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ORIGINAL PAPER

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Allogeneic hematopoietic cell transplantation as treatment for hematological malignancies: a review

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Abstract Allogeneic hematopoietic cell transplantation (HCT) was originally developed as a form of rescue from high-dose chemoradiotherapy, which is given both to eradicate malignancy and provide sufficient immunosuppression for allogeneic engraftment. The first attempts of allogeneic HCT in humans met with little success. However, a better understanding of the complexities of the human leukocyte antigen (HLA) system has allowed selecting compatible sibling donors, and the development of postgrafting immunosuppressive regimens has helped prevent serious graft-versus-host disease, thereby changing the role of allogeneic HCT from a desperate therapeutic maneuver to a curative treatment modality for many patients with malignant hematological diseases. In addition, the establishment of large registries of HLA-typed volunteers has permitted finding suitable unrelated donors for many patients without family donors. Further advances in the immunogenetics of HLA, especially typing by molecular techniques, have improved results after unrelated HCT, which have begun resembling those obtained with HLA-identical sibling grafts, at least in young patients. Important advances have also been made in the prevention and treatment of infectious complications and in other areas of supportive care. Since the late seventies, it has been recognized that allogeneic immunocompetent cells transplanted with the stem cells, or arising from them, mediated therapeutic anti-tumor effects independent of the action of the high-dose therapy, termed graft-versus-tumor (GVT) effects. This has prompted the recent development of non-myeloablative conditioning regimens for allogeneic HCT that have opened the way to include elderly patients and those with comorbid conditions. Remaining challenges include further advances in the prevention and treatment of both severe graft-versus-host disease and infections. Also, progress in adoptive transfer of T cells with relative tumor specificity and disease-targeted therapy with agents such as Imatinib, Rituximab or radiolabeled monoclonal antibodies would make allogeneic HCT even more effective.

Keywords Allogeneic hematopoietic cell transplantation · Hematological malignancies · Immunogenetics · Conditioning regimens · Immunosuppressive drugs

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Introduction: History of allogeneic hematopoietic cell transplantation

Bone marrow is an organ that is exquisitively sensitive to radiation, and exposure to high doses of total body irradiation (TBI) leads to death from marrow failure. The era of hematopoietic cell transplantation (HCT) began with the recognition that marrow lethality from external photon beam irradiation in mice could be prevented by shielding their spleens with lead [30]. Subsequent studies showed that intravenous infusion of marrow or spleen cells after TBI in mice and rats could also prevent death [42]. The reasons for the marrow protective effect of these experimental interventions were subject to controversy, but in the mid-1950s firm proof was obtained in several laboratories that the protection was due to transplantable hematopoietic stem cells (reviewed in [41, 83]).

Researchers and clinicians alike were excited by these discoveries, which suggested that transplantation of marrow from healthy donors could be turned into treatment for human patients with acquired lack of marrow function, with inborn errors, and hematological malignancies. In the latter case, the intensity of cytotoxic anticancer agents could be increased beyond the range that was toxic to the bone marrow cells, thereby potentially increasing their efficacy. In the second half of the 1950s and in the early 1960s, results of murine studies of HCT led to attempts at translating the discovery into the clinic. In 1955, Thomas and colleagues [80] pioneered early studies of human marrow grafting. However, transplantations were, for the most part, unsuccessful with most patients dying of allograft failure or progressive disease, and only one patient engrafting transiently. In 1970, Bortin [7] reviewed data from approximately 200 human allogeneic human marrow grafts published in the 1950s and early 1960s and confirmed that very few had been successful. Often patients failed to engraft or, if they engrafted, developed fatal graft-versus-host disease (GVHD). The feasibility of crossing the "allogeneic barrier" in humans was seriously doubted. It had become evident that graft-versus-host (GVH) reactions in random-bred animals including humans were incomparably more violent than in inbred rodents. As the experimental hematologist and cell biologist Dirk van Bekkum stated in 1967, "Many errors in extrapolation from the laboratory experiment to the patient have been made and much time was lost before it became evident that the GVH reaction in primates (random-bred large animals) including man is incomparably more violent than in (inbred) rodents" [41, 83]. Many researchers abandoned the concept that allogeneic HCT could be used to treat human patients. However, subsequent studies in dogs and in nonhuman primates renewed a sense of confidence that allogeneic bone marrow transplantation might one day become a therapeutic option for patients with hematological disorders. The outbred nature and the wide genetic diversity of dogs made them a particularly suitable animal model for preclinical studies.

In 1968, two studies demonstrated that dog leukocyte antigen (DLA) compatibility between donors and recipients improved the outcome of allogeneic HCT [17, 72]. Moreover, although GVHD had been previously observed in major histocompatibility complex-mismatched mice and unrelated monkeys, the canine studies clearly showed that GVHD could occur even across minor histocompatibility barriers. Effective drug regimens were developed to contain GVHD [73] and immunosuppressive therapy could frequently be discontinued after 3–6 months of treatment due to the establishment of mutual graft/host tolerance. These observations encouraged further trials of allogeneic HCT between HLA-matched human siblings.

Conventional high-dose allogeneic HCT

From 1969 to 1975, clinical studies were carried out in patients with immunodeficiency diseases, severe aplastic anemia and refractory advanced leukemias and lymphomas using grafts from HLA-matched sibling donors. In 1975, a review article by the Seattle marrow transplant team described the results in 100 patients with leukemia/lymphoma and aplastic anemia given allogeneic HCT after failure of conventional therapy [82]. Despite high transplant-related mortality, the demonstration of long-term disease-free survival was encouraging.

In 1977, Thomas et al. [77] reported that leukemic patients in fair general condition at the time of the transplantation had significant higher disease-free survivals than those in poor condition and that patients with advanced refractory leukemia/lymphoma continued to have high disease recurrence rates of approximately 75% despite the high-dose conditioning regimens. These observations suggested that transplantation should be carried out earlier in the course of the disease, while patients were still in good medical condition and had lower tumor burdens. In 1979, two reports of transplantation for acute myeloid [78] and lymphoblastic [81] leukemias in first remission showed greatly improved results, with 2-year overall survivals of 60% and 50%, respectively. In 1986, two studies reported that a majority of patients with chronic myeloid leukemia in chronic phase could be cured by chemo-irradiation and marrow transplantation from HLA-matched sibling donors [26, 79].

Conditioning regimens

The second half of the 1960s saw the development of high-dose conditioning regimens. These consisted of maximally tolerated doses of either cyclophosphamide or TBI alone [82]. While many patients relapsed with their original malignancies, two girls with acute lymphoblastic leukemia given HLA-matched grafts from their brothers after 1000 cGy TBI showed unusual leukemic transformation of the engrafted male marrow cells, which eventually resulted in the deaths of both patients. Based on the available knowledge, we hypothesized that destruction of the leukemic cells by TBI had induced the production of either a fully active oncogenic virus or of a helper virus which collaborated with a defective endogenous virus in the graft to produce malignant transformation of donor cells [69]. We then decided to reduce the tumor burden before TBI with another agent, known to have a lower potential for virus induction than radiation. The agent chosen was cyclophosphamide, 60 mg/kg per day for 2 days. After combining cyclophosphamide with TBI, leukemic transformation of donor cells was rarely seen.

In 1983, busulfan (another alkylating agent that killed cells by cross-linking DNA) was combined with cyclophosphamide as an alternative to TBI-based regimens [60]. Ten years later, a randomized study including patients with chronic myeloid leukemia in chronic phase demonstrated comparable results when cyclophosphamide was combined with TBI or with busulfan [12]. However, a comparison of the same two regimens in patients with acute myleloid leukemia in first remission showed the cyclophosphamide/TBI regimen to be superior [65].

A number of approaches were tried to improve preparative regimens. Numerous studies found considerable variability in plasma busulfan concentrations following a set oral dose [62]. By adjusting subsequent doses based on the metabolism of the initial dose, and

thereby achieving a targeted busulfan plasma concentration, both under treatment with increased relapse rates and excessive toxicity were avoided [62].

GVHD

The magnitude of GVHD in humans was not fully appreciated until long-term engraftment of donor marrow was achieved. GVHD has been classified into two syndromes: acute GVHD, occurring within 3 months of transplantation (Table 1, 2), and chronic GVHD (Table 3), developing thereafter [39, 55]. Despite HLA matching and the use of postgrafting immunosuppression with methotrexate (MTX), approximately half of the patients developed acute GVHD, consistent with earlier findings in experimental dogs [17]. Major improvements in both GVHD prevention and patient survival were seen when the antimetabolite MTX was combined with the T cell activation inhibitors cyclosporine (CSP) or tacrolimus (FK506) given for 180 days with a short course of MTX given on days 1, 3, 6 and 11 after HCT [57, 70, 71]. Table 4 shows results of two multicenter randomized studies comparing FK506 plus MTX versus CSP plus MTX for prevention of GVHD after allogeneic HCT. These drug combinations have become the most widely used methods to prevent GVHD, although combining the purine synthesis inhibitor, mycophenolate mofetil (MMF), with either CSP or FK506 also appears promising [88].

Other investigators have focused on removing T cells from the hematopoietic grafts as a way of preventing GVHD. While the technique efficiently prevented GVHD, initial trials revealed higher incidence of infections, graft rejection and relapse as well as the occurrence of Epstein-Barr virus (EBV)-induced lymphoproliferative malignancies [46]. Some

Table 1 Clinical grading of acute GVHD: severity of individual organ involvement [55] (*GVHD* graft-versus-host disease)

Grade	Skin	Liver	Gut[a]
+	Maculopapular eruption involving less than 25% of the body surface	Bilirubin (2.0–3.0 mg/100 ml)	≤1,000 ml of liquid stool/day[b] (≤15 ml of stool/kg per day)[c]
++	Maculopapular eruption involving 25–50% of the body surface	Bilirubin (3–5.9 mg/100 ml)	>1,000 ml of stool/day[b] (>15 ml of stool/kg per day)[c]
+++	Generalized erythroderma	Bilirubin (6–14.9 mg/100 ml)	>1,500 ml of stool/day[b] (>20 ml of stool/kg per day)[c]
++++	Generalized erythroderma with bullous formation and often with desquamation	Bilirubin >15 mg/100 ml	2,000 ml of stool/day[b] (≥25 ml of stool/kg per day)[c]

[a] Nausea, vomiting or anorexia caused by GVHD is assigned as +; patients with visible bloody diarrhea are at least ++
[b] In the absence of medical cause
[c] For pediatrics patients

Table 2 Clinical grading of the severity of acute GVHD [55]

Overall grade	Skin	Liver	Gut	Functional impairment
0 (none)	0	0	0	0
1 (mild)	+ to ++	0	0	0
2 (moderate)	+ to +++	+	+	+
3 (severe)	++ to +++	++ to +++	++ to +++	++
4 (life threatening)	++ to ++++	++ to ++++	++ to ++++	+++

Table 3 Seattle revisited: clinical criteria for limited and extensive chronic GVHD [39] (*BSA* body surface area)

Clinical limited
1. Oral abnormalities consistent with chronic GVHD, a positive skin or lip biopsy, and no other manifestations of chronic GVHD
2. Mild liver test abnormalities (alkaline phosphatase ≤2× upper limit of normal; AST or ALT ≤3× upper limit of normal,and total bilirubin ≤1.6) with positive skin or lip biopsy, and no other manifestations of chronic GVHD
3. Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of BSA, dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy, and no other manifestations of chronic GVHD
4. Ocular sicca (Schirmer's test ≤5 mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of chronic GVHD
5. Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of chronic GVHD

Clinical extensive
1. Involvement of 2 or more organs with symptoms or signs of chronic GVHD, with biopsy documentation of chronic GVHD in any organ
2. Karnofsky or Lansky Clinical Performance scores <60%, ≥15% weight loss, and recurrent infections not due to other causes, with biopsy documentation of chronic GVHD in any organ
3. Skin involvement more extensive than defined for clinical limited chronic GVHD, confirmed by biopsy
4. Scleroderma or morphea
5. Onycholysis or onychodystrophy thought to represent chronic GVHD, with documentation of chronic GVHD in any organ
6. Decreased range of motion in wrist or ankle extension due to fasciitis caused by chronic GVHD
7. Contractures thought to represent chronic GVHD
8. Bronchiolitis obliterans not due to other causes
9. Positive liver biopsy; or abnormal liver function tests not due to other causes with alkaline phosphatase >2× upper limit of normal, AST or ALT >3× upper limit of normal, or total bilirubin >1.6, and documentation of chronic GVHD in any organ
10. Positive upper or lower GI biopsy
11. Fasciitis or serositis thought to represent chronic GVHD and not due to other causes

Table 4 Results of two randomized multicenter studies comparing tacrolimus (FK506) plus MTX versus CSP plus MTX for prevention of GVHD (*MTX* methotrexate, *CSP* cyclosporine)

Author	Donors	No. of patients	GVHD prophylaxis regimen	Grade II–IV acute GVHD	Chronic GVHD	2-year overall survival
Ratanatharathorn [57]	HLA-identical siblings	329	FK506 + MTX versus CSP + MTX	32% vs 44% (P=0.01)	56% vs 49% (NS)[a]	47% vs 57% (P=0.02)[b]
Nash [50]	HLA-matched unrelated donors	180	FK506 + MTX versus CSP + MTX	56% vs 74% (P=0.0002)	76% vs 70% (NS)	54% vs 50% (NS)

[a] The incidence of extensive chronic GVHD was significantly lower in the FK506 plus MTX group (P=0.03)
[b] The 2-year overall survival was the same for patients with nonadvanced disease

of these complications can now be prevented by the use of more refined methods of T cell depletion or by the use of pre-emptive donor lymphocyte infusions (DLI; reviewed in [3]). In patients transplanted for chronic myeloid leukemia, monitoring for both residual disease and re-emergence of recipient T cells has allowed treatment with subsequent DLI to initiate graft-versus-tumor (GVT) responses without causing GVHD [16].

Table 5 HLA locus: effect of matching on survival and severe GVHD incidence after allogeneic bone marrow transplantation with unrelated donors [28]

HLA locus	Matching	Impact of matching on survival	Impact of matching on severe GVHD incidence
A	Recommended	Improved	Decreased
B	Recommended	Improved	Uncertain
C	Recommended	Improved	Uncertain
DRB1	Recommended	Improved	Decreased
DQB1	Recommended	Uncertain	Decreased
DPB1	Uncertain	Uncertain	Uncertain

Allogeneic HCT using alternative donors

Very few HLA-matched unrelated transplants were carried out in the 1970s when matched donors were found fortuitously, and the heterogeneity of the HLA haplotypes underlined the need for large panels of HLA-typed volunteer donors. The creation of the Anthony Nolan Foundation in England preceded the establishment of such programs in other countries. Today, more than 50 registries of potential HLA-typed adult hematopoietic stem cell donors exist in 40 countries and include more than 7.5 million volunteers [29]. The HLA phenotypes available in most registries worldwide have been summarized within the database of Bone Marrow Donor Worldwide [29]. The minimum acceptable match was originally defined serologically (HLA-A, HLA-B and HLA-DR loci) and confirmed by mixed leucocyte culture non-reactivity, and required compatibility for at least five of six HLA antigens [28]. However, transplantation outcomes could be improved by matching strategies that increased the degree of HLA compatibility above this minimum, and it has become possible to select unrelated donors who were matched with their respective recipients serologically for HLA-A, B, C and at DRB1 and DQB1 at the allele level [28] (Table 5). Results with such unrelated HCT have begun resembling those seen with grafts from HLA-identical siblings, at least in patients transplanted below age 50 years. Further, progress has been made with regard to haplo-identical HCT, by infusing mega doses of purified hematopoietic stem cells [1].

Stem cell sources

In the past, bone marrow was the source of hematopoietic stem cell for most allogeneic HCT. However, the development of recombinant human hematopoietic growth factors such as recombinant human granulocyte colony-stimulating factor (G-CSF) that could “mobilize” stem cells from the marrow and increase their concentration in the peripheral blood, has allowed the use of peripheral blood mononuclear cells (PBMC) as stem cell source. Prospective randomized studies suggested that using stem cells derived from G-CSF-primed PBMC (G-PBMC) instead of marrow was associated with faster hematopoietic recovery, similar incidence of acute GVHD, and perhaps more chronic GVHD, and increased overall survival [5, 6, 14, 61] (Table 6).

More recently, cord blood has been used as a source of transplantable stem cells [25, 84]. Potential advantages included more rapid availability and, because cord blood is relatively deficient in T cells, the possibility that a greater degree of HLA mismatching might be tolerable. Disadvantages of cord blood have been slower engraftment and an increased

Table 6 Randomized studies comparing marrow and G-PBMC as stem cell source for allogeneic HCT with a HLA-identical related donor (*HCT* hematopoietic cell transplantation, *G-PBMC* granulocyte colony-stimulating factor-primed peripheral blood mononuclear cells, *n* number of patients)

Author (reference)	*n*	Incidence of grade II–IV acute GVHD (%)			Incidence of extensive chronic GVHD (%)			2-year % of overall survival		
		Marrow	G-PBMC	*P* value	Marrow	G-PBMC	*P* value	Marrow	G-PBMC	*P* value
Blaise [6]	100	42	44	NS	8	34	<0.03	65	67	NS
Bensinger [5]	172	57	64	NS	32	37	NS	54	66	0.06
Schmitz [61]	350	39	52	0.01	54[a]	67[a]	0.007	65	65	NS
Powles [54]	39	58[b]	68[b]	NS	40[a]	44[a]	NS	63	70	NS
Couban [14]	228	44	44	NS	30	40	NS	60[c]	68[c]	0.04

[a] Both limited and extensive GVHD
[b] Grade I–IV acute GVHD
[c] Overall survival of 30 months

risk of graft failure. Factors associated with survival after cord blood transplantation were the number of cells infused per kilogram of recipient, patient age and the degree of HLA-match with the donor. Unfortunately, the low cell content of most cord blood units has limited the current use of cord blood transplantation to children and perhaps smaller adults.

GVT effect

Reduced risks of relapse in patients with GVHD

A GVT effect was first suggested by Barnes et al. in 1956 [2]. They observed that mice receiving syngeneic HCT and injection of congenic leukemic cells after TBI almost uniformly died from leukemia, whereas mice receiving allogeneic transplants developed GVHD but had a lower incidence of leukemic deaths. The authors suggested that a reaction of the donor spleen cells might kill cancer cells. The initial evidence for GVT effects in humans came from studies reporting reduced leukemic relapse rates in allografted patients who developed acute [85] and/or chronic [86] GVHD compared with those who did not (Fig. 1). The GVT effect was confirmed by other investigators who observed increased risks of relapse in patients receiving T cell-depleted grafts and in recipients of syngeneic transplants [22]. More recently, lower relapse rates were described in male patients receiving HCT from female donors for chronic myeloid leukemia, acute myeloid leukemia and acute lymphocytic leukemia, after controlling for GVHD occurrence [56]. This observation suggested that minor histocompatibility antigens encoded by Y-chromosome genes contributed to selective GVT effects.

Donor lymphocyte infusions (DLI)

Further evidence for the GVT effects came from observations that infusions of donor lymphocytes could induce complete remissions in patients with hematological malignancies who had relapsed after allogeneic HCT [35, 64]. Two large multicenter studies have re-

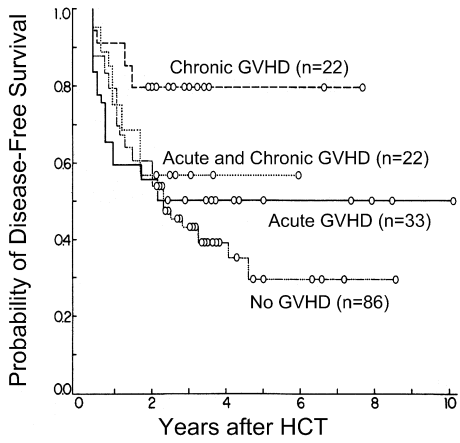

Fig. 1 Disease-free survivals of patients with hematological malignancies given cyclophosphamide and TBI, HLA-matched related HCT, and MTX for GVHD prevention. Shown are data in patients with and without acute and chronic GVHD (*TBI* total body irradiation, *HCT* hematopoietic cell transplantation, *MTX* methotrexate, *GVHD* graft-versus-host disease) (modified from [86], Copyright 1981 Massachusetts Medical Society. All rights reserved)

Table 7 Results of donor lymphocyte infusion as treatment of relapse after HLA-matched related HCT

	No. of complete response/no. of evaluable patients (%)	
	EBMT [37]	North America [13]
Chronic myeloid leukemia		
Cytogenetic/molecular relapse	40/50 (80)	3/3 (100)
Hematological relapse	88/114 (77)	25/34 (74)
Accelerated phase/blast crisis	13/36 (36)	5/18 (28)
Acute myeloid leukemia/myelodysplastic syndrome	15/58 (26)	8/44 (18)
Acute lymphoblastic leukemia	3/20 (15)	2/11 (18)
Multiple myeloma	5/17 (29)	2/4 (50)
Non Hodgkin lymphoma	–	0/6 (0)

viewed the results of DLI in more than 300 patients with relapse [13, 36] (Table 7). DLI induced complete remissions in 64% of patients with chronic myeloid leukemia and in 20–40% of patients with multiple myeloma, acute myeloid leukemia, and myelodysplastic syndromes. In patients with chronic myeloid leukemia, response rates were highest when lymphocytes were infused in early cytogenetic relapse (79%) and lowest when used to treat accelerated phase or blast crisis (19%). It has been speculated that the relatively high responses of chronic myeloid leukemia to DLI might be due both to the slow evolution of the disease and the fact that dendritic cells, the most potent antigen-presenting cells, were part of the leukemic clone [36]. The GVT reactions might not have sufficient time to be effective in patients with more rapidly progressing diseases. Moreover, one study suggested that BCR/ABL expression might increase the susceptibility of leukemic cells to immune cytolysis [4].

One major complication of DLI has been GVHD [13, 36, 37]. Acute GVHD occurred in about 60% of the patients (grades 3–4 in about 20%) and was significantly correlated with achievement of complete responses. Chronic GVHD, occurring in about 60% of the patients (extensive in 30%), also correlated with responses. However, some complete responses were seen in the absence of GVHD, suggesting GVT responses were directed at targets other than those involved in GVHD. The risk of GVHD increased with increasing numbers of donor lymphocytes infused [37]. It was possible to reduce the GVHD risk without impairing the GVT effect by depleting $CD8^+$ lymphocytes from DLI [9] or by starting with lower doses of infused T cells and increasing the dose in a stepwise fashion in case of no response [15]. One other complication of DLI has been marrow aplasia that occurred in about 20% of the patients, most often when marrow cells were at least partially of recipient origin. Marrow aplasia resolved spontaneously in 50% of the patients, and marrow function was restored in the majority of the remaining patients by G-CSF and/or infusion of donor hematopoietic stem cells.

Responses to DLI were most often associated with conversion from a mixed donor/host chimeric state to complete donor hematopoiesis [13, 36, 37], suggesting that GVT reactions were directed against polymorphic minor histocompatibility antigens (mHA) expressed on both normal and leukemic marrow cells rather than against tumor-specific antigens.

Allogeneic HCT after reduced intensity or non-myeloablative conditioning

Due to regimen-related toxicities, the use of conventional ablative allogeneic HCT has been restricted to younger and medically fit patients. This was unfortunate since the median ages for patients with hematological malignancies such as acute and chronic leukemias, lymphomas, myelodysplastic syndromes and multiple myeloma ranged from 65 to 70 years [49]. In an attempt to extend the use of allogeneic HCT to older patients and those with comorbid conditions, several groups of investigators have carried out allogeneic HCT after reduced intensity conditioning regimens [23, 33, 63] or after truly non-myeloablative regimens [10, 11, 47, 68], in which the burden of tumor eradication was shifted toward GVT effects.

A number of reduced intensity or truly non-myeloablative conditioning regimens are shown in Table 3. In patients with slowly progressing diseases, such as chronic lymphocytic leukemia, low-grade non-Hodgkin lymphoma, chronic myeloid leukemia in first chronic phase or with more aggressive diseases in complete remission, a non-myeloablative regimen might be sufficient to achieve engraftment, and GVT effects might be able to cure malignant disease. However, prior reduction of tumor load might be required in patients with aggressive diseases, such as acute leukemias, multiple myeloma, high-grade lymphoma or Hodgkin disease who were not in complete remissions at the time of the transplant.

Reduced intensity conditioning regimens

Most reduced intensity conditioning regimens have combined purine analogs, such as fludarabine, cladribine or pentostatin and alkylating agents, usually cyclophosphamide, busulfan or melphalan. The rationale for using purine analogs was their ability to inhibit mixed lymphocyte reactions in vitro and to produce lymphopenia and substantial immunosuppression in vivo. In 1997, Giralt et al. [23] reported engraftment of HLA-identical related transplants after a reduced intensity conditioning regimen combining fludarabine 30 mg/m^2 per day for 4 days, cytarabine 1 g/m^2 per day for 4 days and idarubicin 12 mg/m^2 per day for 3 days. Postgrafting immunosuppression consisted of CSP plus steroids. Initial engraftment was greater than 90% and transplant-related mortality around 20%. Giralt et al. [24] subsequently reported a more intense regimen combining fludarabine (120–125 mg/m^2) and melphalan (140–180 mg/m^2) for patients with advanced leukemia, multiple myeloma or renal cell carcinoma. Postgrafting immunosuppression was carried out with FK506 + MTX. Non-relapse mortalities at 100 days and 1 year after the transplant were approximately 20% and 40%, respectively [24]. The Jerusalem group developed a protocol that combined fludarabine 30 mg/m^2 per day for 6 days, anti-thymocyte globulin (ATG) 5–10 mg/kg per day for 4 days, and oral busulfan at 4 mg/kg per day for 2 days. Postgrafting immunosuppression consisted of CSP alone. This regimen resulted in achievement of full donor chimerism in the majority of rather young patients and low transplant-related mortality [52]. Or et al. [52] reported results of HCT after this conditioning regimen in 24 patients with chronic myeloid leukemia in first chronic phase. Median patient age was 35 years (range 3–63 years). Nineteen patients received HCT from HLA-matched family members (16 siblings and 1 father) and 5 patients from HLA-matched unrelated donors. The 5-year probability of progression-free survival was 85% [95% confidence intervals (CI) 70–100%]. The United Kingdom group for non-myeloablative transplantation used another regimen which

combined Campath-1H (100 mg/m^2), melphalan (140 mg/m^2) and fludarabine (150 mg/m^2) [8, 38]. Postgrafting immunosuppression consisted of CSP. This regimen allowed engraftment with low incidences of GVHD and transplant-related mortality in both HLA-matched related and unrelated recipients. The Spanish group for non-myeloablative transplantation studied a similar combination of melphalan (140 mg/m^2) and fludarabine (150 mg/m^2), but without Campath-1H. Postgrafting immunosuppression consisted of MTX plus CSP. Retrospective comparison between the United Kingdom and the Spanish prospective studies in patients with lymphoproliferative disorders showed that Campath-1H significantly reduced the incidence of grade II–IV acute GVHD (from 49% to 9%, $P<0.001$) but was associated with a higher incidence of CMV reactivation. The 2-year probabilities of event-free survival were 34% in patients who received Campath-1H versus 39% in patients who did not [53] (Table 8).

Cyclophosphamide-based regimens

Studies in rats, dogs, and rhesus monkeys in 1969/70 showed that cyclophosphamide, although non-myeloablative, was a powerful immunosuppressive drug that enabled stable engraftment of transplanted allogeneic hematopoietic cells. In 1971, Santos et al. [59] reported that conditioning with cyclophosphamide alone allowed engraftment in patients with advanced leukemia, although tumor cells were not completely eradicated and disease recurred in all patients. While cyclophosphamide was abandoned as the sole conditioning regimen in patients with hematological malignancies, it has remained the conditioning regimen of choice for patients with aplastic anemia (reviewed in [76]).

More recently, Childs et al. [10, 11] combined cyclophosphamide (120 mg/kg) and fludarabine (125 mg/m^2). Postgrafting immunosuppression consisted of CSP. The patterns of engraftment varied considerably among their patients but most often full donor chimerism was achieved earlier among T cells than among granulocytes, and the achievement of full donor T cell chimerism preceded GVHD and anti-tumor responses. Transplant-related mortality was 12% at 1 year in metastatic renal cell carcinoma patients. Spitzer et al. [68] showed that mixed chimerism could be achieved after a regimen of cyclophosphamide (50 mg/kg per day for 3–4 days), thymic irradiation (700 cGy in patients who had not previously received mediastinal radiation therapy) and ATG (30 mg/kg per day for 3 days). However, approximately 30% of the patients subsequently rejected their transplants.

Non-myeloablative conditioning regimen with 200 cGy TBI with or without fludarabine

The following paragraphs review the non-myeloablative approach that was developed first in a preclinical canine model in Seattle [75] and then directly translated into multi-center clinical studies [45, 47, 51].

Preclinical canine studies

Two immunological barriers must be overcome in allogeneic HCT. One is the rejection barrier or host-versus-graft (HVG) reaction and the other is the GVH reaction. In the major histocompatibility complex-identical setting, both HVG and GVH reactions are medi-

Table 8 Examples of reduced intensity or non-myeloablative conditioning regimens (*pts* patients, *NRM* non-relapse mortality, *2-CDA* cladribine, *ATG* anti-thymocyte globulin, *CSP* cyclosporine, *FK506* tacrolimus, *MTX* methotrexate, *BM* bone marrow, *OS* overall survival, *DFS* disease-free survival, *PFS* progression-free survival, *NR* not reported)

Center	Preparative regimens	Postgraft immunosuppression	No. of patients (median age in years)	Diseases	GVHD		NRM (days after transplant)	Outcome
					Acute (grade 2–4)	Chronic		
Anderson [24]	Fludarabine 25 mg/m^2 per day (or 2-CDA 12 mg/m^2) for 5 days Melphalan 140–180 mg/m^2	FK506 + MTX	86 (52)	Hematological malignancies.	49%	68%	37% (at 100 days)	–730-day OS: 28% –730-day DFS: 23%
United Kingdom [38]	Fludarabine 30 mg/m^2 per day for 5 days Melphalan 140 mg/m^2 CAMPATH-1H 20 mg/day for 5 days	CSP +/– MTX	44 (41)	Hematological malignancies. 19 pts had a previous failed transplant.	3/44. 1 after DLI	NR	11% (at 365 days)	–360-day OS: 73% –360-day: 71%
Jerusalem [52]	Fludarabine 30 mg/m^2 per day for 6 days Busulfan (p.o.) 4 mg/kg per day for 2 days ATG 5–10 mg/kg per day for 4 days	CSP +/– MTX	24 (35)	Chronic myeloid leukemia in first chronic phase.	75%[a]	55%	3 pts (days 116, 499 and 726)	–1725-day DFS 85%
Anderson [34]	Fludarabine 25 mg/m^2 per day (or 2-CDA 12 mg/m^2 per day) for 5 days or Fludarabine 30 mg/m^2 per day for 3 days Cyclophosphamide 1 g/m^2 per day for 2 days or 750 mg/m^2 per day for 3 days +/– Rituximab	FK506 + MTX	20 (51)	Lymphomas.	20%	64%	2 (at day 45 and before 300 days)	–730-day DFS: 84%
National Institutes of Health [11]	Fludarabine 25 mg/m^2 per day for 5 days Cyclophosphamide 60 mg/kg per day for 2 days	CSP	15 (50)	Hematological + solid malignancies.	10/15 pts. 1 after DLI	NR	2 pts (days 59 and 205)	–8/15 pts survived between 121 and 409 (median, 200) days
Boston [68]	Cyclophosphamide 50 mg/kg per day for 3–4 days ATG 30 mg/kg per day x 3 days or 15 mg/kg per day for 4 days Thymic irradiation 700 cGy[b]	CSP	21 (44)	Hematological malignancies.	12 pts. 6 after DLI.	NR	2 pts (days 77 and 180)	At a median follow-up of 445 days: –11 pts were surviving –7 pts were surviving free of progression
Seattle [58]	TBI 200 cGy +/– fludarabine 30 mg/m^2 per day for 3 days	CSP + MMF	453 (55)	Hematological malignancies.	48%	44%[c]	22% (at 730 days)	–730-day OS: 51% –730-day PFS: 37%

[a] Grade 1–4
[b] In patients who have not received previous mediastinal irradiation
[c] Extensive chronic GVHD

ated by T cells. Therefore, it was hypothesized that optimizing postgrafting immunosuppression could reduce not only GVH but also HVG reactions, thereby enabling a decrease in the intensity of the pre-transplant conditioning regimen needed for sustained engraftment.

A dose-response relationship between TBI dose and allogeneic marrow engraftment has been demonstrated. A single TBI dose of 920 cGy was sufficiently immunosuppressive to allow engraftment of DLA-identical littermate marrow in 95% of dogs not given postgrafting immunosuppression [74]. When the dose was successively decreased to 450 cGy, only 41% of dogs had stable engraftment [74, 89]. However, the addition of postgrafting CSP for 5 weeks, led to stable engraftment in all dogs conditioned with 450 cGy TBI [89]. Further reduction of the TBI dose to 200 cGy resulted in transient engraftment followed by autologous recovery despite postgrafting CSP [75]. The addition of a short course of methotrexate to CSP led to sustained donor engraftment in two of five dogs [75], while a regimen combining MMF and CSP led to stable engraftment in 10 of 11 dogs studied [75]. However, when the TBI dose was further decreased to 100 cGy, stable long-term engraftment did not occur, demonstrating a delicate balance between host and donor cells [75].

Clinical trials

The clinical trials were carried out jointly by a group of collaborators located at the Fred Hutchinson Cancer Research Center, University of Washington, Children's Hospital and Regional Medical Center, and Veterans Administration Medical Center (all in Seattle, WA); Stanford University (Palo Alto, CA); City of Hope National Medical Center (Duarte, CA); University of Leipzig (Leipzig, Germany); University of Colorado (Denver, CO); University of Torino (Torino, Italy); University of Arizona (Tucson, AZ); Baylor University (Dallas, TX); University of Utah (Salt Lake City, UT); Oregon Health Sciences University (Portland, OR); and, more recently, the Medical College of Wisconsin (Milwaukee, WI); and Emory University (Atlanta, GA). Transplantation candidates have been exclusively patients with hematological diseases treatable by allogeneic HCT who were ineligible for conventional allogeneic HCT because of age and/or concomitant diseases or preceding extensive therapies, such as failed autologous or allogeneic HCT.

The initial transplant regimen was the same as that developed in dogs and consisted of 200 cGy TBI given on day 0, with postgrafting MMF, 15 mg/kg orally given twice a day from day 0 to day 27, and CSP, 6.25 mg/kg orally given twice a day from day −1 to day 35 [47]. The stem cell source was G-PBMC, and donors were HLA-identical siblings. The transplant regimen was remarkably well tolerated, with the majority of eligible patients having their transplants in the outpatient setting. Typical side effects of conventional myeloablative HCT including new-onset alopecia, diarrhea, veno-occlusive disease of the liver, and mucositis were absent. Nine of the 44 first patients (20%) given this regimen had nonfatal graft rejections [47]. To reduce the risk of graft rejection, fludarabine 30 mg/m^2 per day for 3 days was added to the 200 cGy TBI, and the rejection rate decreased to 3%. In addition, the CSP administration was gradually extended through days 56–180, depending on the aggressiveness of the patients' underlying diseases.

Next a multi-institutional study was initiated using 10/10 HLA-matched unrelated donors. The conditioning regimen included 200 cGy TBI preceded by fludarabine 30 mg/m^2 per day for 3 days [45]. The postgrafting immunosuppression with MMF was extended to

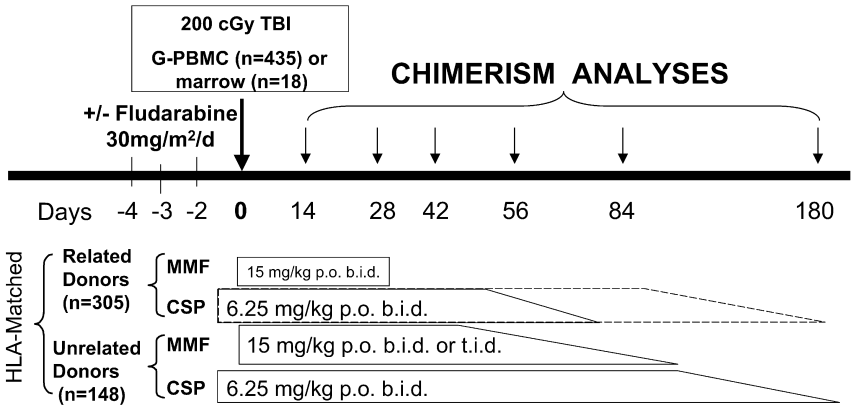

Fig. 2 Non-myeloablative HCT regimen for patients with malignancies given grafts from HLA-matched related and unrelated donors (*MMF* mycophenolate mofetil, *CSP* cyclosporine, *G-PBMC* granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells

40 days with taper to day 96 and CSP was given for 100 days with taper through day 180. Durable engraftment was observed in 85% of G-PBMC (*n*=71) and 56% of marrow recipients (*n*=18). Based on this observation, all subsequent unrelated recipients were given G-PBMC grafts.

Results in the first 453 patients

Results of the first 453 patients transplanted for hematological malignancies were recently reviewed [58]. HCT regimens are illustrated in Fig. 2. Median patient age was 55 years (range 5–74 years), and median follow-up was 696 days (range 82–1,795 days). Three hundred five (67%) patients received grafts from HLA-matched related donors and 148 (33%) grafts from HLA-matched unrelated donors. Stem cell sources were marrow in 4% (all from unrelated donors) and G-PBMC in 96% of patients. Diagnoses included multiple myeloma (*n*=116), myelodysplastic syndromes or myeloproliferative disorders (*n*=82), non-Hodgkin lymphomas (*n*=79), acute myeloid leukemia (*n*=59), chronic lymphocytic leukemia (*n*=44), chronic myeloid leukemia (*n*=37), Hodgkin disease (*n*=26) and acute lymphoblastic leukemia (*n*=10). Sixty-six percent of patients with unrelated donors and 38% of those with related donors had aggressive and advanced stage diseases at transplantation. Graft rejection occurred in 5% of patients with related donors, mostly among those conditioned with 200 cGy TBI alone, and in 16% of patients with unrelated donors. Thirty-four percent of patients had grade II, 10% grade III and 4% grade IV acute GVHD, and 44% of patients had chronic GVHD requiring therapy. Three hundred thirty two patients had measurable disease at HCT, and 56.5% of them achieved complete (49%) or partial (7.5%) remissions after HCT. Typically, remissions occurred over extended periods of time, and some patients achieved complete remissions beyond 1 year after HCT (Table 9).

Table 9 Results of allogeneic HCT after a non-myeloablative regimen consisting of 200 cGy TBI with or without fludarabine (30 mg/m^2 per day for 3 days) in 453 patients transplanted for hematologic malignancies [58] (*MRD* HLA-matched related donor, *URD* HLA-matched unrelated donor, *NRM* nonrelapse mortality)

Patients	% of patients with acute GVHD			% of patients with chronic GVHD	% of mortality (2 year Kaplan-Meier estimates)					% of survival (2 year Kaplan-Meier estimates)	
	II	III	IV		Relapse/ progression	NRM				Overall	Progression free
						Overall	GVHD ± Infection	Infection	Other		
Total (*n*=453)	34	10	4	44	26	22	11.2	6.7	4.1	51	37
MRD (*n*=305)	31	10	5	43	23	22	12.5	6.5	3.0	54	40
URD (*n*=148)	42	9	3	45	32	22	8.5	7.1	6.4	45	31

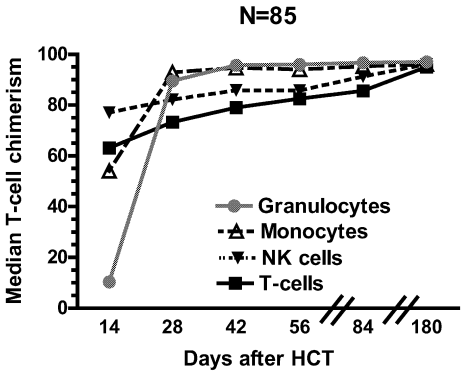

Fig. 3 Kinetics of engraftment after HCT in 85 patients conditioned with 200 cGy TBI with or without fludarabine (30 mg/m^2 for 3 days) and given grafts from HLA-matched related donors

Engraftment kinetics

We analyzed the kinetics of donor engraftment in peripheral blood cell subpopulations from 120 patients [40]. While most patients rapidly developed high degrees of donor engraftment, they remained mixed donor/host chimeras for up to 180 days after HCT (Fig. 3). Generally, donor T cell chimerism lagged behind myeloid chimerism. A history of chemotherapy before HCT and G-PBMC as a stem cell source were associated with higher degrees of donor chimerism. Having less than 50% donor T cell and natural killer (NK) cell chimerisms on day 14 were associated with an increased risk of graft rejection. Conversely, more than 90% donor T cell chimerism on day 28 after HCT was associated with an increased risk of acute GVHD. Of interest, there was a positive association between the level of NK cell-donor chimerism and improved progression-free survival. These data suggested that monitoring mixed chimerism early after HCT might help predict outcomes and/or allow early interventions to prevent graft rejection, GVHD or disease progression.

Comparing HCT after myeloablative and non-myeloablative conditioning

A number of retrospective studies have compared outcomes after myeloablative and non-myeloablative conditioning. The hematological changes after non-myeloablative were much milder than seen after myeloablative conditioning (Fig. 4). Seventy-seven percent and 37% of the ablative recipients versus 0% and 4% of the nonablative recipients, respectively, required platelet and red blood cell transfusions [87]. Liver and lung toxicities were significantly reduced with nonablative conditioning. The cumulative incidence of bilirubin >4 mg/100 dL was 48% at 100 days in 1,419 consecutive ablative recipients versus 26% at 200 days in 193 consecutive nonablative recipients [27]. The 120-day cumulative incidence of idiopathic pneumonia syndrome was 8.4% in 917 ablative recipients versus 2.2% in 183 nonablative recipients [21]. There were significantly fewer bacterial infections in nonablative recipients, and the incidence of CMV-disease was significantly lower during the first 100 days [31, 32]. However, the 1 year probabilities of CMV disease for CMV-seropositive recipients were similar among ablative and nonablative recipients, stressing the importance of CMV monitoring for the 1st year after HCT.

Mielcarek et al. [48] compared GVHD in 52 ablative recipients to that among 44 nonablative recipients. Recipients in both groups were age matched, with median ages of

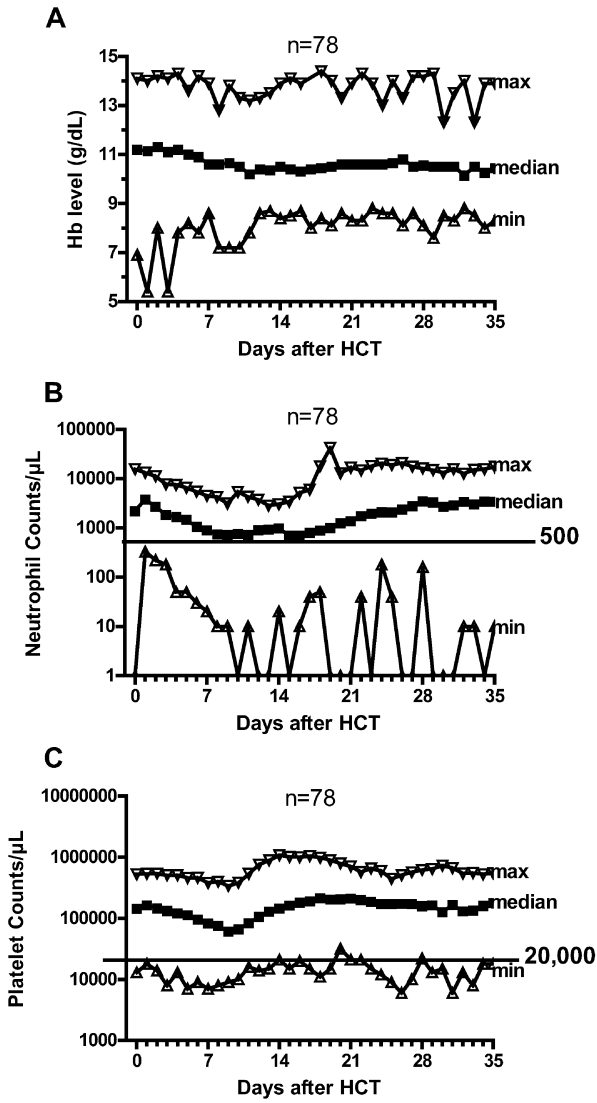

Fig. 4 Hematological changes in 78 patients with hematological malignancies conditioned with 200 cGy TBI with or without fludarabine (30 mg/m^2 for 3 days) and given HLA-matched related or unrelated grafts

54 years in the ablative and 56 years in the nonablative groups. Grafts were from either related or unrelated donors who were serologically matched for HLA-A,-B and -C and allele-level matched for HLA-DRB1 and -DQB1. Postgrafting immunosuppression was MTX plus CSP (n=48) or MMF plus CSP (n=4) for ablative patients and MMF plus CSP for all nonablative patients. The cumulative incidences of grade II-IV acute GVHD were 85% in ablative recipients versus 64% in nonablative recipients (p=0.001), but there were no differences in the cumulative incidences of extensive chronic GVHD (71% versus 73%, respectively). The median times to initiation of corticosteroids were 0.9 months in the ablative group versus 3.0 months in the nonablative group (P<0.001), and fewer systemic immunosuppressants were used in nonablative recipients the first 3 months after

Table 10 Definition of Life-threatening GVHD [19]

1. Death related to GVHD or its treatment (if the cause of death was while on prednisone >1 mg/kg per day, this is considered death related to GVHD treatment).
2. Severe disability due to GVHD
   a) Respiratory insufficiency (secondary bronchiolitis obliterans or bronchiolitis obliterans organizing pneumonia and need for supplemental oxygen >7 days)
   b) Hospitalization for control of GVHD >60 days
3. More than 3 major infections in a calendar year (sepsis, pneumonia, meningitis, encephalitis) while on systemic immunosuppression
4. Suicidal gesture or hospitalization because of suicidal threat secondary to morbidity of GVHD or its treatment

HCT (P=0.04). One-year survival was 50% in the ablative group versus 68% in the nonablative group (P=0.04). In addition, risk factors for life-threatening GVHD (as defined in Table 10) were analyzed among 171 consecutive patients who received related or unrelated grafts after nonablative conditioning [19]. Overall, 26% of patients developed life-threatening GVHD, the incidence of which was similar among related and unrelated recipients. In a Cox regression analysis, factors that were significantly associated with life-threatening GVHD were: diagnosis of myeloid malignancies (P=0.01), DLI for persistent, progressive or recurrent malignancies (P=0.05) and, preceding non-severe acute GVHD (P=0.002).

Maris et al. [44] compared immune reconstitution after ablative and nonablative regimens. During the first 6 months, absolute counts of lymphocyte subsets were similar in both groups but counts of CMV-specific T-helper lymphocytes were higher at days 30 and 90 in the nonablative group. Ablative patients had higher naive CD4 and CD8 counts than nonablative patients at 1 year after HCT probably reflecting lower counts of recent thymic emigrants; this finding might be related to older age and/or later onset of GVHD (and steroid use) among nonablative recipients.

Sorror et al. [66] analyzed transplantation-related toxicities following HLA-matched unrelated HCT in 134 concurrent patients given either ablative (n=74) or nonablative (n=60) conditioning using the National Cancer Institute common toxicity criteria grading. Additionally, the effects of pre-transplant comorbidities, graded according to the Charlson Comorbidity Index (CCI) score, on outcome were investigated. Even though nonablative recipients were older, had more often advanced disease, had more extensive prior therapies including failed ablative HCT, and had higher CCI scores at HCT, they experienced significantly less gastrointestinal, hepatic and hemorrhagic grade III–IV toxicities compared to patients concurrently transplanted with a myeloablative conditioning. Estimated 100-day and 1-year non-relapse mortalities were 18% and 32% in ablative recipients compared to 12% and 20% in nonablative recipients, respectively. After adjusting for differences in age, CCI scores, number of prior therapies, and prior ablative HCT, ablative recipients had higher odds ratios for overall grade IV toxicities (9.4, P=0.0001), day-100 non-relapse mortality (3.6, P=0.07) and 1-year non-relapse mortality (3.0, P=0.04). Multivariate analysis showed higher pre-transplant CCI scores to result in both increased toxicity and mortality after HCT.

Fungal infections

Fukuda et al. [20] analyzed risks and outcomes of invasive fungal infections in 163 HCT nonablative recipients. The 1-year cumulative incidences of proven or probable invasive fungal infections, invasive mold infections, invasive aspergillosis and invasive candidiasis were 19%, 15%, 14% and 5%, respectively. Invasive mold infections occurred a median of 107 days (range 4–282 days) after HCT. In multivariate analysis, risk factors for mold infection were grades III–IV acute GVHD (HR=2.8, P=0.04), chronic extensive GVHD (HR=3.7, P=0.04) and CMV disease (HR=13.3, P<0.001). Eighty-four percent of mold infections occurred after the onset of acute GVHD. Mold infections contributed to 9% overall mortality. Outcomes after diagnosis of mold infection were dependent on GVHD treatment requirements. The 1-year survivals for patients with invasive mold infections whose corticosteroids doses were <2 mg/kg per day compared to ≥2 mg/kg per day were 44% versus 11%, respectively.

Results in specific diseases

Feinstein et al. [18] reported outcomes in 18 patients with de novo (n=13) or secondary (n=5) acute myeloid leukemia in first complete remission who received HCT from HLA-identical sibling donors. Median patient age was 59 years (range 36–73 years). Conditioning consisted of 200 cGy TBI alone (n=10) or combined with fludarabine (n=8). Two rejections were observed in patients not given fludarabine, and 1 of the 2 died with relapse. At a median follow-up of 766 days, 7 patients have remained in complete remission. The 1-year probabilities of transplant-related mortality, overall survival and progression-free survival were 17% (95% CI 0–35%), 54% (95% CI 31–78%) and 42% (95% CI 19–66%), respectively.

Sorror et al. [67] described outcomes in 47 patients with chronic lymphocytic leukemia who received HCT from HLA-matched related (n=33) or unrelated (n=14) donors after conditioning with 200 cGy TBI alone (n=19) or combined with fludarabine (n=28). Median patient age was 58 years (range 47–68 years). The median number of prior regimens was 4 (range 1–12). Ninety-four percent of patients were refractory to at least 1 regimen, including 77% who were refractory to fludarabine. Three patients experienced graft rejection. One of the 3 died of aplasia and the 2 others are surviving with autologous marrow recovery and progressive disease. With a median follow up of 32 months (range 6–69 months), overall response and complete response rates were 57% and 43%, respectively. Seven patients died from progressive disease, 9 from infections with or without GVHD, 2 from cardiac problems, 1 from multiorgan failure, 1 from metastatic lung cancer, and 1 from rejection and aplasia. Estimated 2-year rates of overall and progression free survivals were 56%, and 49%, respectively.

Maloney et al. [43] summarized results in 54 multiple myeloma patients who first received cytoreductive autologous HCT after 200 mg/m^2 melphalan followed by allogeneic HCT after 200 cGy TBI. Patients were 29–71 years (median 52 years) old and had previously treated stage II or III multiple myeloma. Forty-eight percent had refractory (35%) or relapsed (13%) disease. Allografts were performed 40–229 days (median 62 days) after the autografts. The 100-day mortalities after autologous and allogeneic HCT were 2% and 2%, respectively. With a median follow-up of 552 days after allografting, 57% of patients achieved complete remissions and 26% partial remissions. Of the 28 patients with respon-

sive disease entering the trial (complete response or partial response), 3 have died and 2 have had disease progression. In contrast, of the 26 patients with relapsed or refractory disease at study entry, 9 have died, either from complications related to GVHD (n=4), disease progression (n=3), pulmonary failure (n=2), lung cancer (n=1), CMV infection (n=1) or encephalopathy (n=1). The estimated 2-year overall and progression-free survivals were 78% and 55%, respectively.

Summary

Allogeneic HCT has changed from a desperate therapeutic maneuver plagued by apparently insurmountable complications to a curative treatment modality for many patients with malignant hematological diseases. Now, cure rates following allogeneic HCT with HLA-matched siblings exceed 85% for diseases such as chronic myeloid leukemia. In addition, the recent development of HCT after non-myeloablative conditioning regimens has opened the way to include elderly patients and those with comorbid conditions with a wide variety of hematological malignancies. Remarkably, a minimally toxic regimen of 200 cGy TBI with or without fludarabine followed by postgrafting immunosuppression with MMF and CSP assured engraftment rates similar to those after myeloablative conditioning. Further development of immunosuppressive drugs might result in better control of HVG reactions. Better understanding of tissue-specific polymorphic minor histocompatibility antigens might result in the development of vaccines that can be used to direct the donor cytotoxic T cells toward tumor targets rather than inducing general GVHD. Combination of non-myeloablative regimen with disease-targeted therapy such as Imatinib, Rituximab or radiolabeled monoclonal antibodies, are likely to make the transplant procedure more effective.

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