# Donor lymphocyte infusions after haploidentical stem cell transplantation with PTCY: a study on behalf of the EBMT cellular therapy & immunobiology working party

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EBMT

ARTICLE

# Donor lymphocyte infusions after haploidentical stem cell transplantation with PTCY: A study on behalf of the EBMT cellular therapy & immunobiology working party

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Donor lymphocyte infusion (DLI) is a treatment option to prevent or treat relapse after allogeneic hematopoietic cell transplantation (HCT). We here report data for 173 patients who received one or multiple DLIs after haploidentical-HCT with post-transplant cyclophosphamide (PTCY) at 47 EBMT centers from 2009 to 2018. Indication for DLI was: prophylactic for 59 (34.3%), preemptive for 20(11.6%), and therapeutic for 93(54.1%). For the prophylactic group, the median number of DLIs was 1 (IQR:1–2.5) with a median first dose of $0.1 \times 10^6$ CD3+ T cell/kg, for the preemptive 2 (IQR:1–3) with $0.5 \times 10^6$ CD3+ T cell/kg, for the therapeutic 1 (IQR:1–3) with $1 \times 10^6$CD3+ Tcell/kg, respectively. OS after first DLI was 61% (46–75%) for prophylactic, 40% (19–61%) for preemptive, and 22% (13–31%) for therapeutic. CI of II-IV aGVHD and cGVHD was 17% (7–27%) and 53% (40–67%) for the prophylactic, 20% (2–38%) and 21% (3–39%) for the preemptive, 17% (9–24%) and 24% (15–33%) for the therapeutic group, respectively. Our data show great variability in the indications and modalities of DLI across responding EBMT centers. Survival rates remain relatively low in patients with active disease. While the cumulative incidence of aGVHD appears acceptable, we showed a high incidence of cGVHD in the prophylactic group, compared with preemptive and therapeutic DLI. These data should be investigated further in prospective clinical trials.

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## INTRODUCTION

Relapse after allogeneic hematopoietic cell transplantation (HCT) remains a major therapeutic challenge and the combination of prophylactic strategies and preemptive intervention could increase survival in patients with high-risk disease [1]. Unmanipulated donor lymphocytes infusion (DLI) has been the first cellular immunotherapy used to tackle relapse, taking advantage of the graft versus leukemia (GVL) effect elicited by donor lymphocytes. DLI has been extensively used since the 1990s in the setting of HLA-matched transplants from sibling donors, showing durable remissions with variable efficacy depending on the type and burden of disease [2–5] In the last years, the use of non T-cell depleted haploidentical stem cell transplantation (haplo-HCT) with post-transplant cyclophosphamide (PTCY) has rapidly increased worldwide, producing comparable clinical outcomes to HLA-matched unrelated donor HCTs in several retrospective studies [6]. The potentially increased antitumor effect [7] and the easily manageable collection of lymphocytes from the donor make this procedure very interesting in the setting of unmanipulated haplo-HCT. Huang et al [8]. firstly tested the efficacy and safety of DLI in patients who relapsed after haplo-HCT using granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells and short-term immunosuppression [8–10]. Data on DLI use in the setting of haplo-HCT using PTCY [11–14] have been reported in single-center studies including few patients with heterogeneous hematological diseases. The optimal dosing and sequencing of DLIs remain largely unknown, with expected variations depending on whether DLI indication is prophylactic, to prevent relapse in high-risk patients, pre-emptive to treat minimal residual disease, or curative to treat clinical or hematological relapse. We

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performed a multicentric retrospective survey across EBMT centers with the aim to investigate the differences in the clinical practice and the outcomes of DLI in haplo-HCT using PTCY.

## PATIENTS AND METHODS

### Study design and definition

This is a multicenter retrospective analysis conducted on behalf of the Cellular therapy and Immunobiology Working party (CTIWP) of the EBMT. The EBMT is a non-profit, scientific society representing more than 600 transplant centers mainly in Europe that are required to report all consecutive stem cell transplantations and follow-ups at least once a year. Data are entered, managed, and maintained in a central database with internet access; each EBMT center is represented in this database. Audits are routinely performed in JACIE-accredited centers to determine the accuracy of the data. EBMT Centres commit to obtain informed consent according to the local regulations applicable at the time of transplantation in order to report pseudonymized data to the EBMT.

Eligibility criteria included all adults (⩾18 years) with hematological malignancies who received DLI after a non-T-cell depleted haplo-HCT using PTCY at EBMT transplant centers from 2009 to 2018. Data were collected through a study-specific questionnaire that was sent to all EBMT member centers with eligible patients or patients missing information regarding PTCY. The questionnaire included information about PTCY schedule, the reason for DLI, the timing from HCT, the schedule of administration of DLI (total number and doses/dose escalation), the time from discontinuation of GVHD prophylaxis to first DLI, and the chemotherapy treatment before DLI. 47 EBMT centers participated and returned the study-specific questionnaires with a total number of 173 patients.

Monitoring of patients for relapse/progression post-transplant was conducted according to the individual centers' protocols. The conditioning regimens were defined according to data reported by the centers as myeloablative (MAC), non-myeloablative (NMAC), or reduced intensity (RIC) based to the the EBMT definition [15]. DLI's were categorized by the center as: prophylactic, in high-risk patients without sign of disease recurrence; preemptive, in patients with minimal residual disease (MRD) positivity after haplo-HCT, or therapeutic, in patients with documented relapse/disease progression.

High-risk disease was defined as unfavorable karyotype or molecular marker at diagnosis, secondary malignancy, induction failure or > CR1, not in complete remission at transplantation. MRD was considered as any cytogenetic or molecular or phenotypic marker previously detected at diagnosis as reported by the centers. Chimerism analyses were performed by PCR amplification of post-transplant recipient DNA isolated from peripheral blood or bone marrow samples. Full donor chimerism was defined as reported by the centers according to their procedures.

### Endpoints

The primary endpoint was overall survival (OS). Secondary endpoints were disease-free survival (DFS) and cumulative incidences of acute GVHD (aGVHD), chronic GVHD (cGVHD), relapse (RI) non-relapse mortality (NRM), and complete remission (CR). OS was defined as the time to death from all causes. DFS was calculated as the time from first DLI to relapse or death, whichever occurred first. aGVHD was graded according to the modified Glucksberg criteria [16] and cGVHD according to the revised Seattle criteria [17]. Cumulative incidences of relapse, NRM, and CR were calculated from the date of DLI to the date of relapse, death in remission, or complete remission. For studying GVHD and CR, death was considered as a competing event.

### Statistical analyses

Median, interquartile range (IQR, 25th to 75th percentile), and range (minimum to maximum) were reported for continuous variables. Numbers and percentages were reported for categorical or binary variables. Intergroup comparisons were performed using Kruskal-Wallis and Chi-squared tests for continuous and categorical variables respectively. Analysis of OS /DFS was performed using Kaplan-Meier methods, analysis of aGVHD/cGVHD/RI/NRM/CR using competing risk methods. The analysis of DFS, RI, and NRM were only performed for patients who were not in relapse/progression at first DLI and part of either the prophylactic or preemptive group. As those in the therapeutic group by definition were in relapse or continuous progression at the time of first DLI, we assessed the cumulative incidence of CR after first DLI for this group with death as a competing event. Outcome probabilities are reported for 2 years, together with 95% confidence intervals. Median follow-up (median, IQR) was calculated using the reverse Kaplan-Meier methodology. Univariate comparisons of outcomes were done using log-rank test for OS and DFS, Gray's test for competing risk outcomes. Survival probabilities and cumulative incidence curves are presented as appropriate.

In order to assess whether time from first allogeneic transplant to first DLI (continuous variable), stem cell source, conditioning regimens (RIC/MAC/NMAC), and immunosuppression status (stopped vs. not stopped 1$^{st}$ DLI) are associated with aGVHD and cGVHD occurrence, cause-specific Cox proportional hazards models were employed. Variables were included in these multivariable models if they were conceptually important or if they approached or attained statistical significance in univariable analysis, leading to the following variables to be included: reason for DLI, disease at diagnosis, patient age at DLI (<40 vs. ≥40), donor age at DLI (continuous variable), disease stage at transplant (Other vs. CR1). With the aim to analyze the impact of repeated administration of DLI to the development of GVHD, cumulative incidences of aGVHD/cGVHD were calculated from 1st until 2nd DLI and from 2nd until 3rd DLI. For a fair comparison, only aGVHD/cGVHD events before a second DLI had to be included in the estimate after first DLI and only events before third DLI in the estimate after second DLI. To estimate this, next DLI and death were competing events in all these analyses. All the analyses were performed using SPSS 25 and R Version 4.1.0 (packages: survival, prodlim and cmprsk).

## RESULTS

### Patient and transplant characteristics

We were able to analyze 173 patients who received one or multiple DLIs after haplo-HCT with PTCY. The median follow-up from first DLI was 31.38 (IQR: 19.55–45.21) months. The majority of patients (65.4%) had a Karnofsky performance status (KPS) of ≥90% at transplant.

For 52 (30.1%) of the patients the disease status at transplant was first complete remission, 37 (21.4%) patients were in second complete remission or beyond; 25 patients (14.5%) were in partial remission and 59 (34.1%) had progressive disease (Table 1). The most common donor-patient relationships were sibling for 38.4%. Peripheral blood was the most frequently used source of stem cells (76.9%).

Conditioning regimens were myeloablative (MAC) for 63 (36.4%), non-myeloablative (NMAC) for 31 (17.9%) and at reduced intensity (RIC) for 79 (45.7%). 62 (35.8%) patients received total body irradiation (TBI).

In combination with PTCY, the GVHD prophylaxis regimens were mainly based on ciclosporin A+ mycophenolate mofetil (65.3%) or tacrolimus + mycophenolate mofetil (27.2%). No additional GVHD prophylaxis was given following DLI. Before DLI, 24.6% and 10.4% of patients experienced grade II-IV aGVHD and cGVHD respectively.

### DLI characteristics

According to transplant center indication, the reason for DLI administration was prophylactic for 59 (34.3%), preemptive for 20 (11.6%), and therapeutic for 93 (54.1%) patients (missing information for 1 patient).

In all three groups, treatments before DLI were extremely different by diagnosis and disease type. Patients with AML and MDS mainly received hypomethylating agents (azacytidine or decitabine) alone or in combination with systemic chemotherapy or targeted therapies (venetoclax, FLT3 ITD inhibitors). For ALL, treatment was mainly chemo-based and or associated with blinatumomab or TKI inhibitors. The majority of HL patients received brentuximab vedotin-based regimens.

In the prophylactic group ($n = 59$), the median age at first DLI was 55.2 years (IQR: 42.7–64.8). The most common diagnoses in this group were AML ($n = 31$, 52.5%) and MDS ($n = 12$, 20.3%) followed by ALL ($n = 5$, 8.5%), HL ($n = 5$, 8.5%), NHL ($n = 5$, 8.5%) and CML ($n = 1$, 1.7%). Seven (11.9%) patients received targeted

**Table 1.** Patient and transplant characteristics.

| | N Missing | | $N = 173$ (%) |
|---|---|---|---|
| Patient sex | | Female | 61 (35.3) |
| | | Male | 112 (64.7) |
| KPS at tx | 14 | <90 | 55 (34.6) |
| | | ≥90 | 104 (65.4) |
| Disease status at tx | | CR1 | 52 (30.1) |
| | | CR2+ | 37(21.4) |
| | | PR | 25 (14.5) |
| | | PD | 59 (34.1) |
| SC source | | BM | 40 (23.1) |
| | | PB | 133 (76.9) |
| Conditioning regimens | | MAC | 63 (36.4) |
| | | NMAC | 31 (17.9) |
| | | RIC | 79 (45.7) |
| TBI given | | No | 111 (64.2) |
| | | Yes | 62 (35.8) |
| Sex mismatch | | F to M | 36 (20.8) |
| | | Others | 137 (79.2) |
| ABO match | | Bidirectional | 9 (5.2) |
| | | Major mismatch | 26 (15) |
| | | Match | 112 (64.7) |
| | | Minor mismatch | 26 (15) |
| CMV match (R/D) | 5 | −/− | 29 (17.3) |
| | | other | 139 (82.7) |
| Relationship to Recipient | 1 | Child | 61 (35.5) |
| | | Other relative | 8 (4.7) |
| | | Parent | 37 (21.5) |
| | | Sibling | 66 (38.4) |
| GVHD prophylaxis regimen | | CSA/MMF/CY | 113 (65.3) |
| | | TAC/MMF/CY | 47 (27.2) |
| | | Other | 13 (7.5) |
| aGVHD II-IV before DLI | 2 | No | 129 (75.4) |
| | | Yes | 42 (24.6) |
| cGVHD before DLI | | No | 155 (89.6) |
| | | Yes | 18 (10.4) |

*IQR* inter quartile range, *m* months, *KPS* Karnofsky performance status, *TX* transplant, *CR* complete remission, *PR* partial remission, *PD* progressive disease, *SC* stem cell, *BM* bone marrow, *PB* peripheral blood, *MAC* myeloablative conditioning, *NMAC* non-myeloablative conditioning, *RIC* reduced-intensity conditioning, *TBI* total body irradiation, *M* male, *F* female, *CMV* cytomegalovirus, *R* recipient, *D* donor, *GVHD* graft versus host disease, *CSA* cyclosporine, *MMF* mycophenolate, *CY* cyclophosphamide, *TAC* tacrolimus.

therapy and or chemotherapy pre-DLI. At first DLI, 46 (78%) patients were still receiving immunosuppression (on tapering) while 13 (22%) had stopped immunosuppression (Table 2).

The median number of DLIs was 1 (IQR: 1–2.5). 31 (52.5%) patients received a single dose, 28 (47.5%) received multiple doses and the maximum dose was five in only 1 patient. The median interval between haplo-HCT and the first DLI was 3.1 (IQR: 2.6–4.4) months. The median interval between the first and the second dose was 2.1 months (IQR: 1.8–2.5), and between the second and the third was 2.6 months (IQR: 1.5–3.0).

The median dose of first DLI was $0.1 \times 10^6$ CD3+ T cell/kg (IQR: 0.1–0.5) and the median second dose was $0.5 \times 10^6$ CD3+ T cell/kg (IQR: 0.5–0.1).

For the preemptive group ($n = 20$), the median age at first DLI was 42.0 (IQR: 25.9–55.4) years. The diagnosis for this group were AML ($n = 7$, 35%), ALL ($n = 5$, 25%), NHL ($n = 3$, 15%), CLL ($n = 2$, 10%), HL ($n = 2$, 10%) and MDS ($n = 1$, 5.0%). At first DLI, 12 patients (60%) had stopped immunosuppression. Chemotherapy/targeted therapy pre-DLI was given in 6 (30%) patients (Table 2). The median number of DLIs was 2 (IQR: 1–3). 12 of 21 (60%) received multiple doses: 12 the 2nd dose, 6 the 3rd dose, 1 the 4th, the 5th, and the 6th dose, respectively. The median interval between transplant and first DLI was 6.1 (IQR: 2.8–9.8) months. The median interval between the first and the second dose was 1.5 months (IQR: 1.2–1.6), and between the second and the third was 1.9 months (IQR: 1.5–2.7). The median CD3+ T-cell dose of first DLI was $0.5\times10^6$ CD3+ T cell /kg (IQR:0.1–1) and the median second dose was $1 \times 10^6$ CD3+T cell /kg (IQR:0.7–1).

For the therapeutic group ($n = 93$), the median age at first DLI was 40.2 (IQR: 27.5–59) years. The diagnoses were AML ($n = 42$, 45.2%), ALL ($n = 18$, 19.4%), HL ($n = 13$, 14.0%), NHL ($n = 9$, 9.6%), MDS ($n = 7$, 7.5%), MM ($n = 3$, 3.2%) and CML ($n = 1$, 1.1%). 62 (69.7%) patients had already stopped immunosuppression at first DLI, and 58 of 93 patients (63%) received chemotherapy or targeted therapy pre-DLI. The median number of DLIs was 1 (IQR: 1–3). 46 (49.5%) patients received multiple doses: 46 the 2nd dose, 25 the 3rd dose, 10 the 4th, and 3 the 5th dose. The median interval between the first and the second dose was 1.2 months (IQR: 1.0–1.8), and between the second and the third was 1.5 months (IQR: 0.8–2.3) (Tables 2, 3). The median dose of first DLI was $1 \times 10^6$ CD3+ T cell/kg (IQR: 0.2–5.1) and the median second dose was also $1.1 \times 10^6$ CD3+ T cell/kg (IQR: 0.5–5). The median interval between haplo-HCT and the first DLI was 7.2 (IQR: 4.9–12.5) months.

### Acute and chronic GVHD after DLI

CI of II-IV aGVHD at 100 days was 17% (95% CI 7–27%) for the prophylactic, 20% (95% CI 2–38%) for the preemptive, and 17% (95% CI 9–24%) for the therapeutic group, respectively ($p = 0.72$) (Fig. 1).

CI of III-IV aGvHD at 100 days, was 7%(95% CI 0–13%) for the prophylactic, 5%(95% CI 0–15%) for the preemptive, and 9%(95% CI 3–15%) for the therapeutic group, respectively.

For cGVHD, the CI at 2 years was 53% (95% CI 40–67%) for the prophylactic group, 21% (95% CI 3–39%) for the preemptive, and 24% (95% CI 15–33%) for the therapeutic group, respectively ($p < 0.001$) (Fig. 1). 2 years CI of extensive cGVHD was 22% (95% CI 10–34%), 11% (95% CI 0–24%) and 14% (95% CI 7–21%) for the prophylactic, preemptive and therapeutic.

The cumulative incidence of aGVHD in a model with 2nd DLI as an additional competing risk is 13% (8–18%) after 100 days since 1st DLI and the cumulative incidence of aGVHD calculated from timing of 2nd DLI onwards is 8% (1–15%) after 100 days since 2nd DLI. For cGVHD these numbers are respectively 22% (15–28%) and 17% (7–26%).

Results from cause-specific Cox proportional hazards models indicate that risk for aGVHD is higher for patients with MAC regimen compared to those received a RIC (HR = 3.32, 95% CI:1.24–8.90, $p = 0.02$), whereas for cGVHD there is no significant impact of conditioning regimen. Furthermore, no significant associations with aGVHD or cGVHD were found according to the type of stem cell source, immunosuppression status (stopped vs. ongoing), and time from haplo HCT to DLI.

**Table 2.** Diagnosis and DLI characteristics.

| | Prophylactic *N* (%) | Preemptive *N* (%) | Therapeutic *N* (%) | Total *N* (%) | *p* |
|---|---|---|---|---|---|
| Reason of DLI | 59 (34.3) | 20 (11.6) | 93 (54.1) | 172 (100) | |
| Median age at first DLI | 55.2 (IQR:42.7–64.8) | 42.0 (IQR:25.9–55.4) | 40.6 (IQR:27.5–59) | 45.6 (IQR: 28.3–60.5) | 0.01 |
| Diagnosis: | | | | | |
| AML | 31(52.5) | 7 (35.0) | 42 (45.2) | 80 (46.5) | 0.01 |
| ALL | 5 (8.5) | 5 (25.0) | 18 (19.4) | 28 (16.3) | |
| MDS | 12 (20.3) | 1 (5.0) | 7 (7.5) | 20 (11.6) | |
| HL | 5 (8.5) | 2 (10.0) | 13 (14.0) | 20 (11.6) | |
| NHL | 5 (8.5) | 3 (15.0) | 9 (9.6) | 17 (9.9) | |
| CLL | 0 (0) | 2 (10.0) | 0 (0) | 2 (1.2) | |
| MM | 0 (0) | 0 (0) | 3 (3.2) | 3 (1.7) | |
| CML | 1 (1.7) | 0(0) | 1 (1.1) | 2 (1.2) | |
| Chemotherapy pre-DLI | 7 (11.9) | 6 (30.0) | 58 (63.0) | 71 (41.5) | <0.001 |
| Stop immunosuppression before DLI | 13 (22) | 12 (60.0) | 62 (69.7) | 87 (51.8) | <0.001 |
| Single infusion | 31 (52.5) | 9 (45.0) | 47 (50.5) | 87 (50.6) | 0.8 |
| Multiple infusions | 28 (47.5) | 11 (55.0) | 46 (49.5) | 85 (49.4) | |

1 case missing for reason for DLI, hence total $N = 172$. *p*-values derived from Kruskal-Wallis rank sum test and Pearson's Chi-squared test.
*DLI* donor lymphocyte infusion, *IQR* inter quartile range, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* myelodysplastic syndrome, *HL* Hodgkin lymphoma, *NHL* non-Hodgkin Lymphoma, *CLL* chronic lymphocytic leukemia, *MM* multiple myeloma, *CML* chronic myeloid leukemia.

### OS, RI, NRM, and DFS after DLI

2 years-OS was significantly different according to the indication for DLI: it was 61% (95% CI 46–75%) in the prophylactic group, 40% (95% CI 19–61%) in the preemptive group, and 22% (95% CI 13–31%) in the therapeutic group ($p < 0.001$) (Fig. 2).

2 years CI of relapse was 25% (95% CI 13–37%) for the prophylactic group, and 61% (95% CI 39–84%) for the preemptive group ($p = 0.002$). 2-years CI of NRM was 15% (95% CI 5–25%) in the prophylactic and 17% (95% CI 0–34%) in the preemptive ($p = 0.87$). 2-years DFS was 60% (95% CI 46–74%) and 22% (95% CI 3–41%) for the prophylactic and preemptive respectively ($p = 0.002$). In the therapeutic group the 2-year CI of complete remission after first DLI is 30% (95% CI: 20–39%).

Among patients that had no immunosuppression before first DLI, 3 out of 13 (23%) patients of the prophylactic and 7 out of 12 (58.3%) of the preemptive presented recurrence of disease. In patients for whom immunosuppression was not stopped (under immunosuppression tapering) at the first DLI, relapse occurred in 10 out of 46 patients (21.7%) of the prophylactic and in 4 out of 8 (50%) of the preemptive group. For patients for who immunosuppression was not arrested and/or under immunosuppression tapering at first DLI, relapse occurred in 10 out of 46 patients (21.7%) of the prophylactic and 4 out of 8 (50%) of the preemptive group.

In the entire population, the main cause of death was relapse/progression of disease for 74.8% of patients, followed by transplant-related complications for 22.4% of patients, and other causes for 2.8%. GVHD was a subcause of death for 7 out of 23 (30%) patients that died because of transplant-related complications.

## DISCUSSION

Donor-derived immune effector cells are important mediators of the graft versus leukemia effect [18, 19]. Administration of the same donor blood lymphocytes collected through apheresis (DLI) has demonstrated clinical efficacy to treat relapse of several blood malignancies when it occurs after allogeneic hematopoietic cell transplantation, although the exact nature of immune effector cells (IECs) contributing to this effect remains unknown. Currently, the feasibility and outcome of DLI in non-T-cell depleted haplo-HCT have been reported only in single-center studies [7]. We here report on a multicentric retrospective survey of DLI administered after haplo-HCT PTCY, with the objective to describe the practical modalities, safety and effectiveness of this approach across EBMT centers.

In our analysis, DLI was used to treat disease relapse in the majority of the patients (as a preemptive or curative strategy), while 34.3% of patients received DLI as prophylactic treatment for high-risk disease assessed before HCT.

The main differences between the groups were the CD3+ T-cell dose, timing between transplant and first DLI, the concomitant treatment associated with the DLI, and the presence of GVHD prophylaxis.

DLI dose varied according to the indication for the DLI use and by transplant center experience. Overall, in agreement with the current practice and the EBMT recommendations for clinical application of haplo-DLI, the dose of CD3+ lymphocytes was higher in the patients with proven relapse, rather than those receiving DLI as prophylactic or preemptive treatment [7].

In our study population, the treatment associated with DLIs was very heterogeneous depending on the type of disease, center policy, and disease status. Several studies demonstrated that DLI-combined treatments showed a clinical benefit by providing synergistic antitumor activity and immunomodulatory effects [20–26]. In the prophylactic setting, the only prospective study that combined azacitidine with DLI showed no survival benefit compared to the historical control [21]. Further data on prospective randomized clinical trials providing high-level evidence of the best treatment associated with DLI, are warranted.

Regarding the efficacy of DLIs, our results highlight several differences depending on the clinical setting. It is well known that patients experiencing disease recurrence after HCT have poor outcome [20] and this was also observed in our study. Patients receiving DLI as curative treatment experienced dismal outcomes compared with those having preemptive or prophylactic DLI, highlighting that the high tumor burden is a predictor of poor response [27].

**Table 3.** Details on DLI doses and intervals between administrations.

| | Prophylactic ($n = 59$) | Preemptive ($n = 20$) | Therapeutic ($n = 93$) |
|---|---|---|---|
| Median number of doses (IQR) | 1 (1–2.5) | 2 (1–3) | 1 (1–3) |
| Median 1st CD3+ T cell dose/kg (IQR) | $0.1 \times 10^6$ (0.1–0.5) | $0.5 \times 10^6$ (0.1–1) | $1 \times 10^6$(0.2–5.1) |
| Median 2nd CD3 +T cell dose/kg (IQR) | $0.5 \times 10^6$ (0.5–1) | $1 \times 10^6$ (0.7–1) | $1.1 \times 10^6$ (0.5–5) |
| Interval between HCT and DLI (months) | 3.1 (2.6–4.4) | 6.1 (2.8–9.8) | 7.2 (4.9–12.5) |
| Median time between the 1st and the 2nd dose (IQR) | 2.1 (1.8–2.5) | 1.5 (1.2–1.6) | 1.2 (1.0–1.8) |
| Median time between the 2nd and the 3rd dose (IQR) | 2.6 (1.5–3) | 1.9 (1.5–2.7) | 1.5 (0.8–2.3) |

1 case missing for reason for DLI, hence total $N = 172$
*IQR* inter quartile range, *HCT* hematopoietic cell transplantation, *DLI* donor lymphocyte infusion.

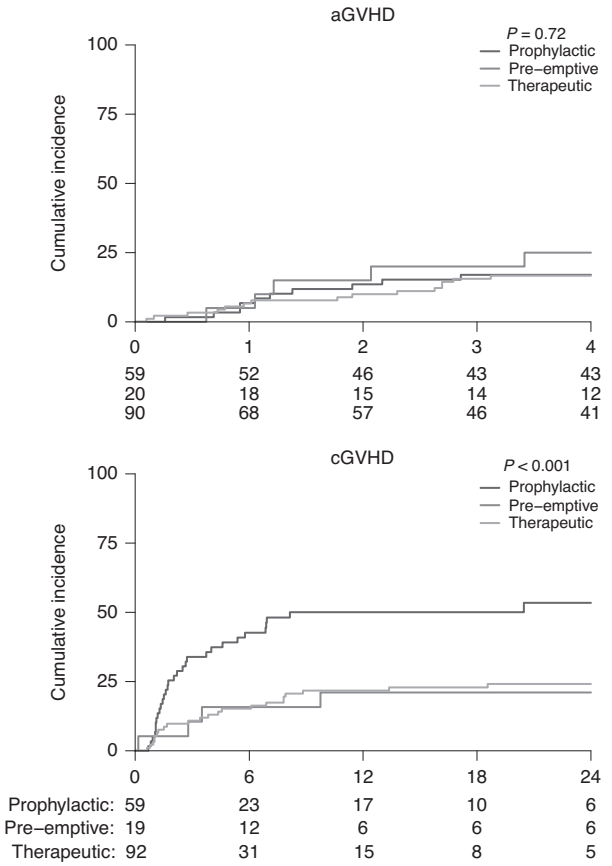


**Fig. 1** GVHD according to the reason of DLI for grade II-IV aGVHD and cGVHD.

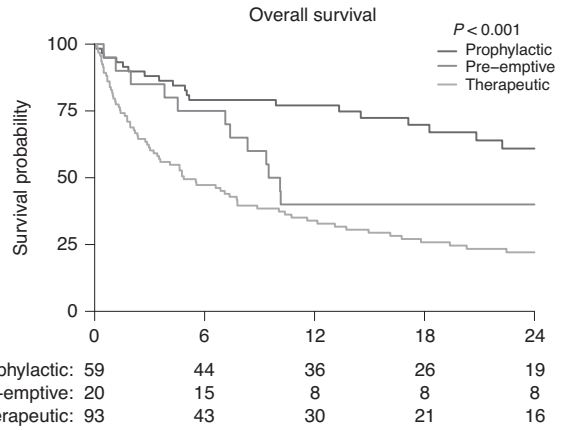


**Fig. 2** OS according to the reason of DLI.

In line with previous reports of DLI in haplo-HCT with PT-CY, the cumulative incidence of grade II-IV aGVHD after DLI was low [11–13, 28] and it appears uniform across the three subgroups. Furthermore, as was observed in a recent study [29], the cumulative incidences of acute and chronic GVHD appear to be lower after second administration compared with after first administration. Unfortunately, due to the small number of patients for each subgroup we were not able to identify which disease could better respond to DLI, and this deserves further investigations.

Our study has limitations related to the retrospective nature of the study. In particular, we report the cases from centers responding to the survey, therefore our analysis is not representative of the policies of all the EBMT centers. We lack details on subsequent events (i.e., hematological toxicities) after the first DLI, which may have an impact on the planned schedule of DLI administration and on the management of tapering of immunosuppression.

We also missed information about chimerism assessment at first DLI in all the subgroups. Furthermore, we included a heterogeneous cohort of patients with different diseases for whom the treatments before DLI were different. As a consequence, the timing and role of concomitant systemic therapies were diverse across centers. Future prospective clinical trials are needed to refine and evaluate this information using harmonized procedures across centers for proper interpretation of medical data.

In conclusion, our results confirm the feasibility of unmanipulated DLI in patients receiving haplo-HCT with PTCY, however with a warning on the high incidence proportion of cGVHD in the prophylaxis group. Survival and disease outcomes appeared better in the early intervention. In patients with active diseases, results remain poor, while patients receiving prophylactic or preemptive DLI showed significantly better survival outcomes. These results support the putative interest of immunotherapy approaches using DLI in haplo-HCT with PTCY, without further manipulation and at relatively low costs. Although there is no comparison between the use of conventional drugs and cell therapies, DLIs represent a universal post-transplant immune therapy, since their activities do not depend on an identified tumor target. In addition, the donor is rapidly available and a single collection and cryopreservation at the time of donation might also be conceivable. In the era of personalized precision medicine, the advancements in biotechnology are making it possible to develop more sophisticated cellular therapies that could improve the efficacy and safety of cellular therapy products [29, 30]. In the setting of haplo-HCT, several groups are studying the efficacy of donor-derived Natural Killer (NK) cells [31], gene-modified immune effector T-cells, and haploidentical chimeric antigen receptor (CAR) T-cell or CAR NK-cell therapies with promising results, but further clinical data are needed.

## DATA AVAILABILITY

The final analysis dataset will be available upon specific request to the Working Party chair.

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## AUTHOR CONTRIBUTIONS

AR and CC designed the study, NS and AR wrote the manuscript, JEM, and LCdW performed the statistical analysis, JDH prepared the data, DB, YC, JV, LC, ZG, JDM, MCA, AK, MA, CHA provided cases for the study, MPM and MDI helped in data interpretation. All authors edited and approved the manuscript.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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