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Competitive Repopulation and Allo-Immunologic Pressure Determine Chimerism Kinetics after T Cell-Depleted Allogeneic Stem Cell Transplantation and Donor Lymphocyte Infusion

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A B S T R A C T

After allogeneic stem cell transplantation (alloSCT), patient-derived stem cells that survived the pretransplantation conditioning compete with engrafting donor stem cells for bone marrow (BM) repopulation. In addition, donor-derived alloreactive T cells present in the stem cell product may favor establishment of complete donor-derived hematopoiesis by eliminating patient-derived lymphohematopoietic cells. T cell-depleted alloSCT with sequential transfer of potentially alloreactive T cells by donor lymphocyte infusion (DLI) provides a unique opportunity to selectively study how competitive repopulation and allo-immunologic pressure influence lymphohematopoietic recovery. This study aimed to determine the relative contribution of competitive repopulation and donor-derived anti-recipient alloimmunologic pressure on the establishment of lymphohematopoietic chimerism after alloSCT. In this retrospective cohort study of 281 acute leukemia patients treated according to a protocol combining alemtuzumab-based T cell-depleted alloSCT with prophylactic DLI, we investigated engraftment and quantitative donor chimerism in the BM and immune cell subsets. DLI-induced increase of chimerism and development of graft-versus-host disease (GVHD) were analyzed as complementary indicators for donor-derived anti-recipient alloimmunologic pressure. Profound suppression of patient immune cells by conditioning sufficed for sustained engraftment without necessity for myeloablative conditioning or development of clinically significant GVHD. Although 61% of the patients without any DLI or GVHD showed full donor chimerism (FDC) in the BM at 6 months after alloSCT, only 24% showed FDC in the $CD4^+$ T cell compartment. In contrast, 75% of the patients who had received DLI and 83% of the patients with clinically significant GVHD had FDC in this compartment. In addition, 72% of the patients with mixed hematopoiesis receiving DLI converted to complete donor-derived hematopoiesis, of whom only 34% developed clinically significant GVHD. Our data show that competitive repopulation can be sufficient to reach complete donor-derived hematopoiesis, but that some alloimmunologic pressure is needed for the establishment of a completely donor-derived T cell compartment, either by the development of GVHD or by administration of DLI. We illustrate that it is possible to separate the graft-versus-leukemia effect from GVHD, as conversion to durable complete donor-derived hematopoiesis following DLI did not require induction of clinically significant GVHD.

INTRODUCTION

The goal of allogeneic hematopoietic stem cell transplantation (alloSCT) in patients with hematologic malignancies is to eradicate the disease by replacing patient hematopoiesis with donor-derived hematopoiesis and introducing donor alloreactive T cells capable of eliminating residual malignant cells. After alloSCT, patient hematopoietic stem cells (HSCs) that survived the pretransplantation conditioning compete with engrafting donor HSCs for bone marrow (BM) repopulation [1]. Patient-derived alloreactive T cells may reject the graft [2], but donor engraftment can be supported by alloreactive donor-derived T cells recognizing nonself antigens on patient immune cells [3,4]. These alloreactive donor T cells can further eliminate patient HSCs and residual malignant cells and

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provide lasting immune surveillance against the malignancy, the graft-versus-leukemia (GVL) effect. However, when nonhematopoietic tissues of the patient are recognized, graft-versus-host disease (GVHD) may develop [5,6].

Allo-immune responses are induced by presentation of antigens to functional alloreactive T cells. To become properly activated, naïve alloreactive T cells require costimulatory signals from activated professional antigen-presenting cells (APCs). Important factors influencing the balance between donor- and patient-derived alloimmunologic pressure include greater genetic disparity between patient and donor encoding more antigens that can induce alloreactivity [7,8]. Activation of professional APCs by tissue damage may increase the risk of GVHD after more toxic myeloablative (MA) compared with less toxic nonmyeloablative (NMA) conditioning regimens [9]. The risk of GVHD decreases over time as the professional patient APCs are gradually replaced by donor-derived APCs [10]. Finally, the recovery of regulatory T cells and de novo lymphopoiesis after transplantation may lead to a state of tolerance [11,12].

Several strategies to modulate the alloimmunologic pressure after alloSCT have been developed. Most patients receive prophylactic systemic immunosuppression with or without a form of T cell depletion (TCD) to prevent rejection and GVHD [13]. The impact of TCD on patient- and/or donor-derived T cells depends on the method (in vivo versus in vitro) and timing (before or after alloSCT) [14–18]. Excessive suppression of donor-derived alloimmunologic pressure against the patient immune cells, HSCs, and tumor cells favors their persistence and eventual dominance, with the risk of graft failure and/or recurrence of the malignancy [19–21]. To improve engraftment or boost the GVL effect, unmodified donor lymphocyte infusion (DLI) can be administered after alloSCT [22–25].

Our strategy of alemtuzumab-based TCD alloSCT followed by standard prophylactic DLI [26] aims to separate the establishment of donor hematopoiesis from the introduction of donor alloreactivity. The delayed introduction of donor alloreactivity allows the induction of a GVL effect without a high risk of GVHD necessitating systemic treatment. In this context, TCD permits analysis of BM repopulation in the absence of strong alloimmunologic pressure. Obviation of the need for prophylactic pharmacologic immunosuppression facilitates analysis of natural immunologic recovery. DLI is administered starting 3 months after alloSCT after the competitive repopulation of the BM and early T cell expansion have taken place. This setting offers the unique opportunity to exclusively analyze the impact of donor alloreactivity introduced by DLI on persisting patient-derived HSCs and T cells. In a cohort of 281 patients, we examined lymphohematopoietic recovery and chimerism kinetics in the BM and circulating immune cells in relation to conditioning and GVHD after TCD alloSCT and DLI.

METHODS

Study Population

This observational study included all adult patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia, or myelodysplastic syndrome in complete morphologic remission after intensive induction therapy who underwent a first BM or G-CSF-mobilized peripheral blood stem cell alloSCT using a standard conditioning and TCD protocol [27–29] at Leiden University Medical Center between 2005 and 2015. Exclusion criteria were use of a haploidentical donor or prescheduled post-transplantation chemotherapy. The study was approved by the Medical Ethical Committee of Leiden University Medical Center (P03.114, P03.173, and P04.003). All patients provided signed informed consent for data collection and analysis. Data were analyzed as of August 2020.

Transplantation and DLI Strategy

MA conditioning consisted of cyclophosphamide (60 mg/kg i.v. for 2 days) with 9 Gy total body irradiation or busulfan (4 × .8 mg/kg i.v. for 4 days). NMA conditioning consisted of fludarabine (50 mg/m^2 orally for 6 days) and busulfan (4 × .8 mg/kg i.v. for 2 days).

Standard in vitro TCD was performed by adding 20 mg of alemtuzumab (Sanofi Genzyme) to the graft prior to infusion [28]. Additional in vivo TCD depended on donor type and conditioning regimen. MA conditioned patients with a 10/10 HLA-matched related donor (RD) did not receive any in vivo TCD; all other patients received 15 mg alemtuzumab i.v. on days -6 and -5 for MA conditioning or on days -4 and -3 for NMA conditioning. NMA-conditioned patients with an unrelated donor (UD) also received rabbit antithymocyte globulin (Sanofi Genzyme) on day -2 (2 mg/kg until April 2010 and 1 mg/kg thereafter). Only MA-conditioned patients with an UD or a 9/10 HLA-matched RD received cyclosporine as GVHD prophylaxis, which was tapered from 1 month and stopped within 3 months post-transplantation.

Preemptive DLI was administered for increasing or persisting mixed chimerism (MC) or the presence of minimal residual disease (MRD), whereas prophylactic DLI was administered regardless of chimerism or MRD status to all patients without clinically significant GVHD indicating alloimmunologic pressure. Preemptive DLI was administered at escalating doses with ≥3 months between infusions until the development of GVHD or disappearance of MRD and/or MC. Prophylactic DLI was introduced in May 2010 and administered to all patients at 6 months post-transplantation in the absence of clinically significant GVHD or relapse (3M T cells/kg for patients with RD and 1.5M T cells/kg for patients with UD). Forty-two patients at high risk of early relapse (within 6 months post-alloSCT) also received prophylactic low-dose DLI (0.3M and 0.15M T cells/kg for RD and UD, respectively) at 3 months [30]. Indications for this early prophylactic DLI were very poor risk AML or high-risk acute lymphoblastic leukemia according to the HOVON criteria [31,32], MRD positivity at time of transplantation, incomplete pretransplantation treatment, therapy-related AML with unfavorable karyotype, AML with persisting underlying disease, and acute leukemia that relapsed early after the previous curative induction chemotherapy. Along with the unmodified DLI, patients could receive modified (purified or genetically modified) T cell products as part of several clinical trials. Interferon could be administered to patients with an increasing number of blasts in the BM (but morphologically below 5%) or with MRD not responding to DLI.

Follow-Up

BM cytology, lymphocyte counts, and BM chimerism analysis were performed at least every 3 months during the first 2 years post-transplantation. In a subset of patients, chimerism was also determined in granulocytes, monocytes, CD4$^+$T cells, CD8$^+$T cells, natural killer cells, and B cells on peripheral blood (Supplementary Data, Methods).

Because we were interested in the kinetics of chimerism following TCD alloSCT and unmodified DLI, we excluded all chimerism and cell count measurements obtained 1 week or longer after infusion of a modified T cell product. Measurements obtained after relapse, interferon administration, chemotherapy, and second alloSCT were excluded as well. Measurements used in the comparison analyses were performed at 6 weeks (actual range, 5 to 7 weeks), 3 months (range, 2 to 4 months), and 6 months (range, 5 to 7 months) after alloSCT. For the clinical outcomes, all patients, including those who received a modified T cell product, were included to assess the overall outcome of our total strategy and to prevent selection bias, as recruitment for trials with modified T cell products started a few weeks after alloSCT.

Definitions

Neutrophil recovery was defined as the first of 3 consecutive measurements with an absolute neutrophil count >.5 × 10^9/L, and nonengraftment was defined as detection of <5% donor BM chimerism without prior neutrophil recovery. The date of relapse was defined as the date of the first recurrence of ≥5% blasts on cytomorphologic BM examination or ≥1% blasts in peripheral blood after alloSCT (confirmed by BM biopsy if possible). Clinically significant GVHD was defined by therapeutic systemic immunosuppression (tIS) for GVHD for at least 14 days or until death or stopped as part of palliative care due to refractory GVHD, or continued use of GVHD prophylaxis beyond 3.5 months. tIS was started for acute GVHD grade II-IV according to the modified Glucksberg criteria, for extensive chronic GVHD according to the Seattle criteria, and for mild GVHD not responding to topical treatment [33,34]. We used tIS instead of the exact grading because our strategy aims to prevent GVHD necessitating systemic treatment. MC was defined as detection of patient DNA at or above the limit of detection, and full donor chimerism (FDC) was defined as undetectable patient DNA. DLI-induced cytopenia was defined as the development of severe neutropenia (<.5 × 10^9/L) after DLI not caused by relapse or infection.

Study Objective and Endpoints

The objective of this study was to determine the relative contribution of competitive repopulation and donor-derived anti-recipient alloimmunologic pressure on the establishment of full-donor lymphohematopoietic chimerism after alloSCT. DLI-induced increase in chimerism and development of GVHD were analyzed as complementary indicators

Table 1
Baseline Characteristics of the Total Cohort and Subgroups Based on Conditioning Intensity and Donor Type

Characteristic	Total (N = 281)	MA, Matched RD (N = 78)	MA, UD or Mismatched RD (N = 99)	NMA, Matched RD (N = 41)	NMA, UD (N = 63)
Age at alloSCT, yr, median (range)	50 (18-73)	43 (18-60)	42 (19-59)	61 (28-72)	63 (40-73)
Disease, n (%)					
AML	188 (67)	47 (60)	56 (57)	33 (80)	52 (83)
Acute lymphoblastic leukemia	76 (27)	26 (33)	39 (39)	5 (12)	6 (10)
Myelodysplastic syndrome	17 (6)	5 (6)	4 (4)	3 (7)	5 (8)
Conditioning regimen, n (%)					
Cyclophosphamide/total body irradiation	171 (61)	76 (97)	95 (96)	0 (0)	0 (0)
Cyclophosphamide/busulfan	6 (2)	2 (3)	4 (4)	0 (0)	0 (0)
Fludarabine/busulfan	103 (37)	0 (0)	0 (0)	41 (100)	62 (98)
Fludarabine/busulfan/cyclophosphamide*	1 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Donor type, n (%)					
10/10 matched RD	119 (42)	78 (100)	0 (0)	41 (100)	0 (0)
9/10 matched RD	2 (1)	0 (0)	2 (2)	0 (0)	0 (0)
10/10 matched UD	120 (43)	0 (0)	63 (64)	0 (0)	57 (90)
9/10 matched UD	39 (14)	0 (0)	33 (33)	0 (0)	6 (10)
8/10 matched UD	1 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Graft source, n (%)					
G-CSF-mobilized PBSCs	266 (95)	69 (88)	94 (95)	41 (100)	62 (98)
BM	15 (5)	9 (12)	5 (5)	0 (0)	1 (2)
Period of alloSCT†					
First transplantation in cohort	1/20/2005	1/20/2005	3/10/2005	8/14/2008	10/6/2009
Before May 2010, n (%)	87 (31)	35 (45)	37 (37)	9 (22)	6 (10)
Since May 2010, n (%)	194 (69)	43 (55)	62 (63)	32 (78)	57 (90)

PBSC indicates peripheral blood stem cells.

* One patient received cyclophosphamide 750 mg/m^2 i.v. for 2 days in the conditioning regimen because a second consolidation course before transplantation was not given.

† Prophylactic DLI has been included in the transplantation strategy since May 2010.

for donor-derived anti-recipient alloimmunologic pressure. The primary endpoint was the level of donor BM and T cell chimerism at 6 weeks and 3 and 6 months after alloSCT. Secondary endpoints were primary engraftment, clinically significant GVHD as a surrogate for alloimmunologic pressure, and BM chimerism kinetics during the first 2 years after DLI. Other secondary endpoints were overall survival, relapse-free survival, cumulative incidence of relapse, and non-relapse mortality during the first 5 years after alloSCT.

Analyses

An algorithm was developed to assess the chimerism response after the first unmodified DLI that patients received while having mixed BM chimerism. We defined this DLI as the "starting DLI" for this analysis (Supplementary Data, Methods).

The probabilities of overall survival and relapse-free survival from alloSCT with 95% confidence intervals (CIs) were calculated using the Kaplan-Meier method. Follow-up from alloSCT was quantified using the reverse Kaplan-Meier method [35]. Cumulative incidences of neutrophil recovery as proxy for primary engraftment and clinically significant GVHD were calculated using competing-risks models (Supplementary Data, Methods).

To evaluate the effects of donor-derived alloimmunologic pressure and DLI on BM repopulation and immunologic recovery, donor chimerism in the BM and T cells was evaluated at 3 and 6 months after alloSCT and compared between groups based on whether patients had developed clinically significant GVHD, had received unmodified DLI without any clinically significant GVHD, or had neither. Because chimerism levels did not follow a normal distribution, groups were compared using the Mann-Whitney *U* test (2 groups) or Kruskall-Wallis test followed by, if applicable, the post hoc Dunn test with Holms adjustment for multiple comparisons (>2 groups). An (adjusted) *P* value <.05 was considered significant.

Software

All analyses were performed in R version 4.0.2 using the survival, cmprsk, prodlim, rstatix, ggplot2, ggpubr, gridExtra, and ggalluvial packages.

RESULTS

Population

A total of 281 patients were included in this study. The patients' baseline characteristics are summarized in Table 1. The median follow-up was 61 months (interquartile range [IQR], 43 to 85 months) after alloSCT. The clinical outcomes of our total strategy of TCD alloSCT followed by DLI are presented in Supplementary Data, Results.

Successful Primary Engraftment after TCD AlloSCT Does Not Depend on MA Conditioning or Donor-Derived Alloimmunologic Pressure

The cumulative incidence of neutrophil recovery was 91% (95% CI, 88% to 94%) at 4 weeks after alloSCT and increased to 99% (95% CI, 97% to 100%) at 2.5 months (Supplementary Figure S3). One patient, who underwent transplantation after MA conditioning, failed to engraft. Two patients died, at 2 and 12 days after alloSCT, before (non)engraftment. Successful engraftment of all 103 evaluable NMA-conditioned patients demonstrates sufficient suppression of the patient immune cells by alemtuzumab, in combination with antithymocyte globulin in case of an UD, to prevent graft rejection.

To evaluate whether strong donor-derived alloimmune responses after alloSCT had a profound role in the primary engraftment in this cohort, we examined the development of clinically significant GVHD before any DLI after alloSCT in the 278 engrafted patients. At 3 months after alloSCT, the cumulative incidence of clinically significant GVHD was 13% (95% CI, 9% to 17%) in the total cohort and only 2% (95% CI, 0 to 5%) after NMA conditioning (Supplementary Figure S4). Together with the 99% probability of engraftment, these data show that

primary engraftment after alemtuzumab-based TCD alloSCT was not impaired after MA or NMA conditioning and in the absence of clinically significant GVHD.

MC Is More Common in the T Cell Compartment Than in the BM

Because engraftment does not necessarily lead to persistent complete (100%) donor-derived hematopoiesis, we investigated chimerism kinetics in the 278 engrafted patients. Among the 223 patients alive without any prior cellular intervention, chemotherapy, interferon, or relapse and with evaluable BM chimerism at 3 months after alloSCT, 59% had FDC. Within the group with MC, the middle 50% (ie, the IQR) had donor chimerism levels between 94% and 98%. To investigate whether the circulating immune cells also were of mixed origin after TCD alloSCT, we measured the level of donor chimerism in 6 immune cell types in a subset of patients, again excluding all samples after cellular intervention, chemotherapy, interferon, and relapse (Supplementary Figure S5; Supplementary Figure S6 and Supplementary Table S2 provide the cell counts in the total cohort). At 3 months after alloSCT, 73% to 78% of the patients showed FDC in the granulocytes, monocytes, natural killer cells, and B cells. The IQR of the donor chimerism values with MC in these cells ranged between 96% and 99%. In contrast, only 22% and 28% of the patients showed FDC in the $CD4^+$ and $CD8^+$T cell populations, respectively, and the IQR of donor chimerism within T cells with MC was 7% to 92%. Even in patients with complete donor-derived hematopoiesis, circulating T cells could be predominantly of patient origin at 3 months post-alloSCT (Figure 1).

Associations between Conditioning Intensity and Clinically Significant GVHD and BM and T Cell Chimerism at 3 Months Post-AlloSCT

Although primary engraftment after TCD alloSCT was not affected by conditioning intensity or donor-derived alloimmunologic pressure, these factors could influence the level of donor chimerism. To investigate the influence of conditioning intensity and alloimmunologic pressure on the development of complete donor-derived hematopoiesis, we compared BM chimerism at 3 months after alloSCT between patient groups defined by conditioning intensity and development of clinically significant GVHD prior to measurement of chimerism (Figure 2A). In the absence of clinically significant GVHD, MA-conditioned patients had significantly higher donor BM chimerism (71% of the patients had FDC) compared to NMA-conditioned patients (32% FDC), showing that MA conditioning promoted the establishment of complete donor-derived hematopoiesis after alloSCT. To evaluate the effect of GVHD on the development of complete donor-derived hematopoiesis, we compared donor BM chimerism between MA-conditioned patients with and those without clinically significant GVHD. Patients with clinically significant GVHD had higher donor BM chimerism at 3 months after alloSCT compared with those without (88% versus 71% FDC; adjusted P= .12).

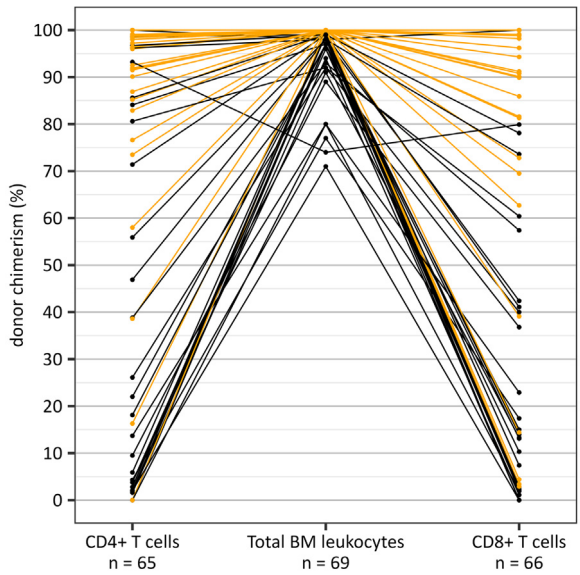

Figure 1. Donor chimerism in the BM and T cells at 3 months. Donor chimerism in the BM, $CD4^+$, and $CD8^+$ T cells at 3 months post-alloSCT without any prior cellular intervention, relapse, chemotherapy, or interferon. Lines corresponding to patients with FDC in the BM are highlighted in orange.

To investigate the influence of conditioning intensity and clinically significant GVHD on T cell chimerism, we compared the level of donor chimerism in $CD4^+$ and $CD8^+$T cells at 3 months between the same groups for all patients with available T cell chimerism (Figure 2B,C). In the absence of clinically significant GVHD, donor chimerism in $CD4^+$T cells and $CD8^+$T cells was significantly higher in MA-conditioned patients (33% had FDC in $CD4^+$ T cells and 41% had FDC in $CD8^+$T cells) compared with NMA-conditioned patients (7% and 12%, respectively). In the MA-conditioned group, there was no significant difference in the level of donor chimerism between patients with and those without clinically significant GVHD: 43% versus 33% had FDC in $CD4^+$T cells (adjusted P = .37) and 43% versus 41% had FDC in $CD8^+$T cells (adjusted P = .74). Together, these data indicate that myeloablative conditioning led to higher donor T cell chimerism after TCD alloSCT, but we did not find a significant effect of clinically significant GVHD on the level of $CD4^+$ or $CD8^+$T cell chimerism at 3 months after alloSCT. This may be explained by the immunosuppressive treatment that almost all patients with GVHD still were receiving at the time of chimerism measurement.

Donor Chimerism in the BM and T Cells Increases after Early DLI

Starting from 3 months, prophylactic and preemptive DLI was administered to induce an alloimmune response against patient-derived hematopoietic cells. To investigate the impact of this alloimunologic pressure by early DLI in the absence of GVHD, we compared donor BM chimerism between 3 and 6 months after alloSCT in patients who received unmodified DLI within 4 months after alloSCT but without any clinically significant GVHD up to 6 months, and in patients without any DLI or GVHD in this period. Of the 71 evaluable patients (51% NMA-conditioned) without any DLI or GVHD during this period, 66% showed FDC at 3 months and 61% did so at 6 months, illustrating that in absence of donor-derived alloimmunologic pressure, mixed BM chimerism remained prevalent after TCD alloSCT. Thirty patients received unmodified DLI within 4 months after alloSCT, 30% after NMA conditioning. Notably, although only 38% of these patients showed FDC in the BM at 3 months after alloSCT, this percentage increased to 63% at 6 months after alloSCT, indicating that early unmodified DLI could increase donor BM chimerism without the concomitant development of GVHD.

To evaluate the impact of early DLI on donor T cell chimerism, we investigated the kinetics of donor T cell chimerism during the first 6 months after alloSCT in patients without any DLI or GVHD during this period and in patients who received DLI (Figure 3). Again, the 18 evaluable patients without any DLI or GVHD showed a stable pattern of MC, and almost all

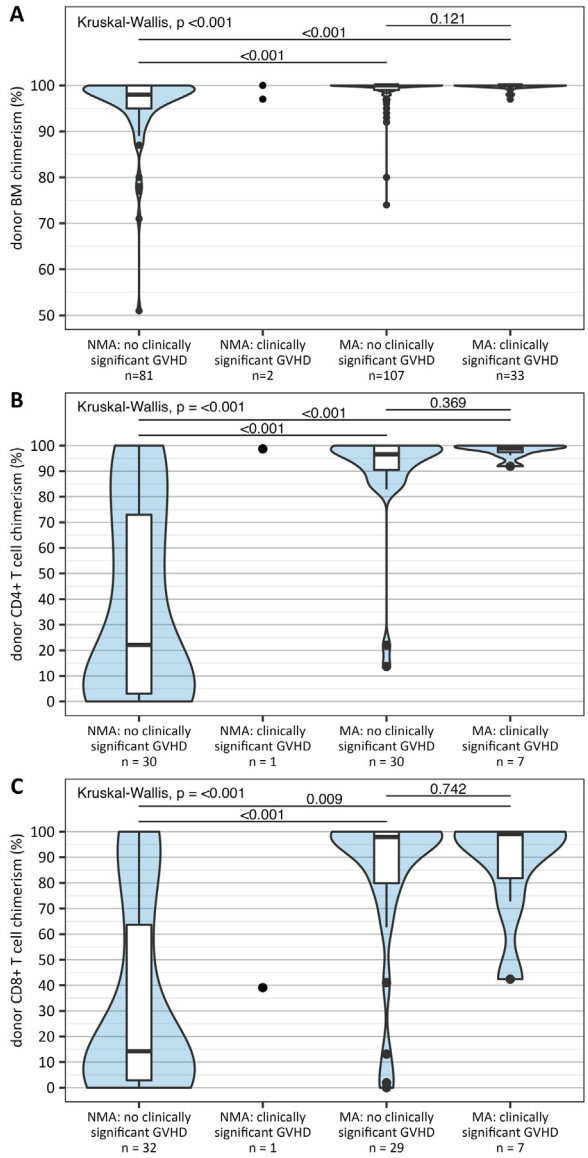

Figure 2. Donor chimerism in the BM and T cells at 3 months according to conditioning regimen intensity and the development of GVHD before the measurement. Donor chimerism in the BM (A), CD4+ T cells (B) and CD8+ T cells (C) at 3 months after TCD alloSCT without any prior cellular intervention, relapse, chemotherapy or interferon. T cell chimerism was measured in a subset of patients. In 7 patients, either the $CD4^+$ or the $CD8^+$ fraction was missing. In all panels, the data are grouped based on conditioning intensity and development of clinically significant GVHD before the 3-month measurement. The boxplots are combined with violin plots showing the kernel probability density, to visualize the distribution of the data. The lower and upper hinges of the boxplots correspond to the 25th and 75th percentiles, respectively. In each panel, the level of donor chimerism was compared among 3 groups, as the 2 NMA-conditioned patients with GVHD were excluded from this test. The *P* values for the pairwise comparisons are adjusted for multiple comparison.

patients with an early DLI without any clinically significant GVHD (n = 8) showed increasing levels of donor $CD4^+$ and $CD8^+$T cell chimerism. To test whether these kinetics led to significant differences in the level of donor T cell chimerism at 6 months after alloSCT and to compare the impact of DLI with the impact of clinically significant GVHD without DLI on chimerism, we compared the levels of 6-month donor chimerism in $CD4^+$ and $CD8^+$T cells between these 2 patient groups, adding a third group comprising the 12 evaluable patients who developed clinically significant GVHD before the 6-month measurement, all without any prior DLI (Figure 4A,B). $CD4^+$T cell donor chimerism was significantly higher in patients with DLI (75% FDC) or clinically significant GVHD (83%) compared to patients without any DLI or GVHD (24% FDC). $CD8^+$T cell chimerism showed a similar trend, with 33% of the patients without any DLI or GVHD having FDC, compared to 50% of those with DLI and 55% of those with clinically significant GVHD. Patients with DLI or clinically significant GVHD had both a lower number of circulating patient-derived T cells and a higher number of donor-derived T cells (Figure 4C,D). Together, these data show that 6-month donor $CD4^+$T cell chimerism is significantly higher in patients with GVHD than in those without GVHD, and that early DLI can increase the level of donor T cell chimerism in the absence of GVHD.

Strategy of Dose-Escalating DLI Can Convert Mixed Hematopoiesis to Durable Complete Donor-Derived Hematopoiesis without Necessarily Inducing Clinically Significant GVHD

To investigate the alloimmunologic effects of our total DLI strategy, we developed an algorithm to quantify BM chimerism responses in the 65 patients with mixed hematopoiesis receiving unmodified DLI without any prior relapse (Methods). Clinical outcomes of all patients who received an unmodified DLI are presented in the Supplementary Data, Results. The median level of donor BM chimerism in these patients at time of the starting DLI was 98% (IQR, 94% to 99%) (Figure 5A). In 7 patients, the BM chimerism response could not be evaluated because of early death from severe GVHD after DLI (n = 4) or early relapse (n = 3) (Figure 5B, Supplementary Table S3). Within 2 months after starting DLI, 15 of the 65 patients (23%) converted to FDC and 9 (14%) showed a partial response with decreasing patient chimerism. Over time and with our dose-escalating DLI protocol, the numbers of patients with a response increased: 38 (58%) showed a response within 4 months and 46 (71%) did so within 7 months after starting DLI. At 25 months, 47 patients (72%) had converted to FDC, including 35 after 1 DLI, and 5 (8%) had shown a partial response. Six patients (9%) with available BM chimerism measurements after DLI did not show any response within this period, of whom 3 relapsed and 2 died within 25 months after the starting DLI. Only 1 patient completed the 25-month follow-up period without showing any chimerism response. Notably, this patient eventually converted to FDC in the BM at 29 months after the starting DLI, 6 months after the fourth DLI.

After complete conversion, 4 of the 47 patients died and 4 relapsed within 25 months after starting DLI. The other 39 patients (83%) with complete conversion were still alive and in complete remission at 25 months after DLI. Only 1 patient occasionally had some detectable patient DNA (Supplementary Figure S7).

To study whether GVHD is required for conversion to complete donor-derived hematopoiesis after DLI, we evaluated the development of clinically significant GVHD in the 47 patients with conversion from MC to FDC and found that 16 (34%) developed clinically significant GVHD within 25 months after starting DLI and 31 (66%) did not (Figure 5C). Together, these data show that our DLI strategy led to durable complete donor-derived hematopoiesis in the majority of the patients with mixed hematopoiesis receiving DLI after TCD alloSCT, without necessarily inducing clinically significant GVHD.

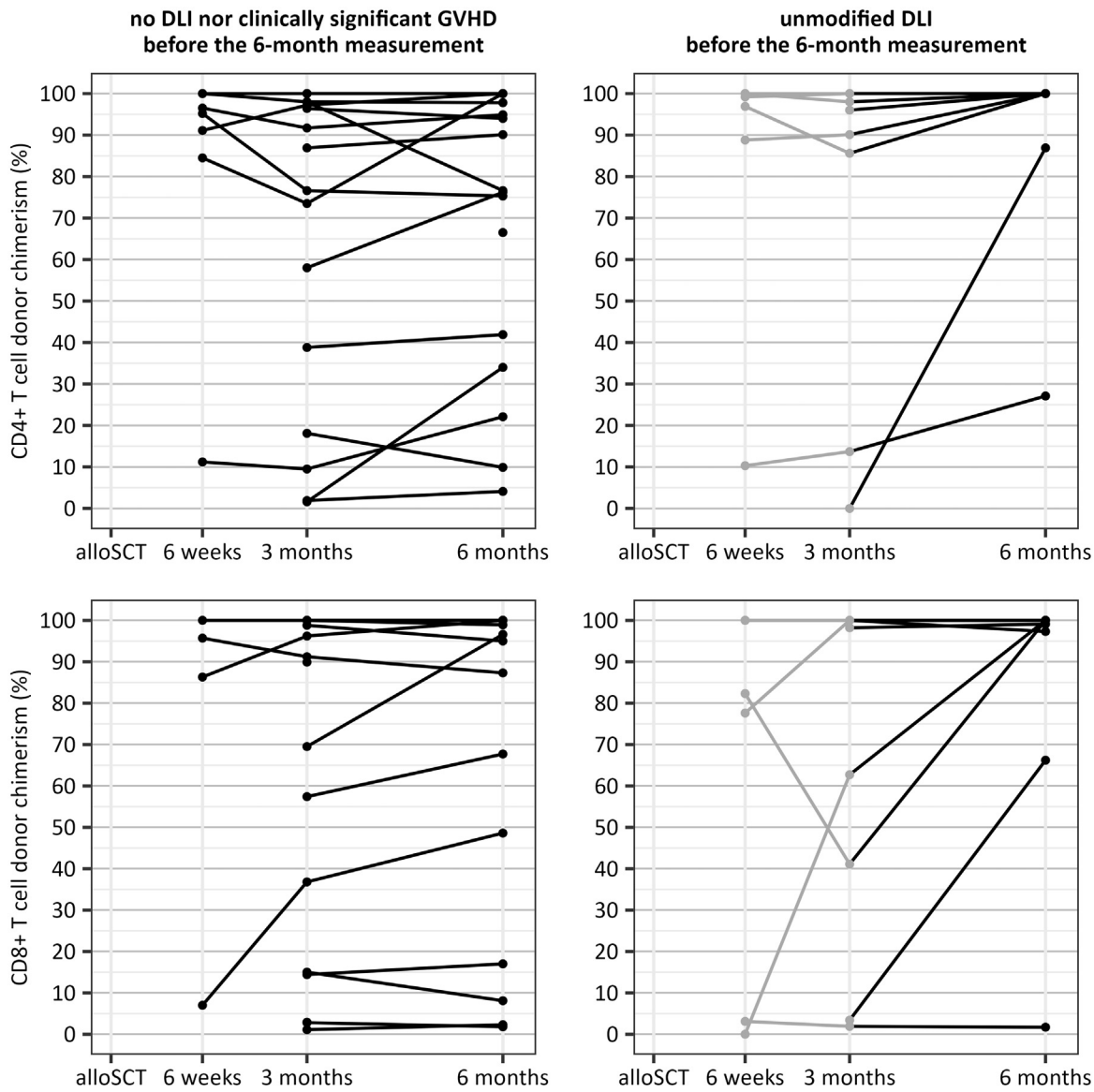

Figure 3. T cell chimerism kinetics during the first 6 months after TCD alloSCT in patients without any clinically significant GVHD nor DLI before the 6-month measurement and in patients who received an unmodified DLI before the 6-month measurement. Patients who died, relapsed, or received chemotherapy, interferon, a second alloSCT, or a modified T cell product before the 6-month measurement were excluded. In the second column, the chimerism measurements done before DLI are in gray, and the measurements done after DLI are in black, to visualize the impact of DLI on the level of donor T cell chimerism.

DISCUSSION

In this study, we leveraged a strategy of TCD alloSCT followed by standard prophylactic DLI to investigate how competitive repopulation and alloimmunologic pressure influence the lymphohematopoietic recovery after alloSCT. The sequential introduction of donor hematopoiesis and alloreactivity enabled us to study these mechanisms separately, which is not possible in T cell-replete alloSCT or TCD alloSCT without standard DLI. Effective suppression of the patient-derived alloimmunity by the conditioning regimens sufficed for sustained engraftment without the need for myeloablative conditioning or evident donor-derived alloimmunologic pressure. However, the development of complete donor-derived hematopoiesis depended on both competitive repopulation and alloimmunologic pressure; the proportion of patients with FD BM chimerism at 3 months was lowest in the NMA-conditioned patients without any GVHD (32%), higher in the MA-conditioned patients without any GVHD (71%), and highest in the MA-conditioned patients who had developed GVHD (88%). In patients without GVHD, an alloimmune response against patient-derived hematopoietic cells could be efficiently induced by DLI even in the absence of concomitant GVHD. Following our total strategy of dose-escalating DLIs, 72% of the patients with mixed BM chimerism at time of DLI converted to complete donor-derived hematopoiesis. Only 34% of converting patients developed clinically significant GVHD after DLI, illustrating that the GVL effect can be separated from GVHD. For the establishment of a completely donor-derived T cell compartment, some alloimmunologic pressure seemed to be required.

Although the level of donor chimerism in CD4+ and CD8+ T cells at 3 months was higher after MA than NMA conditioning, only 33% and 41% of the MA conditioned patients without any GVHD had FDC in these subsets, respectively. However, 83% of the patients who had developed GVHD, and 75% of the patients who had received an early DLI without developing any GVHD,

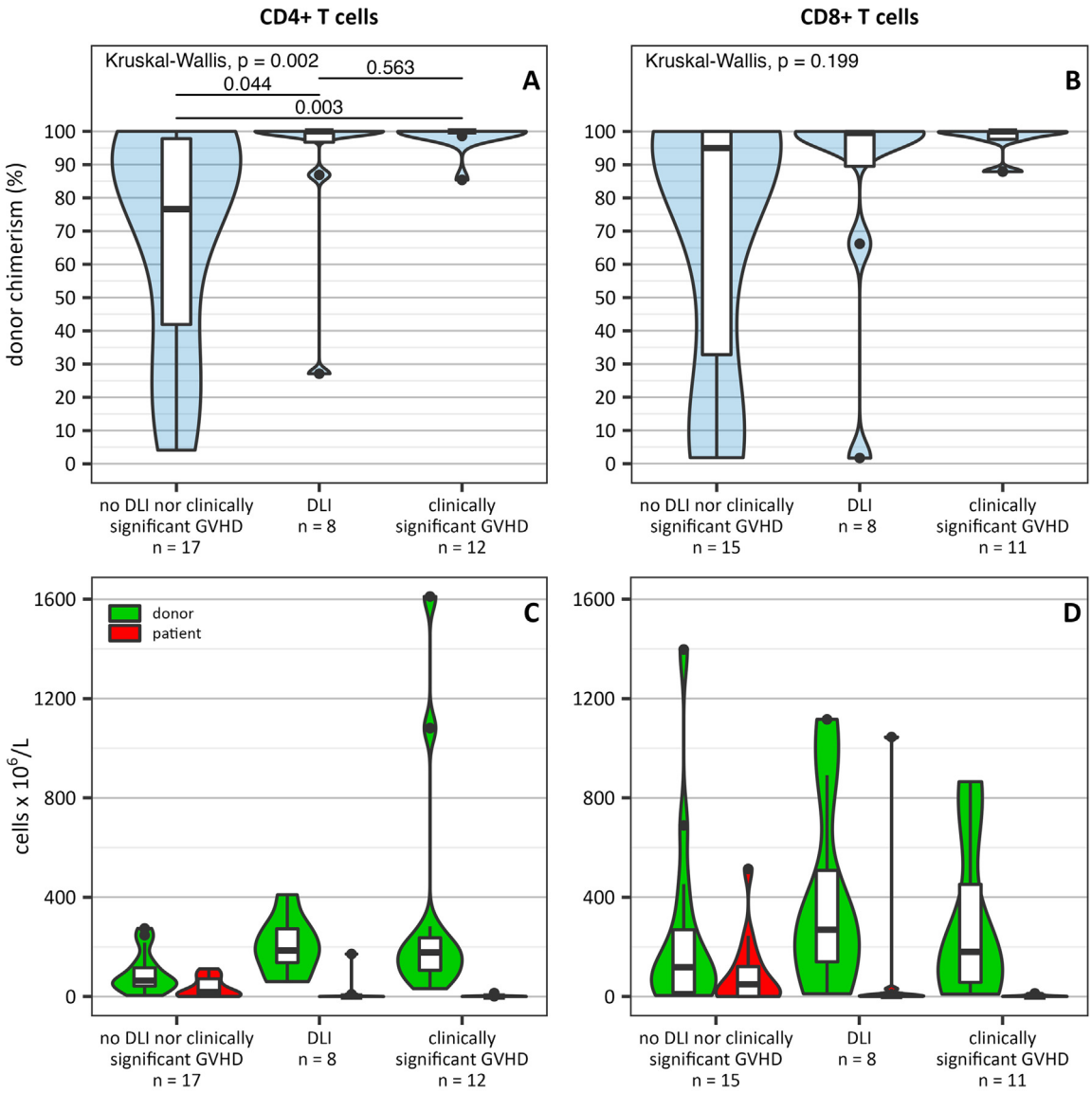

Figure 4. T cell chimerism and patient/donor-specific counts at 6 months in patients with DLI, GVHD or neither. $CD4^+$ and $CD8^+$ T cell chimerism (A and B) and patient/donor-specific counts (C and D) at 6 months after alloSCT in patients without prior DLI or clinically significant GVHD, patients with DLI before this measurement, and patients who had developed clinically significant GVHD after alloSCT without prior DLI. Patients who relapsed or received chemotherapy, interferon, a second alloSCT, or a modified T cell product before the 6-month measurement were excluded. The *P* values for the pairwise comparisons in (A) are adjusted for multiple comparison.

had FDC in the $CD4^+$T cells at 6 months after alloSCT, compared to 24% of the patients without prior DLI or GVHD, showing that DLI also could convert mixed T cell chimerism to FDC in the absence of GVHD. Together, these data indicate that the establishment of complete donor-derived hematopoiesis can be the result of competitive repopulation, but that donor-derived alloimmunologic pressure is needed for the development of FD T cell chimerism.

Because competitive repopulation can be sufficient to induce FD BM chimerism, the presence of FDC itself does not prove occurrence of an alloimmune response against patient hematopoietic cells or achievement of a meaningful GVL effect. This conclusion can explain why the value of FDC in predicting relapse remains controversial in different settings of alloSCT. For instance, Konuma et al. [36] did not observe any association between FDC in the BM and relapse after MA single-unit umbilical cord blood transplantation. Owing to the MA conditioning and the relatively low alloimmunologic pressure after cord blood transplantation [37], the achieved FDC might have been mainly the result of competitive repopulation. In contrast, Koreth et al. [38] showed that having <90% donor chimerism in the BM or peripheral blood increased the risk of relapse after T cell-replete alloSCT following NMA conditioning. As in this case, the competitive repopulation probably played a more limited role, FDC was more likely a result from donor-derived alloimmunologic pressure. The chimerism kinetics also can indicate whether alloreactivity played a role. Although FDC early after transplantation in the absence of GVHD may reflect the outcome of competitive repopulation, conversion from stable MC to FDC is most likely the result of an alloimmune response, leading to low relapse rates after chimerism conversion from MC to FDC, as observed in this study and as reported by others [39–41]. Therefore, not only the level of donor chimerism, but also the clinical setting and the

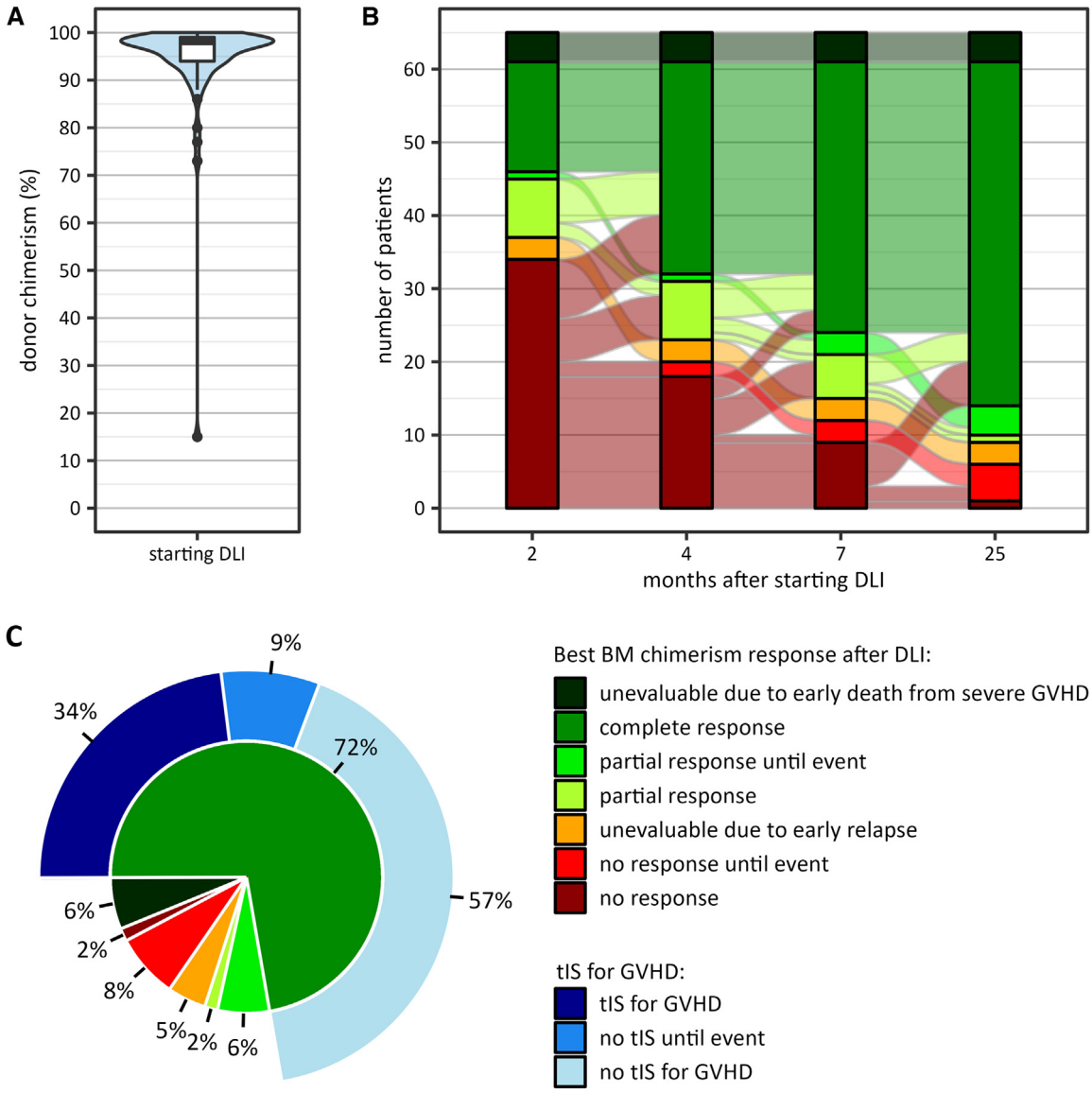

Figure 5. Alloimmune responses in the 65 patients with mixed hematopoiesis receiving unmodified DLI. Patients who relapsed or received chemotherapy, a second alloSCT, or a modified T cell product before DLI or who continued GVHD prophylaxis after DLI were excluded. (A) The level of donor BM chimerism at time of DLI initiation. (B) The best BM chimerism response achieved by different time points after the first unmodified DLI. The events that terminated the evaluation period (death, relapse, chemotherapy, interferon or second alloSCT) are described in Supplementary Table S3. Note that recurrence of MC or terminating events occurring after a response are not shown in this plot. The current response, which considers these possibilities, is shown in Supplementary Figure S7. (C) Distribution of the best BM chimerism responses at 25 months after initiation of DLI (inner circle) and the use of tIS for GVHD by the converted patients during this period (outer ring). Five of these patients did not start tIS for GVHD but relapsed (n = 4) or died (n = 1) during this period.

chimerism kinetics, should be considered when using chimerism to monitor the presence of donor-derived alloimmunologic pressure and thereby the GVL effect in patients.

Because a conversion to FDC can be observed only in patients who have MC to start with, the ideal cell lineage for monitoring alloreactivity needs to show stable levels of MC after alloSCT in the majority of patients. Owing to the persistence of long-living patient-derived T cells, mixed T cell chimerism is common after TCD alloSCT, and can exist in patients with a completely donor-derived hematopoiesis [41–44]. Therefore, changes in T cell chimerism potentially could be used as marker for alloreactivity more often than BM chimerism. Applicability depends on the transplantation strategy. For instance, mixed T cell chimerism is common after $CD34^{+}$-selected alloSCT [46], whereas Carnevale-Schianca et al. [45] observed 97% FDC at 28 days after MA alloSCT with post-transplantation cyclophosphamide.

An important question is whether DLI can induce a sufficient GVL effect without needing to induce clinically significant GVHD as well. In concordance with our data, others have shown that conversion from MC to FDC can occur in the absence of GVHD after DLI, and that this conversion significantly decreases the risk of relapse [24,41,47,48]. The accumulating evidence that DLI can be effective in preventing relapse even without the induction of GVHD encourages further investigation into how the risk of GVHD after DLI can be decreased without losing the beneficial GVL effect. Several DLI modification strategies are being investigated that either remove cell subsets that are important for the development of GVHD (eg, depletion of $CD8^{+}$T cells) or select only immune cells that

target hematopoietic cells [49]. The toxicity of unmodified DLI can be reduced by administering prophylactic immunosuppression around DLI or by decreasing the initial DLI dose for patients with a higher risk of severe GVHD [50].

In conclusion, we examined how the fundamental processes of BM repopulation and alloimmunologic pressure shape the lymphohematopoietic recovery after TCD alloSCT and DLI. The suppression of the patient-derived alloimmunologic pressure by the conditioning suffices for sustained engraftment without requiring intensive myeloablation or donor-derived alloimmunologic pressure. We show that competitive repopulation can be sufficient to reach complete donor-derived hematopoiesis, but that some alloimmunologic pressure is needed for the establishment of a completely donor-derived T cell compartment, either by the development of GVHD or by administration of DLI. We illustrate that it is possible to separate GVL from GVHD, as conversion to durable complete donor-derived hematopoiesis following DLI did not require the induction of clinically significant GVHD.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.12.022.

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