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Low intensity regimens with allogeneic hematopoietic stem cell transplantation as treatment of hematologic neoplasia

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ABSTRACT

Conventional myeloablative conditioning regimens for allografting rely on the use of toxic myeloablative and immunosuppressive therapies to achieve engraftment and control of hematologic neoplasias. Unfortunately, these regimens have resulted in substantial morbidity and mortality. Preclinical and pilot clinical studies have shown that conditioning regimens can be reduced in intensity (resulting in reduced morbidity and mortality) since stem cell allografts can create their own space in the host's bone marrow. Initial promising results with these attenuated conditioning regimens confirm that such an approach is feasible in patients with hematologic neoplasias and genetic diseases ineligible for conventional allografting because of age and / or organ toxicity. The combination of high-dose therapy / autografting followed by a low intensity conditioning regimen (Flu-Cy protocol) and donor mobilized hematopoietic stem cell infusion (mini-allografting) may ultimately be useful in advanced resistant hematologic neoplasia. Finally, these initial promising results with attenuated conditioning regimens have been achieved in transplants with HLA-identical siblings. In the future the main goal will be to explore non-toxic conditioning regimens in the context of transplants from related MHC-mismatched or unrelated MHC-matched donors by increasing the patient's immunosuppression.

Key words: low intensity regimens, chimerism, hematologic neoplasias

Traditionally, allografting has relied on a combination of myeloablative and immunosuppressive therapies, which results in substantial morbidity and mortality. These regimens must be restricted to young patients who are in a good medical condition. To circumvent the problems inherent to the toxicity and treatment-related deaths associated with allografting, the possibility of achieving engraftment of donor hematopoietic stem cells (HSC) after low intensity conditioning regimens has recently been assessed.[1,2] Of course, these regimens cannot solve the problems arising from post-transplant relapses. The basic observation which serves as the rationale for non-myeloablative stem cell transplantation originates from the documented therapeutic potential of adoptive transfer of alloreactive donor lymphocytes to eradicate resistant malignant host cells escaping maximally tolerated doses of chemoradiotherapy, an observation that has provided an option for cure of patients with a large variety of hematologic malignancies,[3-5] especially chronic myeloid leukemia (CML).[4-11] The observation that these less intensive regimens based on fludarabine have resulted in engraftment of allogeneic cells in hematologic malignancies raises the possibility that such conditioning might even be useful in achieving a graft-versus-tumor effect.[1,2] Besides, the first preliminary data seem to demonstrate that these low intensity regimens can decrease severe acute graft-versus-host disease (GvHD).[1,12,13,25] In this review, we will discuss the general concepts and the preliminary results achieved in clinical studies.

The general concepts

It is well recognized that the graft-versus-leukemia (GvL) effect is important in preventing relapse after allografting. The concept of a GvL effect is supported by the observation that patients with chronic phase CML who relapse after allografting can achieve a complete cytogenetic and molecular remission following donor lymphocyte infusion (DLI).[4-11,14] Among the major leukemias treated with allografting, AML is more affected by GvL than ALL and indolent lymphoid malignancies also appear to be susceptible to graft-versus-malignancy effects.[15-17] Pilot studies in solid tumors have reported antitumor responses in patients with GvHD suggesting a graft-versus-adenocarcinoma effect.[18-20] Moreover, better GvL effects may be obtained by amplification of the alloreactive capacity of donor lymphocytes by *in vivo* administration of recombinant human interleukin-2 (rIL-2).[5] As recently stated, this occurs because, after relapse, leukemia recurs in host-derived cells but residual normal hematopoiesis and immunity remain largely donor-derived.[21] The infused donor lymphocytes are

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therefore not subject to rejection, but acute GvHD develops in up to 80% of cases. After DLI, responding patients may suddenly become hypoplastic followed by recovery from donor-derived HSC and return to complete chimerism.[4,5,8] This long interval between DLI and clinical response is presumably related to the time required for the proliferating relevant alloreactive cells[22] to reach a critical mass, sufficient to eradicate the malignant cells. Considering that older and resistant patients represent the group with the worst prognosis following conventional myeloablative therapy, novel therapeutic options need to be explored. The success of DLI in inducing remission in patients with CML who relapse post-transplantation suggests that alternative approaches must be explored. A new perspective could derive from a combination of an immunosuppressive non-myeloablative regimen followed by allogeneic HSC transplantation to achieve engraftment. Therefore, DLI could be given to induce complete remissions in patients with resistant disease who do not develop GvHD. All these patients could receive high intensity regimens followed by autologous stem cells rescue before in order to reduce the neoplastic burden.[23] Fludarabine and 2CDA are the crucial drugs in these less intensive conditioning regimens. In particular, fludarabine is an antileukemic and effective immunosuppressive agent, as recently established at the MD Anderson Cancer Center.[24] It has been demonstrated that non-myeloablative chemotherapy using fludarabine combinations are immunosuppressive enough to allow engraftment of allogeneic blood progenitor cells.[25] This has led several groups of investigators to develop less toxic allotransplantation regimens that rely on a graft-versus-tumor effect rather than chemoradiation therapy for complete eradication of malignant cells. These non-myeloablative approaches can be divided roughly into three categories: 1) moderate immunosuppression pre- and post-transplant; 2) pre-transplantation host immunosuppression combined with post-transplantation immunosuppression directed at host and donor immune cells; 3) high-dose therapy and autologous stem cell transplantation followed by moderate immunosuppression and allogeneic stem cell transplantation. In all these settings, allotransplantation is the platform for subsequent adoptive immunotherapy of the underlying malignancies using donor lymphocytes.

Moderate immunosuppression pre- and post-transplant

Focusing on myelotoxic therapy

Investigators in Houston used fludarabine-containing regimens in high-risk patients with hematologic malignancies.[1]

a. FLAG-Ida and 2 CDA-AraC conditioning regimens. Results obtained in 25 patients were recently updated (22 AML, 3 MDS). The median age of the group was 61 years (range, 24-74). Most patients were refractory or beyond first salvage therapy, with only 6 patients receiving transplants in first remission, or untreated first relapse. The median number of prior therapies was 2 (range, 1-5), and the median percentage of bone marrow blasts was 10%. Fourteen patients received fludarabine, idarubicin and cytarabine, while 11 patients received a combination of chloroadenosine (2CDA) and cytarabine (Ara-C) in the doses and schedules presented in Figure 1. Other characteristics of the patients and their treatment are summarized in Table 1. There was only 1 regimen-related death (from pulmonary hemorrhage and multiorgan failure) among the 25 patients. Twenty patients recovered neutrophil counts of > 0.5×10^9/L at a median of 11 days post-transplantation (range, 9-21) and 17 achieved platelet transfusion independence at a median of 15 days (range, 8-78). Fifteen patients achieved complete remission after transplantation (defined as < 5% bone marrow blasts, with neutrophil and platelet recovery). Chimerism analysis in patients achieving a complete remission revealed that on day 30, 12 of the 15 patients had >80% donor cells as detected by either cytogenetics or restriction fragment polymorphism techniques. At 90 days, of the 10 patients remaining in CR, 6 had >80% donor cells. At 1 year, of the 3 patients in CR, 2 had >80% donor cells. Nine patients failed to respond to therapy and one patient died of graft failure. Five patients developed ≥ grade 2 GvHD, with 2 having grades 3-4 and 1 dying from this complication. The overall survival at 1 year for these very poor risk patients was 28%. No significant differences were seen in survival between patients receiving either the fludarabine or the 2CDA regimen (Figure 2) but, as one would expect, patients without peripheral blood blasts or those with <10% bone marrow blasts did significantly better (Figure 3). Nine patients with chronic myelogenous leukemia also received a non-myeloablative regimen with fludarabine. The characteristics of these patients and their treatment are summarized in Table 2. All patients had neutrophil and platelet recovery, and only one patient died from transplant-related complications (grade 4 GvHD). The 7 recipients of cells from related donors showed evidence of donor cell engraftment at the time of initial engraftment, while the 2 patients who received cells from unrelated donors

Figure 1. Dose and treatment schedules for FLAGIda, 2CDA / Ara-C and Flu-Cy protocols.

	D-5	*D-4*	*D-3*	*D-2*	*D-1*	*D-0*
FLAG-Ida						
Fludarabine mg/m²	30	30	30	30		
Ara-C gm/m²	2	2	2	2		
Idarubicin mg/m²	12	12	12			
2CDA/Ara-C						
2CDA mg/m² CI	12	12	12	12	12	
Ara-C gm/m²	1	1	1	1	1	
Flu-Cy						
Fludarabine mg/m²		30	30	30		
Cyclophosphamide mg/m²		300	300	300		

Table 1. Patient and treatment characteristics of MD Anderson Cancer Center patients with AML/MDS receiving FLAG-Ida or 2CDA/Ara-C.

No.	25	
Age (years)	61	(27-74)
Time to transplant (days)	361	(77-1807)
Diagnosis		
AML	22	
MDS	3	
Stage at BMT		
CR1 or Untreated Rel 1	6	
>Untreated Rel 1 or Ref	19	
# Prior Rx	2	(1-5)
% BM Blast	10	(1-95)
% PB Blast	4	(0-98)
Preparative regimen		
FLAG-Ida	14	
2CDA/AraC	11	
GvHD prophylaxis		
CSA/MP	13	
FK/MTX	11	
None	1	
Donor type		
5/6 sib	6	
6/6 sib	18	
Syngeneic	1	
Stem cell source		
BM	23	
PBSC	2	

Table 2. Patient and treatment characteristics of CML patients receiving FLAG-Ida and allogeneic progenitor cell transplantation.

N	9	
Age (years)	55	(42-67)
Time to transplant (days)	866	(400-1249)
Stage at BMT		
Late CP	5	
Transformed Phase	4	
Prior IFN	9/9	
Donor type		
6/6 Sibling	7	
6/6 MUD	2	
Stem cell source		
BM/PB	3/6	
GvHD prophylaxis		
CSA/CSA+MP	2/4	
FK/MTX	3	

had autologous reconstitution without ever demonstrating donor cell engraftment. All 4 patients transplanted in advanced phase relapsed and have failed to respond to salvage therapy with

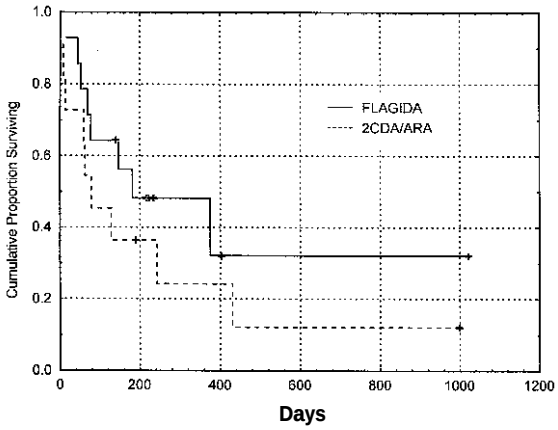
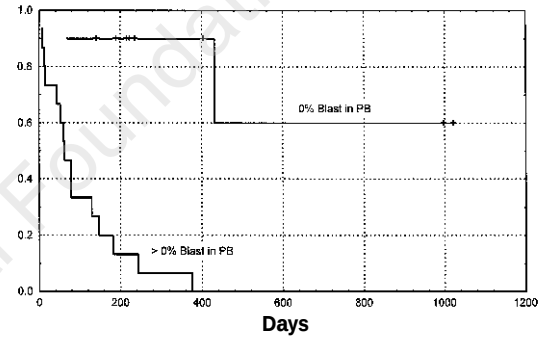


Figure 2. Survival according to preparative regimen.



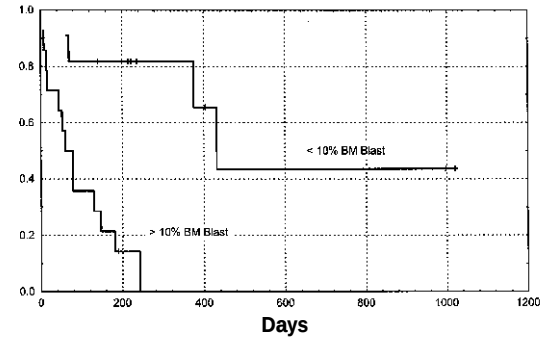


Figure 3. Survival according to tumor burden at time of transplant.

immunosuppression withdrawal, interferon, donor lymphocyte infusions, or second transplantation. Two of the 4 patients in chronic phase who had evidence of donor cell engraftment are alive in complete cytogenetic remission at 12 and 18 months post-transplantation, one with and one without donor lymphocyte infusions. This initial experience with FLAG-Ida and 2 CDA/Ara-C in patients with myeloid malignancies revealed that engraftment of

Table 3. Patient and treatment characteristics of patients receiving melphalan/purine analog combinations.

N	86
Age (years)	52 (22-70)
Sex	52 M/34 F
Diagnosis	
AML/MDS	34/9
CML	27
ALL/lymphoma	3/13
Status at BMT	
Early leukemia (CR1/CP1)	1/6
Untreated relapse 1	10
≥CR2	6
Transformed	21
Refractory or relapse> 1	42
# Prior therapies	3 (0-8)
Prior BMT	24
Comorbid conditions	
Age > 50	48
Muga < 50%	5
PFT's < 50%	4
GPT > 120	11
Prior BMT/ or >3 Rx	24/21
PS=2/infection	14/10
Time to transplant	547 days (27-6626)
Donor type	
6/6 related	39
5/6 related	7
6/6 MUD	40
Stem cell source	
BM	52
PB	34
CMV status	
(Neg/Neg)	6
Preparative regimens	
FM 180	66
FM 140	12
2CDA/M 180	8
GvHD prophylaxis	
CSA/MP	3
FK/MP	2
FK/MTX	81

allogeneic progenitor cells could be achieved with non-myeloablative purine analog containing regimens; however, long-term disease control was achieved only in patients with a relatively low tumor burden who were transplanted early in the course of their disease. Likewise, these regimens may not be sufficiently immunosuppressive to allow engraftment of unrelated donor cells in the setting of CML. Therefore other novel non-myeloablative regimens needed to be developed.

b. Fludarabine-melphalan conditioning regimens.
The combination of melphalan with purine analogs has been used at M.D. Anderson Cancer Center as a more intense, but still non-myeloablative regimen for patients considered poor candidates for conventional myeloablative regimens. Eighty-six patients with a variety of hematologic malignancies received one of three melphalan/purine analog containing preparative regimens. Patient and treatment characteristics are summarized in Table 3. Seventy-seven patients had neutrophil recovery at a median of 14 days (range, 9-35), 55 patients recovered platelet transfusion independence at a median of 21 days (range, 9-118). All engrafting patients except 1 had documentation of >80% donor cell engraftment by day 30, with one instance of autologous reconstitution and 1 case of secondary graft failure. In this group of very poor prognosis patients ineligible for conventional transplant the 100 day transplant-related mortality (TRM) was 42% (36/86) with 4/8 patients in the 2CDA/melphalan arm dying from multiorgan failure, leading to this treatment arm being closed. The overall survival for all patients was 29% at 2 years and disease-free survival was 23%. Patients transplanted in CR1/CP1 or untreated first relapse (good and intermediate risk groups) did significantly better than patients with more advanced disease, with no difference being seen between recipients of related or unrelated donor cells (Figure 4). Therefore fludarabine/melphalan combinations can allow engraftment of allogeneic progenitor cells, including those obtained from matched unrelated donors. This strategy can produce long-term disease control in patients with hematologic malignancies transplanted early in the course of their disease with acceptable risk and toxicity in patients ineligible for conventional myeloablative transplant therapies. Treatment-related mortality from GvHD and disease recurrences remain the most common causes of treatment failure in this patient population.

Results of mini-transplants for lymphoid malignancies

The use of allogeneic transplantation is limited in patients with lymphoid malignancies such as chronic lymphocytic leukemia or lymphomas because they typically affect older patients. Khouri *et al.* at the M.D. Anderson Cancer Center have evaluated the induction of a graft-versus-lymphoma effect as primary therapy for patients with lymphoid malignancies who are considered poor candidates for conventional transplant techniques.[25] Nine patients, of whom eight were over 50 years, were treated. All patients, 5 with advanced CLL and 4 with transformed lymphoma, received one of two preparative regimens (fludarabine/cytoxan or fludarabine/Ara-C/platinum). Mixed chimerism was observed in 6 of 9 patients with a percentage of donor cells ranging from 50% to 100% one month post-transplantation. No regimen-related deaths were observed, and 4 patients achieved complete remission, one after donor lymphocyte infusions.

Focusing on immunosuppressive therapy pre-transplantation

Investigators at the Hadassah University Hospital in Jerusalem focused on induction of a window of immunosuppression (step 1) followed by induction of host-versus-graft tolerance accompanied by GvL effects mediated by donor lymphocytes infused with the mobilized blood stem cells (step 2) or DLI given later as an outpatient procedure (step 3), reasoning that the same approach may open new therapeutic options for safer treatment of malignant and non-malignant diseases in all age groups with minimal

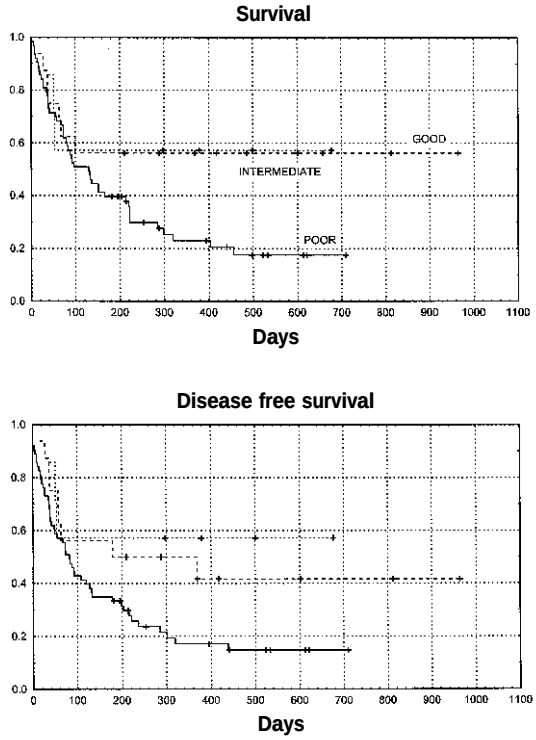


Figure 4. Overall survival and disease free survival in patients receiving melphalan / purine analog combinations.

and well-controlled immediate and late procedure-related toxicity and mortality.[27] Intensive pre-transplant immunosuppressive therapy was accomplished with a combination of fludarabine 30 mg/m^2 for 6 days, busulfan 4 mg/kg for 2 days and fresenius anti-T-lymphocyte globulin (ATG) 5-10 mg/kg/day for 4 days (Table 4). Following the conditioning, each patient received one or two infusions of G-CSF mobilized blood stem cell collections. Low dose cyclosporin A (CSA) (3 mg/kg/day) was used as the sole anti-GvHD prophylaxis for <100 days. The initial study included 26 patients with standard indications for allogeneic BMT; acute leukemia (n=10), chronic leukemia (n=8), non-Hodgkin's lymphoma (n=2), myelodysplastic syndrome (n=1), multiple myeloma (n=1) and genetic diseases (n=4).[2] The benign immediate post-transplant outcome suggested that this attenuated conditioning regimen was extremely well tolerated, with no severe procedure-related toxicity. G-CSF mobilized blood stem cell transplantation with standard doses of CSA as the sole anti-GvHD prophylaxis resulted in stable partial (n=9) or complete (n=17) chimerism. In nine patients absolute neutrophil count (ANC) did not decrease below 0.1×10^9/L whereas two patients never experienced ANC $<0.5\times10^9$/L. An ANC $\geq0.5\times10^9$/L was accomplished within 10-32 (median 15) days. Platelet counts did not decrease below 20×10^9/L in 4 patients who did not require any platelet support; overall platelet counts $>20\times10^9$/L were achieved within 0-35 (median 12) days. Fourteen patients experienced no GvHD at all; severe GvHD (grades 3 and 4) was the single major complication and the cause of death in 4 patients, occurring following early discontinuation of CSA. Relapse was reversed by allogeneic cell therapy in 2/3 cases, currently with no residual host DNA (male) as detected by cytogenetic analysis and PCR. At an observation period extending over 1 year (median 8 months), 22 of 26 patients (85%) treated with allogeneic hematopoietic stem cells were alive, 21 (81%) were disease-free. The actuarial probability of disease-free survival at 14 months was 77.5% (95% confidence interval 53-90%).

A total of 48 patients (age: 1-63 yrs) were reported more recently.[25] Forty of these patients had hematologic malignancy: CML 11 (one juvenile); AML 12; ALL 6; resistant lymphoma 7 (NHL 5; HD 2); MDS 3; multiple myeloma 1, and 8 patients had a non-malignant disorder. Procedure-related toxicity was minimal with continuous oral feeding possible because of the lack of severe mucositis. Early engraftment was documented in all patients: in 16/48 and 4/48 patients the granulocyte count never dropped below 0.1 and 0.5×10^9/L, respectively: 5/48 never required platelet transfusions. Severe GvHD occurred in 8/40 with hematologic malignancy and in 0/8 with non-malignant disease. Relapse occurred in 7/40 with a hematologic malignancy but reversed with donor lymphocyte infusion in 4/5 (2 not treated because of GvHD).

Table 4. Allogeneic non-myeloablative blood stem cell transplantation for malignant and non-malignant diseases. HLA matched siblings and MUD.

Days	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1	0	1
Fludarabine (30 mg/m^2/d)	F	F	F	F	F	F						
Busulfan (4 mg/kg/d)					B	B						
Fresenius ATG (5 or 10 mg/kg/d)							A	A	A	A		
PBSC						P	P					
Cyclosporin A						C				⟶		

1. Cyclosporin A 3 mg/kg IV should be given starting on day –1 prior to transplant: attempt to discontinue within 3 months pending no GvHD. 2. Administer as many as possible stem cells, preferably blood stem cells.

Overall, 32/40 and 8/8, respectively, are alive and disease-free (overall 40/48). A spontaneous uncomplicated pregnancy occurred in one patient with AML less than 12 months after allografting.

The most recent report on the cumulative experience with an attenuated conditioning regimen in 77 patients (63 with a large variety of hematologic malignancies and 14 with non-malignant diseases) indicated that cure of otherwise fatal leukemia became feasible while minimizing early and late procedure-related toxicity and mortality, thus enabling safer cure for infants and children without impairment of growth and development. Similarly, this conditioning regimen was well tolerated by elderly individuals, who were excluded until recently from BMT programs because of increased risk of procedure-related toxicity and mortality. Furthermore, durable engraftment was achieved in all 11 patients receiving bone marrow or blood stem cell allografts from matched unrelated donors, with a relatively low incidence of GvHD.[28] Likewise, the attenuated conditioning regimen was well-tolerated by recipients of second stem cell allografts after failure of a primary procedure of autologous bone marrow or blood stem cell transplantation, suggesting that this regimen can provide an option for cure for patients with resistant disease failing to get benefit from maximal tolerated doses of chemoradiotherapy.[29] Moreover, replacement of host immunohematopoietic cells by donor ones and eradication of malignant or genetically abnormal stem cells and their products may be accomplished without the need for supportive blood product transfusions, with no episodes of septic fever, and avoiding a nadir of aplasia, in 10-25% of the patients, which contrasts with the pattern of recovery of hematopoiesis following standard myeloablative conditioning, hence the former is possible on an outpatient basis. Taken together, induction of immunosuppression (step 1) followed by induction of host-versus-graft tolerance accompanied by GvL effects mediated by donor lymphocytes infused with the mobilized blood stem cells (step 2) or DLI given later as an outpatient procedure (step 3), may offer new therapeutic options for the rational treatment of malignant and non-malignant diseases in patients of all ages.

Investigators in Boston used CY 150 to 200 mg/kg along with ATG and thymic irradiation prior to HLA-identical sibling BMT in 21 patients with advanced, refractory hematologic malignancies. Grade II-IV GvHD was seen in only 1 of 21 patients not receiving DLI. Prophylactic DLI were given to patients in whom no suspicion of GvHD was present by day 35. Seven patients were alive and free of disease progression 105 to 548 (median 445) days after transplantation. Durable chimerism was seen in about 90% of patients receiving HLA-identical and mismatched transplants with this protocol, and lasting mixed chimerism has been demonstrated >1.5 years in extensively documented remissions obtained in this group of patients with advanced and refractory disease, suggesting that this is a promising approach to achieving disease eradication, possibly with less GvHD than is seen with conventional transplants.[30]

Briefly, we can conclude that the more intensive non-myeloablative regimens (fludarabine/busulfan and fludarabine/melphalan) although less intensive than conventional myeloablative regimens can still be associated with toxicities, in older and heavily pretreated debilitated patients, and that many patients in these categories have benefited from a treatment strategy that until recently they were considered ineligible for. Thus, physicians should now be tailoring the preparative regimen to the disease and patient rather than trying to treat all comers with the same preparative regimen. The results achieved support the general concept that future regimens may rely less on intensive cytotoxic therapy and more on allogeneic effects to eradicate malignant cells.

Pre-transplantation host immunosuppression combined with post-transplantation immunosuppression directed at both host and donor immune cells

In the last few years, the Seattle group has pionereed studies to determine whether post-grafting immunosuppression (to prevent GvHD) can be used to suppress host immunity, thereby promoting allogeneic engraftment.[21,26,32] This hypothesis has been tested by Storb *et al.* on healthy dogs which were given marrow grafts and post-grafting immunosuppressive therapy with mofetil mycophenolate (MMF) and cyclosporin A (CSA) after conditioning with 4.5 Gy. Marrow and lymph node samples obtained from unirradiated areas showed stable mixed chimerism as early as 4 weeks after transplantation and lasting for the entire observation period.[26] This finding supports the hypothesis that grafts can create marrow space, most likely through subclinical GvH reactions, and suggests that pre-transplantation irradiation can be replaced by more specific and less toxic means of host T-cell suppression.

Based on the efficacy and lack of toxicity of the canine transplants, the Seattle group designed an outpatient allografting protocol for human patients over 50 years old with hematologic malignancies.[26] The goal was to establish mixed chimerism that could serve as a platform for subsequent DLI. Immunosuppression consisted of TBI 2 Gy delivered as a single fraction. Of eight patients treated, five had a follow-up of 3 to 12 months and three within one month of transplantation. Diagnoses were chronic lymphocytic leukemia (n=2), myelodysplastic syndrome (MDS) evolving into acute myelogenous leukemia (AML) (n=1), AML-CR (n=2), multiple myeloma (n=2) and MDS/refractory anemia with excess blasts (RAEB) (n=1). Their ages ranged from 53 to 66 years except for a 42 year old male with MDS/RAEB who had advanced liver cirrhosis. Four patients had therapy administered entirely in an outpatient setting. Only one patient's granulocyte count fell to less than 5×10^9/L for 2 days, and his platelet nadir was 60×10^9/L. Acute GvHD occurred in one patient. Donor T-cell engraftment at days 28 and 56 after transplantation occurred in all patients. Four patients were eligible for initial DLI. Patient #1 developed grade 2 acute GvHD. After tapering down prednisone and CSA, there was a GvL reaction resulting in complete molecular (polymerase chain reaction) remission in marrow and blood by 7 months after

transplantation. Patient #3 (MDS/AML-CR) developed skin GvHD after DLI and was treated with corticosteroids. At 4 months after DLI, 69% of his T-cells were of donor origin and he has remained without evidence of AML. Patient #2 has progressive RAEB with loss of donor chimerism, and patients #4 and 5 (both myeloma) have stable disease but rejected their grafts shortly after DLI.

Given these results, the Seattle team hypothesizes that current toxic transplant regimens can be replaced by non-toxic host-immunosuppression before and host-donor immunosuppression after transplantation, which controls both HvG and GvH reactions and results in stable mixed donor host hematopoietic chimerism. This approach is safe enough to be carried out in the setting of out-patient care.

High-dose therapy and autologous stem cell transplantation followed by moderate immunosuppression and allogeneic stem cell transplantation

Given that fludarabine-based conditioning regimens can make engraftment of allogeneic donor hematopoietic stem cells (HSC) possible, investigators in Genoa have tried to verify whether high-dose therapy/autografting with protocols appropriate for underlying diseases followed by allografting conditioned by only an immunosuppressive regimen could be combined in order to achieve the reduction of tumor burden after autografting and the control of residual disease with immune-mediated effects after allografting.[23] Immunosuppression consisted of fludarabine 30 mg/m^2 with cyclophosphamide 300 mg/m^2 both delivered daily for three days. Cyclosporine 1 mg/kg i.v. from day -1 to day 10 (and subsequently given orally) and methotrexate 8 mg/m^2 i.v. on days 1, 3, 5 were given. Granulocyte colony-stimulating factor-mobilized HSC from serologically and molecularly identical sibling donors were reinfused on day 0. Chimerism studies were performed every 10 days for the first month and every 15 days in subsequent months. The 23 patients treated had a follow-up of 330 (range, 66-72) days. Diagnoses were metastatic breast cancer (n=4), advanced resistant Hodgkin's disease (n=10) or non-Hodgkin's lymphoma (n=5), refractory anemia with excess of blasts (RAEB-t) (n=2), accelerated/blastic phase-CML (BC-CML) (n=2). The median age of the patients was 36 years (range, 19-60) (Table 5). In only two patients did the granulocyte count fall to less than 1×10^9/L and the platelet nadir was 20×10^9/L. Acute GvHD occurred in 10 patients but was generally mild except for three patients who experienced grade 3 gastrointestinal and liver disease. Fourteen patients achieved complete donor HSC engraftment at a median of 115 days (range 53-180). Seven patients obtained mixed chimerism and two patients (RAEB-t and BC-CML) re-engrafted autologous (Table 6). Thirteen patients were eligible for DLI at a median dose of 1×10^7 CD3$^+$ cells/kg. The outcome is the following: breast cancer: 2/4 alive in good PR at 420 and 630 days. These patients achieved and maintained complete chimerism; they developed grade III gastrointestinal/liver acute GvHD after DLI and in one case a reduction of bone metastases was demonstrated; Hodgkin's disease: alive 7/10. Two patients achieved CR for the first time; 2 patients maintained CR achieved with autografting, 3 patients achieved CR for the third and fourth times (Table 7). The median survival from allografting is 330 days (range, 66-780); non-Hodgkin's lymphoma: 3/5 alive after 240 to 630 days (median, 270 days). One patient resistant to first/second line therapy achieved his first CR after mini-allografting maintained for 12 months; subsequently, the patient relapsed and DLI were given a few weeks ago in an attempt to obtain a new remission. At the time of writing the patient is in partial remission. Three patients achieved good PR after autografting and CR (CR-2, CR-4, and CR-5) after mini-allografting and DLI. One patient with large cell lymphoma in progressive disease after first and second line chemotherapy achieved PR after autografting, maintained for a few months after mini-allografting. Subsequently, the patient relapsed and died of lymphoma (Table 7); RAEB-t: 2/2 alive at 330 and 450 days. Both patients

Table 5. Clinical and treatment characteristics of Genoa patients receiving the Flu-Cy protocol.

Patients	23
Age, median (years)	36 (19-60)
Sex	9M/14F
Diagnosis	
Hodgkin's disease	10
Non-Hodgkin's lymphoma	5
Metastatic breast cancer	4
CML (AP/BP)	2
RAEB-t	2
Prior treatment courses median (range)	3 (1-5)
Autografted (ASCT)	21
Interval ASCT/mini-allo median, days	40 (30-1149)
Stage of disease (HD/NHL)	
Partial response	1
First relapse	2
Relapse >2	6
Progressive disease	6
Donor age (years) median (range)	38 (19-69)
Donor gender	13M/10F
CMV status (D/R)	neg/neg
GvHD prophylaxis	CSA/MTX

Table 6. Results: engraftment after Flu-Cy protocol.

Patients	23
WBC < 1×10^9/L	2/23
Platelets < 20×10^9/L	2/23
Complete chimerism (100% donor)	14/23 (61%)
Mixed chimerism (20-90% donor)	7/23 (30%)
Autologous recovery	2/23 (9%)

Table 7. Clinical results in Hodgkin's disease and non-Hodgkin's lymphoma.

Pts.	Dx	Status		Status		Outcome (months)
		Pre-auto	Post-auto	Pre-allo	Post-mini-allo	
ZM	HD	PRD	PR	PD	PD	Died in PD/cGVHD (liver) (20)
BG	HD	REL-2	PR	PR	CR-3	Alive in CR-3 (+23)
GI	HD	PRD	PRD	PRD	PRD	Died in PRD (2)
CP	HD	REL-2	PR	PR	CR-3	Died in CR-3 (brain aspergillus) (4)
RS	HD	REL-4	CR-5	REL-5	PR	Alive in PR (+5)
LM	HD	REL-1	CR-2	CR-2	CR-2	Alive in CR-2 (+12)
MR	HD	CR-2	CR-2	CR-2	CR-2	Alive in CR-2 (+8)
MC	HD	PRD	PR	PR	CR-1	Alive in CR-1 (+8)
IP	HD	REL-4	PR	PR	CR-4	Alive in CR-4 (+6)
GC	HD	PRD	PR	PR	CR-1	Alive in CR-1 (+4)
BR	NHL	PRD	PR	PR	CR-1	Alive in PR (+18)
RC	NHL	PRD	PR	PR	PD	Died in PD (5)
GS	NHL	REL-4	PR	PR	CR-5	Alive in CR-5 (+4)
MG	NHL	REL-1	PR	PR	CR-2	Alive in CR-2 (+3)
SV	NHL	REL-3°	PR	PR	CR-4	Alive in CR-4 (+4)

*Pt: patients; Dx: diagnosis; HD: Hodgkin's disease; NHL: non-Hodgkin's lymphoma; PRD: primarily refractory disease; CR: complete remission; PR: partial remission; REL: relapse; PD: progressive disease; GPR: good partial remission; *CR-2 induced by MOPP; OE: on evaluation; °these patients had received a previous autograft.*

received only mini-allografting. One patient had t(1;3)(p36; q21) and achieved complete chimerism with a normal karyotype for three months after which she relapsed. This case was extensively described in a previous publication.[31] AP/BP-CML: 2/2 alive at 480 and 780 days. The patient with p190 AP-CML developed grade 2 acute GvHD that was associated with the development of full donor HSC engraftment. After tapering down CSA, the patient developed granulocytopenia and thrombocytopenia followed by normalization of blood values and complete molecular (polymerase chain reaction) remission in marrow and blood by 780 days after mini-allografting. The patient is now off CSA and steroids and has returned to work.[31] Table 8 shows the outcome of these patients; the Kaplan-Meier survival plot is shown in Figure 5. In conclusion, mini-allografting, alone or after tumor debulking with autografting, when employed in older patients or in young patients resistant to first and second line therapies with the main goal of exploiting GvL effects, is feasible using mainly non-toxic immunosuppressive conditioning regimens. It is extremely important to underline that side effects of these therapies were extremely rare and mixed/complete chimerism was established in the majority of the patients after allografting. If these results are confirmed, immunosuppression regimens alone should be successful in establishing stable engraftment and controlling neoplasia, particularly if the regimens are preceeded by autografting.

Table 8. Outcome.

Patients	23
Alive	16 (70%)
Follow-up, median (days)	330 (range, 66-780)
Died	6
≤ d+100	3 (HD: 1; breast: 2)
> d+100	4 (HD: 2; NHL: 2)

HD: Hodgkin's disease; NHM: non-Hodgkin's lymphoma.

Conclusions

Future protocols will certainly focus on attempts to exploit the therapeutic potential of alloreactive donor lymphocytes, while trying to reduce or minimize procedure-related toxicity and mortality. Attenuated conditioning regimens are likely to provide an option for

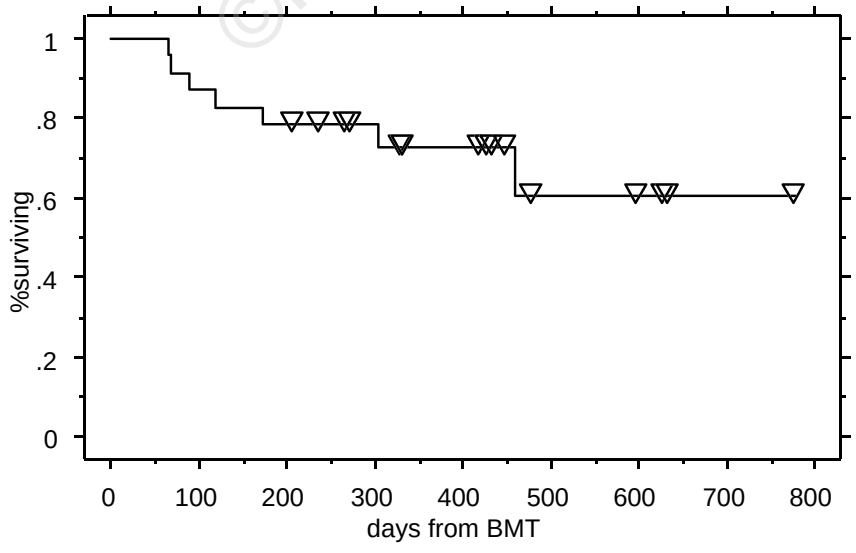


Figure 5. Kaplan-Meier survival plot in 23 patients receiving the fludarabine-cyclophosphamide regimen.

cure in patients who until recently were excluded from bone marrow transplantation programs, including elderly individuals with a large variety of hematologic malignancies and possibly metastatic solid tumors as well. At the other end of the spectrum, optimal variations of these less intensive regimens are likely to provide a curative option for infants, children and young adults with malignant and non-malignant indications for stem cell transplantation, reducing the acute toxicity and risk of mortality on the one hand, and avoiding late complications such as impaired growth and development, cataract formation and multiple endocrine adenopathies, sterility included, on the other. It remains to be seen whether effective antigen non-specific GvL and GvT effects can be accomplished independently of GvHD, or if tumor specific approaches can be developed in the future which improve beneficial anti-tumor effects while reducing anti-host responses. Such a noble goal will remain the subject of future studies.

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