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Multicenter Experience Using Total Lymphoid Irradiation and Antithymocyte Globulin as Conditioning for Allografting in Hematological Malignancies

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A non myeloablative conditioning with total lymphoid irradiation (TLI) and antithymocyte globulin (ATG) was shown to protect against graft-versus-host disease (GVHD). To evaluate the effects of TLI-ATG in a multicenter study, 45 heavily pretreated patients, median age 51, with lymphoid (n = 38) and myeloid (n = 7) malignancies were enrolled at 9 centers. Twenty-eight patients (62%) received at least 3 lines of treatment before allografting, and 13 (29%) had refractory/relapsed disease at the time of transplantation. Peripheral blood hematopoietic cells were from HLA identical sibling (n = 30), HLA-matched (n = 9), or 1 antigen HLA-mismatched (n = 6) unrelated donors. A cumulative TLI dose of 8 Gy was administered from day −11 through −1 with ATG at the dose of 1.5 mg/kg/day (from day −11 through −7). GVHD prophylaxis consisted of cyclosporine and mycophenolate mofetil. Donor engraftment was reached in 95% of patients. Grade II to IV acute GVHD (aGVHD) developed in 6 patients (13.3%), and in 2 of these patients, it developed beyond day 100. Incidence of chronic GVHD (cGVHD) was 35.8%. One-year nonrelapse mortality was 9.1%. After a median follow-up of 28 months (range, 3-57 months) from transplantation, median overall survival was not reached, whereas median event-free survival was 20 months. This multicenter experience confirms that TLI-ATG protects against GVHD and maintains graft-vs-tumor effects.

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KEY WORDS: Graft-versus-host disease, Nonmyeloablative conditioning, Total lymphoid irradiation/antithymocyte globulin

INTRODUCTION

The introduction of reduced-intensity and nonmyeloablative conditionings for allogeneic transplantation has allowed older and medically unfit patients to benefit from this potentially curative procedure [1]. However, the use of less toxic conditionings did not significantly have an impact on the incidence of

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graft-versus-host disease (GVHD) and its treatment-related mortality [2-4]. In particular, the incidence of grade II to IV acute GVHD (aGVHD) after nonmyeloablative conditionings ranged from 20% up to 65% of patients and, in about half of these, its complications, especially in steroid refractory GVHD, were fatal [1,2,5-8]. A different clinical approach that spares the bone marrow (BM) was proposed in recent years to prevent aGVHD. It consists of a nonmyeloablative preparatory regimen based on murine models of BM transplantation that uses fractionated total lymphoid irradiation (TLI) and antithymocyte globulin (ATG). In this model, the combination TLI-ATG alters the host immune profile to favor regulatory natural killer T (NKT) cells that suppress GVHD by polarizing conventional T cells toward secretion of noninflammatory cytokines and by promoting the expansion of donor CD4+ CD25+ regulatory T cells [9-13]. This animal model was first translated into a clinical pilot study on 37 patients [14]. This single-center experience was recently updated on a total of 111 patients [15]. To confirm their findings in a multicenter study and to evaluate which subsets of patients may most benefit from this approach, we conducted a prospective phase II clinical trial through the Gruppo Italiano Trapianto di Midollo Osseo (GITMO; http://ClinicalTrials.gov; NCT01081405).

MATERIAL AND METHODS

Patients

Between November 2007 and August 2010, 45 consecutive patients were enrolled at 9 Italian transplantation centers (Table 1). Inclusion criteria included hematologic malignancies in whom an allograft is warranted, age ≤70 years, and Karnofsky performance status ≥60%. Exclusion criteria included age above 70 years, Karnofsky performance score below 60%, severe abnormal organ function (heart, kidney, lung, and liver), pregnancy, seropositivity for human immunodeficiency virus, and presence of active nonhematologic malignancies. Patient comorbidities were evaluated according to the hematopoietic cell transplantation (HCT) comorbidity index score [16]. Eligible donors were either related or unrelated HLA identical donors who accepted to donate granulocyte-colony stimulating factor mobilized peripheral blood hematopoietic cells. One antigen HLA-mismatched donors were allowed. All patients signed informed consent upon enrollment. The protocol was approved by the institutional review boards of the participating centers in accordance with the Declaration of Helsinki (http://ClinicalTrials.gov; NCT01081405).

Table 1. Patient Characteristics

No. of patients	45
Median age at transplantation, yr (range)	51 (23-68)
Median follow-up, mo (range)	28 (3-57)
Male/female	30/15
Disease	
CLL	13
NHL[a]	10*
Hodgkin lymphoma	8
AML/RAEB	4
Multiple myeloma	4
Acute lymphoblastic leukemia	3
Myelodysplastic syndrome	3
High-risk/low-risk disease	39/6
Median time from diagnosis to transplantation, mo (range)	31 (4-146)
Previous lines of treatment	
0	1
1	9
2	7
≥3	28
Disease status at transplantation	
CR	17
PR	15
Refractory of progressive disease	13
HCT comorbidity index	
0	29
1-2	9
≥3	7
Donor type	
Related	30
HLA-matched URD	9
HLA-mismatched URD	6

CLL indicates chronic lymphocytic leukemia; NHL, non-Hodgkin lymphoma; AML, acute myeloid leukemia; RAEB, refractory anemia with excess of blasts; CR, complete remission; PR, partial remission; HCT, hematopoietic cell transplantation; URD, unrelated donor.

[a]Histologies included 5 follicular lymphomas, 2 diffuse large cell lymphomas, 1 Burkitt lymphoma, 1 lymphoblastic lymphoma, and 1 lymphocytic lymphoma.

Treatment Plan

TLI was planned as follows: a whole body computed tomography scan was obtained for every patient, and target volumes were outlined in sequential axial computed tomography images. The clinical target volume corresponded to all lymphatic stations (bilateral neck, bilateral axillas, mediastinum, paraortic, bilateral common iliac, external and internal iliac, inguinal lymph nodes), and spleen. The Waldeyer ring was excluded in order to reduce potential toxicity. A planning target volume was then generated adding a 10-mm isotropic margin. The neck, thoracic, and abdominal organs at risk were then contoured. TLI was delivered over 10 fractions for a total dose of 8 Gy, from day −11 through −1, with a linear accelerator and 6- to 10-MV photons, using a 3D-conformal technique consisting of 3 sequential individually shaped antero posterior and postero anterior fields (mantle, upper part of inverted Y, and lower part of inverted Y). Portal images were acquired on the first day and then every 3 days. ATG (thymoglobuline, genzyme) was administered at the dose of 1.5 mg/kg/day (from day −11 through −7). Primitive hematopoietic stem cell infusion was scheduled on day 0. Postgrafting

immunosuppression consisted of oral cyclosporine and mycophenolate mofetil. Oral cyclosporine was given at 12.5 mg/kg/day from day −3 until day +56, then tapered to day 180 in the absence of GVHD. Doses were adjusted to maintain blood trough levels of about 500 ng/mL throughout the first month posttransplantation unless clinical side effects occurred. Oral mycophenolate mofetil was given at 30 mg/kg/day divided into 2 doses from day 0 until day 28 or at 45 mg/kg/day divided into 3 doses from day 0 until day 40 and then tapered through day 56 in the setting of related and unrelated donor (URD) transplantation, respectively.

All patients received standard prophylaxis against viral, bacterial, and fungal infections. Cytomegalovirus (CMV) reactivation was monitored through levels of CMV antigenemia and/or serum CMV DNA and treated with ganciclovir or foscarnet as clinically indicated.

Chimerism Analysis

Chimerism analyses of peripheral blood T cells and unfractionated marrow were carried out at days 28, 56, 90, 180, and 360 posttransplantation, and yearly thereafter with fluorescence in situ hybridization in sex-mismatched pairs or PCR-based analyses of polymorphic microsatellite regions in sex-matched pairs, as previously described [17]. Mixed chimerism was defined as between 5% and 95% peripheral blood donor T cells, and full chimerism was defined as greater than 95% donor T cells. Graft rejection was defined as donor CD3-positive cells 5% or less at any of the assessed time points after achieving posttransplantation donor chimerism.

GVHD and Relapse Risk

The aGVHD was diagnosed according to the recent indications of the National Institute of Health, which include clinical features as criteria to discriminate acute from chronic GVHD (cGVHD) and graded according to common criteria [18-20]. The cGVHD was graded as previously described [21]. Hematologic diseases were classified as at low risk of relapse (chronic myelogenous leukemia in first chronic phase, refractory anemia with no blast excess, acute myeloid leukemia, and lymphoblastic leukemia in first remission) or at high risk of relapse (all other diseases) [22]. Disease relapse or disease progression was defined as recurrence of disease after complete remission (CR) and partial remission (PR) and progression of persistent disease, respectively. Standard treatment, as per institutional guidelines of the participating centers, with/without donor lymphocyte infusions (DLIs) were allowed to treat progression or relapse posttransplantation. DLIs were administered in the absence of clinical GVHD manifestation and after a rapid taper and discontinuation of the immunosuppression.

Statistical Analyses

Primary endpoints were overall survival (OS) and event-free survival (EFS) from transplantation. OS was defined as the time from transplantation to death from any cause. EFS was defined as the time from transplantation to progression, relapse, or death, whichever occurred first. Alive patients without progression or relapse were censored at the date of last contact. Survivals were analyzed by the Cox proportional hazards model, comparing the 2 arms by the Wald test and calculating 95% confidence intervals. Univariate analyses were performed for the following variables: age (>50 vs ≤50 years), gender (male vs female), type of donor (matched unrelated vs sibling donor), number of previous chemotherapy lines (>2 vs ≤2), HCT comorbidity index score [16] (≥1 vs 0), donor chimerism at day +90 and +180 (>95% vs ≤95%), occurrence of aGVHD or cGVHD (any vs none), and disease status at transplantation (CR + PR vs refractory/progressive disease). Occurrence of aGVHD and cGVHD were treated as time-dependent variables. Cumulative incidences of developing aGVHD, cGVHD, nonrelapse mortality (NRM), and relapse incidence (RI) were determined using the Fine and Gray competing risk regression model. Competing events were defined as relapse and death without GVHD for aGVHD and cGVHD, death without relapse for NRM, and as relapse for RI [23]. Patient characteristics were tested using the Fisher exact test for categorical variables and the Mann-Whitney test for continuous ones. All reported *P* values were 2-sided at the conventional 5% significance level. Data were analyzed as of September 2011 by SPSS 19.0.0 (Chicago, IL) and R 2.13.1 software (The R Foundation for Statistical Computing, Wien-A).

RESULTS

Patient Characteristics

Patient characteristics are reported in Table 1. All patients were ineligible for a myeloablative conventional allograft either because of age or because of comorbidities. Of the 45 consecutive patients prospectively enrolled, 38 patients (84%) had lymphoid malignancies, and 7 patients (16%) had myeloid malignancies. The vast majority of patients (78%) had advanced and heavily pretreated diseases. Only 1 patient with transfusion-dependent chronic myelogenous leukemia with no blast excess received an allograft upfront without previous chemotherapy.

Engraftment

Median numbers of CD34+ and CD3+ T cells infused were 6.0×10^6/kg (range, 1.1-12.9) and 2.5×10^8/kg (range, 0.2-4.0) recipient body weight, respectively. Sustained donor chimerism >95% was achieved in 24% of the evaluated patients (n = 37) at day +90

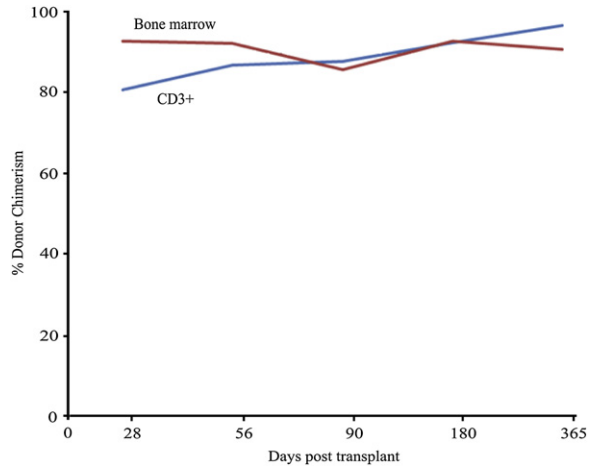

Figure 1. Donor chimerism evaluated on bone marrow and on CD3+ T cells over time.

and in 48% at day +180 (Figure 1). Of the 32 patients evaluated at 1 year, 79% were full donor chimeras. Graft rejection occurred in 2 patients, at day +56 in a patient who underwent transplantation from an HLA-matched URD and at day +180 in a patient who underwent transplantation from an HLA-matched related donor. There was no difference in OS and EFS between patients with donor chimerism >95% and those with a lower chimerism at 3 and 6 months post-transplant (OS: $P = .2$ and .1; EFS $P = .3$ and .3). One patient who underwent transplantation from a mismatched URD, who experienced an abrupt decrease of donor CD3+ T cell chimerism at day +60 received off-protocol DLIs followed by grade II aGVHD.

Transplant-Related Toxicity and Mortality

Overall, grade II to IV aGVHD developed in 6 patients, including grade III GVHD in 3 patients after an HLA-matched sibling donor transplantation and grade IV GVHD in a patient after an HLA-matched URD transplantation. Overall, the cumulative incidence was 13.3% (Figure 2A), 4 of 6 patients (8.9%) within day 100, given that 2 patients experienced late-onset (beyond day +100) aGVHD. At a median follow-up of 28 months (range, 3-57 months), 50% of patients with aGVHD died from NRM, vs 12% of those who did not develop aGVHD ($P = .018$). Patients with aGVHD did not relapse, whereas 39% of the patients without aGVHD did. Cumulative incidence of cGVHD was 35.8%, only 1 patient had received an allograft from an URD (Figure 2B). RI at 1 year was 7.1% in patients with cGVHD and 33.3% in patients without ($P = .048$).

At a median follow-up of 28 months (3-57 months) from transplantation, NRM was 17.2%, and 1-year NRM was 9.1% (Figure 2C). Of note, 3 of 7 deaths occurred after the first 12 months posttransplantation due

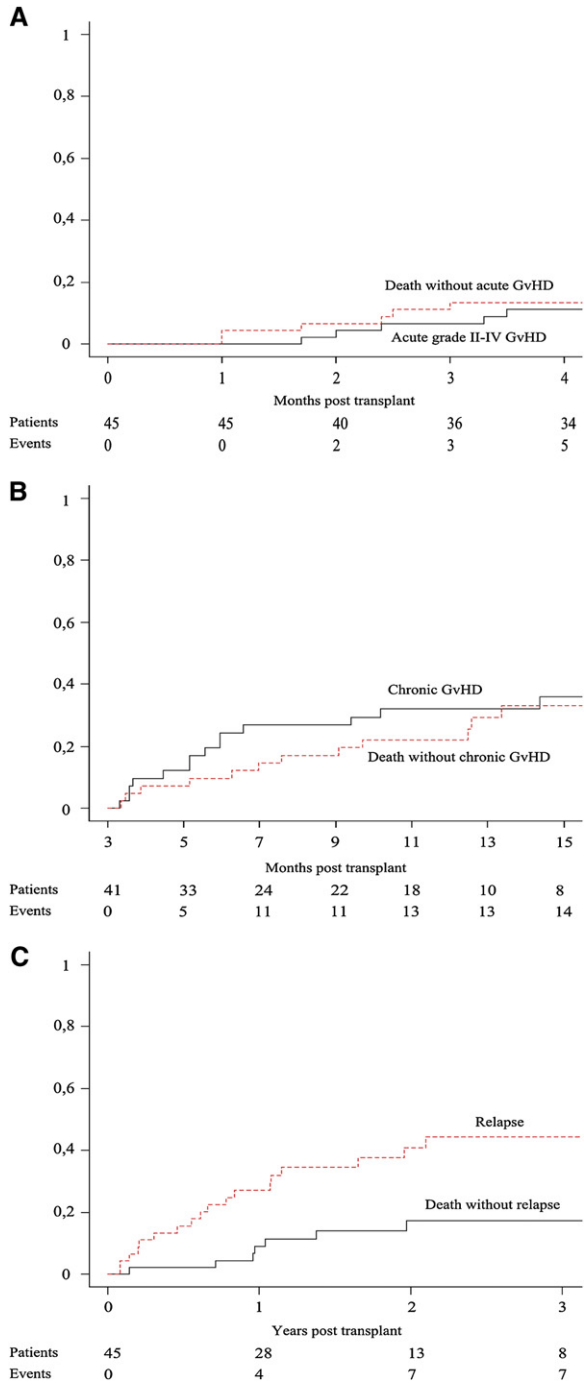

Figure 2. (A) Cumulative incidence of acute grade II-IV graft-vs.-host disease (solid line) and competing events (dotted line). (B) Cumulative incidence of chronic graft-vs.-host disease (solid line) and competing events (dotted line). (C) Cumulative incidence of non relapse mortality (solid line), and competing events (dotted line).

to viral encephalitis, cGVHD-related complications, and due to diffuse large cell lymphoma in a patient originally treated for Hodgkin lymphoma. In this patient, Epstein–Barr virus-associated lymphoma was ruled out. Other causes of death included aGVHD (n = 3) and graft rejection complications (n = 1) (Table 2). CMV reactivation occurred in 20 of 45 patients (44%)

Table 2. Causes of Death

Causes of Death	No. of Patients
Graft rejection	1
aGVHD	3
Viral encephalitis	1
Secondary diffuse large cell lymphoma	1
cGVHD	1
Disease relapse or progression	9

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

after a median of 1.2 months (range, 0.2-11.3 months) from transplantation, whereas only 1 patient experienced Epstein–Barr virus reactivation at day +165.

Response

Only 6 of 45 patients (13%) were at low risk of relapse. Although advanced, disease at the time of transplantation was chemosensitive in 32 of 45 patients (71%; 17 CR and 15 PR). The overall posttransplantation response rate was 73%. Ten of 15 patients who were in PR at transplantation and 5 of 13 of those patients with relapsed/refractory disease achieved CR, for an overall CR rate of 64% (29 of 45 patients). Five of 45 patients (11%) achieved PR: 3 patients maintained a pretransplantation PR, whereas 2 patients had relapsed/refractory disease at transplantation. Only 6 of 29 patients (21%) who achieved posttransplantation CR eventually relapsed, whereas disease recurred in 4 of 5 patients (80%) who achieved PR. Overall, cumulative incidence of relapse/progression was 27.1% at 1 year and 44.3% at 36 months. Patients with chronic lymphocytic leukemia (CLL) and lymphomas mostly benefited from allografting with a CR rate of 70.9% (22 of 31 patients) posttransplantation as compared to a CR rate of 50% (7 of 14 patients) with other diseases.

OS and EFS

At a median follow-up of 28 months (range, 3-57 months) from transplantation, the median OS was not reached, whereas the median EFS was 20 months (Figure 3A and B). By univariate analysis, there was a trend toward a better OS and in those patients who underwent transplantation from a URD (hazard ratio [HR], 2.34; $P = .095$). The only significant variables associated with better EFS were disease in CR or PR at the time of transplantation (HR, 0.41; $P = .033$) and the development of cGVHD (HR, 0.02; $P = .031$; Table 3).

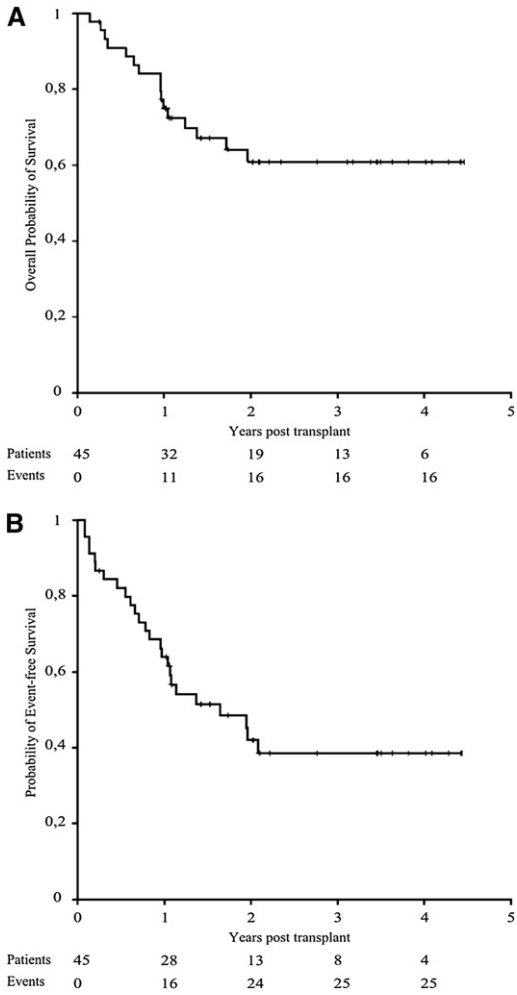

Figure 3. (A) Overall survival from transplant (B) Event-free survival from transplant.

DISCUSSION

Though transplant-related mortality has drastically decreased in the past decade due to the development of less toxic conditioning regimens and, partly, to improved supportive care, GVHD and its treatment remain one of the major areas where progress is needed to further improve transplantation clinical outcomes [24,25]. The clinical use of TLI as an immune-modulating strategy that altered T cell mediated immune responses was first reported in patients treated for Hodgkin lymphoma [26]. Moreover, in the late 1970s, preclinical models described TLI as a nonmyeloablative conditioning that altered the recipient immune system and resulted in long-term survival after allogeneic BM and skin grafts [27-29]. The immune-modulating effects of TLI, with/without ATG, aimed at protecting against GVHD and at favoring transplantation tolerance after allografting and solid organ transplantations, were studied in several preclinical animal models. It was observed that TLI-ATG modifies the balance of host T cell subsets favoring regulatory NKT cells, constitutively more resistant to irradiation through the p53/Bcl-2 apoptotic pathway, over host conventional T cells. The complex interplay between residual host IL-4 secreting NKT cells and donor IL-10 secreting CD4+CD25+

Table 3. Univariate Analyses of Risk Factors in 45 Patients Conditioned with TLI/ATG

	Univariate Analysis			
	OS		EFS	
Variable	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age: >50/<50 yr	2.65 (0.91-7.70)	.074	1.58 (0.71-3.50)	.264
Sex: male/female	1.18 (0.41-3.40)	.760	0.88 (0.39-1.99)	.751
Donor: MUD/MRD	2.34 (0.86-6.35)	.095	1.82 (0.80-4.14)	.156
Previous CT lines: at least 3/0-2	0.72 (0.27-1.94)	.517	1.19 (0.51-2.77)	.692
HCT-comorbidity index: >1/0	2.27 (0.85-6.10)	.103	1.72 (0.77-3.84)	.190
Disease status at transplantation: CR-PR/no response	0.65 (0.24-1.79)	.402	0.41 (0.18-0.93)	.033
aGVHD[a]: yes/no	5.02 (0.41-61.24)	.207	1.59 (0.22-11.28)	.644
cGVHD[a]: yes/no	0.11 (0.01-5.98)	.276	0.02 (0.01-0.69)	.031
Day-180 chimerism: at least 95%/<95%	0.31 (0.06-1.54)	.151	0.59 (0.21-1.66)	.316

TLI indicates total lymphoid irradiation; ATG, antithymocyte globulin; OS, overall survival; EFS, event-free survival; HR, hazard ratio; CI, confidence interval; MUD, matched unrelated donor; MRD, matched related donor; CT, chemotherapy; HCT, hematopoietic cell transplantation; CR, complete remission; PR, partial remission; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.
[a]Treated as a time-dependent variable.

regulatory T cells determines a Th2 bias that suppresses the GVHD capacity of conventional donor T cells. However, these mechanisms do not prevent direct tumor cell lysis by donor CD8+ T cells. Thus, graft-vs-tumor effects are not abrogated [9-13].

These preclinical findings were adapted to human HCT by the Stanford group in the attempt to reduce clinical GVHD and improve long-term survival [14,15]. The preliminary findings were first reported on a cohort of 37 patients with lymphoid malignancies or acute leukemias. Only 2 of 37 patients developed aGVHD after HCT. Patients with lymphoid malignancies changed their disease status from PR to CR after transplantation. Major tumor shrinking was also observed outside the TLI radiation fields suggesting strong graft-vs-lymphoma effects [14]. In their recently updated and extended single-center experience, 111 patients with lymphoid and myeloid malignancies underwent transplantations from matched related donors (n = 61) or URDs (n = 50) [15]. At the time of transplantation, most patients were heavily pretreated and were at high risk of relapse. The probability of aGVHD (grade II-IV) by day 100 was 2% in related and 10% in URD transplantations, whereas the cumulative risk of extensive cGVHD at 3 years was 28% and 26%, respectively [15]. After a median follow-up of 665 days, 67 of 111 patients (60.3%) were alive. Overall, the 3-year probability of OS and EFS was 60% and 40%, respectively [15]. These findings, in a heavily pretreated patient cohort at high risk of relapse, showed that graft-vs-tumor effects were maintained.

To our knowledge, the here-reported GITMO experience is the first sizable multicenter trial that prospectively used the TLI-ATG conditioning. Although some differences can be observed, overall, our findings confirm the Stanford experience. Full-donor chimerism was slowly achieved if compared with what was described in other studies in which nonmyeloablative conditionings were used [8,22,30]. However, only 2 patients eventually rejected their graft and, importantly, a delayed achievement of full-donor chimerism did not seem to have an impact on patient survival (Table 3). This discrepancy may be explained by the relatively rapid achievement of high donor chimerism seen in most of our patients by the third month post-transplant. The overall cumulative incidence of aGVHD was 13.3%. However, day-100 aGVHD was 8.9%, which is in keeping with the Stanford experience, 2% and 10% in related and URD transplantations, respectively. Delayed onset of aGVHD has been described as not infrequent after reduced-intensity conditionings [2,31]. In light of the occurrence of aGVHD symptoms beyond day 100, a new classification has also been proposed by a consensus panel [19,20]. In our study, delayed aGVHD occurred in 2 of 6 patients, which may explain the slightly higher overall incidence as compared with what was published by the Stanford group in which only aGVHD by day 100 was reported [15]. Cumulative incidence of cGVHD was 35.8%. Interestingly, only 1 patient had received an allograft from a URD.

At a median follow-up of 28 months, overall NRM was 17.1%, whereas 1-year NRM was 9.1%. Potentially curative graft-vs-tumor effects were confirmed. Most patients in CR or in PR, and some additional patients with refractory/progressive disease at transplantation, maintained or achieved CR after the allograft despite the lack of any possible cytoreductive effect due to the conditioning regimen. In our study, the efficacy of immune-mediated antitumor activity was more evident in patients with CLL and lymphomas. Even though the reduced sample size did not permit a Cox multivariate analysis, we still observed that a lower tumor burden (patients in CR or PR;

Table 3) at the time of transplantation and the development of cGVHD were significantly associated with better EFS by univariate analysis.

The clinical association between GVHD and antitumor effects was initially reported in the early 1980s [32]. These findings were also reported in several more recent reports [33-36]. It is interesting to notice that TLI-ATG seemed to significantly decrease aGVHD but not cGVHD to the same extent, suggesting both biological and clinical implications. First, these findings underline the well-documented different pathophysiology between aGVHD and cGVHD, and second, the current difficulty in separating cGVHD from graft-vs-tumor effects in the clinical setting.

Cumulative incidence of relapse/progression was 27.1% at 1 year and 44.3% at 36 months. Overall, the risk of relapse or progression after nonmyeloablative transplantations remains an issue. A combined approach with a sequential use of an allograft followed by consolidation/maintenance with monoclonal Abs or newly developed targeted drugs may lead to more curative strategies. Novel treatment plans may include posttransplantation targeted therapies with ofatumomab (anti-CD20) for patients with non-Hodgkin lymphomas and CLL, or brentuximab vedotin (anti-CD30) for Hodgkin lymphoma, tyrosine kinase inhibitors for Ph+ acute leukemias, and new agents such as lenalidomide or bortezomib/carfilzomib for multiple myelomas [37-42].

In conclusion, our multicenter experience confirms previous single-center reports. TLI-ATG is protective against aGVHD and maintains graft-vs-tumor effects mainly associated with cGVHD. However, new avenues of research should be sought to reduce the risk of disease recurrence. Moreover, further studies should aim at prospectively comparing different nonmyeloablative conditioning regimens.

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Authorship Statement: Giuseppe Messina and Benedetto Bruno contributed to the initial conception and designed the study and wrote the manuscript. Giuseppe Messina, Luisa Giaccone, Moreno Festuccia, Giuseppe Irrera, Ilaria Scortechini, Roberto Sorasio, Federica Gigli, Irene Cavattoni, Andrea Riccardo Filippi, Fabrizio Carnevale Schianca, Massimo Pini, Antonio M. Risitano, Carmine Selleri, Alessandro Levis, Nicola Mordini, Andrea Gallamini, Rocco Pastano, Marco Casini, Massimo Aglietta, Mauro Montanari, Giuseppe Console, Mario Boccadoro, Umberto Ricardi, and Benedetto Bruno provided the study materials or patients and collected and assembled the data. Giuseppe Messina, Benedetto Bruno, Luisa Giaccone, Moreno Festuccia, and Roberto Passera analyzed and interpreted the data. All authors gave final approval to the manuscript.

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