haematologica
Journal of the Ferrata Storti Foundation

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Received: December 23, 2024.
Accepted: April 17, 2025.

Citation: Giuliano Filippini Velázquez, Jan Frederic Weller, Anna Rubeck, Tobias Arndt, Stefan Schiele, Markus Mezger, Claudia Lengerke, Wolfgang Bethge, Martin Trepel, Gernot Müller, Maximilian Christopeit and Christoph Schmid. Prophylactic and pre-emptive donor lymphocyte infusion in patients with acute myeloid leukemia and myelodysplastic syndrome: validation of current recommendations and proposal of a modified outcome assessment.
Haematologica. 2025 Apr 24. doi: 10.3324/haematol.2024.287206 [Epub ahead of print]

Prophylactic and pre-emptive donor lymphocyte infusion in patients with acute myeloid leukemia and myelodysplastic syndrome: validation of current recommendations and proposal of a modified outcome assessment

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Disclosures: The authors declare no conflicts of interest

Data availability: All data generated or analyzed during this study are included in this published article and its supplementary information files. Additional data are available from the corresponding author upon reasonable and justified request.

Authors´ contributions: GFV and CS obtained ethical approval and performed literature research. GFV and JFW collected data from clinical charts. MM, CL, WB, MT, MC, and CS contributed clinical data and provided logistical support for data collection. AR, TA, SS, and GM performed statistical analyses; AR served as the primary statistician, supervised by GM. GFV, JFW, and AR created and edited tables and figures; figures were mainly prepared by AR with support from TA and SS. GFV, JFW, AR, MC, and CS were involved in study conception, data analysis, and interpretation. MC and CS supervised the study as senior authors. GFV and CS drafted the manuscript; JFW, AR, WB, MM, and MC revised it critically. All authors approved the final version and submission.

Running Title: Treatment success after pro/preDLI for AML/MDS

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Abstract

Prophylactic and pre-emptive donor lymphocyte infusion (pro/preDLI) is used to prevent haematological relapse of AML and MDS after allogeneic stem cell transplantation. For lack of prospective trials, outcome reports, risk factor analyses and published recommendations for DLI administration had to rely on registry studies, frequently limited by inconsistent reporting and missing data. Therefore, we performed an extensive chart review on recipients of pro/preDLI in two German centers to investigate the clinical applicability of current guidelines in a well-defined cohort. Beyond, as outcome after pro/preDLI is unsatisfactorily described by conventional parameters, we constructed a model for *treatment success*, defined as leukaemia-free survival (LFS) without intensive immunosuppressive treatment for Graft-versus-Host-Disease (GvHD).

Eighty-three patients had received proDLI (n=36), preDLI for incomplete chimerism (preDLI-IC, n=27) or for persisting minimal residual disease/molecular relapse (preDLI-MRD, n=20). According to current guidelines concerning initial T cell doses and timing of DLI, 42% of patients had received DLI as recommended (standard-intensity), whereas 30%/28% had received DLI in lower/higher cell doses and/or at a later/earlier time point (low-/high-intensity).

Two-year rates of overall survival (OS), LFS, relapse incidence and non-relapse mortality within the entire cohort were 80%/67%/27%/8%. One-year rates of high-grade acute/chronic GvHD were 34%/27% among all patients and 53%/33% after high-intensity DLI. One-year *treatment success* rate were 72%/69% after low-/standard intensity, in contrast to 34% after high-intensity DLI. Apart from advanced disease at alloSCT, high-intensity DLI was the major risk factor for lower OS (HR=6.12), LFS (HR=5.43), higher aGvHD (HR=2.51), and lower treatment success (HR=0.41), supporting adherence to current recommendations.

Introduction

Recurrence of the underlying malignancy remains the most common cause of treatment failure in high-risk acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) undergoing allogeneic stem cell transplantation (alloSCT). (1) After haematological relapse, less than 1/3 of patients achieve long-term remissions. (2-4) Therefore, for patients in complete haematological remission (CHR) after alloSCT with a high risk of relapse, prevention strategies are essential.

Donor lymphocyte infusion (DLI) are given after alloSCT to reinforce the Graft-versus-Leukaemia (GvL) reaction. (5) In overt haematological relapse, therapeutic effects of DLI were limited. (6) In contrast, the efficacy of DLI given in complete haematological remission (CHR) was demonstrated after pre-emptive application (preDLI) to patients with incomplete donor chimerism (IC), minimal residual disease (MRD) and molecular relapse, or as pure prophylaxis for patients with a high-risk of relapse, based on genetics or advanced stage at alloSCT (proDLI). (7-9) Long-term survival rates between 40% and 80% have been reported. (10-13) Pro/preDLI are thus considered effective strategies for relapse prevention in high-risk myeloid malignancies, especially for patients lacking targeted treatment options for post-SCT maintenance. (14, 15)

The major clinical drawback of pro/preDLI is the risk to induce Graft-versus-Host disease (GvHD), that might be difficult to manage, require prolonged immunosuppressive treatment (IS), and can cause considerable morbidity and mortality. (12) Thus, the art of DLI consists of identifying the sweet spot in which pro/preDLI can be implemented both safely and effectively. In an approach towards standardization of the procedure, an international expert panel on behalf of the European Group for Blood and Marrow Transplantation (EBMT) has provided consensus-based recommendations for indication, timing, and doses of DLI. (16) However, the level of evidence of such recommendations is limited to a certain extent, given that prospective trials are scarce in the setting of DLI, and most data come from retrospective registry analysis, which differ substantially in their inclusion criteria and methods, and are frequently limited by inconsistent reporting or missing data. Acknowledging these limitations, systematically increasing the number of patients with well documented, detailed clinical courses before, during and after DLI has been claimed as a prerequisite for a better understanding and improved clinical application. (16) Accordingly, we performed an exhaustive chart review and analysis of patients with AML and MDS with increased risk of post-transplant relapse, who had received DLI in CHR in two German transplant centers. The goal of the study was to assess the role of recommended doses and schedules of DLI for established long-term clinical outcome parameters.

Another challenge in the field of alloSCT and particularly after pro/preDLI, is to define clinically relevant outcome parameters. Interpretation of overall survival (OS) and leukaemia-

free survival (LFS) might be difficult outside of a randomized prospective trial, and outcome variables such as cumulative incidence of GvHD or GvHD-free, relapse-free survival (GFRS), do not consider that certain events such as GvHD might be transient and therefore of subsidiary importance for the final evaluation of treatment outcome. This is of particular relevance in patients with a high-risk of relapse, who might be ready to accept a mild degree of GvHD or low-dose immunosuppression (IS), as long as haematological relapse can be avoided. To address this problem, multistate models, consisting of different states and transitions have been proposed, as they offer a more comprehensive assessment with the advantage of capturing not only the final clinical outcome, but also assessing temporary states, such as GvHD. (17) These models are able to consider both sequential events and transient, i.e. non-absorbing states. Therefore, in a second part of our study, we constructed a multistate model to illustrate both transient and definitive clinical events occurring after pro/preDLI. Further, we introduced the modified clinical outcome parameter *treatment success*, which we defined as being free of leukaemia, without GvHD requiring more than low-dose immunosuppressive medication, allowing unrestricted quality of life.

Methods

We included all consecutive adult patients from the centres in Augsburg and Tübingen, Germany, fulfilling the following criteria: **(1)** AML or MDS in CHR after alloSCT from matched sibling-, matched/mismatched unrelated, and haploidentical donors (MSD/MUD/MMUD/HAPLO) **(2)** proDLI or preDLI applied between 2007-2021 **(3)** no anti-leukaemic therapy for relapse prevention after alloSCT other than DLI, **(4)** follow-up after DLI1 ≥100 days. DLI for haematological relapse or viral infections were excluded. All patients provided informed consent into the use of their clinical data for scientific purposes. The study was approved by the Ludwig-Maximilian-University Munich ethics board (Nr: 22-0865)**.**

DLI

By local standards, DLI consisted of unmodified CD3+ lymphocyte concentrates. Routinely, DLI were collected after alloSCT in a single, unstimulated apheresis, with the first portion (DLI1) administered immediately and the remaining cells cryopreserved in pre-defined, escalating doses. Alternatively, DLI were harvested at stem cell collection, cryopreserving all portions. As suggested, (11, 18) prerequisites for pro/preDLI included **(1)** Stop of immunosuppressive medication ≥4 weeks before DLI1, **(2)** Absence of active GvHD, **(3)** No history of GvHD grade III/IV after alloSCT, **(4)** No active infection.

Pro/preDLI administration varied by local standards and over time. Pre-DLI was repeated until achievement of complete chimerism (CC) or MRD-negativity. Subsequent DLI were

withheld in case of GvHD grade I/II. No further DLI were given after development of GvHD grade >II. No prophylactic IS was applied.

DLI intensity

Starting in 2019, expert panels from the EBMT recommended doses and intervals for pro/preDLI (19, 20) that have recently been updated by the EBMT Practice Harmonization and Guidelines Committee **(Table 1)**. (16) To validate these recommendations, we defined the variable *DLI intensity* and retrospectively assigned patients to either having received standard-, low-, or high-intensity DLI based on time from alloSCT to DLI1, and the CD3+ cell dose used for DLI1. Accordingly, standard-intensity DLI was defined by CD3+ cell counts and time intervals from alloSCT to DLI1 as recommended. High-intensity DLI was defined as higher CD3+ cell count for DLI1 or first application earlier after alloSCT than recommended, and low-intensity DLI was defined by lower CD3+ cell count for DLI1 or a longer interval from alloSCT than recommended.

Definitions

ProDLI: DLI in CHR with CC and undetectable MRD. **PreDLI-MRD/ preDLI-IC:** Pre-emptive DLI for MRD or IC (without MRD). **Standard-dose IS:** Immunosuppressive treatment per international guidelines for acute (a) or chronic (c) GvHD. (21) **Low-dose IS:** Oral Ciclosporin A (CSA) ≤50mg/day, or Tacrolimus (TAC) ≤1mg/day, and/or prednisolone ≤20mg/day. **Treatment success:** Being alive in CHR without GvHD or with mild cGvHD requiring no IS or low-dose IS, without subjective quality-of-life impairment. Further definitions in **Supplementary methods**.

Statistics

Endpoints included OS, LFS, relapse incidence (RI), LAD, NRM, a/cGVHD and treatment success. Follow-up was calculated from the date of DLI1. Standard tests were used for differences in variable distribution, outcome probabilities, and risk factor analysis (see **Supplementary methods** for details). A Markov multistate model was constructed for assessment of clinical events following DLI over time **(Figure 1)**.

Results

Patient characteristics

Eighty-three patients (AML, n=75; MDS, n=8, median age 58.7 [range: 24.5–76.1] years) were included **(Table 2)**. At time of alloSCT, all patients had fulfilled ≥1 of the following criteria defining high-risk disease: unfavourable genetics according to ELN 2022 (22) (n=48),

secondary AML (n=2), primary induction failure (n=18), persistent MRD/molecular relapse (n=5), or haematological relapse (n=4) after conventional therapy, refractory disease or relapse after first alloSCT (n=8). Overall, 49 (59%) had an active disease at the last alloSCT. Donor types where MSD (n=20, 24%), MUD 10/10 (n=47, 57%), MMUD 9/10 (n=10, 12%) and HAPLO (n=6, 7%).

No uniform conditioning for alloSCT was used, however 57% of patients had received a sequential protocol based on the FLAMSA-RIC regimen. (18) **(Supplementary table 1)**. In-vivo T cell depletion (TCD) for GvHD-prevention was performed in 72 patients (87%), using rabbit anti-thymocyte globulin (ATG) in 68 patients, and post-transplant cyclophosphamide (PTCy) in 4 (more details in **Supplementary results**). Post-transplant IS included a calcineurin-inhibitor in 93%, either in combination with mycophenolate mofetil (MMF) or methotrexate, to be tapered in the absence of GvHD from day +35 in haploidentical, and from day +56 in HLA matched donor settings**.**

DLI characteristics

Fifty-six (67%) patients received unstimulated donor lymphocytes that were collected by a separate apheresis after alloSCT, 27 (33%) patients received DLI collected at the time of donor stem cell harvest.

ProDLI was given in 36 (43%) patients, preDLI-IC in 27 (33%), and preDLI-MRD in 20 (24%), respectively. Overall, median time from alloSCT to DLI1 was 5.9 (range: 1.1–42.9) months; 6.6 (range: 3.8–16.3) months for proDLI, 5.6 (range: 3.9–15.4) months for preDLI-IC; and 5.7 (range: 1.1–42.9) months for preDLI-MRD. Median number of infusions was 3 (range: 1-5). Reasons to limit numbers of DLI included GvHD (44%), treatment response (31%), physicians' decision/per protocol (17%), disease progression (8%). Median number of CD3+ cells/kg at DLI1 was $0.2x10^6$ (range: 0.02–10.0).

As described above, we retrospectively categorized pro/preDLI intensity based on recent international recommendations **(Table 1).** Standard-intensity DLI had been given to 35 (42%) patients, 23 (28%) had received high-intensity, and 25 (30%) low-intensity DLI**.** At DLI1, high-intensity DLI contained a median CD3+ cell count/kg of $2.4x10^6$, significantly different from the standard- ($0.2x10^6$), and low-intensity DLI ($0.2x10^6$; $p<0.001$). Similarly, the median interval from alloSCT to DLI1 was shorter for high-intensity DLI (4.7 months) than for standard-(5.4 months) and low-intensity DLI (8.4 months; $p<0.001$; **Table 2)**.

Response to pre-emptive DLI

Forty-seven patients received preDLI, of which 39 (83%) showed a primary response (preDLI-IC: 22/27 [82%]; preDLI-MRD 17/20 [85%]). Only 1/22 (5%) patients initially

responding to preDLI-IC developed haematological relapse thereafter, in contrast to 5/17 (29%) of responders to preDLI-MRD.

Survival and causes of death

Median follow-up from DLI1 among surviving patients was 40 months. The 2-year OS/LFS for the entire cohort was 80% (95%-confidence interval [CI95]: 71-90%) and 67% (CI95: 57-78%). Two-year OS/LFS were 81% (CI95: 69-96%)/ 70% (CI95: 56-88%) for proDLI, 88% (CI95: 77-100%)/ 74% (CI95: 59-93%) for preDLI-IC and 65% (CI95: 46-93%)/ 48% (CI95: 29-80%) for preDLI-MRD. The overall 2-year cumulative RI (regardless of DLI-type) was 26% (24% after proDLI, 19% after preDLI-IC, and 49% after preDLI-MRD). The 2-year NRM for the whole population was 8% **(Table 3, Figure 2)**.

At LFU, 29 patients (35%) had died. Leukaemia was the major cause of death (n=20). Nine patients died in remission. GvHD induced by DLI was lethal in only one patient, however all patients dying in remission had developed prior GvHD at some point after DLI. Other causes of NRM (n ≤2 each) were infections, liver failure from iron overload, pulmonary hypertension, and secondary neoplasia.

Outcome according to DLI-intensity

The 2-year OS/LFS were 87% (CI95: 75-100%)/ 72% (CI95: 58-89%) after standard-intensity DLI, 92% (CI95: 82-100%)/ 88% (CI95: 76-100%) after low-intensity, and 54% (CI95: 35-82%)/ 30% (CI95: 15-62%) after high-intensity DLI. The 2-year RI after standard-intensity DLI was 22%, 8% after low-intensity, and 55% after high-intensity DLI. The 2-year NRM after standard-intensity DLI was 6%, 4% after low-intensity, and 14% after high-intensity DLI **(Table 3, Figure 3)**.

GvHD following DLI

The 1-year cumulative incidence of aGvHD grades I-IV, II-IV, and III-IV were 50% (CI95: 30-60%), 34% (CI95: 24-44%), and 16% (CI95: 9-24%), respectively. The 1-year cumulative incidence of limited, and moderate/severe cGvHD were 16% (CI95: 9-25%) and 27% (CI95: 18-38%). Median time from DLI1 of aGvHD and cGvHD onset were 2.3 (range: 0.1–9.0) and 5.2 months (range: 0.2–25.8), respectively. Of 32 patients requiring standard IS for acute or chronic GvHD, 25 (78%) could discontinue after a median treatment duration of 10.7 months (range: 0.7–121). At LFU, seven patients (8%) still required low dose IS as defined above. These patients had a median duration of IS-treatment of 17.5 months (range: 9-121). The median time between standard IS onset and transition to low-dose IS was 8.7 months (range: 1.3-16.7).

With respect to DLI intensity, the 1-year cumulative incidence of aGvHD grades II-IV after standard-intensity DLI was 29% (CI95: 15-44%), 24% (CI95: 10-42%) after low-intensity DLI, and 53% (CI95: 30-72%) after high-intensity DLI. The 1-year cumulative incidence of cGvHD moderate/severe after standard-intensity DLI was 30% (CI95: 15-45%), 20% (CI95: 7-38%) after low-intensity DLI, and 33% (CI95: 14-54%) after high-intensity DLI **(Table 3)**.

End-organs affected by GvHD and response to IS treatment

Clinically significant GvHD requiring systemic IS treatment most often affected skin (31%), liver (19%), and oral mucosa (14%). Other organs affected in less than 10% of patients were lower-/upper GI tract (7%/6%), joints/muscles (7%), eyes (5%) and lung (5%). Rare manifestations included autoimmune haemolytic anemia (AIHA), nail dystrophia, sexual organ affection, and serositis (all 1%, **Supplementary figures 1-2)**.

Regarding treatment response of GvHD from the different organs in 32 patients requiring systemic IS (most frequently based on steroids and a calcineurin inhibitor), we observed an overall response rate of >80% in most organs. Treatment refractory GvHD rarely occurred but was observed in the lower GI tract (n=2), eyes (n=1), oral mucosa (n=1), liver (n=2), and skin (n=2, **Supplementary table 2)**.

Risk factor analyses

As shown above, no major differences on outcome parameters were observed between standard- and low-intensity DLI. Therefore, the two cohorts (n=60) were combined for risk factor analysis and compared to patients receiving high-intensity DLI (n=23). UVA of risk factors for OS, LFS, NRM and GvHD are shown in **Supplementary table 3.**

In MVA, active disease before alloSCT and a high-intensity DLI were associated with worse outcome. For OS, LFS, and RI, active disease was associated with a HR of 2.81 (CI95: 1.2-6.5, p=0.018); 2.88 (CI95: 1.2-6.4, p=0.010); and 3.19 (CI95: 1.3-7.7, p=0.011), respectively. High-intensity DLI was associated with a HR of 6.1 (CI95: 2.7-13.6, p<0.001); 5.43 (CI95: 2.6-11.2, p<0.001); and 4.77 (CI95: 1.9-11.4), p<0.001), for OS, LFS and RI, respectively. A multivariable risk factor analyses for NRM could not be performed due to the low number of events.

High-intensity DLI was the only significant risk factor for acute GvHD II-IV (HR 2.51 [CI95: 1.2-5.2], p=0.015). Low numbers of affected patients precluded a risk factor analysis for cGvHD. Results from MVA for clinical outcome parameters are shown in **Table 4**.

Although MRD status after alloSCT was not a significant factor for RI in MVA, we conducted an exploratory risk factor analysis excluding patients that had received preDLI-MRD (n=20) to analyse the effects of DLI-intensity on outcome parameters in a more homogeneous cohort. In this selected subgroup (proDLI and preDLI-IC, n=63), results obtained in the entire

cohort were confirmed, with active disease before alloSCT and a high-intensity DLI remaining significant risk factors for worse OS and LFS. Beyond, DLI-induced GvHD (acute or chronic, any grade, calculated as time-dependent covariate) was associated with a significant reduction of RI among these patients (HR 0.27 [CI95: 0.08-0.9], p=0.039)**.** Again, low number of events precluded a risk factor analysis for NRM. (See **Supplementary table 4** for details**).**

Finally, consistent with the results described above, among recipients of preDLI-MRD (n=20), relapse rates were 0% after low-/standard intensity DLI, and 67% after high-intensity DLI.

Exploratory analyses of DLI after haploidentical, and HLA-mismatched alloSCT.

Sixteen patients had received DLI after alloSCT from a haploidentical or a 9/10 HLA mismatched donor. Clinical outcome in this selected cohort was not remarkably different from the whole cohort of patients. A detailed description is provided in **Supplementary table 5.**

Multistate model analysis and proposal of treatment success as outcome parameter

A multistate Markov model was constructed to evaluate important clinical events after DLI. **Figure 4** shows the probabilities over time to be in the previously described absorbable and non-absorbable states, out of which the states marked in green represent *treatment success* as defined above. Accordingly, in the entire cohort the 1- and 2-year probability for *treatment success* was 61% (CI95: 49-71%), and 71% (CI95: 60-80%) respectively, thereby increasing over time due to improving/resolving GvHD. With respect to DLI-intensity, the rates of treatment success at 1- and 2 years were 69% and 76% after standard-intensity DLI, and 72% and 84% after low-intensity DLI, respectively. In contrast, *treatment success* rate after high-intensity DLI was 34% at 1 year (2-year analyses not possible due to low number of events; **Table 3**). In MVA, active disease before alloSCT (HR 0.55 [CI95: 0.3-0.8], p=0.002), and a high-intensity DLI (HR 0.4 [CI95: 0.2-0.8], p=0.016) were associated with a significantly reduced probability of *treatment success* **(Table 4,** see **Supplementary table 3** for UVA**).**

Discussion

Patients with high-risk AML and MDS achieving CHR after alloSCT require effective and safe relapse prevention strategies. Particularly in patients without targeted treatment options, pro/preDLI is a frequently used strategy, but carries the risk of severe, potentially life-threatening GvHD, or might be detrimental for the patients' quality of life (QoL). The concept to separate GvL effects from GvHD through delayed DLI administration until establishment of complete donor chimerism and by escalating dose schedules has optimized DLI use and

mitigated (severe) GvHD risk. As a general problem in the field, the lack of prospective trials, as well as missing data and inconsistencies within retrospective registry studies (e.g. concerning cellular composition of the inoculum, cell-subset selection, timing and dosing) complicate the interpretation of reported results and treatment standardization. During recent years, expert panels have developed consensus-based recommendations for the use of pro/preDLI focusing on CD3+ doses and the interval from alloSCT to the first DLI. Yet, these guidelines remain limited by the absence of systematic validation studies and the overall low degree of supporting evidence.

Against this background, we took advantage of an extremely detailed documentation of 83 consecutive pro/preDLI recipients at two transplant centres that have used DLI for relapse prevention in high-risk AML and MDS for many years. Variations in local DLI standards over time allowed us to compare different strategies with respect to timing and cell dosing facilitating validation of current recommendations. Overall outcomes in our study confirmed published data on pro/preDLI, (12) demonstrating the representativeness of our cohort. With respect to DLI intensity, earlier application or higher CD3+ cell doses were clearly associated with an increased risk of clinically significant GvHD and inferior OS/LFS. Hence, in the setting investigated here (infusion of unmodified CD3+ cells without GvHD prophylaxis), higher CD3+ cell doses or application earlier than recommended time intervals from alloSCT (16) **(Table 1)** should definitively be avoided. An estimated higher relapse risk, even in case of MRD or molecular relapse, may not justify the decision to increase pro/preDLI-intensity, since it does not improve outcomes, but substantially increases the risk of GvHD and its associated toxicity.

In contrast, excellent outcome could be demonstrated, when DLI was applied following recommended schedule and dosing. Among patients receiving either standard- or low-intensity DLI, 2-year OS/LFS were 92%/88% after low-, and 87%/72% after standard-intensity DLI, respectively, underscoring the safety and the promising outcome that can be obtained by following current recommendations. Although overall incidence of GvHD after pro/preDLI was considerable (50%), systemic IS was only required by about 2/3 of affected patients, and 78% could either discontinue IS over time or switch to low-dose IS. All patients requiring low-dose IS at LFU (8%) reported no or minimal complaints related to GvHD or its treatment.

Clinical results were comparable among patients receiving DLI as recommended and those receiving low-intensity DLI, suggesting –within the limitation of small numbers- the possibility of eventually further reducing recommended cell doses. Alternative DLI modifications, such as G-CSF mobilized DLI, infused as early as day +30 after alloSCT together with ongoing or newly initiated immunosuppression (23, 24) or low-dose DLI, repeated without dose

escalation up to a median number of 8 infusions over a period of up to 36 months, (25) have been proposed.

In a second part of our study, we applied a Markov multistate model to illustrate the clinical course after pro/preDLI over time and introduced the outcome variable *treatment success* to allow for a more real-life based estimate of patient outcomes. In the DLI setting, the value of classical endpoints might be limited due to their inability to consider that certain events, in particular GvHD, might either be transient or at least be well controlled in a way that they do not impair the QoL of affected patients. Patients with a high-risk of relapse might be ready to accept a certain degree of GvHD or low-dose immunosuppression, as long as haematological relapse can be avoided. In contrast, induction of severe GvHD might profoundly reduce QoL, even in the absence of leukaemia relapse, thereby questioning DLI as a relapse prevention strategy with acceptable side effects.

Taking advantage of the pioneering work by the group from Leiden, (17) we analyzed *treatment success* as outcome parameter, which was defined as being free of leukaemia without GvHD requiring more than mild immunosuppressive medication, and unrestricted QoL. With a median follow-up of 40 months from DLI1, treatment success at 2 years was achieved by 71% of patients, with a considerable proportion of patients entering the success state after developing transient high-grade GvHD. Within the model, both the direct transition from start to final success and the transient state of “standard-dose IS for GvHD” contributed most to the differences in outcome between the two intensity groups (**Supplementary table 6**). Beyond, the model also showed how a subset of patients that relapsed early after pro/preDLI (hence not fulfilling the definition of *treatment success*) was alive at LFU, reflecting the potential of the model for further analyses of long-term outcomes even after relapse.

Our model differs from the application introduced by the colleagues from Leiden, which had been designed to describe how alloSCT outcomes are influenced by subsequent DLI. In contrast, our model was developed to consider transient events occurring after DLI. In general, the outcome parameters illustrated by multistate models are more flexible and informative than the rigid endpoints such as LFS or the cumulative incidence of relapse or GvHD/RI, which are terminal. By allowing a more real-life based description of *treatment success* following pro/preDLI, the approach underscores the model’s applicability for the description of events and outcome parameters in the context of maintenance treatments after alloSCT.

Regarding the influence of DLI intensity on *treatment success*, in the cohort of patients who had received pro/preDLI in line with current recommendations, *treatment success* after 2 years was 76% and 84% among those receiving low-intensity DLI (differences not significant), with limited requirement of standard IS for treatment of GvHD, and very low NRM

rates. Exploratory analyses showed successful and early discontinuation (>90%) of IS for GvHD in patients receiving standard- or low-intensity DLI.

Limitations of our study include its relatively small sample size, the essential restriction to the *in-vivo* TCD setting, and the retrospective nature, which did not allow to identify why other transplant recipients had not been assigned to pro/preDLI. Hence, no comparative analysis of the clinical efficacy of pro/preDLI could be performed. Further, a certain heterogeneity in the DLI1-application, i.e. cryopreserved vs. fresh infusion, with some products obtained in the context of G-CSF stimulation of the donor needs to be accounted for. Nevertheless, although theoretically an influence of these inconsistencies on efficacy and safety of DLI cannot be excluded, this is not supported by the literature. (5, 26) In addition, due to the study design, that excluded patients who had received additional medical treatment, no data about potential synergisms between DLI and other types of maintenance therapy can be provided, although they are suggested by published data. (5, 27) In particular, in patients with *FLT3*-mutations, the use of sorafenib or gilteritinib might confer synergistic effects for relapse prevention. (28, 29) Finally, patients' QoL has not been systematically evaluated using questionnaires or scores established in the alloSCT setting, which, however, have not been validated in the context of DLI.

In summary, with respect to overall outcome, our results confirm previous observations on pro- and preDLI with high rates of *treatment success*. Adherence to current EBMT recommendations (16) can significantly reduce the risk of GvHD and its associated morbidity and mortality, and leads to superior outcome. The application of a multistate model might help to more precisely describe the clinical course and treatment success of DLI recipients.

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Table 1. Practical recommendations of pro/preDLI by timing and cell dose for the first DLI after alloSCT (Standard-intensity DLI)

DLI indication/ time since alloSCT	Donor type		
ProDLI	**MSD (CD3+ cells/kg)**	**MUD (CD3+ cells/kg)**	**MMUD and HAPLO (CD3+ cells/kg)**
3 months	0.1×10^6	0.1×10^6	0.1×10^6
6 months	1×10^6	1×10^6	0.5×10^6
PreDLI-IC/ preDLI-MRD			
3 months	$0.1\text{-}0.5 \times 10^6$	0.1×10^6	0.1×10^6
6 months	$1\text{-}3 \times 10^6$	1×10^6	0.5×10^6

DLI: Donor lymphocyte infusion, preDLI-IC: pre-emptive DLI for incomplete chimerism, pre-emptive DLI for minimal residual disease or molecular relapse, proDLI: prophylactic DLI, alloSCT: allogeneic stem cell transplantation, MSD: matched-sibling donor, MUD: matched-unrelated donor, MMUD: mismatched-unrelated donor, HAPLO: haploidentical donor, CD: cluster of differentiation, kg: kilogram. **Modified according to (16).**

Table 2. Patient and DLI characteristics according to DLI-intensity

		DLI intensity				
Variable	**N**	**Overall n=83**	**Low-intensity n=25**	**Standard-intensity n=35**	**High-intensity n=23**	**p-value[1]**
Diagnosis, n (%)	83					0.7
AML		75 (90%)	22 (88%)	31 (89%)	22 (96%)	
MDS		8 (10%)	3 (12%)	4 (11%)	1 (4%)	
Centre, n (%)	83					0.10
Augsburg		36 (43%)	15 (60%)	14 (40%)	7 (30%)	
Tübingen		47 (57%)	10 (40%)	21 (60%)	16 (70%)	
ELN + IPSS classification, n (%)	83					0.7
Low		6 (7%)	3 (12%)	2 (5.7%)	1 (4.3%)	
Intermediate		29 (35%)	10 (40%)	12 (34%)	7 (30%)	
High		48 (58%)	12 (48%)	21 (60%)	15 (65%)	
Patient age in years (median, range)	83	59 (24-76)	62 (53-64)	58 (51-63)	58 (53-64)	0.6
Patient sex, n (%)	83					0.1
female		31 (37%)	10 (40%)	13 (37%)	8 (35%)	
male		52 (63%)	15 (60%)	22 (63%)	15 (65%)	
Number of alloSCT, n (%)	83					0.9
alloSCT1		75 (90%)	22 (88%)	32 (91%)	21 (91%)	
alloSCT2		8 (10%)	3 (12%)	3 (9%)	2 (9%)	
Donor Type, n (%)	83					<0.01
Matched sibling		20 (24%)	0 (0%)	9 (26%)	11 (48%)	
Matched unrelated (10/10)		47 (57%)	15 (60%)	22 (63%)	10 (43%)	
Mismatched unrelated (9/10)		10 (12%)	7 (28%)	3 (8%)	0 (0%)	
Haploidentical		6 (7%)	3 (12%)	1 (3%)	2 (9%)	
Donor Age (median, range)	81	39 (28-46)	37 (30-44)	32 (25-47)	45 (33-54)	0.093
CMV patient and donor, n (%)	83					0.5

Donor neg. / Recipient pos.		18 (22%)	7 (28%)	9 (26%)	2 (9%)	
any other		54 (65%)	15 (60%)	22 (63%)	17 (74%)	
Unknown		11 (13%)	3 (12%)	4 (11%)	4 (17%)	
TCI score, n (%)	83					0.3
Low (1-2) / RIC		7 (8%)	2 (8%)	2 (6%)	3 (13%)	
Intermediate (2,5-3,5)		25 (30%)	11 (44%)	8 (23%)	6 (26%)	
High (>3,5) / MAC		51 (62%)	12 (48%)	25 (71%)	14 (61%)	
T cell depletion, n (%)	83					<0.01
ATG		68 (82%)	22 (88%)	34 (97%)	12 (52%)	
No depletion		11 (13%)	1 (4%)	0 (0%)	10 (44%)	
ptCY		4 (5%)	2 (8%)	1 (3%)	1 (4%)	
Stage at alloSCT, n (%)	83					0.6
Complete remission		34 (41%)	11 (44%)	12 (34%)	11 (48%)	
Active disease (upfront alloSCT, refractory, partial remission)		49 (59%)	14 (56%)	23 (66%)	12 (52%)	
Year of alloSCT (median, range)	83	2016 (2014-2019)	2016 (2014-2019)	2016 (2014-2019)	2016 (2009-2019)	0.4
Stage 30 days after alloSCT, n (%)	83					<0.01
CR with minimal residual disease or incomplete chimerism		41 (49%)	12 (48%)	10 (29%)	19 (83%)	
Molecular CR and full chimerism		42 (51%)	13 (52%)	25 (71%)	4 (17%)	
Acute GvHD after alloSCT, (before DLI) n (%)	83	34 (41%)	13 (52%)	14 (40%)	7 (30%)	0.3
Chronic GvHD after alloSCT (before DLI), n (%)	76	5 (7%)	4 (17%)	1 (3%)	0 (0%)	0.068
Karnofsky Performance Score before DLI, n (%)	81					0.046
<90		13 (16%)	1 (4%)	8 (24%)	4 (18%)	
90 - 100		68 (84%)	24 (96%)	26 (76%)	18 (82%)	
Indication for DLI, n (%)	83					<0.01
proDLI		36 (43%)	16 (64%)	18 (52%)	2 (9%)	

preDLI-IC		27 (33%)	5 (20%)	13 (37%)	9 (39%)	
preDLI-MRD		20 (24%)	4 (16%)	4 (11%)	12 (52%)	
Time to DLI1 (median months, range)	83	5.9 (1.1-42.9)	8.4 (3.8-22.3)	5.4 (3.9-29.6)	4.7 (1.1-42.9)	<0.01
Total number of DLI, n (%)	83					0.12
1		20 (24%)	3 (12%)	9 (26%)	8 (35%)	
2		18 (22%)	5 (20%)	7 (20%)	6 (26%)	
3		30 (36%)	12 (48%)	12 (34%)	6 (26%)	
4		9 (11%)	5 (20%)	4 (11%)	0 (0%)	
5		6 (7%)	0 (0%)	3 (9%)	3 (13%)	
DLI1 Cell Dose (10^6 CD3+ cells / kg) (median, range)	83	0.20 (0.02-10.0)	0.2 (0.02-0.5)	0.2 (0.2-1.0)	2.4 (0.5-10.0)	<0.01

[1]Fisher's exact test; Kruskal-Wallis rank sum test; Pearson's Chi-squared test

AML: acute myeloid leukaemia, MDS: myelodysplastic syndrome, AlloSCT: allogeneic stem cell transplantation, GvHD: Graft-versus-Host Disease, DLI: Donor lymphocyte infusion, ELN: European LeukemiaNET, IPSS: International Prognostic System Score, CMV: Cytomegalovirus, TCI: Transplant Conditioning Intensity, RIC: reduced intensity conditioning; MAC: myeloablative conditioning, ATG: Anti-Thymocyte Globuline, PTCy: Post-transplant Cyclophosphamide, CR: complete remission, preDLI-IC: pre-emptive DLI for incomplete chimerism, preDLI-MRD: pre-emptive DLI for minimal residual disease or molecular relapse, proDLI: prophylactic DLI, CD: cluster of differentiation, kg: kilogram.

Table 3. Summary of clinical outcome according to DLI intensity

	DLI intensity			
	Overall (n=83)	**Low-intensity (n=25)**	**Standard-intensity (n=35)**	**High-intensity (n=23)**
Outcome parameter	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**
2-year OS	80% (71-90%)	92% (82-100%)	87% (75-100%)	54% (35-82%)
2-year LFS	67% (57-78%)	88% (76-100%)	72% (58-89%)	30% (15-62%)
2-year RI	26% (17-36%)	8% (1-23%)	22% (10-39%)	55% (29-76%)
2-year NRM	8% (3-15%)	4% (0.3-17%)	6% (1-17%)	14% (3-33%)
1-year aGvHD (II-IV)	34% (24-44%)	24% (10-42%)	29% (15-44%)	53% (30-72%)
1-year cGvHD moderate/severe	27% (18-38%)	20% (7-38%)	30% (15-45%)	33% (14-54%)
1-year treatment success	61% (49-71%)	72% (49-86%)	69% (50-83%)	34% (15-55%)
2-year treatment success	71% (60-80%)	84% (60-94%)	76% (56-88%)	na

DLI: Donor lymphocyte infusion, OS: overall survival, LFS: leukaemia-free survival, RI: relapse incidence, NRM: non-relapse mortality, IS: Immunosuppression, GvHD: Graft-vs-Host Disease, aGvHD: acute GvHD, cGvHD: chronic GvHD, na: not-applicable

Table 4. Multivariable analyses of OS, LFS, RI, GvHD and treatment success

Variable	HR	95% CI	p-value
Overall survival (OS)			
Stage before alloSCT			
Complete remission (baseline)	-	-	-
Active disease	**2.81**	**1.2-6.5**	**0.018**
DLI intensity			
low or standard (baseline)	-	-	-
High	**6.12**	**2.74-13.6**	**<0.001**
Indication for DLI			
proDLI or preDLI-IC (baseline)			
preDLI-MRD	1.92	0.78-4.75	0.159
Stage at d30 after alloSCT			
Molecular CR, full chimerism (baseline)	-	-	-
CR with minimal residual disease or incomplete chimerism	1.27	0.54-2.97	0.589
Leukaemia-free survival (LFS)			
Stage before alloSCT			
Complete remission (baseline)	-	-	-
Active disease	**2.88**	**1.29-6.40**	**0.010**
DLI intensity			
low or standard (baseline)	-	-	-
High	**5.43**	**2.64-11.2**	**<0.001**
Relapse incidence (RI)			
Stage before alloSCT			
Complete remission (baseline)	-	-	-
Active disease	**3.19**	**1.31-7.78**	**0.011**
DLI intensity			
low or standard (baseline)	-	-	-
High	**4.77**	**1.99-11.4**	**<0.001**
Indication for DLI			

proDLI oder preDLI-IC (baseline)	-	-	-
preDLI-MRD	1.44	0.59-3.53	0.420
DLI-induced GvHD			
No GvHD (Baseline)	-	-	-
GvHD	0.46	0.18, 1.20	0.110
acute GvHD grades II-IV			
Patient age (every 10 years increase)	1.62	0.95-2.75	0.077
DLI intensity			
low or standard (baseline)	-	-	-
High	**2.51**	**1.20-5.27**	**0.015**
Treatment success			
Stage before alloSCT			
Complete remission (baseline)	-	-	-
Active disease	**0.55**	**0.38-0.81**	**0.002**
DLI intensity			
low or standard (baseline)	-	-	-
High	**0.41**	**0.2-0.84**	**0.016**

OS: overall survival, LFS: leukaemia-free survival, RI: relapse incidence, NRM: non-relapse mortality, IS: Immunosuppression, GvHD: Graft-vs-Host Disease, aGvHD: acute GvHD, DLI: Donor lymphocyte infusion, proDLI: prophylactic DLI, preDLI-IC: pre-emptive DLI for incomplete chimerism, preDLI-MRD: pre-emptive DLI for minimal residual disease or molecular relapse, alloSCT: allogeneic stem cell transplantation, CR: complete remission.

Legends to figures

Figure 1. Structure of the multistate model

At time of their first DLI, all patients started in a state being *alive, without GvHD and without relapse* **(1)**. From there, patients could transition into the following states: *Standard dose IS for GvHD* **(2)**, *Relapse* **(5)** or to *being alive without having received IS for GvHD nor experiencing relapse* **(6)**. Although clinically possible, a transition between state **(1)** and *NRM* **(4)** was not modeled because this transition was not observed in our cohort. Possible transitions for patients in the non-absorbing state **(2)** included either *stop IS or ongoing low dose IS* **(3)**, *NRM* **(4)** or *relapse* **(5)**. From non-absorbing state **(3)** patients could pass over to *relapse* **(5)** or *NRM* **(4)**. Patients with a relapse (non-absorbing) could only transition to *LAD* **(7)**. The cumulative incidence of treatment success was assessed in a competing risk model with relapse, death or Standard dose IS regarded as competing events.

DLI: donor lymphocyte infusion, IS: immunosuppression, GvHD: Graft-versus-Host Disease, NRM: non-relapse mortality, LAD: leukaemia-associated death.

Figure 2. Overall and leukaemia-free survival, and cumulative incidence of relapse incidence, and non-relapse mortality by DLI indication

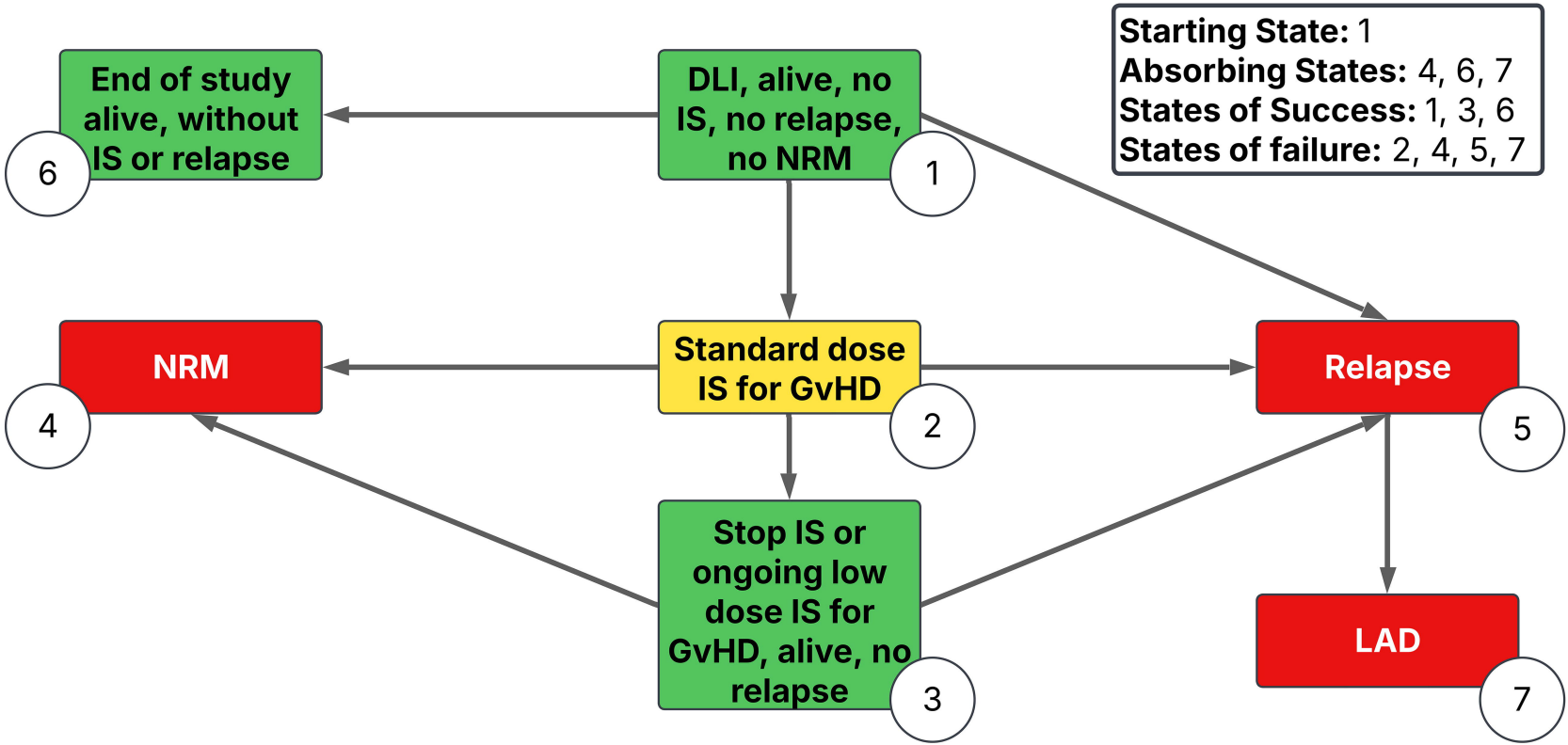
DLI: donor lymphocyte infusion, preDLI: pre-emptive DLI, MRD: minimal residual disease or molecular relapse.

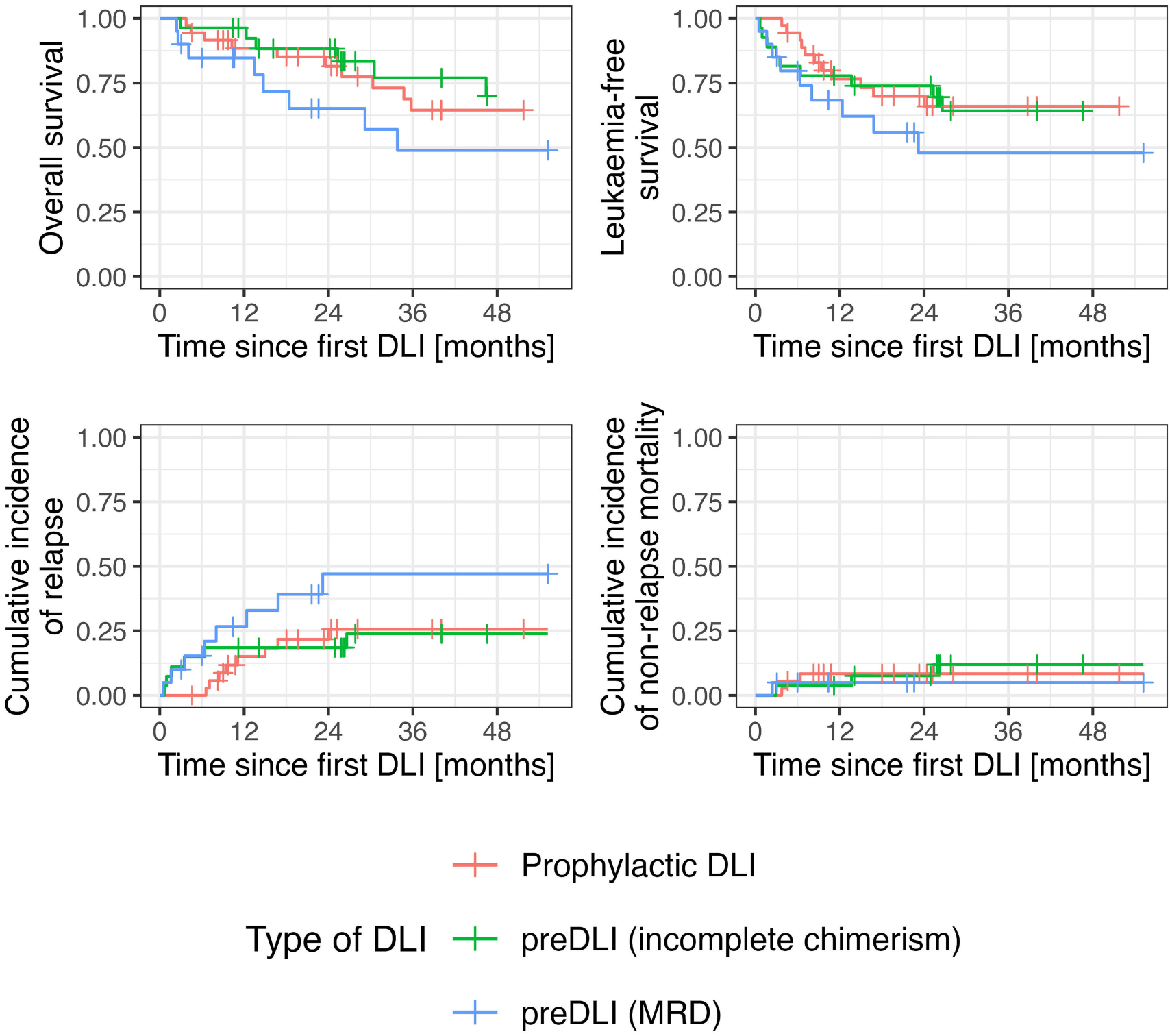
Figure 3. Overall and leukaemia-free survival, and cumulative incidence of relapse incidence, and non-relapse mortality by DLI intensity (definition of DLI-intensity in methods)

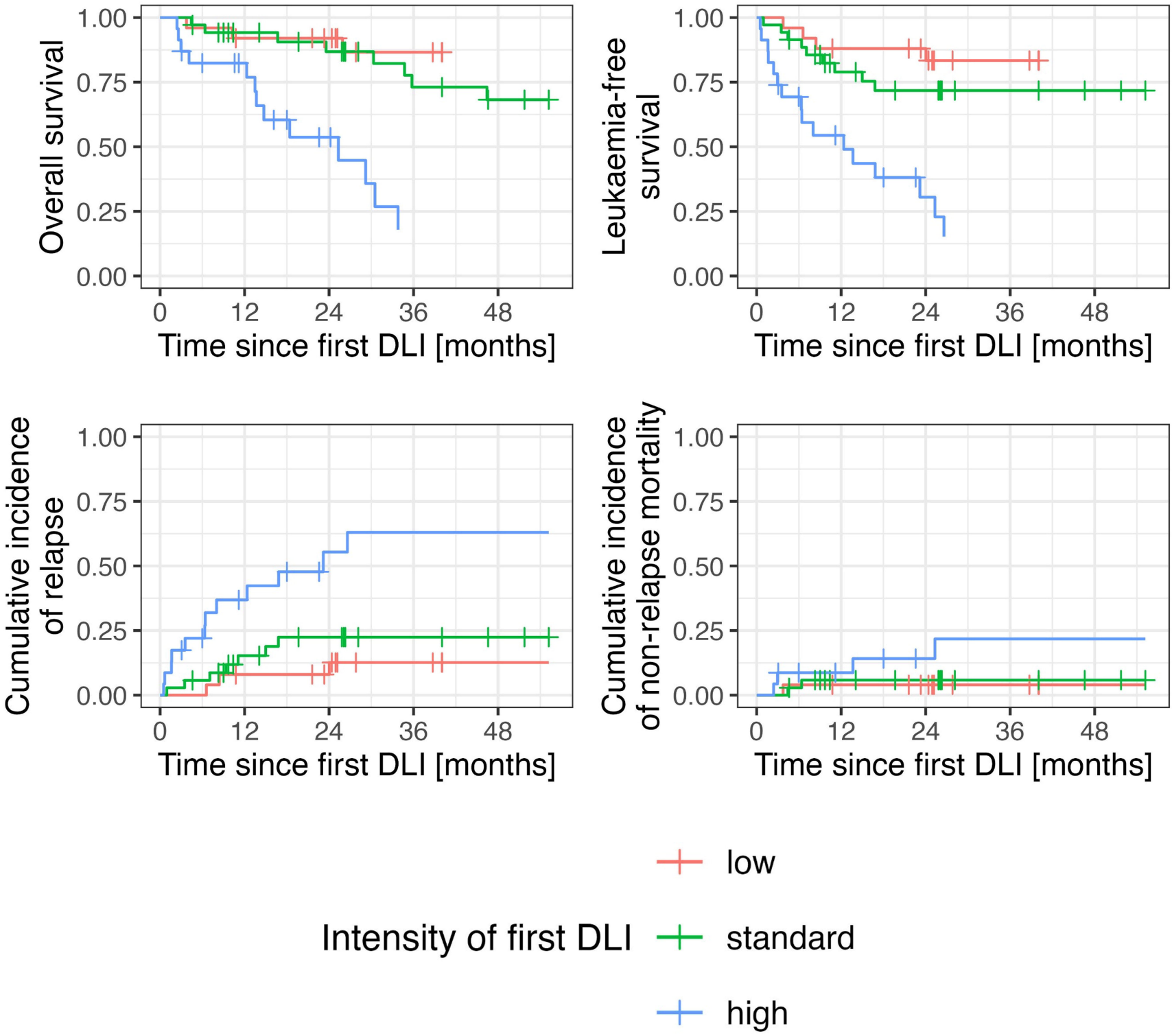
DLI: donor lymphocyte infusion.

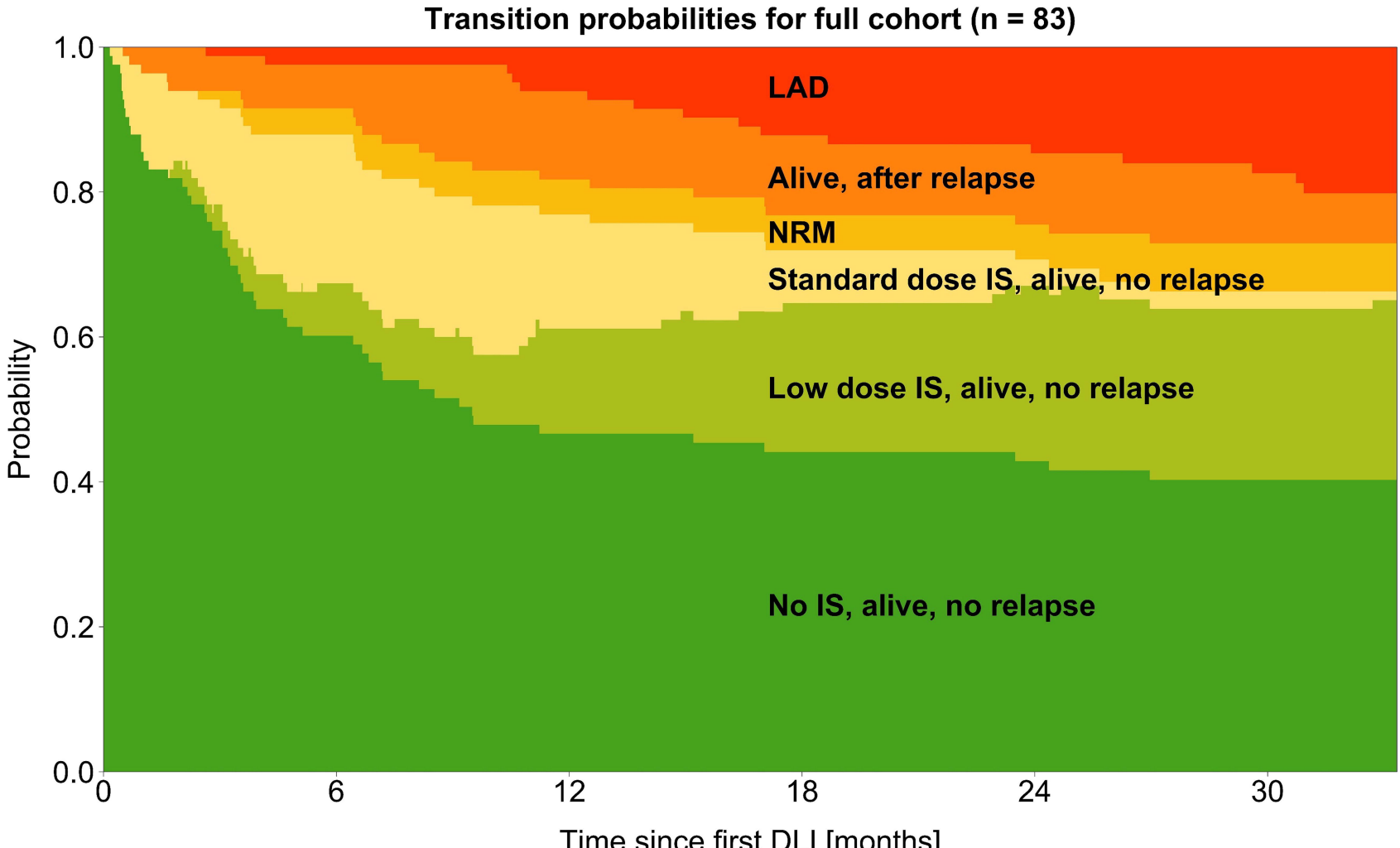
Figure 4. Multistate model for the analysis of clinical events after pro/preDLI over time

The green areas represent states fulfilling the criteria for treatment success, defined as being alive, without relapse and with no or only low-dose Immunosuppression (IS) for Graft-vs-Host Disease. LAD: leukaemia-associated death, NRM: non-relapse mortality. DLI: donor lymphocyte infusion.


Starting State: 1
Absorbing States: 4, 6, 7
States of Success: 1, 3, 6
States of failure: 2, 4, 5, 7
End of study alive, without IS or relapse
6
DLI, alive, no IS, no relapse, no NRM
1
NRM
4
Standard dose IS for GvHD
2
Relapse
5
Stop IS or ongoing low dose IS for GvHD, alive, no relapse
3
LAD
7


Overall survival
1.00
0.75
0.50
0.25
0.00
0
12
24
36
48
Time since first DLI [months]
Leukaemia-free survival
Cumulative incidence of relapse
Cumulative incidence of non-relapse mortality
Type of DLI
Prophylactic DLI
preDLI (incomplete chimerism)
preDLI (MRD)


Overall survival
1.00
0.75
0.50
0.25
0.00
0
12
24
36
48
Time since first DLI [months]
Leukaemia-free survival
Cumulative incidence of relapse
Cumulative incidence of non-relapse mortality
Intensity of first DLI
low
standard
high


Transition probabilities for full cohort (n = 83)
LAD
Alive, after relapse
NRM
Standard dose IS, alive, no relapse
Low dose IS, alive, no relapse
No IS, alive, no relapse
Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
30
Time since first DLI [months]

Supplement to

Prophylactic and pre-emptive donor lymphocyte infusion in patients with acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) - Validation of current recommendations and proposal of a modified outcome assessment

By Giuliano Filippini Velazquez, Jan Frederic Weller, Anna Rubeck et al.

Supplementary methods

Definitions

CHR and **relapse** were defined as published (Döhner et al., Blood 2022). **CC/IC (complete/incomplete donor chimerism):** 100%/<100% donor signals in bone marrow (BM) or peripheral blood (PB). **MRD (minimal residual disease):** detection of a disease related marker either in cytogenetic- or molecular genetic analyses in BM or PB. **LAD (leukaemia-associated death)**: all deaths following relapse. **NRM (non-relapse mortality):** death without prior evidence of relapse. **OS (overall survival):** time from DLI1 to date of death and last follow-up (LFU). **LFS (leukaemia-free survival):** time between DLI1 and date of relapse, death or LFU. **Response** was defined as MRD negativity after preDLI-MRD, and as conversion of IC to CC for preDLI-IC, respectively. Obviously, response could not be defined in proDLI.

MRD assessment

MRD was assessed by fluorescence-in-situ hybridization (FISH), next-generation-sequencing (NGS) and polymerase chain reaction (PCR) according to local standards. Levels of detection for each MRD marker varied according to local pre-established analytic assays, which are based on the recommendations for monitoring of measurable residual disease provided by the European LeukemiaNet (Döhner et al., Blood 2022). For the Augsburg cohort, FISH, NGS and PCR analyses were performed by an external institution: “Münchner Leukämie Labor (MLL)”, based in Munich, Germany. For the Tübingen cohort, NGS and PCR were also performed by MLL, whereas FISH was in part performed by another institution: “Medizinisches Versorgungszentrum Dr. Eberhard & Partner”, Dortmund, Germany.

Measurement of donor chimerism

Donor chimerism analyses were performed with multiplex PCR or PCR amplification of short tandem repeat sequences (Bader, Beck et al., BMT 1998).Detection levels for each MRD marker and chimerism values varied according to local pre-established analytic assays (Döhner et al., Blood 2022). For the Augsburg cohort, chimerism testing was performed by

“Labor AgenDix”, Dresden, Germany which uses multiplex PCR with a level of detection up to 1% for receiver residual cells. For the Tübingen cohort, analyses were performed using PCR amplification of short tandem repeat sequences with levels of detection for IC between 0,1-1% (Bader, Beck et al. BMT 1998).

Statistics

Patient- and treatment-related characteristics at the time of alloSCT and DLI1 were summarized using median and range for continuous, and frequency and percentage for categorical data. Differences of variable distribution between groups were tested using chi^2 test for categorical and t-test for continuous variables.

Endpoints of interests were OS, LFS, relapse incidence (RI), LAD, NRM, and treatment success, as previously defined. Follow-up was calculated from the date of DLI1. The Kaplan-Meier method was used to compute the probabilities of OS and LFS, along with their respective 95% confidence intervals (CI95). Cumulative incidences of relapse and NRM were jointly estimated in a competing risks model with relapse and death acting as events.

Additional endpoints were the cumulative incidence of a/cGvHD, which were defined as time between DLI1 and the first clinical sign of a/cGvHD and estimated in a competing risk model with death or relapse acting as competing events. We considered IS-requiring GvHD (grade II-IV aGvHD; moderate/severe cGvHD) as a parameter for the clinical relevance of DLI-induced GvHD, with the associated morbidity and potential mortality,

The cumulative incidence of treatment success was assessed in a competing risk model with relapse, death or Standard dose IS regarded as competing events.

To identify risk factors for OS and LFS, univariable and multivariable analyses (UVA/MVA) were performed using Cox models. For a/cGvHD, RI, NRM and treatment success, a Fine and Grey model was fitted, including NRM, relapse and a/cGvHD after DLI as time-dependent variables. Factors reaching a significance level $p < 0.1$ in UVA were included in the respective MVA. The cut-off for statistical significance was set at 0.05. Statistical analyses were performed using the software IBM SPSS Statistic 25 and R version 4.3.1 using the packages ‘survival’, ‘cmprsk’ and ‘mstate’ (de Wreede et al., Comput Methods Programs Biomed. 2010).

Supplementary results

In vivo T cell depletion

In-vivo T cell depletion (TCD) for GvHD-prevention was performed in 72 patients (87%), using rabbit anti-thymocyte globulin (ATG) in 68 patients (Neovii, formerly Fresenius 20mg/kg for 10/10 MUD, 10mg/kg for MSD, and Thymoglobulin [Sanofi] 7.5mg/kg for one 9/10 MUD, days -3 to 1, respectively), and post-transplant cyclophosphamide (PTCy 50mg/kg at days +3 and +4) in 4.

Supplementary Table 1 – conditioning regimen

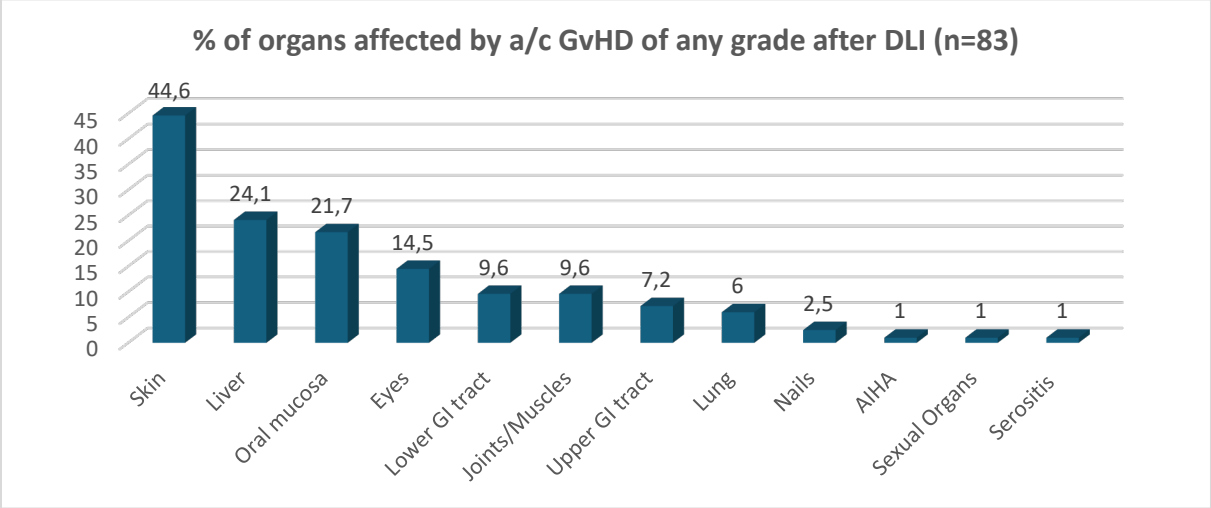
TCI-Score	MAC vs RIC	Conditioning regime
low (1 - 2)	**RIC**	Fludarabin 150, Treosulfan 30g
		Fludarabin 150, Busulfan 6,4
		Fludarabin 120, Treosulfan 30
		Fludarabin 120, Melphalan 140
		Clofarabin 150, Cytarabin 100, Treosulfan 30
intermediate (2,5 - 3,5)	**RIC/ MAC**	Flamsa Busulfan 6,4 (-Bu)
		Flamsa Busulfan 6,4 (Flamsa-Bu)
		Fludarabin 150, BCNU 400, Melphalan 140
		Fludarabin 150, BCNU 300, Melphalan 110
		Fludarabin 150, Treosulfan 36
		Fludarabin 150, Melphalan 140
		Fludarabin 150, Busulfan 12, 8
		Fludarabin 120, Melphalan 140, TBI 4Gy
		Fludarabin 120, TBI 8Gy
		Clofarabin 150, Fludarabin 150, Melphalan 110, Cyclophosphamide 29
		Clofarabin 120, Fludarabin 150, Melphalan 110, Cyclophosphamide 29
		Fludarabin 150, Idarubicin 12, BCNU 300, Melphalan 110
		Fludarabin 150, Cytarabin 8, Mitoxantron 20, Busulfan 6,4
		Fludarabin 120, Carmustin 400, Melphalan 140
high (> 3,5)	**MAC**	Flamsa, Fludarabine 60, Busulfan 8
		Busulfan 12,8, Cyclophosphamide 120
		Flamsa, Busulfan 8, Cyclophosphamide 80
		Fludarabine 180, Busulfan 12,8
		Flamsa, Fludarabine 60, Busulfan 8
		Flamsa, TBI 4Gy, Cyclophosphamide 120
		Flamsa, TBI 4Gy, Cyclophosphamide 80
		Flamsa, TBI 2Gy, Cyclophosphamide 80
		Flamsa, TBI 2Gy, Cyclophosphamide 29
		Fludarabine 120 Melphalan 140 Thiotepa 10, TBI 7Gy
		Melphalan 140, Fludarabin 100, TBI 8

		Flamsa TBI 4Gy
		Flu 120 Mel 140 TBI 8Gy
		Flamsa Treosulfan
		Flamsa Bu 6,4 Cy 120
		Flamsa Treosulfan 30

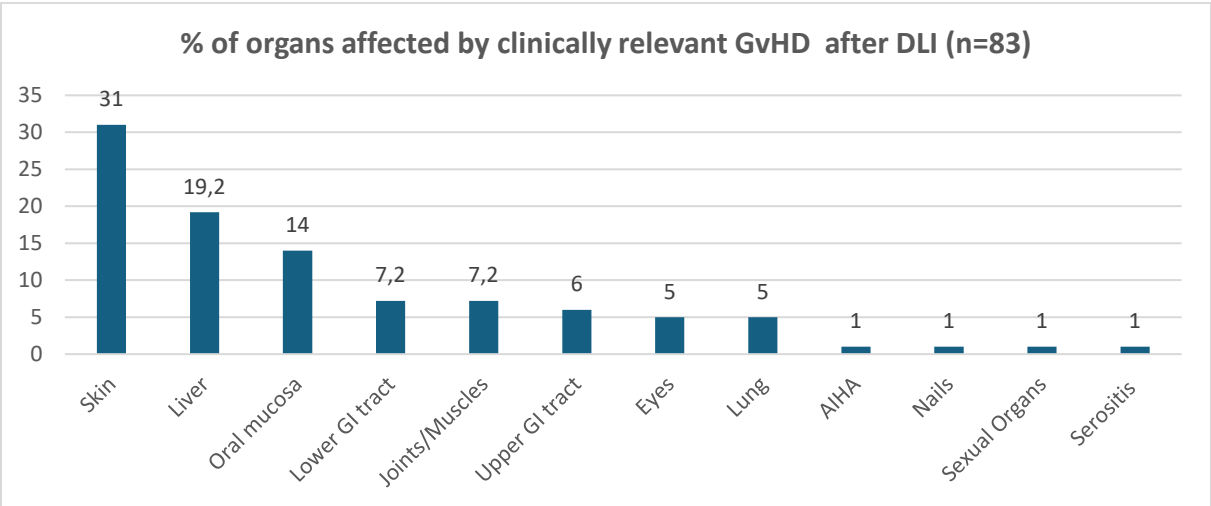
TCI: transplant conditioning intensity, MAC: myeloablative conditioning, RIC: reduced intensity conditioning, FLAMSA: Fludarabine, Amsacrine, Cytarabine, Cy: cyclophosphamide, TBI: total body irradiation.

Supplementary figures 1 & 2. End-organ affection by DLI-induced GvHD

Supplementary figure 1



Supplementary figure 2



Among all patients (n=83) receiving DLI, the most common organs affected by acute or chronic GvHD of any grade included skin, liver, oral mucosa, and eyes **(supplementary figure 1)**.

However, clinically significant GvHD (acute GvHD grades II-IV or chronic GvHD moderate/severe) that required systemic immunosuppressive treatment (IS) most often affected skin (31%), liver (19%), and oral mucosa (14%). Other organs that were affected each in less than 10% of patients were lower-/upper GI tract (7%/6%), joints/ muscles (7%), eyes (5%) and lung (5%). Rare manifestations included autoimmune haemolytic anemia (AIHA), nail dystrophia, sexual organ affection, and serositis (all 1%), **supplementary figure 2.**

Supplementary table 2. GvHD response to IS treatment by organ affected

Rates of response to IS treatment by organ affected (only patients with GvHD II-IV)												
	Total patients	**Total number of patients with affected site**		**Complete response to IS**		**GvHD improved to IS, but was persistent/ ongoing**		**Overall response to IS**	**No response (refractory GvHD to IS)**		**n.a.**	
Site (organ affected)	**n**	**n**	**%**	**n**	**%**	**n**	**%**	**% OR**	n	**%**	n	**%**
Skin	32	26	81,3	14	**54**	9	**35**	**89**	2	**8**	1	3
Lower GI tract	32	6	16,8	4	**67**	0	**0**	**67**	2	**33**	0	0
Liver	32	16	50	10	**62,5**	4	**25**	**87,5**	2	**12,5**	0	0
Upper GI tract	32	5	15,6	3	**60**	2	**40**	**100**	0	**0**	0	0
Oral mucosa	32	12	37,5	6	**50**	4	**33**	**83**	1	**8**	1	8
Eyes	32	4	12,5	0	**0**	3	**75**	**75**	1	**25**	0	0
Joint/ Muscles	32	6	18,8	1	**17**	5	**83**	**100**	0	**0**	0	0
AIHA	32	1	3,1	1	**100**	0	**0**	**100**	0	**0**	0	0
Lung	32	4	12,5	2	**40**	2	**40**	**80**	0	**0**	1	20
Nail dystrophia	32	1	3,1	0	**0**	1	**100**	**100**	0	**0**	0	0
Sexual Organs	32	1	3,1	0	**0**	1	**100**	**100**	0	**0**	0	0
Serositis	32	1	3,1	1	**100**	0	**0**	**100**	0	**0**	0	0

GvHD: graft versus host disease, IS: systemic immunosuppression, GI: gastrointestinal, Overall response: complete response + clinical improvement, but persistent/ongoing GvHD), n.a.: non applicable / missing.

Standard systemic immunosuppressive treatment was based on prednisolone with or without the addition of a CNI (tacrolimus, ciclosporin A) or mycophenolate mofetil. Only 8 patients required additional treatment which included ruxolitinib, ibrutinib, ECP, or etarnecept.

Regarding the response rates of GvHD from the different organs to the immunosuppressive treatment (n=32 of patients requiring systemic IS), we observed an overall response (OR, including complete responses and improvement but ongoing mild GvHD) of >80% in most organs (skin 89%, liver 87%, upper GI tract 100%, oral mucosa, 83%, Joints/muscles, 100%,

lungs 80%). Treatment refractory GvHD was observed in the following affected organs: lower GI tract (n=2, 33% of affected), eyes (n=1, 25% of affected), oral mucosa (n=1, 8% of affected), liver (n=2, 12% of affected), and skin (n=2, 8% of affected), **see supplementary table 2.**
Among patients that received additional treatment (ruxolitinib, ibrutinib, extracorporeal photopheresis, etanercept), OR was seen in 100% (CR, n=2; improvement but ongoing GvHD, n=6).

Supplementary table 3 – Univariable analysis of risk factors

Overall Survival

Variable	N	HR	95% CI	p-value
Stage before alloSCT	83			
CR (Baseline)		-	-	-
Active disease		2.29	0.98, 5.33	0.054
Diagnosis	83			
AML (Baseline)		-	-	-
MDS		0.38	0.05, 2.85	0.349
ELN + IPSS risk group	83			
Low (Baseline)		-	-	-
Intermediate		2.05	0.26, 16.0	0.495
High		2.28	0.30, 17.3	0.426
aGvHD after alloSCT before DLI	83			
Yes (Baseline)		-	-	-
no		1.42	0.63, 3.18	0.401
cGvHD after alloSCT before DLI	76			
Yes (Baseline)		-	-	-
No		NA	NA	NA
Extramedullary disease before alloSCT	83			
Yes (Baseline)		-	-	-
No		1.40	0.19, 10.4	0.741
TCI Score	83			
Low (Baseline)		-	-	-
intermediate		1.05	0.23, 4.81	0.952
high		0.69	0.16, 3.08	0.630
Stage at d30 after alloSCT	83			
molecular CR, full donor chimerism (Baseline)		-	-	-
CR with minimal residual disease or IC		1.72	0.79, 3.74	0.174
Type of DLI	83			
proDLI (Baseline)		-	-	-
preDLI-IC		0.83	0.32, 2.15	0.703
preDLI-MRD		1.69	0.68, 4.21	0.261
Type of DLI combined	83			

proDLI oder preDLI-IC (Baseline)		-	-	-
proDLI-MRD		1.82	0.79, 4.20	0.159
Chimerism by groups	82			
>99% (Baseline)		-	-	-
90-99%		0.80	0.24, 2.72	0.726
<90%		1.85	0.43, 8.06	0.411
DLI intensity	83			
low or standard (Baseline)				
high		5.36	2.41, 11.9	< 0.001
GvHD after DLI	83			
No GvHD (Baseline)		-	-	-
GvHD		1.15	0.50, 2.63	0.743
IS requiring GvHD	83			
No IS requiring GvHD (Baseline)		-	-	-
IS requiring GvHD		1.38	0.62, 3.08	0.431

Leukaemia-Free Survival

Variable	N	HR	95% CI	p-value
Stage before alloSCT	83			
CR (Baseline)		-	-	-
Active disease		2.38	1.08, 5.26	0.032
Diagnosis	83			
AML (Baseline)		-	-	-
MDS		0.58	0.14, 2.42	0.450
ELN + IPSS risk group	83			
Low (Baseline)		-	-	-
intermediate		2.13	0.27, 16.6	0.473
high		3.02	0.40, 22.6	0.281
aGvHD after alloSCT before DLI	83			
Yes (Baseline)		-	-	-
no		1.20	0.58, 2.48	0.627
cGvHD after alloSCT before DLI	76			
Yes (Baseline)		-	-	-
no		NA	NA	NA
Extramedullary disease before alloSCT	83			
Yes (Baseline)		-	-	-
no		0.91	0.22, 3.82	0.896
TCI Score	83			
Low (Baseline)		-	-	-
intermediate		1.79	0.40, 7.96	0.442
high		1.02	0.23, 4.48	0.976
Stage at d30 after alloSCT	83			
molecular CR, full donor chimerism (Baseline)		-	-	-
CR with minimal residual disease or IC		1.69	0.83, 3.47	0.150

Type of DLI	83			
proDLI (Baseline)		-	-	-
preDLI-IC		1.11	0.48, 2.58	0.803
preDLI-MRD		1.71	0.72, 4.06	0.226
Type of DLI combined	83			
pro DLI oder preDLI-IC (Baseline)		-	-	-
preDLI-MRD		1.63	0.75, 3.54	0.218
Chimerism by groups	82			
>99% (Baseline)		-	-	-
90-99%		1.37	0.51, 3.65	0.531
<90%		1.98	0.59, 6.67	0.271
DLI intensity	83			
low or standard (Baseline)		-	-	-
high		4.78	2.34, 9.78	< 0.001
GvHD after DLI	83			
No GvHD (Baseline)		-	-	-
GvHD		1.17	0.54, 2.57	0.69
IS requiring GvHD	83			
No IS requiring GvHD (Baseline)		-	-	-
IS requiring GvHD		1.31	0.61, 2.81	0.494

Relapse incidence

Variable	N	HR	95% CI	p-value
Stage before alloSCT	83			
CR (Baseline)		-	-	-
Active disease		2.80	1.06, 7.41	0.038
Diagnosis	83			
AML (Baseline)		-	-	-
MDS		0.84	0.20, 3.45	0.810
ELN + IPSS risk group	83			
Low (Baseline)		-	-	-
intermediate		1.02	0.11, 9.27	0.980
high		2.34	0.29, 18.7	0.420
aGvHD after alloSCT before DLI	83			
Yes (Baseline)		-	-	-
no		1.21	0.51, 2.92	0.660
cGvHD after alloSCT before DLI	76			
Yes (Baseline)		-	-	-
no		NA	NA	NA
Extramedullary disease before alloSCT	83			
Yes (Baseline)		-	-	-
no		0.62	0.14, 2.69	0.530
TCI Score	83			
Low (Baseline)		-	-	-
intermediate		2.83	0.39, 20.8	0.310

high		1.85	0.26, 13.2	0.540
Stage at d30 after alloSCT	83			
molecular CR, full donor chimerism (Baseline)		-	-	-
CR with minimal residual disease or IC		1.60	0.69, 3.71	0.270
Type of DLI	83			
proDLI (Baseline)		-	-	-
preDLI-IC		1.01	0.34, 2.95	0.990
preDLI-MRD		2.25	0.88, 5.76	0.090
Type of DLI combined	83			
pro DLI oder preDLI-IC (Baseline)		-	-	-
preDLI-MRD		2.25	0.95, 5.29	0.064
Chimerism by groups	82			
>99% (Baseline)		-	-	-
90-99%		1.64	0.48, 5.59	0.430
<90%		1.57	0.33, 7.42	0.570
DLI intensity	83			
low or standard (Baseline)		-	-	-
high		4.57	2.01, 10.4	< 0.001
GvHD after DLI	83			
No GvHD (Baseline)		-	-	-
GvHD		0.38	0.15, 0.97	0.043

Non-relapse Mortality

Variable	N	HR	95% CI	p-value
Stage before alloSCT	83			
CR (Baseline)		-	-	-
Active disease		1.06	0.32, 3.58	0.920
Diagnosis	83			
AML (Baseline)		-	-	-
MDS		NA	NA	NA
ELN + IPSS risk group	83			
Low (Baseline)		-	-	-
intermediate		NA	NA	NA
high		NA	NA	NA
aGvHD after alloSCT before DLI	83			
Yes (Baseline)		-	-	-
no		0.97	0.28, 3.40	0.960
cGvHD after alloSCT before DLI	76			
Yes (Baseline)		-	-	-
no		NA	NA	NA
Extramedullary disease before alloSCT	83			
Yes (Baseline)		-	-	-
no		NA	NA	NA
TCI Score	83			

Low (Baseline)		-	-	-
intermediate		0.81	0.09, 7.52	0.860
high		0.39	0.04, 3.79	0.410
Stage at d30 after alloSCT	83			
molecular CR, full donor chimerism (Baseline)		-	-	-
CR with minimal residual disease or IC		1.45	0.42, 5.07	0.560
Type of DLI	83			
proDLI (Baseline)		-	-	-
preDLI-IC		1.30	0.35, 4.86	0.700
preDLI-MRD		0.47	0.05, 4.27	0.500
Type of DLI combined	83			
pro DLI oder preDLI-IC (Baseline)		-	-	-
preDLI-MRD		0.42	0.05, 3.42	0.420
Chimerism by groups	82			
>99% (Baseline)		-	-	-
90-99%		0.63	0.07, 5.76	0.680
<90%		1.81	0.21, 15.4	0.590
DLI intensity	83			
low or standard (Baseline)		-	-	-
high		2.32	0.62, 8.65	0.210

Acute GvHD

Variable	N	HR	95% CI	p-value
Patient age/10 years	83	1.57	0.93, 2.66	0.090
Donor type grouped_MSD vs the rest	83			
MSD (Baseline)		-	-	-
any other		0.59	0.26, 1.30	0.190
Donor sex_combination	83			
Female in male (Baseline)		-	-	-
any other		1.42	0.40, 4.96	0.590
Stage before alloSCT	83			
CR (Baseline)		-	-	-
Active disease		0.78	0.37, 1.61	0.500
CMV serology status (neg/neg vs other)	73			
neg/neg (Baseline)		-	-	-
other		1.54	0.55, 4.26	0.410
T cell depletion	83			
ATG (Baseline)		-	-	-
ptCY		NA	NA	NA
no TCD		0.74	0.08, 7.00	0.790
TCI Score	83			
Low (Baseline)		-	-	-
intermediate		0.39	0.11, 1.42	0.150
high		0.39	0.12, 1.27	0.120

aGvHD after alloSCT before DLI	83			
Yes (Baseline)		-	-	-
no		1.05	0.49, 2.23	0.900
cGvHD after alloSCT before DLI	76			
Yes (Baseline)		-	-	-
no		0.73	0.15, 3.47	0.690
Type of DLI	83			
proDLI (Baseline)		-	-	-
preDLI-IC		0.81	0.34, 1.92	0.630
preDLI-MRD		1.25	0.51, 3.08	0.630
Type of DLI combined	83			
pro DLI oder preDLI-IC (Baseline)		-	-	-
preDLI-MRD		1.37	0.60, 3.10	0.450
DLI intensity	83			
low or standard (Baseline)		-	-	-
high		2.33	1.12, 4.86	0.024
Time from alloSCT to DLI	83			
below median (Baseline)		-	-	-
above median		0.54	0.26, 1.13	0.100
Cell dose (CD3+) for DLI1	83			
above median (Baseline)		-	-	-
below median		0.65	0.31, 1.35	0.250

Chronic GvHD

Variable	N	HR	95% CI	p-value
Patient age/10 years	83	1.35	0.87, 2.09	0.180
Donor type grouped_MSD vs the rest	83			
MSD (Baseline)		-	-	-
any other		0.47	0.20, 1.11	0.085
Donor sex_combination	83			
Female in male (Baseline)		-	-	-
any other		1.89	0.46, 7.87	0.380
Stage before alloSCT	83			
CR (Baseline)		-	-	-
Active disease		0.51	0.22, 1.16	0.110
CMV serology status (neg/neg vs other)	73			
neg/neg (Baseline)		-	-	-
other		0.86	0.34, 2.19	0.750
T cell depletion	83			
ATG (Baseline)		-	-	-
ptCY		NA	NA	NA
no TCD		0.99	0.10, 9.54	0.990
TCI Score	83			
Low (Baseline)		-	-	-
intermediate		0.39	0.12, 1.30	0.130
high		0.31	0.10, 0.94	0.039

aGvHD after alloSCT before DLI	83			
Yes (Baseline)		-	-	-
no		1.02	0.44, 2.35	0.970
cGvHD after alloSCT before DLI	76			
Yes (Baseline)		-	-	-
no		0.63	0.15, 2.66	0.530
Type of DLI	83			
proDLI (Baseline)		-	-	-
preDLI-IC		0.95	0.36, 2.52	0.920
preDLI-MRD		1.21	0.44, 3.35	0.720
Type of DLI combined	83			
pro DLI oder preDLI-IC (Baseline)		-	-	-
preDLI-MRD		1.23	0.50, 3.06	0.650
DLI intensity	83			
low or standard (Baseline)		-	-	-
high		1.28	0.54, 3.06	0.580
Time from alloSCT to DLI	83			
below median (Baseline)		-	-	-
above median		1.13	0.49, 2.58	0.770
Cell dose (CD3+) for DLI1	83			
above median (Baseline)		-	-	-
below median		0.63	0.28, 1.44	0.270

Treatment success

Variable	N	HR	95% CI	p-value
Patient age/10 years	83	1.02	0.81, 1.28	0.890
Donor type grouped_MSD vs the rest	83			
MSD (Baseline)		-	-	-
any other		1.29	0.73, 2.29	0.380
Donor sex_combination	83			
Female in male (Baseline)		-	-	-
any other		1.02	0.60, 1.74	0.930
Stage before alloSCT	83			
CR (Baseline)		-	-	-
Active disease		0.61	0.41, 0.92	0.018
CMV serology status (neg/neg vs other)	83			
neg/neg (Baseline)		-	-	-
other		0.52	0.15, 1.75	0.290
T cell depletion		0.52	0.15, 1.75	0.290
ATG (Baseline)	83			
ptCY		-	-	-
no TCD		0.61	0.28, 1.31	0.200
TCI Score		0.83	0.41, 1.68	0.610
Low (Baseline)	83			
intermediate		-	-	-

high		0.85	0.56, 1.29	0.430
aGvHD after alloSCT before DLI	76			
Yes (Baseline)		-	-	-
no		0.57	0.37, 0.89	0.014
cGvHD after alloSCT before DLI	83			
Yes (Baseline)		-	-	-
no		0.86	0.56, 1.31	0.480
Type of DLI		0.64	0.33, 1.25	0.200
proDLI (Baseline)	83			
preDLI-IC		-	-	-
preDLI-MRD		0.69	0.37, 1.29	0.250
Type of DLI combined	83			
pro DLI oder preDLI-IC (Baseline)		-	-	-
preDLI-MRD		0.44	0.23, 0.84	0.013
DLI intensity	83			
low or standard (Baseline)		-	-	-
high		1.33	0.86, 2.05	0.200
Time from alloSCT to DLI	83			
below median (Baseline)		-	-	-
above median		1.48	0.94, 2.32	0.087

AlloSCT: allogeneic stem cell transplantation, DLI: Donor lymphocyte infusion, preDLI-IC: pre-emptive DLI for incomplete chimerism, preDLI-MRD: pre-emptive DLI for minimal residual disease or molecular relapse, proDLI: prophylactic DLI, GvHD: Graft-versus-Host Disease, aGvHD: acute GvHD, cGvHD: chronic GvHD, OS: overall survival, LFS: leukaemia-free survival, RI: relapse incidence, NRM: non-relapse mortality, CR: complete remission, IS: Immunosuppression, ELN: European LeukemiaNET, IPSS: International Prognostic System Score, CMV: Cytomegalovirus, TCI: Transplant Conditioning Intensity, RIC: reduced intensity conditioning; MAC: myeloablative conditioning, ATG: Anti-Thymocyte Globuline, PTCy: Post-transplant Cyclophosphamide, NA: not applicable (analyses not possible).

Supplementary table 4 - Multivariable analyses of risk factors for OS, LFS, RI, and GvHD after pro/pre DLI excluding preDLI-MRD (only proDLI + preDLI-IC, n=63)

Variable	HR	95% CI	p-value
OS			
Stage before alloSCT			
CR (Baseline)	-	-	-
Active disease	**3.97**	**1.27, 12.4**	**0.018**
DLI intensity			
low or standard (Baseline)	-	-	-
High	**3.42**	**1.19, 9.83**	**0.023**
LFS			
Stage before alloSCT			
CR (Baseline)	-	-	-
Active disease	**4.14**	**1.45, 11.8**	**0.008**
DLI intensity			
low or standard (Baseline)	-	-	-
high	**3.94**	**1.56, 9.90**	**0.004**
RI			
Stage before alloSCT			
CR (Baseline)	-	-	-
Active disease	3.96	0.94, 16.7	0.061
DLI-induced GvHD (acute or chronic, any grade)			
No GvHD (Baseline)	-	-	-
GvHD	**0.27**	**0.08, 0.94**	**0.039**
acute GvHD grades II-IV			
Patient age (every 10 years increase)	1.62	0.95, 2.75	0.077
DLI intensity			
low or standard (Baseline)	-	-	-
high	**2.51**	**1.20, 5.27**	**0.015**

OS: overall survival, LFS: leukaemia-free survival, RI: relapse incidence, NRM: non-relapse mortality, IS: Immunosuppression, GvHD: Graft-vs-Host Disease, aGvHD: acute GvHD, DLI: Donor lymphocyte infusion. preDLI-IC: pre-emptive DLI for incomplete chimerism, preDLI-MRD: pre-emptive DLI for minimal residual disease or molecular relapse, proDLI: prophylactic DLI, alloSCT: allogeneic stem cell transplantation, CR: complete remission.

Supplementary table 5 – Characteristics of patients receiving DLI from a haploidentical (n=4) or a MUD 9/10 donor (n=12, total n=16)

Variable		Total number of patients (N=16)
Patient age in years	median (range)	60 (24-67)
Patient sex	Male	7 (44%)
	Female	9 (56%)
Diagnosis	AML	14 (87%)
	MDS	2 (13%)
Risk group ELN	Low risk	1 6%)
	Intermediate risk	5 (31%)
	High risk	8 (50%)
	Not applicable (MDS)	2 (12%)
Stage at alloSCT	CR	6 (37%)
	Active Disease	10 (63%)
Donor sex combination	Female in male	3 (19%)
	Any other	13 (81%)
Last number of alloSCT	1	13 (81%)
	2	3 (19%)
Indication for DLI	Prophylactic	10 (63%)
	Pre-emptive for IC	5 (31%)
	Pre-emptive for MRD	1 (6%)
DLI intensity	Low	10 (63%)
	Standard	4 (25%)
	High	2 (12%)
Total number of DLI	1	4 (25%)
	2	3 (19%)
	3	6 (37%)
	4	3 (19%)
Acute GvHD grade II-IV after DLI	Yes	4 (25%)
	No	12 (75%)
Median time to aGvHD	Months (range)	1.6 (0.9-3.8)
Chronic GvHD (moderate/severe) after DLI	Yes	2 (12%)
	No	14 (88%)
Median time to cGvHD	Months (range)	3.7 (1.2-18)
Relapse	Yes	6 (37%)
	No	10 (63%)
Dead	Yes	6 (37%)
	No	10 (63%)

Sixteen patients had received DLI after alloSCT from a haploidentical donor or a 9/10 HLA mismatched donor **(supplementary table 5)**. In this selected cohort, DLI was given in low-, standard-, and high-intensity in 10 (63%), 4 (25%), and 2 (12%) patients, respectively. The median number of CD3+ cells/kg at DLI1 was 0.2 (range: 0.02 – 1.0). The median time of DLI1 from alloSCT was 6.9 months (range: 3.9-16.36).

Acute GvHD grades II-IV was seen in 4 (25%) patients with a median onset time after DLI1 of 1.6 months. Chronic GvHD moderate/extensive developed in 2 (12%) patients with a median onset time after DLI1 of 3.7 months.

The median overall survival for all patients at data cut-off was 36 months. The 2-year OS was 81% (95% CI: 64-100). The 2-year RI was 31% (95% CI: 13-55). In total, 6 patients developed a relapse (4 after proDLI) or the AML progressed soon after preDLI (n=2). One patient whose AML was refractory to preDLI-IC has achieved complete remission after salvage treatment (including subsequent DLI) and remain in remission > 2 years from relapse. The 2y-NRM was 6% (95% CI: 0-18). One patient (60 years old) developed aGvHD grade IV of the skin, mouth, gastrointestinal tract and eyes after 3 infusions of prophylactic DLI. The GvHD was responsive to the immunosuppressive treatment, but he died of a pulmonary infection.

Taken together, neither the inclusion of donor type into the risk factor analysis as done in the paper, nor the more detailed analysis of patients with mismatched donors provided here did suggest an influence of donor type on overall clinical outcome. This again supports the value of the published guidelines for DLI, where different recommendations are provided for the related, matched unrelated and mismatched donor setting.

Supplementary table 6 - Role of different state-transitions within the multistate model

Transitions (states) in the low/standard-Intensity DLI group (n=60)

Number of patients in transition

from/to	start	IS	success	NRM	relapse	LAD	no event	total entering
start	0	19	32	0	9	0	0	60
IS	0	0	18	1	0	0	0	19
success	0	0	0	4	1	0	45	50
NRM	0	0	0	0	0	0	5	5
relapse	0	0	0	0	0	9	1	10
LAD	0	0	0	0	0	0	9	9

Transitions (states) in the high-Intensity DLI group (n=23)

Number of patients in transition

from/to	start	IS	success	NRM	relapse	LAD	no event	total entering
start	0	12	3	0	8	0	0	23
IS	0	0	8	0	4	0	0	12
success	0	0	0	4	0	0	7	11
NRM	0	0	0	0	0	0	4	4
relapse	0	0	0	0	0	11	1	12
LAD	0	0	0	0	0	0	11	11

DLI: donor lymphocyte infusion, IS: Standard immunosuppression for GvHD, NRM: non-relapse mortality, LAD: leukaemia-associated death

Two transitions (respectively states) contributed mostly to the differences of final treatment success between the two DLI intensity cohorts: Primarily, it is the direct transition from the start state (1) to **"being alive without having received IS for GvHD nor experiencing relapse"** (6), which was observed in 53% of patients receiving low/standard dose DLI, but only 13% of those receiving high intensity DLI. The second discriminating state for final treatment success is the transient state **"standard dose IS for GvHD"** (2): In the low intensity group, only 1/19 patients in this state (5%) develops NRM, whereas 18/19 (95%) patients show transition to the success state **"stop IS or ongoing low dose IS"** (3) out of which 13/19 (68%) remain there by the end of study and achieve final treatment success. In contrast, in the high intensity cohort, transition from the **"standard dose IS for GvHD"** state (2) to the success state **"stop IS or ongoing low dose IS"** (3) is only achieved by 8/12 (67%) of patients, and only 4/12 (33%) achieve final treatment success.

Supplementary result: Immune reconstitution and changes in lymphocyte subpopulations after pro/preDLI

No systematic monitoring of immune reconstitution/ counts of peripheral lymphocytes was performed, since formally patients had already developed post-transplant immune reconstitution after alloSCT before DLI was performed. Therefore, representative data were available only for a minority (n=11) of the patients included in this study. To analyse the effects of DLI on changes in lymphocyte subpopulations we added the data from 15 patients that received DLI more recently but were not included in the study due to limited follow-up, for which analyses of immune reconstitution have been performed extensively in the context of another trial. For these patients, data on immune cell subtypes in peripheral blood were available between 60 days before the first DLI until 120 days after the first DLI.

In total, these 26 patients that had received prophylactic or pre-emptive DLI in escalating doses according to local standards. Donor types were MSD, MUD 10/10, MUD 9/10, and Haploidentical in 8 (31%), 12 (46%), 2 (8%), and 4 (15%), respectively. The median time from SCT to the first DLI (DLI1) was 7.2 months (range: 4.5-29.6). The median number of infusions was 3 (range: 1-5). Five (19%) patients received only 1 infusion, meaning 81% received at least 2 infusions.

Immune cell subtypes that were analysed by immunophenotyping in the peripheral blood included CD19-, CD4-, CD8-, and NK cells. The median time from the baseline analysis before DLI1 was 14 days (range 0-59), and the median time from DLI1 to the post-DLI1 analysis was 63 days (range: 7-104). The values measured at baseline and after DLI are described in **supplementary table 7:**

	Baseline (range)	**After last DLI**	**Difference**
CD19	148/ µl (0-997)	201.5/ µl (91-1303)	+29%
CD4	144/ µl (65-637)	154/ µl (43-707)	+7%
CD8	384/ µl (17-2710)	361/ µl (41-2700)	-6%
NK cells	230/ µl (55-857)	239/ µl (75-898)	+4%

In summary, in this selected cohort of patients, only the concentration of CD19 cell showed a substantial overall increase after DLI, whereas no changes were noted for T and NK cells. Further analyses regarding correlation of the baseline/delta values of immune cells and clinical outcome parameters are limited by the low number of patients.

Whereas immune reconstitution and changes of lymphocyte subtypes by DLI was not in the focus of the present analysis, we had investigated early changes (i.e. within one week after DLI) in detail in a previous study (Schmaelter et al., Hemato 2021, 2, 692–702. https://doi.org/10.3390/hemato2040046). In the somewhat heterogenous cohort studied there, we observed an overall increase of CD8+ and CD56+ cell counts and significant changes in memory and activated CD8+ subsets as well as CD56+ cells. In addition, higher initial cell

doses were associated with increased overall numbers and various subsets of CD8+, CSD4+ and CD56+ cells. However, available samples did not allow to investigate persistence of these changes over a longer time period.