypharmacy is a common scenario in transplant recipients and pharmacists who can assist in understanding drug interactions, drug levels, and oversight of the use and availability of these drugs are an important asset to a program. Medications used for the transplant procedure are expensive and often require ongoing use in order to minimize complications. Access to and availability of certain drugs for a prolonged period are important considerations. Drugs for infection prophylaxis and treatment (including antibiotics and antifungal and antiviral agents) and drugs for GVH-D prophylaxis (calcineurin inhibitors, methotrexate), as well as corticosteroids and a range of other immunosuppressant agents are all included in the required list of drugs, particularly for allogeneic HSCT. Chemotherapeutic agents used for conditioning, and associated anti-emetic agents, are also included in this list.

Radiology services with standard X-ray and computed tomography or magnetic resonance imaging are also critical components of ancillary clinical services. The availability of these services at the same institution is critical to facilitate care, as imaging evaluations are frequent and are used for routine clinical decision-making. Special radiology services, including interventional radiology for the insertion of central line access, are preferred but not critical, as these procedures can be performed by other services. However, the placement of central access catheters is a minimal requirement, so services with expertise in performing this intervention should be available. Though it is discouraged and rarely needed, central line placement may be required for donors, for peripheral blood stem cell collection through leukapheresis, and having services with this expertise is critical to minimize life-threatening risks with this procedure.

Stem cell collection capability, either through bone marrow harvest or the collection of mobilized peripheral stem cells, is another obvious requirement, but might differ for autologous and allogeneic HSCT programs. Autologous HSCTs are mainly done using mobilized peripheral blood stem cells, and allogeneic HSCT, despite some differences, can be done with either approach. Thus, programs that decide to start with autologous HSCT are required to have leukapheresis services available for stem cell collection. Allogeneic HSCT programs may decide on only one type of stem cell collection first in order to contain costs.

Personnel

Specialized personnel are critical for planning, for patient and donor evaluations, and for overseeing the transplant procedure and post-transplant care. Even in regions with large numbers of transplant centers, there are no formal certifications for HSCT physicians. However, transplant physicians are often hematologists and/or oncologists who have trained in HSCT units. There is no agreed minimal training period for these individuals; however, for those with no previous exposure to the management of patients undergoing HSCT, a minimum of 6–12 months of additional training in HSCT should be undertaken, depending on their previous subspecialty training in hematology. Training considerations must also be made in the context of the presence of other HSCT programs in the city/country, or whether a new HSCT program is the first in a region. In the latter scenario, 6 months of training might not be sufficient and other approaches to support the implementation of the HSCT program must be in place. The HSCT program must have at least one physician who has been adequately trained in HSCT for 6–12 months. Ongoing twinning/communication with more experienced programs might be a consideration for continuing training and support in the initial years. Similarly, a cell processing laboratory director who will oversee all activities related to the cellular product is required to have experience in HSCT graft processing. Such a person could be from a hematology, blood banking, oncology, or immunology background. Nursing care is the most critical component of the HSCT program. Nurses with experience in oncology practice with competencies in chemotherapy handling and infection control should be sought for the HSCT program. As mentioned above, the inclusion of a clinical pharmacist or equivalent professional is also critical for the HSCT team. Ongoing training of these professionals is important and twinning or on-site training at more experienced training programs is an essential activity for newly implemented programs.

Quality and Data Management

In HSCT, quality management and improvement are automatically viewed as accreditation standards. Although accreditation is an important objective for the development of a transplant program, it is not a minimal requirement upon implementation, although it is important that some components included in accreditation standards are in place from the beginning. Clinical protocols or algorithms for commonly observed complications help to standardize practices and minimize risks to individual patients. Examples of some of these protocols include, but are not limited to: commonly used conditioning regimens, approaches to patients with fever, treatment of neutropenic fever, prevention and treatment of GVH-D, transfusion in transplant recipients, post-transplant vaccination guidelines, donor selection, and algorithms for allogeneic HSCT.

Another important aspect of accreditation standards is communication and the coordination of care, which are invaluable for the HSCT program. Some elements, including a coordinator who can assist with donor searches and pre-transplant recipient work-ups, are important to standardize these approaches and to minimize the time the recipient awaits transplant.

Understanding transplant outcomes is an important activity of transplant centers. Although the requirement to register cases with international transplant registries can be daunting as a minimal requirement, understanding the important variables to be captured and setting up a plan to collect and store these data is a critical activity.

Beyond Minimal Requirements for HSCT Programs

Once HSCT programs are established, how they evolve, in terms of application and areas of focus, varies. Natural progression leads to increased capacity to offer transplants to a larger number of patients, which by itself requires more resources and increases complexity. Another approach for established HSCT programs is to specialize in a particular application or indication. Regardless of the model, HSCT programs need to have a continuing process improvement approach and strive to add some additional requirement. Once the program has performed a number of transplants, decision for accreditation and a follow-up plan needs to be in place. The importance of accreditation depends on the region. In certain countries, the ability to continue doing transplants, either with insurance contracts, as in the United States, or by registration with the government healthcare system to perform HSCT, as in Europe, and the ability to participate in clinical trials, are all dependent on whether the center is accredited by national or international accreditation agencies, such as FACT and JACIE. For other centers, the accreditation process is important to standardize all the processes associated with the transplant and to improve quality. However, given the demanding nature of this process, the timing and necessity for accreditation need to be considered with respect to the cost and time investment required for establishing an HSCT program.

Another important activity, which can lead to program quality improvement, is the development of a process for understanding HSCT outcomes. This is done by registering all HSCT data with national or international outcomes databases. Outcome registries have harmonized the types of data and time points required to understand transplant outcomes. Participation in these registries is another invaluable activity that has an impact not only locally but also on the overall transplant community.

Conclusions

Minimal requirements for infrastructure, ancillary services, personnel, and quality management can assist in prioritization for initiating HSCT programs. We must recognize, though, that some of these suggestions for minimal requirements have

not necessarily been challenged with prospective studies and are recommended based on existing practices in centers worldwide. Thus, alternative approaches to facilitate transplantation could be considered, providing that the broad principles are followed, in terms of having suitably trained personnel in a tertiary health care setting. We must appreciate that groups that perform highly complex HSCT procedures in areas with scarce resources may employ alternative approaches that adapt to the challenges that these groups encounter. Assessment of the difficulty of implementing these minimal requirements and the safety margins required for an elective HSCT program is necessary in order to refine these recommendations.

Twinning or association with more experienced programs, either through personal training or using telemedicine, can also be considered as a minimal requirement to assist in decision-making and personnel training. Once established, HSCT programs can add additional elements to meet the planned trajectory for the program. Accreditation and capturing of outcome data are integral next steps to be considered for established good HSCT programs. An organized stepwise approach in establishing an HSCT program is important to make sure all elements are in place, facilitating future expansion and maximizing patient outcomes and donor safety.

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Chapter 4
Inpatient HSCT Unit

Simone Cesaro

Introduction

The location of a hematopoietic stem cell transplant (HSCT) unit should allow easy access to out-of-unit diagnostic and treatment facilities, such as radiology, radiotherapy, an intensive care unit, and a blood bank. Moreover, an HSCT unit must comply with structural and system requirements. In particular, rooms, and spaces must be adequate for the type and volume of treatment activity and must adhere to the standards for the safety and comfort of patients, caregivers, visitors (if any allowed), and healthcare personnel, according to national laws or international recommendations [1].

The establishment of an HSCT unit must consider the following general requirements for spaces or rooms:

- Space or location for secretarial activity related to patient admission, discharge, registration, and storage of clinical charts or patients' files
- Location or dedicated space for the storage of health instruments or facilities; for instance, portable electrocardiogram or ultrasound machine, X-ray machine
- Dedicated areas for deposit of dirty materials
- Toilets for healthcare personnel, separate from those for the patients
- Toilets for caregivers or visitors (if any allowed), separate from those for the patients and the healthcare personnel
- Sink and tap in every location or room to allow hand-washing for healthcare and hospital personnel and caregivers before and after approaching the patient. The use of a non-manual tap opening is highly recommended

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- Medical oxygen system complying with national law or international recommendations for the safety of the patient, caregivers, visitors (if any), and healthcare personnel
- Medical system for respiratory tract aspiration
- Waterbed (or possibility of acquiring one quickly) to prevent bedsores
- Changing rooms with shower and toilet for the staff, in the same unit or building, or in an adjoining unit, with a double cabinet to separate work clothes or uniforms from ordinary clothes.

Medical and nursing assistance shall be guaranteed all day long with the possibility that nurses can consult the transplant physician in charge during working days, at night, and on public holidays. The ratio of nurses to patient beds may vary according to the type of patient (adult or pediatric, young or old), type of transplant (autologous or allogeneic), type of conditioning regimen (myeloablative or reduced intensity), and performance status of the patient. Generally, a ratio of one nurse to every two to three transplant beds is reasonable to allow adequate assistance in a high-demand unit such as an HSCT center. The days, times, and names of healthcare personnel who are in charge during the day or the week shall be clearly indicated in order to allow family members to talk with the doctors or nurses to get information on the patient. The nursing coordinator shall have a safety key (passkey) to open any location, room, or window of the unit that can be locked.

Patient Area

In the patient area, floors and walls up to 2 m in height shall be easily washable and disinfectable. All cabinetry and furniture shall have smooth, nonporous, cleanable, wipeable surfaces that are resistant to scratching. No decorative water features, fish tanks, plant boxes, or containers with live or dried plants are allowed. Every location or room shall have a sink and tap for hand washing.

The locations or rooms required for daily activity are:

- Working location or room for nurses for activity related to patient assistance
- Location or room for doctors that can be used as a meeting room for the daily briefing
- Location or room for the nursing coordinator
- Location for medical visits and invasive surgical or medical procedures (bone marrow aspiration, bone marrow biopsy, lumbar puncture)
- Room for medical doctor on duty during the night (if provided)
- Location or room for drug storage

Moreover, the patient area shall include a relaxation room for caregivers or parents or other family members and, in a pediatric unit, adequate spaces for playing, school activity, and socialization for self-sufficient children and adolescents.

The essential instruments for patient assistance and treatment are: emergency cart with drugs for resuscitation, Mayo or Guedel cannula, Ambu ventilator, pocket mask, cardio monitor and defibrillator; surgery cart for patient medication; pharmacy cart for the daily therapy, and machine for lifting an (adult) patient if they are uncooperative.

The patient room shall allow adequate comfort and respect the patient's privacy and have windows that provide natural light. Room windows shall have fixed sashes and must be well sealed to prevent external air, dust, or small-insect infiltration. Electric lighting shall be adjustable to allow good visibility for the patient and the healthcare staff during routine assistance and procedures, but also to favor the sleep and the comfort of the patient. The room door shall have a viewing panel to facilitate observation and control by the nursing staff; as well, panels or curtains should be available in the room to cover the viewing panel and protect the privacy of the patient.

All rooms shall be equipped with at least one power point for each bed, a system for oxygen gas and an aspiration system, as well as a cardio monitor to control vital parameters such as respiratory rate, peripheral oxygen saturation, heart rate, and blood pressure. Internet network access by telephone line or WiFi is recommended to facilitate the collection of the patient data in the patient's clinical file and to allow the patient to communicate with family, friends, or school, or to maintain some work activity during hospital admission. The minimum size of a single-bed room is at least 12–14 m^2, and for a double-bed room it is 24 m^2, excluding the bathroom. The bathroom doors should open outwards or be sliding. In a pediatric unit, in considering the size of the room, space must be allowed for a parent or tutor who is usually allowed to stay all day, and the room shall be equipped with a bed or sofa bed for the parent/carer. Every room shall be equipped with a wheel-bed with a scale to allow monitoring of the weight of a non-self-sufficient critical patient and to facilitate the transportation of the patient from the room. The room equipment shall also include a bedside table, a patient wardrobe made of easily washable and disinfectable material, a light and acoustic call facility, and courtesy and safety lights in the room and bathroom.

Infections are among the major complications in patients who have undergone HSCT and they still represent a major cause of morbidity and mortality, especially in the early period after HSCT. Consequently, the establishment of an HSCT unit requires specific attention to preventive infection measures [2, 3]. As with other immunocompromised patients, the diffusion of nosocomial infections in HSCT patients is controlled by isolation of the patient in a single room to avoid contact with other infectious patients, by using barrier precautions (gloves, gown, mask, cap), and by hand disinfection by healthcare or caregiver personnel to avoid the direct transmission of potential pathogens to the patient; also healthcare workers with transmittable diseases (herpes, gastroenteritis, respiratory tract infections) are excluded from taking care of transplanted patients, and water- or food-borne infections are prevented by cooking food and filtering drinking water. These measures are effective against nosocomial and community bacterial and viral infections, but they are insufficient to prevent nosocomial fungal disease. In particular, mold infections

are started by the inspiration of fungal spores and subsequent colonization of the respiratory tract, followed by spore germination, proliferation to hyphae, and tissue invasion. Therefore, the HSCT patient needs to be placed in a protective environment equipped with air-filter systems capable of high efficiency, >99.97%, in removing air particles <0.3 μm in diameter (high-efficiency particulate air [HEPA] filter) from the room. Moreover, the room air pressure needs to be higher than that in the other rooms or spaces of the unit in order to create airflow. The pressure differential is maintained by supplying a greater volume of air than is extracted via the exhaust vent within an airtight room. Typically the intake air is HEPA-filtered, to provide additional protection, and delivered through a diffuser that facilitates air mixing. To maintain a positive pressure of 10 Pa relative to the corridor, all openings within the room must be properly sealed to avoid air leaks. An isolation room lobby and an en-suite bathroom are usually incorporated in this system (Fig. 4.1).

Although evidence supporting the effectiveness of protective environments is not based on well-executed randomized trials, most authorities and expert committees recommend the use of these environments. The guidelines approved and sponsored by several scientific associations, such as the Center for International Blood and Marrow Transplant Research (CIBMTR), the National Marrow Donor Program

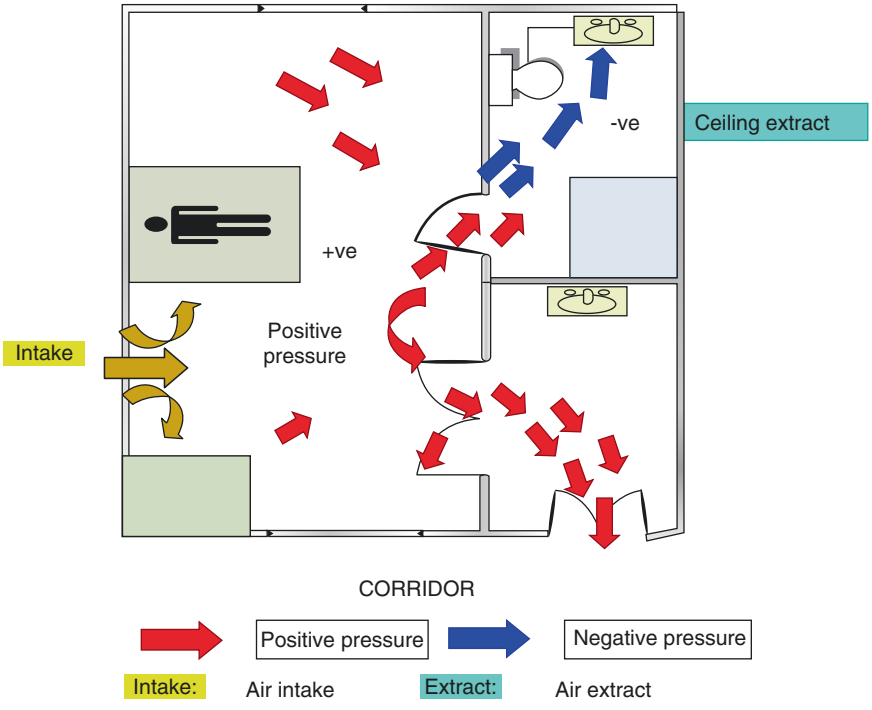

Fig. 4.1 Isolation in a positive-pressure room. The airflow is directed from the patient to the hospital corridor and exterior. The patient is protected from air ingress from the corridor. Extraction of the air is done in the bathroom

(NMDP), the American Society for Blood and Marrow Transplantation (ASBMT), the Canadian Blood and Marrow Transplant Group (CBMTG), the Infectious Diseases Society of America (IDSA), the Society for Healthcare Epidemiology of America (SHEA), the Association of Medical Microbiology and Infectious Diseases (AMMI), the Centers for Disease Control and Prevention (CDC), and the European Group for Blood and Marrow Transplantation (EBMT) have clear recommendations with respect to hospital room design and ventilation [4–10]. Recommendations on a protective environment and ventilation for an HSCT room shall include:

- The room must be equipped with HEPA filters with 99.97% efficiency for removing particles ≤0.3 μm in diameter
- The filters should be replaced regularly based on manufacturers' recommendations
- Ventilation must be performed with ≥12 air exchanges/h
- The direction of airflow is that air intake occurs at one side of the room and air exhaust occurs at the opposite side
- The air pressure differential between the patient's room and the hallway, ≥2.5 Pa, must be consistently positive
- Rooms must be well-sealed (e.g., gaps between the walls and windows, outlets, floor, and ceiling must be filled) to prevent infiltration of air from outside the room that could allow entry of spores and hinder the maintenance of the proper pressure differential
- Continuous-pressure monitoring is needed, especially while rooms are occupied
- Adopt room monitoring systems with an alarm that is activated when the pressure differential between any protective environment room and adjacent hallway or anteroom falls to less than 2.5 Pa, to alert staff to possible engineering failures
- Use self-closing doors to maintain constant differential pressure
- Perform visual monitoring of the HSCT recipient even when the doors are closed, through windows installed in either the door or the wall of the HSCT recipient's room (or by internal video-monitoring)

Established risk factors for healthcare-associated fungal infections are hospital building works such as excavation, demolition, recarpeting, and the installation of new fit-outs. Molds are ubiquitous in soil, water, decaying vegetation, walls, and ceilings and their spores can be aerosolized or dispersed for extended periods or travel long distances in the air. To prevent fungal outbreaks in places with ongoing construction, patients, healthcare workers, family, and other visitors should avoid such construction or renovation sites. The HSCT area should be sealed from the outside and the efficiency of the filtration system should be monitored frequently to determine the appropriate time for replacement. Moreover, the use of high-efficiency masks is recommended for patients who must go outside protected HSCT areas, e.g., to radiotherapy or radiology units, while building construction is ongoing.

If there is an occurrence of an airborne infectious disease (e.g., influenza, varicella) while the patient is in a positive-pressure room, there is a risk of spreading the infection to other patients or to all the staff, because the contaminated airflow occurs toward the corridor of the unit and the closest locations. The isolation room lobby

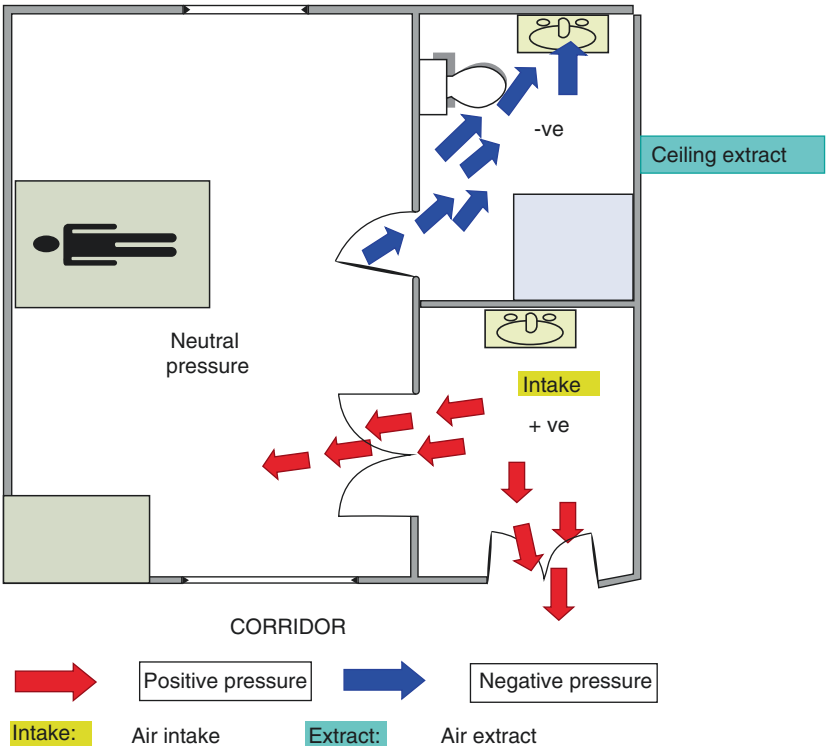

Fig. 4.2 Isolation in a neutral-pressure room. The neutral pressure in the patient's room is obtained from the balance between air intake in the anteroom and air extraction in the bathroom

may act as a barrier, but it cannot totally prevent the egress of air. The consequence is that an immunosuppressed patient in a positive-pressure room who develops an airborne infection must be moved, to minimize risk to other patients. In the past, HSCT rooms were protected with a reversible positive- or negative-pressure switch mechanism, so that the room could operate under either positive or negative pressure. This design is not recommended now, because of the risk that a lack of training by healthcare personnel or the lack of an appropriate operating procedure can result in the activation of the wrong option, nullifying the desired protective effect.

Recently, to overcome the inconvenience of isolating the patient in a positive-pressure room to prevent the spread of airborne infections, a model of isolation in a neutral-pressure room has been proposed. In this model, there is a positive-pressure anteroom lobby with an extensive number of air changes per hour, which has a positive pressure relative to the corridor and prevents corridor air from entering the room. The patient's room is at neutral pressure and air extraction is via the bathroom (Fig. 4.2).

Also, in this model, the neutral-pressure area must be well sealed and it is important that the side doors of the room remain closed. Although this isolation model is feasible from an engineering point of view, data are limited regarding its clinical validation.

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Chapter 5
Outpatient HSCT Unit

Jeff Szer

Introduction

Transplant numbers continue to increase worldwide, both for autologous and allogeneic transplantation [1]. This reality continues to put increasing pressure on transplant programs to accommodate a growing number of eligible patients, and much of the increased activity will flow through to the outpatient department. After successful transplantation, patients will spend much more time in an ambulatory care than in an inpatient setting. A variety of transplant procedures themselves may also be undertaken in part or in whole in the outpatient setting, and this chapter will outline the requirements for the ambulatory service required to result in optimal outcome of the patients. Components of this include efficient routine management, including assessment, delivery of medications, early management of complications, and ready access to inpatient beds if required. This will often require a close relationship with the emergency department and bed management system of the institution.

The outpatient area itself should be adequately staffed with clerical and nursing staff to ensure that patients are aware of where and when they need to be at all times, have a concept of waiting times, and have the assurance that any emergent problems arising while the patient is in the area are dealt with efficiently and safely.

The general descriptions that follow reflect an optimal structure. It is recognized that there is a wide variety of available infrastructure and staffing levels in different institutions offering or planning to offer stem cell transplantation services across the world [2]; nevertheless, an understanding of what optimal care looks like can help to optimize an actual department, given local constraints.

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Clinic Rooms

Clinic rooms are required for the medical, nursing, and allied health evaluation of patients at selected visits. Not all patients may appear to require an assessment in a clinic room, but a quiet area with examination and record-keeping resources where each patient coming through the outpatient facility can be evaluated is necessary. Outpatient departments which have had unstructured booking systems have found that patients often wait for lengthy periods, occupying a treatment or procedural area that could have been more efficiently used, only to later be told that no such procedure or treatment is required on the day. If the area is shared with other hematology or oncology patients, a unified booking system taking into account the specific needs of transplant patients is essential.

A clinical evaluation prior to any treatment can assist in the efficient utilization of other parts of the department, as well as facilitate the early identification of previously unsuspected problems that might need additional intervention. For example, a quick questioning of a patient may reveal an increase in diarrhea or the development of pruritus in an allogeneic recipient that might prompt closer attention to the possible development of graft-versus-host disease at the initial part of the visit rather than at the end. Ideally, patients will have had appropriate routine or previously requested tests done with results available prior to this consultation so that the day's management can be planned most efficiently.

Essential components of a clinic room include a desk, ready access to contemporaneous investigation results and previous medical records including treatment delivered, a quiet area for history-taking, and an examination couch with adequate lighting and patient access. In addition, essential clinical equipment such as a stethoscope, ophthalmoscope, and sphygmomanometer will be in the room. These rooms should make adequate hand hygiene for staff simple and intuitive, with appropriate placement of either sinks or hand-cleaning solutions, or both. Oxygen must be available, as well as adequate access for emergency teams in the event of a rapid clinical deterioration while the patient is in the room.

For patients who need to be transferred from the clinic rooms to the inpatient or emergency area, specific criteria such as hemodynamic instability, unremitting fever, ongoing requirement for intravenous support, etc. need to have been established and need to be easily available for reference by the treating staff [3].

The waiting area for clinic rooms should be large enough to account for the patients who are expected, as well as accompanying carers, but an efficient booking system will ensure that this area need not be excessively large relative to the number of clinic rooms or treatment chairs. Patients who may be at higher risk of acquiring infections should spend the least possible time in a shared waiting area and patients at risk of infecting others (for example, those with a productive cough and those with likely viral infections such as varicella-zoster or influenza) should also be moved out of such a waiting area as quickly as possible, if necessary to the emergency department or an inpatient area.

Increasingly, clinic rooms in the outpatient department are used for undertaking long-term follow-up clinics, which are proving to be an essential component of the lifelong care of transplant recipients [4]. An essential function delivered here is the provision of post-transplant vaccination protocols, a sample of which is shown in Table 5.1.

Table 5.1 A sample post-transplant vaccination flow sheet

Vaccine	Months post-transplant							Comment
	6	7	8	12	24	25	26	
Diphtheria, tetanus, pertussis, polio								
Boostrix IPV or Adacel polio	√		√	√				Diphtheria and tetanus booster (ADT) at 10–20 years after first dose
Streptococcus pneumoniae								
Prevenar 13 conjugate	√		√	√				
Pneumovax 23					√			Booster of Pneumovax 23 repeated every 5 years after first dose
Haemophilus influenzae type B								
Hiberix	√		√	√				
Neisseria meningitidis								
Menveo (ACWY Conjugate)	√		√					Doses to be given at least 2 months apart
Hepatitis B								
H-B-Vax II (dialysis formulation)	√	√		√				Dialysis formulation recommended in immunocompromised patients. If unavailable consider single-strength adult vaccine (Engerix-B 1.0 mL) in each arm at each dosing schedule
								Consider checking HepBsAb titer 4–8 weeks after course. If titer ≤10 IU/L repeat course
								Consider re-check at 12-month intervals to assess need for booster
Influenza								
Seasonal influenza vaccine	√	√						Annual vaccination. Consider two flu vaccinations (4 weeks apart) for first flu vaccination for all patients after BMT. Then only one dose annually, except in patients who are on ongoing immunosuppression
								Counsel household members to be immunized.
Measles, mumps, and rubella								
Priorix (MMR)						√		Not to be administered if on immunosuppression or with active graft-versus-host disease
								May be given at the same time as Varilrix or 4 weeks post
								Do not administer within 4 weeks of pneumococcal polysaccharide vaccine
								Check MMR Ab titer 4–8 weeks post-dose and if undetectable consider repeat dose

(continued)

Table 5.1 (continued)

Vaccine	Months post-transplant							Comment
	6	7	8	12	24	25	26	
Varicella								
Varilrix						√	√	Not to be administered if on immunosuppression or with active graft-versus-host disease
								May be given at same time as MMR or 4 weeks post
								Do not administer within 4 weeks of pneumococcal polysaccharide vaccine
								To achieve an immune response a second dose should be given 4 weeks post-first dose (longer interval acceptable if required) *BMT* bone marrow transplant, *HepBsAb* hepatitis B surface antibody

This sample schedule is adapted from the Royal Melbourne Hospital BMT Procedure Manual and was designed for adult patients only; it was adapted from international guidelines [5], taking local factors into consideration

A system of ensuring the timely administration of and recording of the details of vaccinations is essential. These clinics are increasingly being led by a nurse, often with advanced practice qualifications. These key staff can ensure appropriate adherence to protocols such as those for vaccination schedules and appropriate timing of routine post-transplant evaluations, such as lung function studies and bone density assessments.

These clinics provide a resource for optimal total care of patients after transplant and provide for the collection of data on the developing comorbidities and quality of life of these patients to serve as a form of quality control in transplant programs [6].

The availability of single-patient clinic rooms for evaluation and examination of patients is regarded as a *minimal* requirement for an outpatient hemopoietic stem cell transplant (HSCT) service.

Procedure Rooms

Rooms where commonly performed procedures can be undertaken, separate from the consultative clinic rooms, are preferably available. At least one bed or chair needs to be isolated from the rest of the treatment facility, providing a place where patients can receive medications that might be hazardous or potentially hazardous to others (e.g., if aerosolized pentamidine is used to treat or prevent pneumocystis infection, this should be done away from other patients and preferably in a negative-pressure room).

Commonly performed procedures include lumbar punctures (diagnostic and for prophylactic insertion of chemotherapy and other agents), the care of central venous

access devices as well as their removal, biopsies (including diagnostic bone marrow biopsies), and dressings. Some institutions may undertake bone marrow harvest procedures in a treatment room set up like an operating room with anesthetic support, although in most cases, these procedures will occur in a formal operating room setting [7].

Procedure rooms as described are regarded as a *preferred* requirement for an outpatient HSCT service.

Infusion Area

A large part of post-transplant care in the outpatient department is the near-routine infusion of fluids to minimize plasma volume depletion in patients whose appetite and ability to self-sustain liquid intake is compromised, particularly early after transplantation or in the context of complications such as gastrointestinal graft-versus-host disease. In addition, electrolyte supplementation may be required and routine drugs such as intravenous immunosuppressives and antiviral agents may be needed. A facility in which these procedures are undertaken may be a part of a larger hematology/oncology infusion center or may be stand-alone for transplant patients, depending on the size of the program. It is important that, apart from well-trained staff and written procedures and protocols, the physical facility has adequate access to the bed (or chair) to enable infusions to be set up and to allow for appropriate staff access to the patient. This facility, like the procedure area, will require oxygen and resuscitation facilities to be available, and, like the rest of the outpatient area, hand hygiene facilities should be prominent. Because this area may often be shared with cytotoxic chemotherapy areas (and may be used as such for outpatient conditioning therapy delivery), procedures to ensure that the correct patient is administered the correct fluids must be in place, as well as procedures to ensure that there is no spillage of fluids with the potential for cross contamination.

With the increasing use of outpatient transplantation, an area set aside for the infusion of hemopoietic stem cells may also be required. Ideally, this would be in a high-efficiency particulate air (HEPA)-filtered separated room, but such facilities may not be readily available; provided there is strict adherence to thawing (for cryopreserved products) and administration procedures, this may be done safely in a general infusion area. On this point, infection control in the outpatient phase of stem cell transplantation is more complicated than putting barrier processes into place within a facility. These outpatients spend considerable time in the community and, in particular, may walk into the facility through a building site if the hospital is undergoing construction or reconstruction. Thought needs to be given to infection control advice, taking these matters into consideration as well [8].

A day infusion area is regarded as an *ideal* requirement for an outpatient HSCT service.

Venous Access

Most patients before and after transplantation will have an implanted central venous access device in situ. Appropriately trained staff with written and readily available procedures for accessing these devices, as well as aseptic techniques, should be the only staff able to access them. Appropriate protocols for the care of these devices should be followed strictly because of the risk of infection related to these devices. As a general principle, central venous access devices should be removed as soon as it is deemed that they are no longer needed for patient care or when the risk of leaving the device in situ significantly exceeds any benefits.

In patients having peripheral venous access, it is important to minimize the trauma of venepuncture and to ensure that staff accessing peripheral veins are of an appropriate level of competency. This includes staff accessing veins for blood sampling for tests. If the apheresis service is co-located, staff performing this service are most often the most expert at venepuncture and may be called on to assist in patients with difficult venous access.

Educator

A nursing education officer who enables maintenance of standards for all procedures in the outpatient facility and who can re-educate nurses new to the area or re-entering the workforce is an important member of the team. The education role extends beyond these professionals to the patient and relatives and ongoing education of the patient, and starting prior to making a decision about proceeding with a transplant through to key time points after transplants will assist with avoiding preventable psychological trauma [9], as well as ensuring compliance with important treatment advice. From a patient education perspective, the ready availability of appropriate literature including, but not limited to brochures, locally produced educational fact sheets, and links to appropriate websites can be a useful educational tool, as well as enabling patients and family members to use waiting times efficiently.

The location of a key educator is not strictly required to be in the outpatient facility, but ideally would be situated nearby so that education can be delivered at the bed (or chair) side when appropriate.

Transplant Coordinator

Ideally, when a unit reaches a certain level of activity, coordinators are required to ensure the smooth running of the service, from scheduling and arranging pre-transplant evaluation of patients and donors to post-transplant care. Some units have two separate coordinators, for pre-transplant workup and scheduling, and for post-discharge care (both of whom are usually nurses with significant experience in

transplantation) and ideally a donor coordinator, who may not necessarily be a nurse but who should have a good understanding of transplant processes and a good relationship with tissue-typing personnel.

Personnel

The ideal staff structure for an outpatient facility is based on a medical director with a staff of senior transplant physicians and advanced medical trainees to undertake the medical assessment of patients coming through the facility, perform necessary procedures, and coordinate care in general. Transplant nurse practitioners with an appropriate scope of practice can not only ensure consistent quality care of the patients, but assist in the professional development of medical and nursing staff. Nursing staff with hematology/oncology or transplant experience are the necessary backbone of a functional outpatient area, although the ideal number of nursing staff per transplant outpatient has not been studied, to my knowledge. It is unlikely that a single nurse could supervise the treatment of more than two or three patients simultaneously. An experienced nurse unit manager to oversee the running of a transplant outpatient facility is ideal.

Outpatient facilities also require staff available for particular and intermittent requirements: consultative medical staff (e.g., infectious disease), wound care specialists, physiotherapists, social workers, and dietitians may all be required at various times. An on-site specialty pharmacist is important for medication education, as well as for advice on drug interactions and ensuring that patients receive the medication that is prescribed, prior to departing the facility.

Conclusions

The outpatient transplant facility is an increasingly important part of the patient journey through an HSCT and its aftermath. Appropriately planned and set up, there are few components of care of these patients that cannot be undertaken safely and efficiently in a well set up ambulatory service, and for many patients, most if not all of the treatment can be undertaken there. Planning and communication between the various disciplines is required to ensure smooth running and avoidance of errors.

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Chapter 6
Indications for Hematopoietic Stem Cell Transplantation in Children

Franco Locatelli and Luisa Strocchio

Introduction

The past few years have seen dramatic changes in the field of pediatric hemato-oncology, due to both significant advances in transplantation techniques and the introduction of new targeted therapies, thus modifying the position of hematopoietic stem cell transplantation (HSCT) in the therapeutic armamentarium for childhood hematologic malignancies.

Guidelines on the indications for allogeneic (allo-HSCT) and autologous HSCT (auto-HSCT) have been released by the American Society for Blood and Marrow Transplantation (ASBMT) [1] and the European Society for Blood and Marrow Transplantation (EBMT) [2] (Table 6.1).

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Table 6.1 Indications for allogeneic and autologous HSCT (adapted from [1, 2])

Disease	Disease status	Allogeneic HSCT		Autologous HSCT	
Hematological malignancies		EBMT	ASBMT	EBMT	ASBMT
Acute lymphoblastic leukemia	CR1 (HR)	S	S	N	N
	CR2	S	S	N	N
	Subsequent CR	S	S	N	N
	No remission	–	S	–	N
Acute myeloid leukemia	CR1 (HR)	S	S	C	N
	CR2	S	S	C	N
	Subsequent CR	S	S	N	N
	No remission	S	S	–	N
	APL, relapse	–	R	–	R
Chronic myeloid leukemia	First chronic phase failing TKI	C	S	N	N
	Accelerated phase	C	S	N	N
	Blast phase	–	S	–	N
Myelodysplastic syndromes	Low risk	S	S	N	N
	High risk	S	S	N	N
	JMML	S	S	N	N
	Therapy related	S	S	N	N
Hodgkin lymphoma	Primary refractory	–	S	–	[a]
	First relapse	C	S	S	[a]
	Second or greater relapse	C	S	S	[a]
Non-Hodgkin lymphoma (NHL)					
T-cell NHL	CR1 (high risk)	C	S	C	N
	CR2	S	S	C	N
	Subsequent CR	–	S	–	N
	No remission	–	S	–	N
Lymphoblastic B-cell NHL	CR1 (high risk)	C	S	C	N
	CR2	S	S	C	N
	Subsequent CR	–	S	–	N
	No remission	–	S	–	N
Burkitt's lymphoma	First or greater relapse	S	S	C	[a]
Anaplastic large cell lymphoma	Primary refractory	–	S	–	[a]
	First relapse	S	S	C	[a]
	Second or greater relapse	S	S	C	[a]
Non-malignant disorders		*EBMT*	*ASBMT*	*EBMT*	*ASBMT*
Severe combined immunodeficiency		S	R	N	N
Wiskott-Aldrich syndrome		S	R	N	N
Chronic granulomatous disease		S	R	N	N

Table 6.1 (continued)

Disease	Disease status	Allogeneic HSCT		Autologous HSCT	
Hematological malignancies		EBMT	ASBMT	EBMT	ASBMT
Severe congenital neutropenia		S	R	N	N
Hemophagocytic disorders		S	R	N	N
Other phagocytic cell disorders		S	R	N	N
Thalassemia		S	S	N	N
Sickle cell disease		S	S	N	N
Severe aplastic anemia	Newly diagnosed	S	S	N	N
	Relapsed/ refractory	S	S	N	N
Fanconi anemia		S	R	N	N
Dyskeratosis congenita		–	R	–	N
Blackfan-Diamond anemia		–	R	–	N
Congenital amegakaryocytic thrombocytopenia		–	R	–	N
MPS-1H Hurler syndrome		S	R	N	N
MPS-1H Hurler Scheie syndrome (severe)		–	–	–	N
MPS-VI Maroteaux- Lamy syndrome		C	R	N	N
Osteopetrosis		S	R	N	N
Globoid cell leukodystrophy (Krabbe)		–	R	–	N
Metachromatic leukodystrophy		–	R	–	N
Cerebral X-linked adrenoleukodystrophy		–	R	–	N
Solid tumors		C	D	S/C	S

[a]Depending on disease chemosensitivity

N not generally recommended, *S* standard of care, *C* clinical option, *D* developmental, *R* rare indication. *HSCT* hematopoietic stem cell transplantation, *CR* complete remission, *HR* high-risk, *APL* acute promyelocytic leukemia, *TKI* tyrosine kinase inhibitor, *JMML* juvenile myelomonocytic leukemia, *NHL* non-Hodgkin lymphoma, *EBMT* European Society for Blood and Marrow Transplantation, *ASBMT* American Society for Blood and Marrow Transplantation, *MPS* Mucopolysaccharidoses

Hematological Malignancies

Acute Lymphoblastic Leukemia (ALL)

Current frontline chemotherapy protocols for children with newly diagnosed acute lymphoblastic leukemia (ALL) can now cure more than 80% of patients [3]. Nonetheless, for subsets of children with high-risk (HR) features, identified by poor early response to therapy and/or genetic characteristics of leukemia cells, as well as for patients who experience disease relapse, outcomes are significantly worse.

The therapeutic advantage of allo-HSCT as a post-remission/consolidation strategy for these patients lies not only in the possibility to administer a more intensive treatment during the conditioning regimen, but also in the antileukemia alloreactions mediated by the graft.

Indications for HSCT in First Complete Remission (CR1)

The role of HSCT as a consolidation strategy in the frontline treatment of pediatric ALL must be considered in the context of a risk-stratified approach, based upon prognostic factors that can drive treatment intensity, with the aim of optimizing outcomes, while reducing unnecessary toxicities. The definition of these prognostic factors (namely, cytogenetic/molecular abnormalities at diagnosis and the response to induction treatment) is the result of the remarkable knowledge gathered from a series of large-scale analyses conducted by international cooperative groups.

HSCT in children with ALL in CR1 is currently reserved for subsets of patients with HR features.

In 2005, the International Berlin-Frankfurt-Münster (BFM) Study Group and the Pediatric Working Party of the EBMT Group reported the results of a cooperative prospective study comparing chemotherapy versus allo-HSCT from a human leucocyte antigen HLA–matched family donor (MFD) for very-HR childhood ALL in CR1, defined by the presence of at least one of the following criteria: (1) failure to achieve post-induction CR; (2) t(9;22) or t(4;11) clonal abnormality; (3) poor response to a 7-day prednisone prephase, associated with T-immunophenotype, white blood cell count (WBC) of 100×10^9/L or greater, or both. Five-year disease-free survival (DFS) was 40.6% in children allocated to chemotherapy and 56.7% in those given HSCT ($p = 0.02$). The 5-year overall survival (OS) estimate in children assigned to chemotherapy or HSCT was 50.1% and 56.4%, respectively ($p = 0.12$) [4].

A large prospective clinical trial has demonstrated that standardized quantitative assessment of minimal residual disease (MRD), using quantitative polymerase chain reaction (PCR) analysis of immunoglobulin gene rearrangements, measured at two time points (TPs) during induction treatment (TP1: day 33; TP2: day 78), can provide risk stratification of children with B-cell precursor (BCP) ALL and affect the choice of post-induction treatment [5]. Patients were considered MRD standard-risk (SR) if negative for MRD at both time points; MRD intermediate-risk (IR) if positive either at day 33 or day 78 and $<10^{-3}$ leukemic cells at day 78; and MRD HR if positive $\geq 10^{-3}$ leukemic cells at day 78. In the multivariate analysis, PCR-MRD was observed to be the most relevant factor for discriminating prognosis. The 5-year event-free survival (EFS) estimates for MRD-SR, MRD-IR, and MRD-HR patients were 92.3%, 77.6%, and 50.1%, respectively, with 5-year OS probabilities of 97.8%, 93.4%, and 60.8%, respectively. High levels of MRD at TP2 were predictive of poor outcome (5-year EFS < 50%). Fast clearance of MRD was associated with a favorable prognosis independently of non-MRD-related risk features, suggesting that, in patients undergoing relatively intensive treatment, if the MRD response is favorable, HSCT may not be indicated, even in the presence of any other combination of risk factors. On the other hand, for patients with a poor MRD response (i.e., MRD $\geq 10^{-3}$ leukemic cells after 2 months of therapy) despite favorable non-MRD risk criteria,

treatment intensification with HSCT may be indicated to compensate for the MRD-derived high risk of relapse.

The outcome of the cohort of HR patients enrolled in the Associazione Italiana Ematologia e Oncologia Pediatrica (AIEOP)-BFM ALL2000 study has been recently reported [6]. The statistical comparison of HSCT versus chemotherapy, accounting for waiting time to transplantation, did not show a significant advantage for HSCT over chemotherapy in terms of DFS. Nonetheless, in the larger subgroup of patients (subgroup 2), characterized by MRD-HR $\geq 5 \times 10^{-4}$ and $< 5 \times 10^{-3}$ leukemic cells at TP2 or by the presence of t(4;11) and prednisone good response, the initial advantage of chemotherapy changed to a disadvantage in favor of HSCT as time increased, due to late relapses after chemotherapy. Patients with T-cell lineage ALL belonging to subgroups 2 and 3 (MRD-HR $\geq 5 \times 10^{-3}$ leukemic cells at TP2 or no remission at day +33 or the presence of t(4;11) and poor prednisone response) seemed to benefit from HSCT in terms of both DFS and OS.

The current approach of the AIEOP-BFM treatment scheme is to emphasize the role of MRD kinetics in the choice of the HSCT strategy in CR1 (see also Table 6.2).

Distinct mention needs to be made of two particular conditions:

- BCR/ABL-positive ALL with poor early response.
- ALL diagnosed within the first 12 months of life ("infant ALL") harboring rearrangements of the mixed-lineage-leukemia (MLL) gene.

In the pre-tyrosine kinase inhibitor (TKI) era, the prognosis of BCR/ABL-positive ALL was dismal, with low survival rates even with the combination of chemotherapy and HSCT. The introduction of TKIs into HR ALL chemotherapy backbones deeply modified the history of BCR/ABL-positive ALL.

Table 6.2 Indications for allogeneic HSCT according to the current BFM-AIEOP ALL 2009 study protocol

	PCR-MRD results				
			MRD-HR		
Risk factor	MRD-SR	MRD-MR[a]	MRD TP2 $\geq 10^{-3}$ to $<10^{-2}$ leukemic cells	MRD TP2 $\geq 10^{-2}$ leukemic cells	No MRD results
No CR day 33	No	MMD	MMD	MMD	MMD
t(4;11)	No	MD	MD	MMD	MD
Hypodiploid karyotype <44 chromosomes	No	MD	MD	MMD	MD
Poor prednisone response T-ALL	No	No	MD	MMD	MD
None of the above-mentioned features	No	No	MD	MMD	No

[a]Including MRD-MR patients who are slow early responders (MRD TP1 $\geq 10^{-3}$ leukemic cells and TP2 $10^{-4/-5}$ leukemic cells)

ALL acute lymphoblastic leukemia, *BFM-AIEOP* Berlin-Frankfurt-Münster-Associazione Italiana Ematologia e Oncologia Pediatrica International, *PCR-MRD* polymerase chain reaction-based minimal residual disease, *TP1* timepoint 1 (day 33), *SR* standard risk, *MR* medium risk, *HR* high risk, *CR* complete remission, *no* no indication for HSCT, *MD* HLA-matched donor, *MMD* HLA-mismatched donor

In 2009, the Children's Oncology Group (COG) reported data on the use of imatinib, progressively increased in five patient cohorts from 42 (cohort 1) to 280 continuous days (cohort 5), combined with an intensive chemotherapy regimen, in 92 BCR/ABL-positive ALL patients aged 1–21 years. The addition of imatinib improved the outcome in cohort 5 patients, who achieved a 3-year EFS of 80%, higher than that of historical controls (35%; $p < 0.0001$) and comparable to that of MFD or HLA-matched unrelated donor (MUD)-HSCT recipients [7].

In 2012, the results of a large collaborative European trial (EsPhALL) were reported [8]. Patients were classified as good risk or poor risk according to early response to induction treatment. Allo-HSCT was recommended for all poor-risk patients, from any type of donor, and for good-risk patients with any genotype-matched donor, and was performed in CR1 in 137 out of 178 (77%) patients. In both the good- and poor-risk groups, the outcomes of patients given imatinib without transplantation appeared to be poorer than those of HSCT recipients.

The current approach in the treatment of Philadelphia chromosome (Ph)-positive ALL emphasizes MRD monitoring in the therapeutic decision-making process. An ongoing combined follow-up study (ClinicalTrials.gov Identifier: NCT01460160), assessing the effect of earlier, continuous exposure to dasatinib, is pursuing the transplant approach in CR1 only in patients who fail to meet predefined MRD criteria and who have an HLA-matched donor.

The prognosis of infant ALL is still relatively poor if compared with that of older children with ALL, achieving EFS probabilities of about 40–50% with current therapies [9]. Treatment protocols developed in the past 10–15 years have been investigating strategies to improve outcomes, such as treatment intensification with hybrid protocols including both lymphoblastic- and myeloid-oriented regimens, or the use of HSCT in CR1 [9, 10]. The potential benefits of HSCT for treating patients in this extremely vulnerable age group must be carefully weighed against the risk of long-term effects of the conditioning regimen on growth and development, requiring us to limit the transplant indication to infants with a poor probability of maintaining remission with chemotherapy alone.

In infants with MLL-positive ALL, a significant difference in DFS between patients receiving HSCT and those given chemotherapy alone was reported by the Interfant-99 Study Group. Furthermore, in the subgroup of infants younger than 6 months and with either prednisone-poor response or leukocytes $\geq 300 \times 10^9$ cells/L, HSCT was associated with a 64% reduction in the risk of failure resulting from relapse or death in CR, while in the remaining patients, no advantage for HSCT over chemotherapy alone was observed [11].

The current International Collaborative Treatment Protocol for Infants (Interfant06; ClinicalTrials.gov Identifier: NCT00550992) identifies three risk groups, based upon MLL status, age, and WBC at diagnosis/prednisone response. All HR patients (infants with MLL rearrangement, age < 6 months, and either WBC $> 300 \times 10^9$/L or prednisone-poor response) are considered eligible for HSCT, whereas in the MR group (remaining MLL-rearranged patients) HSCT is indicated only in those with MRD level $> 10^{-4}$ leukemic cells at the end of consolidation.

Indications for HSCT in Second (CR2) or Subsequent Complete Remission

While the prognosis of newly diagnosed childhood ALL has dramatically improved, the outcome of children with relapsed ALL remains unsatisfactory. At relapse, about 30–50% of the children can be rescued with high-dose chemotherapy regimens, in most cases followed by allo-HSCT.

Factors identified to be predictors of outcome in relapsed ALL, and thus critical in the identification of patients who can be rescued with chemotherapy alone and those in need of allo-HSCT, are the time to relapse (very early, early, or late), the site of relapse (isolated bone marrow [BM], combined, or isolated extramedullary relapse), and the immunological lineage of the disease (BCP vs. T-lineage ALL) [12]. Combining these risk factors, a classification into four different risk groups has been proposed to stratify patients with relapsed ALL in order to deliver risk-adapted treatments. Details of this classification, together with its prognostic impact, are reported in Table 6.3.

Allo-HSCT from an MFD is able to guarantee a higher EFS probability in comparison to that achieved with second-line chemotherapy [13]. Some studies suggested that the advantage of HSCT over chemotherapy alone could be limited to specific subgroups, e.g., patients with HR relapse (S3/4 group) or IR relapse [14], or patients experiencing disease recurrence within 36 months from diagnosis and receiving a total body irradiation-based conditioning regimen [15].

In patients with a standard risk profile (SR or S1/2), HSCT should be offered to those with BM involvement and MRD poor response after salvage induction therapy.

Thanks to the dramatic advances achieved in the field of allo-HSCT, outcomes after MUD-HSCT now approach those obtained in the MFD setting [16]. A matched-pair analysis from the BFM group, comparing MUD-HSCT with chemotherapy for children with ALL in CR2, documented a significantly higher EFS probability for the HR subgroup (44% vs. 0%), but not for IR patients (39% vs. 49%) given HSCT [17].

Table 6.3 BFM classification of relapsed childhood ALL (modified from [12])

S1–S4 group	Patients (%)	Definition of relapse	5-Year OS with chemotherapy (%)	5-Year OS with HSCT (%)
S1	5	1. Late extramedullary relapses	60–70	Not employed
S2	55	1. Early extramedullary relapses	40	60
		2. Very early extramedullary relapses		
		3. Non-T late BM relapses		
		4. Non-T combined early/late relapses		
S3	15	1. Non-T early BM relapses	<5	30
S4	25	1. Very early BM relapses	<5	25
		2. Very early combined relapses		
		3. T-phenotype BM relapses		

BM *bone marrow*, *OS* overall survival, *very early relapse*, <18 months from diagnosis*; early relapse*, >18 months from diagnosis, but <6 months from treatment discontinuation; *late relapse*, >6 months from treatment discontinuation; *S1-S4:* stratification groups (*S1,* standard risk*; S2,* intermediate risk*; S3, S4,* higher risk)

Current outcomes after umbilical cord blood transplantation (UCBT) have been observed to be similar to those obtained with unrelated BM grafts [18]. A retrospective analysis of children with ALL given unrelated UCBT reported to the Eurocord Registry documented a 4-year EFS of 44%, with high levels of pre-HSCT MRD predicting an increased risk of relapse [19].

The significant advances also achieved in the haploidentical setting have significantly broadened the applicability of HSCT, with outcomes currently approaching those obtained in the matched-donor setting.

In patients with ALL in CR3, the use of sole chemotherapy is associated with a very high risk of subsequent relapse; however, it has to be mentioned that allo-HSCT can also result in a considerable risk of transplant-related mortality (TRM), due to the pre-existing cumulative treatment toxicity.

Acute Myeloid Leukemia (AML)

The past two decades have seen a significant improvement in the outcomes of children with newly diagnosed acute myeloid leukemia (AML) [20], as a result of multiple factors, including advances in supportive care, progressive acquisitions of cytogenetic/molecular markers that have refined patient risk stratification, and the broad use of HSCT as consolidation strategy [21].

As in ALL, the therapeutic potential of HSCT results from both the possibility of delivering an intensive treatment before the allograft and the immunologic effect of the graft towards residual AML.

Indications for HSCT in First Complete Remission (CR1)

Allo-HSCT has been shown to be the most effective post-remission therapy for children with AML in CR1 when an MFD is available, in particular in patients with HR features [21], in whom transplantation is able to lower the relapse incidence to an extent comparable to that in SR children [22]. Thanks to the introduction of high-resolution HLA-typing, allowing a dramatic improvement in outcomes after transplantation from unrelated volunteers, indications for MUD-HSCT now partially coincide with those for MFD-HSCT.

In protocols in which the sole indication for HSCT was the availability of an MFD, a higher DFS was documented in patients transplanted in CR1, in comparison with patients receiving sole chemotherapy, without any difference in OS [23, 24].

More recently, a risk-stratified approach is being used, and candidates for HSCT in most current cooperative protocols are identified by the presence of HR features (i.e., unfavorable cytogenetic/molecular characteristics of leukemia cells and/or poor MRD clearance during induction therapy) [20].

Indications for HSCT in pediatric AML in CR1 are summarized in Table 6.4.

Genetic characterization of AML blast cells represents a major criterion for risk assessment at diagnosis, as first documented by the Medical Research Council (MRC) AML 10 trial [34].

Table 6.4 Indications for HSCT and proportion of patients given allo-HSCT in CR1 in recently reported pediatric AML trials (modified from [25])

Protocol	HSCT indications	Donor	Allo-HSCT (%)	Reference number
AIEOP AML 2002/01	HR patients (all patients except those with t(8;21) and inv.(16) and those in morphologic CR after the first of two induction courses)	MFD	29	[22]
		Auto-HSCT if MFD not available		
BFM 2004	HR patients (all patients except those with FAB M1/M2 with Auer rods, FAB M4eo or favorable cytogenetics [t(8;21) or inv.(16)] and blasts in BM on day 15 < 5%, FAB M3). From 2006 only no CR after 2nd induction	MFD	18	[26, 27]
COG CCG-2891	All patients with an available MFD	MFD	15	[28]
JPLSG AML99	IR and HR patients (all patients except for those with t(8;21) and WBC < 50,000/μL, inv.(16), or age < 2 years without HR factors)	MFD for IR patients MFD or MUD for HR patients	15	[29]
LAME 89/91	All patients with an available MFD	MFD	23	[30]
MRC AML 12	All patients except for those with t(8;21), inv.(16), t(15;17), or FAB M3, irrespective of BM status after course 1	MFD	11	[31]
NOPHO 2004	Poor response to induction (>15% blasts at day 15 after 1st induction or no CR after 2nd induction) or *MLL* rearrangements other than t(9;11)(p21;q23)	MFD or MUD	13	[32]
	From 2009: poor response to induction, only			
St Jude AML 02	SR patients with an available MFD	MFD for SR patients	25	[33]
	HR patients (monosomy 7, *FLT3*-ITD, t(6;9), FAB M7, treatment-related AML, AML secondary to MDS or >25% blasts after induction I or persistent MRD after three courses of therapy)	MFD or MUD for HR patients		

AML acute myeloid leukemia, *AIEOP* Associazione Italiana di Ematologia e Oncologia Pediatrica, *BFM* Berlin-Frankfurt-Münster, *CCG* Children's Cancer Study Group, *COG* Children's Oncology Group, *JPLSG* Japanese Paediatric Leukaemia/Lymphoma Study Group, *LAME* Leucemie Aique Myeloide Enfant, *MRC* Medical Research Council, *MDS* myelodysplastic syndromes, *MFD* HLA-matched family donor, *MLL* mixed-lineage-leukemia, *NOPHO* Nordic Society for Pediatric Haematology and Oncology

Core-binding factor abnormalities, such as t(8;21) or inv.(16), identified a group of patients with a relatively favorable prognosis, while in patients lacking these favorable changes, the presence of a complex karyotype, monosomy 5, del(5q), monosomy 7, or abnormalities of 3q was found to predict a poor outcome.

More recently, other cytogenetic/molecular prognostic markers were identified [25]. In the favorable group, t(1;11)(q21;q23), normal karyotype with *NPM1* mutation, and double mutant *CEBPA* were reported. Among adverse cytogenetic features, the following abnormalities have been associated with poor prognosis: del(7q); KMT2A (MLL) aberrations, excluding t(9;11)(p21;q23) and t(11;19)(q23;p13), t(9;22)(q34;q11), −17; and abnormalities of 12p, t(6;9), t(7;12), del(12p). A very poor outcome has been reported in the presence of the NUP98/NSD1 fusion gene, often associated with Fms-like tyrosine kinase 3 (FLT3)-internal tandem duplication (ITD) [35].

Considering that morphological CR is achieved in more 90% of children after induction therapy, but that relapse occurs in 30–40% of patients, the monitoring of MRD during treatment may allow the identification of patients at higher risk of relapse. A benefit of HSCT compared with chemotherapy alone has been reported in patients with poor MRD clearance, in particular when MRD levels remain above 1% after the first induction course [33]. For this reason, MRD monitoring has been included in many current protocols for the treatment of newly diagnosed pediatric AML, in order to refine patient stratification to receive HSCT in CR1.

The outcomes of children with HR-AML in CR1 given either auto- or allo-HSCT (based on the availability of an MFD) in the AIEOP AML 2002/01 Study Protocol were recently reported. Patients with M7 FAB subtype, complex karyotype or FLT3-ITD, were eligible for HSCT from alternative donors. The 8-year probability of DFS was 73.8% for recipients of MFD allografts, while for patients given MUD-HSCT, DFS was 75.5% in BM recipients, 53% in peripheral blood stem cell (PBSC) recipients, and 92.3% when UCB cells were employed (overall $p = 0.0035$) [36].

Indications for HSCT in Second (CR2) or Subsequent Complete Remission

Allo-HSCT represents the best chance of cure in children with AML in CR2. Patients with favorable cytogenetic/molecular characteristics, long duration of CR1, not receiving HSCT in CR1, and with good response to reinduction therapy have a higher probability of being rescued by transplantation in CR2 [37].

Patients not given HSCT in CR1 and who receive HSCT in CR2 have a 5-year OS approaching 60%, whereas in those relapsing after HSCT performed in CR1, poor outcomes have been reported [38].

Acute Promyelocytic Leukemia (APL)

Given the excellent results obtained since the introduction of all-*trans*retinoic acid (ATRA) in the treatment of APL, HSCT is currently not indicated in CR1. In patients with relapsed/refractory APL, the current role of HSCT as post-remission/consolidation strategy is controversial, as most reports of HSCT for APL in CR2 were published before the introduction of arsenic trioxide (ATO). Furthermore, as relapse incidence is very low in the ATRA and ATO era, randomized trials to compare different consolidation approaches in CR2 appear hardly feasible.

Experience with HSCT in treating pediatric relapsed APL is limited, the majority of data having been obtained from small retrospective studies. Data from the largest published series documented a 5-year EFS in the order of 70% for both auto- and allo-HSCT, with an incidence of TRM after auto- and allo-HSCT of 0% and 19%, respectively, all treatment-related deaths occurring in the early study period, before 1996. Relapse occurred in 27% of autografted patients and 10% of allo-HSCT recipients [39]. Even though the success of allo-HSCT is hampered by a higher risk of TRM, if compared with auto-HSCT, its use can provide a lower relapse incidence, probably due to the Graft versus Leukemia (GvL) potential of the donor graft against residual APL.

An expert panel of members from the COG and the International BFM Study Group recently published recommendations for the management of relapsed and refractory childhood APL. The authors suggest considering allo-HSCT in patients with prior ATO exposure, in patients with short duration of CR1, in patients with primary refractory disease, in those in second or further relapse, or those not achieving molecular CR after four salvage cycles. Auto-HSCT appears to be a reasonable option for treatment consolidation for ATO-naïve patients who achieve a second molecular CR after four salvage cycles [40].

Chronic Myeloid Leukaemia (CML)

In the pre-TKI era, allogeneic HSCT was the standard of care for children with Ph+ chronic myeloid leukemia (CML). The introduction of TKIs into the treatment of Ph + CML deeply modified the history of the disease, leading to a significant decrease in the use of HSCT. Nonetheless, based on currently available data, no certain evidence of the complete eradication of the Ph + clone by prolonged treatment with TKIs exists. Furthermore, the long-life expectancy of pediatric patients, entailing the need for potentially life-long treatment, renders the alternative choice between TKIs and transplantation controversial.

Current algorithms for the management of children with newly diagnosed CML in chronic phase (CP) include frontline treatment with hydroxyurea and a first-generation TKI, with a switch to a second-generation TKI in cases of failure to obtain an acceptable response. Allo-HSCT is reserved for patients who experience progression or relapse or persistently high levels of the BCR/ABL fusion transcript on second-generation TKI treatment. For children presenting with CML in accelerated phase or blast crisis, initiation of TKI therapy is recommended, followed by allo-HSCT once a reversion to chronic phase has been obtained [41].

Myelodysplastic Syndromes and Myeloproliferative Neoplasms

Myelodysplastic syndromes (MDSs) encompass a group of clonal disorders of HSCs and their precursors, characterized by peripheral cytopenia, dysplasia in one of the myeloid lineages with ineffective hematopoiesis, and a variable propensity to

evolve towards acute leukemia. The classification of pediatric MDSs includes low-grade forms (refractory cytopenia of childhood; RCC) and advanced MDSs; namely, refractory anemia with excess blasts (RAEB) and RAEB in transformation (RAEB-t). MDSs are rare in children, accounting for about 5% of hematologic malignancies, and they can be part of the natural evolution of inherited BM failure syndromes.

As childhood MDSs show relatively poor responses to conventional chemotherapy [42] and pre-transplant chemotherapy is not associated with improved outcomes [43], HSCT should be considered early in the course of the disease. Commonly accepted indications include advanced MDSs (i.e., RAEB and RAEB-t), MDS secondary to chemo-radiotherapy, and RCC associated with either cytogenetic anomalies (e.g., monosomy 7, complex karyotype) or severe neutropenia or transfusion dependence [43].

The results of the European Working Group on Childhood MDS (MDS) 98 study, which enrolled 97 patients with RAEB, RAEB-t, and myelodysplasia-related AML given HSCT from an MFD ($N = 39$), MUD ($N = 57$), or alternative family donor ($N = 1$), were recently reported. The 5-year probability of OS was 63%, with a 21% cumulative incidence of TRM and relapse. Factors associated with increased TRM were age at HSCT >12 years, time from diagnosis to HSCT longer than 4 months, and occurrence of acute or extensive chronic graft-versus-host disease (GVH-D) [43].

Monosomy of chromosome 7 or partial deletion involving its long arm [del(7q)] are recurrent chromosomal aberrations in RCC and have been reported to be associated with a significantly higher probability of progression to advanced MDS [44]. Moreover, a significantly better probability of survival has been shown in patients transplanted before evolution to advanced MDS in comparison to patients experiencing disease progression (76% vs. 36%, respectively, $p = 0.03$) [44].

For this reason, children with RCC and monosomy 7, del(7q), or a complex karyotype should be offered transplantation from either an MFD or a MUD early in the course of the disease. Conversely, children with RCC and normal karyotype or chromosomal abnormalities other than monosomy 7, del(7q) or a complex karyotype may experience a long, stable disease course, allowing a “watch and wait” approach. By virtue of the low TRM rates of MFD-HSCT, transplantation may be recommended for children with an available HLA-identical sibling. For patients lacking such a donor but experiencing transfusion dependence, severe neutropenia, or infections, transplantation from a MUD should be offered. A valid alternative is represented by immunosuppressive therapy (IST), with cyclosporine, antithymocyte globulin (ATG), and steroids.

Juvenile myelomonocytic leukemia (JMML) is an aggressive clonal hematopoietic disorder of infancy and early childhood, with features straddling myeloproliferative neoplasms and MDS. Approximately 90% of children with JMML carry either somatic or germline mutations in genes involved in the RAS/mitogen-activated protein kinase (MAPK) pathway, such as PTPN11, NRAS, KRAS, CBL, or NF1. Although spontaneous resolution has been rarely described, allogeneic

Table 6.5 Indications for HSCT in genetic subgroups of JMML (modified from [45])

	PTPN11	K-RAS	N-RAS	NF1	CBL
Germline mutations	"Watch and wait" (Noonan syndrome)	"Watch and wait" (Noonan syndrome)	"Watch and wait" (Noonan syndrome)	HSCT (neurofibromatosis type 1)	"Watch and wait" HSCT only if disease progression occurs (CBL syndrome)
Somatic mutations	HSCT from either an MFD or a MUD	HSCT from either an MFD or a MUD	HSCT from either an MFD or a MUD for most patients		

HSCT remains the treatment of choice for most JMML patients, being able to cure more than 50% of such patients. Prompt HSCT is recommended for all children with JMML and NF-1, somatic PTPN-11 mutations, and K-RAS mutations, and for the majority of children with somatic N-RAS mutations (Table 6.5). Conversely, because spontaneous regression of myeloproliferation has been observed in children with germline CBL mutations, as well as in Noonan syndrome patients, a "watch and wait" strategy is appropriate in these cases [45].

Disease recurrence is the main cause of treatment failure in patients given allogeneic HSCT for JMML. Thus, strategies aimed at optimizing the GvL effect, such as, whenever possible, a rapid tapering and discontinuation of GVH-D prophylaxis after transplantation, are recommended in children with JMML.

Pediatric Lymphomas

Given the excellent outcomes achieved with current risk-adapted first-line therapy for both pediatric Hodgkin lymphoma (HL) and non-Hodgkin lymphomas (NHLs), there is no indication for HSCT during frontline treatment for either of these entities [46]. However, primary refractory disease or relapse can occur in up to 10–15% of children, for whom a dismal prognosis has been reported [47]. For those patients, both autologous and allogeneic HSCT have become part of salvage therapy strategies.

Data on children with lymphoma treated with high-dose chemotherapy followed by autologous stem cell rescue, as well as data on allo-HSCT, are limited to small case series, with heterogeneous pre-transplant chemotherapy and conditioning regimens. Historically, auto-HSCT has been preferred to allo-HSCT because of easier stem cell availability and a lower rate of TRM [46]. In a large EBMT registry-based analysis, including both pediatric and adult patients, and comparing allo-HSCT with auto-HSCT, the advantage of allo-HSCT, in terms of disease recurrence, was counterbalanced by a high incidence of treatment-associated complications,

resulting in a higher OS after auto-HSCT [48]. Nonetheless, with recent advances in allo-HSCT techniques (including high-resolution HLA-typing, improvements in supportive care, and the implementation of less toxic conditioning regimens), this approach is being increasingly used in children with lymphomas.

Hodgkin Lymphoma

In adult patients, high-dose chemotherapy followed by the infusion of autologous HSCs has been shown to be superior to chemotherapy alone in randomized controlled trials including relapsed and primary refractory HL [49]. The improvement in progression-free survival (PFS) was particularly evident in patients with disease recurrence within 1 year after the end of treatment (41% for auto-HSCT vs. 12% for chemotherapy alone, $p = 0.008$), but was still significant for patients with later relapse (75% vs. 44%, $p = 0.025$). Based on these results, auto-HSCT has also been increasingly used as salvage therapy in children with poor-risk features. Indeed, even among patients with HR HL with a first relapse, salvage therapies including auto-HSCT can result in long-term cure in approximately 50% of cases [50].

In a recent retrospective analysis from the Center for International Blood and Marrow Transplant Research (CIMBTR) on 606 Childhood, Adolescent and Young Adult (CAYA) patients, performance status at the time of HSCT, no extranodal involvement, and chemosensitivity were associated with a significantly improved PFS, while patients with time from diagnosis to first relapse shorter than 1 year had a significantly inferior PFS [51].

Due to a reported higher rate of TRM in allo-HSCT than in auto-HSCT [48], the role of allo-HSCT in HL is still controversial, both in adults and the CAYA population. However, a meta-analysis showed a reduced (up to 5–10% lower) non-relapse mortality (NRM) with increased PFS and OS (up to 15–20% higher) in recent studies (i.e., those starting accrual in 2000 or later) [52]. The largest study reporting data for children and adolescents given allo-HSCT showed an NRM of 21%, with comparable results after reduced-intensity conditioning (RIC) or a myeloablative conditioning (MAC) regimen [53]. Relapse incidence was increased after RIC compared with MAC, thus resulting in a better PFS for patients given MAC (40 vs. 30%, $p = 0.02$). Of note, while no difference in outcome was observed between MFD and MUD-HSCT, the use of mismatched donors significantly reduced PFS after HSCT. Unmanipulated haploidentical BM transplantation with post-transplantation cyclophosphamide showed good results in patients with advanced HL [54].

Non-Hodgkin Lymphoma (NHL)

In children and adolescents the four most frequent subtypes of NHL are Burkitt (BL), lymphoblastic (LBL), diffuse large B cell (DLBCL), and anaplastic large cell lymphoma (ALCL). Despite very good results obtained with first-line therapies, with long-term EFS up to 90%, depending on histological subtype [55], the

prognosis of relapsed or refractory NHL is dismal, with the only exception being ALCL [56]. In adults, auto-HSCT has been proven to be superior to chemotherapy alone for the treatment of relapsed NHL [57], but no clear indications exist for selecting autologous or allogeneic HSCT.

The role of auto- and allo-HSCT is also still unclear in children with NHL. A recent registry-based study examined the role of HSCT in 182 patients affected by BL, LBL, DLBCL, and ALCL, given autologous (N = 90) or allogeneic HSCT (N = 92) from an MFD (N = 43) or a MUD (N = 49) [58]. After adjusting for disease status, no difference in 5-year EFS was observed between allo- and auto-HSCT for BL, DLBCL, or ALCL, while the outcome of relapsed/refractory LBL was superior after allo-HSCT [58].

A promising approach is the combination of MAC auto-HSCT, followed by a RIC allo-HSCT, which has been reported to allow a 10-year EFS of 70% [59].

Some children with NHL have a pre-existing condition predisposing to lymphoma (e.g., cancer predisposition syndromes or primary immune deficiencies). Because these patients suffer from increased treatment-related toxicities (leading to an inferior survival rate), special vigilance should be exerted when they are receiving chemotherapy or undergoing auto- or allo-HSCT [60].

Non-malignant Disorders

Primary Immune Deficiencies

Since the first successful attempt was made to cure primary immune deficiencies (PIDs) with HSCT, many significant changes have been made in transplant indications and techniques for these disorders. While the management of some PIDs is still based on conservative approaches, for other disorders HSCT is now becoming a widely accepted treatment strategy.

Taking into consideration the wide clinical heterogeneity of patients, the consensus of the EBMT and the European Society for Immunodeficiencies (ESID) is that each case should be carefully evaluated for indications and transplant strategy, in a center with significant experience [61].

Apart from severe combined immune deficiencies (SCIDs), for which there is a clear recommendation for HSCT [62], transplantat indications for non-SCID PIDs are being debated. Among the non-SCID PIDs, successful HSCTs have been performed in Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), hemophagocytic syndromes (such as hemophagocytic lymphohistiocytosis [HLH] and X-linked lymphoproliferative syndromes [XLP1 and XLP2], CD40-ligand deficiency, DNA repair disorders (such as ligase 4 deficiency, Cernunnos syndrome, and Nijmegen breakage syndrome), DOCK8 deficiency, and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome [63].

Until recent years, the availability of an HLA-identical related donor was one of the main factors influencing the choice of transplantation in PIDs. However, the introduction of high-resolution molecular HLA-typing [64], together with

the optimization of graft manipulation techniques, has broadened transplant access for these disorders [65]. Prognosis after HSCT for PIDs (influencing the decision to offer a transplantation) is dependent on the molecular defect at the basis of the disorder, disease status, donor type, HSC source, and the conditioning regimen [66].

Further, the increasing interest in gene therapy for the cure of PIDs is likely to render the therapeutic decision-making process and the definition of clear indications for HSCT more complex in coming years.

Hemoglobinopathies Disorders

The past 30 years have witnessed significant advances in supportive care and interventional therapies for thalassemia major (TM) and sickle cell disease (SCD). This has led to improved quality of life and survival rates for TM and SCD patients in many high-income countries, but has simultaneously brought about new medical needs associated with the progressive development of chronic disease and/or treatment-related complications. Conversely, in developing countries these disorders still represent a relevant cause of childhood mortality.

While the recent advances in gene-therapy approaches are likely to allow the forthcoming translation of promising preclinical and clinical evidence into a viable reality, at present allogeneic HSCT is the only consolidated possibility of definitive cure for hemoglobinopathies.

The widest experience of HSCT in these diseases has been obtained using BM cells harvested from an HLA-identical sibling donor. In this setting, major recently published studies report OS and DFS probabilities of over 90% and 85%, respectively, for TM, and more than 90% and 80%, respectively, for SCD [67, 68].

In 2014, a consensus document with recommendations on current HSCT strategies for TM and SCD was published by an expert panel selected by the EBMT Paediatric Diseases Working Party and Inborn Error Working Party [69].

Thalassemia Major

As indicated by the Pesaro experience [70], the disease status at the time of transplantation, and thus the timing of HSCT, appear to be critical to outcome in TM. Indeed, the identification and the adoption, in clinical practice, of three risk classes identified on the basis of three criteria, namely, hepatomegaly, liver fibrosis, and regularity of iron chelation, have been shown to influence HSCT outcomes. [70].

The EBMT recently reported data from a retrospective study of 1493 TM patients given allo-HSCT, with the best results observed in recipients of MFD-HSCT, in whom 2-year OS and EFS probabilities were 91% and 83%, respectively, while the 2-year estimates of both OS and EFS in the MUD-HSCT subgroup were 77%. A significant threshold age of 14 years for optimal results was identified [71].

Based on these considerations, TM children with a suitable, unaffected, HLA-identical sibling should be offered HSCT at an early disease stage, before the development of treatment-related complications and/or tissue damage associated with iron overload. Unfortunately, for the majority of patients, a suitable MFD is not available, leading to the need for alternative transplantation strategies.

Thanks to the dramatic advances achieved in the field of allogeneic HSCT, outcomes after MUD-HSCT in TM now approach those obtained in the MFD setting, provided that the donor selection is performed using high-resolution molecular typing for HLA class I and II *loci* and according to strict criteria of donor/recipient compatibility (i.e., full match or single allelic disparity for HLA-A, B, C, DRB1, and DQB1 *loci*). Moreover, a significantly increased risk of graft rejection has been described in the presence of non-permissive HLA-DPB1 mismatches in the host-versus-graft (HvG) direction, with a lower probability of DFS in patients given HSCT from donors with at least one HLA-DPB1 non-permissive disparity [72].

Unrelated UCBT holds the potential to broaden the access to HSCT to patients lacking an MFD or MUD, and this procedure appears appealing in non-malignant diseases by virtue of a suggested lower risk of GVH-D. Nevertheless, discordant results have been reported in the experience with unrelated UCBT in TM, with high rates of graft failure, largely attributable to low HSC content in cord blood units (CBUs) [73]. Based on currently available experience, unrelated UCBT appears to be a suboptimal strategy in TM, unless it is performed in the context of clinical trials aimed at exploring specific treatment platforms of ex-vivo UCB graft manipulation.

Although experience with haploidentical HSCT in children with TM is limited and this type of allograft is not routinely recommended, currently explored platforms hold the potential to extend the access to HSCT to the proportion of TM patients lacking both an HLA-matched related and unrelated donor [65, 74].

Sickle Cell Disease

While transfusion dependency is currently considered an indication for HSCT in TM, a general agreement on indications and timing for HSCT in SCD is less defined.

Indications for allogeneic HSCT in SCD include: (1) stroke or central nervous system event lasting longer than 24 h, acute chest syndrome with recurrent hospitalizations or previous exchange transfusions; (2) recurrent vaso-occlusive pain (more than two episodes per year over several years) or recurrent priapism; (3) impaired neuropsychological function with abnormal cerebral magnetic resonance imaging (MRI) scan; (4) stage I or II sickle lung disease; (5) sickle nephropathy (moderate or severe proteinuria or a glomerular filtration rate 30–50% of the predicted normal value); (6) bilateral proliferative retinopathy with major visual impairment in at least one eye; (7) osteonecrosis of multiple joints; and (8) red-cell alloimmunization during long-term transfusion therapy [69].

More recently, additional risk factors have been suggested and these are being considered in the evaluation of the risk/benefit ratio for transplantation in SCD (see also Table 6.6) [75].

Table 6.6 Indications for HSCT in SCD balanced with donor availability

HLA-identical donor (BM or CB)	HLA-matched-unrelated donor or unrelated CB	HLA-haploidentical donor
– Stroke	– Recurrent stroke	– Recurrent stroke despite adequate chronic transfusion therapy and/or hydroxyurea
– Elevated TCD velocity	– Elevated TCD velocity/ worsening cerebral vasculopathy	– RBC alloimmunization in patients with indication for chronic transfusion therapy
– Recurrent acute chest syndrome	– Recurrent acute chest syndrome despite supportive care	
– Recurrent VOC	– Recurrent VOC despite supportive care	– Pulmonary hypertension
– Recurrent splenic sequestration	– RBC alloimmunization in patients with indication for chronic transfusion therapy	– Inability to tolerate supportive care though strongly indicated, e.g., RBC alloimmunization, severe VOC, and inability to tolerate hydroxyurea
– Pulmonary hypertension/ tricuspid regurgitation jet velocity > 2.5 m/s	– Pulmonary hypertension	
– Osteonecrosis/AVN		
– RBC alloimmunization		
– Silent stroke with cognitive impairment		
– Recurrent priapism		
– Sickle nephropathy		

BM bone marrow, *CB* cord blood, *TCD* transcranial Doppler, *VOC* veno-occlusive crisis, *AVN* avascular necrosis, *RBC* red blood cell, *SCD* sickle cell disease

A further aspect to mention is that donor-host hematopoietic mixed chimerism after HSCT is not a rare finding in patients with hemoglobinopathies. As documented for both TM and SCD, the development of stable mixed chimerism in non-malignant disorders maintains the potential to correct the phenotypic expression of the disease [76]. This observation has provided a rational basis for considering RIC regimens in patients with hemoglobinopathies, with the aim of promoting stable engraftment of at least a threshold fraction of donor cells, sufficient to correct the abnormal hemoglobin phenotype, while reducing toxicity.

Acquired Severe Aplastic Anemia

Acquired aplastic anemia is a disorder characterized by BM failure and peripheral blood pancytopenia, assumed to result from an immune-mediated destructive mechanism that may be triggered by environmental exposures. First-line allo-HSCT is

considered the treatment of choice if an HLA-identical sibling donor is available. For patients lacking an MFD, IST consisting of ATG, cyclosporine, and steroids is employed as frontline treatment strategy.

HSCT from a well-matched unrelated donor is currently considered a rescue option for children who have failed IST, with OS and EFS approaching 80% and 70%, respectively [77].

The results of two recently reported retrospective studies suggest a potential benefit also of upfront HSCT from unrelated donors in children affected by severe aplastic anemia (SAA) [78, 79]. In the first analysis of 29 children given frontline MUD-HSCT, outcomes were similar to those observed in a historical control group given MFD-HSCT (2-year OS: 96% in the upfront MUD-HSCT group and 91% in the MFD-HSCT group, $P = 0.30$; 2-year EFS 92% in the upfront MUD-HSCT group and 87% in the MFD-HSCT group, $P = 0.20$) and superior to IST (OS 94%, $P = 0.68$; EFS 40%, $P = 0.0001$) and MUD-HSCT post-IST failure (OS 74%, $P = 0.02$; EFS 74%, $P = 0.02$). Similar outcomes were reported in 42 children and adolescents (estimated failure-free survival rate of the frontline HSCT group 91.3% vs. 30.7% in the frontline IST group, $P < 0.001$).

Alternative options, such as UCBT [80] or haploidentical HSCT [81], may be considered in patients lacking a matched related or unrelated donor and failing IST.

Constitutional Bone Marrow Failure Syndromes

Fanconi Anemia

Fanconi anemia (FA) is a genetically and phenotypically heterogeneous disorder, variably characterized by congenital somatic abnormalities, BM failure, and predisposition to clonal disorders. HSCT currently represents the only possibility of cure, having the potential to correct the hematologic manifestations associated with FA, as well as to prevent/treat myeloid malignancies. Due to the peculiar chromosome fragility and hypersensitivity to DNA interstrand cross-linking agents that characterize this disorder, conditioning regimens that are specifically developed for FA patients are employed.

In the therapeutic decision-making process for patients with FA, multiple factors should be taken into consideration. Indeed, the risk of developing BM failure and hematologic malignancies increases with age, and a variety of factors, such as the recipient's age, extent of prior treatments, and disease stage have been shown to negatively affect the outcome of HSCT [82].

Commonly accepted absolute indications for HSCT are severe BM failure with transfusion dependence, and clonal evolution to HR MDS (i.e., RCC with HR chromosomal abnormalities or advanced MDS) or AML. Relative indications that may lead to the choice of transplantation in the presence of an MFD can also be moderate isolated cytopenias with evidence of progression towards transfusion dependence and low-risk MDS (i.e., RCC with no chromosomal abnormalities or low-risk chromosomal abnormalities).

Dyskeratosis Congenita

Dyskeratosis congenita (DC) is an inherited disorder characterized by mucocutaneous abnormalities, BM failure, and predisposition to cancer, resulting from mutations in genes involved in telomere maintenance.

HSCT represents the only chance of definitive cure for the hematologic abnormalities associated with DC, but it is, unfortunately, associated with significant early and late morbidity. As in FA, due to the underlying defect in genome maintenance, RIC protocols are required for DC. Transplantation should be performed at centers experienced in treating DC, considering the risk of graft failure and early mortality, as well as long-term complications such as diffuse vasculitis and lung fibrosis.

Diamond-Blackfan Anemia

Diamond-Blackfan anemia (DBA) is a disorder associated with mutations in genes that encode for ribosomal proteins, clinically characterized by hypo-regenerative anemia with absent or decreased BM erythroid precursors, which may be associated with somatic abnormalities. Conservative therapy in DBA includes chronic transfusions and corticosteroids. HSCT may be offered to patients who develop transfusion-dependence or other cytopenias.

Data from the Diamond-Blackfan Anemia Registry of North America and AIEOP indicate OS probabilities of 72–74% after MFD- or MUD-HSCT, and 17% after HSCT from alternative donors [83, 84].

Considering the incomplete penetrance of DBA, disease-causing mutations may be present in subjects without an evident DBA phenotype, rendering genetic analysis of any potential related donor mandatory.

Severe Congenital Neutropenias and Inherited Thrombocytopenias

The category of severe congenital neutropenias includes a variety of hematologic disorders characterized by severe neutropenia, with a high risk of developing severe and life-threatening bacterial infections from early infancy.

More than 90% of patients respond to treatment with recombinant human (rHu) granulocyte colony-stimulating factor (G-CSF), obtaining neutrophil counts higher than 1.0×10^9/L. Allogeneic HSCT remains the only currently available treatment for patients with severe congenital neutropenia (Kostmann disease) refractory to rHuG-CSF or those who develop clonal evolution into MDS or leukemia [85].

HSCT also represents the only possibility of cure in congenital amegakaryocytic thrombocytopenia (CAMT), an autosomal recessive disorder caused by mutations of the gene encoding for the thrombopoietin (TPO) receptor (c-MPL), clinically characterized by early-onset thrombocytopenia (at birth) with reduced or absent BM megakaryocytes, and eventual progression to BM failure [86].

Inborn Errors of Metabolism

Inborn errors of metabolism (IEMs) are disorders derived from the deficiency of enzymes that play a key role in metabolic pathways. The consequent progressive accumulation of toxic metabolites within different cells/tissues leads to multisystemic impairment. The observation that the enzymatic activity in deficient cells could be restored by mixing, in culture, normal cells and fibroblasts derived from patients affected by mucopolysaccharidoses, led to the first attempts at HSCT in this kind of IEM. Moreover, unlike in enzyme-replacement therapy, donor-derived monocytes are able to cross the blood-brain barrier, thus alleviating/arresting central nervous system damage.

To date, more than 2000 transplants have been performed worldwide in patients with IEMS, with results showing that not all IEMs can benefit from HSCT [87]. A possible partial explanation for this observation could lie in the fact that HSCT seems to induce a response only in some tissues, probably due to the suboptimal delivery of the target enzyme in non-responder tissues. In IEMs, the timing of transplantation appears to be critical for outcome, as late HSCT may be ineffective in preventing disease progression [88]. In particular, for patients who have already developed central nervous system involvement or those with advanced disease, HSCT is contra-indicated. The use of donors who carry the enzymatic defect is not recommended, because the delivery of the target enzyme in recipient tissues is suboptimal. The full-donor chimerism rate was found to be significantly higher in recipients of UCBT as compared with patients receiving either BM or peripheral blood transplantation.

Solid Tumors

Because of continuous improvements in multimodal therapy and supportive care, the outcomes of children with solid tumors have constantly improved in the past few decades. However, some of these tumors, although initially chemosensitive, have a dismal prognosis. Against this background, both auto- and allo-HSCT have been employed for the treatment of HR solid tumors [89], with the former strategy being the most widely used (only 446 allogeneic transplant procedures were registered at the EBMT until 2011). However, with the exception of neuroblastoma (for which randomized trials have been conducted, showing a clear advantage of auto-HSCT versus sole chemotherapy) [90], prospective trials are lacking. From registry data, the following findings can be inferred [91]:

- Outcomes of HSCT performed during first-line treatment are significantly better than those observed after transplantation in relapsed patients.
- Patients with good response at the time of transplantation (i.e., complete response, very good partial response, and partial response) have, not surprisingly, an improved outcome when compared with those with an unsatisfactory response.

– Recent years have seen a trend towards a reduction of TRM.
– Peripheral blood autologous stem cells represent the currently most often used HSC source.
– Total body irradiation has shown no advantage for any of the solid tumor indications; however, busulfan coupled with melphalan increased survival in neuroblastoma and Ewing sarcoma.

Tumors for which there is a general indication for auto- or allo-HSCT are listed in Table 6.1.

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Chapter 7
Indications for Allogeneic Hematopoietic Stem Cell Transplantation in Adults

Narendranath Epperla, Mehdi Hamadani, and Mary M. Horowitz

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has evolved to become a frequently used and effective therapy for many malignant and non-malignant hematological disorders considered incurable with standard therapies [1]. Over the past two decades, there was a steady increase in the use of allogeneic HSCT and continued evolution in its technology. Despite the advent of novel agents and targeted therapies, allogeneic HSCT remains the only curative option for several life-threatening blood disorders.

Allogeneic HSCT involves multiple steps, starting with the administration of a conditioning regimen (chemotherapy and/or radiation). The purpose of the conditioning regimen generally is to provide varying degrees of disease control (depending on its intensity) and to eliminate host immune cells (capable of rejecting even human leucocyte antigen [HLA]–matched donor cells). The ability of the donor hematopoietic stem and progenitor cells (HSPCs) to restore hematopoiesis in the

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recipient permits the administration of substantially higher doses of cytotoxic therapy than would otherwise be possible in the higher intensity (myeloablative) allogeneic HSCT setting. Although originally regarded primarily as a way of rescuing patients from conditioning therapy-induced marrow aplasia, it is now well known that alloreactive donor immune cells contribute substantially to disease eradication, by exerting potent graft-versus-malignancy effects. The conditioning therapy is followed by the infusion of donor HSPCs. This is generally (but not always) followed by prolonged (several months) therapy with immune suppressive agents to prevent (or treat) graft-versus-host disease (GVH-D) and prophylactic antibiotics to reduce the risk of infectious complications.

Disease-Specific Indications for Allogeneic Transplantation

The indications for allogeneic transplantation can be subdivided into two broad groups – malignant and non-malignant hematological conditions (Figs. 7.1 and 7.2).

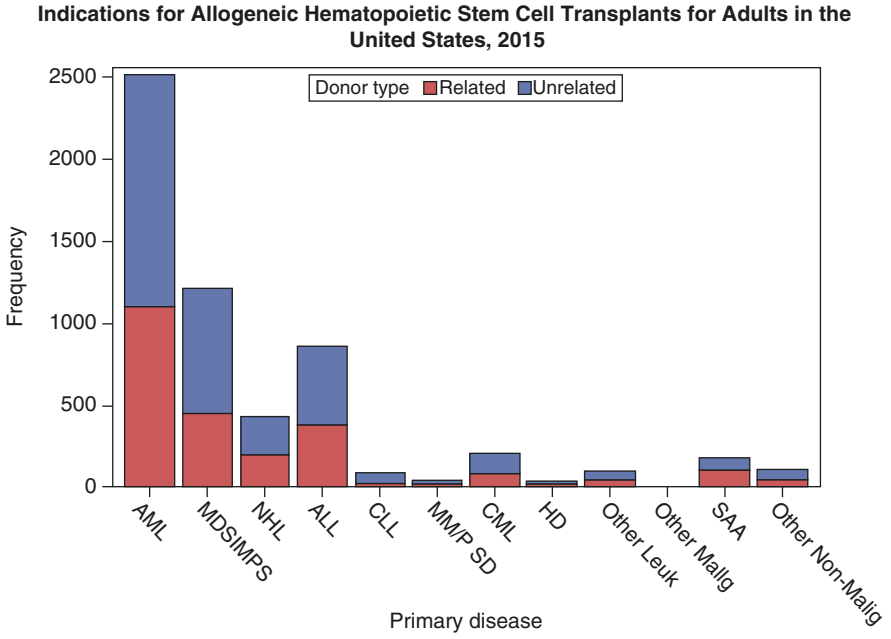

Fig. 7.1 Indications for allogeneic hematopoietic stem cell transplantation in adults (United States data reported to Center for International Blood and Marrow Transplant Research [CIBMTR]), 2015

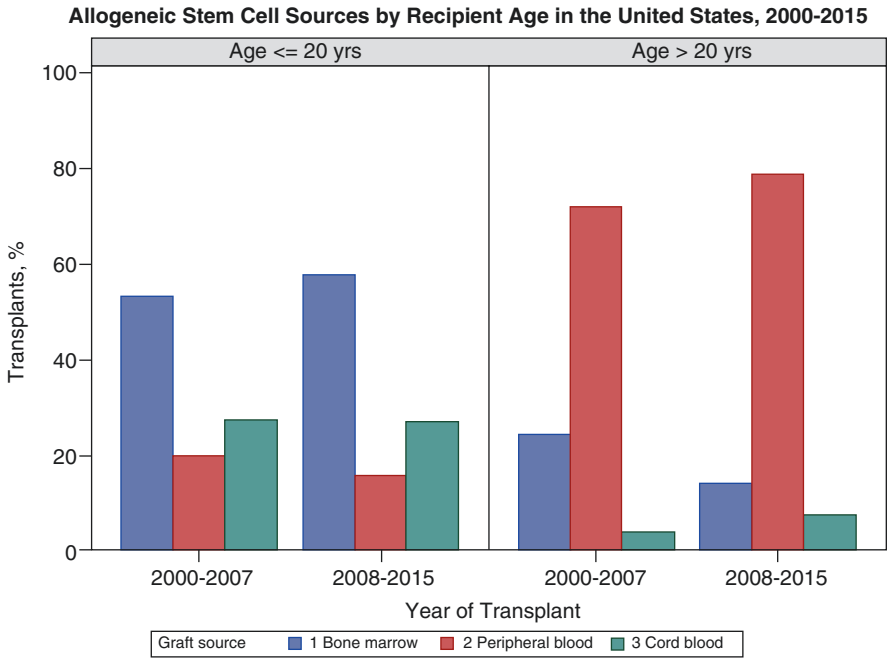

Fig. 7.2 Allogeneic hematopoietic stem cell transplantation (HSCT) graft sources, by donor type vs. donor age, 2000–2015

Malignant Hematological Conditions

Leukemias

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) represents a heterogeneous group of high-grade myeloid neoplasms with variable outcomes. Though remission induction is an important first step in the management of AML, additional treatment strategies are essential to ensure long-term disease-free survival (DFS). Allogeneic HSCT represents an effective anti-leukemia therapy in AML, providing the possibility of cure with potent graft-versus-leukemia effects.

AML can be stratified into good- or favorable-risk [(t(8;21) and inv.(16)/t(16;16)], intermediate-risk [cytogenetically normal-AML with *NPM1* mutated/FLT3-ITD-positive, *NPM1* wild-type/FLT3-ITD-negative, t(8;21)/inv. (16) with c-KIT mutation and all other abnormalities not classified as favorable or adverse risk] or poor-risk [abnormalities of chromosome 3q (abnl 3q), deletions of 5q (−5q), monosomies of chromosome 5 or 7 (−5/−7), complex karyotype and monosomal karyotype] [2].

Two separate meta-analyses (the Dutch-Belgian Haemato-Oncology Co-operative Group [HOVON] and the Swiss Group for Clinical Cancer Research [SAKK group] and Koreth et al.) concluded that overall survival (OS) was improved following allogeneic HSCT compared with chemotherapy in AML, and that this advantage was most obvious for patients with high- or intermediate-risk cytogenetics and was not present in patients with favorable-risk disease [3, 4]. Thus, for medically fit AML patients in first complete response (CR1) with poor- and intermediate-risk cytogenetics, allogeneic HSCT should be considered a standard option. Matched related donor (MRD) allogeneic HSCT is preferred, but in the absence of a matched sibling, it is certainly reasonable to consider matched unrelated donor (MUD) HSCT [5–8]. The German-Austrian Acute Myeloid Leukemia Study Group showed that transplantation might have an important role in a molecular subset of patients with cytogenetically normal AML. Patients with FLT-3 ITD mutation or those without FLT-3 ITD but with wild-type NPM1 and CEBPA derived benefit from an allogeneic HSCT performed in CR1. However, there was no benefit of allogeneic HSCT in patients with NPM1 mutation without FLT-3 ITD [7]. In double-mutant CEBPA, allogeneic HSCT in CR1 improved DFS without impacting OS [9].

AML is a disease of older adults, with a median age of 70 years. Yet most prospective, randomized studies on allogeneic HSCT have excluded patients older than 60 years. Moreover, AML in this age group has an increased probability of poor prognosis, because the cytogenetic profile is more often unfavorable and the AML is more likely to have evolved from previous myelodysplastic syndrome (MDS) or is therapy related. The Center for International Blood and Marrow Transplant Research (CIBMTR) analyzed the outcome of 545 patients with AML transplanted in CR1 between 1995 and 2005 and found no effect of age on relapse, non-relapse mortality (NRM), DFS, or OS [10]. The outcome of 94 patients aged 60–70 years with AML receiving reduced-intensity conditioning (RIC) allogeneic HSCT in CR1 (as reported by the CIBMTR) was compared with the outcome in 96 matched patients uniformly treated with chemotherapy protocols of the Cancer and Leukemia Group B (Table 7.1 summarizes large registry studies comparing RIC against myeloablative HSCT). Allogeneic HSCT was associated with a significantly lower risk of relapse, higher NRM, and longer leukemia-free survival at 3 years. Although the OS was better for HSCT recipients, the difference was not statistically significant [11]. The good outcomes with allogeneic HSCT in older patients were recently validated in a multicenter prospective trial [12].

Allogeneic HSCT, including carefully planned alternative donor allografts, is the preferred option for most medically fit patients with AML in CR2 [13]. Allogeneic HSCT offers the best prospect of long-term DFS for patients with relapsed/refractory AML beyond CR2 [14, 15]. A myeloablative conditioning (MAC) regimen is the standard-of-care for AML patients who are able to receive it. The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0901 trial randomized patients with AML/MDS to allogeneic HSCT with RIC versus MAC regimens. The trial was suspended after enrolling 272 out of the planned 356 subjects when early results indicated significantly reduced relapse risk in favor of MAC regimens [16]. However, RIC transplants remain reasonable for those not eligible for MAC.

Table 7.1 Retrospective registry-based comparisons of NMA/RIC vs. myeloablative allogeneic HSCT

Group	Disease	Donor	*N*RIC vs. MAC	NRMRIC vs. MAC	Relapse	Comments
EBMT [82]	MDS or sAML >50	MUD 39%	315 vs. 407	32% vs. 44% at 4 years	41% vs. 33% at 4 years	Survival 31% at 4 years. RIC predicted for greater relapse but lower NRM in multivariate model. Wide variety of different conditioning regimens used
EBMT [83]	MM	MUD 12%	320 vs. 196	24% vs. 37% at 2 years	27% vs. 54%	NRM lower after RIC but relapse risk is double
EBMT [84]	AML	Sibling	215 vs. 621	22% vs. 32% at 3 years	45% vs. 27% at 3 years	Relapse rate higher and NRM lower in RIC but OS similar (41% vs. 45%) in both groups
EBMT [85]	CLL	MUD 22%	73 vs. 82	19% vs. 26%	28% vs. 11%	Similar NRM but higher relapse risk after RIC
EBMT [86]	HL	MUD 13%	89 vs. 79	23% vs. 46% at 1 year	57% vs. 30%	Relapse rate higher and NRM lower in RIC, but OS similar
CIBMTR [87]	Follicular NHL	Sibling	88 vs. 120	23% in both at 1 year	17% vs. 8%	RIC associated with higher risk of relapse, but similar NRM, while lower KPS impacted on NRM. OS was similar
EBMT [88]	ALL	Sibling	127 vs. 449	21% vs. 29%	32% vs. 38%	NRM lower after RIC, but higher relapse rate. Leukemia-free survival similar to MAC
CIBMTR [89]	AML/MDS	MUD/sibling	1448 vs. 3731	3-Year NRM similar	Lower risk of relapse in myeloablative group	Overall and disease-free survival was highest for myeloablative group

ALL acute lymphoblastic leukemia, *AML acute myeloid leukemia, CIBMTR* Center for International Blood and Marrow Transplant Research, *CLL* chronic lymphocytic leukemia, *EBMT* European Group for Blood and Marrow Transplantation, *HL* Hodgkin lymphoma, *HCST* hematopoietic stem cell transplantation, *KPS* Karnofsky performance score, *MAC* myeloablative conditioning, *MDS* myelodysplastic syndrome, *MM* multiple myeloma, *MUD* matched unrelated donor, *NMA* non-myeloablative, *NHL* non-Hodgkin lymphoma, *NRM* non-relapse mortality, *OS* overall survival, *RIC* reduced-intensity conditioning, *sAML* severe AML, *CTCL* cutaneous T-cell lymphoma

Assessment of minimal residual disease at different time points is another area of active research. Retrospective data suggest the best post-transplant outcomes are in those AML patients without evidence of flow cytometrically detectable minimal residual disease [17].

Recommendations:

1. AML in CR1: The standard option for poor-risk cytogenetic patients and intermediate-risk patients other than NPM1 + ve/FLT3-ITD-negative patients. Not recommended for favorable-risk patients. Allogeneic transplant should be strongly considered for all intermediate- and high-risk medically fit patients older than 60 years of age (Table 7.2).
2. AML in CR2 and beyond: Allogeneic HSCT is the preferred option.
3. Primary refractory AML: Allogeneic HSCT represents the only curative option for these patients. MAC transplants can be considered in fit patients with active disease at the time of transplantation.
4. Therapy-related AML or AML with MDS-related changes: Strongly consider in all medically fit patients.

Myelodysplastic Syndrome

Allogeneic HSCT is a curative option for adult patients with MDS and outcomes are better if the transplantation is performed before progression to AML. National Comprehensive Cancer Network (NCCN) guidelines recommend consideration of allogeneic HSCT early for high-risk MDS (i.e., intermediate-2/high-risk International Prognostic Scoring System [IPSS]) with MRD or MUD HSCT based on CIBMTR data, derived from MAC transplantation [18]. For patients who are ≥60 years old with intermediate-2/high-risk IPSS, early allogeneic HSCT with RIC is preferred [19]. An evidence-based policy statement from the American Society for Blood and Marrow Transplantation (ASBMT) also recommended early allogeneic HSCT in MDS patients with intermediate-2/high-risk IPSS [20]. Hypomethylating agents may be used as a bridge to transplant while awaiting donor availability, but these agents should not be used to delay HSCT. Chronic GVH-D and relapse are still the major challenges after HSCT. There is an ongoing BMT CTN trial (BMT CTN 1101) that is designed to confirm the benefit of allogeneic HSCT in high-risk patients by using a genetic randomization design. Results from this key trial will carry important ramifications for continued insurance coverage for this procedure in MDS patients in the United States.

Recommendations

Allogeneic HSCT is the standard of care for intermediate-2 or high-risk MDS with MRD or MUD HSCT (ideally before progression). There is a paucity of data regarding alternative donor transplantation in this setting (Table 7.2).

Table 7.2 Allogeneic HCST: Conditioning regimen, graft source, and donor type and timing of HSCT for specific disease states

Disease	Conditioning regimen	Graft source	Acceptable donor	Timing of allogeneic HSCT	Comments
Acute myeloid leukemia	MAC superior to RIC in younger, fit patients	BM preferred over PB (especially for standard-risk patients undergoing MUD ablative HSCT)	MRD > MUD (alternative source acceptable in patients without available adult donors)	– AML in CR1 with intermediate- or high-risk disease[a] – AML in CR2 and beyond – Primary refractory AML – Therapy-related AML or AML with MDS-related changes	RIC transplants remain a potential option for those not eligible for MAC
Myelodysplastic syndrome	MAC might be superior to RIC in younger, fit patients	BM preferred over PB (especially for standard-risk patients undergoing MUD ablative HSCT)	MRD or MUD Limited data for alternative donors	Early HCST in patients with intermediate-2 or high-risk MDS	RIC is preferred for patients who are ≥60 years old with intermediate 2/ high-risk IPSS
Acute lymphoblastic leukemia	MAC preferred in younger, fit patients	BM preferred over PB (especially for standard-risk patients undergoing MUD ablative HSCT)	MRD or MUD (alternative source acceptable in patients without available adult donors)	– ALL in CR1 with standard risk, young adult (<35 years) and high-risk disease (including Ph-positive ALL) – ALL in CR2	
Chronic myelogenous leukemia	MAC preferred in younger, fit patients	BM preferred over PB[b] (especially for standard-risk disease)	MRD or MUD	– CML in first or subsequent chronic phase patients who are either intolerant or refractory to two to three different TKIs – CML in blast phase	RIC may be an acceptable alternative to MAC in older patients (>50 years) or those with medical comorbidities

(continued)

Table 7.2 (continued)

Disease	Conditioning regimen	Graft source	Acceptable donor	Timing of allogeneic HSCT	Comments
Chronic lymphocytic leukemia	RIC preferred	PB	MRD or MUD (alternative source acceptable in patients without available adult donors)	– Standard-risk CLL failing at least two of the three novel agents (ibrutinib, idelalisib, venetoclax) – High-risk CLL after failing two lines of therapy and showing an objective response to BCR inhibitors or to a clinical trial – Transformation to a high-grade lymphoma after demonstrating response to anthracycline-based chemotherapy	
Myeloproliferative neoplasms (except CML)	Limited data to recommend conditioning intensity	PB favored especially in myelofibrosis	MRD or MUD Limited data with alternative donor sources	– Primary MF with intermediate-2 or high-risk DIPSS – Secondary MF – CMML with high-risk disease	RIC is the regimen of choice in patients who are older than 55 years.
Diffuse large B-cell lymphoma	RIC preferred	PB reasonable	MRD or MUD (alternative source acceptable in patients without available adult donors)	– DLBCL with relapse after autologous HCST – Subset of DLBCL with chemorefractory disease	
Follicular lymphoma	RIC preferred	PB reasonable	MRD or MUD (alternative source acceptable in patients without available adult donors)	– FL with relapse after autologous HSCT – Heavily pretreated FL (e.g., more three lines of prior therapies, bone marrow involvement, and/or refractory disease)	
Mantle cell lymphoma	RIC preferred	PB reasonable	MRD or MUD (alternative source acceptable in patients without available adult donors)	– MCL with relapse after autologous HSCT – MCL with chemotherapy-refractory disease	No data suggest clear benefit of MAC over RIC in patients with MCL

Mature T-cell or NK/T-cell lymphoma	RIC preferred	PB reasonable	MRD or MUD	– T-cell lymphoma with relapse after autologous HSCT	
			Limited data for alternative donor transplantation	– Relapsed or refractory T-cell lymphoma	
				– Progressive or refractory stage IIB-IV CTCL after failure of biologic agents and at least one line of chemotherapy	
Hodgkin lymphoma	RIC preferred	PB reasonable	MRD or MUD (alternative source acceptable in patients without available adult donors)	Relapsed or progressive Hodgkin disease patients (who have failed autologous HSCT and therapy with at least brentuximab vedotin or nivolumab)	
Multiple myeloma	RIC preferred	PB reasonable	MRD or MUD	– In relapsed refractory disease in the setting of a clinical trial	Allogeneic HSCT with RIC needs to be considered only in the setting of a clinical trial, in the relapsed refractory setting
			Limited data available for alternative-donor transplantation	– In primary plasma cell leukemia	
Aplastic anemia	RIC preferred	BM preferred	MRD or MUD	– In newly diagnosed severe (acquired/ idiopathic) aplastic anemia in young patients	
				– Relapsed or refractory severe aplastic anemia	
				– Can be considered as a treatment option for *select* patients with Fanconi anemia and dyskeratosis congenita	

[a]Other than those who are positive for NPM1 mutation and with negative FLT3-ITD status

[b]For patients with accelerated or blast crisis, mobilized PBSC may be preferred due to the more robust graft-versus-tumor effects

BCR B-cell receptor, *RIC* reduced intensity conditioning, *MAC* myeloablative conditioning, *BM* bone marrow, *PB* peripheral blood, *MRD* matched related donor, *MUD* matched unrelated donor, *HSCT* hematopoietic stem cell transplantation, *AML* acute myelogenous leukemia, *MDS* myelodysplastic syndrome, *ALL* acute lymphoblastic leukemia, *CML* chronic myelogenous leukemia, *CLL* chronic lymphocytic leukemia, *MF* myelofibrosis, CMML chronic myelomonocytic leukemia, *CTCL…*, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *MCL* mantle cell lymphoma, CR complete response, *Ph* Philadelphia chromosome, *DIPSS* dynamic international prognostic scoring system, *IPSS* international prognostic scoring system, *TKI* tyrosine kinase inhibitor, *NK* natural killer, *PBSC* peripheral blood stem cell

Acute Lymphoblastic Leukemia

Clinically, acute lymphoblastic leukemia (ALL) patients are categorized into Philadelphia chromosome (Ph)-positive ALL and Ph-negative ALL, for management purposes. While Ph-positive ALL confers higher risk, patients with Ph-negative ALL who are older than 35 years or with an elevated white blood cell (WBC) count (>30 × 10^9/L for B-cell lineage; >100 × 10^9/L for T-cell lineage) at diagnosis, low hypodiploidy/near triploidy, t(11q23)/mixed lineage leukemia (MLL) rearrangements, and complex karyotype (≥5 chromosomal abnormalities) also fall into a high-risk group [21, 22].

Allogeneic HSCT in first remission from related or unrelated donors is generally accepted as the most effective available therapy for adult patients with Ph-positive ALL. For adults with Ph-negative ALL, the appropriate timing of HSCT is more controversial, despite the largest prospective study (Medical Research Council [MRC]/Eastern Cooperative Oncology Group [ECOG]) and a meta-analysis suggesting a survival advantage for those assigned to transplant in first CR [23, 24]. Allogeneic transplantation is currently considered by NCCN expert consensus as the best curative therapy for adult patients with high-risk features (as detailed earlier). An evidence-based policy statement from the ASBMT recommended allogeneic HSCT for standard-risk, young (<35 years) adults in first CR and high-risk ALL in first CR. Though no prospective data are available comparing RIC with MAC, CIBMTR data suggest that RIC is an acceptable option for patients who are not candidates for MAC [25]. MRD and MUD HSCT generally provide comparable outcomes [20, 26]. For those without suitable matched adult donors, alternative donor sources should be considered.

Recommendations

1. ALL in CR1: Allogeneic HSCT is indicated for standard-risk, young adult (<35 years), and high-risk ALL, including Ph-positive ALL (Table 7.2).
2. ALL in CR2: Allogeneic HSCT is the preferred option.
3. Post-transplant relapse is a major cause of treatment failure; novel therapies such as blinatumomab for Ph-negative disease and tyrosine kinase inhibitors (TKIs) for Ph-positive disease may improve outcome.
4. Chimeric antigen receptor T-cells directed against CD19 are a potentially exciting advance, especially as a bridge to transplant in patients with relapsed/refractory disease.

Chronic Myelogenous Leukemia

Before the advent of TKIs, allogeneic transplantation was considered a standard treatment option for all eligible patients early after diagnosis, in first chronic phase. After the remarkable success of imatinib (and subsequent-generation TKIs), allogeneic transplantation is now used only for more advanced stages of the disease. In the current

era, HSCT should be considered early for chronic myelogenous leukemia (CML) patients whose initial presentation is in blast phase. Early transplantation in CML patients presenting in accelerated phase, but with brisk and optimal response to TKIs, is controversial and cannot be considered as a standard therapy. In TKI-treated CML patients with a suboptimal response, relapse, or intolerance to two to three different TKIs, referral to a transplant center should be strongly considered. HSCT should also be considered for patients who have bcr-abl mutations that predict for non-response to all TKIs except ponatinib (e.g., T315I mutation). It is important to establish a monitoring plan for early signs of progression and for mutation screening, because outcomes are significantly better if HSCT is performed prior to transformation to advanced-phase disease. Definitions of suboptimal response and failure with TKIs and guidelines for monitoring have been developed [27]. European LeukemiaNet guidelines recently addressed the timing of allogeneic HSCT in CML [27]. Allogeneic HSCT was recommended in all CML patients presenting in blast phase, and for the accelerated-phase patients who do not achieve an optimal response. HSCT transplantation was also recommended for TKI-treated chronic-phase patients subsequently progressing to accelerated or blast phase, after achieving optimal disease control. For patients in chronic phase, the recommendation was to reserve allogeneic HSCT for those who are resistant or intolerant to at least one second-generation TKI, and for patients developing T315I mutation. MAC is the preferred regimen whenever possible. RIC may be an acceptable alternative to MAC in older patients or those with medical comorbidities [28].

Recommendations

1. CML in first or subsequent chronic phase: Allogeneic HSCT for patients who are either intolerant or refractory to two to three different TKIs. It is important to note that poor response to TKIs does not predict a poor response to HSCT (Table 7.2).
2. CML in second chronic phase: Allogeneic HSCT should be considered, especially in the subset achieving second chronic phase after entering into a blast- or accelerated-phase disease.
3. CML in blast phase: Allogeneic HSCT is the recommended option (standard of care).
4. In TKI-resistant mutations: Allogeneic HSCT is the recommended option.

Chronic Lymphocytic Leukemia

HSCT offers the only potentially curative approach to the treatment of chronic lymphocytic leukemia (CLL), but it is suitable only for a minority of patients and is associated with significant treatment-related mortality and morbidity. Before the approval of highly active agents for CLL (e.g., ibrutinib, idelalisib, venetoclax), the European Group for Blood and Marrow Transplantation (EBMT) consensus group [29] recommended allogeneic HSCT as a reasonable treatment option for younger patients with

17p deletion not achieving a CR with first-line therapy, 17p-deleted patients with relapsed disease, or in patients relapsing (within 12 months) after purine analogue therapy. Patients who relapse within 24 months after having a response to purine analogue-based combinations, patients with TP53 abnormalities, and those with Richter's transformation requiring treatment are also considered candidates for transplant evaluation. The ASBMT recently published updated guidelines regarding the role and timing of allogeneic HSCT in the era of highly effective novel agents. For high-risk CLL, allogeneic HSCT is recommended in patients relapsing after treatment with B-cell receptor (BCR) inhibitors or not responding to this treatment. While for standard-risk CLL, allogeneic HSCT should be deferred until the patient has shown treatment failure with at least two of the three currently available highly active novel therapy agents (ibrutinib, PI3K inhibitors, venetoclax). In patients with Richter transformation, allogeneic HSCT is recommended upon demonstration of an objective response to anthracycline-based chemotherapy. Filgrastim-mobilized peripheral blood is the preferred stem cell source and RIC is the recommended conditioning regimen whenever allogeneic HSCT is indicated in CLL [30].

Recommendations

1. Standard-risk CLL: Allogeneic HSCT is recommended in patients failing treatment with at least two of the three currently available novel agents (ibrutinib, idelalisib, venetoclax) (Table 7.2).
2. High-risk CLL: Allogeneic HSCT is recommended for patients who fail two lines of therapy but show an objective response to BCR inhibitors or BCL2 inhibitors or to a clinical trial.
3. Transformation to a high-grade lymphoma: Allogeneic HSCT is recommended after achieving an objective response to anthracycline-based chemotherapy.

Myeloproliferative Neoplasms (Other than CML)

The only curative option for patients with myeloproliferative neoplasms is allogeneic HSCT. In general, allogeneic HSCT is not indicated in polycythemia vera and essential thrombocythemia unless there is disease progression to myelofibrosis, MDS, or secondary leukemia [31]. Given the lack of alternative therapeutic options to reverse marrow fibrosis, allogeneic HSCT is a reasonable approach for primary myelofibrosis with intermediate-two and high-risk according to the Dynamic International Prognostic Scoring System (DIPSS). Myelofibrosis post-essential thrombocythemia or post-polycythemia vera should also be considered an indication for allogeneic HSCT for all patients less than 65 years of age [32]. The introduction of Janus kinase (JAK) inhibitors in the treatment of myelofibrosis may help to improve constitutional symptoms and to reduce spleen size before transplantation. An evidence-based policy statement from the

ASBMT recommended consideration of allogeneic HSCT for intermediate-/high-risk patients with primary myelofibrosis and secondary myelofibrosis [20]. The CIBMTR evaluated the outcomes of patients with myelofibrosis who received a MAC regimen and reported superior outcomes for MRD transplants [33]. In patients who are older than 55 years RIC is the regimen of choice [34].

Allogeneic HSCT is the only therapeutic modality that can substantially alter the natural history of chronic myelomonocytic leukemia (CMML), resulting in cure in a small proportion of patients. Patients with high-risk disease based on prognostic scoring systems (high Dusseldorf or CMML-specific prognostic scoring system [CPSS] score) should be offered allogeneic HSCT. Based on the data from a single-center retrospective study, the type of conditioning regimen does not seem to impact outcomes [35].

Recommendations

1. Primary myelofibrosis: Allogeneic HSCT is indicated in primary myelofibrosis, especially in the subset with intermediate-two or high-risk DIPSS (Table 7.2).
2. Secondary myelofibrosis: Allogeneic HSCT is indicated in all eligible patients with secondary myelofibrosis.
3. Chronic myelomonocytic leukemia: Allogeneic HSCT should be considered; especially in the subset with high-risk disease (high Dusseldorf or CPSS score).

Lymphomas

Diffuse Large B-Cell Lymphoma

Autologous transplantation is the treatment of choice for relapsed or refractory diffuse large B-cell lymphoma (DLBCL) that has demonstrated evidence of chemosensitivity to salvage therapies. Allogeneic HSCT is an option for DLBCL patients relapsing after an autologous HSCT (especially in those with good performance status, chemosensitive disease, and >1 year between autologous and allogeneic HSCT) [36]. CIBMTR data suggest that even a small subset of DLBCL patients who are refractory to chemotherapy can be salvaged by allogeneic HSCT [37]. There is no benefit of MAC over RIC in patients with DLBCL [36–38]. The role of allogeneic HSCT in biologically high-risk patients with DLBCL (e.g,. c-myc rearranged, double-hit, double-protein expressors) warrants investigation.

Recommendations

1. DLBCL with relapse after autologous HSCT: Allogeneic HSCT is indicated (Table 7.2).
2. Allogeneic HSCT can be considered in the subset of DLBCL with chemorefractory disease.

Follicular Lymphoma

The only prospective BMT CTN study comparing autologous vs. allogeneic HSCT in relapsed follicular lymphoma was terminated because of slow accrual [39]. A systematic review by the ASBMT noted the lack of high-quality evidence regarding indications for allogeneic HSCT in follicular lymphoma [20, 40]. The consensus panel recommended autologous HSCT for relapsed disease or transformed follicular lymphoma. In the allogeneic setting, RIC was considered an acceptable alternative to MAC regimens. The panel found no differences in outcomes between HLA-identical sibling donors and MUDs. Given the efficacy of rituximab-based salvage treatments and autologous HSCT for follicular lymphoma, allogeneic HSCT is generally used for patients who have failed, are likely to fail, or are unable to proceed to salvage autologous HSCT. However, late application of allogeneic HSCT may be less effective, especially for chemotherapy-refractory disease. The NCCN guideline panel recommended autologous transplantation in second or third remission as a standard consolidative strategy for relapsed follicular lymphoma [41]. Recent CIBMTR data suggest that autologous and allogeneic HSCT, when applied as the first HSCT approach, provide comparable outcomes in follicular lymphoma; however, risk of relapse is substantially lower, and NRM significantly higher after allogeneic HSCT. Among patients who survived for 2 years, those receiving allogeneic HSCT were more likely to remain alive and disease-free [42, 43]. CIBMTR data suggest no clear benefit of allogeneic HSCT over autologous HSCT in follicular lymphoma undergoing transformation to DLBCL [44].

Recommendations

1. Follicular lymphoma with relapse after autologous HSCT: Allogeneic HSCT is indicated (Table 7.2).
2. Allogeneic HSCT might be the preferred option for heavily pretreated follicular lymphoma (e.g., more than three lines of prior therapies, bone marrow involvement, and/or refractory disease).

Mantle Cell Lymphoma

NCCN guidelines recommend autologous transplantation as an adjunct to initial therapy in eligible patients [41]. No prospective studies have evaluated allogeneic HSCT in relapsed/refractory mantle cell lymphoma (MCL) and there are no data supporting its role as upfront consolidation in chemotherapy-sensitive disease. However, given the poor prognosis of recurrent MCL and the curative potential of allogeneic HSCT, it is a reasonable option for patients relapsing after upfront autologous transplantation or for those with chemotherapy-refractory disease [45, 46]. RIC regimens are reasonable in patients with MCL.

Recommendations

1. MCL with relapse after autologous HSCT: Allogeneic HSCT is indicated (Table 7.2).
2. MCL with chemotherapy-refractory disease: A subset (25%) of patients can attain durable remission after allogeneic HSCT.

Mature T-Cell and Natural Killer (NK)/T-Cell Lymphomas

In mature T-cell and NK/T-cell lymphomas, patients with chemosensitive disease after first-line therapy, consolidation with autologous or allogeneic HSCT remains a matter of debate. A recent small German study randomizing peripheral T-cell lymphoma (PTCL) patients to autologous vs. allogeneic HSCT after first-line therapy had to be prematurely closed because of an interim analysis suggesting a low probability that the primary endpoint (25% event-free survival improvement by allogeneic HSCT) could still be met [47]. Allogeneic HSCT consolidation in T-cell non-Hodgkin lymphoma (NHL) in remission after frontline therapies is controversial. NCCN guidelines recommend consideration of allogeneic HSCT in the relapsed or refractory T-cell lymphomas [41]. The European Society for Medical Oncology (ESMO) also recommends allogeneic HSCT in the relapsed or refractory T-cell lymphomas (T-cell lymphoma – NOS, ALK-negative anaplastic large cell lymphoma, and angioimmunoblastic lymphoma). There is no difference in outcomes based on donor type (MRD vs. MUD) or regimen intensity (MAC vs. RIC) [48]. Unpublished registry observations suggest a possible role for allogeneic HSCT in first remission in a subset of very-high-risk mature T-cell or NK/T-cell lymphomas (e.g., advanced extranodal NK/T-cell lymphoma, nasal type, aggressive NK-cell leukemia, high-risk angioimmunoblastic lymphoma, hepatosplenic gamma/delta T-cell lymphoma).

The prognosis is dismal in patients with stages IIB to IV mycosis fungoides and Sézary syndrome with conventional therapy [49]. Though autologous HSCT can induce remissions, the responses are short-lived. On the other hand, allogeneic HSCT can offer these patients a potentially curative option (although there are no well-designed comparative trials in this setting) [50].

Recommendations

1. T-cell lymphoma with relapse after autologous HSCT: Allogeneic HSCT is indicated.
2. Relapsed or refractory T-cell lymphoma: Allogeneic HSCT may be the only curative modality in this setting (Table 7.2)
3. Cutaneous T-cell lymphoma: Allogeneic HSCT is considered in the setting of progressive or refractory stage IIB-IV disease after the failure of biologic agents and at least one line of chemotherapy.

Hodgkin Lymphoma

Allogeneic HSCT is curative in Hodgkin lymphoma patients with prior autologous HSCT, especially if they had chemosensitive disease after salvage chemotherapy at the time of transplant [51]. The EBMT showed that there was no benefit of MAC over RIC allogeneic HSCT in Hodgkin lymphoma patients who relapsed after autologous HSCT [52].

Recommendation

Relapsed or progressive Hodgkin disease: Allogeneic HSCT is reasonable in patients who have failed autologous HSCT and have also failed therapy with at least brentuximab vedotin or nivolumab (Table 7.2).

Plasma Cell Dyscrasias

Multiple Myeloma

Allogeneic HSCT may be a curative option for patients with advanced multiple myeloma (MM). A meta-analysis of five randomized trials did not show any benefit of tandem auto-allo HSCT over tandem autologous HSCT in patients with myeloma [53]. In the relapsed setting, the data are limited to single-institution retrospective studies. These studies suggest that allogeneic HSCT (generally with melphalan-based RIC regimens) is associated with favorable outcomes, especially in those who were young and in complete remission at the time of transplant [54, 55]. Given the lack of consistent survival benefit in both newly diagnosed and relapsed MM, the use of allogeneic HSCT in MM should ideally be restricted to well-designed clinical trials. Of note, BMT CTN trial 1302 was recently launched to evaluate the role of early allogeneic HSCT in high-risk patients with MM, followed by maintenance with MLN9708 (ixazomib).

Data pertaining to transplant in primary plasma cell leukemia (pPCL) are limited, given the rarity of this disorder (pPCL accounts for only 1% of all plasma cell disorders). One of the largest published experiences on HSCT for pPCL comes from the CIBMTR. In their study, the outcomes of 147 patients with pPCL who received autologous HSCT ($n = 97$) or allogeneic HSCT ($n = 50$) within 18 months after diagnosis were reported. Though allogeneic HSCT was associated with a significantly lower relapse rate, it was associated with a higher risk of NRM and no OS benefit [56]. A recent prospective study has shown durable remissions in a subset of PCL patients following allogeneic HSCT [57]. Considering the dismal prognosis of pPCL with standard approaches, a tandem auto-allo HSCT in this setting is a reasonable consideration.

Recommendation

1. Consider allogeneic HSCT with RIC in the setting of a clinical trial, in the relapsed refractory setting.
2. Tandem auto-allo HSCT is not recommended as consolidation after frontline therapy (Table 7.2).
3. Allogeneic HSCT should be considered for pPCL.

Non-malignant Hematological Conditions

Aplastic Anemia

Acquired (or Idiopathic) Aplastic Anemia

MRD allogeneic HSCT is the treatment of choice for young patients with severe aplastic anemia. Conditioning with cyclophosphamide and anti-thymocyte globulin (ATG), and GVH-D prophylaxis with a calcineurin inhibitor and methotrexate represent the standard of care for HSCT in acquired aplastic anemia [58, 59]. EBMT data show that the outcomes of patients aged 30–40 years and 40–50 years are similar. However, a careful assessment of comorbidities should be made prior to HSCT, to determine fitness for up-front HSCT, instead of first-line immunosuppressive therapy, in the age group of 35–50 years [60].

For patients who lack an MRD, immunosuppressive therapy remains the treatment of choice. However, 30–40% of the patients will eventually relapse or are refractory to immunosuppressive therapy and thus will be considered for transplantation using an MUD [61]. In severe aplastic anemia, bone marrow is the recommended stem cell source, from a sibling or unrelated donor, for allogeneic HSCT [62]. While alternative-donor HSCT can be considered in the absence of a matched sibling or unrelated donor after failure of immunosuppressive therapies [63, 64], limited data are available for this scenario. All patients should be screened for donor-specific antibodies to mitigate the risk of primary graft rejection. For cord blood unit transplantation, a minimum cell dose of 4×10^7/kg total nucleated cells (frozen) is recommended [60]. The ASBMT panel recommends allogeneic HSCT in newly diagnosed severe aplastic anemia and in those with relapsed or refractory severe aplastic anemia [20].

Recommendations

1. Newly diagnosed severe aplastic anemia: Allogeneic HSCT is indicated in young patients. Bone marrow is the recommended stem cell source for sibling or unrelated donor HSCT (Table 7.2).
2. Relapsed or refractory severe aplastic anemia: Allogeneic HSCT is the preferred therapy option.

Sickle Cell Disease

Early experience for HSCT in sickle cell disease was limited to those with severe disease and an available MRD. However, the definition of severe disease has been in flux over the years. Currently, the generally accepted indications for allogeneic HSCT in sickle cell disease include history of a clinically significant neurological event (e.g., stroke) or any neurological deficit lasting >24 h, history of ≥2 episodes of acute chest syndrome per year for 2 years despite supportive care measures, history of ≥3 severe pain crises per year for 2 years despite supportive care measures, and/or ≥8 packed red blood cell transfusions per year for ≥1 year (to prevent vaso-occlusive complications and tricuspid valve regurgitant jet velocity ≥ 2.7 m/s on echocardiogram) [65–67].

MRD allogeneic HSCT for sickle cell disease has built on the seminal work of the French, Belgian, and United States groups, while maintaining excellent survival with reduction of the toxicity or intensity of pre-allogeneic HSCT conditioning regimens [68–70]. While these successful outcomes were encouraging, several important impediments to the broader utilization of HSCT were illustrated by these and more recent studies. First, very few individuals had suitable related donors available. It is estimated that only 14% of individuals with sickle cell anemia will have an MRD and 19% will have a well-matched unrelated donor in the National Marrow Donor Program (NMDP) registry ([90, 71]). Secondly, the treatment itself carries a high risk of morbidity and mortality, and a high risk of infertility [72] in transplant survivors. These considerations have historically dampened the enthusiasm for this curative therapy [73, 74]. Accordingly, the EBMT and CIBMTR have reported only 611 and 627 patients, respectively, receiving transplantations for sickle cell disease, as of 2013 [75]. The report "Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members", published in 2014 by the National Heart, Lung and Blood Institute (NHLBI), concluded that there is a need for additional research to address the potential risks of this therapy before HSCT can become a standard therapy option [76]. There is currently a BMT CTN trial trying to address this issue (BMT CTN 1503 is comparing allogeneic HSCT to standard of care in adolescents and young adults with severe sickle cell disease). Another BMT CTN trial (BMT CTN 1507, led by Drs. Michael Debaun, Mark Walters, and Rob Brodsky) will explore the use of haploidentical transplants in children and adults with sickle cell disease.

Recommendations

Transplantation for individuals with severe disease should be considered, ideally in the context of a clinical trial.

Thalassemia

Allogeneic HSCT is the only definitively curative option for patients with thalassemia major. Thalassemia patients are categorized into three classes depending on the degree of hepatomegaly (greater than or not greater than 3 cm), the

presence or absence of portal fibrosis in the pre-transplant liver biopsy, and the quality of chelation therapy (regular or irregular). Class 1 patients have none of the risk factors, while class 2 has one or two, and class 3 has all three of these adverse risk factors [77]. Adult patients have more advanced disease and treatment-related organ damage, mainly due to prolonged exposure to iron overload and/or exposure to hepatitis C virus. Consequently, most adult patients belong to the class 3 risk group. Of note, unlike class 3 younger patients, adult patients treated with the same conditioning regimens had a low graft rejection rate (4%), but higher NRM rates (approximately 25%) [78]. The major factor determining transplant outcome in adults is the presence of iron overload [79], and with improved medical therapy over the past few years [91], HSCT outcomes may improve.

Results of HSCT in thalassemia using MUDs, especially in class 1 and class 2 patients, are similar to those obtained with MRD. Advances in transplantation biology have made it possible to perform haploidentical stem cell transplantation in patients with thalassemia who lack an MRD or unrelated HLA-matched donor, with very encouraging results [80].

Recommendations

Allogeneic HSCT in adults can be offered to suitable patients, provided they have been well chelated since infancy. Ideally, such patients should be evaluated in centers with expertise for thalassemia transplantation.

Other Indications

Allogeneic HSCT outside of a clinical trial is not recommended for autoimmune diseases in general (e.g., rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, etc), except for patients with autoimmune thrombocytopenia, autoimmune hemolytic anaemia, and Evans' syndrome refractory to at least two lines of treatment (including rituximab and thrombopoietin receptor agonists for immune thrombocytopenia) [81].

Conclusion

Over the past two decades allogeneic HSCT has indeed undergone significant advances, with improvements in NRM and survival (Figs. 7.3 and 7.4) and evolution in the practice for selecting the intensity of conditioning regimens (Fig. 7.5). Improvements in supportive care, high-resolution allele level HLA-typing, and the use of alternative donor allografts and RIC have expanded the use of allogeneic HSCT. Despite the advent of the novel agents over the past decade, indications for allogeneic HSCT continue to grow, given its curative potential for advanced

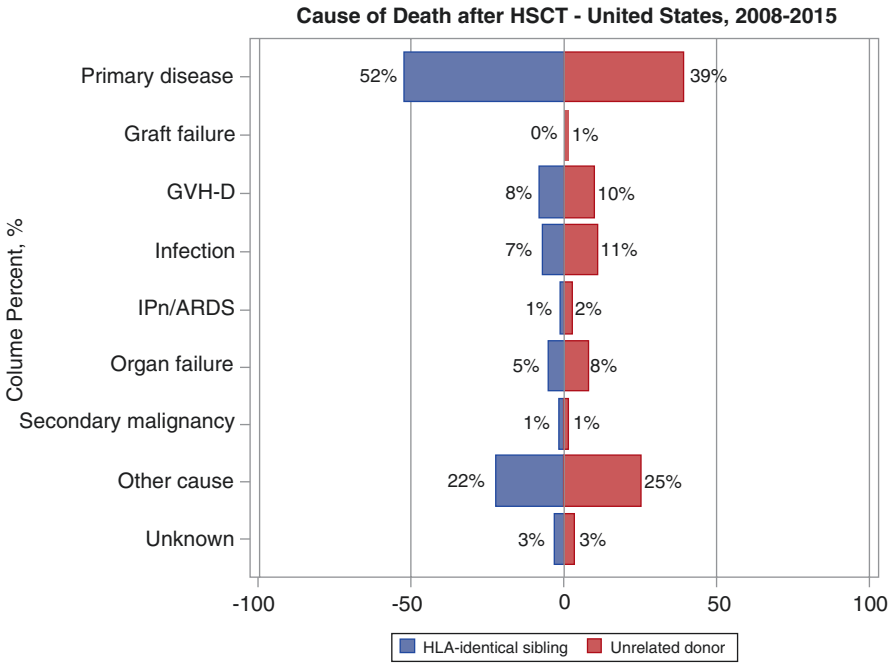

Fig. 7.3 Causes of death after transplantation, 2008–2015

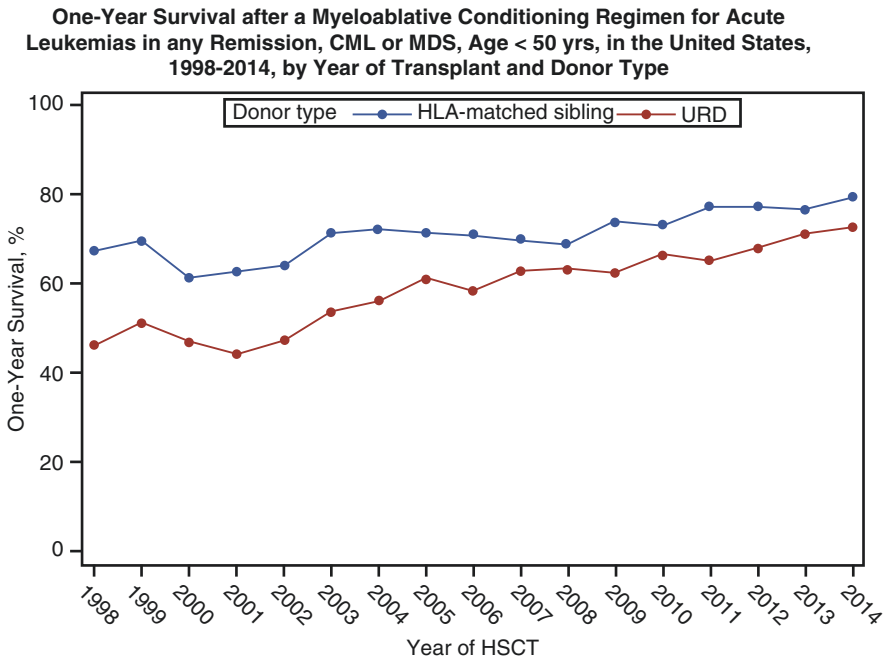

Fig. 7.4 One-year survival after myeloablative HSCT in younger patients (related vs. unrelated donor)

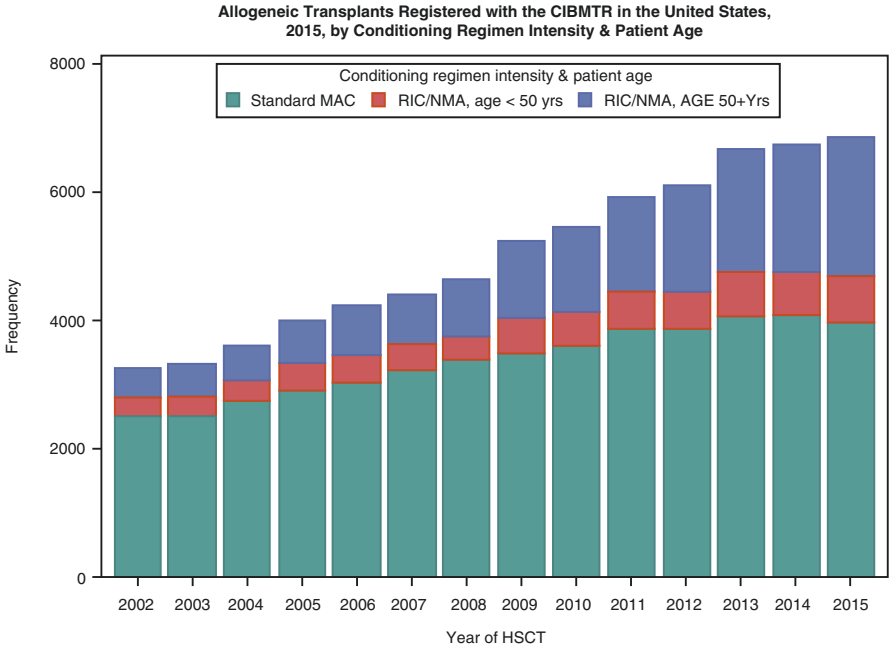

Fig. 7.5 Conditioning regimen intensity and patient age—changing trend over time (2002–2015)

hematological malignancies. A variety of novel post-allogeneic HSCT immunotherapeutic strategies are now being explored, to reduce the risk of post-transplant relapse. The next decade of studies will likely establish personalized post-transplant maintenance strategies, with the goal of achieving minimal residual disease negative state.

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Chapter 8
Immunogenetics Laboratory

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Introduction

The history of allogeneic hematopoietic stem cell transplantation (HSCT) is inextricably bound to histocompatibility and immunogenetics (H&I). The discovery of the major histocompatibility complex (MHC) in 1958 was a prerequisite for the successful engraftment of allogeneic hematopoietic stem cells, first in animals and then in humans [1]. The increasing clinical success of allogeneic HSCT for the treatment of malignant and non-malignant blood disorders up to the present day has been made possible partly by advances in tissue-typing technologies, from serological human leukocyte antigen (HLA) typing through molecular techniques with increasing resolution power towards the end of the twentieth century up to next-generation sequencing (NGS) HLA typing in recent years [2]. These developments are reflected by the increasing level of specialization required for personnel involved in H&I, on the one hand, and clinical transplantation on the other, with the two fields strongly intertwined, but no longer as united as in the early days.

Services provided by H&I laboratories to transplant centers include HLA typing, antibody testing, and chimerism and engraftment monitoring, all of which are detailed in this chapter. The results of these analyses need to be critically evaluated in relation to the clinical needs for each individual patient. Thus, when setting up an H&I laboratory, it is important to keep in mind that the service should not be limited

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to the delivery of the test result, which must be obtained under controlled and well-documented conditions, but should also include expert consultation by the H&I specialist with the clinical transplant team. This factor dictates specific requirements for the staff and facilities of the H&I laboratory, as outlined below.

Staff and Facilities

Staff at the supervisory level (i.e., laboratory director and technical supervisor) must have specific expertise obtained by several years of full-time training in human H&I testing. Procedures for supervising the training of incoming personnel must be clearly defined and documented. Moreover, staff must participate in continuing education specific to the H&I field. These considerations must be taken into account in determining the size of the staff, which must be adequate for the anticipated workload to be carried out within an acceptable time frame for urgent donor searches.

Facilities must be adequate to allow for the physical separation of pre- and post-polymerase chain reaction (PCR) areas. The flow of any type of material (samples, pipettes, plastic ware, laboratory books, laboratory coats, etc.) from post-PCR to pre-PCR areas must be rigorously excluded to reduce the potential for the contamination of new samples with previously amplified DNA. This factor must also be considered for the equipment, which must include dedicated freezers and centrifuges for the pre- and post-PCR areas. Regular maintenance of all equipment must be organized and documented. The integration of robotics secures the traceability of each specimen at each step of the process and minimizes the risk of contamination and sample mix-up. However, the decision on whether to implement this costly and maintenance-intense instrumentation must be carefully weighed against logistical considerations, including the anticipated sample throughput. Equipment also includes adequate informatics software for test-result generation and reporting, as well as for sample identification and traceability. All software must be secured by proper validation, updating, and back-up filing.

Services to the Transplant Center

The services and relevant techniques offered by the immunogenetics laboratory must be adequate for the clinical needs of the transplant center. There are no fixed rules; however, the requirements should be based on the most recent available evidence [3–5] (Table 8.1) and agreed upon with the clinical transplant team. This agreement must be documented in a written transplant protocol signed by both parties, which is to be subject to regular documented review by both sides. All techniques must be documented in written standard operating procedures (SOPs), which must be available at the bench where the tests are being carried out, and reviewed at least annually by the supervisory staff.

Table 8.1 Recommendations for histocompatibility and immunogenetics (H&I) services according to hematopoietic stem cell transplantation (HSCT) donor type

	HSCT donor	HLA typing[a]	Antibody[b]	Chimerism[c]
Related	Genotypically matched sibling[d]	First-field[*]	No	Outside HLA
	Phenotypically matched sibling[e]	Second-field	No	Outside HLA
	Genotypically haploidentical[d]	First-field	Yes	In/outside HLA
	Phenotypically haploidentical[e]	Second-field	Yes	In/outside HLA
Unrelated	Matched unrelated volunteer	Second-field	Optional[f]	In/outside HLA
	Umibilical cord blood	Second-field	Yes	In/outside HLA

The Table contains recommendations; the final decision should be made in accordance with the most recent available evidence [3–5] by the H&I specialists in agreement with the clinical transplant team, and documented in a dedicated transplant protocol

[a]HLA typing should be performed for the loci HLA-A, B, C, DRB1. Additional typing for the loci HLA-DRB3/4/5, DQA1/DQB1, and DPA1/DPB1 is optional

[b]Pre-transplant crossmatch with the selected donor; in the case of a positive crossmatch, selection of a different donor-specific antibody (DSA)-negative donor based on antibody screening and identification

[c]By short tandem repeat (STR) or quantitative polymerase chain reaction (qPCR) targeting of polymorphic genes outside the HLA system and, optionally, inside the HLA system after HLA-mismatched HSCT to address the possibility of HLA loss relapses [6, 7]

[d]Segregation of both HLA haplotypes must be unequivocally proven by family studies, including resolution of recombination where relevant

[e]Where segregation of both HLA haplotypes cannot be unequivocally proven by family studies

[f]In the case of HLA-A, B, C, or DR mismatch if pre-transplant crossmatch is positive, antibody screening and identification is needed for the selection of a DSA-negative donor. In the case of HLA-DP mismatch, identification of HLA-DP-specific antibodies by single antigen beads (SAB) is optional

[*]See Figure 8.1

Services and Specific Techniques in the Immunogenetics Laboratory

The three main services of the immunogenetics laboratory are HLA typing, antibody testing, and chimerism and engraftment monitoring. The basic principles of the main techniques used for these purposes are described below, along with their respective practical pros and cons.

HLA Typing Technologies

Resolution Levels and Ambiguities

The best choice of HLA typing techniques depends on the level of resolution, required to meet the clinical needs of the transplant center (Table 8.2). Resolution levels reflect HLA nomenclature, details of which can be found on the ImmunoGenetics (IMGT)

Table 8.2 HLA typing techniques

	Serology[a]	Polymerase chain reaction (PCR)-SSP[b]	PCR-SSP[b]	PCR-SSO[c]	PCR-SSO[c]	SBT Sanger[d]	SBT NGS[e]
Resolution	Low	Low	Medium-high	Low	Medium-high	High	High
Maximum throughput	~20/day/person	~10/day/person	5–10/day/person	~40/day/person	~40/day/person	~100/week[f]	Min. 200/week[f]
Workload	Moderate	Moderate	High	Low	Low	High	High
Complexity[g]	Very low	Low	Low	Moderate	Moderate	High	Very high
Time[h]	1 day	1 day	1 day	1–2 days	1–2 days	~4 days	6–10 days
Interpretation	Very easy	Easy	Moderate	Moderate	Complex	Complex	Very complex
Cost/sample	Low	Low	High	Low	Moderate	Moderate-high[f]	Moderate-high[f]

[a]By commercially available complement-dependent cytotoxicity (CDC)
[b]By commercially available sequence-specific priming (SSP)
[c]By commercially available sequence-specific oligonucleotide (SSO)
[d]By commercially available sequence-based typing (SBT)
[e]By commercially available next-generation sequencing (NGS)
[f]Highly dependent on sample throughput, the degree of automation of SBT procedures, and depreciation on investment for the instruments required
[g]Complexity for implementation and for routine use
[h]Average time from sample registration to the generation of the final report of the human leucocyte antigen (HLA) type

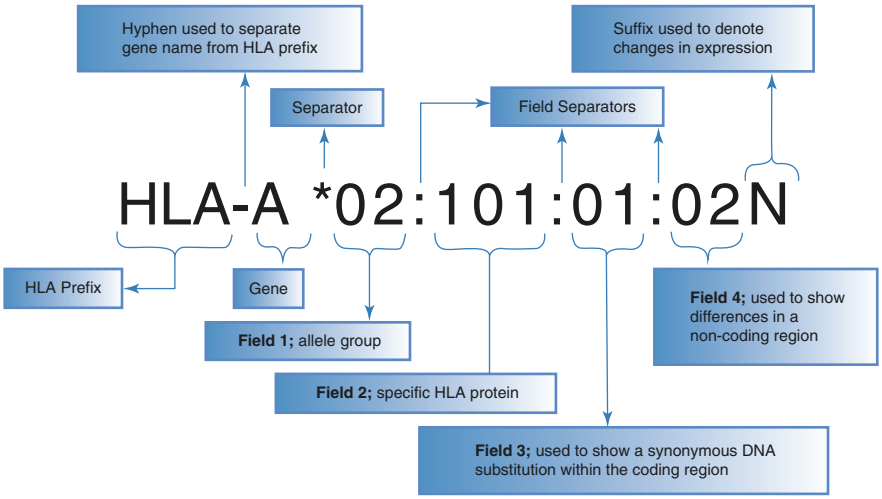


Fig. 8.1 Human leucocyte antigen (HLA) nomenclature and typing resolution. Shown are all components of an HLA allele name for the example of HLA-A*02:101:01:02:01N. This figure was kindly provided by Professor Steven GE Marsh, Anthony Nolan Research Institute, London, United Kingdom (www.hla.alleles.org) [8]. Low- and high-resolution typing resolves allele groups to field 1 (first-field typing) and field 2 (second-field typing), respectively. In addition, accreditation standards for high resolution require the identification of all *Null* alleles (alleles with suffix N). Serological typing is generally equivalent to first-field typing; however, certain serological splits can be solved only by second-field typing at the molecular level

website (www.ebi.ac.uk/ipd/imgt/hla/nomenclature/index.html), as well as in the dedicated literature [8]. The nomenclature, in turn, reflects the structure of the HLA antigen, in particular the polymorphic peptide antigen binding groove, and its coding and non-coding genomic correlates (Fig. 8.1). Typing for HSCT purposes generally does not require resolution beyond the Second-field, however, the reesolution of non-expressed *Null* alleles (suffix N) is needed. Low resolution corresponds to the first-field, which generally reflects serological typing; however, certain serological splits can only be resolved at the Second-field. High-resolution typing is defined as "a set of alleles that encode the same protein sequence for the region of the HLA molecule called the antigen binding site and that exclude alleles that are not expressed as cell-surface proteins" [9]. High resolution is sometimes difficult to achieve due to the impressive number of HLA alleles known to date (N = 17,166 according to Nomenclature Release 3.29.0.1 from 2017-08-18; www.ebi.ac.uk/ipd/imgt/hla/docs/release.html), which can give rise to ambiguous typing results that vary in number according to the typing method used. A list of ambiguous allele combinations that arise despite the sequencing of exons 2 + 3 for HLA class I or text on 2 of HLA class II can be found at http://www.ebi.ac.uk/ipd/imgt/hla/ambig.html. Laboratories may choose to report intermediate levels of resolution, such as common and well documented (CWD) alleles [10, 11] or "G" group alleles with identical nucleotide sequences across the exons encoding the peptide-binding domains. There are no fixed rules for these strategic decisions, which, however, must be taken in accordance with the transplant team's requirements and documented in the transplant protocol as mentioned above.

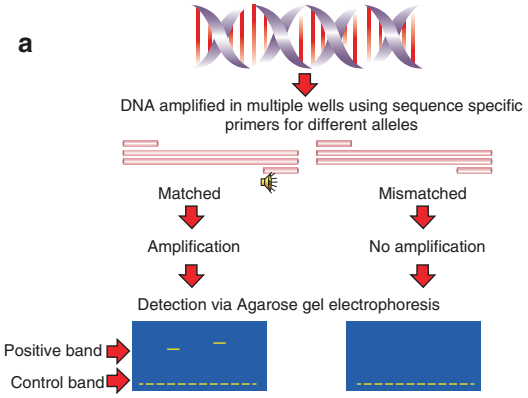
Serology

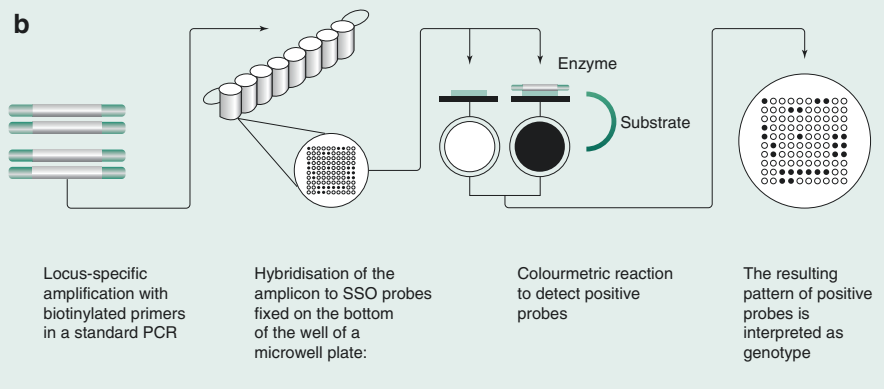
Historically, tissue typing has been performed with the complement-dependent cytotoxicity test (CDC), using a defined set of sera containing HLA-specific antibodies [12]. Serologic HLA typing is therefore also referred to as CDC technique. This technique has the advantage that it detects the expressed protein on the cell surface, thereby allowing the indirect definition of so-called *Null* alleles present at the genomic level. However, the number of HLA antibody epitopes and, hence, the resolution level of the CDC technique, is limited. Apart from certain antigenic splits, the CDC technique corresponds to molecular first-field typing, although many first-field alleles, in particular those of low expression loci such as HLA-C and DPB1, cannot be serologically defined. Over the past two decades, DNA-based tissue typing has almost completely replaced serological typing in HLA laboratories.

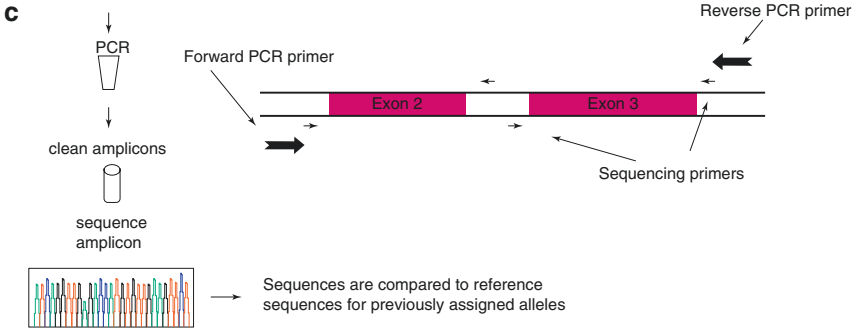
PCR-SSP

PCR-based typing using sequence-specific primers (SSP) has become routine in HLA laboratories since the mid-1990s [2]. These assays can be used to obtain low, intermediate, or high HLA typing resolution results. The PCR-SSP technology uses a variety of primers to cover multiple single-nucleotide polymorphisms (SNPs) at a given HLA locus (Fig. 8.2a). Subsequently, the amplified products are visualized by gel electrophoresis, and the HLA type assignment is based on the combination of positive primer mixes. Commercial kits including pre-aliquoted lyophilized SSP primer mixes (including primers for an internal control), as well as dedicated

Fig. 8.2 Basic principles of molecular HLA typing by polymerase chain reaction sequence-specific priming (PCR SSP), sequence-specific oligonucleotide (SSO), or sequence-based typing (SBT). (**a**) PCR SSP. An extendable number of primers located in different single-nucleotide polymorphisms (SNPs) of the HLA alleles is used to amplify products of varying length. The amplification is then generally visualized by gel electrophoresis. The combination of amplified reactions is used by software to assign the two HLA alleles present in the sample. Potential difficulties lie in the weak amplification of specific or non-specific (cross-reactive) alleles, which need to be interpreted with caution. (**b**) PCR SSO. A generic PCR product amplifying all alleles of the HLA locus in question is hybridized with an extendable number of probes corresponding to different SNPs in the amplified region. Visualization is generally carried out enzymatically (use of labeled PCR primers) either on membranes or in a cytometer device. The combination of positive probes is used by software to assign the two HLA alleles present in the sample. Potential difficulties lie in weak signals from specific or non-specific (cross-reactive) probes, which need to be interpreted with caution. Unlike SSP, only SNPs residing within the amplified region can be probed by SSO. (**c**) SBT. A PCR product using either generic locus-specific or HLA allele- or allele group-specific primers is subsequently sequenced, either by Sanger sequencing or by next-generation sequencing (NGS). The combination of polymorphic sequences is used by software to assign the two HLA alleles present in the sample. Like SSO, only SNPs residing within the amplified region can be probed by SBT; however, identification of new SNPs residing in areas of the gene not yet reported as polymorphic is readily possible with this method. For this reason, with the advent of NGS typing, the number of HLA alleles identified has been increasing exponentially


a
DNA amplified in multiple wells using sequence specific primers for different alleles
Matched
Mismatched
Amplification
No amplification
Detection via Agarose gel electrophoresis
Positive band
Control band


b
Enzyme
Substrate
Locus-specific amplification with biotinylated primers in a standard PCR
Hybridisation of the amplicon to SSO probes fixed on the bottom of the well of a microwell plate:
Colourmetric reaction to detect positive probes
The resulting pattern of positive probes is interpreted as genotype


c
PCR
clean amplicons
sequence amplicon
Forward PCR primer
Reverse PCR primer
Exon 2
Exon 3
Sequencing primers
Sequences are compared to reference sequences for previously assigned alleles

interpretation software, are currently available from a variety of different companies. The test is easy to perform and needs little technical equipment (i.e., thermocycler and gel electrophoresis device). Another advantage is the relatively high power of resolution, owing to the specificity given by two different SNPs at a time (one for each primer). However, the PCR-SSP method is not suitable for high sample throughput due to the relatively high number of manual pipetting steps.

PCR-SSO

PCR sequence-specific oligonucleotide probe (SSO) technology entered the HLA field in the mid-1990s [2] and started with assays using membrane-bound HLA locus-specific PCR products that were hybridized with probes corresponding to individual SNPs in different HLA alleles ("forward SSO"). This allowed the simultaneous analysis of large numbers of samples, but was work-intensive and time-consuming. Moreover, since only a single SNP could be probed at a time, the resolution level of forward SSO was inferior to that of SSP. Before long, methods to bind the probes to solid phases, followed by hybridization with amplified PCR products, were developed ("reverse SSO"; Fig. 8.2b). In addition, progress in probe development allowed the analysis of more than one SNP with a single probe, making the resolution level of SSO more similar to that of SSP. However, an intrinsic resolution restriction of the SSO method is that polymorphisms outside the amplified region cannot be resolved.

A number of commercial SSO kits with different solid phases are currently on the market, with Luminex colored microsphere beads read by a small cytometer device being among the most commonly used. Like SSP, today low, intermediate, and high resolution levels can be achieved by SSO.

Sequence-Based Typing (SBT)

The PCR sequencing-based typing (SBT) approach relies on the same basic amplification principles as PCR SSP (allele or allele group-specific amplicons) or PCR SSO (generic HLA locus-specific amplicons) [2]. In SBT, however, the read-out step consists in direct sequencing of the entire amplified product, thereby enabling the detection of all-including previously unknown-polymorphisms of the gene (Fig. 8.2c). Sequencing by classical Sanger methods requires relatively simple technology and has been facilitated by numerous commercial kits that provide different amplification primers (generic or allele/group-specific), as well as dedicated interpretation software. Thus, the resolution power of SBT is generally higher than that of SSO or SSP; however, it should be noted that SBT has a similar limitation regarding the achievable level of resolution, which is limited to polymorphisms within the amplified region. Moreover, the process is rather complex and not easily amenable to very high throughput. Over the past few years, NGS approaches have also been developed for HLA typing [13]. NGS is based on the massive parallel sequencing of thousands of individual amplified molecules. The assignment of these molecules to an individual and/or to a given PCR product is achieved by specific bar-coding. The advantages of this method are at least twofold: first, it solves all typing

ambiguities resulting from the difficulty of assigning SNP combinations to either of the two alleles present in a heterozygous sample (so-called *cis*/*trans* ambiguities), due to single-molecule sequencing. Second, it is amenable to very high throughput, because coverage of a few thousand single-molecule sequences is generally sufficient to obtain accurate HLA typing, but a single NGS run can identify up to one million sequences, thereby enabling complete 6-locus typing of about 50 samples in a single run. The massive amount of generated data can be interpreted only by fully automated software, which is now commercially available from different companies.

Table 8.2 summarizes the above-described HLA typing strategies and lists the advantages and disadvantages of the different methodologies for a typical medium-sized laboratory. It should be noted that current quality standards require the use of Conformité Européenne (CE) or equivalent marked test kits for diagnostic clinical use, posing serious limits to the routine application of the in-house test kits that were in common use previously.

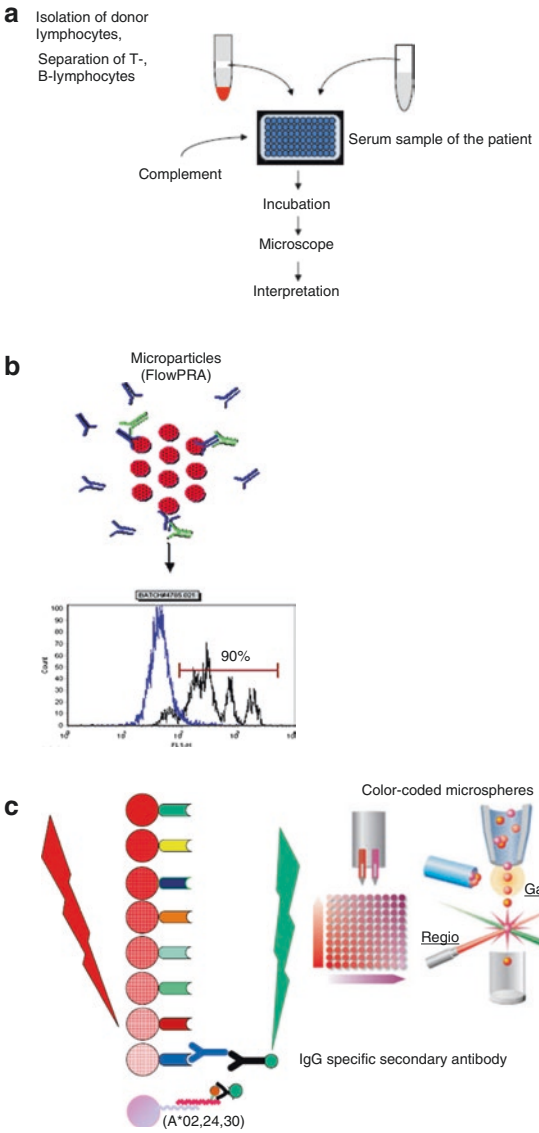
Antibody Testing

Antibodies to allogeneic HLA antigens can be induced during pregnancy, but also in specific clinical conditions such as those in patients with multiple blood transfusions. These antibodies are also called panel-reactive antibodies (PRA) and, if specific for HLA antigens carried by the donor, donor-specific antibodies (DSA). DSA are most commonly recognized for their role in antibody-mediated rejection episodes in solid-organ transplants, but have recently also been shown to be detrimental in HLA-mismatched HSCT, including haploidentical HSCT [14]. Therefore, testing for DSA is highly recommended prior to the selection of an HLA-mismatched donor.

Antibody testing is divided into three applications: cross-matching, which tests the reactivity of patient antibody with donor cells (generally performed separately on T cells for HLA class I antibodies and B cells for HLA class II antibodies) and, hence, the presence of DSA; antibody screening, which tests for the presence or absence of anti-HLA antibodies in the patient serum; and antibody identification, which determines the specificity of any anti-HLA antibodies detected in an antibody screening. Three main methods are used for antibody testing: CDC, flow cytometry and bead arrays.

CDC

CDC relies on the same principles as serological HLA typing, whereby the patient serum is incubated with target cells, followed by complement-mediated lysis of antibody-labeled cells and dye-based visualization of live and dead cells (Fig. 8.3a). CDC is a standard method for all three applications in antibody testing (cross-matching, antibody screening, and antibody identification). Its drawbacks are the relatively time-consuming approach, which is not easily amenable to automation; relatively low sensitivity; and the detection of clinically irrelevant antibodies reacting with non-HLA cell-surface molecules.


a
Isolation of donor lymphocytes,
Separation of T-, B-lymphocytes
Serum sample of the patient
Complement
Incubation
Microscope
Interpretation
b
Microparticles (FlowPRA)
90%
c
Color-coded microspheres
Gating
Regio
Signal
IgG specific secondary antibody
(A*02,24,30)

Flow panel reactive antibodies (PRA)

With PRA, antibodies can be identified by incubating the patient serum with donor cells for cross-matching, or incubating the patient serum with a cell panel expressing the most frequent HLA antigens. Like CDC assays, these tests will detect both HLA and non-HLA specific antibodies. However, more specific assays are available using panels of beads coated with single HLA antigens ("single antigen beads"; SAB) for antibody screening and identification, followed by detection via a fluorescence-labeled secondary immunoglobulin G (IgG) antibody (Fig. 8.3b).

Bead Arrays

Bead array techniques are based on the same principle as flow PRA; however, these techniques take advantage of Luminex technology for visualization, using color-coded microspheres that can be distinguished by a dedicated dual-laser flow-based detection instrument (Fig. 8.3c). Luminex SAB assays are regarded as the most sensitive and specific tests for the detection of HLA specific antibodies.

Chimerism and Engraftment Monitoring

The accurate and sensitive determination of chimerism status after allogeneic HSCT is of great clinical importance, because the detection of an increase in mixed chimerism is considered a risk factor for adverse events, including graft rejection and disease relapse.

Chimerism monitoring after HSCT can target any polymorphic gene differences between patient and donor. These include variable number tandem repeats (VNTR) and short tandem repeats (STR), as well as SNPs or insertion-deletion (indel) poly-

Fig. 8.3 Methods for antibody screening and identification. (**a**) Complement-dependent cytotoxicity (CDC). Patient serum is incubated with donor T-cells (for HLA class I antibodies) or donor B-cells (for HLA class II antibodies). CDC will occur only after antibody binding; dye-based visualization of cellular vitality is therefore read out for the presence of donor-specific antibodies. Immunoglobulin (Ig) M antibodies are discriminated from IgG by the use of dithiothreitol (DTT), which disrupts the disulfide bonds present in IgM but not in IgG antibodies. (**b**) Flow panel reactive antibodies (PRA). Patient serum is incubated with a panel of HLA-typed T-cells (for HLA class I antibodies) or B-cells (for HLA class II antibodies) covering the most frequent HLA antigens, followed by staining with CD3 or CD19 and anti-IgG secondary antibody, each labeled with different fluorochromes. Alternatively, single antigen-coated fluorescent beads with individual purified HLA antigens can also be used. The read-out is the mean fluorescence intensity (MFI) of positive cells. (**c**) Bead array. Patient serum is incubated with color-coded microspheres, followed by a labeled secondary antibody, and analyzed by a dedicated dual-laser flow-based detection instrument. The amount of anti-HLA antibody attached to the microspheres is directly proportional to the MFI of the secondary label, while the HLA specificity is indicated by the color of the corresponding microsphere

morphisms throughout the genome. SNPs in the HLA system, and/or in minor histocompatibility antigens, can also be used for chimerism purposes; however, due to the SNP density in the MHC, the targeting of MHC SNPs for this purpose is challenging and currently is not common practice, although protocols for this purpose have recently been developed [7]. It should be noted, however, that leukemia blasts are characterized by genomic instability, which can alter the SNP landscape of the patient prior to HSCT or at relapse thereafter [15]. Therefore, the chromosomal location of polymorphisms targeted by the chimerism assay used should be known to the H&I laboratory and, where relevant, discussed with the laboratory that performs minimal residual disease testing (see Chap. 14).

Short Tandem Repeats (STRs)

STRs are 2- to5-bp repeats of variable length, distributed throughout the genome at different loci. Their diagnostic use was initiated in forensic medicine, and was adapted to HSCT monitoring in the 1990s. This semi-quantitative approach relies on multiplex amplification of different STR loci in a single PCR, followed by size resolution in capillary gel electrophoresis, which allows the discrimination of amplicons differing in length by only very few bases. The relative amount of donor or patient DNA is then determined by calculating the area under the peak (AUP) for each specific STR (Fig. 8.4a). In order to minimize the risk of competition between amplification primers, the amount of target genomic DNA used for STR analysis is generally 1–2 ng. This limits the sensitivity of this method to about 5%, although lower sensitivities, ranging between 0.8 and 1.6%, have been reported for certain protocols [16]. Different commercial kits for up to 20 different STR loci are currently available. Their use and interpretation is easy and does not require sophisticated software. Therefore, the use of STRs is, to date, still the most common method for chimerism assessment after HSCT.

Quantitative Real-Time PCR (qPCR)

Quantitative PCR (qPCR) is based on the amplification of a polymorphic SNP or indel followed by hybridization with a specific Taqman probe labeled with a 5'reporter and a 3'quencher dye which is cleaved by the 5' to 3' exonuclease activity of the Taq polymerase. The increasing fluorescence of the reporter dye correlates with the accumulation of the PCR product, which, in turn, is detected by a dedicated light cycler in real time, i.e., quantitatively) (Fig. 8.4b). The cycle at which the light overcomes the background fluorescence is defined as the threshold cycle (Ct) and is set at the beginning of the exponential phase. The relative amount of patient- or donor-specific DNA is calculated by the normalization of the Ct values to a housekeeping gene, which is a constantly and uniformly expressed common denominator (ΔCT), followed by calculating the relation to pure patient or donor DNA ($\Delta\Delta$Ct). The sensitivity of qPCR depends on the amount of input genomic DNA, which can

be increased to up to several hundred nanograms, as the PCR is mono- and not multiplex, as in STR. Therefore, qPCR can detect chimerism with a sensitivity of less than 0.1% [17]. However, the method becomes inaccurate at mixed chimerism values in higher ranges. This problem has recently been overcome by applying the qPCR system to droplet digital PCR (ddPCR), in which qPCR reactions are performed in thousands of droplets, each containing a single molecule of target DNA; this is followed by the counting of amplicon-containing droplets in a dedicated device [18]. Both classical qPCR and ddPCR require some dedicated technical equipment (light cycler and microdroplet generator and reader, respectively); however, the ease of interpretation and the high sensitivity render this method attractive, especially for laboratories interested in the early detection of mixed chimerism as a sign of leukemia relapse post-HSCT [17, 19].

External Proficiency Testing

Participation in external proficiency testing (EPT) is an essential requirement for any laboratory supporting HSCT. The testing and reporting of unknown samples gives the laboratory the opportunity to demonstrate that it is capable of successfully completing the HLA typing, and, if appropriate, the antibody testing, that is necessary to match patients and donors. Also, if appropriate, EPT should be performed for post-engraftment monitoring. Successful participation in EPT schemes is a mandatory requirement for laboratories seeking accreditation.

The European Federation for Immunogenetics (EFI) External Proficiency Testing Committee (EPTC) aims to provide information on EPT schemes to all H&I laboratories and to harmonize EPT schemes for H&I. On its website, it provides standards for EPT providers, as well as a list of EPT providers for laboratories (http://www.efiweb.eu/efi-committees/ept-committee.html).

Laboratory Accreditation

The purpose of laboratory accreditation is to certify that a laboratory meets the requirements of a given set of standards. The rationale is that if these standards are met there is a level of assurance that the service provided is appropriate and of an acceptable level of quality.

For laboratories supporting HSCT there are different options for accreditation. Such laboratories can be accredited to a set of standards that can be applied generically to laboratories working in different pathology disciplines. The most common example is accreditation to International Organization for Standardization (ISO) 15189 medical laboratories—requirements for quality and competence. Accreditation to these standards provides assurance that the laboratories operate within a defined quality management system and that the service meets the requirements of the users.

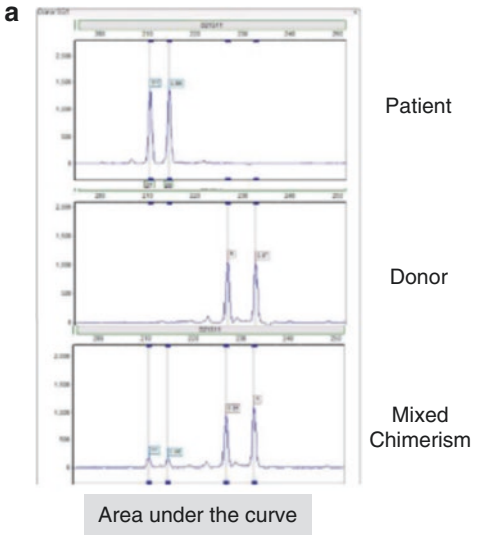

a
Patient
Donor
Mixed Chimerism
Area under the curve
b
Insertion patient
Deletion donor
R = Reporter
Q = Quencher
Forward Primer
R
Probe
Q
5'
3'
3'
5'
5'
3'
5'
Reverse Primer
Patient
Mixed Chimerism
ΔΔCT

Fig. 8.4 Methods for chimerism and engraftment monitoring. (**a**) Short tandem repeats (STRs). Various (typically at least 13) different STRs on different human chromosomes are amplified from genomic patient and donor DNA in a multiplex PCR reaction, and separated by capillary gel electrophoresis. STRs giving rise to peaks of different length in the patient compared with the donor are informative for chimerism. The relative amounts of patient and donor DNA present in each sample correspond to the relation between the area under the peak (AUP) of patient- and donor-specific peaks. In order to avoid competition between primers in the multiplex PCR, very low amounts (typically 1–2 ng) of genomic DNA are used as a template, thereby limiting the sensitivity of the assay to approximately 5%. (**b**) Quantitative polymerase chain reaction (qPCR). Various (typically at least 19) different insertion-deletion (indel) or single-nucleotide polymorphisms (SNPs) on different human chromosomes outside and/or inside HLA, are each amplified in a dedicated qPCR reaction using a Taqman probe labeled with a 5'reporter and a 3'quencher dye. A non-polymorphic housekeeping gene is amplified as reference. The amount of template DNA is proportional to the fluorescence released by cleavage of the Taqman probe through the exonuclease activity of Taq polymerase amplifying the specific PCR product. The cycle threshold of the indel- or SNP-specific qPCR is then related to that of the reference qPCR (ΔCT), and the quantity of DNA in the post-transplant sample is related to that of patient or donor DNA pre-transplant, to calculate the percentage of donor chimerism (ΔΔCt). Instead of a Taqman probe, detection can also be based on the incorporation of double-stranded DNA binding dyes such as SYBR Green. Because each qPCR assay is performed separately, the amount of genomic DNA template can be increased to up to several hundred nanograms (typically 50–100 ng), thereby increasing the sensitivity of the assay to at least 0.1%. qPCR is also amenable to transfer to droplet digital (dd) PCR systems, whereby thousands of microdroplet qPCR reactions are performed in parallel, followed by the counting of positive droplets in a dedicated counter, further increasing the accuracy of the assay, especially for high percentages of chimerism

However, there are also specific accreditation/certification schemes for H&I laboratories. These are schemes organized by professional bodies in the field of immunogenetics. They use H&I-specific standards, which are regularly reviewed and updated, taking into account the latest clinical and technical advances in the field of immunogenetics and transplantation. The schemes offered by the EFI and the American Society for Histocompatibility and Immunogenetics (ASHI) fall into this category.

A key feature of EFI and ASHI accreditation is that the standards define minimum requirements for the resolution of HLA typing for specific clinical categories, including related and unrelated HSCT. Of particular importance is the requirement that, for unrelated HSCT, laboratories must HLA type patients and donors at high resolution. This requirement is based on extensive published evidence showing that matching at the allele level is a key factor in HSCT outcomes. This detail is not contained within the ISO standards, which are generic across multiple disciplines. Figure 8.5 outlines the typical process that is needed to meet the detailed requirements of the EFI standards.

Both EFI and ASHI accreditation schemes are similar in the way they operate, with 3-year cycles requiring an on-site inspection every third year, along with annual submission of documents by the laboratories. Evidence of satisfactory participation in EPT and of continuing education is included in the essential requirements at each annual review.

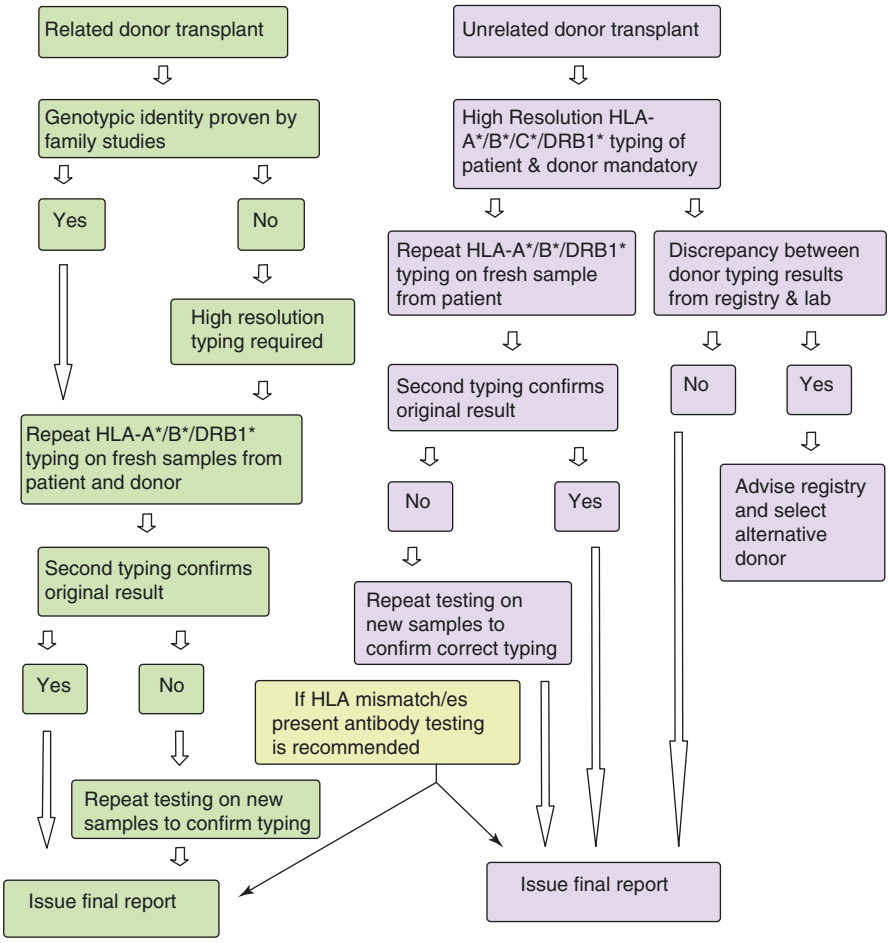


Fig. 8.5 HLA testing process for donor search in accordance with standards for accreditation. Shown is the process required for definitive typing of related or unrelated donors, including confirmatory typing. The criteria for donor selection must be agreed upon with the transplant center in a written transplant protocol (see also Chap. 10 on donor selection)

H&I-specific accreditation is regarded as an important requirement for laboratories supporting transplant units by a number of organizations involved in HSCT, including the World Marrow Donor Association (WMDA), the National Marrow Donor Program (NMDP), and the Joint Accreditation Committee International Society for Cellular Therapy [ISCT] European Group for Blood and Marrow Transplantation [EBMT] (JACIE). The number of accredited laboratories worldwide also indicates the widespread acknowledgement that H&I-specific accreditation provides benefits to the laboratory and, importantly, to the patients those laboratories serve. In 2016, over 260 laboratories in more than 30 countries held EFI accreditation and around 200 laboratories in 12 countries held ASHI accreditation.

Summary

Efficient collaboration between H&I specialists and transplant clinicians is crucial for the clinical success of allogeneic HSCT. The spectrum of services to be provided by the immunogenetics laboratory includes tissue typing and antibody testing for donor selection, as well as chimerism and engraftment monitoring post-transplantation, according to the clinical requirements. The choice of test procedures also has to take into account different logistical issues, such as expected sample throughput, working facilities, and costs. All procedures need to be carried out under rigid quality requirements, and need to be extensively documented and continuously controlled. Laboratory accreditation/certification by professional bodies such as EFI or ASHI is a mandatory requirement for a number of HSCT organizations worldwide. The set-up of an immunogenetics laboratory in support of an HSCT team is therefore critically dependent on the availability of specifically trained staff and adequate facilities.

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Chapter 9
Stem Cell Source

Christian Chabannon and Annalisa Ruggeri

Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for a variety of severe malignant and non-malignant hematological diseases affecting children or adults, and is now broadly used worldwide, in high- as well as in low- to middle-income countries [1].

In the context of human autologous transplantation, the infusion of peripheral blood stem cells (PBSCs) reduces the length of aplasia induced by the administration of high-dose chemotherapy, and thus limits the frequency and severity of clinical events associated with severe and prolonged aplasia, mainly febrile neutropenia. It is, however, nearly impossible to demonstrate that HSCs present in the graft contribute to long-term hematopoietic reconstitution, because their progeny are indistinguishable from the progeny of residual quiescent stem cells spared by cytotoxic agents; indeed, examples of situations in which the autologous graft was accidentally

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lost demonstrate that patients can recover blood counts after receiving combinations of high doses of cytotoxic agents without stem cell support [2].

In the setting of allogeneic HSCT, the establishment of hematopoietic chimerism after transplantation is proof of the existence of HSCs in the cell product collected from the donor. While autologous HSCT almost uniformly uses peripheral blood grafts collected from patients previously treated with a variety of mobilization regimens, procedural aspects vary greatly for allogeneic HSCT [3]. The choice of the donor and of the collection procedure will have significant consequences for the cellular composition of the collected cell products and for numerous clinical and biological endpoints in the recipient. For adult donors, bone marrow (BM) was historically the preferred stem cell source; because of the introduction of hematopoietic growth factors in the pharmacopeia, the use of PBSCs from both related and unrelated donors has progressively increased, and this is nowadays the most widely used source of stem cells, at least for adult donors [3]. In many countries, regulations prevent the administration of granulocyte-colony-stimulating factor (G-CSF) to children; as a consequence, significant BM collection activity remains in pediatric transplant programs. Cord blood units (CBUs) represent the third allogeneic stem cell source; they are collected, banked, and distributed in the context of a stringently different organization than that used for living related or unrelated donors.

Donors can either be related or unrelated to the recipient. Related donors are usually easily accessible through their affected relative. The logistics for related donor search, recruitment, and collection are easier, quicker, and cheaper, although far from being harmonized, because this is the responsibility of each transplant program. Unrelated donors can be accessed through national registries that have been progressively networked, allowing for international queries when a patient is in need of finding a donor. The Bone Marrow Donors Worldwide (BMDW) database (www.bmdw.org) currently lists more than 28 million registered donors. However, the probability of finding a suitable unrelated donor for a designated patient varies greatly, depending on the ethnic background of the potential recipient, because ethnic groups are not equally or proportionally represented in national and international registries [4]. The delay in the identification of an unrelated donor is usually longer than that for the identification of a related donor, and the two delays must be added when trying to identify a suitable HLA-matched donor, because most institutions will only launch the search for an unrelated donor when the conclusion has been reached that no suitable related donor can be found. Unrelated donors are living adult volunteer or donors, or are newborns in the name of whom parents have consented to cord blood donation following a normal pregnancy and delivery; public cord blood banks total more than 690,000 CBUs worldwide (see https://wmda.info or www.bmdw.org).

Until recently, the field of allogeneic HSCT was dominated by the quest for a "perfect match" between donor and recipient; the increasing accuracy of HLA typing with modern molecular techniques is now approaching full sequencing, and this

has both confirmed the high degree of polymorphisms in HLA genes and antigens and made this quest even more complex (for details of HLA-typing and the organization of an immune-genetics laboratory, refer to Chapter 8). This approach mostly allowed for the use of HLA-identical siblings—a not-so-frequent situation in families with a small number of children given the Mendelian inheritance of HLA genes and haplotypes—or for the use of matched unrelated-identical donors, a rare situation that can, however, benefit a majority of recipients of Caucasian ancestry, given the total and still increasing number of volunteer donors registered worldwide, as mentioned above [4]. The introduction of cord blood transplantation was the first breach in the dogma, because it was rapidly demonstrated that partially mismatched CBUs could successfully engraft myeloablated recipients, provided that the infused cell dose was above a sufficient threshold [5].

More recently, the demonstration that a modification in the immune-suppressive regimen—mostly the use of post-transplant cyclophosphamide—allows for the use of mismatched related donors, and specifically of haplotype-mismatched related donors, has opened new avenues [6]. Parents, children, and 50% of brothers and sisters are haplotype-mismatched with a potential recipient, notwithstanding potential recombinations that may occur during meiosis. Under such circumstances, the lack of a donor for proceeding to allogeneic HSCT becomes an exception, rather than the rule; indeed, most situations are characterized by the existence of several potential donors. There is an urgent need for robust algorithms that will help select the best donor, taking into account such criteria as donor age, sex, cytomegalovirus (CMV) status, and willingness to donate, but also taking into account the presence or absence of donor-specific antibodies (DSA) in alloimmunized recipients [7]. There has been a growing interest in the use of haplo-identical donors, as shown by data in registries [8], at the expense of cord blood transplantation, because the clinical results, in terms of disease control and overall survival, appear to be comparable [9, 10]. The next and ongoing step will be to determine whether haplo-identical donors may be a substitute for unrelated HLA-matched donors. The increasing use of haplo-identical donors automatically results in a higher proportion of allogeneic transplants being performed with a blood or marrow transplant, or even combined blood + marrow [11], although the advantages of using two combined stem cell sources in this context remain to be firmly established. In the near future, such procedures may also reverse the ratio of unrelated to related donations, the former having become more frequent, a situation seen as a major incentive for the international harmonization of practices and the deployment of quality management systems across transplant programs.

Current algorithms for donor selection still favor the search for an HLA-identical donor as the first step. When no HLA-identical sibling is identified or can be solicited, the search for a matched unrelated donor can be conducted simultaneously with the search for a suitable cord blood unit, and the typing of other family members for a haplo-identical donor search. The final choice will, in part, depend on physicians' preferences and the transplant center's experience with the use of alternative donors.

Collection

Trained and qualified personnel, using validated procedures, must perform all BM harvests, cord blood collections, and PBSC collections, as required by the Foundation for the Accreditation of Cellular Therapy (FACT) and the Joint Accreditation Committee for the International Society of Cellular Therapy and European Society for Blood and Marrow Transplantation (JACIE) (FACT-JACIE) International Standards for Hematopoietic Cellular Therapy (now in version 6.0; accessible at www.jacie.org); by accreditation systems; and by local, national, and international regulations. However, only peripheral blood collection relies on an automated procedure, where a processor separates the various populations of blood cells based on their physical properties (sizes and densities). Collection efficiency can be individually monitored through the implementation of algorithms that use simple physical and biological measurements, including blood volume and circulating $CD34^+$ cell numbers [12–14]. Thus, the collection procedure can be tailored to individual situations, allowing the operator to modulate the volume of blood to be processed in an inverse relation with the number of circulating $CD34^+$ cells, with the goal of meeting predefined collection target figures within the shortest possible procedure (see Table 9.1): the higher the number of circulating $CD34^+$ cells, the lower the total volume of blood to be processed, although for the sake of simplicity, the volume of blood to be processed is usually a one-, two- or three-fold amount of the actual blood volume of the person, as computed by the cell separator from simple parameters including height, weight, and sex. The generation of recently marketed cell separators has not yet proven that these allow for the collection of improved cellular products as compared with historically used devices, although their design facilitates the training of nursing personnel [15, 16]. Cell separators may allow for a choice of procedural variations, none of them being clearly superior in terms of quality of the collected cell product; however, other criteria, such as the volume of extracorporeal blood, may be used to decide on the procedure, especially when it comes to collection for low-weight children, a situation that mostly occurs in the autologous context.

Table 9.1 Cellular composition and collection targets for various cell sources in the context of hematopoietic stem cell transplantation

	Volume collected	$CD34^+$ cell content	$CD3^+$ cell content	Target cell dose
Bone marrow collection	10–20 mL/kg	~ 2–3 × 10^6/kg	~ 25 × 10^6/kg	2 × 10^8 TNC (total nucleated cells)/kg
Peripheral blood stem cell (PBSC) collection = apheresis product(s)	150–400 mL	~ 2–8 × 10^6/kg	~ 250 × 10^6/kg	2–5 × 10^6 $CD34^+$/kg (autologous collection) > 4x10^6 CD34+/kg (allogeneic collection)
Umbilical cord blood collection = cord blood unit (CBU)	80–160 mL	~ 0.2 × 10^6/kg	~ 0.5–2 × 10^5/kg	>3 × 10^7 TNC/kg

PBSC collection is possible only when donors have previously received a mobilization treatment that transiently increases the percentage and frequency of circulating $CD34^+$ progenitors. G-CSF is the only agent marketed for this use; its use is not systematically allowed for pediatric donors in many countries. G-CSF requires daily subcutaneous injections; while it can be combined with the administration of acutely myelosuppressive agents for patients undergoing autologous collection and transplantation, it is obviously used as a single agent for donors. There is no consensus on whether the dose should be administered on a single occasion daily, whether in the morning or in the evening, or whether it should be split, with mid-dose administered in the morning and mid-dose administered in the evening; thus, practices vary from one center to another. The administration of G-CSF carries its own side effects and contraindications [17]; frequent immediate side effects include bone pain, myalgias, fever, and other reversible symptoms, and the prophylactic use of analgesics is usually recommended. Rare but severe side effects include allergic manifestations and splenic ruptures; for these reasons, some centers and registries recommend that the first administration of G-CSF be performed at the hospital rather than at home. A number of G-CSF biosimilars have been marketed in the past decade, with recommendations from both the European Society for Blood and Marrow Transplantation (EBMT) and the World Marrow Donor Association (WMDA) not to substitute these for princeps molecules for donor mobilization. Despite all these caveats, the administration of G-CSF to healthy donors has thus far turned out to be safe in the middle- to long-term, although improved follow-up is still necessary to thoroughly detect events that may occur at low frequencies, especially in relatives of patients affected with hematological diseases [18]. The use of a mobilization treatment in donors also means that collected cells and progenitors are not obtained in homeostatic conditions; as an illustration, changes in immune cell subsets that result from G-CSF administration have been widely explored, with contradictory results and no robust conclusion that G-CSF may contribute to mitigation or flaring of the immune phenomenon in the recipient. Changes in immune cells that are mobilized with pharmacological treatment for $CD34^+$ cell mobilization and collection are the main reason why there is no marketing authorization in the United States and in Europe for plerixafor, a CXCR-4 antagonist that is widely used to rescue poor-mobilizer patients who are candidates for autologous collection and transplantation; in the allogeneic context it is recommended that plerixafor not be used outside of clinical research protocols [19]. Finally, there is significant inter-individual variability in donors' response to G-CSF; clinical and biological factors underlying this variability include age, body mass index, ethnic origin, and likely some genetic determinants [20–22].

Bone marrow collection and cord blood collection are largely manual procedures, performed by trained physicians and midwives, respectively. Because BM collection is now performed for a minority of allogeneic HSCTs, training and maintenance of competencies has been an issue for most transplant programs over the past decade [23], leading the FACT-JACIE International Standards for Hematopoietic Cellular Therapy to request minimal yearly activity to maintain competencies; although aspiration from the posterior iliac crest carries no technical difficulty in

itself, it is important that collecting physicians refrain from collecting too much volume at each aspiration, which speeds up the procedure, but results in BM cell dilution with blood cells, and a lessened content of hematopoietic progenitors [22]. It is rarely necessary to further the procedure with additional collection from the anterior iliac crest.

Cord blood collection is usually performed after a normal delivery, through the catheterization of the umbilical veins, allowing blood to flow out of the placenta by gravity. It is important to collect the highest possible volume, because the volume largely correlates with the total nucleated cell count, which nowadays remains the main predictor—together with HLA compatibility—of cord blood transplantation outcome. The volume of cord blood that can be collected largely depends on the midwife's skills, but also on pregnancy-related characteristics, including mother's age at birth and baby weight. A peculiar aspect of cord blood use is the fact that collection and transplantation are temporarily dissociated: thus, only cord blood collections that meet predefined specifications are banked, with a small proportion of these CBUs to be released and distributed for transplantation, months or years after they have been collected. The majority of cord blood collections are ultimately discarded, and will never be listed in national or international registries.

Complete and accurate clinical and biological screening of patients and donors prior to their admission to the collection facility is mandatory to protect the comfort and safety of the person undergoing collection, prevent the transmission of occult or known infections or diseases of other natures to the recipient, and to facilitate the execution of the collection procedure (for details of pre-donation workup, see Chapter 10). The process is strictly regulated at the European level by Directives # 2004/23/EC, 2006/17/EC, and 2006/86/EC, with their contents being transcribed into national regulations, which results in some discrepancies between national regulations. Donor screening must include a medical questionnaire, medical examination, and biological tests, the nature of which are defined by national and international regulations; results of biological testing are only valid for a limited duration, usually 30 days prior to collection. Pregnancy testing of women of childbearing potential is mandatory within 7 days prior to the start of G-CSF treatment or the recipient conditioning regimen, whichever comes earlier. It is important that the collection facility is extensively informed of these results, and that the facility participates in the management of unplanned deviations, because this will affect the delivery and administration of the graft to the recipient.

The different techniques that can be used to collect allogeneic grafts will result in very different cellular products (see Table 9.1 for the cellular composition of the three different types of grafts). The highest number of hematopoietic progenitors—as identified through $CD34^+$ cell counting, a robust although crude surrogate marker for hematopoietic stem cells—is observed with PBSCs, while CBUs contain much smaller numbers. The numbers of $CD34^+$ cells in a CBU and in apheresis products vary by as much as tenfold; however, hematopoietic recovery or the establishment of hematopoietic chimerism is not ten times longer after cord blood transplant than

after PBSC transplantation [24], although retrospective comparative studies with BM transplantation (BMT) in adults [9, 10, 25] have demonstrated delayed neutrophil and platelet recovery in CBU recipients; this suggests that there is no strict correlation between cell dose and major clinical endpoints in the recipient. However, PBSCs also contain the highest numbers of T- and B-lymphocytes [26]; meta-analyses of randomized trials comparing BM and PBSCs as stem cell sources for HLA-identical and -matched unrelated donor transplantation have unequivocally concluded that the highest incidence of chronic graft-versus-host disease (GVH-D) occurs in PBSC recipients [27], suggesting that the cell composition of the infused cell product can influence the clinical outcome, and that the choice of cell source cannot be considered as neutral. Consistently, already mentioned retrospective comparative studies [9, 10, 25] have demonstrated the decreased incidence of acute or chronic GVH-D in recipients of CBU transplantation, as compared with recipients of BM transplantation. Because transplant outcome does not exclusively depend on engraftment and immune recovery, we will not extensively review here the numerous comparative studies that report on overall survival and disease-free survival following allogeneic transplantation with different sources of stem cells; these data will be presented elsewhere in this book.

However, because the low content of total nucleated cells (TNCs) and $CD34^+$ cells in CBU represents a definitive limitation in their use for allogeneic transplantation, several approaches have been evaluated as a means to improve engraftment and immune recovery. Ex-vivo expansion of hematopoietic stem cells is briefly described in the conclusion to this chapter, but implies extensive and expensive manufacturing of primary collected cells with no robust and definitive demonstration that it will consistently improve engraftment and immune recovery in routine clinical practice. The combination of several CBUs is technologically simpler and more accessible for academic cell-processing facilities and transplant programs. A few published reports evaluate double CBU transplantation: one report compared results in a large group of 536 patients with malignant diseases transplanted with an HLA 8/8 allele-matched related or unrelated donor, 1 allele-mismatched unrelated donor, or double umbilical cord blood transplantation (UCBT), but delayed neutrophil and platelet recovery was still demonstrated after double UCBT, and leukemia-free survival was similar in all groups [28]. Furthermore, an intriguing and consistent observation was the dominance of one of the two CBUs, the second one contributing to transient engraftment at best.

Processing

The vast majority of HSCTs performed nowadays rely on the intravenous infusion of cell grafts that qualify as “minimally-manipulated cell products”, and as such are regulated at national levels as “cell transplant” and not as medicinal products (in the European context these are not regulated as Advanced Therapy Medicinal Products

[ATMPs]). Processing is performed in cell-processing facilities that usually operate on relatively small scales, serving the local adult and pediatric programs. Allogeneic BM and PBSCs are usually collected and infused within 24 h; only major or minor ABO incompatibilities impose significant processing, with the need to deplete erythrocytes from the BM obtained from a donor with major ABO incompatibility, or the need to reduce plasma in the case of minor ABO incompatibility. In the case of ABO incompatibility, there is a lack of harmonization regarding the acceptable amount of residual erythrocytes in the processed BM, with published figures as high as 5 mL/kg of recipient body weight, but more widely acceptable figures are in the range of 0.2 mL/kg. Unrelated CBUs are collected, cryopreserved, stored, and distributed as a thawed product by licensed and often FACT/NetCord-accredited cord blood banks. The procedure for cryopreservation is very much like the procedure for the cryopreservation and thawing of autologous PBSCs, and relies on controlled-rate freezing after the addition of a cryoprotectant, of which the most widely used is dimethylsulfoxide (DMSO). In order to optimize the use of storage resources, most CBUs are nowadays miniaturized to a final volume of 26 mL. Little progress has been made in identifying new cryoprotectants or in the optimization of the freeze-and-thaw procedures. As technically simple as they appear, these processing procedures are far from being harmonized across transplant centers, because processing facilities have been implementing them for years, with more or less significant technical variations, and without formal initial validation and continuous monitoring, as are now required by modern quality management systems and made mandatory by accreditation processes or regulations, including good manufacturing practices (GMP).

Distribution and Delivery; Administration to Patient

Similarly to processing, distribution is the responsibility of the local processing facility. There is significant variability at this step. For cryopreserved products, most programs still use a simple thawing procedure, carried out at the bedside by transplant nurses and physicians; the procedure relies on a waterbath in which the product is immersed immediately before reinfusion to the patient. Despite the published evidence that the possibility of thawing cryopreserved autologous products in the cell-processing facility, using water-free devices and automated procedures, does not compromise hematopoietic recovery [29], few programs take advantage of this procedure, which allows for biological controls of the actual cell product that is reinfused to the patient. The delivery of unrelated CBUs, however, relies on the intervention of the cell-processing facility, which receives the frozen CBUs from the cord blood bank prior to the initiation of the conditioning regimen that is delivered to the recipient, ensures temporary storage before thawing and—when necessary—carries out dilution of the cell product immediately before delivery and reinfusion [30]. For other allogeneic cell products obtained from living related or unrelated donors, donor collection and recipient administration are usually

synchronized; non-substantial manipulations, quality controls, and appropriate labeling are performed immediately after collection in the processing facility, and the product is delivered within hours after the end of collection, with no need for cryopreservation.

Conclusion and Perspectives

While HSCT is now a widely accepted medical procedure used to treat a variety of severe malignant or non-malignant conditions that primarily affect the hematopoietic tissue and while thousands of preclinical and clinical reports have been published, it is puzzling that little is known regarding the relation between the cellular composition of the graft and the clinical and biological events triggered by engraftment. This is because engraftment represents a complex biological phenomenon, where both graft- and recipient-related factors affect the establishment of chimerism. Also, we lack the tools to follow the fate of donor-derived stem, progenitor, and mature cells in the recipient over time; the homing of infused stem cells, for example, cannot be easily monitored in human recipients. It is, however, likely that the variability in clinical outcome could be reduced through the harmonization of practices and procedures; this is one of the goals of the FACT-JACIE accreditation process, which implies the deployment of a quality management system, including but not limited to policies, standard operating procedures (SOPs), audits, reporting, and other basic tools of quality management. Nevertheless, the limited experience with the central manufacturing of more-than-minimally manipulated cell grafts suggests that the variability of the final product and the recipient clinical outcome also result from variabilities in donor characteristics, which themselves impact on the characteristics of the collected cell products. So far, engineered hematopoietic stem cells have met with little clinical success beyond the demonstration of feasibility. In the UCBT setting, multiple strategies under clinical investigation have aimed to overcome the low total cell and stem cell dose provided by a single CBU. These strategies focus primarily on methods to increase the cell dose of a cord blood graft, and include the use of ex-vivo expanded CBUs [31], the systemic addition of mesenchymal stem cells [32], and the use of agents to enhance cord blood homing to the marrow [33].

Recently published data on cellular therapies that nowadays qualify as ATMPS, such as CAR-T cells, have confirmed the cellular variability of extensively manufactured medicinal products derived from human primary material [34], as well as the absence of a simple dose-response relation, thus confirming that the dose-response relationship observed with conventional drugs cannot be easily transferred to the field of cellular therapies. Such limitations may disappear only with the design of even more advanced technologies that will produce off-the-shelf cellular therapy products from standardized raw or primary biological material such as fully characterized human cell lines. This still appears as a relatively distant perspective for HSCT.

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Chapter 10
Donor/Recipient Selection, Work-Up, and Safety

Joerg P. Halter, Nina Worel, and Jakob R. Passweg

Introduction

Decisions on whether to perform an autologous or allogeneic hematopoietic stem cell transplantation (HSCT) are based on different aspects. Nowadays transplant teams can choose among a wide variety of different conditioning regimens of variable intensity and potential for tissue damage, although some regimens might not be applied in all centers due to the unavailability of total body irradiation or certain drugs. Hence, the final decision is based on multiple criteria: disease risks, including remission status; genetic risk categories; patient comorbidities; and for allogeneic HSCT, donor characteristics, including degree of human leucocyte antigen (HLA)-match, donor age and gender, and the presence of donor-specific HLA antibodies or other donor characteristics, including any donor health disorders.

Even though some reduced-intensity regimens lead to only limited tissue damage, patients undergoing reduced-intensity transplantations still need to be fit enough to tolerate potentially occurring transplant-associated complications.

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Patient Selection and Work-Up

Recipient evaluation and selection for becoming a candidate for an autologous or allogeneic HSCT depends on several factors that impact the risk-benefit assessment. Most important to consider are the disease status at the time of transplantation and the patient's health status. Today a number of scores are available to estimate the risk-benefit ratio, based on disease risk (Dana-Farber/Center for International Blood and Marrow Transplant Research disease risk index [DRI]), transplant risk (European Society for Blood and Marrow Transplantation [EBMT] risk score), and patient-related risk factors (hematopoietic stem cell transplantation-specific comorbidity index [HSCT-CI], pretransplant assessment of mortality [PAM] score). These different scores are not perfect and may not be suited for every individual decision; they, nevertheless, provide a framework within which decisions may be made.

Beyond these scores, further important issues to consider when preparing recipient work-up are:

- When patients are referred for HSCT it is important to provide enough time to explain the HSCT procedure, starting from basics, in words patients can follow depending on their knowledge and education. Educational material from the transplant center or professional societies may help.
- Patients need the opportunity to ask questions and to participate in the decision for this treatment. Because intensive care and life support may be needed during transplantation, patients should also have the possibility of expressing their attitudes toward limits of care.
- Issues of fertility and the possibilities of cryopreservation of reproductive tissue may need to be discussed
- Many patients referred to transplant centers live at some distance from the transplant center. Hence, the planning of post-transplant care after returning home should already be considered before they start HSCT. This includes a careful assessment of whether patients and caregivers are able to read and/or understand and follow written or spoken orders.
- Financial restrictions may jeopardize optimum care. Lack of funding by authorities or insurance companies may delay or preclude HSCT. But even if insurance coverage is available, funding will not cover all important drugs, auxiliary devices, supplemental enteral nutrition, and travel costs, etc.

Underlying Disease and Remission Status

Many different disease entities are currently treated with HSCT, based on variable evidence. Furthermore, even if a disease can basically be cured by HSCT, there are clear impacts of sensitivity to chemo−/radiotherapy and especially of the remission status on transplant outcome. Guidelines on the indications for both autologous and allogeneic HSCT are regularly published by the EBMT and the American Society

for Blood and Marrow Transplantation (ASBMT) [1, 2], including a classification by disease and remission status based on level of evidence if appropriate. These statements are regularly updated to include new data. Still, it is important to consider that the recommendations are mainly based on the experiences and availability of alternative treatments in the United States and Europe. Recommendations might need to be adapted in areas with limited resources (e.g., lack of availability of tyrosine kinase inhibitors [TKIs] for chronic myelogenous leukemia in chronic phase 1 (CP1), difficulties in transfusion support for hemoglobinopathies, lack of availability of eculizumab for paroxysmal nocturnal hemoglobinuria).

The DRI is helpful for estimating adult patients' survival based on disease-related parameters (https://www.cibmtr.org/ReferenceCenter/Statistical/Tools/Pages/DRI.aspx) [3].

The EBMT risk score allows the estimation of the patient's prognosis for overall survival based on disease- and transplant-related factors [4].

Patient Clinical Assessment and Comorbidities

The patient's health condition is an important predictor of non-relapse mortality (NRM), which can be estimated by the HSCT-CI; http://www.hctci.org) [5] or PAM score; http://pamscore.org) [6]. These scores have been validated for several scenarios, but may need additional validation if used in centers with different characteristics or if used for diseases different from those initially approved.

When pre-transplant patient work-up focuses on comorbidities most pathologic findings will not lead to cancelation of the HSCT, but may lead to efforts to improve organ functions pre-transplant or modification of the transplant regimen (Table 10.1).

Performance status and age: With the use of reduced-intensity and non-myeloablative regimens, "chronological" age is no longer considered a strong limiting factor. Comorbidities, either related to or independent of previous therapies, at the end much better reflect the "biological age" of a patient and seem to have a bigger prognostic impact [7]. Clearly comorbidities become more prevalent with increasing age. The performance score gives a rough estimate of the patient's physical fitness. Currently most centers request a pre-transplant Karnovsky performance status (KPS) of at least 70% and recommend geriatric assessments in patients over the age of 70 years.

Cardiovascular system: Cardiovascular fitness is a precondition for successful HSCT. Previous or planned exposure to anthracyclines and high-dose cyclophosphamide, thoracic radiotherapy, volume overload, and septicemia may impair cardiac function before or during HSCT. A left ventricular ejection fraction of at least 45% is generally regarded as a prerequisite before starting conditioning. Arterial hypertension should be controlled and end-organ damage excluded or adequately treated. An electrocardiogram (ECG) is recommended to clarify conduction or rhythm abnormalities.

Table 10.1 Suggested minimum criteria for patient suitability for HSCT beyond remission status (adapted from Hsu JW et al. Patient evaluation before transplantation. In: Wingard JR, Gastineau DA, Leather HL, Snyder EL, Szczepiorkowski ZM, eds. Hematopoietic Stem Cell Transplantation: A Handbook for clinicians, 2nd edition, Bethesda, MD: AABB, 2015)

	Autologous HSCT	Allogeneic HSCT (wide range to include intensive and reduced-intensity regimens)
Upper biologic age limit (years)	70	45–70 (exceptionally up to 75)
KPS	≥60–70	≥60–70
LEV (%)	≥45	≥40–≥50
Cardiac rhythm	No uncontrolled tachy- or bradyarrhythmia	No uncontrolled tachy- or bradyarrhythmia
Pulmonary function tests:		
FEV1 DLCO	≥60% ≥60%	≥50–80% ≥50–80%
LFT (ALT, bilirubin)	≤2× ULN	≤2–3× ULN
Creatinine clearance	No limit, consider dose reductions if <60 mL/min	For intensive conditioning, ≥60 mL/min; no limit for RIC/NMA, consider dose reductions if <60 mL/min
Uncontrolled infections	Absent	Absent
Pregnancy test	Negative	Negative

HSCT Hematopoietic stem cell transplantation, *KPS* Karnofsky performance score, *LEV* left ventricular ejection fraction, *FEV1* forced expiratory volume in 1 s, *DLCO* diffusing capacity of the lungs for carbon monoxide, *LFT* liver function test, *ALT* alanine aminotransferase, *ULN* upper limit of normal, *RIC* reduced-intensity conditioning, *NMA* non-myeloablative conditioning

Respiratory system: Although pulmonary toxicity is more frequent after intensive conditioning, it still remains an issue after reduced-intensity regimens. Pretransplant identification of patients with impaired lung reserve by spirometry—and if possible by measuring the diffusing capacity of the lungs for carbon monoxide (DLCO)—is therefore mandatory, because these patients are at increased risk of the need for respiratory support and have an increased risk of NRM. Furthermore, a decreased pulmonary function test (PFT) predicts the risk for late declines in lung function. The need for smoking cessation must be emphasized.

Gastrointestinal and hepatic function: Assessment of the gastrointestinal tract should focus on potential locations of bleeding and infections, and monitoring for impaired nutrition status.

Elevated liver function tests (transaminases, alkaline phosphatase) and prior liver damage, including hepatitis virus infections (determined by careful assessment) have become established risk factors for sinusoidal obstruction syndrome of the liver and may require preventive measures and/or modification of the conditioning regimen. Iron overload and viral infections are important factors that may require the start of treatment before conditioning.

Renal function: Many drugs applied during HSCT have nephrotoxic potential (antibiotics, antifungals, and calcineurin and mechanistic target of rapamycin [mTOR] inhibitors). Adjustments of drug dosages are generally required if creatinine

clearance is below 60 mL/min. Decreased creatinine clearance is also a risk factor for post-transplant kidney failure. Early involvement of nephrologists is recommended if renal replacement therapy is anticipated or needed.

Infectious diseases: These might be one of the most important reasons, beyond disease status, for delaying or canceling HSCT. Active search should include pulmonary or hepatosplenic fungal or bacterial infections and florid dental infections, as well as viral infections (e.g., human immunodeficiency virus [HIV], hepatitis B virus [HBV]). Other viral infections may not delay HSCT, but may have an important impact on prophylaxis regimens or post-transplant monitoring if there is a risk of reactivation or primary infection (e.g., with Hepatitis C virus [HCV], cytomegalovirus [CMV], Epstein Barr virus [EBV], Herpes simplex virus [HSV], Varicella zoster virus [VZV], positive HBV serostatus).

The presence of upper respiratory tract viral infection at the beginning of conditioning may be a reason to postpone transplantation (respiratory syncytial virus [RSV], metapneumovirus, others).

Other comorbidities: These include, basically, every other organ system, as well as mental health issues such as anxiety, depression, and a history of non-compliance and/or substance abuse, including alcohol abuse.

Donor Selection, Work–Up, and Safety

Donor Selection

Donor selection is based on a number of criteria, with the most important being HLA-matching. During the past few years there has been rapid progress in HLA-mismatched HSCT, so that it has become difficult to recommend one single algorithm for selecting a donor. Centers need to define what kind of HLA-matching they accept for HSCT: HLA genotypically matched sibling donors, HLA-matched unrelated donors, single HLA mismatched donors, and/or haploidentical donors. The inclusion of further histocompatibility-associated criteria (such as killer-cell immunoglobulin-like receptors [KIR]-mismatch (https://www.ebi.ac.uk/ipd/kir/ligand.html) or models of permissive mismatching, HLA-DP expressor status, non-inherited maternal antigens [NIMA], and others) is often weighted against other donor characteristics that have an impact on transplant outcome or transplant processes; these characteristics include donor sex, age, prior exposure to viruses (mainly CMV), blood group, donor's preference to donate bone marrow (BM) or peripheral blood stem cells (PBSCs), donor's health disorders and consecutive risk for HSC collection or transmission of diseases by transplantation, donor's timely availability to donate, and the transplant center's resources and experience. Specific patient characteristics, such as alloantigen sensitization (HLA antibodies, human platelet antigen [HPA] antibodies, and antibodies against blood group antigens), may also have an impact on donor selection. The priority ranking of donor qualities is likely to be different for different patients and depends on patient characteristics,

disease and disease status; donor characteristics and stem cell source; time limits; and available resources, as well as the teams' experience. Hence, transplant teams need to define the decision algorithms in standard operating procedures (SOPs) for different indications, based on current knowledge and center-specific characteristics, such as experience and the availability of techniques for graft manipulation or conditioning (total body irradiation, measurement pharmacokinetics, etc.).

Responsibility for Donor Selection, Informed Consent, Medical Evaluation, and Donor Clearance

To ensure the decision for HSC donation is voluntary and to maximize donor safety, it is important to follow the basic principle of divided responsibility for donor and recipient care [8]. This includes:

Responsibility for obtaining donor's informed consent: Donors need to give their informed consent once or several times during the process, starting before HLA-typing and finally leading to the donation procedure. It is strongly recommended (and is increasingly mandatory by regulations) that, to avoid conflicts of interest, informed consent has to be obtained by healthcare providers familiar with the donation procedure, but without involvement in direct patient care. Conflicts of interest may also affect parents who are the legal representatives of pediatric or mentally handicapped donors who donate exclusively for a sick relative (most often a sibling, i.e., a child with the same parents); hence, today an independent donor advocate is recommended by an increasing number of professional societies and standard committees to allow an unbiased medical evaluation and consent process.

Responsibility for donor selection: The final decision for donor selection, based on HLA- and non-HLA criteria, is the responsibility of the transplant team. Neither the HLA-laboratory nor—if involved—the donor registry for unrelated donors, nor the collection team can take over this responsibility, but all of these entities may assist by providing information and advice to the transplant team in order to select the best donor.

Responsibility for donor's medical evaluation, final donor clearance, and donation procedure: After a donor has given informed consent and has been selected by the transplant team, it is the responsibility of the collection team—and if applicable the donor registry—to proceed with the donor work-up, provide donor final clearance, and perform the HSC collection. Again, to allow unbiased care, providers responsible for donor care should not be involved in the direct care of the recipient.

Even if the principle of divided responsibility cannot be met yet in some centers due to lack of resources, teams need to be aware of the risk of conflicts of interest and should take preventive measures wherever possible to avoid this risk as much as possible.

Confirmation of the donor's final clearance is a prerequisite for starting conditioning therapy in the recipient. It is the responsibility of the transplant team that they start the conditioning only after they have received this document.

Donor Evaluation and Work-Up

This chapter discusses donor evaluation and work-up for adult and pediatric BM or PBSC donors. For cord blood donors, special considerations may apply.

The donor evaluation for medical suitability ensures that: (a) the product is free of transmissible disease and (b) the donation procedure is safe for the donor and it is highly likely that an adequate number of HSCs can be collected in a timely manner.

Today, PBSCs, collected by apheresis, are the most frequently used stem cell source. PBSCs are almost uniquely used in autologous HSCT and mostly used in allogeneic HSCT, although it is still a matter of debate, depending on different indications, whether PBSCs or BM are preferable (cf. Chapter 9). From the perspective of graft safety, the donor work-up remains the same, independent of whether a BM or PBSC donation is planned. However, from the perspective of donor safety, although the donor work-up might be largely identical for BM and PBSCs, some differences between the collection procedures need to be taken into account when making the final decision on donor suitability.

Many aspects of donor evaluation are guided by regulations (national, international); local requirements (e.g., testing for infectious diseases); donor's health disorders; and the availability of resources for testing, with the need to balance costs against risk/benefit.

A number of guidelines from various authorities and societies are available that focus mainly on safety for unrelated donors and/or product safety. Helpful resources can be found at:

- https://wiki.wmda.info
- http://www.jacie.org/standards; http://www.factwebsite.org/Standards
- http://www.fda.gov/BiologicsBloodVaccines/default.htm

Less guidance is available for decision-making on the suitability of related donors who do not meet the characteristics of unrelated donors but may still be able to donate. Therefore the Worldwide Network for Blood and Marrow Transplantation (WBMT) Standing Committee on Donor Issues recently published two consensus recommendations on suitability criteria for pediatric and elderly donors, as well as related donors with health disorders who would not qualify as unrelated donors [9, 10].

Furthermore, it has to be considered that most available guidelines have been edited by European, North American, Japanese, or Australian/New Zealand organizations and therefore may fail to fully cover the special circumstances in other regions. For example, the recommended temporary deferral of donors after they have travelled to areas with endemic infections (e.g., malaria, chagas disease, Zika virus, etc.) might not be reasonable for transplant programs working in these areas. These issues were extensively addressed in an additional WBMT Donor Outcome Committee Workshop in 2016. Consensus recommendations are currently being prepared for publication.

The medical evaluation consists of several parts. These are: donor screening questions and personal history, physical examination, laboratory examinations, and further examinations as appropriate (e.g., radiologic examinations, ultrasound, ECG, and other cardiologic examinations, additional examinations). The aim of this evaluation is to detect donors who have an increased risk of transmission of diseases and/or have an increased risk of donation-related adverse events.

Personal History and Physical Examination

Personal history includes questions on general health, and past and current health disorders, including a past history of hematological, immunological, or malignant disease. Smoking and consumption of alcohol and substances causing addiction, as well as current medical treatments, give important information on donors' health status and risk behavior. Family history may point to hereditary disorders that are not yet clinically apparent. Systemic history must include questions on allergies and cardiovascular, respiratory, and neurologic symptoms and histories of neck and/or back injuries and pain. Although some of these questions are more specific for one type of donation (BM vs. PBSC), it is reasonable to ask all donors all of these questions, because the decision on which type of donation will be preferred may depend on these answers. For BM donation, there should be a complete history of previous anesthesias and their course and donors should be asked whether they have a personal or family history of malignant hyperthermia. For PBSC donation, donors should be asked whether they have previous experience with apheresis donations. History should include autoimmune disorders; splenic disorders, such as traumatic or atraumatic splenic conditions, including infections (EBV, CMV, malaria); and episodes of thrombocytopenia or thromboembolic disorders.

Questions intended to improve graft safety include:

- Vaccination history with live vaccines (e.g., oral polio, VZV, measles-mumps-rubella [MMR], yellow fever) during the previous weeks.
- Travel history to areas with: (a) endemic infectious diseases that are otherwise not routinely tested for, or their incubation time is within the window period (malaria, dengue, human T-lymphotropic virus [HTLV]) or (b) rare strains of infectious agents that are not routinely detected by current screening tests (e.g., rare strains of HIV).
- Blood transfusion history or treatment with other medical products of human origin, to assess the risk of blood-derived infectious diseases.

Physical examination encompasses a complete general examination. An additional focus needs to be made on anatomic abnormalities that may interfere with positioning for HPC collection [e.g., contractures or scars in the cubital region, obesity that may interfere with iliac crest puncture or venous punctures (cubital region or regions for catheter placement), pelvic skeletal abnormalities, etc.]. For

BM donation, a careful oropharyngeal assessment should be done, as well as an inspection for skin or skeletal abnormalities of the pelvis that may interfere with access to the iliac crests. For PBSC donation, venous access (veins, skin changes) needs to be checked early.

Laboratory Examination

Laboratory examinations focus on the donor's health disorders and the transmission of infectious or non-infectious diseases (Table 10.2). The minimum number of analyses required might differ for different donors, related to the donor's risk of infectious diseases, the donor's health, the results of the personal and family histories and physical examination, and the area of residence or travel history.

Table 10.2 Laboratory evaluation of donors

• Complete blood and differential count
• Blood chemistry: electrolytes, BUN, creatinine, ALT, alkaline phosphatase, bilirubin, LDH, glucose, protein, albumin, blood sedimentation rate or C-reactive protein
• Urine analysis
• Blood coagulation test
• ABO blood group and Rh type (type and screen-test) complete blood group typing
• Serum electrophoresis and immunofixation and immunoglobulin levels are recommended
• For female donors: pregnancy assessment
• Screening for hemoglobin S (HbS; either by hemoglobin analysis or by obtaining donor's origin and history) and if positive for HbS-beta thalassemia, sickle cell (SC) or other complex sickle cell hemoglobinopathies
• If transplant indication is a hereditary disorder, related donors need to be checked for carrier status that may affect transplant outcome
• Infectious disease agents:
– HIV-1, HIV-2
– Hepatitis B, hepatitis C
– Treponema pallidum (syphilis)
– CMV (unless already documented during donor selection process)
– Additional tests as indicated to minimize the possibility of transmission of infectious diseases (or as requested by national guidelines and/or authorities):
Hepatitis E
HTLV I/II
West Nile virus
Trypanosoma cruzi (Chagas)
Others (based on medical/travel history, including risk assessment for infectious diseases depending on area of residence, time, and season of stay in this area and changing geographic spread of infectious diseases)

BUN Blood urea nitrogen, *LDH* lactate dehydrogenase, *HIV* human immunodeficiency virus, *CMV* cytomegalovirus, *HTLV* human T-lymphotropic virus

Other Examinations

Depending on the donor's personal history and the findings of the clinical and laboratory examinations, further examinations may be indicated to exclude or further characterize health disorders. These examinations may include:

- Chest-X-ray or computed tomography (CT) scan
- ECG, echocardiogram, ergometry, diagnostic cardiac imaging or catheterization
- Pulmonary function test
- Ultrasonography
- Endoscopy
- Others.

Adverse Events Associated with HSC Donation and Donor Safety

Both BM collection and PBSC collection by apheresis are associated with adverse events. Most of these events are harmless (although bothersome when present), of mild to moderate intensity, and transient. Serious adverse events are rare, but they do occur. These include: (1) death, (2) life-threatening events, (3) events requiring in-patient hospitalization or prolongation of existing hospitalization owing to World Health Organization (WHO) grade 3 or 4 toxicity, and (4) events that result in significant disability/incapacity [11].

Frequent side effects associated with BM collection or the apheresis of filgrastim- or lenograstim-stimulated PBSCs are well described [12, 13] and seem to be similar for unrelated and related donors. Bone pain is the most frequent adverse event with both types of donation (up to 80%), mostly of mild to moderate intensity, with a peak before and during (PBSC) or after donation (BM). Other frequent side effects include fatigue, insomnia, anorexia, nausea, and dizziness. Headache and flu-like symptoms with myalgia are also common in PBSC donation. Overall, the donation-associated discomfort is similar for BM and PBSC donors in terms of the pattern and maximum intensity of symptoms, with different times of onset and recovery. Usually the adverse events do not lead to a premature stopping of the donation procedure. Donors who are obese, older, or female are more prone to experience this donation-associated discomfort [12].

Rarely, severe adverse events do occur with BM or PBSC donation [12–17], including very rare fatal complications (Table 10.3). Readers may be also referred to the Notify Library (http://www.notifylibrary.org) for more information on severe adverse events associated with HPC donation.

Although there are no prospective studies, it is reasonable to suggest that careful donor evaluation is able to minimize the risk of donation-associated adverse events.

Table 10.3 serious adverse events associated with HPC donation

With bone marrow donation:
• Associated with risks of anesthesia: Arrhythmias with/without cardiac arrest, myocardial infarction, stroke, pulmonary edema, PE, malignant hyperthermia, anaphylaxis
• Local complications: wound infection (local, systemic); osteomyelitis; fractures; nerve, bone, or tissue injury; hemorrhage with compression of tissue and severe pain; chronic pain
• (blood loss with need for allogeneic transfusion—sometimes classified as SAE)
With PBSC donation:
• Catheter-related: Bleeding, thrombosis, pneumo−/hematothorax
• Related to apheresis procedure: Hypocalcemia, thrombocytopenia and anticoagulation with/without bleeding, need for priming with allogeneic blood
• Associated with biologic actions of G-CSF: Allergic reactions/anaphylaxis, splenic rupture, respiratory distress/acute lung injury, triggering or flares of inflammatory diseases, thrombosis (arterial, venous), sickle cell crisis

PE Pulmonary embolism, *SAE* significant adverse event, *PBSC* peripheral blood stem cell, *G-CSF* granulocyte-colony-stimulating factor

Standard operating procedures (SOPs) for collection methods, including definition of the maximum volume collected, the volume processed, and number of donations help to further increase donor safety.

Long-term Follow-up after HSC Donation

The long-term safety of HSC donation after growth factor administration needs to be further investigated. Theoretical concerns about late effects after the administration of traditional G-CSF formulations (filgrastim, lenograstim) have not yet been confirmed [18]. The long-term follow-up of related donors, as well as that of donors after multiple donations or donors after the use of new mobilizing agents still presents challenges.

Subsequent Donations

Subsequent donations of HPC or donor lymphocytes may become necessary for a variety of reasons (poor graft function; persistent minimum residual disease; relapse; or to improve donor chimerism, enhance graft-versus-tumor effect [especially after reduced-intensity conditioning], or to generate virus-specific cytotoxic T-lymphocytes). Although data on subsequent donations are limited, current experience suggests that collection yields tend to be slightly lower and donor's adverse events are similar on the first and second donations. Donor evaluation for subsequent

donations remains the same as that for the initial donation, but includes experience of previous donations. Collection teams should have a policy for the way they handle donors for subsequent donations, including the time intervals and maximum number of donations employed. Currently only recommendations for unrelated donors are published [19].

Pediatric Donors

Collections from pediatric donors for related patients have been performed since the beginning of HSCT. Special considerations have recently been addressed [10]. Children can donate BM safely, but may need allogeneic blood for volume replacement. Tolerance of PBSC collection is similar to that in adults, but the need for central venous catheters or allogeneic blood (for priming) is more frequent. Hence, centers need to define their SOPs for pediatric donors separately. Serious adverse events are rare [17]; however, it was recently shown that the health-related quality of life in pediatric donors may be less than previously suggested and this needs further attention [20].

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Chapter 11
Essential Requirements for Setting Up a Stem Cell Processing Laboratory

Mickey B.C. Koh, Mark Lowdell, and Tom Leemhuis

Introduction

One of the key components of a successful and sustainable hematopoietic stem cell transplant (HSCT) program is the presence of a robust and reliable cell processing laboratory (CPL). The overarching purpose of the CPL is to adequately support either an autologous-only transplant program or a combined autologous/allogeneic program. At present, stand-alone programs performing only allogeneic transplants are less common.

The fundamental function of the CPL is to preserve, as much as possible, the integrity and quality (including viability and sterility), as well as the quantity, of the hematopoietic stem cell (HSC) graft product that it receives. This function should be performed in an accurate, reproducible, and validated manner that adds confidence to the clinical transplant program. Many of the principles, such as process control, traceability of validated transport conditions, product testing, release criteria, quarantine, consistency, automation, and quality systems, as well as personnel proficiency, are derived from blood-component processing in transfusion services.

Once established, these principles and essential elements can be used as a template for the future expansion of numbers and capabilities, such as manipulation of the graft (CD34 selection, T-cell subset depletion). This chapter is therefore focused

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on getting these principles right—and the planning and forethought that should be invested from the start.

Although HSCT is a global activity and is regulated in different ways and with different requirements around the world, the essential requirements for a processing laboratory to provide autologous and allogeneic HSCs for therapy are the same. Similarly, accreditation of stem cell processing laboratories varies widely across the world, so we will discuss the global standards set by the Foundation for the Accreditation of Cellular Therapy (FACT)/JACIE (Joint Accreditation Committee of the International Society for Cellular Therapy [ISCT] and the European Group for Blood and Marrow Transplantation [EBMT], rather than try to present the statutory licensing procedures applied by individual countries.

Establishing a Quality Management System

When planning a new processing laboratory, the capital budget is usually the first challenge but, in fact, the creation of a quality management system (QS) with associated document control should be the first deliverable. This is essential, because the QS is used to control the design and equipment of the facility and the subsequent qualification of the unit and the procedures.

When building a QS for the first time, the mantra should be "proportionate and lean". Take advice from colleagues (especially those who run blood banking and pathology laboratories, where QSs have become a way of life over the past 30 years, and from other stem cell transplant laboratories). It is good practice to compare and adapt existing QS plans and, currently, platforms and discussion groups do exist for the exchange of such information, as well as the discussion of new and innovative practices. These can be country-specific organizations like the Quality Manager Forum in the United Kingdom or international organizations like the Alliance for Harmonization of Cell Therapy Accreditation and the Worldwide Network for Blood and Marrow Transplantation (WBMT).

Most QSs contain three types of documentation: policies, standard operating procedures (SOPs), and forms. A labeling system should be designed for each type of documentation, such as P/001/01—Policy number 1 version 1. The policy document defines how an aspect of your laboratory works. SOPs describe what you do to ensure that the policies can be delivered. Forms are used to track events resulting from an SOP. The first SOP to be written should describe how to write documents (SOPs, forms, policies) and how document numbering is applied. Ideally, appoint the task of document numbering to a single individual (quality manager) who can then assign document numbers and keep control of new versions. Every document should state who wrote it, who approved it, when it was implemented, and when it needs to be reviewed. The document should explain which staff members need to be trained to carry out its requirements, and their individual training needs to be recorded in their personal training record (which is an example of a form).

Laboratory Design

Capital expenditure will cover the initial facility build cost and the equipment needed for the laboratory, but management of the design, build, fit-out, and subsequent validation is best performed within a documented "change control" process; in some countries, this is a requirement for the licensing of the facility. Change control is the way you ensure that the introduction of a new process, piece of equipment, or even a whole facility is systematically managed.

The change control will list all of the critical aspects of the project and identify the staff members responsible for each aspect, it will also give dates for the proposed completion of each task, so that the inter-relationship of tasks can be seen and appreciated by the staff members involved. An essential part of the change control process is the risk assessment for each step, which should be documented, and the risk mitigated where possible or accepted as a risk and managed in a planned process.

- Crucial Considerations or Critical Aspects
  - What products are going to be made, how many per week, and how long is the working day?
  - How large does the laboratory/facility need to be?
  - What Local and International standards such as the widely accepted International Organization for Standardization (ISO) standards does the laboratory need to meet?
  - What is the planned flow of staff, materials, and finished product?
  - How are raw materials, starting materials, and finished products quarantined if needed?
  - How much storage space is needed and at what temperatures?
  - Will there be a need for liquid nitrogen (LN)? How close can the bulk tank be located to the facility (LN_2 piping is expensive and inefficient. There are physical limits on the height to which LN_2 can be pumped)
  - If mechanical freezers are to be used for some or all of the storage, how will these be backed-up (CO_2 or LN_2)
  - A list of critical equipment for the new facility and specifications for each item
  - A qualification process to be used during and at completion of the project to determine whether the design specification has been met

- A validation process to cover:
  - Installation (IQ)—does the laboratory/facility meet the design specification and is each piece of equipment properly installed?
  - Operation (OQ)—does the laboratory/facility work as planned when a process is run?
  - Process (PQ)—does a specific process produce the product that meets the product specification?

- A list of documents is to be provided by the contractor to the end user to ensure that ongoing routine maintenance can be performed and emergency responses to breakdowns can be provided. It is a good idea to require the contractor to provide a 6- or 12-month service initially to cover breakdowns and to perform the first annual routine maintenance.

The change control will help to identify all of the likely capital expenditure required to build, equip, and validate the new laboratory, so this process becomes an essential part of the business plan for determining the budget. It is a "live" document until the very end of the project, when it can be closed and signed off by the laboratory director. In many cases the authority regulating the stem cell laboratory will ask to see the change control for the new build and to see who signed it as "closed".

When first establishing a transplant program, and if financial or physical constraints are an issue, a relatively small dedicated space, or even a clearly defined shared space, can be sufficient for a cell processing laboratory, provided that product safety risks, such as cross-contamination or product mix-ups, are taken into consideration by the design and location. The laboratory should be located as far from potential contaminants as possible and in as clean a space as possible. When space is shared with a hospital laboratory, locating the CPL adjacent to the blood bank can be advantageous, because the equipment needed, procedures used, product safety focus, and staff training needs are similar. One must also plan ahead for any projected growth of the program and its consequent need for an expansion of processing capabilities.

The FACT/JACIE standards do not prescribe a minimum air quality standard, but they do require that the space is of adequate size and appropriately ventilated, with surfaces that can be easily cleaned; these standards also require that the facility is secure from the entry of non-accredited personnel. In many countries, a CPL will be required to meet minimum air quality standards, so ensure you are aware of the legal requirements applying to your facility. Remember to factor into the design adequate lighting, restricted access, sufficient supply storage space, readily cleanable and impervious work surfaces, a separate product storage area, and a separate office.

It is a good principle to keep processing areas separate from support areas, such as stores/inventory areas. Ideally, separate workstations and rooms should be dedicated to a single product at a time, so as to prevent cross-contamination and minimize the chance of accidental product mix-ups. Each workstation should contain a class II microbiological safety cabinet (MSC) providing ISO 5 air quality, at least 2 metres of bench space, a plasma extractor, and a centrifuge, with access to a refrigerator, a −70 °C (or colder) freezer, and a microscope nearby. The number of workstations needed would depend on the number of products anticipated to be processed in a day and on the number of staff available for processing.

Storing products in a mechanical freezer requires a consistent and reliable electrical power source; if the likelihood of power interruptions is high, it is safer to store the products in an LN_2 freezer. If products are to be stored in tanks requiring liquid nitrogen, the facility must either be designed to facilitate the regular delivery of liquid nitrogen supply dewars or, if possible, designed with a vacuum-insulated delivery system connected to an outside storage tank of considerable capacity.

All cleaning protocols and schedules should be clearly outlined and validated. The temperature and humidity of the laboratory space should be controlled (and monitored) to the extent necessary to maintain proper storage conditions for reagents and supplies, and for employee comfort. Bio-hazardous waste and sharps can usually be disposed of according to hospital practices, and several lockable file cabinets are usually required for storing facility documents and product records securely.

Process Flow

The laboratory should be designed to minimize the chance of product contamination or cross-contamination. This can be achieved by designing a product work flow that is as close to unidirectional as possible. Starting materials, such as patient or donor apheresis products, should enter one end of the processing suite and, where possible, the finished products should leave through a separate route.

Also make use of information technology (IT); this can facilitate electronic data and record storage, thereby minimizing the need for physical space. Product traceability and inventory can be made more robust by leveraging on IT systems. An example is the widely adopted ISBT128 labeling system, which provides for consistency worldwide.

Processing Capabilities

Cryopreservation and Storage

The CPL's primary roles in supporting an autologous transplant program are: graft characterization, cryopreservation, and secure storage, together with transport, product thawing, and outcome analysis procedures. Autografts are collected prior to the patient receiving high-dose therapy, then they are cryopreserved and stored frozen for anywhere from several days to a few weeks, months, or even years [1].

Storage at 2–8 °C maintains acceptable hematopoietic progenitor cell viability for only relatively short periods of time (48–72 h) [2]; therefore, cryopreservation is required for longer storage periods. Most cryopreservation protocols involve volume reduction, the addition of a cryoprotectant solution (usually dimethylsulfoxide [DMSO]), controlled-rate (slow) freezing, and storage in protective metal canisters at vapor-phase LN_2 temperatures (≤ -160 °C).

Before freezing the cells, it is best practice to place the bag of patient's cells in an overwrap bag that can be vacuum-sealed. The "double-bagging" is a requirement in many countries to minimize the risk of the cross-contamination of products in storage. Hence, a vacuum wrapper is another essential piece of equipment, and most laboratories use a commercial food vacuum wrapper. The double-wrapped cells are now ready for cryopreservation and two options are available; controlled-rate versus "dump" freezing.

Detailed cryopreservation protocols for controlled-rate freezing are available from published articles and chapters in books [3–5] and from the ISCT and American Association of Blood Banks (AABB) professional association websites. Procedures for performing these steps are far from standardized; however, strict adherence to aseptic technique is universally required. The use of closed systems for product handling greatly reduces the risk of contamination.

The preferred method practiced by most established centers makes use of a controlled-rate freezer, where vapor-phase nitrogen (VPN) is added to a chamber containing the stem cell product in a controlled manner to reduce the temperature by approximately 1 °C/min down to −80 °C over about 90 minutes before transferring to long-term VPN storage below −155 °C [6]. The alternative is to place the bag of cells into a polystyrene box and thence into a mechanical freezer at −70 to −80 °C, where the cells will slowly freeze. Mapping studies using temperature probes within these boxes have confirmed that "dump" freezing can achieve a rate similar to that of controlled freezing, and the quality of products is the same with respect to long-term viability and patient engraftment [7, 8], provided temperature fluctuations are avoided. However, for longer-term storage (years), cryopreservation in VPN is still the preferred method [9].

A start-up CPL should demonstrate their chosen cryopreservation protocol results in terms of an acceptable post-thaw viability ($\geq \approx 70\%$) prior to performing their first transplant. Once established, the laboratory should routinely monitor outcomes and watch for adverse trending as part of their quality plan.

Transfer of the cryopreserved cells to long-term storage is best done within a "dry shipper", which is pre-charged with LN_2 to ensure an internal temperature of below −155 °C. This dry shipper can be used both to transfer frozen products from the controlled freezer to long-term storage and then from long-term storage to the patient's bedside when needed for re-infusion. It is very valuable to have two dry shippers, because they can be used for short-term quarantine storage when a product has to be processed and stored before the results of compulsory screening tests for infectious diseases are available.

Products are best thawed in close proximity to the patient in a sterile overwrap bag and a 37 °C water bath, not letting the product warm past ambient temperature, and minimizing the DMSO exposure time prior to infusion [10]. If feasible, washing the products in the laboratory to remove DMSO may reduce the incidence and severity of adverse reactions [11–13]. Products can be thawed (one at a time) in the laboratory and transported to the hospital in a cooler if the products can still be infused within 15–20 min of thawing; or if the cryoprotectant is removed, products may be stored at refrigerator temperatures for up to 3 h.

Allogeneic Capabilities

The laboratory needs to have a larger range of graft manipulation capabilities if it also supporting an allogeneic transplant program. Many allogeneic products can be infused directly, without processing, other than sampling for sterility and performing cell counts and determination of CD34+ cell content. In addition, the laboratory

may need to perform either red blood cell (RBC) depletion or plasma depletion on ABO-incompatible bone marrow products and plasma depletion of mobilized peripheral blood products. Red cell depletion is rarely required for peripheral blood stem cell (PBSC) grafts, as there is usually minimal or acceptable red cell contamination, which is not the case for bone marrow harvests. There are a few ways to accomplish the RBC depletion; most laboratories perform either a gravity sedimentation procedure after adding hetastarch [14, 15], or else they process the marrow on an apheresis machine, collecting the buffy coat layer, as is done during a stem cell collection. Depending on the amount of donor plasma present, the donor's isoagglutination titers, and donor/recipient blood types, plasma depletion may or may not be required for minor ABO-mismatched products [14, 16]. Plasma depletion can be accomplished with one or two centrifugation steps and by removing the majority of the plasma prior to infusion.

The preparation and processing of donor lymphocyte infusions (DLIs) is sometimes required, either prophylactically as part of a planned protocol or in an unplanned process to treat disease post-allogeneic transplant, and the CPL needs to be familiar with the processing of DLIs are generally collected via apheresis as part of the allogeneic graft collection, or separately, if there is disease relapse and the process was previously unplanned.

Cellular Therapy Product Characterization

The stem cell graft, whether allogeneic or autologous, is critically dependent on the CD34 cell count, which is a surrogate marker for hematopoietic stem cell activity and its regenerative capacity [17]. This is applicable to both PBSC and bone marrow collections. In addition, the total nucleated cell count is preferred for bone marrow grafts, as these grafts contain a wide range of cell types; historically, the total nucleated cell count was used before the advent of CD34 enumeration. It has been demonstrated that the blast cell count and the number of immature myeloid cells in the blood are predictive of the timing of collections and harvest numbers, and ultimately of engraftment [18, 19].

Although the minimum number of CD34+ cells required for engraftment has not been firmly established, most investigators accept a minimum of 2×10^6 CD34+ cells/kg for optimal engraftment [20]. When possible, higher doses, of 5×10^6 CD34+ cells/kg, are preferred, because they are associated with faster engraftment, reduced incidence of infection, and reduced need for transfusions, especially in transplantation for non-malignant diseases such as aplastic anemia, where the graft failure rate is higher.

A more specialized flow cytometry analyzer is required for CD34+ cell detection. This analyzer is required for characterizing the graft and for the enumeration of peripheral blood CD34 counts during mobilization. Adhering to published guidelines for the CD34 analysis is critically important, because the stem cells represent a small percentage of the total number of cells and must be carefully enumerated [21–23]. The use of a "single-platform" method for the enumeration of viable

CD34+ cells is recommended for many low-volume start-up laboratories. The laboratory requirements would be a dilution buffer, sample tubes, syringes, and needles, a viability dye, and fluorescently labeled monoclonal antibodies to CD34 and to CD45 (and to CD14 if assessing mononuclear cells), although currently there are kits available that contain all the necessary reagents for the "single-platform" method. External quality control (QC) testing in a National External Quality Assessment Service scheme is highly recommended.

Most regulatory agencies require a sample for cellular therapy product sterility testing for aerobic and anaerobic bacteria and fungus to be taken prior to product infusion. This testing is best done in collaboration with a hospital microbiology laboratory that can also provide antibiotic sensitivity testing on any organisms found. Line infections or bacteremia at the time of collection are the most common sources of contamination for autologous peripheral blood products. Likewise, during bone marrow harvest, skin contaminants are not infrequently introduced. Therefore, we recommend testing HSC products before and after processing, just prior to either infusion or cryopreservation, to facilitate investigation of the cause of any identified contamination. This is especially important in a start-up situation, because the laboratory has to know that they are not introducing contaminants.

A typical HSC product testing plan is provided in Table 11.1. The testing plan should be designed to prove the product's identity, purity, potency, and (most importantly) safety. Note that all product release testing for fresh infusion products needs to be performed immediately, so that the product can be infused in a

Table 11.1 Quality control testing for hematopoietic stem cell (HSC) products

Attribute	Test method	Specification
Donor screening	Summary of records; donor eligibility form	Donor eligible
Infectious disease testing	Certified laboratory	Negative (exclusive of cytomegalovirus; CMV)[a]
Infusion volume	Measurement	≤20 mL/kg/infusion
DMSO volume	Calculation	≤1 mL/kg/day
Total nucleated cell (TNC) count	Automated cell counter	As measured
Red blood cell (RBC) content (if ABO-incompatible)	Automated cell counter	≤20–30 mL/adult infusion
CD34+ cell count	Flow cytometry	$\geq 2 \times 10^6$/kg
CD3+ cell count (if allogeneic)	Flow cytometry	As measured
Viability (pre-freeze)	Flow cytometry	≥80%
Sterility	Bacterial culture	No growth
Sterility	Fungal culture	No growth
Final product labeling	Observation	Labeled correctly

[a]Infectious disease testing of autologous products is not universally required worldwide. Consult national regulations

timely manner. Products can still be infused if they do not meet pre-determined acceptance criteria, but only if there is an urgent medical need and only with documentation from the transplant physician and/or the approval of the medical director.

Personnel

Education and training of staff is critical for the establishment and operation of a CPL. Staff with formal education in a biological science, preferably a laboratory-based discipline, with some experience in clinical hematology and/or blood banking, are most suited for the work that is required. Attention to detail, strict aseptic technique, a focus on quality, and an understanding of the importance of each product are also key attributes.

A minimum of two trained laboratory technicians is required; not only to cover absences due to illness, but also owing to the variability of the workload in transplant programs. A second individual is also essential for the verification of procedures and of product and patient identity. Mistakes can be fatal for the patient; having two people reviewing records prior to product release minimizes the likelihood that a mistake will occur. It is also essential that one person work with only one stem cell product at any one time to prevent mix-up of samples. Quality Control (QC) testing of the product can be performed by the cell processing staff if necessary, or it can be contracted out to the hospital's microbiology, flow cytometry, and/or hematology laboratories.

Ideally, an individual should be separately hired to concentrate primarily on quality systems and regulatory tasks, such as reviewing charts, inspecting raw materials, releasing products for infusion, doing process improvement projects, reviewing incidents, and performing internal regulatory compliance audits. This will provide a framework of quality for the laboratory and improve objectivity and impartiality, thereby leading to more consistent outcomes and the reduction of avoidable errors. This person needs to report quality parameters to the clinical program director and to the hospital's overall quality management personnel regularly, and should be supervised either by the clinical program director or by someone in the hospital's compliance office. In addition, staff will also be required for operational issues such as raw-material purchasing, facility cleaning, and equipment calibration.

Equipment

The equipment requirements for a CPL are fairly minimal, and are listed in Table 11.2. The equipment listed as shared is necessary for the CPL's use; however, because of expense, maintenance considerations, and low-volume use, such

Table 11.2 Essential cell processing laboratory (CPL) equipment

CPL equipment		
Biosafety cabinet	Refrigerator	Balance (scale)
Controlled-rate freezer	Centrifuge (with carriers to hold 600-mL blood bags)	Freezer (≤−70 °C)
Plasma extractor	Tubing sealer	Liquid nitrogen (LN_2) storage freezer
Cryo-transporter (−80 °C) or liquid nitrogen dry shipper	Micropipettes (100 uL and 1000 uL)	Sterile connecting device
Water bath	Hemostats	Tubing stripper
Personal computer	Tube racks	Filing cabinet(s)
Bag sealer		
Shared equipment		
Flow cytometer	Hematology analyzer	Label printer

equipment can often be shared with another laboratory that is within reasonable proximity. More specialized equipment would be required for more complex stem cell processing, such as T-cell depletion. Critical equipment should be maintained and calibrated on a regular basis. Backup equipment should be identified when only one device is in use by the laboratory. Each piece of equipment, including that designated as backup equipment, should be qualified prior to use [24]. If an uninterruptible emergency power supply is available, the critical pieces of equipment should be connected to that supply. Refrigerators and freezers that store patient products or critical reagents should be in a secure location, with only authorized personnel having access and, if at all possible, this equipment should be connected to a continuous temperature-monitoring system with alarms that notify key personnel when temperatures are out of range. All freezers will ultimately fail; therefore access to a backup freezer, or another contingency plan in the event of a freezer outage, is highly recommended.

Supplies and Reagents

A list of supplies and reagents that will be needed is provided in Table 11.3. All reagents that will be in contact with the product need to be sterile and infusion-grade. All supplies need to be sterile and disposable. Reagents can be dispensed into single-use containers prior to use, in order to minimize waste. All reagents and supplies need to be inspected prior to use and stored in controlled (and monitored) environments, separate from potentially harmful research reagents, and it is important to document the specific lot numbers used during processing. Careful materials management requires significant effort, but contributes greatly to error prevention and overall product quality.

Table 11.3 Essential cell processing laboratory supplies

Cryobags	Transfer packs (300; 600 mL)	Syringes (1, 3, 10, 30, 60 mL)
Safety needles; couplers	Spike-to-needle, spike-to-spike adapters; stopcocks	Alcohol swabs, iodine swabs, syringe caps, sterile swabs
Labels, laminating tags; zip ties	15, 50, 175 mL conical tubes	Pipette tips
Biohazard sample bags	Dimethylsulfoxide (DMSO)	Dry ice
Cryovials, microtubes	Hetastarch	Dextran
Sterile overwrap bags	Plasmalyte (or equivalent)	ACD-A
Human serum albumin	Hetastarch	Heparin
70% IPA; bleach; bactericidal and fungicidal detergent	Biohazard bags; sharps containers; garbage bags; trash can	

ACD-A; Anticoagulant Citrate Dextrose Solution, Solution A
IPA; Isopropanol

Clinical Correlates

It is important that the processing laboratory has a close relationship with the clinical transplant program and is viewed as an integral part of the transplant team. The laboratory should be represented at clinical transplant planning meetings so that expected collection dates and transplant dates are clearly communicated. The apheresis efficiency should be analyzed regularly and harvest numbers correlated against CD34 enumeration in the peripheral blood. Laboratory staff should have access to engraftment data that can and must be correlated with the stem cell collection data (CD34 count, total nucleated dose, and viability). Any incidents or adverse events arising from the graft product should be systematically reviewed. Often, one of the transplant physicians serves as the medical director of the CPL, providing oversight and guidance for laboratory staff.

Traceability, Vigilance, and Quarantine

Regardless of whether the laboratory supports an autologous or an allogeneic transplant program, strict product labeling, transportation, and product-tracking procedures need to be in place, from the beginning through to infusion, to prevent product mix-up errors and to maintain product integrity while delivering the product to the patient. Complete traceability is essential and IT systems, as well as ISBT labeling, will aid in this traceability. As discussed above, there should be systematic reporting of unexpected and adverse events. If possible, near misses and any other relevant incidents should also be reported. If possible, the lessons and principles learned from the hemovigilance systems pioneered in blood banking should be adopted. Also, non-punitive reporting (not only by the laboratory staff but also by the clinical

and hospital staff} of all adverse and unexpected events; issues related to transport, delivery, and thawing; and other clinical issues that might be relevant to the graft product should be actively encouraged [25].

A robust quarantine and discard as well as an informed consent system should also be in place as part of the quality infrastructure to prevent cross-contamination and maintain good use of the cryopreservation storage space.

Regulation and Accreditation of Stem Cell Laboratories

Until the mid-1990's, stem cell transplant products were largely unregulated in any systematic and consistent manner across the world. Nearly every clinical stem cell transplant program had its own dedicated processing laboratory and there was little comparison of the quality of stem cell transplant products between laboratories. The introduction of CD34 enumeration and stem cell mobilization for the production of PBSCs led to many centers setting up CD34 monitoring of patients' blood during mobilization, and some laboratories did participate in early external quality assurance schemes [26]. In 1995 the Council of Europe held a meeting to discuss the need for statutory legislation to control stem cell transplant products; this coincided with the establishment of FACT in the United States in 1996 and the beginning of global standards for cell therapies.

Today the joint FACT/JACIE standards and associated guidelines form a central tenet for stem cell transplant laboratories across the world. These standards have a section that exclusively concentrates on stem cell processing and the product itself. The standards in this section are not statutory requirements, but they rarely fail to reach legal requirements in countries where legislation is in place. Across the European Union, routine stem cell transplant products (autologous and allogeneic) are regulated under the European Union Tissue and Cells Directives (EUTCD), which set minimum standards for procurement, processing, storage, labeling, transport, and traceability. These Directives have been enacted into law in each European Union member state and each state has created authorities that inspect and license each laboratory at least every 2 years.

The joint FACT/JACIE standards are being increasingly used by laboratories and clinical programs as a measure of quality and benchmarking, and many international centers (across Asia and the Middle East) have now achieved accreditation status too.

In addition to FACT/JACIE, other organizations are also involved in the area of stem cell processing; and American Association of Blood Banks (AABB) runs an accreditation scheme that follows on from their trusted and respected history of accrediting blood banks.

In the United States, routine stem cell transplant products from autologous or related-allogeneic donors are regulated by the Food and Drug Administration (FDA) under section 361 of the Public Health & Safety Act, which is akin to the EUTCD. In contrast to the European Union, the United States FDA regulates unrelated cord

HSCT products, including cord blood transplants from banks such as the New York Cord Blood Bank, under section 351 of the Public Health Service Act; this regulation treats these products more like cellular medicines, such as chimeric antigen receptor CAR-T cells or cultured mesenchymal cells for tissue repair.

In Australia, the Therapeutic Goods Administration (TGA) regulates allogeneic HSCT products as "biologics", while in other countries, such as Japan, the use of hematopoietic stem cells for transplantation is enshrined in an Act under the responsibility of the National Government.

These examples demonstrate the complexity of the ways that HSCT is regulated in different parts of the world, and every new stem cell processing laboratory needs to be aware of the legislative framework under which it will be working. However, the standards applied to the screening, processing, labeling, transport, and storage of HSCT products are largely harmonized internationally, and this permits the global exchange of allogeneic products from related and unrelated donors.

Laboratories should also be aware of the legal and international framework for the importing and exporting of hematopoietic stem cells. This is often done via donor registries that are part of the WMDA. The WMDA also have an accreditation system for these registries.

A useful resource are two Aides Memoires developed by the World Health Organization (WHO) and its affiliated organization, the WBMT, for National Health Authorities; these should be of use to any organization intending to start a hematopoietic stem cell transplant program. One Aide Memoire outlines the minimal elements that should be considered in starting a program, including, among others, legislative issues, quality systems, and infectious disease prevention, while the other Aide Memoire outlines the key safety requirements needed for such a program, with emphasis on donor criteria and graft product processing.

Conclusions

This chapter has attempted to outline the essential considerations to consider when starting a cell processing laboratory. It has intentionally assumed that various constraints may be in place (financial, physical space, staffing) and has offered a practical guide on how to navigate such constraints. There is a concerted attempt by the WHO and WBMT to actively encourage transplantation activity worldwide, and the clinical indications for HSCT continue to increase. Cord blood processing is covered in a separate chapter of this book and is therefore not discussed here.

In parallel to this increase is the development of more complex cell therapy protocols that are now added to the standard backbone of HSCT (cord blood as source, T cell depletion of haplo-identical transplants, CAR-T cell manufacture). Hematopoietic stem cells are also being increasingly used in a variety of non-hematological indications, such as in cardiac repair and neurological diseases as part of the regenerative medicine spectrum. Other cell types, such as mesenchymal stromal cells, are also now increasingly being produced in former CPLs that have been

transformed into stringent Good Manufacturing Practice (GMP) Bio-Processing Facilities that are almost akin to drug-manufacturing facilities. Nonetheless, even here, the fundamental principles apply and the evolution of the CPL into a facility producing novel advanced therapeutic medicinal products can be facilitated by ensuring that these initial principles and fundamentals are correct.

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Chapter 12
Supportive Care

Rafael F. Duarte and Isabel Sánchez-Ortega

Introduction

Advances in supportive care have probably contributed the most to the improvement of the outcomes of autologous and allogeneic HSCT over the years. Thus, the organization and delivery of supportive care is one of the key elements required to establish a successful new transplant unit. Elements related to supportive care are present in nearly all aspects of the establishment of a new transplant unit.

Previous chapters in this book on the structure of the transplant program and the transplant unit have covered important elements of the professional teams that support the healthcare of patients undergoing transplantation, and important elements of the isolation requirements for infection control during hospitalization, as well as important elements of the structure and operation of the ambulatory care of transplant recipients. Other chapters address important aspects of supportive care, such as transfusion medicine and laboratory support for patient management, including antimicrobial testing, drug monitoring, chimerism studies, and disease evaluation. In this chapter, we aim to focus on a number of particular elements of supportive care, with the combined view of providing general guidance on recommended practices and on their implementation in a new transplant unit.

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Intravenous Access

The transplant process requires long-term multilumen venous access for chemotherapy administration, supportive care management (blood sampling, antibiotics, analgesics, antiemetics, blood components, total parenteral nutrition), and infusion of hematopoietic cells. There are different types of central venous access: tunneled catheters (Hickman, Broviac, and Groshong), non-tunneled catheters, peripherally inserted central catheters (PICCs), and port-a-caths. In recent years, PICCs have become increasingly popular due to the ease of placement at the patient's bedside by trained nursing personnel. However, PICCs may also be associated with a higher incidence of complications.

Three anatomical sites are commonly used to place central venous catheters: the subclavian, jugular, and femoral veins, all of them with potential major complications, including infectious, thrombotic, and mechanical complications. Bloodstream infections are the most common complication of central venous catheters. The estimated incidence of 5 per 1000 patient-days and mortality rates between 3% and 25% vary according to the type of catheter, frequency of manipulations, and disease-related factors. In vitro studies have demonstrated that catheters made of polyethylene or polyvinyl chloride are less resistant to the adhesion of microorganisms than those made of Teflon, silicone, or polyurethane. To minimize the incidence of infections, long-term intravenous catheters with a minimum number of ports should be placed with the patient in a surgery or radiology suite or a similarly outfitted procedure room. The use of ultrasound guidance may help to reduce the number of cannulation attempts and mechanical complications.

The Centers for Disease Control and Prevention guidelines for preventing intravascular catheter-related infections recommend using a subclavian site, rather than a jugular or a femoral site, in adult patients, if possible. The subclavian insertion site has the lowest bacterial bioburden and it is relatively protected against dressing disruption. A recent multicenter study showed that catheterization of the subclavian vein was associated with a lower risk of catheter-related bloodstream infection and symptomatic deep-vein thrombosis, albeit with a higher risk of mechanical complications, primarily pneumothorax, when compared with jugular or femoral vein catheterization.

The expected duration of catheterization is also important, as the cumulative risk of infectious and thrombotic complications increases with time of catheter exposure. Therefore, catheters should be removed as soon as they are no longer essential or when any sign of malfunctioning, phlebitis, or infection develops.

To cover the catheter site, a sterile gauze or sterile, transparent, semi-permeable dressing should be used. The catheter insertion site must be evaluated daily by palpation through the dressing or by inspection if a transparent dressing is in use. Hand hygiene is essential before and after palpating the catheter insertion site, as well as

before and after replacing or dressing an intravascular catheter. Dressings should be replaced every 2 days for gauze dressings, and at least every 7 days for transparent dressings, and dressings should be replaced if they become damp, loosened, or soiled.

Intensive Care Access

Critically ill transplant recipients may require transfer to an intensive care unit (ICU) to receive specialized care such as mechanical ventilation, vasopressor support, or renal replacement therapy. The rates of admission vary widely, from 9% to 57%, depending on the type of transplant and the reporting center, and are significantly higher for allogeneic than for autologous transplant recipients. The commonest reason for the ICU admission of allogeneic transplant recipients is acute respiratory failure, followed by severe sepsis and septic shock, neurological failure, acute kidney injury, and other factors (bleeding; cardiac and liver dysfunction). Infections represent approximately two-thirds of ICU admissions. However, infectious and noninfectious causes frequently occur in combination and multiorgan failure is often multifactorial. Bacterial infection is the leading cause of organ failure during neutropenia, while viral infections, invasive aspergillosis, and other opportunistic infections are also likely to occur after engraftment in high-risk patients.

Historically, mortality rates for transplant recipients requiring management in an ICU have been very high. However, the outcome has improved significantly due to advances in transplantation itself, as well as advances in critical care medicine, such as the use of reduced-intensity conditioning regimens, improvements in supportive care and in the management of sepsis and respiratory failure, and earlier consultation and referral of patients to the ICU team.

Due to the high mortality rate in certain subgroups of transplant recipients (up to 100% in neutropenic cases with multiorgan failure requiring mechanical ventilation), centers should provide a decision-making process for ICU admission and, when required, early admission should be imperative. Early mortality of allogeneic transplant recipients admitted to the ICU is especially influenced by the number of organ failures. Patients with one organ failure should be considered for admission to the ICU in an early time frame if required. The availability of ICU outreach teams helps in optimizing patient management in conventional rooms in the transplant unit prior to transfer to the ICU. However, admission to the ICU is not recommended when referral to the ICU is delayed in patients with multiorgan failure or when patients have uncontrolled graft-versus-host disease (GVH-D) with respiratory failure, or in patients with relapsed disease if further treatment is not an option (Table 12.1).

Table 12.1 Prognostic factors for intensive care unit (ICU) outcome in transplant recipients

Pre-transplant patient characteristics	HSCT-CI
	Age >60 years
Transplant-related characteristics	GVH-D
	Intensity of conditioning regimen
	Human leucocyte antigen (HLA) mismatch
ICU characteristics	Bilirubin, urea, creatinine levels, platelet count
	Renal replacement therapy
	Mechanical ventilation
	Vasopressors
	Time between HSCT and ICU admission

HSCT-CI Hematopoietic stem cell transplant comorbidity index, *GVH-D* graft-versus-host disease, *HSCT* hematopoietic stem cell transplantation

Before ICU admission, a thorough individual assessment must be performed and the patient's and family's preferences should also be a priority. However, discussion with patients and families about ICU admission should be undertaken before an acute clinical situation develops. Finally, as for many aspects of supportive care, close protocoled collaboration between hematologists and intensive care physicians is crucial.

Antiemetic Support

Chemotherapy-induced nausea and vomiting can result in anorexia, nutrient depletion, and metabolic imbalances, and can lead to functional disability and impaired quality of life. Causes of nausea and vomiting other than conditioning regimen toxicity include electrolyte imbalance, uremia, concomitant drugs, gastroparesis, GVH-D, and infectious complications.

The incidence and severity of nausea and vomiting related to chemo/radiotherapy may vary according to the intensity of the conditioning regimen, the combination of drugs, the dosage administered, the schedule, the route of administration, and the patient's individual experience. Table 12.2 summarizes the emetogenic potential of some chemotherapeutic agents that are frequently used in conditioning regimens.

In patients receiving highly emetogenic chemotherapy (i.e., myeloablative conditioning regimens), which would otherwise cause vomiting in nearly all cases, the establishment of antiemetic prophylaxis and treatment reduces nausea and vomiting to less than one-third of cases.

Prevention should be an essential part of antiemetic protocols. To provide maximal protection, antiemetic therapy protocols for moderate- and high-risk emetogenic agents should start before the administration of chemotherapy and should be continued for up to 2–3 days after the last dose of chemotherapy.

Table 12.2 Emetogenic potential of some chemotherapeutic agents frequently used in conditioning regimens

High risk (>90%)	Moderate risk (30–90%)	Low risk (10–30%)	Minimal risk (<10%)
Intravenous administration			
Cisplatin	Idarubicin	Cytarabine (low dose) 100–200 mg/m^2	Alemtuzumab
	Doxorubicin <60 mg/m^2		
Carmustine >250 mg/m^2	Melphalan, busulfan	Brentuximab-Bedotin	Rituximab
Cyclophosphamide >1500 mg/m^2	Cyclophosphamide ≤1500 mg/m^2	Doxorubicin (liposomal)	Vincristine
Doxorubicin ≥60 mg/m^2	Carmustine ≤250 mg/m^2	Etoposide	Cytarabine <100 mg/m^2
	Cytarabine >200 mg/m^2	Methrotexate >50–<250 mg/m^2	Fludarabine
Oral administration			
Moderate to high risk		*Low to minimal risk*	
Busulfan (≥4 mg/day)	Etoposide	Busulfan <4 mg/day	Melphalan
Cyclophosphamide (≥100 mg/m^2/day)	Lomustine (single day)	Cyclophosphamide (<100 mg/m^2/day)	Fludarabine

Antiemetics may have potential drug-drug interactions and side effects, and these should be carefully considered when establishing the unit's antiemetic protocols. Before prescribing any new agents, drug information and the concomitant drugs administered should be reviewed for interactions and side effects. The following recommendations summarize guidelines by the National Comprehensive Cancer Network (NCCN; www.nccn.org) for antiemetic treatment and the prevention of emesis; these guidelines can be used as a potential basis to guide the establishment of an antiemetic policy in a new transplant unit.

High Emetic Risk: Prevention of Acute and Delayed Emesis

Neurokinin-1 antagonist AND 5-HT3 antagonist AND steroid:

Aprepitant 125 mg PO once on day 1 and 80 mg PO daily on days 2, 3
OR Fosaprepitant 150 mg IV once on day 1
AND
Dexamethasone 12 mg daily PO/IV (individualize dose according to patient's characteristics and extend the course as clinically appropriate; usually until end of conditioning regimen plus 2 days)
AND
Granisetron 2 mg PO once or 0.01 mg/kg (max 1 mg) IV once
OR Ondansetron 16–24 mg PO once or 8–16 mg IV once

Consider using an H2 blocker or proton pump inhibitor to prevent dyspepsia
Lifestyle measures, such as eating small frequent meals and eating food at room temperature can help to alleviate symptomatology
A dietary consult may also be useful

Treatment for Breakthrough Chemotherapy-Induced Nausea and Vomiting

The general principle is to add an agent from a different drug class:

- Lorazepam 0.5–2 mg PO/IV every 6 h.
- Haloperidol 0.5–2 mg PO/IV every 4–6 h.
- Metoclopramide 10–20 mg PO/IV every 4–6 h.
- Change 5HT3 antagonist.

Prevention of Total Body Irradiation (TBI)-Induced Emesis

Pretreatment for each day of radiotherapy:

Granisetron 2 mg PO daily OR ondansetron 8 mg PO BID-TID +/− dexamethasone 4 mg daily PO.

Mucositis

Mucositis is characterized by mucosal damage, ranging from mild inflammation to extensive ulceration affecting any part of the alimentary tract. Oral mucositis occurs in most hematopoietic transplant recipients treated with high-dose chemo/radiotherapy and may cause significant mordibity and mortality. Other causes of mucositis include GVH-D, drugs (antibiotics, magnesium, etc) and infection.

Mucositis has been associated with an increased risk of opportunistic infections (mostly bacteriemia associated with the breakdown of mucosal barriers), use of opioid analgesics, nutritional deficiencies, an increased need for parenteral nutrition, and prolonged hospitalization. Moreover, mucositis significantly impairs quality of life and has a considerable economic impact. Thus, the management of mucositis and its consequences requires a multidisciplinary team.

To prevent complications, patients should receive basic oral care recommendations before transplantation, including evaluation by a dental professional and treatment of risk factors (periodontal disease, gingivitis, deep caries, pulp infections) to reduce the risk of secondary infections. Moreover, patients should receive training about daily routine mouth care during and after transplantation: this includes daily use of a soft toothbrush; bland rinses (normal saline, sodium bicarbonate, and a saline and sodium bicarbonate mixture) three to four times daily; avoidance of tobacco, alcohol, and irritating foods; and the use of water-based moisturizers to protect lips and maintain adequate hydration.

The following recommendations summarize the guidelines issued by the Multinational Association of Supportive Care in Cancer (MASCC) and the International Society of Oral Oncology (ISOO) for the management of oral mucositis in cancer patients (available at www.mascc.org/mucositis-guidelines), as a potential basis to guide the establishment of a mucositis policy in a new transplant unit.

Oral Mucositis

Prevention

- Oral cryotherapy (e.g., ice cubes) for patients receiving high-dose melphalan or TBI.
- Recombinant human keratinocyte growth factor-1 (KGF-1/palifermin) is recommended for autologous hemotopoietic stem cell transplant (HSCT) recipients.
- Low-level laser therapy.

Treatment

- Topical anesthetics may provide short-term pain relief; however, patient-controlled analgesia with morphine is recommended. An alternative is transdermal fentanyl.
- Oral GVH-D treatment: local immunosuppressive agents (tacrolimus, cyclosporine), local steroids (triamcinolone, clobetasol propionate, budesonide), or intraoral psoralen and ultraviolet A (PUVA).
- Treatment of dry mouth (sugarless gum or sweets; frequent water sipping; non-alcoholic mouthwashes; and lip balm or systemic sialogogues, such as pilocarpine hydrochloride).
- Treatment of infections (*Candida*, cytomegalovirus [CMV], herpes).
- Nutritional support (see below).

Gastrointestinal Mucositis

Treatment

- Maintenance of adequate hydration.
- Treatment of diarrhea: If loperamide is ineffective, add octreotide.
- Oral sulfasalazine may reduce the incidence and severity of radiation-induced enteropathy.
- Ranitidine or omeprazole to prevent epigastric pain.
- Treatment of gastrointestinal CMV.
- GVH-D treatment: Methylprednisolone. Failure to respond or refractory recurrence requires second-line treatment. Anti-infectious prophylaxis should be considered for patients on high-dose steroids.

Nutritional Support

Nutritional intervention is highly important before, during, and after hematopoietic stem cell transplantation. Pre-transplant nutritional status has an impact on post-transplant outcomes. Thus, three nutritional prognostic factors (obesity, diabetes mellitus, and liver dysfunction) are included in the hematopoietic stem cell transplantation-comorbidity index.

During transplantation, transplant recipients may develop severe gastrointestinal symptoms, leading to inadequate oral nutrition and gastrointestinal absorption and subsequent malnutrition. Besides, it is essential to follow nutritional status long-term, as patients may experience nutritional and metabolic problems, such as malnutrition and metabolic syndrome, long-term after transplant. Therefore, centers should provide a systematic nutritional protocol for all transplant recipients.

During admission, oral intake; body weight; exocrine pancreatic function; levels of magnesium, calcium, vitamin D, vitamin B12, and zinc; and protein-losing enteropathy, among other factors, should be assessed and patients should receive nutritional support, dietary advice, and adequate energy and protein requirements.

Transplant recipients may require nutritional support with enteral feeding or total parenteral nutrition when a long period of insufficient oral intake is anticipated due to severe mucositis or gastrointestinal GVH-D. As suggested by ESPEN guidelines (European Society for Clinical Nutrition and Metabolism; www.espen.org), nutritional support should be started early and stopped when oral dietary intake approaches energy and protein requirements, which generally occurs at engraftment. When possible, oral or enteral nutrition should be promoted in order to prevent mucosal atrophy. However, nausea, vomiting, and mucositis may prevent the insertion and subsequent tolerability of nasogastric tubes. Moreover, patients receiving enteral nutrition may have digestive discomfort, such as nausea and gastric repletion. Parenteral nutrition allows a better control of fluids, electrolytes, and nutrient administration and it can be administered via a central venous catheter. However, it is associated with more frequent hyperglycemia, liver dysfunction, and catheter-related complications than enteral nutrition.

Diet in HSCT Recipients with Gastrointestinal GVH-D

Gastrointestinal GVH-D is associated with malnutrition; malabsorption; and deficiencies of vitamin D, vitamin B12, zinc, and magnesium. Multidisciplinary treatment by hematologists, dietitians, endocrinologists, and nurses should include early nutritional assessment, nutritional support, and follow-up of micronutrient status.

The oral diet for such patients should be adjusted to the severity of the GVH-D, but in general, patients should avoid fat, fiber, and lactose. Oral foods can be introduced using a stepwise oral upgrade diet.

Step 1	Bowel rest; glutamine-supplemented parenteral nutrition
Step 2	Liquid oral diet
Step 3	Solid food; lactose-free, low fiber, fat-reduced
Step 4	Slowly increase the amount of solid foods. Lactose-containing products are often the last to be tolerated.

Pain Control

Pain management is an essential part of oncologic treatment; goals include optimizing analgesia, minimizing adverse effects, and avoiding aberrant drug taking. In the setting of HSCT, a major cause of pain is mucositis induced by the conditioning regimen; however, other causes include pain related to late complications, acute and chronic GVH-D, and infections, as well as neuropathic pain.

Assessment of pain must be quantified with a rating scale (e.g., a 1- to 10-point scale) and reassessment of pain intensity must be performed at specified intervals to ensure that the analgesic therapy is providing the maximum benefit with as few adverse effects as possible. Guidance based on NCCN and European Society of Medical Oncology (ESMO) requirements provides details of definitions of pain levels and appropriate management to support the development of protocols for the new unit.

Mild Pain (1–3 points): Management should primarily be based on non-opioid analgesics:

- Acetaminophen 650–1000 mg/6 h (daily maximum 4 g/day) if liver function is normal. Caution regarding hepatic toxicity with chronic administration.
- Non-steroidal antiinflamatory drugs (NSAIDs): For chronic use, caution for risk of renal, gastrointestinal, or cardiac toxicities; thrombocytopenia; or bleeding disorders. Monitor toxicities with blood tests.
  - Ibuprofen 400 mg/6 h (daily maximum 3200 mg) or naproxen 220–550 mg/8–12 h (daily maximum 1500 mg). If necessary, consider short-term use of ketorolac 15–30 mg/6 h IV for a maximum of 5 days.
- Consider titrating short-acting opioids (see below).

Moderate (4–6 points) **and severe pain** (7–10 points)

- Titrate short-acting opioids. In cases of severe pain, titrate rapidly.
  - 5–15 mg short-acting oral morphine sulfate or equivalent; or 2–5 mg IV or subcutaneously.
  - Reassess efficacy and adverse effects 60 min after administration by the oral route or 15 min after administration by the IV route.

    If pain is unchanged or increased: Increase dose by 50%–100%.
    If pain is decreased but not adequately controlled: Repeat dose.
    If pain is alleviated and controlled: Continue at current effective dose.

- If pain is inadequately controlled after two to three cycles despite adequate dose titration (calculate dosage increase based on total opioid dose received in the previous 24 h) or if there are persistent adverse effects, consider opioid rotation, specific pain syndrome problems, and pain specialty consultation.
- Titrate opioid with caution in patients with risk factors such as impaired renal or hepatic function, chronic lung disease, upper airway compromise, sleep apnea, and poor performance status.

- If opioid dose reduction is required, reduce by 10%–25% with subsequent re-evaluation and further dose adjustment.
- Adverse effects of opiods, such as constipation, nausea, pruritus, respiratory depression, or sedation should be anticipated and managed aggressively.

For **persistent pain**, initiate a regular schedule of opioid with rescue dose as needed, consider adding or adjusting adjuvant analgesics, provide management of constipation and provide psychosocial support.

For **refractory pain** consider referral to a pain specialist and/or the use of interventional strategies.

Neuropathic pain: Adjuvant analgesics and anticonvulsivants are normally used for neuropathic pain, in combination with an opioid in moderate/severe cases:

- Adjuvant analgesics:
  - Tricyclic antidepressants (amitriptyline, imipramine, desipramine). Start with lower dose and increase every 3–5 days if tolerated. Adverse anticholinergic effects such as sedation, mouth dryness, and urinary hesitancy may occur.
  - Duloxetine and venlafaxine.
- Anticonvulsivants combined with an opioid:
  - Pregabalin or gabapentin: Dose increments of 50–100% every 3 days. Dose adjustment for renal insufficiency.

Management of Drug Toxicity

Hematopoietic transplant recipients receive multiple lines of medication during the transplant process, including conditioning regimen radio-chemotherapy, immunosuppressive drugs, antimicrobials, analgesics, and antiemetics. In addition, many current transplant candidates are already on multiple medications for other conditions prior to transplant, such as anti-hypertensive drugs, statins, and others. Therefore, before administering any new drug, it is essential to review the drug prescribing information, the contraindications, interactions with other drugs, and the requirement for adjusting the dose in cases of renal or hepatic impairment.

Some transplant-specific drugs require specific measures to prevent toxicity:

Thiotepa: As this drug may be excreted through the skin in sweat, patients should shower, bath, or take a sponge bath four times a day while receiving thiotepa and should use only water or a gentle non-soap cleanser to wash the skin. The first shower should be taken 3–4 h after receiving the first dose and this bathing plan should be continued for 36 h after the last dose. After bathing, patients should put on clean undergarments and loose-fitting clothes and should not apply any lotions or creams for up to 36 h after the last dose.

Cyclophosphamide: The risk of developing hemorrhagic cystitis after cyclophosphamide is dose-dependent and the incidence depends on the preventive measures adopted. Occasionally, other agents, such as ifosfamide, busulfan (especially if associated with cyclophosphamide), or VP16; or TBI, have also been implicated. Hydration

and diuresis are essential to prevent cyclophosphamide-induced hemorrhagic cystitis. The recommended daily dose for hydration is 3 L/m^2. In addition, if administered, the daily dose of mesna should be 1.0–1.5 × the daily dose of cyclophosphamide, administered IV as a continuous infusion or as bolus injections, starting before the first cyclophosphamide dose and continued for up to 24 h after the last dose.

Busulfan: High levels of busulfan in the cerebrospinal fluid can produce seizures; therefore, prophylactic anticonvulsant therapy with phenytoin must be administered routinely. Moreover, oral doses of busulfan should be adjusted according to blood levels, because of the high inter- and intra-patient variability in its effects and the risk of hepatotoxicity, and to prevent complications such as hepatic veno-occlusive disease.

Cytarabine: Reversible corneal toxicity and hemorrhagic conjunctivitis have been reported following high doses of cytarabine. These effects may be prevented or diminished by adding prophylaxis with topical corticosteroid eye drops.

Total Body Irradiation: The effects of TBI depend on the total dose, dose rate, and fractionation. Fractionation reduces the incidence and severity of acute and late complications in normal tissue. Furthermore, several parts of the body, usually the lungs or the eyes, must be protected with lead blocks to reduce organ-specific toxicity. Immediate side effects of TBI include nausea, vomiting and, typically, parotid swelling; therefore, adequate preventive measures are recommended.

Melphalan: When the drug is administered at high doses, ensure its excretion by the use of aggressive hydration and furosemide, as appropriate.

Anti-thymocyte globulin (ATG), rituximab, and other monoclonal antibodies: As the use of these agents may be limited by infusional side effects (anaphylaxis, cytokine release syndrome), pre-medication (corticosteroids +/− antihistamine +/− acetaminophen) may be given 30 min prior to their administration.

Establishment of Anti-infective Policies

Oportunistic infections are the main complication in immunocompromised hosts, such as hematopoietic stem cell transplant recipients. Whether occurring directly, or indirectly through the course of other transplant complications (e.g., GVH-D), infections are perhaps the main cause of non-relapse mortality after transplant. Previous chapters in this book have described in detail the requirements that need to be implemented for the design and development of a new transplant unit, with regard to antimicrobial isolation, high-efficiency particulate air (HEPA) filtering and positive air pressure, infection-control procedures, and microbiology laboratory testing.

Unlike the other recomendations described above for complications such as mucositis, antiemetic treatment, or pain relief, addressing the specific protocols and recommendations for the prophylaxis, diagnosis, and treatment of the multiple types of bacterial, fungal, viral, and other infections that may affect transplant recipients is beyond the scope of this chapter. In particular, this is because such protocols and recommendations must be adapted and tailored to the characteristics of the

transplant program, including the type of transplant procedures, the microbiological environment and epidemiology, the isolation characteristics and infection control of the new unit, and the availability of various drugs in different regions and countries. Multiple international professional guidelines for the anti-infective management of transplant recipients are available to guide local policies. Beyond the variability among centers and guidelines, effective anti-infective management can only be adequately implemented and adapted to patient needs through multidisciplinary teams that bring together expertise from multiple professionals, such as hematologists and transplant physicians; infectious disease specialists; clinical microbiologists, including medical mycologists and virologists; radiologists; and organ and system specialists for particular infective syndromes. This is indeed a universal recommendation that applies to all transplant units for anti-infective management, and would ideally also apply as well to other aspects of supportive care.

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Chapter 13
Transfusion

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Introduction

Hematopoietic stem cell transplantation (HSCT) with blood type (ABO and Rh) incompatibility between a recipient and an allogeneic donor is referred to as blood type–incompatibility transplantation. In HSCT, the compatibility of human leukocyte antigen (HLA) type and cell count are prioritized, and blood type incompatibility develops in approximately 40–50% of transplantations [1]. In particular, in cord blood (CB) transplantation, blood type incompatibility often develops because CB transplantation units with a high cell count and high CD34+ cell count are preferentially used. In blood type–incompatibility transplantation, the blood type of the transfused concentrated/packed red blood cells (RBC product), platelet-rich plasma (platelet product), and fresh frozen plasma (FFP) that does not adversely affect a recipient either before or after engraftment of donor-type blood should be selected.

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Consensus has not been reached on the effect of blood type–incompatibility transplantation on the engraftment rate of RBCs and platelets, the frequency of graft-versus-host disease (GVH-D), the non-relapse mortality, the disease-free survival, or the overall survival [2–7].

Considerations Regarding Blood Products for Transfusion

First, to prevent the transmission of infection by transfusion, the blood for transfusion must be tested for infections, including hepatitis B virus, hepatitis C virus, syphilis, human T-cell leukemia virus type I/II, and human immunodeficiency virus I/II. In many countries, nucleic acid amplification tests, in addition to antigen/antibody tests, have been introduced to improve detection sensitivity. In some epidemic infections in limited areas over limited or unlimited periods, countries in the area should reinforce donor questionnaires (i.e., include questions about infections such as Zika virus, West Nile virus, malaria, hepatitis E, and others), with or without performing tests for such infections. The presence of cytomegalovirus (CMV) and anti-CMV antibodies (Abs) in the blood products may be tested for CMV-seronegative recipients, as discussed below. However, even with these efforts, the blood products may still not have been perfectly cleared of infection and recipients shall be notified of this fact.

For the HSCT recipient, blood transfusion from the HSCT donor is basically considered to be contraindicated, so as to avoid engraftment failure due to immunological sensitization. Similarly, blood transfusion from family donors should better be avoided. The fresh donor lymphocytes in transfused blood may engraft and proliferate in an immunosuppressed host and cause transfusion-associated GVH-D (TA-GVH-D). Although TA-GVH-D is not common, once it occurs, the reported mortality rate is nearly 90% [8]. Both autologous and allogeneic HSCT recipients are at increased risk of the development of TA-GVH-D if blood that is not properly manipulated is transfused [9, 10]. TA-GVH-D can be avoided by gamma irradiation and filtration of the blood product. The gamma irradiation of RBC and platelet products induces chemical crosslinks in the DNA of the irradiated donor lymphocytes, preventing their proliferation; therefore, it is a reliable method to prevent TA-GVH-D. The recommended dose is 25 Gy to the internal midplane of a free-standing irradiation instrument canister, with a minimum of 15 Gy at any other point within the canister.

Many transfusion services worldwide routinely provide leukocyte-reduced RBC and platelet products by using leukocyte reduction filters, resulting in the decreased occurrence of febrile non-hemolytic transfusion reactions. The incidence of CMV infection is significantly reduced in HSCT recipients who are CMV-seronegative before transplant and who are exposed only to CMV-seronegative blood products. Therefore, tests for CMV seropositivity in donor blood products should be considered for CMV-seronegative patients. CMV is understood to exist in leukocytes, especially in granulocytes. Leukocyte reduction filters are therefore also helpful,

although they have not convincingly demonstrated efficacy as a substitute for the use of CMV-seronegative blood products.

Washed RBCs are prepared by removing the plasma components remaining in the above RBC products and replacing these components with saline. Washed platelets are prepared by removing the plasma components, and replacing them with acid-citrate-dextrose formula A (ACD-A)-containing saline or bicarbonate Ringer solution [11]. The appropriate bicarbonate Ringer solution and ACD-A solution ratio should be checked with careful pH monitoring. Washed platelets are employed in patients for whom platelets of the required blood type are not available (high titer of anti-RBC antibodies; >×128), or when serious adverse effects such as anaphylactic reactions develop during the intravenous injection of platelet products.

Preparation of Autologous Blood Transfusion for Bone Marrow Harvest in a Bone Marrow Donor

Bone marrow (BM) donors require autologous blood transfusion at BM harvest, because a median volume of 1200 mL (450 ~ 1900mL) of BM is harvested for an adult recipient [12]. In healthy donors for BM transplantation, autologous blood transfusion is recommended to avoid the adverse effects of allogeneic blood transfusion. The timing of autologous blood harvest, and the properly calculated volume, should be scheduled by the HSCT coordinator. Generally, autologous blood products do not require irradiation and leukocyte-reduction filtration.

ABO Blood Type in Transplantation

Transplantation with ABO Blood Type Compatibility

For transplantation with ABO blood type compatibility, transfusion with an RBC product, platelet product, or FFP is performed following the usual procedures. For safety, the blood transfusion products should be filtered with a leukocyte-reduction filter, and irradiated at 15–25 Gy.

Transplantation with ABO Blood Type Incompatibility

For transplantation with ABO blood type incompatibility, on and after the initiation of conditioning, blood transfusion products are, in principle, selected as shown in Tables 13.1 and 13.2. As shown in Fig. 13.1, different blood types for transfusion should be used depending on the time course of HSCT and the change in the patient's blood type.

Table 13.1 RBC products[a]

Recipient blood type	Donor blood type			
	A	B	O	AB
A	A	O	O	A or O
B	O	B	O	B or O
O	O	O	O	O
AB	A	B	O	AB

[a]In the case that red blood cells (RBCs) are engrafted after transplantation and the recipient's blood type is completely converted to the donor's blood type, as determined by blood type tests (forward and reverse blood group test and titration test of anti-A and anti-B antibodies), donor-type RBC products can be transfused

Table 13.2 Platelet products and frozen plasma[a]

Recipient blood type	Donor blood type			
	A	B	O	AB
A	A	AB	A	AB
B	AB	B	B	AB
O	A	B	O	AB
AB	AB	AB	AB	AB

[a]In the case that platelets are engrafted after transplantation and the recipient's blood type is completely converted to the donor's blood type, determined by blood type tests (forward and reverse blood group test and titration test of anti-A and anti-B antibodies), donor-type platelets should be transfused

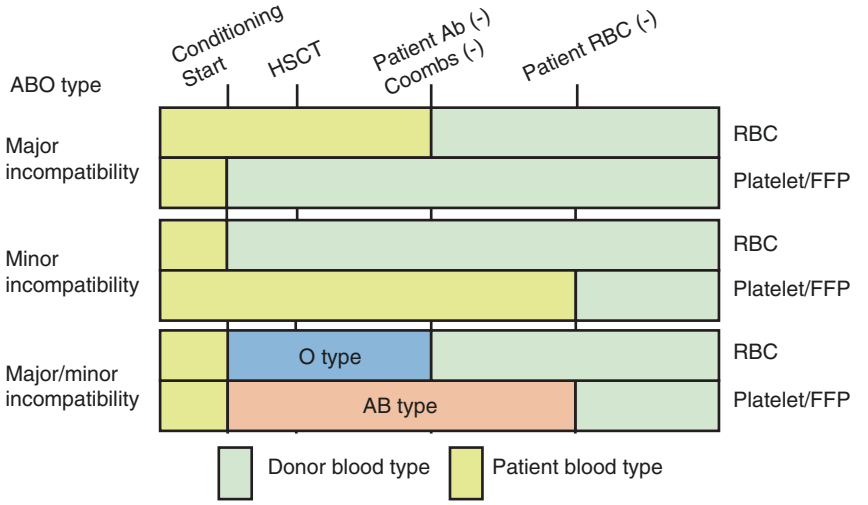

Fig. 13.1 Selection of blood transfusion products in hematopoietic stem cell transplantation (HSCT). Recipient's antibody (−): Time when ABO antibodies in the patient to donor-type red blood cells (RBCs) are not detected. Patient's RBCs (−): Time when Recipient's type RBCs are not detected. In all cases, it is important to determine the Recipient's blood type, by blood type testing (forward and reverse blood group tests and titration test of anti-A and anti-B antibodies) and Coombs test, performed regularly after HSCT

Major Incompatibility

Major incompatibility means the presence of antibodies against the donor's blood type in the recipient's plasma.

Major incompatibility in a BM transplant requires RBC reduction to remove RBCs from the BM cells collected from the donor, to prevent the infusion of large numbers of incompatible-type RBCs. Generally, peripheral blood stem cells (PBSCs) and frozen CB do not require RBC reduction, because of the low percentage of RBC contamination and the low percentage of frozen punctured RBCs, respectively.

The transfused platelet product and FFP should not include antibodies that suppress donor erythropoiesis, and the transfused RBC products should not be susceptible to anti-RBC antibodies present in the recipient's plasma that would cause hemolysis.

In BM/PBSC transplantation, the median time for RBC engraftment after transplantation with major blood type incompatibility is 32 days, which is longer than that with blood type compatibility (median time: 21 days) or minor incompatibility (median time: 21 days). The continuing production of antibodies by remaining lymphocytes in the patient may cause late recovery and the hemolysis of RBCs. After transplantation, the blood type titer should be determined periodically.

The condition in which white blood cells (WBCs) and platelets engraft and proliferate, while RBCs do not reach engraftment for several months to a year or longer, is termed post-transplantation pure red cell aplasia (PRCA) [1, 13, 14]. Several treatments may be effective for PRCA, such as the employment of drugs inculding erythropoietin and steroids; plasmapheresis; and antibody removal by immunoadsorption [15–18].

Minor Incompatibility

Minor incompatibility means the presence of antibodies against the recipient's blood type in the donor's plasma.

Processing of cells to be transplanted: In transplantation, plasma in BM and PBSCs should be removed from the hematopoietic stem cell suspension collected from the donor. In particular, if the titer of anti-RBC antibodies in BM or PBSCs is 256 × or higher, plasma must be removed. CB does not require plasma reduction, because anti-RBC antibodies (immunoglobulin [Ig] M) are not produced in the newborn baby.

Minor incompatibility, in which donor-derived antibodies are directed against antigens on the recipient's RBCs, may cause delayed hemolysis within 1–2 weeks after HSCT, especially in recipients of peripheral blood stem cell transplantation (PBSCT); delayed hemolysis is less common in BMT recipients. This phenomenon is caused by transient antibody production by passenger immunocompetent donor lymphocytes and is not due to the passive transfer of antibodies during BMT and

PBSCT [19]. Its frequency ranges from 15% to 71% depending on the study, and most cases resolve spontaneously. However, in severe cases, hemolytic crisis may cause transplantation complications such as renal disorder, late engraftment, thrombotic microangiopathy, and GVH-D, and hemolytic crisis may be difficult to differentiate from these conditions [20–22]. When the patient shows post-HSCT severe hemolysis with abnormal laboratory data, including Coombs positivity, elevated anti-RBC antibodies (IgM and IgG), elevated lactate dehydrogenase (LDH), elevated bilirubin, and elevated blood urea nitrogen (BUN), and decreased haptoglobin, immediate diagnosis and aggressive treatment, including steroid pulse therapy, is essential.

In RBC product transfusion in minor incompatibility of blood type, the donor's blood type should be selected such that the patient's RBCs are not hemolysed and the transfused RBCs are not hemolysed by the donor's antibody-producing cells.

- Example: In the case of an A-type donor and AB-type recipient, a B-type donor and an AB-type recipient, and an O-type donor and an A-, B-, or AB-type recipient→ donor-type RBCs and recipient type platelets and frozen plasma are selected for transfusion.

Major/Minor Incompatibility

Major/minor incompatibility means the presence of antibodies against the donor's RBCs in the recipient's plasma and antibodies against the recipient's RBCs in the donor's plasma.

In the transplant product, RBCs and plasma are removed from the hematopoietic stem cell suspension of BM and PBSCs collected from the donor. The WBC-rich part (mononuclear cells or buffy coat) of BM cells should be separated by density gradient centrifugation. For PBSCs, when the hematocrit in the harvested PBSCs is less than 3–5%, only plasma should be removed.

In blood transfusion, an appropriate blood type satisfying major and minor incompatibility should be selected; specifically, O-type RBCs and AB-type platelets and frozen plasma should be selected.

- Example: In the case of an A-type donor and B-type patient and a B-type donor and A-type recipient → O-type RBCs and AB-type platelets and frozen plasma are selected for transfusion.

Recipients who receive transplantation from a donor whose blood type shows major/minor incompatibility may experience the above-mentioned complications, which occur in both major and minor incompatibility transplantations. Be aware that donor-derived passenger B-cell hemolysis might occur after HSCT, as described above.

Blood Transfusion after Engraftment

In the case that RBCs and platelets are engrafted after transplantation and the recipient's blood type is completely converted to the donor's blood type (determined by tests for blood type [forward and reverse blood group tests] and tests for sufficient antibody titer), donor-type blood product is transfused. After transplantation, the determination of blood type (forward and reverse blood group tests) and antibody titer (tests for anti-A and anti-B antibodies) and the performance of the Coombs test should be carried out subsequently as needed and the results recorded on the medical chart.

Transfusion in Transplantation with Rh Incompatibility

Rh is reported to include five serological factors, C, c, D, E, and e, and a factor D is thought to be critical for antigen-presenting.

In the case of an Rh (−) donor and Rh (+) recipient

After the engraftment of transplanted cells in the BM, if Rh (−) donor cells produce anti-Rh antibodies, the remaining Rh (+) RBCs of the recipient may be hemolysed.

In the case of an Rh (+) donor and Rh (−) recipient

For an Rh (−) recipient, an Rh (−) donor is generally selected as the first choice for HSCT; however, if such a donor is not available, an Rh (+) donor is acceptable. In such cases, the relevant recipient must be checked as to whether he/she has anti-Rh antibodies before transplantation. If the result is positive, RBCs must be strictly removed from the donor's cells. After transplantation, the transfusion of Rh (−) RBCs should be continued until anti-Rh antibodies disappear from the recipient. In most cases, new anti-Rh antibodies are not produced after transplantation, and Rh (+) blood transfusion often causes no problem.

Other Blood Type Incompatibilities (Irregular Antibody-Positive Cases)

Irregular antibody-positive cases are not uncommon, because many patients have received blood transfusion before transplantation for hematologic diseases such as aplastic anemia and leukemia, which are the primary reasons for the transplantation.

Transplantation to an irregular antibody-positive recipient from a donor who is positive for the relevant antigen is deal as a blood type–major incompatibility transplantation. Transplantation from a woman donor who is positive for a pregnancy-related irregular antibody to a recipient with the relevant antigen is comparable to ABO-type minor mismatch. Therefore, before proceeding to transplantation, both recipient and donor must undergo screening tests for irregular antibodies and, if positive, cold- and warm-antibody types should be differentiated and antibodies should be identified. If the irregular antibodies are warm-type antibodies with the potential for exerting adverse effects on blood transfusion, cell processing or cautions similar to those for ABO blood-type mismatch are required; these are described as follows:

1. If a recipient who is positive for irregular antibody receives an HSCT from a donor with the relevant antigen-positive RBCs, RBC removal, which is used for ABO type major mismatch, should be performed.
2. Caution should be exercised, because hemolysis may be caused by the injection of the small amount of remaining RBCs even after RBC removal processing.
3. If a recipient receives an HSCT from a donor who is positive for both an irregular antibody and for the relevant antigen-positive RBCs, plasma should be removed from the HSC (BM and PBSC) suspension, similar to the procedures used for ABO type mismatch.

Application of Platelet Transfusion in Transplant Patients

As transplantation conditioning causes far deeper BM suppression than common chemotherapies, platelet transfusion is definitely indicated, and should be initiated when the platelet count is 20,000/μL or lower.

The amount of platelet transfusion required is $2 \sim 3 \times 10^{11}$/adult or $0.4 \sim 1 \times 10^{11}$/infant, to keep a platelet count of 20,000/μL or more as the target for the maintenance of treatment. One unit of platelet product is derived from one unit of transfusion blood. In the case of transplantation for an anti-HLA antibody-positive patient, communication with the blood center, through the blood transfusion department, to ensure the availability of an HLA-compatible donor and an adequate HLA-compatible platelet supply should take place before HSCT is performed. In the case of transplantation for a platelet-specific antibody-positive patient, the availability of platelet cross-match test-negative donors and an adequate compatible platelet supply should be confirmed before transplant. If the expected increase in platelet numbers is not obtained after platelet infusion, blood should be drawn from the vein of the patient's opposite arm, within 30 min after the platelet infusion, to test the platelet count; this is followed by the calculation of platelet count increment. If the platelet count is lower than that before transfusion or is not increased at all, the presence of anti-HLA antibody is strongly suspected and the HLA locus/loci should be identified. Patients who are anti-HLA antibody-positive

often experience fever during transfusion. If anti-HLA antibody-positive patients receive frequent random platelet transfusions, not only anti-HLA antibody increased and the transfusion rendered ineffective, but also transfusion-related acute lung injury (TRALI) may develop as a complication, which may become fatal. If the platelet count is not elevated after platelet infusion, even by a small degree, an infectious disease or the consumption of platelets in the presence of BM hypoplasia is suspected; however, examination of anti-HLA antibodies is indispensable. Anti-platelet antibodies include anti-HLA antibody and anti-platelet-specific antibody (e.g., Siba, Sibb). If platelet transfusion efficacy is not achieved without the detection of anti-HLA antibody, the presence of anti-platelet-specific antibody is suspected.

The effective increase in platelets after platelet transfusion is reported to be 73.4% in patients with ABO blood type match and 55% in patients with ABO blood type mismatch, because ABO-type antigens are also slightly expressed on platelet cell membranes. Products of HLA-compatible platelets with ABO-type mismatch are allowable; however, if a patient has an extremely high anti-A or anti-B titer (agglutinin titer) or if blood transfusion is ineffective, it is preferable to transfuse HLA-compatible and ABO-type matched platelets.

Prediction and Evaluation of Transfusion Efficacy

Approximately one-third of transfused platelets stay in the spleen, while the remaining two-thirds circulate in the blood, contributing to an increase in platelet count. Therefore, the increase in platelets can be estimated based on the number of transfused platelets, with the following formulation being used: estimated increase in platelets (/μL) = [total transfused platelet count/circulating blood volume (mL) × 10^3] × 2/3.

However, the platelet count increment changes depending on the presence or absence of complications and anti-alloantibodies; the effect of the platelet increment is assessed based on the corrected count increment (CCI). The CCI can be calculated using the platelet count before transfusion and 1 or 20 h after transfusion, as follows: CCI (/μL) = [platelet count increment (/μL) × body surface area (m^2)]/ total platelet count (×10^{11}).

If the CCI 1 h after transfusion is higher than 7500–10,000/μL, the efficacy of platelet transfusion can be considered favorable. A platelet product contains 3 × 10^{11} platelets or more.

The hemoglobin (Hb) value improved by the transfusion of RBC product can be calculated using the following formula: Expected Hb increment = transfused Hb amount (g)/circulating blood volume (dL).

For example, if RBC product derived from 400mL blood (Hb amount of 50–60 g) is transfused to an adult with a body weight of 50kg (circulating blood volume: 37 dL), the expected Hb increment is 1.5 ~ 1.6g/dL.

Tests when the Planned Transplantation Patient is Anti-HLA Antibody-Positive

In some cases, a planned transplantation patient is anti-HLA antibody-positive. A high anti-HLA titer may not only cause adverse effects of blood transfusion, but may also affect the engraftment of the transplanted HSCs. In such cases, the following tests should be considered:

1. Anti-HLA antibodies are examined with panel lymphocytes in the blood center or laboratory. If the planned patient is anti-HLA antibody-positive, the doctor in charge should contact the blood transfusion department, to ask the blood center or laboratory for the details of the test result.
2. The patient's existing anti-HLA antibodies are required to be preliminarily checked for response to the donor's lymphocytes, and a doctor in the blood transfusion department should be informed of the relevant cases.
3. During transplantation conditioning, the existing anti-HLA antibody may decrease or disappear. Therefore, anti-HLA antibodies should be re-checked during the transplantation conditioning period. If anti-HLA antibody disappeared, HLA-compatible platelets may be no longer required.
4. In the above examinations (especially tests 1 and 2), if a patient has a high titer of anti-HLA antibody against the donor's antigens, backup transplantation cells should preferably be prepared for cases of rejection, or donor source should be re-considered.

Tests when the Donor is Anti-HLA Antibody-Positive

A low titer of anti-HLA antibodies is identified in approximately 1% of the general population of female donors (aged 25 years or older). This means that a donor can be anti-HLA antibody-positive. When donors are examined, particularly if the donor is a woman with a history of pregnancy or blood transfusion, the donor should be checked for anti-HLA antibody.

1. A doctor in charge should preliminarily contact the blood transfusion department, because, after transplantation cell collection, plasma should be removed.
2. Around the time when cells are engrafted after transplantation, the production of anti-HLA antibodies in the donor's lymphocytes may have started; in such cases, HLA-compatible platelet transfusion can be used. Caution should be taken to monitor for extremely elevated anti-HLA antibody titers caused by the irresponsible transfusion of random platelet products during that period, because the elevated titer may cause TRALI.

Preparation of Blood Type Sheet for Transplantation

In practical hematopoietic stem cell transplantation, if the orders for blood products during transplantation conditioning are changed, it is important that the attending physicians in the transplantation team and the blood transfusion department share the information of the change in the order. A blood type sheet for transplantation, as shown in Format 13.1, is useful for recording any changes in the orders for blood products. For patients who experience multiple transplantations or those for whom are multiple transplantations are planned, obtaining information on the blood type of previous donors is clearly important. In such cases, the blood transfusion department in the hospital should be consulted.

Conclusion

To avoid severe adverse effects and infections, blood transfusion pre-, during, and post-HSCT requires extreme care to be taken in regard to the blood types of the donor and the recipient and the selection and management of blood products.

Name of the Patient	
Patient ID	
Disease indication	

HSCT Date: yy/mm/dd

PATIENT		DONOR
Disease status:		Stem Cell Source : CB ( ) BM ( ) PBSC ( )
		Related ( ) Unrelated ( ) Autologous ( )
Blood type: Rh:		Blood type: Rh :
Blood type for Transfusion	Pre HSCT RBC ( ), PC or Plasm a ( )	
	Post HSCT RBC ( ), PC or Plasma ( ) RBC WASH( ), PC WASH ( )	

Format 13.1 Blood type for transfusion related to HSCT

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Chapter 14
Laboratory Support

Hildegard T. Greinix

Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative therapeutic option for selected patients with hematologic and oncologic diseases. In recent years transplant-associated morbidity and mortality has declined, due to improved donor selection and human leukocyte antigen typing in the allogeneic setting, as well as intensified prophylactic measures, including monitoring for infectious diseases and using pre-emptive treatments. During aplasia, transfusions of red blood cells (RBCs) and platelets are required until donor-derived hematopoietic regeneration ocurs. Transfusions may be complicated by transfusion-transmissible infections, including viral and bacterial ones. In contrast to healthy individuals, HSCT recipients, due to their profound immunodeficiency, are at risk of developing severe viral infections when given blood components from cytomegalovirus (CMV)-positive donors. Therefore, thorough screening of potential blood and stem cell donors is of the utmost importance.

During conditioning for HSCT and aplasia, various laboratory analyses are required to avoid severe organ toxicities related to chemotherapy, anti-infective measures, and/or immunosuppressive medications. Therefore, laboratory facilities that cooperate closely with HSCT units and are available for 24 h daily are an important component of the infrastructural requirements that allow safe hematopoietic stem cell grafting and reduce the risks associated with HSCT that, otherwise, can negatively impact patients' outcomes.

In resource-constrained countries or settings, laboratory screening is complicated by deficiencies in infrastructure, transportation, training, financial support, and quality systems. Highly sensitive, yet expensive and technically demanding

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laboratory methods such as nucleic acid testing (NAT) are not routinely available in some areas. Even automated serologic testing platforms require formal training, reagent management, and rigorous quality systems to ensure output reliability. Therefore, for blood screening, rapid diagnostic tests (RTDs) are frequently used due to the lack of availability of more sophisticated technologies in clinical routines. In view of the profound immunodeficiency and thus, vulnerability, of HSCT recipients this practice has to be considered very risky, and HSCT centers are advised to improve their laboratory infrastructure prior to the initiation of hematopoietic stem cell grafting.

This chapter will focus on laboratory analyses that should be available at all HSCT sites, describing various methods for the assessment of blood groups, blood-transmissible diseases, infectious complications during HSCT, measurement of hematopoietic stem cells after harvesting, and assessment of minimal residual disease (MRD) after HSCT.

Serologic Testing of Blood Groups

A blood group is a classification of blood based on the presence and absence of antibodies and inherited antigenic structures on the surfaces of RBCs. These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Blood groups are inherited, and the most important blood group systems are ABO and Rhesus D (RhD) antigen, which determine an individual's blood type (A, B, AB, and O, with Rh positivity or Rh negativity). Because the transfusion of incompatible RBCs results in severe acute hemolytic reaction with hemolysis, renal failure, and shock, and because patients during HSCT require RBC transfusions during aplasia, serologic testing of blood groups in donors and recipients has to be available at every transplant site. Serologic testing of blood groups is based on the in vitro reaction of an antigen on the surface of an individual's RBCs with its specific antibody (anti-A and anti-B serum) resulting in the formation of antigen-antibody complexes, complement activation, and hemagglutination.

Infectious Disease Testing

Infectious complications remain an important cause of post-transplant morbidity and mortality and have been more frequently observed after allogeneic than after autologous HSCT. The implementation of preventive policies and the timely diagnosis of established infectious disease have resulted in improved patients' outcomes and thus, are highly recommended. Infectious risks vary depending on the time during HSCT. During aplasia with severe mucosal damage after conditioning, infection-related mortality is mainly due to severe bacterial sepsis, pneumonia, and fungal infections. The period from the initial hematopoietic engraftment until day +100

after HSCT is characterized by cell-mediated immune deficiency with decreased numbers and function of specific and non-specific cytotoxic cells leading to CMV reactivation and the occurrence of other viral infections, such as those with adenovirus and enteric and respiratory viruses. In the late post-transplant period, immunologic deficiency due to chronic graft-versus-host disease (GVH-D) and impairment of immune reconstitution is the main risk factor for infections with encapsulated bacteria (*Streptococcus pneumoniae* and *Haemophilus influenzae*), as well as fungal infections in the case of prolonged corticosteroid treatment.

Besides quickly and reliably identifying infectious agents in the recipients of hematopoietic stem cells, the screening of potential blood cell as well as stem cell donors is of the utmost importance for the outcome of HSCT.

Testing Methods for Use in Bacterial Infections

In the case of clinical symptoms of bacterial infection, specimens obtained from sites of suspected infection, such as blood, urine, spinal fluid, and other body fluids, should undergo phenotypic typing methods to isolate bacterial strains and to assess biochemical characteristics known to vary within a given species [1], as shown in Table 14.1. Phenotyping may involve colony morphology, color, odor, and other macroscopic features, as well as the qualitative and quantitative assessment of isolates regarding their growth in the presence of specific substances (e.g., metabolites, drugs, bacterial toxins, or bacteriophages) and their expression of specific molecules such as surface antigens. All methods require strict standardization of experimental conditions, because phenotypes are quite susceptible to changes in environmental conditions. Biotyping assesses biochemical characteristics known to vary within a given species. Typeability is usually excellent and stability is dependent on the species and characteristic under consideration. The methods are usually technically easy and inexpensive and all tests can be performed on large numbers of isolates even in small laboratories. Commercial systems facilitating the measurement of large panels of isolates have been developed using versatile redox technologies and enabling the quantification of various biochemical reactions by color readings [2, 3]. In this way strains within a species can be distinguished. Furthermore, phenotype reaction arrays are available that are useful tools in addition to DNA and proteomic technologies. The reproducibility of biotyping is organism- and character-dependent and is rarely 100%.

Antimicrobial susceptibility testing can be performed either by drug diffusion in solid growth media or by drug diffusion in liquid media, using a variety of measurement systems. Antibiogram-based typing with appropriate selection of drugs can be applied to most species and has immediate clinical consequences for guiding therapeutic decisions. Discrimination of species is dependent on the diversity, stability, and relative prevalence of the detectable acquired resistance mechanisms in assessed isolates. This discrimination is also dependent on the number of antimicrobials investigated.

Table 14.1 Current phenotypic and genotypic typing methods for bacterial infections

Method	Principle	Comments
Phenotypic typing	Grouping of organisms according to macroscopic features (colony morphology, color, etc.), growth of isolates, and expression of specific molecules	Requires standardization of experimental conditions to avoid change of organism phenotypes
Biotyping	Assessment of biochemical characteristics known to vary within a given species, ability to distinguish among strains within a species	Excellent typeability, easy and inexpensive methods, suitable for large-scale analyses. Reproducibility is organism- and character-dependent
Antibiogram-based typing	Antimicrobial susceptibility testing	Can be applied to most species
		Important for selection of therapy. Discrimination is dependent on the diversity, stability, and prevalence of detectable resistance mechanisms in the studied isolates. Similar resistance patterns may be due to convergent evolution
Serotyping	Typing of isolates with sera reactive with surface antigens	Widely used, with adequate quality control very reproducible results. Standardization of preparation and testing conditions is important. Typeability and discrimination are variable and complicated by cross-reactions
Genome analysis by array hybridization	Whole-genome sequencing of strains	Detailed bacterial typing. Not yet suitable for routine clinical use, costly, limited access
Plasmid typing	Genotyping of bacterial species	Typeability and discrimination are variable depending on species. Used in combination with antimicrobial susceptibility testing
Single nucleotide polymorphism (SNP) genotyping	Determination of nucleotide base present in a given isolate to define relationships among isolates of homogeneous pathogens	Analysis of nucleotide polymorphisms that are rare along the bacterial chromosome; very efficient, costly

Serotyping has been the most important phenotypic method since the early days of microbiology. Most typing sera react with surface antigens [4]. Typeability and discrimination, complicated by cross-reactions, are variable [5]. With adequate quality control of both reagent and method, serotyping can be a reproducible method of wide applicability. However, the standardization of preparation and testing conditions is important for obtaining reliable results. Genetic instability, horizontal gene transfer, and convergence due to natural or vaccine-driven herd immunity limit the power of serotyping methods.

Genotypic typing methods assess variation in the genomes of bacterial isolates regarding composition, overall structure, or precise nucleotide sequence. Basic

genetic analysis of the molecular events, including mutations, deletions, and insertions, associated with pattern variation is the preferred approach for measuring interstrain relationships, but this is neither always required nor generally feasible [1]. For most clinically relevant microorganisms, whole genome arrays have been developed based on the available whole genome sequences. Probes may be polymerase chain reaction (PCR) products of defined length, but synthetic oligonucleotides are more frequently used. These platforms facilitate bacterial typing in extreme detail. Currently, this method, i.e., whole genome arrays, is not suitable for daily clinical use yet, and costs and accessibility also remain challenging.

Plasmid typing assesses the number, size, and/or restriction endonuclease digestion profiles after agarose gel electrophoresis of these bacterial extrachromosomal genetic elements. It has been used for the typing of many bacterial species, frequently in combination with testing of antimicrobial susceptibility to assess whether an antibiotic resistance gene is plasmid-borne and can be transferred [6].

Single-nucleotide polymorphism (SNP) genotyping involves the determination of the nucleotide base that is present in a given isolate at defined nucleotide positions known to be variable within the population. This method has been primarily used to define the relationships among isolates of homogeneous pathogens, such as *Mycobacterium tuberculosis*, *Escherichia coli*, or *Salmonella enterica* serotype Typhi [7–9].

Testing Methods for Use in Fungal Infections

Invasive fungal infections constitute a serious threat to immunocompromised individuals, including HSCT recipients. Fungal microbes are abundant in nature and frequently colonize various human mucosal surfaces, evading host defenses. Under conditions of impaired immune responses or a break in host barriers, fungi are able to invade normally sterile areas of the human body, causing severe infections that are often lethal [10, 11]. In order to effectively eliminate invasive fungal infections (IFIs), early diagnosis and species identification are of the utmost importance. Furthermore, prophylactic strategies have improved patients' outcomes during recent years [12].

The most commonly used diagnostic techniques are summarized in Table 14.2.

The diagnosis of invasive candidiasis requires biopsy of the involved tissue, followed by staining, culture, and histopathology. Blood cultures remain the gold standard for the diagnosis of candidemia, but they take 1–3 days to grow and an additional 1–2 days for identification of the organism [13]. The ß-glucan assay has been used as a screening test for various fungal infections because ß-D-glucan is a major component of the fungal cell wall of candida spp., aspergillus spp., fusarium spp., and Pneumocystis jirovecii. In a meta-analysis including patients with hematologic malignancies, this test had an excellent specificity but a low sensitivity for the diagnosis of IFI [14].

Table 14.2 Laboratory tests used for diagnosis of invasive fungal infections

Organism	Diagnostic test	Optimal specimen type	Sensitivity %	Specificity %	Comments
Candida spp.	Cultures	Blood	50–60	95	Gold standard, may take up to 3 days for positive result
	Beta-glucan assay	Serum	78–81	87–92	False positive in other IFIs, used as screening test for various fungal infections
	CAGTA assay	Serum	77–89	91–100	Not affected by candida colonization or intake of antifungal agents
	PCR	Blood, other body fluids	80–100	90–100	Also used for identification of specific gene mutations conferring resistance to antifungal drugs
Aspergillus spp.	Histopathology	Various	100	100	Most accurate test is tissue biopsy
	Culture	Various	30–68	72–100	Gold standard, low sensitivity
	Galactomannan assay	Serum, BAL fluid, CSF	71 for serum, 90 for BAL	89 for serum, 94 for BAL	False positive in Histoplasma sp. and Fusarium sp. infections and fungal colonization, false negative in steroid therapy, optimal cutoff not yet established
	Beta-glucan assay	Serum	55–95	77–96	False positive in other fungal and gram-negative bacterial infections and dialysis, screening test for various fungal infections
	Lateral-flow device antigen detection	Serum, BAL fluid	48–100	100	Interpretation is subjective
	PCR	Blood, BAL fluid	86–89	92–94	High variability of results, standardization required
Pneumocystis spp.	Histopathology	Sputum, BAL fluid	33–100	100	Methenamine silver stain on BAL fluid is current gold standard. Toluidine blue stain on induced sputum is most cost-effective
	Beta-glucan assay	Serum	95	86	Excellent screening test for high-risk patients, not useful for monitoring response to therapy
	PCR	BAL fluid, sputum	99	90	High sensitivity and specificity with real-time PCR

Cryptococcus spp.	Cultures	CSF	>95	100	Gold standard, but takes 3–7 days for positive result
	Histopathology	CSF	75	100	India ink stain often used as a screening test
	Cryptococcal antigen test	CSF, serum	97 for CSF, 87 for serum	93–100	Most accurate on CSF
Histoplasma capsulatum	Culture	Tissue, BAL fluid, other body fluids	85	100	Gold standard, but takes 2–4 weeks
	Histopathology	Tissue, BAL fluid	76	100	Unacceptably low sensitivity
	Antibody tests	Serum	75	100	Low sensitivity when only one test performed
	Antigen test	Urine, serum	88–92	100	Most accurate test but cross-reactivity with other dimorphic fungi
Blastomyces dermatitidis	Culture	Sputum, BAL fluid, tissue	86 for sputum, 92 for BAL fluid	100	Gold standard, takes a long time to grow
	Histopathology	Various	46 for sputum, 90 for tissue	100	Broad-based budding
	Antibody test	Urine	57–88	37–100	Cross-reactivity with other dimorphic fungi
	Antigen test	Urine	93	99	Most accurate test, but has cross-reactivity with other dimorphic fungi
Coccidioides spp.	Culture	Sputum, tissue	90	100	Can grow within a week, but identification can take longer
	Histopathology	Sputum, tissue	31–42	100	Spherule detection
	Antibody assays	Serum	95	99	Most commonly used test

BAL Bronchoalveolar lavage, *CAGTA* Candida albicans germ tube antibody, *CSF* cerebrospinal fluid, *PCR* polymerase chain reaction, *IFI* invasive fungal infection

Invasive aspergillosis is proven by the demonstration of fungal hyphae in tissue biopsy specimens. The sensitivity of culture for the diagnosis of aspergillosis is low, and was only 25–50% in HSCT recipients [11, 15]. Histopathology has the advantage of detecting both the invasion of various tissues by fungi and the host response or tissue necrosis. It is almost always performed in combination with cultures to clarify whether a positive culture is the result of infection, colonization, or contamination. The sensitivity of ß-D-glucan testing ranges from 55% to 95% and the specificity ranges from 77% to 96% for patients with hematologic malignancies suffering from invasive aspergillosis [16, 17]. The galactomannan assay is fairly specific and sensitive for the diagnosis of invasive aspergillosis, although galactomannan can also be found on the cell walls of Histoplasma capsulatum and fusarium spp. This test has the highest sensitivity in patients with hematologic malignancies or those who have undergone HSCT [18, 19]. Because its sensitivity increases even more with sequential testing, it is often used in combination with culture for the definitive diagnosis of a fungal infection [20, 21].

Pneumocystis jirovecii can be visualized by methenamine silver staining of bronchoalveolar lavage fluid (BAL) specimens, where diagnostic accuracy is better compared with sputum [22]. In an analysis of patients with pneumocystis pneumonia, toluidine blue staining of induced sputum samples reportedly was the most cost-effective of the staining methods, while the performance of BAL increased the cost without significantly affecting the percentage of patients who were successfully treated [23]. In a meta-analysis, the ß-glucan assay performed on serum had sensitivity and specificity of 95% and 86%, respectively, for the diagnosis of pneumocystis pneumonia [24].

Cryptococcal disease is diagnosed primarily with cerebrospinal fluid (CSF) cultures, which grow mucoid colonies within 3–7 days; as well, staining of the CSF with India ink allows visualization of the cryptococcal cells under the microscope. The most accurate screening method is the cryptococcal antigen test, which has high sensitivity and specificity when performed with CSF [25].

In recent years molecular methods such as PCR have been more frequently used in fungal disease diagnostics, with their simplicity and short turnaround being the most important advantages [26]. Because fungi, however, are frequent colonizers of human surfaces, it is challenging to decide whether identified fungal DNA is the result of colonization or whether it represents an active fungal infection. Therefore, real-time PCR was introduced, allowing quantification of the amount of DNA [27]. Besides potential contamination revealing false-positive results, technical issues such as methods for DNA isolation, choice of primers, and lack of international standardization have been challenging [28]. Recently, the European Aspergillus PCR Initiative (EAPCRI) issued recommendations for optimal PCR performance from whole blood, based on a study with compliant centers being able to detect at least 50 conidia of Aspergillus spp. and achieving an average sensitivity and specificity of 89% and 92%, respectively [29, 30]. A variety of sensitivity and specificity values have been reported for the use of PCR in invasive candidiasis. PCR methods for the identification of specific gene mutations that can confer resistance to known antifungal agents have been described [31]. Multiplex PCR can detect a wide vari-

ety of fungi at once in the same specimen by using primers specifically designed to amplify a region that is conserved among different fungal genera. Superior sensitivities and specificities above 80% have been reported in whole blood, serum, or BAL fluid [32, 33].

In summary, the PCR method is still in need of standardization, but it offers the potential of being able to identify the presence of fungal pathogens within human fluids, define the species, quantify the infection, and detect antimicrobial resistance markers.

Fluorescence in-situ hybridization (FISH) is a technique that uses fluorescent probes to identify target areas on the genomes of microbial pathogens in human samples, which can then be detected by fluorescence microscopy. This method has been used as an adjunct to culture or PCR and has high accuracy for the identification of Candida sp. infections from blood culture bottles [34]. Its reliability will have to be demonstrated after standardization of this molecular method in future studies.

Testing Methods for Use in Viral Infections

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are major causes of chronic liver disease, including cirrhosis and hepatocellular carcinoma. Immune dysfunction arising from hematologic diseases, intensive chemotherapy, immunosuppressive therapy, and HSCT can result in the exacerbation or reactivation of HBV infection [35]. In areas of high HBV endemicity where almost 15% of the patients receiving HSCT are hepatitis B surface antigen (HBsAg)-positive prior to transplantation, HBV reactivation, occurring in more than half of the patients, can significantly impact the post-transplant prognosis, with increased severity of liver injury and increased hepatitis-related mortality [36, 37]. Antiviral therapy for the suppression of HBV, initiated prior to HSCT and continued thereafter under close monitoring of HBV DNA, reportedly improved the safety of HSCT [38]. Because HBV and HCV can be transmitted by blood transfusion and by hematopoietic stem cells, the testing not only of patients but also of potential donors for HBV and HCV is mandatory, as recommended by various scientific and regulatory organizations [39–41].

For the diagnosis of chronic HBV and HCV infections, as well as human immunodeficiency virus (HIV) infection, in adults and children above the age of 12 months, a quality-assured serological assay such as an enzyme immunoassay (EIA), chemoluminescence immunoassay (CLIA), or electrochemoluminescence assay (ECL) should be applied in accordance with the manufacturer's instructions. EIAs and CLIAs are based on the use of immobilized antibodies and/or antigens in a solid phase, which form immune complexes with target analytes present in a sample. Both methods reveal immune complexes through either color generation or light emitted by a chemical reaction. Immunoassays allow high-throughput testing and demonstrate sensitivities and specificities approximating 100%. Therefore, the

automated serological assay is the most commonly used method for transfusion screening in many countries. Antigen/Antibody combination EIAs, which detect both antigens and antibodies, have improved screening and diagnostic testing, specifically for the detection of HCV and HIV [42].

Observed HBsAg-negativity indicates no serological evidence of HBV infection, whereas in the case of HBsAg-positivity a quantitative HBV DNA nucleic acid test is recommended to further guidance on who to treat with antiviral agents. NAT uses in-vitro amplification of a pathogen-specific sequence of DNA or RNA for the detection of pathogens and allows the identification of infectious donors in the pre-seroconversion window period. Risk estimates suggest that individual donation NAT has reduced the time from infection to detection for HIV and HCV from 22 and 70 days (using antibody assays) to 6 and 5 days, respectively [43]. Following a reactive HCV antibody serological test result, quantitative or qualitative RNA NAT is recommended as the preferred testing strategy to diagnose viremic infection. Therefore, the currently in use Food and Drug Administration (FDA) and American Association of Blood Banks (AABB) guidelines include mandatory NAT testing in addition to the serological testing for HIV and hepatitis [44, 45]. This strategy has proven feasible and has provided safe hematopoietic stem cell transplants even in countries with high prevalences of hepatitis B and C [46].

In world regions with a large percentage of the population showing evidence of prior exposure to HBV with a high burden of occult HBV infection, the use of HBsAg alone for screening either in blood transfusion or transplantation services reportedly did not eliminate the risk of HBV transmission [47]. In these regions NATs should be introduced mandatorily for the routine screening of donors and recipients in transplantation services. Because individual donor testing using NAT is more sensitive than testing in pools of blood donors, NAT should be the preferred method for this purpose [48].

Although NAT would confer benefits, the lack of infrastructure and technical expertise, coupled with high costs, still precludes its more widespread implementation in resource-constrained countries. There, rapid diagnostic test (RDTs) have frequently been used for the screening of blood donors, as these single-use test kits usually do not require electricity or a formal laboratory infrastructure [49]. RDTs can be applied to a variety of specimens, including whole blood (finger-stick or venipuncture), serum, plasma, and saliva. RDTs are based either on particle agglutination, solid-phase tests, immunofiltration, or immunochromatography [50, 51]. RTDs have enabled large-scale testing by minimally trained workers and, thus, have been adopted for donor infectious screening in countries and settings where the skill base, time, or resources do not support the use of EIAs or more sophisticated technologies [49]. However, the effectiveness of RDTs is dependent on the test quality and conditions of use and there is a lack of technical guidelines in the field. Evaluations of their use in transfusion screening in Africa revealed a high variability in performance, especially when used for testing for HBV and HCV [49]. Therefore, RDTs for donor infectious screening should not be standard in clinical facilities performing HSCT.

Another policy against transfusion-transmissible infections is to select low-risk donors both for blood donation and HSCT. This includes elaborate donor work-ups

Table 14.3 Donor screening

Infectious disease	Recommended validated assay
HIV	HIV-1,2 antibody, p24 antigen, HIV RNA
Hepatitis B	Hepatitis B surface antigen and antibody, hepatitis B core antibody, hepatitis B DNA
Hepatitis C	Hepatitis C antibody, hepatitis C RNA
HTLV I + II	HTLV I + II antibody
Syphilis	Validated serological testing algorithm
CMV	CMV antibody

Tests have to be performed within 30 days prior to hematopoietic stem cell donation
CMV Cytomegalovirus, *HTLV* human T-lymphotropic virus, *HIV* human immunodeficiency virus

with detailed assessment of medical conditions and lifestyles, as well as donor counseling regarding the transmission of infectious diseases [40]. Table 14.3 summarizes the recommended donor assessment regarding infectious risks as published by the World Marrow Donor Association [40].

CMV occurring either as a primary infection or reactivation is known to significantly influence the outcomes of HSCT. Because CMV infection usually precedes CMV disease, and in view of the poor prognosis of CMV disease despite treatment, pre-emptive therapeutic strategies during HSCT have been developed to reduce the risk of CMV disease. To detect CMV infection before progression to overt disease, patients have to be monitored for CMV in peripheral blood (PB) at least weekly with a sensitive method until day +100 after HSCT, or for a longer time in the case of prolonged GVH-D or previous CMV reactivation. For many years the detection of the CMV antigen CMVpp65, indicating CMV replication and thus, viral antigenemia, has been in clinical use [52] and this is recommended as a minimum requirement for CMV testing. Antigenemia testing detects CMV antigen pp65 in leukocytes by immune staining with monoclonal antibodies. This test is semiquantitative and rapid. Using DNA/RNA-based methods, the detection of CMV in blood is achieved earlier, allowing timely treatment and the reduction of CMV disease and overall mortality [53]. The quantification of the viral load by real-time or light-cycler technologies is of clinical importance besides offering timely results, because higher levels of CMV DNA are indicators of a higher risk of CMV disease. Because the risk of CMV disease is very low after autologous HSCT, systematic CMV screening is not recommended in this patient population, except in high-risk patients such as those receiving CD34-selected stem cell grafts or patients given fludarabine or alemtuzumab.

Therapeutic Drug Monitoring

Immunosuppressive drugs such as cyclosporine A (CsA) , tacrolimus, sirolimus, and everolimus are widely used for the prevention of allograft rejection in solid-organ transplantation and in GVH-D after HSCT. All have a narrow therapeutic

window, and large intra- and inter-patient variabilities in their pharmacokinetics have been observed. Therefore, therapeutic drug monitoring (TDM) is essential for guiding dosing to ensure that blood concentrations are kept within the target range in transplant recipients. Low drug concentrations can result in GVH-D, while high concentrations can lead to nephrotoxicity, arterial hypertension, or transplant-associated microangiopathy. Reliable, accurate, and precise test methods are, therefore, essential to effectively monitor drug levels and to make proper dose adjustments.

In an attempt to standardize the practice of CsA measurement, recommendations for CsA monitoring involve analyzing trough concentrations of the drug in ethylenediaminetetraacetic acid (EDTA)-treated whole blood, using a method specific for the parent compound [54]. Several analytical procedures for monitoring drug levels in blood are currently available, including a number of immunoassays and chromatographic analytical methods [55, 56]. Both radioimmunoassays and monoclonal antibody-based immunoassays have been frequently used clinically. In the latter, whole-blood samples are combined with analyte-specific (e.g., CsA-specific) biotinylated antibody and labeled analyte derivatives. Formation of the respective immune complex depends on the drug concentration in the sample. After the addition of streptavidin-coated magnetic microparticles, the immune complex becomes bound to the solid phase through the interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Application of voltage to the electrode induces chemiluminescent emission, which is measured by a photomultiplier [57]. Immunoassays have been frequently used for TDM because they are fast; however, cross-reactivity of the immunoassay antibodies with inactive CsA metabolites is of concern as it hampers interpretation of the analytical results and affects analytical specificity.

In clinical scenarios with the accumulation of drug metabolites, such as liver disease, a validated high-performance liquid chromatography (HPLC) assay should be the method of choice and should be available in centers dealing with such samples. HPLC in combination with electrospray tandem mass spectrometry is now largely used because of its high specificity compared with immunoassay methods and its capability to simultaneously analyze a range of immunosuppressive drugs that are frequently used in combination with CsA [58].

Immunophenotyping for $CD34^+$ Cell Enumeration

The aspiration of bone marrow (BM) produces a mixture of marrow cells from the bone cavity and capillary blood. Even though the dilution of marrow cells with PB is variable, the adequacy of BM collection is determined by the nucleated cell count of the mixture, with the aim of obtaining over 2.0×10^8 nucleated cells/kg recipient

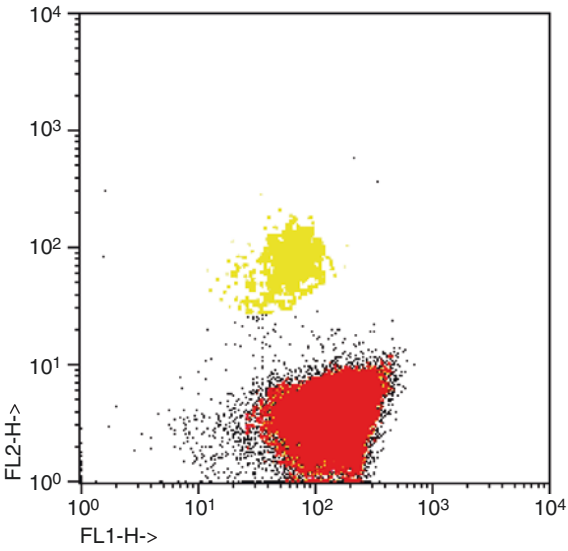

Fig. 14.1 CD34⁺ cell enumeration

body weight. Since the 1990s, hematopoietic stem and progenitor cells (HSPCs) have been characterized by flow cytometric methods using CD34 as a cell surface marker, based on the knowledge that HSPCs express CD34 and CD33 differentiation antigens [59]. For dual-color direct immunofluorescence analysis by flow cytometry, a small aliquot (50 uL) of heparinized whole PB or leukapheresis cell suspension is incubated with a mixture of CD34 fluorescein Isothiocyanate (FITC)-conjugated monoclonal antibody (mAb) and CD33 phycoerythrin (PE)-conjugated mAb for 25 min. After RBC lysis, samples are analyzed by flow cytometry gating on viable cells positioned in the lympho-monocytic area of the forward-scattered light (FSC)/side-scattered light (SSC) dot plot (Fig. 14.1). At least 10,000 cells should be acquired and the frequency of the cells expressing CD34 and/or CD33 antigens is calculated as the percentage of all analyzed cells. Nonspecific binding of the FITC-conjugated mAb to HSPC accounting for CD34⁺ cell numbers has to be ruled out using a PE-conjugated isotype-matched irrelevant antibody control, as described [60].

Since 1996 the two-color International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol focusing on CD45 and CD34 has served as the standard for CD34⁺ cell enumeration [61]. The addition of 7-Aminoactinomycin D (7-ADD) to define cell viability, and the use of beads for standardized quantification expanded the test to a single-platform three-color analysis tool to enumerate CD34⁺ cell numbers in BM, PB, and cord blood [62, 63]. Whereas, in the clinical setting, the most important cell population for use in HSCT is the CD34+ one, research projects investigating multicolor immunophenotyping have meanwhile identified various stem cell subpopulations in CD34⁺ sources, with a currently

unknown impact on engraftment kinetics and potential for immune reconstitution after HSCT [64].

Minimal Residual Disease Testing

Despite HSCT, relapse remains the major obstacle to cure for patients with hematologic malignancies. In recent years technology to detect MRD has matured and currently different methods are available for diagnosing relapse, either very early prior to the onset of clinical symptoms or for the prediction of relapse depending on the MRD results obtained. Assessing MRD status allows timely clinical interventions, including the discontinuation of immunosuppression and/or additional immunotherapeutic interventions for ultimate disease eradication. Optimal methods for

Table 14.4 Detection of minimal residual disease

Detection method	Target	Sensitivity %	Clinical use	Comments
Pathology examination	Cellular morphology	5	Standard clinical practice	Defines CR
Cytogenetics	Chromosome structure	1–5	Standard clinical practice, upfront risk stratification, defines clonal evolution and therapy-related clonal changes	Labor-intensive, limited sensitivity
FISH	Specific genetic markers	0.08–5	Rapid assay for known marker	Evaluates single cells
Multi-color flow cytometry	Surface antigen expression	0.01–1	Rapid test, applicable for most patients	Requires more markers to increase sensitivity and specificity
PCR for chromosome aberrations	mRNA sequence	0.0001–0.1	Rapid, sensitive, limited to patients with known fusion gene transcripts	Positive test may not be clinically meaningful
PCR for immunoglobulin/ TCR genes	DNA sequence	0.001–0.1	Sensitive for patients with B- and T-cell diseases	Time-consuming at diagnosis, individualized disease marker
High-throughput sequencing of multiple Ig genes	IGH CDR3	0.0001	Rapid, sensitive for patients with lymphoid malignancies	Accurate, fast, detection of multiple clones

FISH Fluorescence in situ hybridization, *PCR* polymerase chain reaction, *TCR* T-cell receptor, *Ig* immunoglobulin, *IGH CDR3* immunoglobulin heavy complementarity determining regions, *CR* complete remission

MRD detection must have specificity for the malignant cell population and must be sensitive enough to detect small numbers of clonal cells in a background of normal cells. The currently used methods are summarized in Table 14.4.

If a karyotypic abnormality is detected at the time of diagnosis, cytogenetic analysis can be used to evalute remission samples. However, the sensitivity is low and the requirement for dividing cells to generate metaphase spreads may lead to a high failure rate, especially in the early phase after HSCT [65, 66].

Fluorescence in-situ hybridization (FISH) uses fluorochrome-labeled DNA probes to detect the deletion, amplification, and translocation of one or several DNA molecules within chromosomes. FISH, when applied to interphase nuclei, is a more sensitive technique than conventional cytogenetics [67–69]. Due to the lack of requirement for dividing cells the failure rate is lower than that with standard karyotyping. Currently, most FISH kits that are used clinically detect one or two genes and involve the use of one or two fluorescence colors at a time. The use of quantitative multi-gene FISH has increased in recent years [68].

Leukemic cells as well as myeloma cells are characterized by aberrant or unusual patterns of antigen expression, termed the leukemia-associated phenotype, which allows MRD analysis by flow cytometry [65, 66, 70, 71]. The sensitivity of this method can be increased by the use of an 8- to 12-color multiparameter flow cytometric panel. Furthermore, inter-laboratory standardization is of critical importance, as addressed by the Euroflow Consortium [72, 73].

Due to their high specificity and sensitivity, PCR-based approaches have high clinical relevance for the detection of MRD in malignant disease with a known chromosomal lesion. Primers are designed to bind to the nucleic acid of each of the gene partners in the translocation, allowing specific detection of the chromosomal lesion. RNA as the primary target has to be converted into complementary DNA (cDNA), using the enzyme reverse transcriptase to allow amplification by PCR. Quantitative real time PCR for *BCR/ABL* has been used since many years to detect minimal residual disease (and predict outcome) in patients with chronic myelogenous leukemia after stem cell transplantation [74]. When a chromosomal lesion is lacking, MRD analysis relies on the detection of clone-specific rearrangements, such as the rearranged immunoglobulin (Ig) or T-cell receptor (TCR) genes. Ig and TCR recombinations occur in the early stages of B-cell and T-cell development. Therefore, each lymphocyte contains unique V (D) J recombinations resulting from random coupling between one of many possible V, (D), and J genes, as well as imprecise joining of gene segments and the addition of nucleotides to the DNA sequence at splice sites [75]. Identical recombinations, therefore, indicate the clonal nature of a population and that the population is not derived from independent cells. The monitoring of Ig/TCR-based MRD in patients with acute lymphoblastic leukemia (ALL) consists of step-by-step analysis of V (D) J DNA recombinations in lymphoblasts and their subsequent detection during follow-up.

Leukemic clonal recombinations can be amplified by PCR and examined by capillary electrophoresis and they can then be isolated by polyacrylamide gel electrophoresis and finally sequenced by direct Sanger sequencing. Because many ALL patients have rearranged IgH or TCR genes, these are clinically very relevant for

MRD detection in ALL) [76]. The detection of clonal IgH and TCR fingerprints and of the most frequent fusion transcripts in ALL has been standardized and has become the gold standard method in clinical practice [77, 78].

The introduction of real-time quantitative PCR approaches (RQ-PCR) applied serially has further improved the assessment of treatment response [79, 80]. Recently, high-throughput sequencing has been established, allowing the sequencing of clonal recombinations of multiple Ig/TCR genes by pooling several PCR systems in one analysis. This way it is possible to identify multiple leukemia-specific clones and subclones simultaneously, faster and with higher sensitivity than with standard methods based on V (D) J sequencing [76, 81].

In summary, MRD analysis has been established as a clinical routine in patients with)ALL, resulting in improved assessment of treatment responses and allowing therapeutic risk stratification, as well as the early detection of relapse [80, 82]. In other hematologic diseases, including acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and myeloma, MRD assessment is still performed within clinical studies to investigate its clinical impact on patients' outcomes. MRD analysis requires well-equipped laboratory facilities; it is costly and should be performed using standardized techniques.

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Chapter 15
Quality Management

Eoin McGrath and Kathy Loper

Introduction

Quality management (QM) emerged from industry in the middle of the twentieth century and has become increasingly well established in medicine over the past 20 years. This trend has been particularly pronounced in the field of bone marrow transplantation, largely through the efforts of professional organizations such as the American Association of Blood Banks (AABB), the European Society for Blood and Marrow Transplantion (EBMT), the American Society for Blood and Marrow Transplantation (ASBMT), and the International Society for Cellular Therapy (ISCT) and their pioneering standards and accreditation schemes, e.g., AABB [1], the Joint Accreditation Committee of the ISCT and the EBMT [2], the Foundation for the Accreditation of Cellular Therapy [2], and NetCord [3].

The Council of Europe Guide to the Quality and Safety of Tissue and Cells [4] describes how quality is achieved through compliance with requirements at three different levels: (1) the legal framework, (2) the QM system (QMS), and (3) the technical requirements specific to each type of tissue or cell that ensure quality, safety, and efficacy. This chapter focuses on the QM system.

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Why is a Quality Management System Important?

Without a QMS, it is difficult for a transplant program to monitor its own performance, ensure consistency of processes, identify areas for improvement, and demonstrate its operational effectiveness to internal and external entities.

There is now evidence that QM in hematopoietic stem cell transplantation significantly affects patient outcome. Gratwohl et al. [5] found that "working towards implementation of a QMS triggers a dynamic process associated with a steeper reduction in mortality… and a significantly improved survival after allogeneic stem cell transplantation."

The Quality Management System

The QMS "is a formalized system that documents processes, procedures, and responsibilities for achieving quality policies and objectives. A QMS helps coordinate and direct an organization's activities to meet customer and regulatory requirements and improve its effectiveness and efficiency on a continuous basis" [1]. For hematopoietic stem cell transplant programs, it is a system to manage and control all aspects related to delivering transplantation as a therapeutic strategy and to help ensure reproducible results. The QMS should be (1) global, to cover all related activities; (2) flexible, so as to adapt to advances in medical and scientific practice or regulatory changes; and (3) it should generate continuous improvement [6].

QM in transplantation can also be understood as the ongoing assessment of the stability, reproducibility, and effectiveness of critical processes in order to continually improve program efficiency and patient outcome. QM assessment findings are compared with pre-established specifications which, when not met, require the implementation of corrective or improvement actions with monitoring through follow-up to determine the effectiveness of any changes made [2].

Establishing a QMS

Among the obstacles to establishing a QMS in healthcare in general, and in a transplant program specifically, is the need to dedicate resources and time to its set-up and maintenance. Against a global backdrop of economic difficulties, QM may fall down the list of priorities. However, the question that should perhaps be asked is not how much a QMS costs but how much will poor quality cost? Table 15.1 below shows some of the consequences of poor quality.

Table 15.1 Costs of poor quality

Waste
Rejects
Testing costs
Inspection costs
Recalls
Excessive overtime
Planning delays
Excessive employee turnover
Development cost of failed product
Excessive system costs
Complaint handling
Late paperwork
Lack of follow-up on current programs
Excess inventory
Unused capacity
Incorrectly completed order

Adapted from DeFeo JA. The Tip of the Iceberg. *Qual Prog*. 2001;**34**:29–37

Regulations

Most countries have a legal framework in which transplantation is regulated by the relevant authorities. A detailed discussion of regulations is outside the focus of this chapter. These legal requirements, where they exist, should be the first reference for any QMS. Where no national legal framework exists, a program should first look to the international best practice guidelines and standards being used elsewhere. These can include the professional societies mentioned above or guidance published by international organizations such as the World Health Organization (WHO) [7] or the Council of Europe (CoE) [4]. Other resources include the documents and links available on the Alliance for Harmonisation of Cellular Therapy Accreditation (AHCTA) website (www.ahcta.org.)

Responsibilities

Quality management is the responsibility of everyone working in the program. One person should be designated to coordinate and manage the quality system. That person will not be able to write all of the procedures and perform all of the necessary monitoring alone. It is the Program Director who is ultimately responsible for the administration of care within the program, including QM. Indeed, the QMS

greatly facilitates how this responsibility is exercised by providing tools and mechanisms for overseeing the operations and processes carried out and for drawing attention to cases where results or outcomes are unexpected. The Director may delegate performance of tasks to other members of the program team, but the *responsibility* cannot be delegated [6].

Components of a Quality Management System

A QMS typically encompasses the following components:

- People and organization
- Facilities, equipment, and materials
- Agreements
- Document and record-keeping
- Product tracking and traceability
- Audits
- Validation and verification
- Investigation and reporting of non-conformance, adverse events, complaints, and reactions

People and Organization

There should be sufficient staff to carry out all defined tasks in compliance with quality and safety requirements. Staff should be trained and competent.

All staff should have clear and concise job descriptions. There should be an organizational chart that describes the structure of the organization with clear delineation of responsibilities, reporting structure, and the delivery of services. Any other departments that provide services to the transplant program should also be represented.

Training and Education

It is important for the organization to ensure that staff have the knowledge, experience, and training to perform the required tasks. There should be a training plan for all staff to maintain a suitable level of competence and to ensure that they are only performing the tasks that fall within their job description.

For every role within the program there are basic educational and experience requirements that must be stated in the job description. All staff should receive job-specific training. Training should be documented and should include areas such as equipment operation, drug administration, etc.

A detailed educational program should be defined for all staff. This should include all educational updates required to meet institutional requirements, as well

as the requirements of external organizations. Retraining requirements should also be defined. Staff should be assessed at a minimum annually. Relevant policies and procedures should be reviewed and updated as practices change.

Competency

Competency is the ability to carry out a task effectively and safely in accordance with established procedures. Competency to perform critical tasks must be assessed and documented. The competency assessment may identify the need for further training, which should be provided before the staff member undertakes those tasks again.

Agreements

If the transplant program interacts with third parties, e.g., services for collection or product processing, these relationships should be managed through written agreements. Agreements clarify who is responsible for each activity or part of a process. All such agreements should be dated and, reviewed regularly. Agreements may also be referred to as service level agreements (SLAs).

SLAs generally refer to external services, not services that are part of the same institution. However, institutional policies vary and facilities may require agreements for some internal services as well.

Documentation and Record-Keeping

Documents serve multiple purposes for the QMS. Documents provide the structure needed for quality assurance through policies and procedures. Completion of forms such as pre-printed orders and worksheets helps to reduce inconsistencies, support QM activities, and inform audit reports, outcome analysis, training records, etc. The quality program should identify documents critical to the program. These critical documents adhere to the document control system discussed later in this chapter.

Every part of the program requires written instructions on how to perform key processes. All personnel in the facility should use these documents to carry out tasks and must ensure that the document in use is the current version. Documents are the foundation of the QMS, because they explain how each of the tasks is undertaken and, when grouped together, make the program run effectively. The program should develop a master procedure for formatting, writing, reviewing, implementing, and controlling documentation. This document is sometimes referred to as the "SOP (standard operating procedure) for SOPs".

Documentation should be version-controlled. Table 15.2 lists examples of items considered to be documents.

Table 15.2 Items classified as "documents" in a quality management system (QMS)

Quality plan, handbook, or manual	Standard operating procedure (SOP) for activities, including management of the quality system itself
Records on operations	Processing records
Records of complaints, audits, and non-compliances	Training and competency records of staff
Forms, worksheets, and labels	Policies

Adapted from the Guide to the quality and safety of tissues and cells for human application [4]

Quality Management Plan

The quality management plan consists of the following items and is often kept in one manual, which may be hard-copy or electronic:

- A brief description of the program's activity, including population served, history, main indications for transplantation, volume of activity, applicable regulatory framework, and key relationships
- Organizational chart or organigram listing the team and their roles/responsibilities
- A description of the QMS—responsibilities, quality management team, education, experience and training requirements, processes for adverse event detection, investigation, reporting and resolution, etc.

Standard Operating Procedures

The program should maintain an SOP manual for each department. The SOP manual is the collection of policies and procedures containing the written detailed instructions required to perform procedures. The SOP manual should be easily accessible by staff.

The first step in SOP development is to map out the respective process: donor and patient pathways and the stem cell product's journey from start to finish, after which the program can develop applicable instructions. Pre-existing applicable hospital procedures may be used. The *quantity* of SOPs is not important. What *is* important is that all of the key processes are clearly described and that staff work according to these SOPs. SOPs should reflect how the program units are really working, not how one thinks they *should* be working.

While the quality manager can *facilitate* drafting the everyone should assume their responsibility in this process. The program should ensure that key staff groups provide input on the documents which are relevant to them.

Table 15.3 Minimal elements required in each standard operating procedure (SOP)

Item	Description
Clear description of the objectives of the procedure	Describes what the procedure is intended to achieve, e.g., safe infusion of cellular product
Description of equipment and supplies used	States what equipment etc. is required for the procedure, e.g., labels, syringes. This section can state "not applicable" (N/A) if no equipment is used
Acceptable end-points and range of expected results where applicable	Details the expected result, e.g., processing should yield at least 2.5×10^6/kg CD34 cells. The SOP should also include instructions on what should be done if the expected result is not achieved
Stepwise description of the procedure	List/describe each step required to complete the procedure. Include any required worksheets or forms used and include examples
Reference to other SOPs or policies	Allows the reader to access related procedures, e.g., an Infusion of Cells SOP states: "step 4: check identity of patient with product", which will reference the policy for "Positive identification of Patients"
Reference section listing appropriate literature, if applicable	Published articles, guidelines, or data to support the procedure or process are listed in this section
Documented approval of each procedure and procedural modification	Each document includes the approval date, signature of the approving individuals, and the effective date
Copy of or links to current versions of orders, worksheets, reports, labels and forms, where applicable	Copies of or references to current versions, where applicable, should be a part of each SOP to ensure that these documents are easily accessible to a reader of the SOP. Alternatively, they can be linked electronically to the SOP. Review of SOPs should include review of the associated labels, forms, worksheets, etc.
Additional information	Some documents might require additional information, such as age-specific considerations, risks from undertaking the procedure, preventive and corrective action in the event of equipment malfunction, etc.

Minimal Elements Required in Each SOP

Each individual procedure shall include the minimum requirements summarized in Table 15.3. The style and layout should be consistent for SOPs, policies, forms, and worksheets. Consistency provides evidence of standardization and integration.

Records

Forms, worksheets, and labels become records once completed. Records contain key information relating to a person or product. Facilities should define the record retention or archival period in accordance with applicable standards and regulations.

Document Control

The purpose of document control is to ensure that only currently approved documents or versions are used. The document control system includes details on how critical documents are conceived, generated, implemented, distributed, reviewed, and stored. Replacement and removal of obsolete documents, including labels, are also managed by document control.

The responsibility for document control resides with the quality manager, and the control of large volumes of SOPs, forms, policies, etc. can be challenging. Numerous electronic solutions are now on the market. All documents should be approved by the respective director(s), author/s, reviewers, and QM. When documents span more than one department or service line, all affected groups should approve the documents.

Product Tracking and Traceability

One of the most important processes in the program is ensuring the safe tracking of the cellular therapy product from donor collection to infusion in the patient. Tracking of a product should be documented and involves several processes, including:

- Correct labeling to facilitate donor/recipient tracking
- Use of unique donor and product identification
- Transportation in a validated shipping container
- Visual inspection of the container(s)
- Retention of records documenting the origin and destination of distributed material
- Receipt of cells, including verification of shipment and the condition of the product upon receipt and during transport.

Written procedures and supporting documents should be developed to ensure that product ordering, labeling, packaging, shipping/handling, and administration are performed appropriately.

Audits

According to the American Society of Quality, audits are "the on-site verification activity, such as inspection or examination, of a process or system…, to ensure compliance to requirements. An audit can apply to an entire organization or might be specific to a function, process, or production step" [2]. An audit is a documented,

systematic evaluation of a facility's QM activities to verify, by examination and evaluation of objective evidence, the degree of compliance with those aspects of the quality program under review.

1. **Audit Criteria Selection**—A variety of areas should be considered, including:
   (a) Accuracy of data in data collection forms
   (b) Donor screening, consent, and testing
   (c) Verification of chemotherapy drug and dose against orders and protocols
   (d) Management of cellular products with positive microbial culture results
   (e) Results of services performed by external facilities or contractors
   (f) Engraftment, morbidity/mortality
2. **Audit Frequency**
   Audit intervals should be defined, and audits conducted, reviewed, and reported on a regular basis, at least annually.
3. **Define Criteria and Set Standards**—Program procedures include measurable outcomes, such as:
   (a) Target turn-around time for sampling
   (b) Target cell yield from peripheral blood stem cell (PBSC) or bone marrow (BM) collection
   (c) Target time to engraftment based upon patient type, transplant type, conditioning regimen used, and cell dose infused
4. **Measure against the Standards—**Procedures and records are assessed to determine whether the center is achieving the targets or measurable outcomes intended.
5. **Summary of Audit Results with Targets/Measurable Outcomes**—The center summarizes the audit results and identifies whether targets are met. Missing or incorrect steps are noted and addressed in the action plan. Audit results inform the program on any potential problems, deviations, or service perceptions.
6. **Formulate Action Plans**—Action plans may be developed at different times during the audit process

Auditing the Improvements

The process of auditing does not end with completion, reporting, and action. Once implemented, changes should be audited as the program seeks continuous quality improvement. Changes and practice modifications should be developed and included in a controlled manner, called change control, to ensure that training and document control policies are followed. This completes the PDSA (Plan-Do-Study-Act) [3] cycle, also known as the Plan-Do-Check-Act, as illustrated in Fig. 15.1. The PDCA was made popular by Dr. W. Edwards Deming, who is considered by many to be one of the fathers of modern quality management.

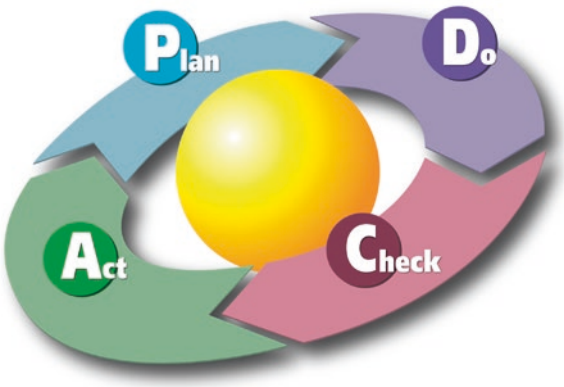

Fig. 15.1 The Plan-Do-Check-Act (PDCA) cycle. By Karn-b - Karn G. Bulsuk (http://www.bulsuk.com). Originally published at: http://www.bulsuk.com/2009/02/taking-first-step-with-pdca.html [CC BY 4.0 (http://creativecommons.org/licenses/by/4.0)], via Wikimedia Commons. Accessed 13/07/2017

Validation and Verification

Validation is the confirmation by examination and provision of objective evidence that particular requirements can consistently be fulfilled. A validated process helps ensure that a cellular therapy product meets its predetermined specifications.

Verification is the confirmation that specified requirements have been fulfilled using a validated process or procedure.

Validation/verification activity needs to be undertaken in accordance with procedures agreed upon by QM and relevant staff that specify all critical steps to be undertaken and the acceptance criteria to be applied. A validation procedure may include aspects of equipment design, installation, initial operation, and performance assessment in the routine environment. A report summarizing the results obtained, any exceptions observed, and conclusions reached should be completed and reviewed by the quality team, or medical director (as applicable) as part of the authorization process prior to implementation.

Investigation and Reporting of Non-conformances, Adverse Events, Complaints, and Adverse Reactions

Examples of non-conformances include deviations from SOPs, errors, and accidents. There should be an SOP in place that defines how the organization manages non-conformances and there should be a record of all non-conformances. Detailed documentation of the investigation, root-cause analysis, and corrective/preventive actions taken are documented. A categorization of critical non-conformances that affect the quality and safety of patients or products is a useful tool for prioritizing corrective actions.

Procedures should be in place to identify appropriate corrective and preventive actions and to inform the relevant authorities as appropriate. Reporting of errors and incidents in a non-punitive context should be encouraged to help achieve practice improvements. Tracking and trending of non-conformances should be carried out to identify common failures and identify areas of concern. Serious adverse events and serious adverse reactions should be reported through a vigilance system, either internal or external.

Complaints

All complaints should be documented, carefully investigated, and managed in a timely manner. The complaints procedure should take into consideration complaints from donors, staff, third-party health professionals, and patients.

A mechanism for categorizing, tracking, and trending complaints is required. Categorization and review of complaints should lead to the assessment of whether the complaint is justified and whether it is related to a potential non-compliance during an audit. In the latter case, the complaint should then be investigated thoroughly, including root-cause analysis and identification of corrective measures.

Recall

There must be an SOP for material and product recall that includes steps for product return, reissue, and notification of suppliers and distributors or registries. For some facilities, product recall may be rare, while for those that distribute products externally, such situations may be more likely to occur, but a contingency plan should be prepared regardless.

Conclusion

Quality management has become an indispensable tool in the management of a complex medical intervention such as hematopoietic stem cell transplantation. QM allows the transplant team to monitor its activities and identify areas for improvement. It aids communication, both within the team and with external service providers. It helps anticipate and respond to adverse events and process improvement to avoid repeated errors. The development of voluntary standards and sample processes by the professionals themselves has been the key factor in driving acceptance of these controls and keeping them relevant to day-to-day practice. New or recently established programs are strongly encouraged to incorporate QM into their plans at an early stage in regard to the most efficient use of resources and as a focus on donor and patient safety.

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Chapter 16
Long-Term Follow-Up Program

Navneet S. Majhail and Shahrukh K. Hashmi

Introduction

There is an increasing recognition of chronic health issues and quality-of-life impairments that autologous and allogeneic hematopoietic stem cell transplantation (HSCT) recipients are susceptible to months to years after transplantation. These late complications are a result of pre-, peri- and/or post-transplant exposures and can contribute to significant morbidity and mortality in long-term HSCT survivors [1]. Lifelong follow-up is recommended for the screening and early detection and management of late transplant effects [2–4]. The number of patients receiving HSCT continues to increase due to newer indications and increasing comfort in transplanting older and sicker patients [5]. Concurrently, early and late survival after transplantation continues to improve over time [6–8]. In combination, this has led to a significant increase in the number of long-term transplant survivors. In the United States alone, the number of HSCT survivors is predicted to surpass 500,000 by 2030 [9].

HSCT survivors need systematic follow-up for the evaluation and management of late complications that can occur months to years after transplantation [1, 10]. Some of this long-term follow-up (LTFU) may need to be conducted by the transplant center, especially if patients need active management of HSCT-related complications such as chronic graft-versus-host disease (GVH-D). There is also an increasing mandate to hold transplant centers responsible for follow-up and survivorship care of their transplant recipients by regulatory agencies such as the Foundation for Accreditation of Cellular Therapy and the Joint Accreditation

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Committee of the International Society for Cellular Therapy and the European Society for Blood and Marrow Transplantation (EBMT). Hence, the presence of a robust LTFU program is an essential component of a successful transplant center. This chapter will review the foundational elements, including the infrastructure and resources, needed to establish an LTFU program at a transplant center. There is no single optimal or recommended model for LTFU programs. Ultimately, LTFU programs have to reflect the size and resources available at a transplant center and cater to the patient population served and their healthcare needs.

Late Complications of HSCT

Although the probability of long-term survival among HSCT recipients who survive in remission for 2–5 years after transplantation is fairly high, their life-expectancy continues to lag behind that of their age- and gender-matched peers from the general population [1, 8, 11–16]. Disease recurrence, chronic GVH-D, organ failure, and subsequent neoplasms are the common causes of late mortality after HSCT [8]. Other late complications may not be associated with mortality, but can contribute to morbidity and impairments in quality of life in HSCT survivors. A variety of pre-, peri- and post-transplant exposures can contribute to the risk of late complications after transplantation. Examples include patient-related factors (e.g., age, gender), lifestyle factors (e.g., smoking), pre-existing comorbidities, treatments for underlying disease prior to HSCT, conditioning regimen chemotherapy and radiation, and post-transplant complications (e.g., GVH-D) and their treatment (e.g., corticosteroids). Overall, certain exposures are associated with risks for specific late complications, and the follow-up care of HSCT survivors can be individualized based on a given patient's exposures through the continuum of his/her treatment course. For instance, chronic GVH-D is associated with a higher risk of secondary squamous cell cancers of the skin, while total body irradiation (TBI) increases risks of breast cancer [17]. Guidelines for screening and preventive practices for HSCT survivors recognize and base their recommendations on the following risk factors and exposures for late complications: age, gender, transplant type (autologous or allogeneic), exposure to TBI or corticosteroids, and presence of chronic GVH-D [2–4]. Table 16.1 highlights the recommended evaluations for the screening and prevention of late complications.

LTFU Care Models

Although there is no established time point when an HSCT recipient transitions from an early to an LTFU phase, generally patients who have survived for 1 year or more following transplantation are considered as HSCT survivors. However,

Table 16.1 Summary of recommendations for screening and prevention of late complications in long-term survivors after HSCT (adapted from [4])

Recommended screening/prevention	6 Months	1 Year	Annually
Immunity			
Encapsulated organism prophylaxis	2	2	2
PCP prophylaxis	1	2	2
CMV testing	2	2	2
Immunizations	1	1	1
Ocular			
Ocular clinical symptom evaluation	1	1	1
Ocular fundus examination	+	1	+
Oral complications			
Clinical assessment	1	1	1
Dental assessment	+	1	1
Respiratory			
Clinical pulmonary assessment	1	1	1
Smoking tobacco avoidance	1	1	1
Pulmonary function testing	+	+	+
Chest radiography	+	+	+
Cardiac and vascular			
Cardiovascular risk-factor assessment	+	1	1
Liver			
Liver function testing	1	1	+
Serum ferritin testing		1	+
Kidney			
Blood pressure screening	1	1	1
Urine protein screening	1	1	1
BUN/creatinine testing	1	1	1
Muscle and connective tissue			
Evaluation for muscle weakness	2	2	2
Physical activity counseling	1	1	1
Skeletal			
Bone density testing (adult women, all allogeneic transplant recipients, and patients at high risk for bone loss)		1	+
Nervous system			
Neurologic clinical evaluation	+	1	1
Evaluate for cognitive development		1	1
Endocrine			
Thyroid function testing		1	1
Growth velocity in children		1	1
Gonadal function assessment (prepubertal males and females)	1	1	1
Gonadal function assessment (postpubertal women)		1	+

(continued)

Table 16.1 (continued)

Recommended screening/prevention	6 Months	1 Year	Annually
Gonadal function assessment (postpubertal men)		+	+
Muco-cutaneous			
Skin self-examination and sun exposure counseling	1	1	1
Gynecologic examination in women	+	1	1
Second cancers			
Second-cancer vigilance counseling		1	1
Screening for second cancers		1	1
Psychosocial			
Psychosocial/QOL clinical assessment	1	1	1
Sexual function assessment	1	1	1

1 = Recommended for all transplant recipients
2 = Recommended for any patient with ongoing chronic GVH-D or immunosuppression
+ = Reassessment recommended for abnormal test results in a previous time period or for new signs/symptoms
HSCT Hematopoietic stem cell transplantation, *PCP* phencyclidine plasma cell pneumonia, *CMV* cytomegalovirus, *QOL* quality of life, *BUN* blood urea nitrogen, *GVH-D* graft-versus-host disease
Adapted from NS Majhail, JD Rizzo, SJ Lee, et al. Recommended screening and preventive practices for long-term survivors after hematopoietic stem cell transplantation. Guidelines of the CIBMTR, ASBMT, EBMT, APBMT, BMTSANZ, EMBMT and SBTMO. Biology of Blood and Marrow Transplantation, 2012, 18(3): 348–371

survivorship-related interventions to prevent and manage risk factors for late complications may be needed prior to 1 year, especially in patients with chronic GVH-D who are on systemic immunosuppressive therapy.

A comprehensive HSCT survivorship program ideally should address the following components of long-term care: (1) surveillance for disease recurrence; (2) surveillance, prevention, and treatment of late complications; (3) screening for new second cancers; (4) routine health maintenance; (5) health promotion and education; (6) psychosocial support; (7) rehabilitation; and (8) financial counseling and reintegration into society (e.g., return to school or work). Furthermore, patients may also need active management of post-transplant complications such as chronic GVH-D. It is beyond the scope of most transplant programs to provide all of these services. However, transplant centers can strive to provide comprehensive survivorship care using coordinated care models, where the transplant center collaborates with other experts and departments within or outside their institution or takes on a role in educating their community providers. The role of a transplant center in this coordinated care model can vary depending on several factors, such as the resources available at the transplant center, how far a patient lives from the transplant center, and the ongoing need to manage post-transplant complications. Irrespective, it is recommended that transplant centers dedicate resources and personnel towards developing LTFU programs for HSCT recipients, especially given the increasing requirement by payers and accrediting organizations that centers focus on survivorship issues.

Survivorship care models that apply to HSCT survivors are summarized below [10, 18]. It is important to emphasize that there is no "ideal" for a transplant center LTFU clinic. Transplant centers can apply variations and combinations of these models based on a given patient's care needs (e.g., distance from the transplant center, need for post-transplant maintenance therapy or presence of HSCT-related complications), the center's practices (e.g., same or different providers/programs for HSCT and hematologic malignancy), and local resources and infrastructure (e.g., availability of a dedicated cancer survivorship program). Ultimately, the focus should be the provision of patient-centered survivorship care that is accessible, affordable, timely, coordinated, high-quality, evidence-based, and individualized to patient needs.

Integrated Care Model

In the integrated care model, survivorship care is provided during routine follow-up for other transplant-related issues at the transplant center. Its implementation can be relatively easy and efficient, as transplant providers can co-manage routine non-transplant, transplant, and survivorship care. The transplant center essentially takes on the role of being the primary provider for coordinating most patient healthcare needs, and close collaboration with other healthcare providers and specialists is needed to ensure the success of this model. Transplant centers need to carefully consider their resources and capacity, as they will need to manage their usual pre- and early peri-transplant HSCT recipient care, in addition to the care of long-term HSCT survivors whose number at a given center will potentially increase over time. It is also important to ensure that the team taking care of long-term survivors has the requisite knowledge, confidence, and expertise in handling chronic GVH-D and survivorship issues. Some transplant centers have separate teams taking care of patients in the early transplant period, while an LTFU team takes over after a given event or time point (e.g., on discharge or at day +100). This care model may be a challenge to apply to patients who live far from the transplant center, although some centers are exploring alternatives (e.g., telemedicine) to facilitate survivorship care. Overall, the increasing complexities of HSCT survivorship care and transplant center capacity are the major challenges to the application of an integrated care model.

Consultative Care Model

In the consultative care model, the patient is referred to a "survivorship clinic" within their institution, which is usually a dedicated group of providers within the transplant program or a standalone general cancer survivorship clinic. In an HSCT-specific survivorship clinic, consultative care is often also provided for the

management of chronic GVH-D. Recommendations for preventive care and management of late complications are provided to the patient's transplant provider, hematologist-oncologist, or primary care providers. Survivorship care may be provided through a single visit at a given time point (e.g., 1 or 2 years post-HSCT) where preventive and follow-up care is discussed, or through multiple visits (e.g., every 6 or 12 months). The availability of specialized providers allows for the delivery of comprehensive survivorship care. The success of this model is contingent upon excellent communication with patients and their transplant and non-transplant healthcare providers.

Transitional Care Model

In the transitional care model, as the name implies, patient care is transitioned at a certain time point post-transplantation (e.g., day +100 or at 1–2 years) to another group of providers, either within the same institution or in the community, who also provide survivorship care. Considering the specialized nature of medical issues and complications faced by HSCT recipients, it is essential that there is a good "sign-off" process when care is transitioned and that it is clarified upfront when the transplant center may need to become involved again. A disadvantage of this model is that community providers may not have the knowledge and experience to handle HSCT-specific survivorship issues, and hence, transplant centers need to focus on their education and ensure ease of communication. This model is frequently employed by transplant centers for autologous HSCT recipients and for allogeneic HSCT recipients who do not have active chronic GVH-D.

Shared Care Model

In a shared care model, survivorship care is provided collaboratively by a group of healthcare providers through the transplant center and the community, including hematologists-oncologists and primary care providers. The roles and responsibilities of each provider and the points of communication and transition are clearly defined. Patients are transitioned to a non-transplant provider at a predefined time point (e.g., day +100), but may be followed by the transplant center at regular time points or may be transitioned back in the event of a medical issue that requires specialized transplant-related care. A variation of this model is the risk-stratified shared care model, where a personalized systematic plan of periodic screening, surveillance, and prevention is based on a given patient's exposures, ongoing medical issues, and risks of late effects [19, 20]. Figure 16.1 highlights two examples of risk-stratified shared care models.

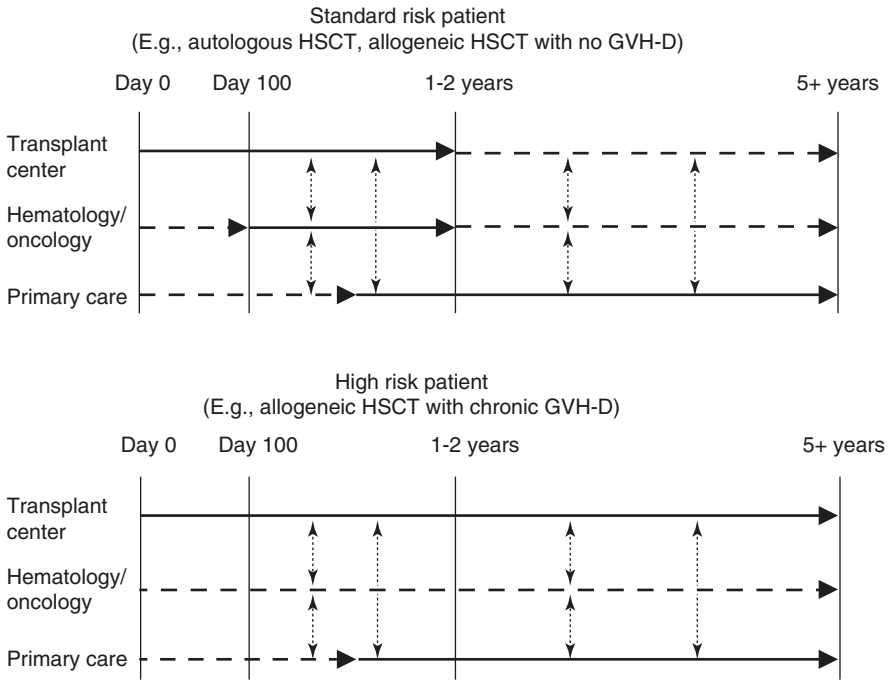

Fig. 16.1 Examples of shared care models for a standard-risk and high-risk hematopoietic stem cell transplantation (HSCT) recipient. *Solid lines* indicate the provider primarily responsible for overseeing care, while *dashed lines* represent providers who are indirectly involved in patient care

Organization and Implementation of an LTFU Program

This section will review the practical aspects of establishing an LTFU program. Table 16.2 identifies the elements that are essential for establishing an LTFU program within a transplant center. The program set-up has to be a well-thought-through process, with a clear delineation of the personnel required and their responsibilities, the ancillary and consultative services needed to provide comprehensive care, the development of educational materials, and the establishment of infrastructure for patient care and research. Additional personnel and resource requirements need to be considered if the LTFU program is going to be structured to care for patients with chronic GVH-D.

The structure of the LTFU program should ultimately reflect the needs of and the capacity/resources available at a given center. For example, the number of personnel and their effort dedicated to the LTFU may be different in adult versus pediatric centers and in small-volume versus large-volume centers, and may depend on the catchment area for referral and the distance patients need to travel to get care, as well as the presence of a standalone cancer survivorship program at the institution.

Table 16.2 Elements required for establishing an LTFU program

Step	Elements
Needs assessment	– Evaluate feasibility and optimal model while considering local resources and capacity
	– Establish business and organization plan describing needs and resources/personnel required
	– Obtain support from program and institutional leadership
Assemble LTFU team	– Identify HSCT, hematology-oncology, and/or primary care providers for staffing LTFU clinic (e.g., physicians, APPs, nurses, social workers and other personnel)
	– Establish core team and determine effort of personnel dedicated to LTFU clinic
Establish administrative and support infrastructure	– Establish work flow for patients
	– Obtain logistical support for patient care (e.g., appointment office, electronic health records support, billing services)
	– Develop templates for documentation and communication with outside providers
	– Establish template and mechanism for dissemination of treatment summary and survivorship care plan
	– Develop or identify educational materials for dissemination to patients/caregivers
Establish consultative services	– Develop collaborations with core departments and identify providers interested in focusing on HSCT survivorship care (e.g., ophthalmology, dermatology, gynecology, cardiology, dentistry, infectious diseases)
	– Develop collaborations with other departments that may serve the needs of HSCT survivors (e.g., reproductive medicine, complementary and alternative medicine, physical therapy, and rehabilitation)
	– Identify provider who will address psychosocial issues (e.g., psychologist, social worker)
Communication	– Establish mechanisms for communicating with referring hematologists-oncologists, primary care providers, and other specialists
Research infrastructure	– Identify areas of interest for research
	– Establish database and infrastructure for collecting relevant LTFU data
	– Establish mechanisms for submitting high-quality data to registries (e.g., CIBMTR, EBMT)

LTFU Long-term follow-up, *HSCT* hematopoietic stem cell transplantation, *APPs* advanced practice providers, *CIBMTR* Center for International Blood and Marrow Transplant Research, *EBMT* European Society for Blood and Marrow Transplantation

In centers that are starting a new program, it is advisable to start in a structured manner and gradually expand services with increasing experience. For instance, a newly established LTFU program may focus first on allogeneic HSCT recipients who are actively entering the survivorship phase before expanding to all prior allogeneic and then autologous HSCT recipients. An important aspect after the establishment of the LTFU program is long-term sustainability and improvement. Institutional leadership, policy makers, and clinic staff quite often face challenges in sustaining newly established longitudinal programs, especially when the programs are funded from within the institution. One key feature of establishing a program is setting up the metrics of success (e.g., quality improvement measures, number of patients served, impact on patient care and outcomes) that can be reviewed at regular intervals (e.g., once a year), so that goal-directed care becomes an essential component of the functioning of the program. Thus, it is imperative that institutional leadership focuses not only on the start-up factors needed for the implementation of an LTFU program, but also on the long-term sustainability of the program itself.

Types of Services Provided

The scope of LTFU care that a transplant center provides can depend on whether their institution has other avenues for providing these services; for example, through a dedicated cancer center survivorship clinic. Irrespective, an essential set of LTFU services that a transplant center can provide are: (1) provision of a treatment summary and survivorship care plan (SCP), (2) assessment and management of psychosocial issues, (3) patient education about late effects of HSCT and their prevention and screening, and (4) referral to appropriate clinical services for additional evaluation and follow-up for preventive care. Most centers should be able to accomplish these services using their existing or minimally additional resources. If resources are available, additional services such as rehabilitation can be provided, or the center can take over the role of coordinating comprehensive survivorship care. Figure 16.2 shows an example of a comprehensive consultative LTFU visit.

Treatment Summary and Survivorship Care Plan

The SCP is a comprehensive summary of a patient's cancer treatment and HSCT course. Ideally, it should include information on diagnosis, pre-transplant therapies, HSCT conditioning, and post-transplant complications. It should also list details of recommended follow-up, preventive practices (including vaccinations), and health maintenance. Finally, the SCP should incorporate patient education on late complications, return to work, and psychosocial issues. The international consensus guidelines for screening and preventive practices in HSCT survivors can be used as a template for the SCP [2–4]. The ultimate aim of an SCP is to enhance patient knowledge about their recommended care, and subsequently influence health behaviors where patients

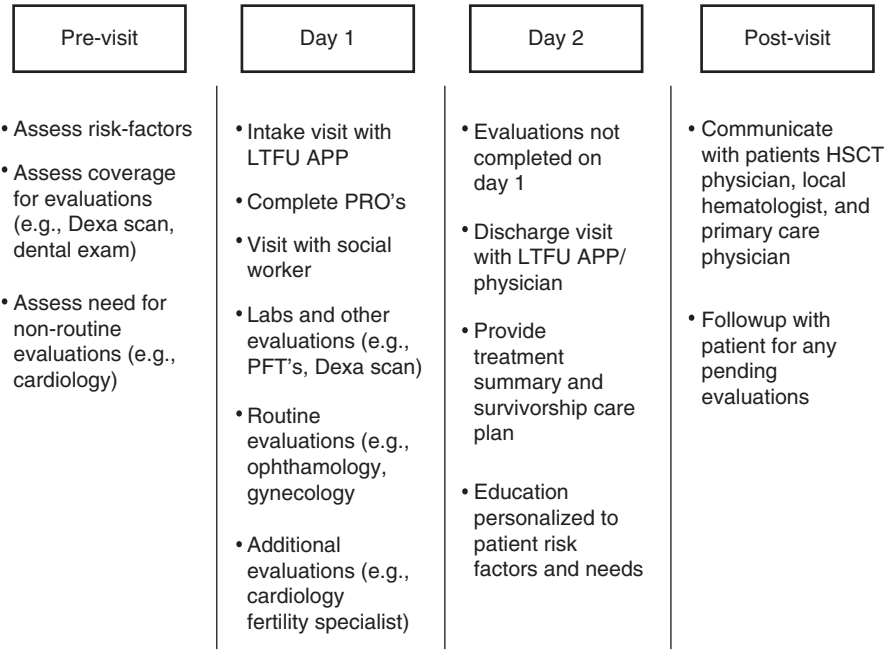

Fig. 16.2 Sample long-term follow-up program (LTFU) visit schedule for a comprehensive consultative survivorship visit

actually receive that care. SCPs have generally been accepted as being an important component of patient care, and some accreditation organizations (e.g., the Commission on Cancer in the United States) are mandating their use, although evidence of their optimal format and implementation in HSCT recipients is lacking. Furthermore, extracting data to generate the SCP, and its dissemination to patients, need resources and personnel. No standard SCP instruments are available for HSCT survivors, and SCPs available for other cancer survivors are difficult to adapt for HSCT survivors. It is most resource-effective to utilize information technology-enabled tools to generate SCPs, although most electronic health record platforms lack this capability. Table 16.3 highlights the infrastructure needed for SCP development and dissemination and the elements that the document needs to cover. Research is ongoing to evaluate whether a centralized HSCT registry (e.g., the Center for International Blood and Marrow Transplant Research) could be employed to effectively use the data it collects from patients to provide an individualized SCP to transplant survivors [21].

Physician- Versus Non-Physician-Led LTFU Programs

High-quality trials have investigated the provision of cancer survivorship care for non-hematologic malignancies by physicians versus that care provided by nurses (including nurse practitioners) and have reported no significant differences in terms

Table 16.3 Development and format of treatment summary and survivorship care plan for HSCT survivors

Data source	– Patient medical records (paper or electronic)
	– Transplant program database
	– Registry data
Format	– Paper
	– Electronic
Generation and dissemination	– Data extracted by coordinator or nurse
	– SCP provided to patient by physician, APPs, or nurse
Treatment summary components	– Patient demographics (identification, age, gender)
	– Details of prior treatments
	– Significant past medical history and comorbidities
	– Diagnosis details (date, disease stage)
	– Transplant details (type, donor source, graft source, prior transplants)
	– Conditioning regimen (drugs, TBI)
	– GVH-D prophylaxis
	– Complications (GVH-D, infections, organ failure)
Care plan components	– Contacts (transplant program, oncologist, primary care physician)
	– Vaccination schedule
	– Recommendations for screening for late organ complications and preventive health
	– Recommendations for routine health maintenance
	– Recommendations for second cancer screening
	– Recommendations for maintaining psychosocial health
	– Diet and nutrition advice
	– Advice on fertility and family planning (if indicated)

SCP treatment summary and survivorship care plan, *APPs* advanced practice providers, *TBI* total body irradiation, *GVH-D* graft-versus-host disease

of survival, recurrence rates, or psychological morbidity [22–26]. Although studies have not specifically addressed this issue in the setting of HSCT, many transplant LTFU programs, especially in the United States, currently provide survivorship care through allied health professionals (nurses, nurse practitioners, or physician assistants). In the absence of data on the long-term success of a program, either a physician or an allied health professional with experience and interest in survivorship can take the lead in the establishment and governance of a well-structured LTFU program.

Conclusion

A dedicated LTFU program at a transplant center can provide value to both the institution and the patients by providing high-quality patient-centered survivorship care and optimizing long-term outcomes. Several models for establishing

HSCT-specific LTFU programs are available. There is no evidence to suggest the benefit of one model over another, and transplant centers have to evaluate their and their patients' needs and the local resources and infrastructure to determine which model best applies to their circumstances.

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Chapter 17
Hematopoeitic Stem Cell Transplantation Outcome Data Management: Importance of Establishing an Institutional Database

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Introduction

In the past four decades, hematopoietic stem cell transplantation (HSCT) has evolved from an experimental procedure to the standard of care in many hematological conditions. It is an increasingly important treatment modality in patients with hematological malignancies and, in many diseases, represents the only curative option. We have recently celebrated one million HSCTs performed all over the world [1].

Transplant outcomes are influenced by many patient- and disease-related factors (such as age, disease stage, and prior treatment), as well as transplant-related factors, such as stem cell source, conditioning regimen, and prophylaxis for graft-versus-host disease (GVH-D). Ideally, most transplant strategies would be evaluated by large randomized clinical trials (RCTs). However, various factors limit the application of randomized trials in HSCT. Many diseases treated with transplants are

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uncommon; thus, single centers may treat only a few patients with a given disorder. This makes RCTs difficult and limits the ability to perform non-randomized (phase II) trials with sufficient power to detect meaningful effects. Small trials, even when randomized, may provide misleading results. On the other hand, patient registries collect data in a comprehensive manner (with few excluded patients) and therefore produce outcome results that may be generalizable to a wide range of patients. These registries also evaluate care as it is actually provided, because care is not assigned, determined, or even recommended by a protocol. Patient registries also offer the ability to evaluate patient outcomes when clinical trials are not practical (e.g., for very rare diseases), and they may be the only option when clinical trials are not ethically acceptable. They are powerful tools when RCTs are difficult to conduct. RCTs are controlled experiments designed to test hypotheses that can ultimately be applied to real-world care. Because RCTs are conducted under strict constraints, with detailed inclusion and exclusion criteria, they are sometimes limited in their generalizability. If RCTs are not generalizable to the population to which the information will be applied, they may not be sufficiently informative for decision-making. Conversely, patient registries that observe real-world clinical practice may collect all of the information needed to assess patient outcomes in a generalizable way. In addition, new transplant technologies are rapidly being introduced, so the results of prospective clinical trials may be obsolete before they are published. Finally, most clinical trials focus on short- and intermediate-term outcomes (1–5 years), while registries can provide long-term information regarding both the patients and donors. Important subjects that can be better addressed by registry studies in the HSCT field include descriptive studies analyzing rare diseases, identification of prognostic and risk factors, comparison of conditioning regimens, determining late consequences of transplant, defining inter-center variability in practice, and developing statistical methodology for transplant outcomes. It is important to note that correctly interpreting all this information from retrospective registry-based studies requires analytic methodology geared to addressing the potential sources of bias that challenge all observational studies. Interpreting patient registry data also requires checks of internal validity and sometimes the use of external data sources to validate key assumptions. Patient registries, RCTs, other study designs, and other data sources should all be considered tools in the toolbox for evidence development, each with its own advantages and limitations.

The growth of HSCT has been accompanied by a coordinated international effort to collect and analyze data on transplant outcomes through international registries. The collection of accurate clinical data is a prerequisite for the delivery of high-quality clinical care [1]. The primary objective of these registries is to define the role and monitor the outcome of treating patients within the countries by any technique involving the use of allogeneic or autologous hematopoietic stem cells. In recent decades, not only has the number of transplants reported to the national and international registries increased, but there has also been a very substantial increase in the complexity of the data requested by national and international transplant bodies and commissioning authorities.

We describe and discuss here some steps and issues involved in establishing HSCT registries; the importance of data collection and quality; issues of ethics and confidentiality, data ownership, and informed consent; and how to analyze trends and outcomes.

Planning an HSCT Registry

There are several key steps in planning an HSCT registry, including articulating its purpose, determining whether it is an appropriate means of addressing the research question, identifying stakeholders, defining the scope and target population, assessing feasibility, and securing funding. It is also helpful to plan for the entire lifespan of a registry, including how and when the registry will end and any plans for transition at that time [2].

Registry Design

An HSCT registry should be designed with respect to its major purpose; that is, to address focused analytical questions to support decision-making. The key points to consider in designing a registry include formulating a research question; choosing a study design; translating questions of clinical interest into measurable exposures and outcomes; choosing patients for study, including deciding whether a comparison group is needed; determining where data can be found; and deciding how many patients need to be studied and for how long. Currently, in the field of HSCT, there are national (such as the French, United Kingdom, Swiss, and Japanese registries) and international registries, such as the Center for International Blood and Marrow Transplant Research (CIBMTR), the European Society for Blood and Marrow Transplantation (EBMT), and Asian-Pacific registries. These registries collect patient, disease, and transplant data, including outcome data. However, there are some "registries" that are intended primarily for descriptive purposes. An example of a descriptive HSCT registry is one in which there is collection of data for epidemiological reports, including demographics and economics, without outcome data that is important for having an overview of the HSCT transplant activity. Many studies have been reported using this type of data. Once these key design issues have been settled, the registry design should be reviewed to evaluate potential sources of bias (systematic error); these should be addressed to the extent that is practical and achievable. The information value of a registry is enhanced by its ability to provide an assessment of the potential for bias and to quantify how this bias could affect study results [2].

Data Elements

The selection of data elements or variables is important for the analysis of primary outcomes. Specific data elements are selected with consideration of established clinical data standards, common data definitions, and whether patient identifiers will be used. It is important to determine which variables related to patients, donors, disease, transplantation, and endpoints are necessary, and which are desirable but not essential. Once data elements have been selected, a data map should be created, and the data collection tools should be pilot tested. Testing allows assessment of the respondent burden, the accuracy and completeness of questions, and potential areas of missing data. Overall, the choice of data elements should be guided by parsimony, validity, and a focus on achieving the registry's purpose [2].

The CIBMTR and the EBMT have agreed to use a common data collection form; namely, the Transplant essential data (TED)-A form or the minimum essential data (MED)-A form. In these forms, there was a consensus for using minimal data elements related to patients, donors, diseases, transplantation, and outcomes, elements that are essential for many studies related to HSCT. More specific data are reported in the research forms of both these international registries, such as the clinical research form [3] or MED-B form [4]. Importantly, definitions of these data elements are listed in the manual of instructions on how to complete the forms of EBMT and CIBMTR. MED-A or TED-A forms and MED-B or research forms as well as the manual of instructions are available at the websites of both organizations [3, 4].

Data Sources

A single registry may integrate data from various sources. The form, structure, availability, and timeliness of the required data are important considerations. Data sources can be classified as primary or secondary. Primary data are collected by the registry for its own direct purposes. Secondary data are collected by a secondary source for purposes other than those of the registry, and may not be uniformly structured or validated with the same rigor as the registry's primary data. Common secondary sources of data linked to registries include medical records systems, institutional or organizational databases, administrative health insurance claims data, death and birth records, census databases, and related existing registry databases [2].

Ethics, Data Ownership, and Privacy

Critical ethical and legal considerations should guide the development and use of patient registries. The Common Rule is the uniform set of regulations on the ethical conduct of human subjects research, and is issued by the national agencies that fund

such research. Institutions that conduct research agree to comply with the Common Rule for federally funded research, and may opt to apply that rule to all research on human subjects conducted within their facilities or by their employees and agents, regardless of the source of funding. The purpose of a registry, the type of entity that creates or maintains the registry, the types of entities that contribute data to the registry, and the extent to which registry data are individually identifiable affect how the regulatory requirements apply. Other important concerns include transparency of activities, oversight, and data ownership [2].

Informed Consent for Registries

The requirement of informed consent often raises different issues for patient registries versus the issues raised for clinical trials. For example, registries may be used for public health or quality improvement activities, which may not constitute "human subjects research." In addition, registries may integrate data from multiple electronic sources and may be linked to biobanks. Institutional review boards may approve waivers or alterations of informed consent (e.g., electronic consent, oral consent) for some registries, depending on the purpose and risk to participants. Established registries that undergo a change in scope (e.g., changes in data-sharing policies, changes to the protocol, extension of the follow-up period) may need to ask patients to "re-consent." When planning informed consent procedures, registry developers should consider several factors, including documentation and format, consent revisions and re-consent, the applicability of regulatory requirements, withdrawal of participants from the study, and the physical and electronic security of patient data and biological specimens [2].

Confidentiality and Legal Concerns for Providers, Manufacturers, and Health Plans

As patient registries are increasingly being recognized as a valuable data source, questions about privacy and the confidentiality of the data arise, particularly when data are desired for litigation or other judicial or administrative proceedings. In addition to patient data, registries often include private, confidential, and/or proprietary information about healthcare providers, manufacturers, and health plans. While significant attention has been paid to protecting the privacy of identifiable patient information, there is no single comprehensive law governing the protection of registry data about healthcare providers, manufacturers, or health plans. Registry developers should consider this issue during the planning phase and clearly articulate the policies and procedures that the registry will follow in the case of a request for registry data (e.g., from litigation attorneys, regulatory authorities, the press, or members of the public) [2].

Data Collection and Quality Assurance

The integrated system for collecting, cleaning, storing, monitoring, reviewing, and reporting on registry data determines the utility of those data for meeting the registry's goals. A broad range of data collection procedures and systems is available. For example, in the EBMT registry, the Project Manager Internet Server (ProMISe) system is used and for the CIBMTR either FormsNet3^{SM} or AGNIS is used. Some systems are more suitable than others for particular purposes. Critical factors in the ultimate quality of the data include how data elements are structured and defined, how personnel are trained, and how data problems (e.g., missing, out-of range, or logically inconsistent values) are handled. Quality assurance aims to affirm that the data are, in fact, collected in accordance with established procedures and that they meet the requisite standards of quality to accomplish the registry's intended purposes and the intended use of the data. For both international registries, the EBMT and the CIBMTR, data management training courses are provided annually. Manuals of instructions for filling in the forms and data element definitions are available on the websites [5].

Requirements for quality assurance should be defined during the registry's inception and creation. Because certain requirements may have significant cost implications, a risk-based approach to developing a quality assurance plan is recommended. Such an approach should be based on identifying the most important or likely sources of error or potential lapses in procedures that may affect the quality of the registry in the context of its intended purpose [2].

Analysis, Interpretation, and Reporting of Registry Data

Analysis and interpretation of registry data begin with answering a series of core questions: Who was studied, and how were they chosen for study? How were the data collected, edited, and verified, and how were missing data handled? How were the analyses performed? Four populations are of interest in describing who was studied: the target population, the accessible population, the intended population, and the population actually studied (the "actual population"). The representativeness of the actual population in relation to the target population is referred to as generalizability.

Analysis of registry outcomes first requires an analysis of recruitment and retention, of the completeness of data collection, and of data quality. Considerations include an evaluation of losses to follow-up; completeness for most, if not all, important covariates; and an understanding of how missing data were handled and reported. Analysis of a registry should provide information on the characteristics of the patient population, the exposures of interest, and the endpoints chosen such as survival, or other HSCT outcomes (for example engraftment, graft versus host disease, relapse, or others). Descriptive registry studies focus on describing the frequency and patterns of various elements in a patient population, whereas analytical

studies concentrate on associations between patients or treatment characteristics and health outcomes of interest. A statistical analysis plan describes the analytical plans and statistical techniques that will be used to evaluate the primary and secondary objectives specified in the study plan. Interpretation of registry data should be provided, so that the conclusions can be understood in the appropriate context and any lessons from the registry can be applied to the target population and used to improve patient care and outcomes [2].

International HSCT Registries

Some major HSCT registries collect HSCT data, analyze outcomes, and have published very important contributions to the field of HSCT.

European Society for Blood and Marrow Transplantation (EBMT)

The EBMT is a non-profit organization that was established in 1974 in order to allow scientists and physicians involved in clinical bone marrow transplantation to share their experience and develop co-operative studies. The EBMT is devoted to the promotion of all aspects associated with the transplantation of HSCs from all donor sources and donor types, including basic and clinical research, education, standardization, quality control, and accreditation for transplant procedures [5]. In the last published annual report (2014 survey), 680 centers from 49 countries were contacted (40 European and 9 affiliated countries); of which 656 teams reported their results on 40,829 transplants. Today, the EBMT registry contains information on more than 500,000 transplants performed [4].

The data from the EBMT registry are entered and maintained in a central database with internet access. Each EBMT center is represented in this database and users from a center can enter, view, modify, obtain reports, and download their own data once the necessary permissions have been granted by the principal investigator of the center. In addition, all EBMT member centers can obtain general overviews of the complete EBMT data. The database is run and accessed through the ProMISe system. ProMISe is the central data management system used by the EBMT. Access to the registry is password-protected through individual accounts, and users are able to enter and retrieve data directly over a secure internet connection. All users access the same data repository, but data visualization is restricted to the user's center data. National registries operating in some countries are integrated in the EBMT data flow by mutual consent and use the same central database.

The MED-A form contains what is considered the minimum essential data. It is mandatory that these data be submitted for all patients, and the submission of MED-A data is a requirement for a center to hold full membership in the EBMT. A

disease-specific MED-B form consists of an initial information sheet common to all diseases and procedures, a detailed disease-specific pre-HSCT section detailing the diagnosis and pre-HSCT treatment, and a follow-up section detailing complications and events that happened after the HSCT. Data checking by the EBMT is a continuous process and proceeds all year around.

Eleven working parties conduct studies in different fields of HSCT. Since 1974, the use of EBMT data and statistical resources has resulted in more than 600 publications in peer-reviewed scientific journals. Inside the EBMT, other specific registries have emerged, such as the Eurocord registry, which collects and analyses outcomes data from cord blood transplants performed in Europe and from cord blood units delivered by European cord blood banks. The Eurocord registry has reported more than 15,000 cord blood transplants and has published important contributions to the field. In addition, the annual activity survey of the EBMT, describing the status of HSCT in Europe and affiliated countries, has become an instrument that is used to observe trends and to monitor changes in technology use. The survey captures the numbers of HSCTs performed in the preceding year from each participating team, categorized by indication, donor type, and stem cell source.

Center for International Blood and Marrow Transplant Research (CIBMTR)

In 2004, the CIBMTR was created after the fusion of the previous International Bone Marrow Transplant Registry (IBMTR), established in 1972, and the National Bone Marrow Donor program (NMDP) registry, established in 1986 for unrelated-donor HSCT. Over 500 institutions in more than 50 countries contribute data to the CIBMTR. Participating centers submit data on their consecutive transplants to the CIBMTR Statistical Center. The Center receives data on more than 12,000 new transplants each year and maintains a database that now includes information on more than 425,000 transplant recipients. The CIBMTR collects data on two levels: registration and research. Registration data include disease type, age, sex, pretransplant disease stage and response to chemotherapy, date of diagnosis, donor type, graft type (bone marrow- and/or blood-derived stem cells), transplant regimen, post-transplant disease progression and survival, engraftment, GVH-D, development of a new malignancy, and cause of death. All CIBMTR centers contribute registration data. Research data are submitted on comprehensive report forms completed for a subset of registered patients in CIBMTR research centers. Research data include detailed pre- and post-transplant clinical information such as disease subtype, tumor size and pathology, sites of disease, non-transplant treatment of the primary disease, performance status, organ function, details of the transplant regimen (including dose and schedule of high-dose therapy), graft manipulation, supportive care, post-transplant toxicities, and functional status. Both the registration and research databases are longitudinal; patients are followed through their transplant centers with yearly updates. Studies are conducted within a Working

Committee structure. They are guided by Chairs who are experts in the relevant field, and by Scientific Directors who are experienced transplant physicians with MS degrees in biostatistics or related fields. MS-level biostatisticians coordinate Working Committee activities and participate in individual studies, and Statistical Directors provide oversight. Investigators from around the world are currently participating in more than 200 CIBMTR studies.

Japanese Registries

There are four HSCT registries in Japan; the Japan Society for Hematopoietic Stem Cell Transplantation (JSHCT), the Japanese Society of Pediatric Hematology, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network; each plays an important role in society by reporting the number and outcomes of transplantations and contributing new findings obtained from studies on individual topics. In 2007, the JSHCT played a central role in developing the "Transplant Registry Unified Management Program (TRUMP)" to enable transplantation institutes to manage patient information with emphases on convenience to institutes, safety of patient information, and quality of data management [6]. While enhancing domestic registries, the program seeks to coordinate with other hematopoietic stem cell transplantation registries around the world to contribute to the development of registries throughout Asia. The JSHCT now has 2300 members, consisting of physicians, nurses, other relevant healthcare practitioners, donor and clinical coordinators, volunteers, and pharmaceutical companies. The JSHCT has a home page (http://jshct.com/) through which one can get more information [7].

Donor HSCT Registries

One of the major landmarks of HSCT was the development of bone marrow registries for treating patients without a human leucocyte antigen (HLA)-identical sibling donor. Shirley Nolan, whose son was diagnosed with Wiskott Aldrich syndrome, established the first unrelated bone marrow registry, Anthony Nolan, in London, in 1973. Following this first donor recruitment drive, the number of bone marrow and peripheral hematopoietic stem cell donors has increased all over the world, with more than 28 million donors now registered, including more than 700,000 cord blood units. In the past 10 years, due to improved definition of HLA-matching and better supportive care, the outcomes of patients transplanted with HLA-matched unrelated grafts (10/10 HLA alleles) have improved substantially and are now comparable to the outcomes after HLA-identical sibling donor transplants. With the increased number of allogeneic HSCTs performed globally, there is a parallel increase in the demand for donors of therapeutic cells. Donor characteristics and collection procedures have undergone major changes during recent decades, and

further changes are foreseen. Information on short- and long-term donor outcomes is of crucial importance to ensure maximal donor safety and availability. Current data, predominantly from unrelated donors, provide reliable information on the frequent early events associated with donation, most of them of mild-to-moderate intensity. Information on the types and relative risks of serious adverse reactions is more limited. However, little data exist on long-term donor outcome. Most of the large retrospective studies of unrelated transplant recipients and events related to donors have been performed by the NMDP in collaboration with the CIBMTR in the United States. Other active donor registries reporting outcome data of recipients and follow-up of donors are the Swiss Donor registry and Anthony Nolan.

Worldwide Network for Blood and Marrow Transplantation (WBMT)

Despite these important registries and their large contribution to the field of HSCT, we know that HSCT activity and its data reporting varies immensely worldwide. In this sense, the WBMT was created as an umbrella organization affiliated as a non-governmental organization (NGO) with the World Health Organization (WHO). The WBMT has taken up the challenge of collecting and disseminating worldwide data on a regular basis. The organizations providing the information to the WBMT are: the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR), the African Blood and Marrow Transplantation Group (AfBMT), the Asia-Pacific Blood and Marrow Transplantation Group (APBMT), Bone Marrow Donors Worldwide (BMDW), the Canadian Blood and Marrow Transplantation Group (CBMTG), the CIBMTR, the EBMT, the Eastern Mediterranean Blood and Marrow Transplantation (EMBMT) Group, the Latin American Bone Marrow Transplantation (LABMT) Group, and the World Marrow Donor Association (WMDA). The first report was published in 2010, based on the global transplant activity in 2006, and this was followed by a report on the data available in 2010 and a retrospective report of the first one million HSCTs (at the end of 2012) in 2015. The number of countries with registries increased from 2 in 1987 to 57 in 2012, and the number of registered donors increased from 3072 in 1987 to 22,346,551 in 2012. Cord blood banks were first established in 1993, in 2 countries, and the number then increased to 18 in 2000 and to 36 in 2012, with 645,646 registered HLA-typed, cryopreserved cord blood products. The use of unrelated donors has increased over time and exceeded family donor transplants in 2006, accompanied by an increase of international transplants across borders to more than 10,000 per year between 2006 and 2012; the exchange of cord blood products across borders has also increased. The use of unrelated donor HSCT and the increase in donor availability have paralleled each other [8].

In summary, progress in the HSCT field would never have been possible without effective prospective databases. Data collection and data analysis are integral parts of therapy and should be considered part of the transplant procedure. Therefore,

transplant centers and national societies must work on developing their own databases in order to study their results and recognize factors that can be improved, ultimately leading to best patient care.

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Chapter 18
Establishing an HSCT Program with Limited Resources

Amr Nassar, Alok Srivastava, Shahrukh K. Hashmi, and Mahmoud Aljurf

Introduction

The establishment of hematopoietic stem cell transplantation (HSCT) programs in developing countries can enhance tertiary care health services. There are various positive attributes that favor the establishment of such a high-profile venture; however, there are also significant obstacles, which need to be dealt with, as outlined below.

In most developing countries, an HSCT program has to compete, for allocation of funds, with other priorities for basic healthcare services, such as the provision of food and sanitation, immunization, and population-control measures, as well as measures for the prevention of communicable diseases.

However, developing countries should have the expertise to offer ”state-of-the-art” treatments, including HSCT, which can provide treatments locally at a much lower cost than abroad.

For the inception of an HSCT program, the bringing together of experienced well trained personnel to lead the program is one of the first crucial steps. The most important logical steps would be to provide financial, legal, ethical, and other support for those local individuals and institutions that have adequate proactivity for developing an HSCT program. The goal is to develop a customized local experience that is unique for each developing country, and also to allow the local dissemination of this experience as it evolves [1].

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While working on establishing an HSCT program in a developing country, one should take into consideration several difficulties. Financial, logistic, and social obstacles, as well as the lack of availability of skilled personnel, top this list of difficulties. Provision of skilled personnel should start with the employment of an experienced and committed leader in the field of HSCT.

Financial Issues and Cost of Transplantation Program

HSCT remains a highly specialized, resource-intense, and costly medical procedure. A recent report from the Agency for Healthcare Research and Quality in the United States showed that HSCT was among the top ten procedures with the highest increase in hospital costs from 2004 to 2007; total United States national costs of HSCT hospitalization increased from US$694 million to US$1.3 billion over this time period [2]. The costs of transplantation can be very variable, depending on many factors, including autologous vs. allogeneic transplantation, conditioning, disease type, donors, source of stem cells (cord vs. bone marrow [BM] vs. peripheral blood stem cells [PBSCs], post-transplant planned donor lymphocyte infusion, post-transplant maintenance chemotherapy, outpatient vs. inpatient transplants, and many other factors. There is no standard cost measurement parameter, but generally most reports regarding the costs of transplantation include at least the cost of the first month of HSCT. A comparison of general costs of transplantation procedures is shown in Table 18.1.

Three main types of economic evaluations provide information that is intended to guide decision-making on the basis of value for money:

(a) Cost minimization
(b) Cost benefit
(c) Cost utility

Cost minimization is commonly practiced in HSCT whenever a lower-cost, equally effective treatment is chosen over more expensive treatments. Cost-benefit

Table 18.1 Costs of transplantation in various countries as reported in the literature

Authors	Year of publication	Country	Cost of allogeneic HSCT procedure (US$)	Cost of autologous HSCT procedure (US$)	Reference number
Saito et al.	2008	United States	128,800		[3]
Sharma et al.	2014	India	17, 914	12,500	[4]
Jaime-Perez et al.	2015	Mexico	12,504		[5]
Saber et al.	2013	Brazil	31, 500 (related) 40,500 (unrelated)		[6]

analysis is almost never used in HSCT because it requires the assignment of monetary units of costs to measure clinical benefits.

Cost-utility analysis is a specific type of cost-effectiveness analysis where outcomes are adjusted to consider health-related quality of life (HRQoL), so that a cure without adverse treatment sequelae is considered more valuable than a cure that results in permanent disability [7].

In order to develop a cost-containment program, it is essential that proof of both clinical and estimated economic effectiveness is required prior to the widespread adoption of new technologies [8]. A technical feasibility analysis prior to the initiation of an HSCT program is optimal. Several factors are linked to the high cost of HSCT, as discussed below.

Patient-Related Factors

When designing a national program for HSCT in a developing country, very few patient-related factors can be assessed for cost reduction. Although there is no consistent significant correlation between costs and patient age, sex, performance status, disease risk, or status, in some more recent studies, advanced-risk disease was shown to be a significant predictor of higher costs [9–13].

In view of the limited resources in developing countries, some health authorities might allocate the limited resources to the best priorities where low-cost inputs yield high dividends. However, there are no solid recommendations and each country has to adopt the policies that satisfy its needs.

Considering the young median age of populations in developing countries, it would be prudent to start providing HSCT procedures to younger patients with curable indications and subsequently advance the transplantation age and disease eligibility with the growth of the program. For curable diseases, a wide variety of outcomes are observed, and for a startup program, a low-risk strategy of not undertaking high-risk transplants should be adopted, e.g., if the relapse risk of a condition is greater than 70% (e.g., in acute myelogenous leukemia [AML] with complex cytogenetics with monosomies), then it would be prudent to exclude this indication from transplantation.

Although it is predicted that the population pyramids of developing countries will be shifting towards older populations, currently the ratio of children vs. the adult population is sufficiently high to warrant targeting the pediatric population as a key priority for the establishment of a new HSCT program.

Transplant Center Experience

Cost reduction and clinical outcomes have been shown to improve with greater institutional experience. However, this economic advantage may be offset as the complexity of the patients being treated increases or more aggressive supportive

interventions are being applied, resulting in a plateau in the improvement curve [14–16]. Building up local experience and selecting cost-effective practices will have a significant effect on both total costs and transplantation outcome.

Human Resources and Continuous Training

At different steps of transplantation, the availability of well trained staff with continuous training and updating of knowledge is a cornerstone of any successful transplant program. Because the team leader is the key to the success of a new program, the institutional leadership needs to hire a team leader with both experience and comprehensive training, using a full fellowship-like program (2–3 years, ideally) to make sure that they start up and lead the HSCT program confidently. The emigration of healthcare professionals from developing countries to developed countries, although helping in the transfer of technology, deprives the developing world of valuable human resources. Countries should strengthen their healthcare system requirements, including those of physical infrastructure and skilled human resources, to meet the multidisciplinary requirements of transplantation, with quality and safety as the fundamental principles. The role of multinational cooperation and twinning with reputable and experienced institutions in developed countries could facilitate the exchange of expertise and training across the globe.

Donor Selection and Human Leukocyte Antigen (HLA) Typing

With advances in immunogenetics and in transplantation immunology research, particularly in the structure and function of the HLA system in the 1990s, new and efficient technologies for HLA typing have emerged, and these technologies continue to improve [17, 18].

According to the guidelines of the World Marrow Donor Association (WMDA) and the European Federation for Immunogenetics (EFI), high-resolution HLA typing should be performed for recipients and donors with matched HLA. In addition, the typing of HLA class I genes should also include locus C, due to the increasingly recognized role of locus C in immune rejection [19, 20].

The technology for HLA typing has evolved from the serological level, to the cellular level, and is currently at the molecular level. Serotyping was the mainstream method for HLA typing and played a critical role in organ transplantations before the 1990s. However, most HLA antisera are polyclonal with low specificity and variable sensitivity.

Therefore, using molecular methods to type HLA at the DNA level has gradually replaced serotyping and cellular typing.

Commonly used DNA-based HLA typing methods (discussed in other chapters of this book in detail) include polymerase chain reaction (PCR)-based sequence-specific primers (PCR-SSP), PCR-based restriction fragment length polymorphism (PCR-RFLP), PCR single-strand conformation polymorphism (PCR-SSCP), PCR sequence-specific oligonucleotide (PCR-SSO), and PCR single-nucleotide polymorphism (PCR-SNP). PCR-SSP genotyping was a commonly used method for HLA typing in clinical laboratories worldwide. PCR-SSP and PCR-SSO methods have a high cost and long operative times; therefore they are rarely used for HLA typing today. PCR-SNP is a simple and fast method with a high resolution, and PCR-SNP is expected to become more popular in HLA typing as the technology continues to improve.

At present, PCR-sequence-based typing (SBT) technology has significant advantages over other HLA typing methods in terms of accuracy, efficiency, and automation. In addition, its operative cost has been greatly reduced [17].

It is recommended that new programs in developing countries with limited resources should start by performing the rather less complicated matched-sibling transplantation, where high-resolution typing may not be absolutely necessary for most of the potential donors. Starting alternative-donor transplantation in a new HSCT center without adequate experience in HSCT could be risky.

Outsourcing HLA typing can be a cost-effective alternative for developing countries with non-availability of an HLA laboratory. Many professional companies are now offering molecular-based HLA typing at competitive prices, particularly for bulk contracts, and thus this option may be explored, although caution has to be exercised in contracting with the appropriate companies whose product portfolios are not just based on standards of care, but are also accredited by global or national accreditation societies/programs (e.g., Clinical Laboratory Improvement Amendments [CLIA] approval certificates, American Association of Blood Banks [AABB] certification, European Union [EU] certification).

Conditioning Intensity for HSCT

Both the intensity and duration of conditioning have been found to affect the cost of transplantation. Large studies have confirmed that the costs of reduced-intensity conditioning (RIC) procedures are lower than those of high-dose regimens, with lower median hospital stays within the first year of the transplantation [11].

Myeloablative allogeneic conditioned (MAC) HSCT is not only associated with acute short-term toxicities, but also with long-term late complications, such as delayed immune reconstitution, infertility, endocrinopathies (especially growth retardation in children), and new malignancies. RIC procedures are expected to have a positive impact on the use of blood products, risk of infections, transplant-related mortality, and length of hospital stay. However, outcomes of different diseases were variable after RIC transplants, with less favorable outcomes observed in aggressive diseases, e.g., for AML/myelodysplastic syndrome (MDS) patients;

indeed, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0901 study accrual was stopped early due to a presumed benefit of MAC compared with RIC, as assessed by an independent Data and Safety Monitoring Board (DSMB) safety review.

Another important factor pertaining to developing countries is the availability of a full radiation oncology facility. Most cancer centers in developing countries do not have provision for total body irradiation, which is currently the standard of care for certain conditioning regimens, particularly for acute lymphoblastic leukemia (ALL). The absence of this modality should not deter the opening up of an HSCT program when there is an imperative need for transplants.

Several recent studies have suggested that a 20%–30% reduction in the dose of full-intensity regimens would markedly reduce toxicity without leading to significant changes in risk of relapse or overall transplantation outcome for certain indications [21–23].

The cost and limited availability of and access to radiation therapy in many developing countries should not be a major obstacle, as alternative non-radiation-based conditioning regimens are available for almost all HSCT indications, with a few exceptions (e.g., B-cell ALL [B-ALL] and T-ALL).

Performing Autologous Stem Cell Transplantation Without Stem Cell Cryopreservation

Cryopreservation of stem cells needs a relatively advanced stem cell processing laboratory. Several reports have been published on the feasibility of cold preservation of granulocyte colony-stimulating factor (G-CSF)-mobilized whole blood or cold preservation of autologous BM (with or without prior administration of G-CSF). The stem cell-containing blood units or BM can be kept in a standard blood bank refrigerator at +4 °C until re-infused to the patient [24–26].

Studies of autologous HSCT for multiple myeloma, using non-cryopreserved stem cells and no G-CSF support, have been published recently by several centers. This technique depends on short conditioning, traditionally used for transplantation in multiple myeloma patients after 1 day of high-dose melphalan or in abbreviated conditioning for lymphoma patients. This technique not only avoids the need for cryopreservation technology, an expensive process that requires special equipment and materials, but also avoids the possible toxic side effects reported with the infusion of dimethylsulfoxide (DMSO) added during cryopreservation. These autologous transplantation techniques were reported to be associated with early engraftment and reduced hospital stay, with significant cost saving and outcome that was comparable to that of conventional conditioning with cryopreserved stem cells in multiple myeloma patients [27–30].

Graft Source

Allogeneic peripheral blood HSCT (PBSCT) was repeatedly reported to offer more rapid neutrophil and platelet recovery than BM HSCT, and this positively reflected on costs of care (about 30% cost reduction using peripheral blood compared with BM as a source of stem cells in some studies) [31–33].

Specific resource savings have been noted in post-chemotherapy factors; primarily in regard to hospitalization, platelet transfusions, and use of growth factors [34, 35].

Unlike transplantation in the autologous setting, in allogeneic HSCT, chronic graft-versus-host disease (GVH-D) is an important late complication and is currently the leading cause of transplant-related mortality (TRM). Most studies have reported a higher incidence of chronic GVH-D with the use of allogeneic PBSCs, which may offset the early cost savings. Optimal donor selection and indications for the use of PBSCs would definitely affect both outcome and cost [36].

In a recent study by the Center for International Blood and Marrow Transplant Research (CIBMTR), the use of PBSCT was the preferred modality for transplanting in aplastic anemia patients in low-income and developing countries, as the PB graft was associated with faster engraftment, less infection, and a lower likelihood of graft rejection in heavily pre-transfused patients than traditionally seen in developing countries [37].

The case is different in autologous stem cell transplantation, where evidence of clinical benefits and cost savings of PB utilization were consistently reported [38–41].

Alternative Donors and Graft Manipulation

The use of alternative donors has emerged as a significant cost driver, even if the costs of stem cell procurement are not included [12, 42]. Alternative-donor HSCT should not be considered as a priority in developing countries for a program in initiation. Of alternative-donor transplants, myeloablative umbilical cord transplants had the highest cost, followed by those for matched unrelated donors.

Practically speaking, the optimal and most cost-effective alternative-donor transplantation modality in developing countries is haploidentical transplantation, using post-graft infusion of cyclophosphamide for GVH-D prevention. A randomized controlled trial directly comparing both the clinical outcomes and the cost-effectiveness of cord blood transplant vs. haploidentical transplantation is ongoing (BMTCTN 1101), and we hope to see the results soon.

The performance of haploidentical transplantation using different methods of T-cell depletion (TCD) in the donor graft is cumbersome, requires advanced stem cell processing technology, and is associated with much higher cost [43].

Drug Costs

Pharmaceutical costs range from 8% to 39% of the total costs of HSCT. Colony-stimulating factors and antimicrobials appeared to be the major contributors to these costs [44, 45]. Several generics are available for fluconazole, and more recently generics also became available for voriconazole [46]. Before acquiring generic drugs, the institution must ensure that appropriate quality assessments are undertaken, including therapeutic drug monitoring if possible.

Pharmaceutical costs are expected to rise continuously, given the changes in HSCT practice in the past decade, with the increasing use of newer immunosuppressive regimens and the high cost of the new anti-infective agents [45]. As mentioned earlier in the chapter, the long-term increase in pharmaceutical costs for patients with chronic GVH-D who may require prolonged immunosuppressive treatment might not be predictable [45].

A biosimilar drug is a copy of an approved original injectable biologic substance whose data protection has expired [47]. The use of well-established biosimilars should be considered for cost containment and for the improvement of availability of the drugs needed for HSCT procedures.

Because the manufacturing processes remain proprietary, biosimilars might not be chemically identical to the originals. If they are properly assessed and clinical effectiveness is proven for one or more of these biosimilar drugs, the reduced costs of drug expenditures would contribute to the financial sustainability of HSCT programs [47–49].

In this area, and as an example, several biosimilar G-CSF molecules are cheaper alternatives to the original brand product. The patent for the original brand of G-CSF expired in Europe in 2006 and in the United States in 2013. The European Medicines Agency (EMA) has approved several biosimilar versions in the past few years [47].

Several G-CSF biosimilars have been repeatedly evaluated for stem cell mobilization for autologous transplantation patients and they have achieved mobilization yields and safety profiles similar to those of the original G-CSF. The speed of both myeloid and platelet recovery with the biosimilars was similar to that seen with the engraftment of stem cells mobilized with the branded G-CSF product [48–54].

The model of the non-inferiority of biosimilar G-CSF to the original branded product can be repeated with other medications, ultimately leading to significant cost savings. Several biosimilars of essential medications used in HSCT are widely available on the market and are being made by very well established pharmaceutical and biotechnical companies [55].

Although intravenous busulfan has recently been demonstrated to be associated with improved outcome, the use of oral busulfan can be considered as an alternative, at a markedly lower cost. This would make a substantial saving in the cost of conditioning chemotherapy, and would make the transplantation procedure available and affordable for larger numbers of patients and more cost-effective for a limited-resources HSCT program [55].

Post Transplantation Factors

Several post-transplantation factors were found to be predictive of higher costs, of which the duration of hospitalization and the occurrence of transplantation complications were the most significant. Programs for post-transplantation care that included home health service and outpatient follow-up systems, where patients could be followed-up either at their own homes or at a hostel, where a well-trained and qualified nurse could monitor those patients who needed less aggressive intervention, were found to reduce the post-transplantation cost significantly [56]. Chronic GVH-D can really drive up the costs, as it generally requires prolonged immunosuppression and multimodal treatments. Having expertise in GVH-D management is crucial to the success of an HSCT program.

Problems with Socioeconomic and Other Factors

In many developing countries, there is high level of acute leukemia case attrition, as a significant proportion of acute leukemia patients would die before being able to access the few referral HSCT centers in the country or region. This will indirectly lead to a relatively larger proportion of HSCTs being done for non-neoplastic indications, such as BM failure syndromes, hemoglobinopathies, and genetic conditions, where the disease nature would permit some delay in performing the HSCT procedure. The time from diagnosis to the actual performance of the HSCT procedure is much longer in developing countries, with the consequences being that the candidates for HSCT are not optimal ones, presenting secondary to advanced disease, and with low performance status, infections, transfusion alloimmunization, transfusional iron overload, and other suboptimal factors. The obvious consequences of this delay will be higher costs of the transplantation procedure and inferior long-term outcomes. In developing countries efforts have to be made to shorten the time from diagnosis of the transplantable disease to the actual performance of the transplantation procedure. This will require not just the establishment of HSCT centers, but the implementation of referral strategies, relevant education, and policy changes within the developing country.

The Human Development Index (HDI) was established by the United Nations (UN) Organization to evaluate a country's socioeconomic achievements in terms of three basic aspects: life expectancy, education, and standard of living [57]. The number of transplantations performed per population (as well as the early- and long-term outcomes) is directly related to the HDI [58–62].

Increasing public awareness of and patient education about essential hygiene measures and infection-control measures, and the provision of social services to enhance patient and family compliance, are important challenges for a successful HSCT in developing countries.

Conclusion and Take-Home Message

The initiation of an HSCT program should be one of the priorities for institutions/healthcare systems in developing countries where conditions that can only be cured by transplantation are endemic. The first step usually includes the development of human resources, and the key for the development of such a program is expert leadership that is committed to the long-term success and quality of the HSCT program. Some key aspects that can lead to success are: careful choice of the priorities for transplantation indications (e.g., not starting with very high-risk diseases), donor selection (starting with fully matched siblings), stem cell source selection (considering haploidentical transplants if a fully matched sibling is unavailable), considering fresh stem cell infusions if applicable (rather than cryopreservation of stem cells), curtailing drug costs via the utilization of generics and biosimilars, and, lastly, focusing on post-transplantation factors and survivorship care so that the overall success of a program can be measured longitudinally with long-term outcomes and cost-effectiveness analysis.

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Chapter 19
HSCT Center's Success is Dependent upon Adequate Staff Education and Training

David D.F. Ma

Introduction

Hematopoietic stem cell transplant (HSCT) commenced four decades ago and in 2013, the Worldwide Network for Blood and Marrow Transplantation (WBMT) reported a milestone of one million transplants achieved globally. Despite this achievement, there is no uniformly accepted education and training (ET) program for transplant center staff. There are recommended ET requirements for some HSCT personnel, such as transplant physicians and apheresis staff, as well as quality assessment programs for HSCT centers in different world regions. It is this discrepancy that may explain published findings that transplant outcomes are dependent on the number of transplants performed per year and improve with the implementation of a quality management system [1–3]. Well-trained and educated transplant staff are thus a vital and precious resource of a transplant center. This chapter puts forward an ET model consisting of four main pillars—with specific considerations for various health professional groups—that fit within a continuous learning loop, incorporating regular appraisal, clinical governance, and quality programs. Case studies are presented to illustrate the salient ideas.

The Pillars of ET

The pillars of this model of ET program apply to all workers in an HSCT center. A high level outline is given here, and it must be noted that the intensity, duration (ranging from weeks to months or years), and assessment of the program depend

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on the qualifications, the experience, and the roles of the individual transplant center staff members.

Pillar 1: Essential Knowledge

The extent and depth of knowledge the staff member needs will depend on their role and prior ET. Training of apheresis staff, for example, will differ from the training of stem cell laboratory staff. An outline of the knowledge required is presented in the list below. Comprehensive lists can be obtained from publications in the chapter reference section and from various transplant societies.

- An understanding of why HSCT is used and which patient groups benefit from it, as well as the risks involved.
- An understanding of hematopoietic stem cells and differentiation of the cell types that create the whole hematopoietic system, including stem cells.
- Awareness of the types of HSCT (allogeneic and autologous) and sources of HSCs (peripheral blood, bone marrow, and cord blood) and their procurement.
- Knowledge of assessment and management of donors.
- Knowledge of chemotherapy, including pharmacological interactions, radiotherapy, immune-modulating agents, and procedures and their benefits and risks.
- An understanding of the management of prolonged pancytopenia, immunodeficiency, and other early and late transplant complications.
- Knowledge of laboratory tests such as flow cytometric assays for $CD34^+$ cells, human leucocyte antigen (HLA) typing, microbial detection assays, molecular genetics, and colony assays, as well as various imaging tests.
- Appreciation of supportive care for transplant recipients and their carers, including, physical, emotional, nutritional, and psychosocial needs.
- Knowledge of quality management systems, and keeping up-to-date on research and development in HSCT and related cellular therapy.
- Awareness of the usefulness of realistic forward planning for establishing and maintaining a successful and sustainable transplant center.

Pillar 2: Practical Skills

The extent and depth of skills needed of the staff member will depend on their role and prior ET. Broadly speaking, the required practical skills include:

- Staff training in hygiene and aseptic techniques and working in low-microbial environments, such as the in-patient ward and stem cell laboratory facilities, similar to the environment of an operating theater.
- Skills in clinical assessment and management of HSCT-related health issues such as graft-versus-host disease and hemorrhagic cystitis.

- Surgical procedures, including central venous catheter (CVC) insertion and associated risks in pancytopenic patients; bone marrow harvests; apheresis; and stem cell processing, cryopreservation, and storage.
- Training in the determination of CD34 and other cell types by flow cytometry, colony and other cellular function assays, and molecular genetic assays such as those used for the detection of engraftment and residual disease.
- Training in ethics, clinical governance, quality management systems, data collection, and research and development.

Pillar 3: Connecting People through Effective Communication [4]

This is an often overlooked, but vital element of ET, especially for team leaders. The operation of an HSCT center depends on a multidisciplinary team, and effective communication among staff is essential.

- Communication should be clear, consistent, and complete. All staff should be familiar with the common terms and jargon used to enable effective communication.
- Tone is important; it should be non-threatening, open, and frank. This helps to encourage open discussions and build team spirit.
- The method and language used needs to vary depending on the audience of the communication.
- It is also essential to be able to communicate effectively with patients/relatives, staff within and outside the HSCT center, funding bodies (non-governmental organization [NGO], government, and industry), and the public at large.
- It is important that staff [5] and patients are provided with an avenue to express anxiety, stress, and grief due to mistakes or death.
- Acknowledging success and providing praise and encouragement for a job well done ensures a harmonious work environment.

Pillar 4: Organizational Skills [6]

The ability to organize and coordinate tasks at all levels is vital especially for team leaders. Strong organizational skills help achieve this.

- It is necessary for the transplant team to have policies and protocols; however, these should be open to modifications pending the discovery of errors and new information.
- Data collection is essential and should include activity and outcome information. This includes benchmarking and participation in external registry/registries.
- It is vital to establish a business case assessment for the transplant center that includes financial assessment and recognition of well-trained staff as precious human capital.

- Leadership training should be available for those in a leadership role.
- An often overlooked aspect of training is the ability to identify positive values in people, and to be aware of staff diversity and staff needs. This skill helps to create amicable team spirit and enhances staff retention.

The Continuous Learning Loop (See Fig. 19.1)

Education and training is a lifelong process that needs to keep pace with advances in science and changes in healthcare.

- Maintenance and improvement of the standards of care require objective analysis and co-operation with others involved in HSCT.
- Ongoing data collection on activity and outcome measures provides information critical to the success of the transplant center and it is therefore necessary for the center to participate in an external quality management system.
- These activities allow identification of risks and errors, and thus protocols and policies need to be altered to reduce risks and improve outcome. There is a saying: "protocol kills"—a major error is to assume that protocols and policies are fixed in stone. Protocols and policies are created by people and may contain errors.

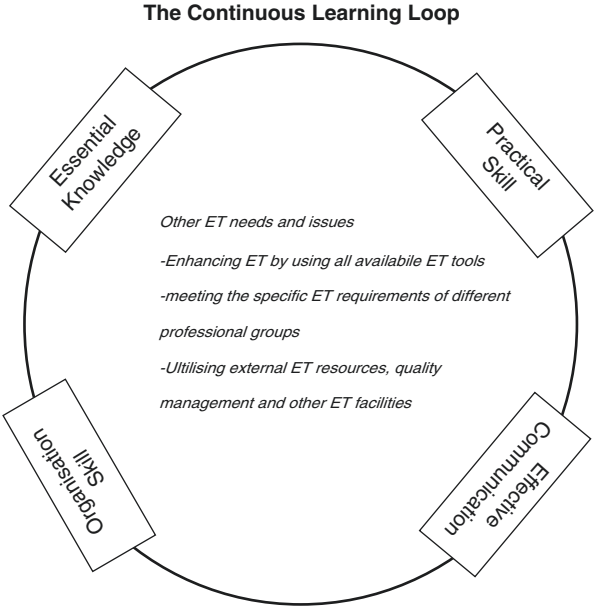

Fig. 19.1 Diagrammatic representation of the proposed model of staff education and training. Hematopoietic stem cell transplant (HSCT) staff are a precious resource for an HSCT center, and the center's success depends on the retention of well-trained, dedicated, and committed staff

- Frontline staff in an HSCT center often work in a very tense and stressful environment. Debriefing and de-stressing techniques need to be part of the program.
- Schedule regular unit discussion meetings, including case reviews, quality management, journal clubs, and presentations of research projects.
- Create a culture where staff are encouraged to speak out on errors and where errors are discussed openly in a non-judgmental manner. The emphasis is on team effort and valuing positive actions.
- Provision of training for trainers and assessors is a vital aspect of ET.

Specific ET Requirements

1. Doctors: Appropriate qualification and license to practice in medicine, with mandatory training and experience in clinical hematology/oncology, should be the prerequisite for a transplant physician [7].
   - Extensive training in HSCT requires, in general, 2 years depending on the person's prior ET. There are specific recommendations for ET and courses for transplant specialists, such as recommendations by the American Society for Blood and Marrow Transplantation (ASBMT) [8] and courses run by the European Society for Blood and Marrow Transplantation (EBMT).
   - Leadership training including skills to delegate tasks/responsibilities should be part of the person's ET, as doctors are required to take on the role of team leader.
   - The ET program therefore needs to include instructions on ways to reinforce positive outcomes, identify and tackle errors, and correct and put in place preventive measures to reduce recurrence.
   - Ability to adapt to changing requirements and advances in HSCT and related areas is vital.
   - Playing an active role in ethics, quality management and research is essential.
   - The ET program needs to provide training on the set-up of a business model regardless of funding sources, including government-funded centers.
   - Ability to deal with psychosocial aspect of patients', carers', and staff needs with empathy is a critical aspect of the role of a doctor, and appropriate training is a key element of an ET program.
2. Nurses: Entry requirements equivalent to those for the doctors should also apply to transplant nurses [9, 10] and scientists/technicians.
   - ET courses usually run for 6–12 months. However, mentorship, i.e., training on the job, is an essential part of the ET program.
   - Training on the job is especially important for those taking care of patients directly, as they are the key frontline members of a successful HSCT center, often working in an extremely busy and stressful environment.

- It is essential that clinical skills, such as observation of vital signs, remain a key element of the nursing ET—these observations are often neglected in the modern era that focuses on high-tech issues, mechanical devices, delivery of medicine, etc.
- Nursing ET needs to include the ability to identify relevant observations and respond appropriately, including identifying the emotional and psychosocial needs of patients and their carers.

3. Scientists and Technicians
   - Being on the staff of an HSCT center is a unique opportunity for laboratory workers to be in a position to process precise and personalized human products for patients.
   - The possible roles include working on hematopoietic stem cell assays and processing [11] and operating blood collection machines to collect stem cells from donors or patients, i.e., apheresis [12, 13].
4. Allied health professionals are valuable staff that ensure the success of a transplant unit; depending on the center's financial resources, they include:
   - Pharmacist—familiar with high-dose chemotherapeutic drugs; drug formulations, i.e., oral, intravenous (IV), and subcutaneous (SC); and drug interactions.
   - Data manager—long-term follow-up and data collection are essential.
   - Social worker, psychologist, dietitian, occupational therapist, and physiotherapist.
5. The forgotten staff
   - This category includes staff that are also essential to the success of a HSCT center, but their ET and their unique roles are often overlooked or taken for granted.
   - Transplant coordinator—essential for the center, proficient in communication and organizational skills, as well as being knowledgeable about HSCT.
   - Infectious disease-control personnel.
   - Domestic staff employed for cleaning, food preparation, and provision of clean water.
   - Engineers and technicians providing an appropriate physical environment, including good air quality, reliable power supply, and communication tools.

Learning and Training Tools

The available tools listed below allow greater flexibility for delivering ET programs for various HSCT staff in all regions of the globe.

- Conventional in-person training and education.
- Video conferencing.
- Online education, training, and assessments.
- Rapid delivery of information and data by electronic means, as well as printed format.

Case Studies: ET in Action

Case Study 1

During my visits for the planning of a new center in the Asia-Pacific region, it was noted that birds flew in and out of the corridors, balconies, and windows of the building and electricity supply ceased abruptly. Clean water and food supplies were unreliable. These seemingly innocent matters being serviced by ancillary/forgotten staff can have devastating effects on transplant patients, such as the loss of stored stem cells needed for their transplant and an increased risk of death due to infection. The team leaders were made aware of these serious matters, and they subsequently educated the engineers in taking appropriate actions, including restructuring of the ward and the installation of a backup electricity generator. Domestic staff and family members were educated on the importance of providing clean water and food. These measures thus reduce infection risks and prevent the loss of electricity supply during stem cell apheresis and stem cell cryopreservation.

Case Study 2: The Continuous Learning Loop

An increase in antibiotic-resistant bacteria and their infection rates was noted in a transplant unit by the infection-control staff. Further investigations revealed proper hand hygiene and isolation procedures were not being followed by staff, including the senior medical staff, nursing staff, and domestic personnel. A refresher campaign about hand hygiene and isolation procedures was launched; this included workshops and notices. Assessments of the effectiveness of the re-education and training campaign, as well as the monitoring of bacterial colonization and infection rates, were performed by anonymous assessors. This cycle of ET followed by evaluation and feedback was repeated until the incidence of bacterial colonization and infection returned to a basal level.

Conclusion

Suitably trained staff are vital to the success of a sustainable transplant program. The required knowledge and practical training of individual transplant staff depends on their role. Their ET is not only about the acquisition of the specific knowledge and skills needed to perform HSCT, it must incorporate organizational skills and the ability to effectively communicate both within and outside the team, with donors and patients and their carers. Continuous learning through a learning loop needs to include objective appraisals of outcome and clinical governance, an external quality management system, and keeping abreast with research and other developments in

the field. Adequate resource and infrastructure support is essential for the success of an ET program.

Transplant is expensive in the short term, but it is cost-effective in the long term. In some regions of the world, it is the only curative treatment for diseases affecting a large section of the population, such as beta-thalassemia major and sickle cell disease.

The goal of ET is to improve the survival and quality of life of transplant patients. One of the less obvious benefits of a successful HSCT center is that it enhances other essential aspects of the healthcare system, including the rational use of safe blood products, control of infection, antibiotic usage, and improvement in the delivery of diagnostic services. Such a center also improves local healthcare services, and ET programs enhance research and development, leading to higher healthcare standards and staff development, and the retention of the skilled staff in their own region; thus increasing the satisfaction of patients, their families, and the community.

To obtain consistent and comparable outcomes for patients globally, all HSCT and related organizations must work together to create a uniform ET model to cement the amazing success of HSCT achieved so far in some regions in the spirit of the United Nations principles of human rights and equality [14].

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Chapter 20
A Global View on Regulatory Issues in Stem Cell Transplantation and Cellular Therapy

Jose R. Nuñez

Introduction

Over the past 50 years, the transplantation of human organs, tissues, and cells has become a worldwide practice that has extended and greatly enhanced the quality of hundreds of thousands of lives. Continuous improvements in medical technology, particularly in relation to organ and tissue rejection, have led to an increase in the demand for organs and tissues. This demand has always exceeded supply, despite a substantial increase in deceased organ donation, as well as greater reliance on donations from living persons in recent years. Stem cell transplantation (SCT), of which more than one million procedures have been performed to date [1], has always occupied a unique position in clinical transplantation. Stem cell transplantation, unlike organ transplantation, can only be done using fully matched donors who, because of the complexity of human leucocyte antigen matching, cannot always be found in the same country or continent as the recipient. Furthermore, hematopoietic stem cells are highly proliferative cells, which can be harvested from the donor without causing major hematopoietic deficits and can be transported for up to 72 h without losing their potential. Approximately 5% of the donor's stem cells are harvested, resulting in regeneration to normal levels within a few weeks in both the donor and the recipient. Hematopoietic stem cells can be transported without cryopreservation, in contrast to organs, for which the outcome is clearly dependent on the period of cold ischemia. This has allowed donor stem cells to be sourced across the world for a given patient, making stem cell transplantation a global effort, for which

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international agreements are necessary. Innovations made in recent years are increasing the importance of cellular therapy for the replacement of damaged tissue or for modulating the immune system (e.g., mesenchymal stem cells). These emerging therapies require regulation at a global level to avoid misuse and to protect both the donor and the recipient.

The need to establish global standards and to avoid commercial trafficking prompted the World Health Organization (WHO) to prepare a document, endorsed by the World Health Assembly (WHA), on Guiding Principles on Human Organ Transplantation, in resolution WHA 44.25 in 1991. Over the past 16 years, these first Guiding Principles have greatly influenced professional codes and practices, as well as legislation, around the world. In the light of changes in practices and attitudes regarding organ and tissue transplantation, the 57th WHA requested the Director General "to continue examining and collecting global data on the practices, safety, quality, efficacy and epidemiology of allogeneic transplantation and on ethical issues, including living donation, in order to update the Guiding Principles on Human Organ Transplantation." The actualized guiding principles WHA 63.22 are intended to provide an orderly, ethical, and acceptable framework for the acquisition and transplantation of human cells, tissues, and organs for therapeutic purposes. Each jurisdiction was asked to determine the means of implementing the Guiding Principles still active today. While they do not apply to blood and blood constituents collected for transfusion purposes, the Principles do apply to cells, tissues, and organs removed from deceased or living persons for the purpose of transplantation, and they cover the entire process from donors to recipients and the follow-up of both.

Guiding Principles WHA63–22

Guiding Principle 1

Cells, tissues, and organs may be removed from the bodies of deceased persons for the purpose of transplantation if:

(a) Any consent required by law is obtained, and
(b) There is no reason to believe that the deceased person would have objected to such removal.

Guiding Principle 2

Physicians determining that a potential donor has died should not be directly involved in cell, tissue, or organ removal from the donor, or in subsequent transplantation procedures; nor should they be responsible for the care of any intended recipient of such cells, tissues, and organs.

Guiding Principle 3

Donation from deceased persons should be developed to its maximum therapeutic potential, but adult living persons may donate organs as permitted by domestic regulations. In general, living donors should be genetically, legally, or emotionally related to the recipients of the organs.

Live donations are acceptable when the donor's informed and voluntary consent is obtained, when professional care of donors is ensured and follow-up is well organized, and when selection criteria for donors are scrupulously applied and monitored. Live donors should be informed of the probable risks, benefits, and consequences of donation in a complete and understandable fashion; they should be legally competent and capable of weighing the information; and they should be acting willingly, free of any undue influence or coercion.

Guiding Principle 4

No cells, tissues, or organs should be removed from the body of a living minor for the purpose of transplantation other than narrow exceptions allowed under national law. Specific measures should be in place to protect the minor and, wherever possible the minor's assent should be obtained before donation. What is applicable to minor's also applies to any legally incompetent person.

Guiding Principle 5

Cells, tissues, and organs should only be donated freely, without any monetary payment or other reward of monetary value. Purchasing, or offering to purchase, cells, tissues, or organs for transplantation, or their sale by living persons or by the next of kin of deceased persons, should be banned. The prohibition on the sale or purchase of cells, tissues, and organs does not preclude reimbursing reasonable and verifiable expenses incurred by the donor, including loss of income, or paying the costs of recovering, processing, preserving, and supplying human cells, tissues, or organs for transplantation.

Guiding Principle 6

Promotion of the altruistic donation of human cells, tissues, or organs by means of advertisement or public appeal may be undertaken in accordance with domestic regulations. Advertising the need for or availability of cells, tissues, or organs, with a view to offering or seeking payment to individuals for their cells, tissues,

or organs, or payment to the next of kin where the individual is deceased, should be prohibited. Brokering that involves payment to such individuals or to third parties should also be prohibited.

Guiding Principle 7

Physicians and other health professionals should not engage in transplantation procedures, and health insurers and other payers should not cover such procedures, if the cells, tissues, or organs concerned have been obtained through the exploitation or coercion of, or payment to, the donor or the next of kin of a deceased donor.

Guiding Principle 8

All healthcare facilities and professionals involved in cell, tissue, or organ procurement and transplantation procedures should be prohibited from receiving any payment that exceeds the justifiable fee for the services rendered.

Guiding Principle 9

The allocation of organs, cells, and tissues should be guided by clinical criteria and ethical norms, not by financial or other considerations. Allocation rules, defined by appropriately constituted committees, should be equitable, externally justified, and transparent.

Guiding Principle 10

High-quality, safe, and efficacious procedures are essential for donors and recipients alike. The long-term outcomes of cell, tissue, and organ donation and transplantation should be assessed for the living donor as well as the recipient in order to document benefit and harm. The level of safety, efficacy, and quality of human cells, tissues, and organs for transplantation, as health products of an exceptional nature, must be maintained and optimized on an ongoing basis. This requires the implementation of quality systems including traceability and vigilance, with adverse events and reactions reported both nationally and for exported human products.

Guiding Principle 11

The organization and execution of donation and transplantation activities, as well as their clinical results, must be transparent and open to scrutiny, while ensuring that the personal anonymity and privacy of donors and recipients are always protected.

Medical Products of Human Origin (MPHO)

Since the endorsement of the Guiding Principles WHA63–22 in May 2010, several new and relevant developments have taken place. Cellular therapy has become an essential option not only for hematological and non-hematological malignancies and non-acquired gene defects, but also for other diseases that are not curable with available drugs. A new category of medical products has appeared, termed medical products of human origin (MPHO). Such products include non-modified cells, as well as substantially manipulated, in-vitro cultured, and/or gene-modified cells classed as advanced therapy medical products (ATMP). MPHO are fundamentally different from other medical products, because they depend on the donation of biological materials from living or deceased persons. MPHO are defined "as substances that are derived wholly or in part from the human body and intended for clinical application".

Concern for the dignity and human rights of the donor requires high ethical standards in the procurement of biological materials. The human origin also entails potential risks to public health and demands appropriate screening and testing.

Guiding principles should cover the following topics:

- Ensuring the ethical and effective procurement, distribution, and use of medical products of human origin by governments.
- Equity in donation in all segments of society in efforts to meet the need.
- MPHO should be used only in situations of proven efficacy and in the absence of alternatives.
- Biological materials from living donors should be taken only with the donor's informed and voluntary consent.
- Exploitation of vulnerable individuals should be avoided and equity in donation should be promoted.
- Donors should be protected against physical and psychosocial risks.
- Information should be provided on the MPHO.
- Equity in access to the benefits of MPHO should be promoted.
- Steps in the development and use of these medical products should be traceable and subject to quality management systems and vigilance and surveillance programs. Reporting of activities will be of the utmost importance.
- Organization and delivery of activities must be transparent and open to scrutiny.

Conclusion

New medical products and new forms of transplantation have become available in recent years. These very potent MPHO often represent the most beneficial and cost-effective therapies for several life-threatening or debilitating conditions. The products range from organs, tissues, blood, cells, and gametes to breast milk, hair, nails, urine, and feces. The human origin of these medical products entails risks for both the donor and the recipient. For this purpose, the WHO has established guiding

principles and is working on a global consensus for the donation and management of blood, blood components, and MPHO.

These "new" guiding principles that would apply to any MPHO will serve as the common ethical framework under which specific tools, guidelines, policies, and strategies should be developed for each of these products.

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Chapter 21
Paving the Way to Hematopoietic Stem Transplantation Worldwide

Yoshihisa Kodera

Introduction

Hematopoietic stem cell transplantation (HSCT) is a cure-oriented therapy for various intractable diseases. After receiving HSCT, many patients can become free of their background diseases without any additional treatment. Because of this, lifelong medical costs for such patients are rather cheaper than those for patients who choose other treatment modalities. Patients can return to social activities within several months after HSCT. For these reasons, HSCT must be initiated and established soon in emerging countries. This chapter, and this whole textbook, offers practical information for new teams who are initiating HSCT, especially in emerging countries.

Section 1: Minimal Essential Requirements for Performing the First HSCT

Some Messages from a Transplant Team in an Asian Country

This textbook is aimed at promoting the initiation of HSCT in emerging countries/regions. The book is also aimed at polishing and reconsidering the basic consensus features of HSCT in advanced countries/regions. This chapter is written by a member of an experienced transplant team in the Asia-Pacific region, a region where both advanced and emerging countries/regions coexist. Therefore, an overview and understanding of HSCT features in both types of regions can be provided.

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To infuse sufficient numbers of hematopoietic stem cells (HSCs) from a sibling donor with a genetically matched human leucocyte antigen (HLA), to give a recipient enough immune suppressive treatment in order to avoid the rejection of grafted stem cells, and to protect the recipient from external infectious agents until the time of hematological recovery are characteristics that form the heart of allogeneic HSCT [1]. Recent advances in HSCT, including autologous HSCT, depend on and learn from these basic principles. In any experienced HSCT institutes, the originators of the teams who initiated HSCT according to these basic principles have obtained reproducibly successful outcomes [2–4]. In this textbook, experts in the field provide the most important and most sophisticated information on HSCT, focusing on fundamental characteristics that are the aim of every transplant team. However, these fundamentals may not necessarily be essential prerequisites for the achievement of the first HSCT in an emerging country. This chapter is intended for new transplant teams who are planning to initiate HSCT in the near future. Some of its contents may summarize or duplicate the information in other chapters in this book, but the summaries of HSCT characteristics here could offer certain benefits to those teams and might encourage their own initiation of HSCT.

Organization of the Transplant Team

A hematologist who has visited an experienced transplant team and observed the entire course of autologous and allogeneic HSCT carried out by this team should be the core physician of the newly formed transplant team. This physician could teach other team members, including nurses and laboratory technicians. Consecutive visits by other physicians, nurses, and other professionals in the same experienced team could be of advantage to the new team. The newly formed team may invite experienced physicians to observe the structure of the team and join in the case conferences after the performance of HSCT by the team. Recently, web case conferencing, including inter- country conferencing, has become possible. The new team may contract with an experienced team for a web case conference. HSCT is a team medical service. Because of this, it is critically important to maintain good communications with other sections of the institute, such as the departments of nursing, blood transfusion, pharmacology, clinical laboratory, pathology, radiology, anesthesiology/operation room, food service/nutrition, and dermatology, and the administrative office. Weekly case conferences with members of these departments are an essential requirement. It is recommended that, from the beginning, each HSCT center has its own written institutional protocol for the implementation of these case conferences.

Provision of a Clean Environment

The provision of a clean environment, which basically consists of clean air, clean water, clean food, and inverted protection from microbes, is essential, especially in areas where the daily life environment does not have modern standards of cleanliness. If the daily environment is not clean enough, stricter isolation of a patient,

by using a class 100 laminar air flow room with gown and masks, might be required. Now that the life environment has become more sophisticated, strict measures to maintain cleanliness are essential.

Patient Selection

For the first case of allogeneic HSCT, patient selection is highly important. It is an established finding that the outcomes of patients after HSCT are better in non-malignant diseases such as aplastic anemia than in malignant diseases such as leukemia, and outcomes are better in younger than in older patients [2–4]. It has also been shown that the outcomes of patients with malignant disease transplanted in remission are better than those in patients transplanted at an advanced disease stage [2–4]. As it is true that a single successful case provides far more useful information than unsuccessful cases and that such a case greatly encourages the transplant team, it would be optimal, for the first allogeneic HSCT, to select a patient with a non-malignant but definitely indicative disease, such as severe aplastic anemia, thalassemia, or sickle cell disease, with such patients usually being of young age [5]. When a team performs the initial transplant for a malignant disease, such as leukemia, selection of a patient in remission with a low comorbidity index [6] is recommended.

Identify a Sibling Donor

To find an HLA genetically matched family donor is relatively easy even in countries where HLA typing laboratories are absent. Transplant teams can access some international laboratories and send blood samples of the patients and the donor candidates, absorbed on filters, for DNA typing. The samples are relatively stable and the cost is relatively low [7].

Informed Consent from Patient and Donor

Explaining the entire expected clinical course and outcome for both the patient and the donor is essential. For this purpose, a new team can use the established review articles published by international registries [2–4]. Providing accurate information on the risks and benefits for the patient and the donor will result in a trustful relationship between the family members and the transplant team.

Patient Decontamination

Selecting a patient who has few clinical signs of infection is essential for the first HSCT. Recently, the strict decontamination of patients has not necessarily been applied by some experienced teams, but this depends on the environment of the

particular HSCT institutes and societies. Carrying out strict oral, dental, and skin (but not necessarily gut) decontamination is recommended for the initial cases for each transplant team [8]. After the decontamination, the patient must be cared for with the availability of sterile water and food.

Patient Preconditioning

For the preconditioning regimen, institutes in emerging countries may choose either a myeloablative conditioning (MAC) regimen or a reduced-intensity conditioning (RIC) regimen, each of which has several modalities [9]. The absence of a total body irradiation facility should not discourage the initiation of HSCT. It has been confirmed that non-irradiation regimens, such as busulfan (BU) + cyclophosphamide (Cy) [10] or BU + fludarabine achieve engraftment rates similar to those achieved with irradiation regimens [11].

Prophylaxis of Graft-Versus-Host Disease (GVH-D)

Consensus has almost been reached regarding the immune suppressive regimens required to control acute GVH-D. Transplant teams may choose either a cyclosporine-A (CyA) + short-term methotrexate (s-MTX) or a tacrolimus (Tac) + s-MTX regimen, both of which have been well established [12]; there are few alternatives at present. Here, it must be mentioned that the team is required to have equipment for the measurement of the blood concentrations of both CyA and Tac. This procedure is critical for maintaining the optimal blood concentrations of these drugs and for avoiding their severe adverse effects.

Stem Cell Harvest

For hematopoietic stem cell harvest, institutes may choose either bone marrow (BM) aspiration or granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs). For BM harvest, the harvest kit and aspiration needle are available at a reasonable price. Nuclear cell count in the harvested BM is the most primitive and the most realistic way to confirm whether there are enough cells for engraftment. For PBSC harvest, there must be blood cell separator equipment. The availability of a flow cytometry laboratory is also essential, as it is necessary to count G-CSF-mobilized HSCs in the peripheral blood product.

Supportive Care

A limited supply of red blood cell (RBC) and platelet concentrate is essential. The team may prepare the concentrates in their institute by using the same equipment as that used for the preparation of PBSCs. It is required to check for blood-borne

infectious microorganisms, such as *Treponema pallidum* (the causative agent of syphilis), and this checking must be done at the same level as that in a sophisticated blood bank.

A list of antibiotics/antifungal drugs must be prepared for the prophylactic or preemptive treatment of infection. Early decisions on treatment, quick response, and immediate identification of the causative microorganisms are important. The prophylactic use of some drugs, such as sulfamethoxazole-trimethoprim (ST compound), which has a broad spectrum, might be effective and have a reasonable cost benefit.

Conclusion of Section 1

The author has tried to outline the HSCT process, the details of which are extensively described in this textbook. HSCT is deeply dependent on national economic status [13]. The governments of some emerging countries may prioritize medical services for, as an example, infectious diseases or nutritional disorders, rather than HSCT. Nevertheless, it is also true that certain diseases that can be cured only by HSCT exist under such circumstances. To have the ability to treat these diseases in one's own country would encourage the population and could prompt positive actions from the government to pave the way for the development of HSCT. The author hopes that a new transplant team would be fully equipped according to the recommendations in this textbook, but the author also encourages the new transplant team to initiate HSCT with the minimal essential requirements, without hesitating to take the first step.

Section 2: Messages from the Worldwide Network for Blood and Marrow Transplantation (WBMT)

Recent data for young aplastic anemia patients who received allogeneic HSCT from HLA genetically matched siblings showed a cure rate of around 90% [14]. This means that the basic technology of stem cell exchange among human beings has been almost fully established. Also, when one compares the medical costs of HSCT and newly developed drugs to cure intractable hematological disease, the whole-life medical costs would be lower for HSCT [15]. The technological benefits and the cost benefits of HSCT are the major reasons why the WBMT has continuously promoted HSCT in emerging countries, as well as in advanced countries, in collaboration with the World Health Organization (WHO).

Since 2007, when the WBMT started, this excellent organization has always paid attention to the status of HSCT at the global level, has gathered annual global survey data, and has organized workshops in emerging countries. For example, the WBMT quickly responded to an episode of the lack of a BM harvest kit in Japan 2009, and they also responded rapidly to the nuclear disaster following the mega-earthquake in Japan in 2011. The WBMT has collected sur-

vey data not only from preexisting international registry systems (the Center for International Blood and Marrow Transplant Research [CIBMTR], the European Society for Blood and Marrow Transplantation [EBMT], the Asia-Pacific Blood and Marrow Transplantation Group [APBMT]), but also from newly established registries such as the Latin American Bone Marrow Transplantation Group (LABMT) and the African Blood and Marrow Transplantation Group (AfBMT), proving that the factor with the most impact for the performance of HSCT is the government expenditure of each country [13]. The WBMT projected that cumulative HSCT numbers in the world would reach one million by the end of 2011 [16]. The WBMT organized workshops in Asia in 2011, Latin America in 2013, Africa in 2014, and Middle-East in 2017, and as a result, several countries in each region have initiated or are going to initiate HSCT. The WBMT now has seven standing committees, the standing committees for recipients and transplantation, donors, cell processing, accreditation, education and dissemination, nuclear accidents, and patient advocacy. It must be mentioned that these standing committees involve delegates from all the 24 member societies. Through these activities, the WBMT provides a human network of scientists who are committed to HSCT worldwide. The WBMT is proud that this textbook was created by these scientists to elucidate the basic requirements that have been reached through our activities in the past decade. Through this textbook, experienced authors provide information and experiences that have been gained in a step-by-step manner over a long time period. New transplant teams will be able to apprehend all the information at once and can choose the most appropriate information for their medical and financial situations.

HSCT is a costly procedure; therefore, we have tried to discuss the aspects of restricted resources that may represent a challenge for the readers of this textbook. It is, nevertheless, also true that HSCT is a cost-effective therapeutic modality from the viewpoint of a recipient's whole life span, so it might be a desirable medical technique not only for emerging countries but also for advanced countries. Also, the creation and maintenance of certain infrastructure entities, such as a stem cell banking system, which is essential so that the opportunity of receiving HSCT can be offered to every eligible recipient, will contribute to creating an ideal atmosphere in all societies.

Section 3: Some Future Aspects

Further Improvement of HSCT Outcomes

Despite the previous information given in this chapter, the outcomes of HSCT for hematological malignancies are still not sufficient, especially in patients who are transplanted at an advanced disease stage. The relatively poor outcomes in these patients are due to three major factors: disease relapse; critical infections, which may cause more severe GVH-D; and organ toxicity, caused by the cumulative effects of chemoradiotherapy performed before the time of HSCT. The most important factor for avoiding these risks is the timing

of the transplant. Certain global eligibility criteria for the timing of HSCT implementation are desirable. For eligible patients, the implementation of HSCT without delay would improve outcomes. To make this possible, the ascertainment of stem cell sources, including stem cells that are HLA-matched, partially mismatched, sourced from haploidentical family members, sourced from national or international cord blood banks, or sourced from international adult donor registries, is essential. When HSCT is appropriately timed, other factors related to outcomes, such as preconditioning regimens, the drugs used for GVH-D prophylaxis and treatment, and the drugs used for the treatment and prophylaxis of infections and organ failures would show their best cost effectiveness.

Fulfilling the Potential Requirements for Current Disease Entities Targeted by HSCT

It is recommended that the potential demands of HSCT in each country/region should be known from the beginning of the implementation of an HSCT program. There is an international consensus about current disease entities targeted by HSCT [17]. Each country/region must estimate the annual incidences of bone marrow failure syndromes, hemoglobinopathies, and congenital immune deficiencies, which are absolute indications for allogeneic HSCT. Next, the numbers of patients with hematological malignancies that cannot be cured by chemotherapy but can be cured by HSCT should be calculated. One may simultaneously estimate the numbers of patients with diseases that can be expected to result in prolonged survival with autologous HSCT. When one estimates such potential demands of HSCT, it must be under the consideration that HSCT is basically a cure-oriented therapy.

Long-term Follow-up of Patients and Donors

The long-term follow-up of patients who have received HSCT is important from two points of view; one is to establish the exact patient outcome data, such as the status of GVH-D, quality of life, and relapse. The other is to identify the problems that must be solved and factors where improvements can be implemented in the next HSCT. Long-term follow up is not necessarily difficult. It should be initiated by the transplant physicians at the usual outpatient clinic. The physicians should maintain good communication with the patients and help the patients to understand the importance of the long-term follow-up, even if they might have a few problems with this. An institutional template of the points to be checked long term is desirable, according to the consensus at the global level [18].

Maintaining donors' safety is essential in allogeneic HSCT. An HSC donor is a healthy individual. So one occurrence of a severe adverse event in a donor may have a catastrophic impact on the further implementation of allogeneic HSCT. The donor's safety should be considered in terms of both early and late events. Early events, some of which have, unfortunately, led to the death of donors during or just

after the harvest of either BM or peripheral blood in the past [19], can be avoided by strict maintenance of donor eligibility criteria and by taking a careful technical approach. To identify late events, the long-term follow-up of donors is required. Some donors may have prolonged pain at the harvest site after the marrow harvest procedure; such pain might be avoided for the next donor by changing the needle size. The information obtained from such follow-up will contribute to the further safety of donors.

Expanding the Targeted Disease Entities

HSCT is a technology-oriented field, but not necessarily an organ- or tissue-oriented one. A new platform, created after allogeneic and autologous HSCT, where the immune system of a patient is renewed, would offer new approaches to treat intractable diseases other than hematological ones, such as autoimmune diseases [20], certain solid cancers [21], and some neurological diseases [22]. Also it must be mentioned that the harmonization of HSCT and currently developed new drugs should bring about new aspects that benefit the patient and reduce medical costs [23].

Toward a Global Outcome Registry

Reporting the outcome of a patient or a donor to an authorized registry system is important not only for creating a database, but also for providing proof of HSCT implementation in each institute. The author recommends that every country has its own national registry at first, and if more than two institutes perform HSCT, then this national registry reports the data to an international registry system. There are three major international patient outcome registry systems; the registries of the CIBMTR, the EBMT, and the APBMT. There are, so far, large differences among the three systems, in terms of the quality and the access. Nevertheless, even if a team or a national registry system reports its case(s) to several international registration systems, the duplicated cases can be recognized by the Global Transplant Center Number (GTCN; created by the WBMT); this number covers many transplant teams worldwide.

Conclusion

The author encourages new teams to initiate HSCT soon, implementing the procedure according to the essential factors for performing HSCT that are described in this textbook. Some, but not necessarily all, of these factors are those that currently advanced teams applied for performing their first HSCT more than half a

century ago. In Section 1 of this chapter, the author has described the minimal essential requirements for initiating HSCT. Once you achieve your own successful cases, their characteristics will serve as the standards for your team, and, in addition, you will recognize what should be improved for the next case. The author believes your unique experiences could contribute to further advances of HSCT worldwide.

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