Escalating-Dose HLA-mismatched donor lymphocyte infusion is safe for the treatment of leukaemia relapse following alemtuzumab-based myeloablative allogeneic stem cell transplantation.

Short Running Title – Safety of HLA mismatched DLI

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Abstract

While the feasibility of using HLA-mismatched unrelated donors as an alternate graft source for haematopoetic stem cell transplantation (HSCT) has been shown, little is known about the safety of HLA-mismatched donor lymphocytes infusion (DLI) for the treatment of relapse. We examined the outcome of 58 consecutive leukaemia patients who received escalating-dose DLI for treatment of relapse after alemtuzumab-conditioned myeloablative unrelated donor HSCT at our institution. High resolution HLA typing on stored DNA samples revealed mismatches in 28/58 patients who were considered HLA matched at the time of transplantation. Following DLI from HLA-matched (10/10) (n=30) or mismatched (7-9/10) (n=28) unrelated donors, we found no significant difference in the incidence of acute graft-versus-host disease (17.2% vs. 23.1%, p=0.59), probability of remission at 3yrs (62.1 vs. 63.9%, p=0.89) or 5-year overall survival (89.8% vs. 77.7%, p=0.22). We conclude that escalating-dose DLI can be safely given to HLA-mismatched recipients following T-depleted myeloablative HSCT.

Introduction

Allogeneic haematopoetic stem cell transplantation (HSCT) remains the only curative treatment strategy for a number of haematological malignancies.[1-3] However for many patients identification of a suitable donor remains challenging. While a 10/10 human leucocyte antigen (HLA)-matched sibling remains the favoured option, 70% of patients do not have a sibling donor[4] and a matched unrelated donor (MUD) is often the preferred alternative. Despite the availability of international donor registries, identification of a fully matched donor still remains problematic, and in 40% no such donor can be identified.[5] The use of increasingly sensitive methods of HLA-typing permits the identification of disparities that would have historically remained unmasked. Modified conditioning regimens, often with T-cell depletion, have facilitated the use of mismatched donors for these

patients,[6,7] albeit with increased transplant related mortality (TRM) and complications.[8] However the combination of T-cell depletion and increasingly sensitive methods of minimal residual disease (MRD) detection results in early relapse detection.[9,10] Donor lymphocyte infusion (DLI) is a safe and powerful strategy to treat post-HSCT relapse in sibling and MUD transplant recipients.[11-14] Indeed registry data suggest that between 16 and 40% of patients undergoing HSCT will receive post-transplant DLI for the treatment of relapse or mixed chimerism. Whilst the use of T-cell depletion may permit HLA mismatch during the initial transplant phase[5,6] and reduce the probability of developing acute graft-versus-host disease (aGvHD),[15,16] the degree to which HLA mismatch influences the probability of response, risk of GvHD post-DLI and overall survival (OS) is unclear.[17] With the advent of increasingly permissive transplant regimens and increasing numbers of mismatched unrelated donors, the question of whether it is safe to give DLI to a mismatched recipient is very pertinent. The aim of this retrospective landmark analysis was to determine the effects of HLA disparity on the probability of developing aGvHD following DLI.

Patients and Methods

Patient Eligibility & Study Design

In this study, we retrospectively performed high resolution HLA typing on stored genomic DNA samples and analyzed the outcome of 58 consecutive patients who underwent escalating-dose DLI for the treatment of relapse following an alemtuzumab-based myeloablative unrelated donor transplant at our institution between December 1995 and August 2010. High resolution HLA typing for HLA-A, B, C, DR and DQ was performed on stored genomic DNA by direct sequencing and the cohort stratified according to the presence or absence of an HLA mismatch. Details of recipient-donor matching are shown in Table 1. The research protocol was approved by the Local Research

Ethics Committee and all patients gave written informed consent in accordance with the Declaration of Helsinki.

Transplant Protocol

All patients received uniform conditioning with 14.4Gy of fractionated total body irradiation on days -4 to -2, intravenous cyclophosphamide (120mg/kg total dose) on days -5 and -6 or etoposide (60mg/kg) on day -5 and in vivo T-cell depletion with alemtuzumab 10mg i.v.daily on days -10 to -6. GvHD prophylaxis consisted of cyclosporine from day -3 onwards and methotrexate (8mg/m^2) on days +2, +4, +8 and +12. Bone marrow (n=45) or peripheral blood stem cells (n= 13) were infused on day 0.

Donor Lymphocyte Infusion Protocol

At relapse, all patients received DLI using a previously established escalating-dose regimen.[10] A starting dose of 1x10^6T cells/kg was given followed by increasing doses at 3-6 monthly intervals (1×10^6, 1×10^7, 5x10^7 and 1×10^8 T cells/kg) in the absence of GvHD.[10] All patients had stopped immunosuppression prior to administration of DLI.

HLA Typing

High resolution HLA typing was performed by DNA sequencing on stored DNA using Invitrogen SeCore(R) kits. Class 1 antigens (HLA-A,B,C) were determined using locus specific primers that sequence exons 2, 3 and 4, whist class 2 antigens (HLA-DRB1 and DQB1) were determined using group specific primers sequencing exon 2 as the only site of significant polymorphisms. The nucleotides and resulting HLA types were determined using multicolour sequence analysis, and

based on the sequence data, the HLA type was assigned using computer software (SBTengine® from GenDx, Netherlands).

Outcome Assessment and Statistical Analysis

The primary endpoint was aGvHD within 100 days of DLI. Secondary endpoints included incidence and severity of chronic GvHD (cGVHD), probability of achieving remission post DLI and overall survival (OS). GVHD was graded using the modified Glucksberg criteria.[18,19] Comparisons of aGvHD and cGvHD outcomes between the HLA-matching categories were made using Chi-squared or Fishers Exact tests. Probabilities of remission were calculated using the cumulative incidence procedure, and the Kaplan-Meier method was used for OS; categories were compared by log-rank or Gray's test. P-values reflect two-sided test results.

Results

Patient Characteristics

Fifty eight patients had stored DNA available for retrospective high resolution HLA typing. Thirty patients were fully matched at all 10 loci, while high resolution HLA typing uncovered mismatches not detected prior to transplantation by low or medium resolution HLA typing in 28 patients (HLA-A n=3, HLA-B n=4, HLA-C n=15, HLA-DR n= 8, HLA-DQ n=12). Mismatches were present at 1, 2 or 3 loci in 18, 6 and 4 patients respectively. Clinical characteristics of the patients, stratified according to the presence or absence of an HLA mismatch, are shown in Table 1. The groups were comparable with regard to baseline and transplant demographics with the exception that there were more male recipients from female donors in the mismatched group compared to the matched group (n=7 vs n=2, p=0.05). There were no significant differences between matched unrelated (MUD) and

mismatched unrelated donor (MMUD) groups in terms of age, sex, indication for transplantation, stem cell source or dose and incidence of acute and chronic GvHD post-HSCT (prior to infusion of DLI).

The median time from HSCT to first DLI was 15 months (range 6-76) in MUD recipients and 16 months (range 5-79) in MMUD recipients (p=0.83).The median number of DLI was 2 (range 1-6) in both groups with a median dose of $11x10^6$/kg (range 1-$361x10^6$/kg) in both groups.

Response to DLI

Indications for DLI included disease relapse (n=57) or mixed chimerism (n=1); 41patients (71%) re-entered complete remission, defined as complete molecular remission in the case of chronic myeloid leukaemia (CML) (Undetectable BCR-ABL1 transcripts by PCR on two consecutive occasions) or complete morphological remission for other forms of leukaemia (<5% blasts on bone marrow morphology). Remission was achieved post-DLI in 22/30 MUDs and 19/28 MMUDs, (probabilities of 62.1% and 63.9% at 3yrs respectively, p=0.89)

Toxicity, GVHD and outcome

Three patients died within 100 days of DLI without evidence aGvHD and were excluded from aGvHD analysis (causes of death included progressive disease, fungal infection and severe haemolytic anaemia). The incidence of aGvHD following DLI was low and not significantly different between MUD and MMUD recipients for all grades; 5 of 29 (17.2%) MUD and 6 of 26 (23.1%) MMUD recipients developed grade 2-4 aGvHD following infusion of DLI (p=0.59) with a median time to aGvHD onset of 38 (range 17-58) and 34 (range 20-82) days from the preceding DLI respectively (p=0.88) (Table 2). Importantly, the rates of aGvHD post-DLI were significantly lower than the rates of aGvHD in the immediate post-transplant period, which were 47% and 50% respectively (also not

significantly different in the two groups, p=0.80) (Table 2). Furthermore, aGvHD in the immediate post-transplant period did not predict the development of aGvHD following infusion of DLI. Of the 28 patients with a history of grade 2-4 aGvHD post-transplant, 6 (21%) developed aGvHD post DLI compared to 5 (16%) of the 30 patients who had no post-transplant aGvHD, with no impact of HLA disparity (p=0.87).

Similarly, we found no significant difference in the rates of overall cGvHD between the groups; 10 of 29 (34%) MUD and 11 of 26 (42%) of MMUD recipients developed cGvHD (p=0.51) following DLI. However, we observed a trend towards more severe cGvHD post-DLI in the MMUD group; 7 of 26 (27%) MMUD recipients developed extensive chronic GvHD compared to only 3 of 29 (10%) MUD recipients (p=0.09).

The OS at 5 years was 86% for the whole cohort, with no significant difference between MUD and MMUD recipients; 89.8% and 77.7% respectively, p=0.22 (Figure 1). The most frequent cause of death in both groups was infection (n=2 in the matched and n=3 in the mismatched group). GvHD accounted for one death in the mismatched group and contributed, along with infection, to thedeath of one of the matched recipients.

An exploratory analysis of the outcomes of DLI from MMUDs at 1 locus (n = 18) versus 2 or more loci (n=10) was also performed. We found no significant difference in the rates of grade 2-4 aGvHD or cGvHD in recipients of DLI mismatched at 1 versus ≥2 loci (31% vs. 11%, p=0.22 and 38% vs. 50%,p=0.53 respectively).

Impact of time to DLI on outcome

Patients receiving DLI within 9 months of HSCT (n=9) had a significantly inferior survival to those receiving DLI more than 9 months after HSCT (n=51) (5 year OS 33.3% and 93.3% respectively, p<0.01) (Figure 2). We found no significant difference in the rates of HLA-mismatch in patients who received DLI within 9 months of HSCT (44.4% vs. 44.9% respectively, p=0.98). Six of 9 patients who

received DLI less than 9 months after HSCT died, all within 12 months of receiving DLI. Importantly, although only 15% of patients received DLI less than 9 months post HSCT, they accounted for 46% of all deaths. Causes of death progressive disease in 3, severe bacterial infection in 1, fungal infection in 1 and severe haemolytic anaemia in 1 patient (Table 3). There was no significant difference in the rates of aGvHD in the two groups (33% vs 19%, p=0.58), although this result was confounded by early deaths post DLI. All three surviving patients achieved complete remission post DLI.

Discussion

Allogeneic HSCT challenges one of the fundamental hallmarks of cancer; the tumour's ability to evade immune surveillance.[20] The success of HSCT depends on achieving the optimal balance between sufficient immunosuppression to allow engraftment and the development of immune tolerance to the graft while establishing adequate GVL effect to generate a sustained attack on the malignant clone. Donor lymphocyte infusion remains a key tool in modulating the balance between the linked GvHD/GVL effect and relapse[9,10] but the associated risk of GvHD and the complications related to its management remain a major challenge for both patients and clinicians. Careful work has previously established safe and effective protocols to maximise the ability of DLI to restore disease control, whilst minimising the risk of GvHD.[14,21] However these protocols were established in the context of HLA-matched sibling[22] and fully matched unrelated donor transplants.[9,10] Whilst there is an increasing literature on transplant regimens permitting the use of mismatched unrelated donors as a source of stem cells, the safety of DLI in this setting remains largely unclear. Existing studies comprise mainly small data sets on modified DLI regimens and more work is therefore needed to establish the safety of such an approach.[16,23]

Here, we examined the outcomes of 58 consecutive MUD transplant recipients who were treated on a uniform escalating dose DLI protocol at our centre. Retrospective high resolution typing revealed mismatches not detected prior to transplantation by low or medium resolution HLA typing in 28

patients. We report that DLI, using an escalating-dose regimen, can be safely given to mismatched transplant recipients following alemtuzumab-based myeloablative allogeneic stem cell transplantation without a significant impact on GvHD or OS.

A large proportion of the patients included in our study were transplanted for chronic phase CML. Whilst in the era of tyrosine kinase inhibitors CML is a less frequent indication for transplantation,[1] the exquisite sensitivity of CML to the graft-versus-leukaemia (GVL) effect has historically provided important insights into the mechanisms of GVL and have helped further our understanding of immunological responses against leukaemia.[24] Moreover, this study primarily addresses the safety and efficacy of DLI many months after transplantation, away from the cytokine storm of high dose therapy and after immune reconstitution and development of tolerance, making the underlying disease less important in the assessment of acute or chronic GvHD. The study of CML patients also allowed us to objectively assess the effect of mismatched DLI on the GVL effect, using a sensitive molecular marker. We found no significant difference in the remission rates between the two groups, indicating that DLI from a mismatched donor is likely to be just as effective at inducing a GVL response as in the HLA-matched setting. In this context, these results are important in that they support safe and effective use of DLI from mismatched donors following T-depleted myeloablative HSCT for CML, and support further exploration of DLI from mismatched donors in other GVL sensitive diseases.

However while we observed that DLI from both MUD and MMUD was safe and effective in patients who relapsed late after their myeloablative T-depleted allograft, its use in the early post-transplantation period was associated with significantly worse outcome. These data are in keeping with previous reports that administration of DLI in the immediate post-transplant period is complicated by a high risk of severe, sometimes fatal, GVHD.[11,25]

In summary, this study demonstrates that DLI, using an escalating-dose regimen, can be safely given to mismatched transplant recipients following alemtuzumab-based myeloablative allogeneic stem cell transplantation, with the ability to deliver a high rate of molecular remission with a low risk of

GVHD. The timing of DLI appears to be crucial with worse outcome seen in those patients receiving DLI in the early post-HSCT period. The advent of targeted therapies such as tyrosine kinase or FLT 3 inhibitors and immunomodulatory drugs such as 5-azacytidine may have relevance for the design of DLI protocols in diseases such as CML, Philadelphia positive ALL, or AML. In these settings, the combination of these drugs might modify relapse kinetics such that DLI, if required from a matched or mismatched unrelated donor, can be delivered safely and effectively.[26,27]

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Authorship

Contribution: AI performed data collection, analysed data and wrote the paper; RB performed research and analysed data; R Sergeant provided reagents and analysed data; DG analysed data and contributed to writing the paper; LF and EB provided sample material and contributed to the paper; D Marin and SM analysed data and contributed to writing the paper, D Milojkovic, M MacDonald, EK, JP, AR, IR, JG, JA and FD provided patient care and contributed to writing of the paper; RS analysed data and wrote the paper; KR designed and supervised the research, analyzed data and wrote the paper.

Conflict-of-interest disclosures

Dr John Goldman is Editor in Chief of Bone Marrow Transplantation. The other authors declare no conflict of interest.

Table 1 – Patient Demographics: CML-CP indicates chronic myeloid leukaemia (CML) in chronic phase; CML-AP, CML in accelerated phase; CML-2nd CP, CML in second chronic phase; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CMV, cytomegalovirus; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen; DLI, donor lymphocyte infusions.

Demographics	**Matched (n=30)**	**Mismatched (n=28)**	p-value
Transplant Characteristics			
Median Age (years)	31 [13-50]	32 [13-52]	0.44
Gender (Male / Female)	19/11	20/8	0.51
Recipient/Donor sex (Matched / Male recipient-Female donor / Female recipient-Male donor)	17/2/11	16/7/5	0.05
Disease			
CML-CP	21	22	
CML-AP	7	2	
CML-2nd CP	2	1	
AML	1	1	
ALL	0	1	0.68
CMV Status - Recipient			
Positive / Negative	20/10	18/9	1.0
CMV Recipient / Donor Match			
Both negative / other	5/25	5/23	0.61
Graft Source			
Marrow / PBSC	24/6	21/7	0.65
HLA Matching			
Mismatch identified by sequencing -			
HLA-A total (antigen / allele)	-	3 (2/1)	
HLA-B total (antigen / allele)	-	4 (1/3)	
HLA-C total (antigen / allele)	-	15 (13/2)	
HLA-DR total (antigen / allele)	-	8 (4/4)	
HLA-DQ total (antigen / allele)	-	12 (5/7)	
Number of Loci -			
1 / 2 / 3		18/6/4	
DLI			
Median time to DLI (Months)	15 [6-76]	16 [5-79]	0.83
Median number of DLI infusions	2 [1-6]	2 [1-5]	0.70
Median cell dose	$11x10^6$/kg	$11x10^6$/kg	0.73

Table 2 – Outcome based on presence or absence of HLA mismatch. HSCT indicates hematopoietic stem cell transplantation; aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; DLI, donor lymphocyte infusions.

Outcome	Matched (n=30)	Mismatched (n=28)	p-value
Graft-versus-host disease post-HSCT			
Post-transplant aGvHD grade II-IV	14 (47%)	14 (50%)	0.80
Post-transplant cGvHD any	10 (33%)	11 (39%)	0.92
Post-transplant cGvHD extensive	3 (10%)	2 (7%)	0.73
Graft-versus-host disease post -DLI			
Post-DLI aGvHD grade II-IV	5 (17%)	6 (23%)	0.59
Post-DLI cGvHD any	10 (34%)	11 (42%)	0.55
Post-DLI cGvHD extensive	3 (10%)	7 (27%)	0.09
Survival post-DLI			
5 year survival	89.8%	77.7%	0.22
Cause of death			
Relapse	1	2	
Infection	2	3	
GvHD	-	1	
Infection/GvHD	1	-	
Other	1	2	

Table 3 – Characteristics of patients who died following DLI: CML-CP indicates chronic myeloid leukaemia (CML) in chronic phase; CML-AP, CML in accelerated phase; CML-2nd CP, CML in second chronic phase; AML, acute myeloid leukaemia; HSCT, haematopoetic stem cell transplant; aGvHD, acute graft versus host disease; cGvHD, chronic graft versus host disease; DLI, donor lymphocyte infusions; AIHA, auto-immune haemolytic anaemia; HLA, human leukocyte antige

Disease	HLA Disparity	Time from HSCT to DLI (days)	DLI received within 9 months of HSCT	aGvHD post HSCT (Grade II-IV)	aGVHD post-DLI (Grade II-IV)	cGVHD post- DLI	Immunosuppression post DLI	Immunosuppression at time of death	Time from DLI to death (days)	Causes of death
CML-AP	Fully matched	87	Yes	None	None	None	Oral presnisolone (<1mg/kg) for AIHA	Prednisolone	41	Progressive disease
CML-CP	Fully matched	268	Yes	None	None	None	None	None	85	Bacterial Infection
CML-AP	Fully matched	395	No	None	Grade III	Extensive	Oral prednisolone (<1mg/kg) Mycophenolate	Mycophenolate	2070	Bacterial Infection cGVHD
AML	Fully matched	474	No	None	None	None	None	None	216	Metastatic breast cancer
CML-CP	Fully matched	1470	No	Grade II	None	None	None	None	3357	Pulmonary oedema Gastrointestinal haemorrhage
CML-AP	1 DQ antigen	139	Yes	None	None	None	None	None	224	Progressive disease (CML-BC)
AML	1 C allele	159	Yes	None	Grade IV	Na	Methylprednisolone (2mg/kg) Daclizumab (3 doses) Alemtuzumab Mesenchymal stromal cells	Prednisolone	108	Progressive disease (AML)
CML-CP	1 C antigen	190	Yes	None	None	None	Methylprednisolone (1mg/kg) for AIHA	Prednisolone	20	Fungal Infection
CML-CP	1 DQ allele	224	Yes	None	None	None	Oral prednisolone (1mg/kg) for AIHA	Prednisolone	41	Severe HA
CML-CP	1 DQ allele	403	No	None	None	None	None	None	679	Osteosarcoma
CML-CP	1 C allele	425	No	Grade II	Grade III	Extensive	Methylprednisolone (1mg/kg) Mycophenolate	Mycophenolate	3541	Bacterial Infection
CML-CP	1 A antigen	430	No	None	None	Extensive	Prednisolone Alemtuzumab Mycophenolate	Mycophenolate	2267	Fungal infection
CML-AP	1 DQ allele	612	No	None	None	Extensive	Oral prednisolone (<1mg/kg) Cyclosporin	Cyclosporin	1018	cGVHD Graft failure

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Figure Legends

Figure 1- Probability of Overall Survival (OS) following DLI based on HLA-matching status

Figure 2- Probability of Overall Survival (OS) following DLI based on timing of DLI (< or > 9 months post HSCT)

Figure 1

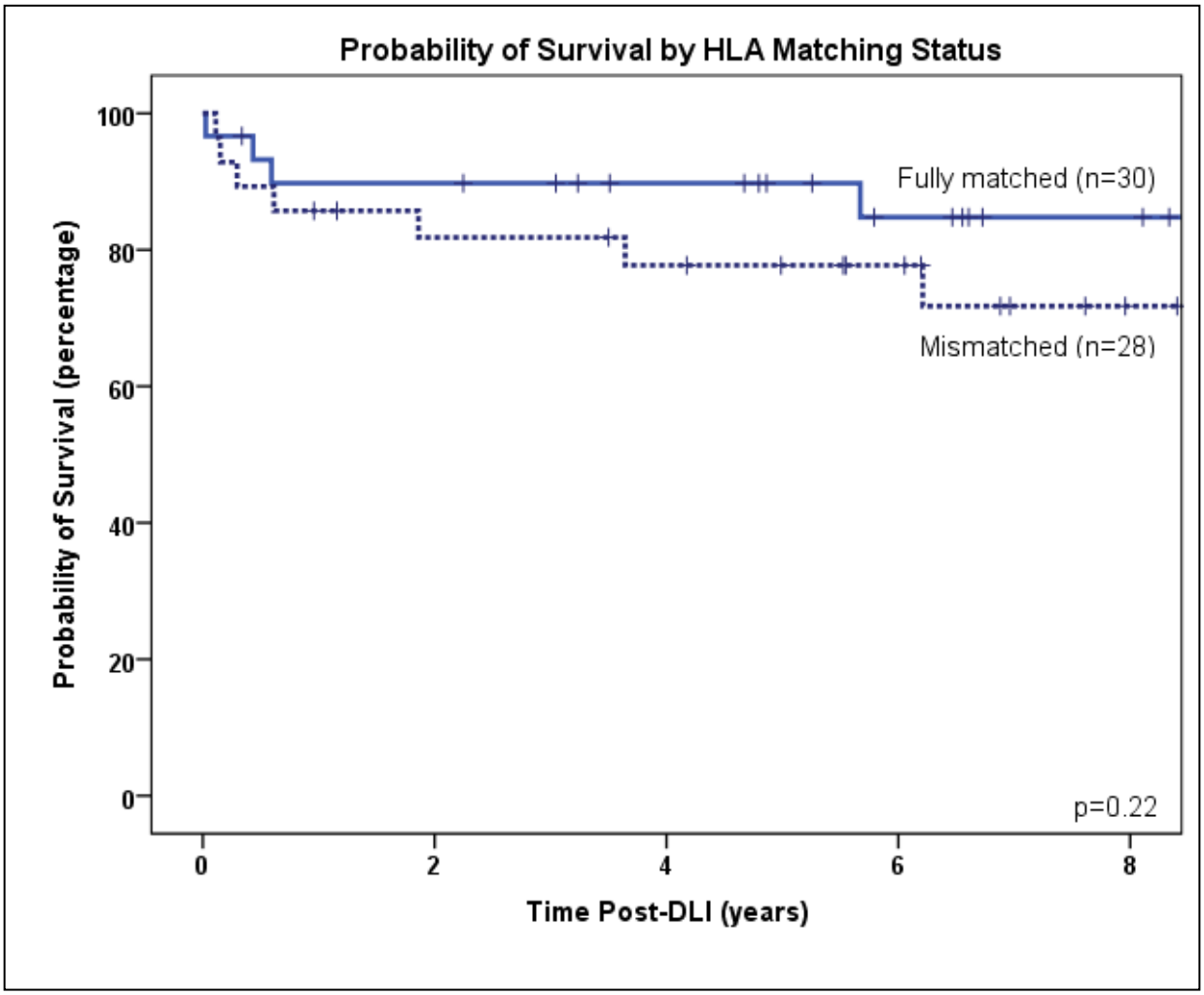
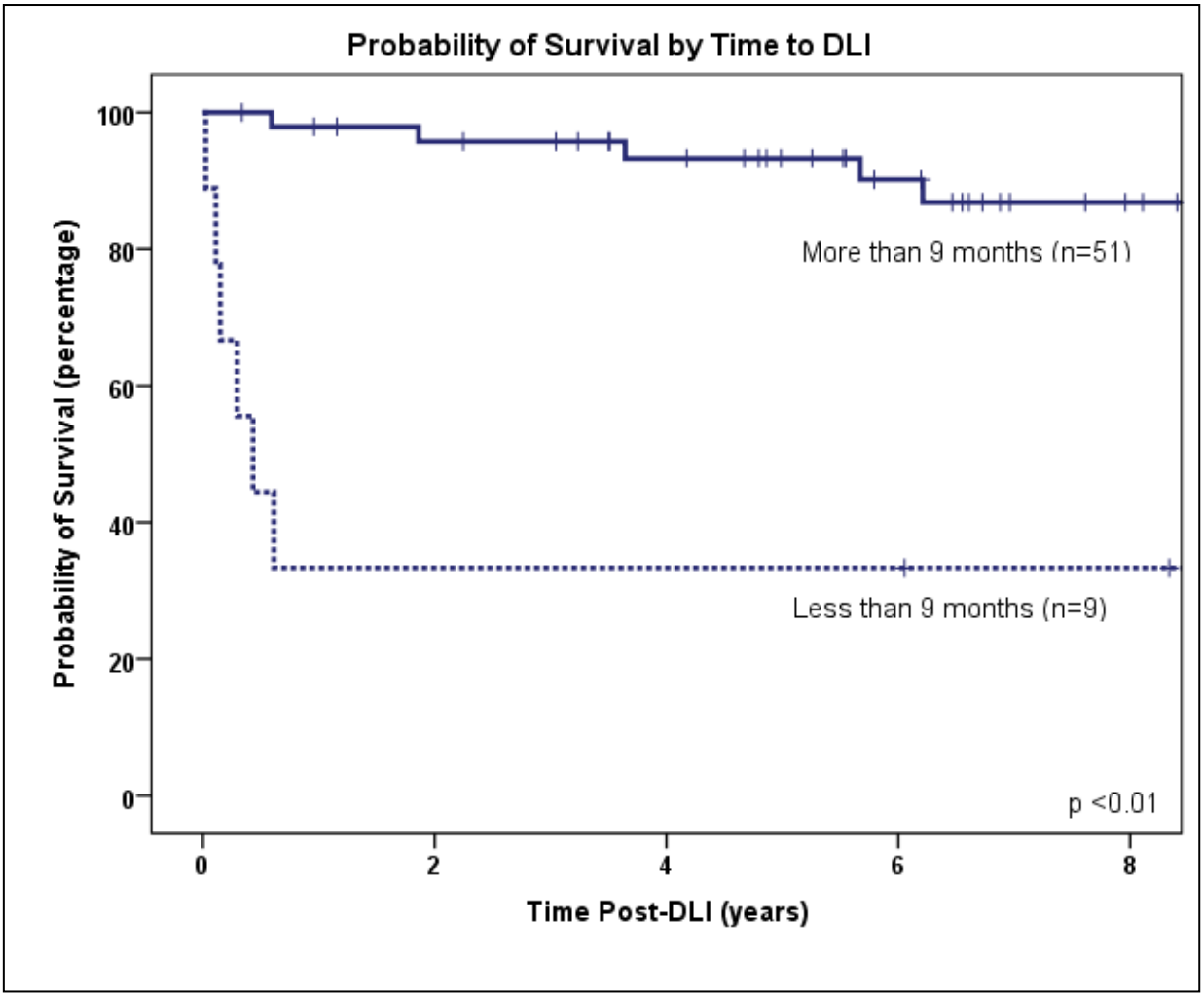

Probability of Survival by HLA Matching Status
Probability of Survival (percentage)
100
80
60
40
20
0
Fully matched (n=30)
Mismatched (n=28)
p=0.22
0
2
4
6
8
Time Post-DLI (years)

Figure 2


Probability of Survival by Time to DLI
Probability of Survival (percentage)
100
80
60
40
20
0
More than 9 months (n=51)
Less than 9 months (n=9)
p <0.01
0
2
4
6
8
Time Post-DLI (years)