Hematopoietic cell transplantation: progress and obstacles

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The use of hematopoietic cell transplantation has expanded and evolved substantially in the last decade. New stem cell sources and stem cell mobilizing agents have been introduced in clinical practice. The incidence of life-threatening complications following autologous stem cell transplant procedures has decreased dramatically. Understanding the immune mediated effect of allogeneic stem cell transplantation has resulted in the development of reduced intensity and non-myeloablative conditioning regimens, allowing transplantation of elderly patients. Long-term complications are starting to emerge, and will gain in importance in the near future.

Key words: stem cell transplantation, autologous, allogeneic

introduction

In 1977, physicians from the Fred Hutchinson Cancer Research Center in Seattle published a paper describing the outcomes of 100 patients with relapsed and refractory acute leukemia who underwent allogeneic bone marrow transplantation from a human leukocyte antigen (HLA)-identical sibling donor [1]. Thirteen patients were alive and in ongoing remission 11 months to 4.5 years after transplantation, thereby demonstrating the potentially curative effect of this procedure. Two years later, the same group reported the outcomes of 19 patients with acute myelogenous leukemia (AML) who were transplanted with marrow from an HLA-identical sibling in first complete remission [2]. Twenty-six years after transplant, seven patients out of this group are still alive and in ongoing remission [3]. These results formed the culmination of work done by many researchers and clinicians since the famous experiments from Jacobson and Lorenz showing that mice could be protected from lethal doses of irradiation by spleen or bone marrow cells [4–6]. Much has changed since those early reports, but many of the problems facing bone marrow transplant recipients are remarkably comparable to the complications reported by those pioneering investigators.

Hematopoietic cell transplantation (HCT) has clearly established itself as an important treatment modality as evidenced by the performance of over 40,000 transplant procedures in the year 2000 [7]. In this paper, we aim to review the presently accepted paradigms pertaining to HCT and discuss the progress that has been made as well as the obstacles that are still limiting the success of this potentially life-saving procedure.

insights in stem-cell biology

The success of HCT is probably the best direct evidence for the existence of hematopoietic stem cells: cells with the unique capacity to produce more differentiated progenitor cells as well as some daughter cells that retain self-renewal capacity. Murine hematopoietic stem cells have been isolated and purified to near homogeneity and long-term lymphohematopoietic reconstitution with a single hematopoietic stem cell has been demonstrated [8]. Although human HCT is commonly referred to as a ‘stem-cell transplantation’, in reality grafts used in HCT procedures contain a mixture of immature and more mature hematopoietic progenitor cells, including (stem) cells with self-renewal capacity. For many years it was believed that adult bone marrow contained only hematopoietic stem cells, however recent developments have challenged this long-held paradigm. It has been known for more than two decades that bone marrow stromal cells are required to support the growth and development of long-term culture-initiating cells in vitro [9]. In vivo the bone marrow, microenvironment is essential in maintaining and regulating hematopoietic stem-cell activity. Multipotent mesenchymal stromal cells (MMSCs) have been isolated from bone marrow and shown to differentiate in vitro into adipose tissue, cartilage, and bone. MMSCs have thus emerged as an important regulator of hematopoietic activity, but also as a precursor for nonhematopoietic tissue, with possible stem-cell properties. An important clinical application of MMSCs within the field of HCT is on the basis of the ability of these cells to down-regulate alloreactive T-cell responses when added to mixed lymphocyte cultures [10]. This makes MMSCs an attractive cell source for therapeutic application in T-cell mediated auto- or alloimmune diseases [such as graft-versus-host disease (GVHD)]. Phase I studies have shown that bone marrow-derived MMSC can be successfully collected.

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culture ex vivo for 4–7 weeks, and administered to patients. Initial results from a phase II trial in patients with severe (grade III–IV) acute GVHD have shown encouraging results with minimal toxicity and some complete responses [11].

Another important development in the area of stem-cell research focuses on malignant stem cells. A small proportion of leukemic blasts have retained or acquired self-renewal properties and these cells are now generally referred to as leukemia stem cells [12]. As all other stem cells, these cells are mostly quiescent (e.g. not going through cell cycle) and therefore relatively resistant to the effects of many chemotherapy agents. Malignant stem cells possess multiple additional mechanisms to increase resistance to chemotherapy and radiation-induced cell death, but might still be sensitive to immune-mediated therapies such as allogeneic HCT [13].

**stem-cell sources**

Mobilized peripheral blood has largely replaced the use of bone marrow as the preferred source of hematopoietic stem cells in autologous transplant procedures and is increasingly used in allogeneic HCT. Peripheral blood stem cells generally produce more rapid hematopoietic reconstitution than marrow-derived stem cells, probably because of the higher content of committed hematopoietic precursor cells. The hematopoietic growth factor granulocyte colony-stimulating factor (G-CSF) is commonly used to increase the number of circulating hematopoietic progenitor cells (identified by expression of the cell surface marker CD34). G-CSF is thought to work through the proliferation of mature neutrophils with the associated increased production of proteases that are able to cleave the proteins that under normal circumstances anchor CD34+ cells to the bone marrow microenvironment. Improved understanding of the roles that adhesion molecules play in maintaining a normal bone marrow microenvironment has resulted in the development of a novel mobilization agent. The bicyclam derivate AMD3100 reversibly competes with and inhibits the interaction between the CXC chemokine receptor 4 (CXCRR4, expressed on the surface of CD34+ cells) and stromal cell-derived factor 1α [14]. AMD3100 is able to mobilize CD34+ cells into the peripheral circulation within hours after a single injection [15]. Combination of AMD3100 and G-CSF appears safe and might be more effective than G-CSF alone for autologous CD34+ cell mobilization [16].

Umbilical cord blood has recently emerged as another rich source of hematopoietic stem cells for transplantation. The minimal risk to the donor and the rapid availability of umbilical cord blood are among the great advantages of this stem-cell source. In addition, HLA matching requirements for cord blood are less stringent as the incidence of both acute and chronic GVHD after cord blood transplantation compares favorable with bone marrow transplantation [17]. The exact reason for the reduced incidence of GVHD remains to be determined, but a commonly accepted hypothesis is that cord blood-derived hematopoietic stem cells are more immunologically naive as their bone marrow-derived counterparts. Slow recovery of absolute neutrophil count and platelet count are the main drawback of cord blood HCT. Both cell dose and extent of HLA matching are important prognostic factors when performing cord blood transplantations, with the best results seen in recipients of cord blood units with a relatively high mononuclear cell dose per kilogram recipient bodyweight and no more than one HLA mismatch [18]. The limited number of cells that can be obtained from a single cord cord has hampered the extension of cord blood transplantation into the population of adult recipients, although the use of mismatched cord blood results in outcomes that are similar to mismatched unrelated bone marrow [19]. Attempts to overcome the impact of low stem-cell doses in cord blood collections have included in vivo expansion and use of two instead of one cord blood grafts [20]. The US Health Resources and Services Administration recently contracted with the National Marrow Donor program to serve as the national Cord Blood Coordinating Center and with the Center for International Blood and Marrow Transplantation Research to establish a Stem Cell Therapeutic Outcomes Database.

**high-dose chemotherapy with autologous HCT rescue**

The effect of autologous HCT is entirely derived from the high-dose conditioning regimen. Most conditioning regimens are comprised of a combination of agents with different mechanisms of action but with hematologic toxicity as the dose-limiting toxicity. The infusion of previously collected and stored autologous hematopoietic stem cells allows for dose escalation beyond the dose-limiting toxicity observed with administration of standard doses. This procedure is particularly attractive for treatment of patients with aggressive but chemo-sensitive malignancies, such as some of the non-Hodgkin’s lymphomas and Hodgkin’s disease. In other malignancies, such as multiple myeloma, high-dose chemotherapy is noncurative, but does improve survival. Unfortunately, malignant stem cells with their inherent resistance to chemotherapy-induced cell damage might survive even the highest tolerated doses of chemotherapy conditioning. Reinfusion of malignant stem cells, inadvertently collected during the stem-cell mobilization process, probably contributes to the high incidence of relapse observed after autologous HCT. Significant improvements in supportive care, in particular early recognition and effective treatment of infectious complications, have greatly improved the safety profile of autologous HCT. In most experienced transplant centers, the 100-day transplant-related mortality associated with autologous HCT is now well <5%. Attempts to further improve outcomes will therefore have to concentrate on more effective ways to eliminate malignant stem cells from both patient and stem-cell graft.

The introduction of (humanized) monoclonal antibodies for therapeutic use may be helpful to achieve this goal. Studies carried out in the 1990s showed that in vitro purging of autologous stem-cell grafts of patients with follicular lymphoma resulted in longer freedom from recurrence in those patients where a graft without evidence of contaminating lymphoma (e.g. negative PCR for bcl2/IgH) could be obtained [21, 22]. In vitro purging is cumbersome and labor intensive, but the availability of monoclonal anti-B-cell antibodies such as rituximab allows for in vivo purging before and after
administration of high-dose chemotherapy and does not appear to add toxicity to the procedure [23, 24]. Equally promising is the use of radioimmune conjugates before high-dose chemotherapy for patients with B-cell non-Hodgkin’s lymphoma. Several phase I/II trials have combined the use of iodine-131 tositumomab or yttrium-90 ibritumomab tiuxetan with high-dose chemotherapy regimens without significant additional toxicity [25–27]. A large randomized trial to establish the efficacy of this regimen is presently being pursued by the Bone Marrow Transplant Clinical Trials Network.

**new developments in allogeneic HCT**

The HLA system plays a critical role in allogeneic HCT. The elucidation of the dog leukocyte antigen system in a canine population allowed for the first systematic experiments studying the application of HCT in an outbred species comparable to humans [28]. Understanding of the HLA system opened the possibility of identifying genotypically identical sibling donors for patients in need of an allogeneic HCT procedure. Lack of available sibling donors was the driving force behind the development of registries of phenotypically identical unrelated donor volunteers for transplantation purposes. Initial studies generally showed a higher complication rate and lower survival after unrelated donor transplants as compared with related donor recipients. Dramatic advances in techniques used to define HLA genes and alloantigens have contributed significantly to improved outcomes in unrelated donor allogeneic HCT [29–31].

Unrelated donor searches and transplants now routinely incorporate allele (DNA) typing of the relevant class I and class II HLA antigens of both donor and recipients, aiming for complete (10/10) allele matches [32]. High-resolution HLA typing has resulted in unrelated donor transplant outcomes that are comparable to outcomes obtained with matched sibling transplants for selected diseases [33].

An equally important development stems from the insight that the effect of an allogeneic HCT is really derived through two different mechanisms: the cytoreductive effect of the high-dose (myeloablative) conditioning regimen and the immune-mediated graft-versus-malignancy (GVM) effect. The importance of the GVM effect was initially inferred from the initial studies generally showed a higher complication rate and lower survival after unrelated donor transplants as compared with related donor procedures. Direct evidence came from studies showing that the infusion of donor lymphocytes can result in sustained remissions in patients who relapse after allogeneic HCT as compared with identical twin transplants and among patients who develop GVHD after allogeneic HCT as compared with patients who do not [34].

Relapse remains a common problem after such nonmyeloablative HCT, in particular for patients who are not in complete remission at the time of transplantation. In an attempt to design a ‘best-of-both worlds’ approach, several investigators have studied reduced intensity conditioning regimens that rely on moderate doses of cytoreductive agents used for conditioning (at doses that are generally not myeloablative but that would result in prolonged deep pancytopenia if not accompanied by stem-cell infusion) combined with the immune-mediated effects of an allogeneic stem-cell graft. Commonly employed regimens combine the immune suppressive effect of the nucleotide analogue fludarabine with alkylating agents such as melphalan or busulfan at doses slightly lower then used in myeloablative transplant regimens. In many cases, the regimen is further modified by addition of an in vivo immune modulating agent such as antithymocyte globulin or alemtuzumab [38–41]. It is clear that the use of nonmyeloablative and reduced intensity conditioning regimens has allowed the extension of HCT to elderly patients and patients otherwise ineligible for allogeneic HCT with low early transplant-related morbidity and mortality. In many studies, the long-term nonrelapse (transplant-related) mortality remains substantial, often the result of late occurring acute GVHD and chronic GVHD. It remains a matter of debate if reduced intensity conditioning regimens have clear advantages over true nonmyeloablative regimens, and if these advantages are limited to patients with residual disease at the time of transplant [42]. The transplant community will need to address the question if it is time to move from a ‘one-size-fits-all’ conditioning approach to a more ‘tailor-made’ conditioning based on disease, disease stage, age, and comorbidities, preferably through randomized clinical trials.

For many patients, finding a suitable donor for allogeneic HCT remains a major challenge, and often a compatible unrelated donor cannot be identified in time to benefit the patient. The use of a cord blood graft is one viable alternative for patients in urgent need of an unrelated allogeneic HCT donor. Another alternative is the use of a donor with only one identical HLA haplotype, usually a parent, sibling, or child. Such a haploidentical donor can be identified rapidly for the vast majority of patients. Previous attempts at such haploidentical transplants were complicated by very high rates of nonengraftment and severe acute GVHD, with most patients dying from transplant-related mortality within a few weeks after the procedure. Recent technological developments have allowed for extensive T-cell depletion of donor grafts to the
extent that acute GVHD is no longer a problem. In addition, the use of intensive immune suppression as part of the conditioning regimen has reduced the incidence of nonengraftment [43]. Most interestingly, results from thus carried out haploidentical transplants have identified a previously unknown antimalignancy effect derived from alloreactive mechanisms involving natural killer (NK) cells [44]. A complex system of activating and inhibitory receptors determines the activation state of NK cells, and knowledge of this system has allowed for the selection of donors, whose receptors mismatch recipient ligands, resulting in NK cell activation. NK cell activation appears to mostly benefit patients transplanted for AML. Unfortunately, the prolonged immune reconstitution that follows T-cell depleted transplants results in a high incidence of infectious complications, and widespread use of haploidentical donor transplants will probably await the development of methods to expedite immune reconstitution.

complications

Neither nonmyeloablative nor reduced intensity regimens have solved the problem of acute and chronic GVHD occurring after allogeneic HCT. Results from animal and human studies have greatly increased our understanding of acute GVHD. This early complication of allogeneic HCT is now viewed as an allogeneic immune response primarily mediated by donor T lymphocytes with specificity against alloantigens expressed by host antigen-presenting cells in an environment of proinflammatory cytokines [45]. These insights have so far not translated in a substantial reduction in the incidence of acute GVHD; however, several promising new agents for prophylaxis of acute GVHD are presently undergoing testing in a large randomized clinical trial coordinated by the Bone Marrow Transplant Clinical Trials Network. In addition, small variations in donor or recipient genes (polymorphisms) that encode for cytokines or other molecules that are critical for initiation or promotion of the inflammatory immune response have been identified, and their effect on the incidence of acute GVHD has been demonstrated [46-48]. This opens the possibility of predicting the risk for severe acute GVHD by genotyping of donor and recipient and adjusting the intensity of the immune suppression regimen accordingly.

Unfortunately, no such progress can be reported in the area of chronic GVHD research. This late occurring complication from allogeneic HCT mostly resembles a systemic autoimmune disease with clinical manifestations consisting of sclerodermatous and lichenoid skin changes, xerostoma, keratoconjunctivitis sicca, and obliterating bronchiolitis. Very recent studies indicate that impaired negative selection of thymic-dependent donor T lymphocytes results in the development of chronic GVHD-like symptoms in mice [49]. Although less immediately life threatening than acute GVHD, the presence of chronic GVHD seriously impacts the health status of long-term transplant survivors [50]. The recent development of consensus criteria to diagnose and study this devastating transplant complication is an important first step in improving long-term quality of life for HCT recipients [51].

Chronic GVHD is by far the most common but certainly not the only long-term toxicity associated with HCT. Infertility due to ovarian failure is expected in most women exposed to high-dose chemoradiation, and azoospermia is the rule in men. Sperm cryopreservation before conditioning is routinely offered to men with preserved pretransplant testicular function [52]. For the minority of women who still have normal ovarian function before starting their conditioning regimen, hormonal suppression might result in resumption of normal ovarian function after HCT [53]. Of great concern is the increased incidence of secondary malignancies that occurs after HCT. The incidence of myelodysplasia and secondary acute leukemia is increased after administration of high-dose chemotherapy with autologous HCT, and seems to be particularly high with the use of TBI [54]. The immune suppression associated with allogeneic HCT results in an increased incidence of skin neoplasms, as well as malignancies of the oral cavity, uterus, thyroid, and breast [55]. In addition, relative to healthy controls, HSCT survivors reported poorer physical, psychological, and social functioning [56]. Long-term follow-up of transplant survivors is often suboptimal as most transplant recipients return to the care of referring oncologists and internist who might not be familiar with the problems that are commonly encountered in this population. Recently published recommendation for long-term follow up of transplant survivors should help to improve this situation [56, 57].

application of autologous HCT in selected diseases

The superiority of high-dose chemotherapy over standard-dose chemotherapy for patients with relapsed chemosensitive diffuse large B-cell non-Hodgkin’s lymphoma was established in the PARMA study, showing a 5-year event-free survival of 46% for patients receiving high-dose chemotherapy versus 12% for standard-dose salvage chemotherapy [58]. Retrospective application of the later developed International Prognostic Index (IPI) scoring system revealed that the survival benefit was limited to patients with an IPI ≥1 [59]. The use of high-dose chemotherapy in first complete remission for ‘high-risk’ patients (as identified by the IPI or comparable scoring system) remains controversial, with multiple studies yielding contradictory results [60]. Interestingly, high-dose chemotherapy has been less commonly employed for patients with relapsed follicular lymphoma, although several studies have shown a survival benefit for patients receiving an autologous HCT [61, 62]. In patients with mantle cell lymphoma, use of high-dose chemotherapy in first complete remission has been associated with an improvement in progression-free survival, but not overall survival (OS) [63]. Importantly, most of these studies were conducted before the commercial availability of monoclonal antibodies, and the results might therefore not be applicable to patients who have received rituximab as a component of initial or salvage chemotherapy.

Multiple myeloma remains an incurable disease, even with the use of high-dose chemotherapy. However, the use of high-dose melphalan with autologous HCT has shown to increase OS in a randomized study [64]. The use of tandem (double) autologous HCT might provide a further survival benefit, in
particular for patients who achieve a suboptimal response to the first transplant procedure [65]. Again, most of these studies were conducted before many of the agents (thalidomide, lenalidomide, bortezomib) that are now routinely used for treatment of newly diagnosed and relapsed multiple myeloma became available, and the superiority of high-dose chemotherapy over some of the newer combination regimens remains to be established.

**application of allogeneic HCT in selected diseases**

Most algorithms for treatment of patients with AML use the results of cytogenetic studies to assign patients to low-risk, standard-risk or high-risk groups. Several studies have used genetic randomization (e.g. the presence or absence of an HLA-identical sibling) to assign patients to treatment with allogeneic HCT in first remission versus multiple cycles of consolidation chemotherapy [66–68]. These studies show that patients with low-risk disease (mostly characterized by balanced translocations involving core binding factors) do not benefit from allogeneic HCT in first complete remission. In addition, patients with acute promyelocytic leukemia generally have an excellent prognosis when all-trans-retinoic acid is incorporated in their treatment regimen, and are in no need of allogeneic HCT. All studies have shown a reduced relapse risk for patients with intermediate or high-risk disease undergoing an allogeneic HCT. This does not always translate in an improved survival, as the mortality associated with allogeneic HCT can be substantial, in particular for elderly patients. The consideration of allogeneic HCT in first remission therefore requires a careful balancing of the anticipated transplant-related mortality versus the reduced risk of relapse. Better characterization of AML risk groups using some of the more recently identified risk factors (FLT3 mutation status, NPM1 mutation status, CEBPa mutation status) or microarray study results might help in restricting allogeneic HCT use to patients at the highest risks for relapse. For patients with relapsed AML who are able to achieve a second remission, allogeneic HCT remains the treatment of choice.

The use of allogeneic HCT for adults with acute lymphoblastic anemia (ALL) remains controversial. A recent evidence-based review concluded that for adults with standard-risk disease in first complete remission, allogeneic HCT yields outcomes similar to chemotherapy consolidation [69]. For patients with high-risk disease there might be an advantage to allogeneic HCT in first remission, although no direct comparison studies have been done. Allogeneic HCT is recommended for all adult patients in second remission. Almost all studies precede the availability of the tyrosine kinase inhibitors imatinib and dasatinib, and the effect of these agents on the role of allogeneic HCT for patients with Philadelphia chromosome-positive ALL remains to be determined.

The bcr-abl tyrosine kinase inhibitor imatinib has largely replaced allogeneic HCT as treatment of choice for patients with chronic myelogenous leukemia (CML) in first chronic phase. Allogeneic HCT is generally reserved for patients with advanced or refractory disease and might also be an option for selected patients who fail treatment with a tyrosine kinase inhibitor [70]. Long-term comparisons of outcomes obtained with imatinib and allogeneic HCT are not available. Recently, presented data show an OS of 89% at 5 years for patients with newly diagnosed CML, significantly superior to the anticipated 5-year OS after allogeneic HCT [71]. These data are, however, not based on an intention-to-treat analysis, and might therefore be biased towards favorable results with imatinib.

**the future of HCT**

In the United States, HCT remains an underutilized treatment modality, and too many patients are not referred for consideration of transplant until they have very advanced disease [72]. Better information of the oncology community and the general public about optimal timing for transplant referral should help to increase access for patients who might benefit from HCT. Analysis of efficacy and cost will allow countries with limited resources to make informal decisions about therapeutic recommendations for patients who might benefit from HCT [73]. Randomized studies will have to answer the issue of the optimal conditioning regimen, stem-cell source, and immune suppression regimen for individual patients. A risk-based approach, allowing for accurate prediction of relapse risk versus transplant-related risks, is already emerging and will probably gain popularity if supported by the results from phase III clinical trials.

Incorporation of monoclonal antibody-based therapy should allow for further improvement in outcomes. A better understanding of normal and malignant stem-cell biology is required to move the field to its next level: elimination of malignant stem cells by using tumor stem cell-specific combinations of chemotherapy, monoclonal antibodies, and cellular immune therapy.

**references**


