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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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

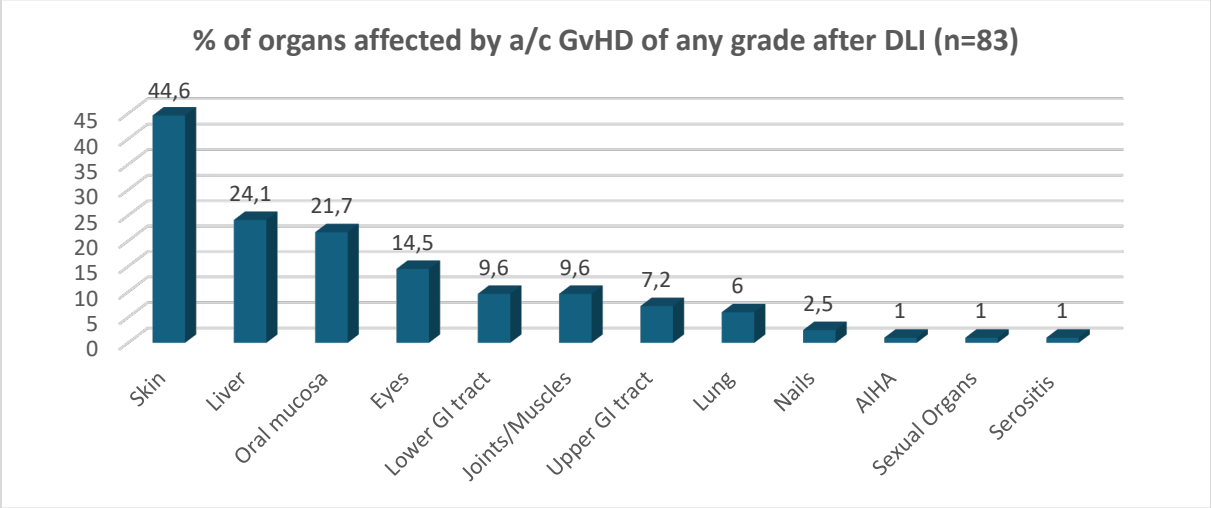
TCI-Score	MAC vs RIC	Conditioning regime
low (1 - 2)	**RIC**	Fludarabin 150, Treosulfan 30g
		Fludarabin 150, Busulfan 6,4
		Fludarabin 120, Treosulfan 30
		Fludarabin 120, Melphalan 140
		Clofarabin 150, Cytarabin 100, Treosulfan 30
intermediate (2,5 - 3,5)	**RIC/ MAC**	Flamsa Busulfan 6,4 (-Bu)
		Flamsa 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

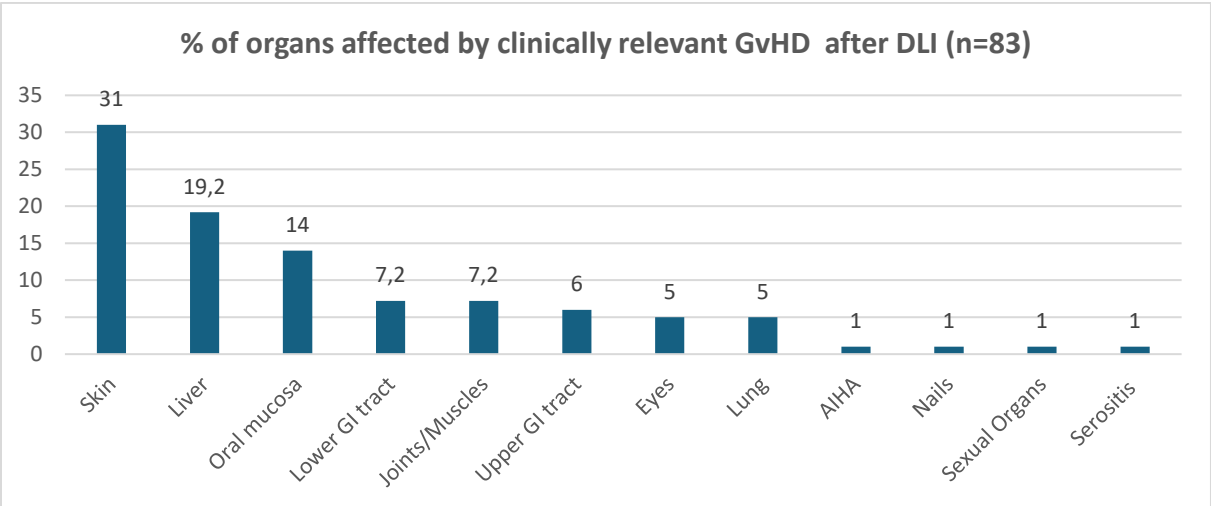
TCI: transplant conditioning intensity, MAC: myeloablative conditioning, RIC: reduced intensity conditioning, FLAMSA: Fludarabine, Amsacrine, Cytarabine, Cy: cyclophosphamide, TBI: total body irradiation.

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.