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Research Article

Treatment of Mixed Chimerism After Hematopoietic Stem Cell Transplantation in Patients with Thalassemia Major

Jianyun Liao[1, 2, *, †], Shimin Liang[3, †], Jingtao Chen[4], Xiaoting Liu[2], Yuqian Xia[1], Jujian He[1], Weiwei Zhang[1], Chaoke Pu[1, 2], Lan He[1, 2], Yuelin He[1, 2], Xiaoqin Feng[2], Xuedong Wu[2], Chunfu Li[1, 2]

[1]Nanfang-Chunfu Children's Institute of Hematology and Oncology, Taixin Hospital, Dongguan, China

[2]Department of Pediatrics, Nanfang Hospital, Southern Medical University, Guangzhou, China

[3]Clinical Medical College of Acupuncture Moxibustion and Rehabilitation, Guangzhou University of Chinese Medicine, Guangzhou, China

[4]Department of Pediatrics, Southwest Medical University, Luzhou, China

Abstract

Background: Incomplete donor cell chimerism often occurs in thalassemia transplant due to host cells remain or reappear overtime, which is termed as mixed chimerism (MC). Objective: To compare the immunosuppression withdrawal (ISW) and donor lymphocyte infusion (DLI) in the correction of mixed chimerism (MC) after thalassemia transplantation. Methods: Eighty-seven patients with post-transplant MC admitted in our center from January 2010 to December 2019 were analyzed. Among them donor cells of 90%-95% and 75%-89% were classified as MC1 and MC2 respectively. MC3 donor cells <75%. The incidence and correction rate of MC, the occurrence rate of graft versus host disease (GVHD), timing of DLI were studied. Results: DLI was associated with higher correction rates and higher GVHD than ISW. In MC1 group, higher GVHD occurred in early and intermediate stage (P = 0.024/0.023) than ISW. In MC2 group, DLI in late stages had higher correction rates than ISW (P = 0.001). Conclusion: ISW was the primary strategy for MC1 patients. DLI should be given to the late-stage MC2 patients quickly. The earlier the treatment is provided, regardless of ISW or DLI, the more likely that patients develop GVHD.

Keywords

Mixed Chimerism, GVHD, Donor Lymphocyte Infusion, Thalassemia, HSCT

1. Introduction

Beta thalassemia major (TM) is an inherited hemolytic anemia caused by the absence or insufficiency of β-globin-chain synthesis due to defect of genes. Currently, allogenic hematopoietic stem cell transplantation (HSCT) remains the only widely available curative therapy for transfusion-dependent thalassemia [1].

*Corresponding author: kinlenli@126.com (Jianyun Liao)

† Jianyun Liao and Shimin Liang are co-first authors.

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The termcomplete chimerism (CC) indicates a total replacement of host-derived cells by donor lymphohaematopoiesis after haematopoietic stem cell transplant (HSCT), while the simultaneous presence in various proportions of both donor- and recipient-derived cells is defifined as MC.

Unlike leukemia, TM patients have a rich-cell marrow and normal immunity. Besides, sensitization to human leukocyte antigen (HLA) usually develops after long-term transfusion, which results in higher graft rejection (GR) and more incomplete donor chimerism than the leukemia patients.

In patients who undergo transplantation to treat a haemoglobinopathy, the probability of graft rejection is correlated with the occurrence of MC in the early stage after HSCT and with the amount of residual host cells (RHC) present in the recipient [2].

The MC should be adoptively and timely treated, if not, GR and cytopenia will occur. So far, immunosuppression withdrawal (ISW) and/or donor lymphocyte infusion (DLI) have been the two major treatment methods, but they both have a risk of acute graft versus host disease (aGVHD). Which of the both is better and what is right timing still remain inconclusive. In this paper, we retrospectively analyzed the risk factors of MC and treatment response in MC post-HSCT in TM patients.

2. Patients and Methods

2.1. Definitions and Standards

The termcomplete chimerism (CC) was defined as fully engraftment of cells derived from donor (donor genetic markers ≥ 95%).

MC was defined as incomplete donor cell chimerism due to host cells remain or reappear (≥ 5%) in bone marrow or peripheral blood after transplantation [3].

MC can be further categorized into three levels based on host-cell ratios: MC1: Host-cells ≤ 10%, MC2: 10% < host cells ≤ 25%, and MC3: host cells > 25% [4].

The time of MC occurrence was defined as early stage (< 90 days post-HSCT), intermediate stage (90-180 days), and late stage (>180 days) respectively.

2.2. Patients and Transplant Procedures

The data of 529 TM patients underwent HLA matched donor HSCT between January 2010 and December 2019 in our centers were retrospectively analyzed.

Among the 529 patients, 302 received grafts from matched related donors, and 63 of them developed MC; 227 cases received grafts from unrelated matched donors, and 24 of them developed MC.

All patients adopted the NF-08-TM HSCT protocol [5] which consisted of cyclophosphamide, fludarabine, busulfan, thiotepa (TT), and anti-thymocyte globulin (ATG). There was a period during which TT was not used in 135 patients due to commercial reasons while the daily dosage of busulfan was increased (from day-4 to day-7). Cyclosporine A, methotrexate, glucocorticoid and/or mycophenolate mofetil were, alone or in combination, used to prevent aGVHD. HLA-A, -B, -C, -DRB1 and DP typing was performed at allele level through allele-specific polymerase chain reaction. Short tandem repeat (STR) and fluorescence in situ hybridization (FISH) methods were selected to detect donor chimerism after sex-matched and sex-mismatched transplant respectively. First donor chimerism was detected at 22 days, thereafter, once every month until the end of one-year after transplantation.

Eighty-seven of 529 TM patients underwent HSCT were diagnosed as MC, with the median age of 5 years (1 to 23 years), male vs. female rate of 58 vs. 29, median follow-up time of 5 years (2 to 9 years), and an incidence of 16.45%.

These patients were divided into ISW group (n=42) and DLI group (n=41).

30 MC1 and 12 MC2 cases fell under ISW group, and 23 MC1, 18 MC2, 4 MC3 under DLI group. All the four patients in MC3 level directly received DLI treatment, and no analysis or comparison was performed between ISW and DLI group.

For MC1 patients, a targeting concentration of immunosuppressive agent was adjusted to 1/2 and 1/4 primary dose at early and intermediate stages and was discontinued at late stage. For MC2 patients, the use of immunosuppressive agent was similar to MC1 patients. The immunosuppressive agent was withdrawn at early stage MC2 and DLI was recommended at intermediate and late stages. For MC3 patients, immunosuppressive agent should be withdrawn as soon as possible no matter it is at which stages. The concentration of immunosuppressive agent may be adjusted based on patients' current situation: with/without significant signs of aGVHD.

2.3. Donor Lymphocyte Infusion Schedule

Fresh donor peripheral blood or lymphocytes obtained by single collection are used for related donor transplantation. Peripheral blood stem cells (PBSC) activated by cryopreserved granulocyte colony-stimulating factor (G-CSF) are used for unrelated donor transplantation.

There is currently insufficient data regarding whether the use of G-CSF mobilized hematopoietic stem cells (HSC) affects the outcome of DLI after stem cell transplantation [6]. A group of patients from Philadelphia [7] conducted a retrospective analysis on 63 patients who received fresh or cryopreserved DLI. Their conclusions were that the use of fresh cells and low-temperature preserved cells did not affect the outcome. Parameters such as thawed CD3 + survival capacity have not been adequately evaluated. The viability of DLI after low-temperature preservation may be affected, which could alter its effectiveness or toxicity. To ensure the efficacy of DLI, the viability of the applied cryopreserved cells should reach at least 60%. If the cell viability is below 60%,

fresh lymphocytes from an unrelated donor will need to be collected for re-mobilization. Our study comprised 41 patients in the DLI group, among whom 29 received fresh donor lymphocyte infusion from related donors and 12 received the infusion from unrelated donors. For two of the unrelated donors whose preserved stem cells had viabilities lower than 60%, fresh lymphocytes were collected from the donors again. The remaining 10 patients were transplanted with cryopreserved stem cells. DLI was initiated with a small dose and then administrated by dose escalation to reduce the risk of aGVHD [8, 9].

Patients were observed for 6 to 8 weeks after each DLI. The DLI dose regimen was specified as follows: for MC1 patients, the initial DLI dose at all stages was lymphocytes of 1×10^7/kg and 0.5×10^7/kg in matched sibling transplants and unrelated donor transplants. Considering that DLI at early and intermediate stages were associated with high aGVHD risk, the immunosuppressive agent was reduced and maintained at 1/4-1/2 level of foregoing dose, while immunosuppressive agents were withdrawn at late stage. For MC2 patients at early stage, the initial DLI was the same as MC1 patients but the immunosuppressive agent was kept lower than 1/4 level of primary dose. For intermediate and late stage MC2 patients, the initial DLI dose was 1.5 and 2.0×10^7/kg in matched sibling donor and 1.0 and 1.5×10^7/kg in unrelated donor transplants and all immunosuppressive agents were withdrawn. For MC3 patients, all immunosuppressive agents are discontinued regardless of transplant type. early stage DLI doses are recommended at 1.0×10^7 / kg, intermediate stage: 2.5×10^7 / kg, late stage: 3.0×10^7 / kg. The second DLI can be administered with an interval of 6-8 weeks. If the percentage of donor cells does not significantly increase or tends to decrease, the dose should be increased by 1.0×10^7/kg for each subsequent dose. (Table 1).

Table 1. *Donor Lymphocyte Infusion Schedule.*

HSCT Type	Stage MC level	early stage	intermediate stage	late stage	Notice
Related	MC1	IA: 1/2 level	IA: 1/4 level	ISW and DLI: 1.0×10^7	The dose of Donor-derive Lymphocytes (lym+ $\times10^7$/kg); DLI repeated every six weeks, base on the percentage of recipient cell; DLI should be discontinued if donor cell chimerism rate increased; If the percentage of donor cells does not increase significantly or tends to decrease, each dose is increased by 1.0×10^7/kg, and so on.
	MC2	IA: 1/4 level	ISW and DLI: 1.5×10^7	ISW and DLI: 2.0×10^7	
	MC3	DLI: 1.0×10^7	ISW and DLI: 2.0×10^7	ISW and DLI: 3.0×10^7	
Unrelated	MC1	IA: 1/2 level	IA: 1/4 level	ISW and DLI: 0.5×10^7	
	MC2	IA: 1/4 level	ISW and DLI: 1.0×10^7	ISW and DLI: 1.5×10^7	
	MC3	ISW and DLI: 1.0×10^7	ISW and DLI: 2.5×10^7	ISW and DLI: 3.0×10^7	

MC: mixed chimerism; MC1: Host-cells ≤ 10%; MC2: 10% < host cells ≦ 25%; MC3: host cells > 25%;
IA: immunosuppressive agent; ISW: Immunosuppressive withdrawen; DLI: Donor lymphocyte infusion.

In the event that aGVHD occurs during ISW or DLI, strengthened immunosuppression treatment should be provided based on aGVHD severity. A second transplantation should be considered if donor chimerism continues to decrease and lower 50%.

2.4. Statistical Analysis Methods

Average values in different groups were compared by Chi-square test or bilateral Fisher exact test. For any analyses, P-value less than 0.05 was considered statistically significant. SPSS 26.0 for Windows was used to perform the above statistical analysis.

3. Results

87 of 529 (16.45%) TM patients underwent HSCT developed MC. Higher MC incidence was observed in the following situations: conditioning regimen without TT, related donor transplantation, and sex-mismatched donor engraftment (Table 2).

56 patients who received a conditioning regimen including TT experienced MC (14.21%), while the MC incidence in those who received a conditioning regimen without TT was 22.96% (P =0.018). (Shown in Figure 1)

aGVHD and bone marrow suppression are the most common complications after ISW and DLI. The median time of aGvHD occurrence was the 4th week (ranged between the 4th and 8th weeks) in ISW group and the 6th week (ranged between the 4th and 8th weeks) in DLI group. Of 83 MC1 and MC2 patients, 28 developed aGVHD. With a total incidence of 33.7%, which were mainly skin and liver aGVHD. GVHD incidences of patients at early, intermediate, and late stages in whole cohort (ISW and DLI group) patients were 57.14%,

33.33%, and 4.55% respectively (P =0.000). (Table 3)

The aGVHD incidences in ISW (9/42) and DLI (19/41) groups were 21.4% and 46.3% respectively. Four patients with aGVHD developed as chronic GVHD, of which 2 were mild and 2 were moderate and all were cured. Six patients receiving DLI (6/41) had a pancytopenia, with an incidence of 14.63%. Three of them were grade II aGVHD. Eight MC patients in the current cohort were not reversed by ISW, but were corrected by DLI. Thus, DLI achieved 100% correction rate. In comparison of the two groups, DLI had a significantly higher correction rate (100% vs. 81.0%, *P*=0.005), but with higher rate of aGVHD occurrence than ISW (46.30% vs. 21.40%, *P* =0.016).

Compared with ISW and DLI in MC1, all early- and mid-stage events in MC1 were corrected by ISW and DLI. However, the incidence of GVHD in the DLI group was higher than that in the ISW group (early stage *P*=0.024, mid-stage *P*=0.023).

Comparing with ISW and DLI in MC2, ISW failed to reverse MC to FDC in 2/5 intermediate stage and 5/5 late stage cases. However, DLI reversed all MC in 18 MC2 patients whenever MC2 occurred. ISW of MC2 induced aGVHD development in 2/2 early-stage and 1/5 intermediate-stage, and 0/5 late-stage patients. DLI led aGVHD development in 2/5 early-stage, 2/5 intermediate-stage, and 1/8 late-stage cases. The incidence of aGVHD occurrence was higher in early stage no matter it was in ISW or DLI group. (Table 4 and Table 5)

Directly discontinuing immunosuppressive agents and using DLI for treatment are recommended in MC3. Due to the small number of patients classified in MC3, there was no comparison between ISW and DLI with MC1/MC2 classification. One patient with MC3 who underwent matched unrelated donor transplantation developed MC in the early stage and was given DLI. The dose of donor lymphocytes infused was 2.0×10^7/kg. Subsequently, the patient developed pancytopenia, but recovered after treatment. The second late-stage patient with MC3 received multiple DLI with a gradually increasing dose (from 1.5×10^7/kg to 5×10^7/kg). The patient developed grade II liver aGVHD 12 weeks after the dose was increased to 5×10^7/kg, but recovered after treatment. The third late-stage patient did not respond to DLI, but successfully underwent donor transplantation after the second mini-transplantation.

The results of the fourth late-stage patient with MC3 remained unchanged after multiple DLI. This patient had normal hemoglobin level while the chimerism level remained at around 75%. Considering that both the donor and recipient had achieved immune tolerance [10], it was deemed that further treatment was unnecessary.

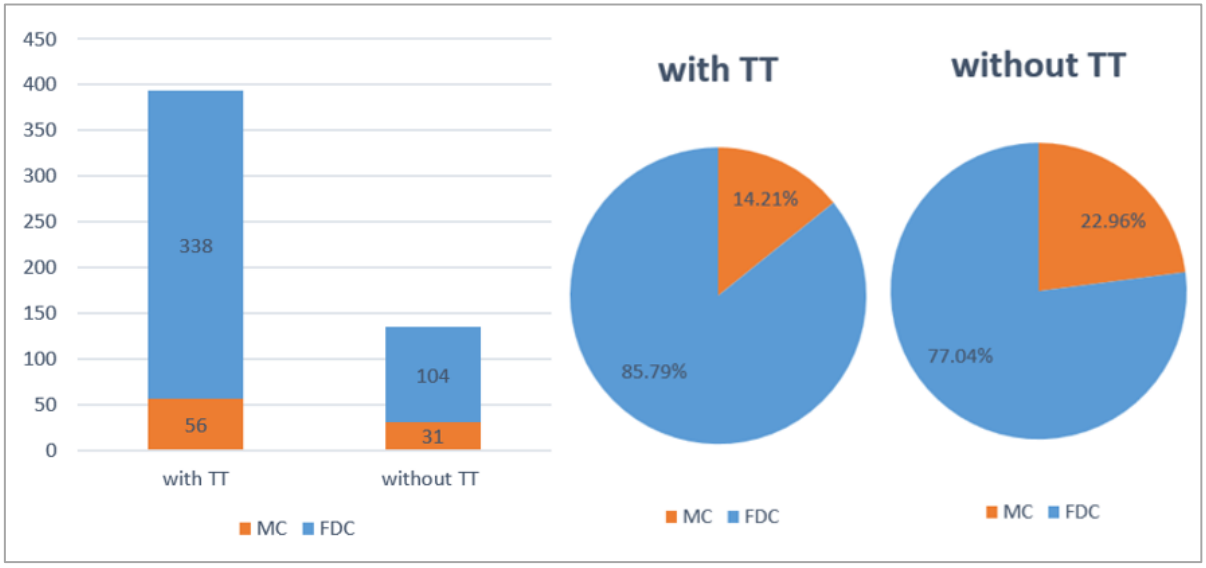

Figure 1. *Comparison of mixed chimerism with Thiotepa and non-Thiotepa.*

Table 2. *Comparison of factor of MC occurring after transplantation (2010-2019).*

	MC	CC	Total	Occurrence rates	*P*
Total	87	442	529	16.45%	/
Age					
Median (years) (range)	Media: 5 (range 1-23)				

	MC	CC	Total	Occurrence rates	*P*
Gender					
Male	58	284	342	16.96%	0.667
Female	29	158	187	15.51%	
Conditioning					
Conditioning with TT	56	338	394	14.21%	0.018
Conditioning without TT	31	104	135	22.96%	
Donor type					
Unrelated donor	24	203	227	10.57%	0.002
Related (Parents+Siblings+Other Relative)	63	239	302	20.86%	
Related Donor type					
Parents+Other Relative	6	19	25	24%	0.687
Siblings	57	220	277	20.58%	
ABO Compatibility					
Compatible	44	226	270	16.30%	
Minor	19	80	99	19.19%	0.787
Major	16	98	114	14.04%	
Major/Minor	8	38	46	17.39%	
Class of risk at transplantation					
Class 1	14	31	45	31.11%	
Class 2	70	396	466	15.02%	0.021
Class 3	3	15	18	16.67%	
Chimerism follow up					
FISH	57	209	266	21.43%	0.002
STR	30	233	263	11.41%	

MC: mixed chimerism; CC: termcomplete chimerism; Cy: cyclophosphamide; Flu: fludarabine; Bu: busulfan; TT: thiotepa; ATG: antithymocyte globulin. BM: Bone marrow; PBSC: Peripheral blood stem cells; HLA: human leukocyte antigens
FISH: fluorescence in situ hybridization; STR: Short tandem repeat.

Table 3. *The occurrence rate of GVHD after Intervention in different stages.*

Time of DLI and ISW	Number of cases			Occurrence rates	χ^2	*P*
	GVHD	No GVHD	Total			
＜90d	16	12	28	57.14%		
90-180d	11	22	33	33.33%	15.251	0.000
＞180d	1	21	22	4.55%		

Table 4. Comparison of correction rate in ISW and DLI group.

Groups	Time of MC	Number of cases			Correction rates	*P*
		CC	MC	Total		
ISW		34	8	42	81%	
DLI		41	0	41	100%	0.005
Total		75	8	83	90.4%	
MC1 of ISW	<90d	12	0	12	100%	/
MC1 of DLI		9	0	9	100%	
MC1 of ISW	90d-180d	14	0	14	100%	/
MC1 of DLI		9	0	9	100%	
MC1 of ISW	>180d	3	1	4	75%	0.444
MC1 of DLI		5	0	5	100%	
MC2 of ISW	<90d	2	0	2	100%	/
MC2 of DLI		5	0	5	100%	
MC2 of ISW	90d-180d	3	2	5	60%	0.444
MC2 of DLI		5	0	5	100%	
MC2 of ISW	>180d	0	5	5	0%	0.001
MC2 of DLI		8	0	8	100%	

ISW: Immunosuppressive with drawer DLI: Donor lymphocyte infusion.

Table 5. Comparison of GVHD in ISW and DLI group.

Group	Time of MC	Number of cases			Occurrence rates	*P*
		GVHD	No GVHD	Total		
ISW		9	33	42	21.4%	
DLI		19	22	41	46.3%	0.016
Total		28	55	83	33.7%	
MC1 of ISW	<90d	4	8	12	33.33%	0.024
MC1 of DLI		8	1	9	88.9%	
MC1 of ISW	90d-180d	2	12	14	14.3%	0.023
MC1 of DLI		6	3	9	66.67%	
MC1 of ISW	>180d	0	4	4	0	/
MC1 of DLI		0	5	5	0	
MC2 of ISW	<90d	2	0	2	100%	0.429
MC2 of DLI		2	3	5	40%	
MC2 of ISW	90d-180d	1	4	5	20%	1
MC2 of DLI		2	3	5	40%	
MC2 of ISW	>180d	0	5	5	0%	1

Group	Time of MC	Number of cases			Occurrence rates	*P*
		GVHD	No GVHD	Total		
MC2 of DLI		1	7	8	12.5%	

4. Discussion

It has been well known that DLI may correct MC but may to induce GVHD and pancytopenia. Relevant literature has reported that GVHD incidence after DLI ranged between 40% and 60% [11]. And 20% patients had a pancytopenia [12]. Nevertheless, there remain unanswered questions regarding what is an adequate timing and cell dose of DLI, and how to do ISW. Post-DLI pancytopenia is inferred to be due to insufficient recovery of hematopoietic function by the ablating recipient cells and accompanying donor stem cells after DLI [13]. The risk of pancytopenia is related to the disease stage during DLI. As shown in the results of DLI patients after intensity reduction, the higher the degree of host chimerism, the higher the risk of pancytopenia. In our study, early and mid-stage MC1 patients received reduced drug treatment, which not only reversed MC but also reduced the incidence of cytopenia.

DLI had a higher correction rate than ISW (100% vs. 81.0%, *P*=0.005), However, the incidence of GVHD was also higher in the DLI group than in the outcome group. (46.3% vs. 21.4%, *P*=0.016), we conclude from our study that MC1 was corrected by ISW and DLI, compared to MC1 ISW and DLI. However, the incidence of GVHD in DLI group was higher than that in ISW group (*P*= 0.024 in the early stage, *P*= 0.023 in the middle stage). In order to avoid GVHD, it is recommended to stop the treatment of immunosuppressant drugs in the early and middle stage of MC1 grade. DLI was not essential in the early stage post-HSCT to reverse MC because the correction rate of ISW, at this point, was not lower than DLI. Our research found that, GVHD is more likely to occur after DLI when donor cells account for higher percentage. A report from Antin et al [14]. also proved that patients with more than 90% donor T cells were more likely to develop GVHD than patients with less than 90% donor T cells. Therefore, we converted MC (particularly MC1) at early stage to CC by gradually reducing immunosuppressive drugs, which also reduced GVHD incidence. This suggests that reduction of immunosuppressive drugs should be considered first for MC1 occurring within 180 days post-transplant.

For intermediate stage MC2, ISW only reversed 2/5 MC but DLI corrected 5/5MC. Although there was no statistical significance when comparing with the both, we still recommend DLI for the intermediate stage MC2 patients because we think this statistical result was due to small events. DLI had a higher correction rate than ISW (100% vs. 0%, *P*=0.001) only in late stage MC2 group. This illustrated that the graft in late stage was more tolerant, while those in early stage was more lethal because the donor-recipient immunotolerance has not fully established. In these stages, in vivo, a number of under-educated alloreactive donor cells, along with new uneducated alloreactive cells from DLI, leading to an unexpected GVHD. Along with alloreactive clones depleted over time after HSCT, the attack ability of donor cells declined and ineffective reversion of MC produced if only using ISW in the late stage. Oppositely, DLI can reverse MC in late stage as new alloreactive donor lymphocytes have not yet been tolerated to the host cells and could kill the residual host cells and then reverse MC.

Other study that GVHD incidences of patients at early, intermediate, and late stages in whole cohort patients were 57.14%, 33.33%, and 4.55% respectively (*P*=0.000), suggesting that the earlier the interference is conducted, the higher incidence of aGVHD (Table 3) will be, regardless of ISW or DLI. Related reports that the earlier the DLI, the higher the incidence of GVHD. In addition, DLI are not usually given within the first 3 months after allo-HCT except in cases of relaps [15].

Our study found out that unrelated donor transplant had lower MC incidence than did the sibling donor transplant after DLI. This was agreed with those reported by Merav [16]. MC occurred more frequently after sibling donor transplant because a sibling's minor HLA was also matched, and consequently, immune-mediated killing to recipient cells was reduced.

The current study showed that MC occurred more frequently in the transplantation included conditioning regimen without TT. Some possible reasons were: first, TT can easily pass through the blood-brain barrier while Cyclophosphamide could not, which, thus, well depleted host lymphocytes remained in central nervous system, and resulted in a more thorough immunosuppression; Second, TT can also eliminate myeloid cells and acquire enough bone marrow suppression. They both promoted full-donor cell engraftment [17].

5. Conclusion

In summary, the current study suggested that more attention to MC post-transplant was crucial in the follow-up of TM HSCT, as MC occurs usually due to TM patients' characteristic of rich marrow cells and HLA sensitization, particularly in matched sibling, cord blood, and conditioning without TT transplants. ISW is the primary strategy for MC1 patients. DLI should be given to late-stage MC2 patients as soon as possible. DLI had a higher correction rate of MC than ISW but also with higher GVHD risk. The earlier DLI is provided,

the higher the risk of GvHD will be.

Ethical Approval

For this type of study formal consent is not required. No ethical issues were addressed in this paper, as we did not study any human or animal subjects, nor did we collect any personally identifiable information or sensitive data.

Author Contributions

Dr. Jianyun Liao and Chunfu Li designed the project, the research line and wrote the manuscript. Shimin Liang and Jingtao Chen reviewed the data and made the statistical analysis. The others performed the research and collected data. All authors reviewed the final version and approved the submission.

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Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare no competing financial interests.

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