Title:

Incremental donor lymphocyte infusion to treat mixed chimerism after allogeneic stem cell transplantation in children with non-malignant diseases

Authors

Maria Gabelli[1,2], Polina Stepenski[3], Giorgio Ottaviano[1], Khushnuma Mullanfiroze[1], Arina Lazareva[1], Irina Zaidman[3], Ehud Even-Or[3], Giovanna Lucchini[1], Robert Chiesa[1], Juliana Silva[1], Stuart Adams[4], Susanne Kricke[4], Maria Finch[1], Annette Hill[1], Rachel Mead[1], Delphine Veys[5], Yael Dinur Schejter[3], Adeeb Naser Eddin[3], Austen Worth[6], Persis J. Amrolia[1] and Kanchan Rao[1].

Affiliations

1. Bone Marrow Transplantation, Great Ormond Street Hospital for Children, London, United Kingdom
2. Pediatric Hematology, Oncology and Stem Cell Transplantation, Azienda Ospedale-Università, Università degli studi di Padova, Padova, Italy
3. Bone Marrow Transplantation, Hadassah Medical Center, Faculty of Medicine, Hebrew University of Jerusalem, Israel
4. Specialist Integrated Hematology and Malignancy Diagnostic Service, Hematology, Great Ormond Street Hospital for Children, London, United Kingdom
5. St John's College, Cambridge, United Kingdom
6. Immunology, Great Ormond Street Hospital for Children, London, United Kingdom

Corresponding author

Maria Gabelli, MD, PhD

Bone Marrow Transplantation, Great Ormond Street Hospital for Children, London, United Kingdom, maria.gabelli@gosh.nhs.uk

Pediatric Hematology, Oncology and Stem Cell Transplantation,, Azienda Ospedale-Università, Università degli studi di Padova, Padova, Italy, maria.gabelli@unipd.it

Conflict of interest

The authors have no competing interests to disclose.

Abstract

Progressive mixed chimerism after hematopoietic stem cell transplant may anticipate graft loss and recurrent disease. Donor lymphocyte infusions (DLI) can revert mixed chimerism (MC) to full or stable donor chimerism but limited data are available on the safety and efficacy of this approach in non-malignant diseases. We report the experience of 2 tertiary centers on escalating dose DLI in children transplanted for non-malignant diseases who developed mixed chimerism. Thirty-two children received DLI for MC. A response was seen in 56.2% of patients: 37.5% achieved complete donor chimerism, 18.7% had an improvement or stabilization of chimerism.

After DLI, acute graft versus host diseases (GVHD) grade II-IV occurred in 12.5%, chronic GVHD in 9.3%, significant cytopenia in 6.2% of patients. Factors associated with response were a longer interval between transplantation and DLI and presence of GVHD post DLI. Five-year overall survival and event-free survival (including second transplant) were significantly worse in patients who did not respond (56% *vs* 100% and 37% *vs* 87%, respectively, $p<0.05$). In conclusion, escalating dose DLI can revert MC in children with non-malignant diseases, avoiding the need for second transplant, with low rate of GVHD and cytopenia. Lineage-specific chimerism should be implemented to guide interventions in these patients.

Article

Mixed chimerism (MC) post hematopoietic stem cell transplantation (HSCT) is observed in children with non-malignant diseases (NMD), especially after reduced intensity conditioning [1–3], and can increase the risk of graft loss (GL) and recurrence of disease [1,4,5]. Data from limited case series indicate that donor lymphocyte infusion (DLI) could revert low levels of donor chimerism to curative levels, averting the need for a second HSCT [6–8]. We conducted a retrospective study in 2 tertiary-care pediatric centers. Thirty-two children with NMD, transplanted between 2010 and 2020, received DLI for MC. Written informed consent for data collection was obtained by parents or legal guardians. Chimerism was monitored monthly, as described [9]. If MC was detected, in the absence of graft versus host diseases (GVHD), IS was tailed and stopped in 2-4 weeks. Decision to proceed to DLI was based on the rate of decline of donor chimerism, on the presence of complications possibly related to MC (e.g. autoimmunity)

and, if available, on lineage-specific chimerism. Escalating DLI was administered at Great Ormond Street Hospital in maximum 3 doses (1×10^7, 5×10^7 and 1×10^8 CD3+ cells/kg) at a minimum interval of 6 weeks [10]. The majority of patients at Hadassah Medical Centre received 1×10^6, 5×10^6 and 1×10^7 CD3+ cells/kg, with some variability due to physicians' preference. DLI was discontinued in patients developing significant acute or any chronic GVHD and in those who responded. Response was categorized as: partial response (PR) if stable chimerism or an increase in donor chimerism >20% with no disease-related symptoms, and complete response (CR) if reversal to full donor chimerism occurred. Categorical and continuous variables were compared using two-tailed Fisher's exact test and Student t-test for unpaired data, respectively. Survival comparison was performed with Log Rank test. Events included death, second transplant or stem cell boost, whichever occurred first.

Characteristics of patients, transplants and DLI are presented in Supplementary Table I.

Median donor chimerism pre-DLI was 44% (range 10-98%). Lineage-specific chimerism was performed in 13 patients: median donor chimerism pre-DLI was 59% (range 22-100%) in the CD3+ T-cell compartment and 35% (range 23-97%) in the myeloid compartment.

Eighteen patients (56.2%) responded: 12 CR (37.5%) and 6 PR (18.7%). Among responders, none required a second HSCT, 2 underwent a stem cell boost because of cytopenia post DLI. All 18 patients are alive with maintained response at last follow-up.

Fourteen patients did not respond to DLI, 10 had an indication for second HSCT. Five patients died (15.6%), all amongst NR: 3 of infections before 2nd HSCT, 2 of toxicity of second HSCT.

Acute GVHD (aGVHD) grade II-IV occurred in 12.5% of patients. Three patients (9.3%) developed mild chronic GVHD (cGVHD), 1 following aGVHD. DLI from a matched unrelated donor (MUD) (as compared to matched sibling donor [MSD] or matched related donor [MRD]) was the only risk factor statistically associated with GVHD in univariate analysis (p 0.01).

Two patients (6.2%) developed significant cytopenia requiring a stem cell boost from the original donor, with resolution. Both these children received DLI from MUD, developed aGVHD and reverted to full chimerism before the boost .

In univariate analysis, a longer interval between HSCT and first DLI was associated with CR (p = 0.03). Presence of aGVHD after DLI was associated with overall response (p 0.02) and, more strongly, with CR (p = 0.004) (Supplementary table II). Due to the limited number of patients, a multivariate analysis was not performed.

Five-year overall survival and event-free survival were significantly higher in patients who responded to DLI than in non-responders (100% *vs* 56%, p<0.05; 87% vs 37% , p <0.05) (Figure 1).

Although the use of DLI is well established in adults with malignant conditions, the experience in children is less well documented, especially in non-malignant pediatric setting.

A report on 27 children with NMD, the majority with hemophagocytic lymphohistiocytosis, showed a CR to DLI in 37% of patients, 18% PR. Response was associated with aGVHD, that occurred in 37% of patients [6]. Another study reported 50% CR and 25% PR after DLI in 32 children with NMD . Response correlated with donor chimerism > 30% at DLI and a DLI dose > 3 x 10^6/kg. In this cohort, aGVHD II–IV affected 37.6% of patients, cytopenia 26.1% and cGVHD 9.7%. Survival did not differ for patients who responded to DLI and non-responders [7].

Our study, combining the DLI experience of 2 tertiary care centers with similar approach, has a wider representation of NMDs compared to previous studies.

The safety profile of escalating dose DLI appeared acceptable, with low rate of aGVHD grade II-IV (12.5%) compared to others [6–8]. This may reflect the selection of donors and recipients: DLI was not administered in the context of HLA mismatched donors or if patients had history of significant GVHD. In addition, we used escalating dose DLI, with a median interval between DLI doses of 6-8 weeks, both factors shown to be associated with a lower risk of aGVHD [10].

Our response rate of 56.2%, with more than a third of patients achieving a CR, is consistent with other reports [6,8], and confirms that a substantive proportion of patients benefits from DLI. In our cohort, responders received DLI later after HSCT than NR; this could indicate that patients with slow progression of MC were more likely to respond to DLI than those who exhibited an early drop in chimerism. Similarly to others, we observed that the presence of aGVHD correlated with CR: increased alloreactivity of T-cells resulted in the beneficial effect of increasing chimerism as well as eliciting undesirable GVHD.

Patients who responded to DLI did not need a second HSCT and showed a better EFS and OS as compared to non-responders. However encouraging, due to the retrospective nature of the study and the small sample size, we cannot attribute this result completely to DLI, as difference in disease biology could have played a role.

Our study presents some limitation. First, it comprises a small number of patients and it is retrospective. Secondly, despite the uniform approach, the DLI doses administered in the 2 centres were different, reducing the generalizability of our findings. Lastly, lineage-specific

chimerism was available only on a minority of patients; although we believe that lineage-specific chimerism is important to guide DLI, as recently suggested by others [4], and merits further investigation.

Despite its limitations, our study shows that DLI is a viable option for children with NMD when a progressive loss of donor chimerism is documented, particularly in the disease-specific lineage. Patients who could benefit most from DLI are those who are free from GVHD, have an available MUD donor and have a slow or late progressive loss in donor chimerism. The risk of cytopenia and the possibility of a stem cell infusion need to be considered before deciding on DLI. Another approach to MC is represented by the administration of CD34+ selected stem cells [11]. This method has lower rate of success but also lower risk of GVHD compared to DLI and could be considered as an alternative in those patients with high risk of GVHD.

With good selection of candidate patients, DLI could avoid a second transplantation in a significant proportion of patients and possibly confer a survival advantage to patients with NMD and progressive MC.

Authorship

MG and KR designed the research study and wrote the manuscript

MG and PS collected patient data

MG and GO analyzed the data

MG, PS, KM, AL, IZ, EEO, GL, JS, RC, MF, AH, RM,YDS, ANE, AW, PJA, KR looked after patients and provided essential data

SA and SK performed chimerism analysis

All authors reviewed and approved the manuscript.

Data availability statement

The datasets of the current study are available from the corresponding author on reasonable request.

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Characteristics of patients	Number of patients (%) Total = 32
Median age at HSCT (range)	1.4 years (0.4-13)
Gender	
Female	12 (37.5%)
Male	20 (62.5%)
Diagnosis	
Severe combined immunodeficiency	8 (25.0%)
Osteopetrosis	4 (12.5%)
Chronic granulomatous disease	3 (9.4%)
CD40-L deficiency	3 (9.4%)
Mucopolisaccaridosis type I	2 (6.3%)
LRBA 1 deficiency	2 (6.3%)
Wiskott-Aldrich Syndrome	2 (6.3%)
APDS1 syndrome	1 (3.1%)
Glanzmann's Thrombasthenia	1 (3.1%)
Osteopetrosis immunodeficiency-lymphoedema	1 (3.1%)
VPS4-primary infantile myelofibrosis	1 (3.1%)
X-linked lymphoproliferative disease	1 (3.1%)
Metachromatic leukodystrophy	1 (3.1%)
TPP2 deficiency	1 (3.1%)
ADA-2 deficiency	1 (3.1%)
HSCT donor	
MUD	15 (46.8%)
MSD	10 (31.2%)
MFD	7 (21.8%)
Source of stem cells	
BM	22 (68.8%)
PB	10 (31.2%)
Conditioning	
Flu/Treo/Thiotepa	16 (50.0%)
Flu/Treo	8 (25.0%)
Flu/Bu	5 (15.6%)
Flu/Mel	1 (3.1%)
Bu/Flu/Thiotepa	1 (3.1%)
Unconditioned graft	1 (3.1%)
Serotherapy	
ATG	17 (53.1%)
Alemtuzumab	14 (43.8%)
none	1 (3.1%)
GVHD prophylaxis	
CSA and MMF	25 (78.1%)
CSA alone	4 (12.5%)
CSA and MTX	2 (6.3%)
none	1 (3.1%)
aGVHD post HSCT	
No	30 (93.7%)
Yes	2 (6.3%)
Median donor chimerism pre DLI (range)	44% (10-98%)
Number of DLI	
3	8 (25.0%)
2	12 (37.5%)
1	12 (37.5%)
DLI Dose (CD3+/kg)	
Median first dose (range)	5×10^6 (9×10^5 – 5×10^7)
Median second dose (range)	5×10^7 (2.2×10^6- 2.3×10^8)
Median third dose (range)	1×10^8 (1×10^7- 1×10^8)
Median cumulative dose (range)	1×10^7 (1×10^6-2.31×10^8)

Median time from HSCT to first DLI (range)	174.5 days (53-904)

Table I

Characteristics of patients, transplant and DLI.

Abbreviations:

ATG anti-thymocyte globulin, BM bone marrow, Bu Busalphan, CSA ciclosporin, CR complete response, DLI donor lymphocyte infusion, Flu fludarabine, HSCT hematopoietic stem cell transplantation, Mel Melphalan, MFD matched family donor, MMF mycophenolate mofetil, MSD matched sibling donor, MTX methotrexate, MUD matched unrelated donor, NR no response, PB peripheral blood, PR partial response, Treo treosulphan

	Responders (CR + PR) n=18	CR only n=12	Non responders (NR) n=14	All responders *vs* NR : *p*	CR *vs* NR: *p*
Median age at HSCT (range)	1.4 years (0.4-13.9)	5.7 years (0.5-13.9)	1.5 years (0.6-12.9)	*ns*	*ns*
HSCT donor					
MUD	10 (55.6%)	9 (75.0%)	5 (35.7%)	*ns*	*ns*
MSD	6 (33.3%)	3 (25.0%)	4 (28.6%)		
MFD	2 (11.2%)	0	5 (35.7%)		
Median donor chimerism pre DLI (range)	44% (10-98%)	53% (26-98%)	48% (12- 83%)	*ns*	*ns*
Median time from HSCT to first DLI (range)	214 days (81-904)	279 days (87-904)	149 days (53-535)	*ns*	***0.03***
Number of DLI					
3	3 (16.7%)	2 (16.7%)	5 (35.7%)	*ns*	*ns*
2	6 (33.3%)	4 (33.3%)	6 (42.8%)		
1	9 (50.0%)	6 (50.0%)	3 (21.4%)		
DLI Dose (CD3+/kg)					
Median first dose (range)	7.5×10^6 (9×10^5-5×10^7)	1×10^7 (9×10^5-5×10^7)	3.5×10^6 (1×10^6-1×10^7)	*ns*	*ns*
Median second dose (range)	2.8×10^7 (2.2×10^6-5×10^7)	5×10^7 (2.9×10^6-5×10^7)	5×10^7 (1×10^7-2.3×10^8)		
Median third dose (range)	1×10^8 (1×10^7-1×10^8)	1×10^8	1×10^8 (1×10^7-1×10^8)		
Median cumulative dose (range)	1×10^7 (1×10^6-1.6×10^8)	1×10^7 (1×10^6-1.6×10^8)	4×10^7 (1×10^7-2.3×10^8)	*ns*	*ns*
GVHD					
aGVHD post DLI	6 (33.3%)	6 (50.0%)	0	***0.02***	***0.004***
cGVHD post DLI	3 (16.7%)	3 (25.0%)	0	*ns*	*ns*
Any GVHD post DLI	8 (44.4%)	8 (66.7%)	0	***0.04***	***0.0003***
Outcome					
Second HSCT	0	0	4 (28.6%)	***0.02***	*ns*
Second HSCT indicated but not performed	0	0	6 (42.8%)		
Second HSCT performed or indicated	0	0	10 (71.4%)	***0 .0001***	***0.0002***
Stem cell boost	2 (11.2%)	2 (11.2%)	0	ns	ns
Deaths	0	0	5 (36.7%)	***0.009***	***0.004***

Table II

Characteristics of responders *vs* non responders.

Abbreviations:

CR complete response, DLI donor lymphocyte infusion, GVHD graft versus host disease, HSCT hematopoietic stem cell transplantation, MFD matched family donor, MMUD mismatched unrelated donor, MSD matched sibling donor, MUD matched unrelated donor, NR no response, ns non-significant, PR partial response.