Infusion of Allogeneic Mesenchymal Stromal Cells After Liver Transplantation: A 5-Year Follow-Up

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Various properties of mesenchymal stromal cells (MSCs) might be particularly of interest after liver transplantation (LT). In this article, we report the long-term results of a prospective, controlled, and first-in-human phase 1 study evaluating the safety of a single MSC infusion after LT. A total of 10 LT recipients treated with standard immunosuppression received 1.5 to 3 × 10^6/kg third-party unrelated MSCs on postoperative day 3 and were prospectively compared with a control group of 10 LT recipients. Primary endpoints were set to prospectively detect potentially delayed adverse effects of MSC infusion, particularly the occurrence of infections and cancers. Secondary endpoints of liver graft and patient survival, graft rejection and function, occurrence of bile duct complications, and development of donor-specific anti–human leukocyte antigen (HLA) antibodies (DSA) against liver or MSC donors were studied. The median follow-up was 85 months. There was no difference in overall rates of infection or cancer at 5 years of follow-up between the 2 groups. There was also no difference in secondary endpoints. The prevalence of de novo liver DSAs related to HLA mismatches was twice as high in the MSC group compared with the control group. All of the de novo class II HLA antibodies against MSCs were linked to a shared HLA mismatch between the liver and MSCs. This study confirms the safety of a single MSC infusion after LT. The potential benefits of MSC injections in the context of organ transplantation have yet to be demonstrated by larger prospective studies. The development of anti-HLA antibodies against an MSC donor should be further evaluated, especially in cases of shared HLA mismatches between graft and MSC donors, despite the fact that no deleterious effect has been detected.

Liver Transplantation 28 636–646 2022 AASLD.
Received July 9, 2021; accepted September 22, 2021.

Mesenchymal stromal cells (MSCs) are multipotent progenitor cells capable of differentiating into various cells and tissues, such as chondrocytes, osteoblasts, and adipocytes.(1) In addition, some MSC characteristics might be particularly of interest in solid organ transplantation, such as their capacity to attenuate ischemia/reperfusion injury, their potential role in tissue regeneration or repair, and their immunomodulation properties.(2-6) Promising preclinical results, including the demonstration of the ability of MSCs to inhibit T cell proliferation and dendritic cell maturation and to induce CD4$^+$CD25$^+$FoxP3$^+$ T regulator lymphocyte expansion,(7) prompted clinical trials using MSC-based therapy after living related or deceased donor transplantation, particularly in kidney transplantation (KT) and in liver transplantation (LT).(2)

Our group initiated 2 prospective clinical trials investigating the safety of injecting allogeneic third-party

Abbreviations: allo-MSC, allogeneic mesenchymal stromal cell; AS, anastomotic stricture; AST, aspartate aminotransferase; BM, bone marrow; CMV, cytomegalovirus; CRP, C-reactive protein; DSA, donor-specific anti–HLA antibodies; GGT, gamma glutamyltransferase; HCC, hepatocellular carcinoma; HLA, human leukocyte antigen; HLA-A, HLA antigen A; HLA-B, HLA antigen B; HLA-C, HLA antigen C; HLA-DRB1, HLA antigen DRB1; HLA-DQB1, HLA antigen DQB1; HSV, herpes simplex virus; INR, international normalized

MSCs after deceased donor KT and LT.[8,9] In theory, among the potential adverse effects, intravenous MSC infusion could be complicated by an immediate toxicity, by a cytokine-release syndrome,[10] and by MSC embolism in the pulmonary vasculature.[11] In addition, as MSCs are potentially immunosuppressive, concerns remain about the potential increased rates of opportunistic infections and cancers after MSC infusion in transplant recipients already receiving standard immunosuppression.[12] The 1-year reports of our 2 studies did not demonstrate an increase in such complication rates either in the KT or the LT cohort,[8,9] adding evidence of MSC short-term safety.[12]

Long-term safety of the clinical use of MSCs has still to be confirmed. In addition to their immunologic risk, it has been suggested that in vitro MSC expansion and culture might generate genomic instability and chromosomal aberrations with a potential risk of MSC neoplastic transformation.[13,14] Another potential long-term adverse effect of MSC injection could be the induction of liver fibrosis.[15] Furthermore, the question of MSC immunogenicity remains debated.[8] Preclinical data suggest that allogeneic MSCs (allo-MSCs) could promote an antidonor immune response in the host.[16] Thus, clinical administration of allo-MSCs could induce the development of anti-MSC donor human leukocyte antigen (HLA) antibodies that potentially could promote rejection, especially in cases of common HLAs between MSCs and graft donors, and could harbor potential issues in cases of the need for retransplantation, particularly in KT.[8,17]

In this article, we report the long-term results of a prospective, controlled, and first-in-human phase 1 study evaluating the safety of a single third-party allo-MSC infusion after LT, the 1-year data of which has been previously published elsewhere.[9] The primary endpoints of this study were set to prospectively detect potential delayed adverse effects of MSC infusion, particularly the occurrence of opportunistic infections and cancers. As secondary endpoints, liver graft and patient survival, graft rejection and function, occurrence of bile duct complications, and development of de novo donor-specific anti-HLA antibodies (DSA) against both liver and MSC donors were studied.

ratio; KT, kidney transplantation; $_{liver}$DSA, DSA against liver donor; $_{liver+MSC}$DSA, DSA against both liver and MSC donors; LT, liver transplantation; LTCG, Laboratory of Cell and Gene Therapy; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; MSC, mesenchymal stromal cell; $_{MSC}$DSA, DSA against MSC; NAS, nonanastomotic stricture; NS, not significant; SSO, sequence-specific oligonucleotides; VZV, varicella-zoster virus.

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This study was supported by the Fonds d'Investissement de la Recherche Scientifique at the University of Liege (CHU ULiege), the Fonds Leon Fredericq of the Medical School of the University of Liege, and through a Senior Research Grant from the European Society for Organ Transplantation. The Laboratory of Cell and Gene Therapy is supported in part by the Fonds National pour la Recherche Scientifique (FNRS) and the "Federal Cancer Plan–Belgium." Morgan Vandermeulen is a Ph.D. fellow sponsored by the FNRS. Pauline Erpicum is a fellow of the FNRS.

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DOI 10.1002/lt.26323

Potential conflict of interest: Nothing to report.

Patients and Methods

STUDY DESIGN

This study was a monocentric, prospective, nonrandomized, controlled, open-label trial.[9] In summary, between March 2012 and February 2014, 10 stable and low-risk LT recipients treated with standard immunosuppression received 1.5-3 × 10^6/kg third-party bone marrow (BM) MSCs on postoperative day 3 ± 2 (MSC group). The protocol of MSC isolation and expansion has been detailed elsewhere.[9,18] MSC donors were unrelated to the recipient and fulfilled generally accepted criteria for allogeneic hematopoietic stem cell donation. MSC expansion cultures were performed and evaluated at the Laboratory of Cell and Gene Therapy (LTCG) of the University Hospital of Liege, CHU ULiège. Briefly, BM was collected in sterile conditions under local anesthesia and put in sterile heparinized syringes. Mononuclear BM cells were then isolated, seeded in sterile tissue culture flasks, cultured in specific medium, and maintained at 37°C in a humidified atmosphere containing 5% CO_2 for a total of approximately 4 weeks. After 2 passages, cells were harvested, washed, and resuspended and then frozen. Before infusion, the MSCs were thawed and diluted

in phosphate-buffered saline, and then injected into the patients within 60 minutes. As quality controls, for each MSC expansion culture we performed flow cytometry analysis to confirm the identity of the MSCs, an evaluation of cell viability using trypan blue exclusion, and microbiology testing. MSC potency was evaluated by determining the percentage inhibition of T cell proliferation in a mixed-lymphocyte reaction essay. MSC differentiation into osteocytes, chondrocytes, and adipocytes was validated in preliminary experiments.[18] No attempt was made to match HLAs between liver graft donors and recipients on one hand and MSC donors on the other.

These patients were prospectively compared with a control group of 10 LT recipients who fulfilled the study inclusion criteria (control group). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, was approved by the local Ethics Committee and by the Belgian Federal Agency for Medicines and Health Products (Eudract no. 2011-001822-81), and was registered at ClinicalTrials.gov (protocol no. NCT 01429038). Written informed consent was obtained from each MSC donor and LT recipient. No organs from executed prisoners were used.

POSTTRANSPLANT MANAGEMENT AND IMMUNOSUPPRESSION

In the phase 2 part of the trial, recipients from the MSC group, who did not develop rejection and had normal graft biopsy, underwent an unsuccessful attempt of immunosuppression withdrawal.[9] All patients were therefore treated according to the local immunosuppression protocol, consisting of low dose tacrolimus (trough levels of between 3 and 6 ng/mL) and mycophenolate mofetil 500 mg with adaptation according to adverse effects. All patients underwent lifelong transplant follow-up and regular outpatient visits with routine blood analyses. No patient had been lost to follow-up, that was fixed at March 21st, 2021. Median follow-up was 85 months with a follow-up of at least 5 years in all surviving patients.

PRIMARY ENDPOINTS: INFECTIONS AND CANCERS

The incidence, timing, and severity of any infection (bacterial, viral, fungal) and any malignant diseases were prospectively recorded in both groups.

SECONDARY ENDPOINTS

Patient and graft survival and biopsy-proven graft rejection rates were prospectively recorded in both groups. Liver graft function (bilirubin, liver enzymes, international normalized ratio [INR]), kidney function (creatinine), C-reactive protein (CRP), and tacrolimus levels were compared at months 12, 36, and 60.

No scheduled long-term graft biopsies were performed, according to the study protocol and to local clinical management. Liver graft biopsies were only performed if clinically indicated (per-cause biopsies) or when a patient underwent unrelated abdominal surgery to repair incisional hernias or hepaticojejunostomy (passage biopsies). These biopsies were blindly compared for fibrosis and for rejection according to the Banff criteria.[19]

Magnetic resonance cholangiopancreatography was performed during the patient follow-up when clinically indicated. Biliary strictures were defined as any stricture requiring endoscopic, percutaneous, or surgical management. Anastomotic stricture (AS) was defined as a stenosis located at the bile duct anastomosis. Non-AS (NAS) was defined as biliary stenosis located in the intrahepatic or extrahepatic bile ducts at least 1 cm above the anastomosis and characterized as extrahepatic if located in the donor's common bile duct or hepatic ducts up to 2 cm above the bifurcation and intrahepatic if located above this level.

Each LT recipient and each liver or MSC donor was genotyped for HLA antigen A (HLA-A), HLA antigen B (HLA-B), HLA antigen C (HLA-C), HLA antigen DRB1 (HLA-DRB1), and HLA antigen DQB1 (HLA-DQB1) based on low/medium resolution molecular typing (Luminex Corp. [Austin, TX]/ Immucor sequence-specific oligonucleotides (SSO) [Immucor Inc., Norcross, GA]); ambiguous results were resolved by means of sequence-specific primer molecular typing (Olerup, Stockholm, Sweden). DSA against MSC ($_{MSC}$DSA) and DSA against liver donor ($_{liver}$DSA) detection and identification were performed using Luminex solid-phase antibody detection technology (Luminex Corp. [Austin, TX] / Immucor LSA [Immucor Inc., Norcross, GA]). HLA antibodies were considered as positive when mean fluorescence intensity (MFI) was >1500 and in accordance with the manufacturer's recommendations. An antibody was considered de novo if not detected before transplantation. An identical

HLA mismatch between the liver recipient and both the MSC and liver donors was considered a shared HLA mismatch. Sera were tested before transplantation and at months 1, 3, 6, and 12 and then at long-term after transplantation. One patient in the control group who died from hemorrhage before month 1 was not included in the mismatch and DSA analyses.

STATISTICAL ANALYSES

Data are presented as median values and ranges, and the differences between groups were evaluated using the Mann-Whitney U test. Proportions were compared using Fisher's exact test. Survival rates were calculated with the Kaplan-Meier curve method and compared with the log-rank (Cox-Mantel) test. A P value <0.05 was considered significant. Data were analyzed using Prism 9.1.0 software for Macintosh OS (GraphPad Software, San Diego, CA).

Results

PRIMARY ENDPOINTS

From transplantation to year 5, there was no significant difference in infection rates between groups (Table 1). In addition to the infections previously described,[9] in the MSC group, 2 patients developed *Clostridium colitis*, 2 others developed biliary infections requiring antibiotics, and 1 suffered from herpetic keratitis. In the control group, 1 patient developed pneumonia and later died from sepsis, 1 suffered from biliary infection requiring antibiotics, and 1 suffered from a resistant *Escherichia coli* urinary tract infection.

There was no difference in the rates of cancer diagnosis between groups (Table 1). In each group, 1 patient developed hepatocellular carcinoma (HCC) recurrence and ultimately died from this recurrence.[9] In the MSC group, 1 patient developed non–small cell lung carcinoma that caused death at posttransplantation month 90. In the control group, 1 patient was diagnosed with prostate adenocarcinoma at month 78, 1 developed a T2 basal skin cancer operated on at month 78, and another died from pulmonary adenocarcinoma at month 21.

TABLE 1. Primary Endpoints

Variables	MSC Group (n = 10)	Control Group (n = 9)	*P* Value
Infection			
Overall	7	9	NS
Fungal	0	0	
Viral			
CMV disease	0	0	
HSV	3	0	
VZV	0	1	
Bacterial			
Wound	0	1	
Urinary	0	3	
Sinusitis	0	1	
Pulmonary	0	2	
Digestive	2	0	
Biliary	2	1	
Cancer			
Overall	2	4	NS
HCC recurrence	1	1	
Lung	1	1	
Prostate	0	1	
Skin	0	1	

NOTE: Fisher's exact test.

SECONDARY ENDPOINTS

The 5-year graft and patient survival rates were 70% and 80% in the MSC and control groups, respectively (not significant [NS]; Supporting Fig. 1). At follow-up, 6 and 5 patients had died in the MSC and control groups, respectively (NS). The cuses of death were malignant diseases in 4 patients (2 in each group), recurrence of primary liver disease in 4 patients (3 in the MSC group and 1 in the control group), septic complications in 2 patients (1 in each group), and 1 patient in the control group died from abdominal hemorrhage. No differences could be detected in liver graft, kidney function, or tacrolimus levels between the 2 groups at years 1, 3, or 5 (Table 2).

No patient in either group developed biopsy-proven acute rejection requiring bolus steroid therapy during the whole follow-up. A total of 13 patients underwent liver graft biopsies (7 and 6 in the MSC and control groups, respectively [passage biopsies, n = 10; per-cause biopsies, n = 3]). The median Banff scores were 3 (1-5) and 1.5 (0-3) in the MSC and control groups, respectively (NS). The median fibrosis scores were 0 (0-3) and 0 (0-1) in the MSC and control groups, respectively (NS). Regarding biliary complications, 6 MSC patients and 3 control patients developed AS that required invasive management by endoscopic dilatation and stenting in 7 patients and by hepaticojejunostomy

TABLE 2. Laboratory Tests

Variables	MSC Group	Control Group	*P* Value
Month 12			
Total bilirubin, mg/dL	0.56 (0.4-0.8)	0.48 (0.28-0.74)	0.22
AST, U/L	31.5 (18-141)	21 (16-55)	0.03
Alkaline phosphatase, U/L	157 (93-253)	140 (83-284)	0.71
GGT, U/L	144 (46-810)	81 (12-183)	0.06
INR	1.06 (0.98-1.28)	1 (0.92-1.18)	0.21
Creatinine, mg/dL	1.04 (0.5-2.4)	1.17 (0.5-1.7)	0.98
CRP, mg/L	6.5 (2.2-25.8)	5.2 (1.2-22)	0.47
Tacrolimus, μg/L	7.5 (1.4-9.5)	7.8 (3.7-13.8)	0.69
Month 36			
Total bilirubin, mg/dL	0.89 (0.34-2.4)	0.56 (0.37-0.77)	0.02
AST, U/L	30 (16-64)	27 (16-34)	0.84
Alkaline phosphatase, U/L	106 (59-214)	98.5 (65-206)	0.86
GGT, U/L	152 (16-447)	44 (14-497)	0.28
INR	1.03 (1-1.14)	1.01 (1-1.06)	0.58
Creatinine, mg/dL	0.99 (0.83-1.78)	1.01 (0.74-1.71)	0.71
CRP, mg/L	5.8 (1.5-27.5)	3.7 (1.4-15.6)	0.44
Tacrolimus, μg/L	7 (4-10.6)	5.4 (1.5-7)	0.18
Month 60			
Total bilirubin, mg/dL	0.72 (0.34-8.58)	0.69 (0.35-0.78)	0.87
AST, U/L	40 (14-112)	20 (12-48)	0.24
Alkaline phosphatase, U/L	158 (64-598)	126 (71-150)	0.20
GGT, U/L	116 (11-580)	44 (25-161)	0.16
INR	0.99 (0.87-1.3)	1 (0.98-1.1)	0.66
Creatinine, mg/dL	1.2 (0.85-8.34)	1.07 (0.65-2.47)	0.59
CRP, mg/L	4.7 (0.8-15.5)	9.6 (2.9-35.8)	0.29
Tacrolimus, μg/L	6.3 (2.7-15.9)	3.9 (1.8-6.2)	0.08

NOTE: Data are presented as median (range; Mann-Whitney *U* test).

after failure of endoscopic treatment in 2 patients. In the MSC group, 1 patient developed NAS requiring retransplantation after failure of hepaticojejunostomy and ultimately died from septic complications.

Regarding immunosuppression at the 5-year follow-up, 6 patients in each group were on tacrolimus. A total of 5 and 6 patients were on mycophenolate mofetil in the MSC and control groups, respectively, and a total of 1 and 2 patients were on everolimus in the MSC and control groups, respectively.

HLA MISMATCHES

Considering the 5 evaluated HLA loci (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1), the total number of HLA mismatches between recipients and donors was 72 in both the control (n = 9 patients alive at month 1) and MSC group (n = 10 patients alive at month 1). The median number of HLA mismatches was 9 (5 for class I and 3 class II loci) and 7 (4.5 for class I and 3 for class II loci) in the control and MSC groups, respectively (Table 3 and Fig. 1).

In the MSC group, the total number of HLA mismatches between recipients and MSC donors was 79. The median number of HLA mismatches was 8 (4.5 for class I and 3 for class II loci; Table 2). A total of 9 patients presented at least 1 shared HLA mismatch between the liver and MSC donors. The total number of shared HLA mismatches was 14 (9 for class I and 5 for class II loci; Table 3 and Fig. 2).

PREFORMED DSA

In each group, 1 patient presented with preformed class I $_{\text{liver}}$DSA before LT. In the MSC group, the preformed $_{\text{liver}}$DSA (HLA*B55:01) persisted up to month 6 with a decreasing intensity (MFI 7500 before LT to 2500 at month 6). In the control group, preformed $_{\text{liver}}$DSA (HLA*A25:01) were cleared from month 1. In the MSC group, 1 patient presented $_{\text{MSC}}$DSA before LT (HLA*A25:01), which persisted up to the month 6 evaluation with a decreasing intensity (MFI 7500 before LT and 1600 at month 6).

DE NOVO $_{\text{LIVER}}$DSA

In the control group, 3 patients developed 1 de novo $_{\text{liver}}$DSA class I during the first 6 months after LT. Another recipient developed 2 de novo $_{\text{liver}}$DSA class II (both with MFI >5000) more than 2 years after transplantation. In total, 5 de novo $_{\text{liver}}$DSA were detected in 4 control recipients during follow-up (Table 2 and Fig. 3A). In the MSC group, 6 patients developed at least 1 de novo $_{\text{liver}}$DSA (all but 1 were HLA class II antibodies) during the first year of follow-up. Among these 6 patients, 3 developed 1 de novo $_{\text{liver}}$DSA and 3 developed 2 de novo $_{\text{liver}}$DSA (Fig. 3A and Table 3). A total of 4 patients with de novo $_{\text{liver}}$DSA class II were with MFI >5000 (Fig. 3A and Table 3).

The prevalence of de novo $_{\text{liver}}$DSA was 6.9% (n = 5) and 12.5% (n = 9) of HLA mismatches in the control and MSC groups, respectively ($P = 0.4$). The prevalence of de novo $_{\text{liver}}$DSA for class I HLA mismatches

TABLE 3. HLA Mismatches and Donor-Specific Anti-HLA Antibodies

Group and Patient No.	Number of HLA Mismatches			Month 1		Month 3		Month 6		Month 12		>Month 12	
	Liver n (class I/II)	MSC n (class I/II)	Shared n (class I/II)	$_{liver}$DSA	$_{MSC}$DSA	$_{liver}$DSA	$_{MSC}$DSA	$_{liver}$DSA	$_{MSC}$DSA	$_{liver}$DSA	$_{MSC}$DSA	$_{liver}$DSA	$_{MSC}$DSA
MSC													
1	9 (6/3)	7 (3/4)	2 (1/1)					DRB1*07 :01 DRB1*11 :01	DRB1*07 :01				
2	10 (6/4)	9 (6/3)	1 (1/0)					B*44:02					
3	5 (3/2)	9 (6/3)	1 (1/0)		**B*57:01**		B*57:01		B*57:01	DQB1*03:01	B*57:01 DQB1*03:01		B*57:01
4	7 (5/2)	8 (4/4)	3 (3/0)										
5	4 (3/1)	6 (4/2)	2 (0/2)										
6	7 (6/1)	9 (6/3)	1 (1/0)										
7	7 (3/4)	6 (4/2)	2 (0/2)	DQB1*06:04	DQB1*06:04							**DQB1*03:01**	
8	9 (5/4)	9 (5/4)	1 (1/0)										
9	7 (4/3)	8 (6/2)	0	DQB1*03:01 DQB1*02:01				**DQB1*03:01** **DQB1*02:01**		**DQB1*03:01** **DQB1*02:01**		**DQB1*03:01** **DQB1*02:01**	
10	7 (4/3)	8 (5/3)	1 (1/0)							DRB1*11 :04			
Total	72 (45/27)	79 (49/30)	14 (9/5)										
Control													
1	5 (3/2)	/	/		/		/		/		/		/
2	10 (6/4)	/	/	A*24:02	/		/		/		/		/
3	7 (4/3)	/	/		/		/		/		/		/
5	9 (5/4)	/	/		/		/		/		/	**DQB1*05:01** **DQB1*06:03**	/
6	5 (3/2)	/	/		/		/		/		/		/
7	9 (6/3)	/	/	C*04:01	/	C*04:01	/	C*04:01	/		/		/
8	8 (5/3)	/	/		/		/		/		/		/
9	10 (6/4)	/	/		/	A*24:02	/		/		/		/
10	9 (5/4)	/	/		/		/		/		/		/
Total	72 (43/29)	/	/										

NOTE: Control group patient 4 died before month 1 and was not included in this analysis. Underlined DSA in cases of $_{liver+MSC}$DSA. Bold DSA in cases of MFI >5000.

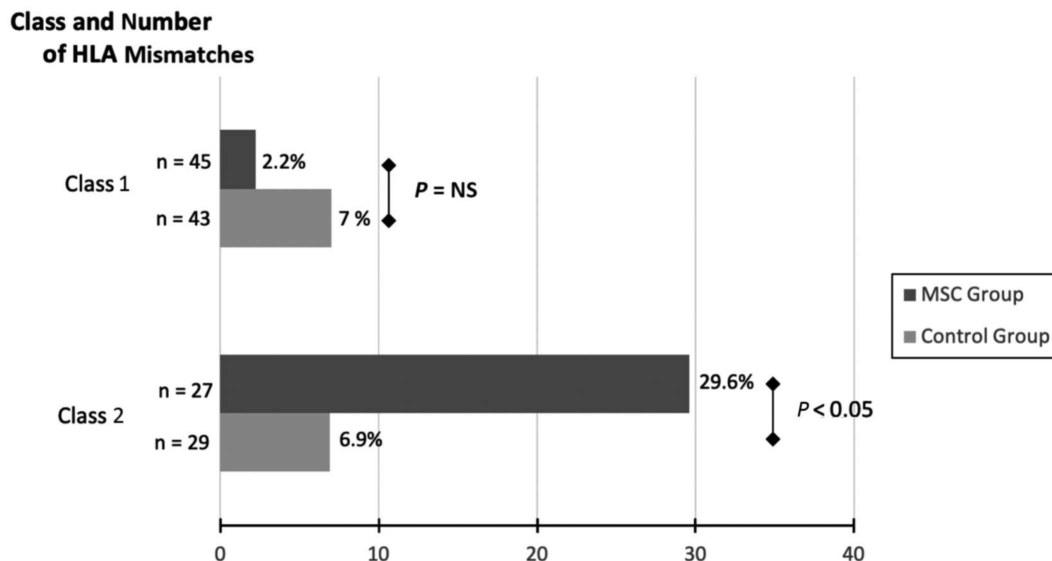

FIG. 1. Prevalence of locus-specific de novo $_{liver}$DSA relative to the number of HLA mismatches in the MSC and control groups (%; Fisher's exact test).

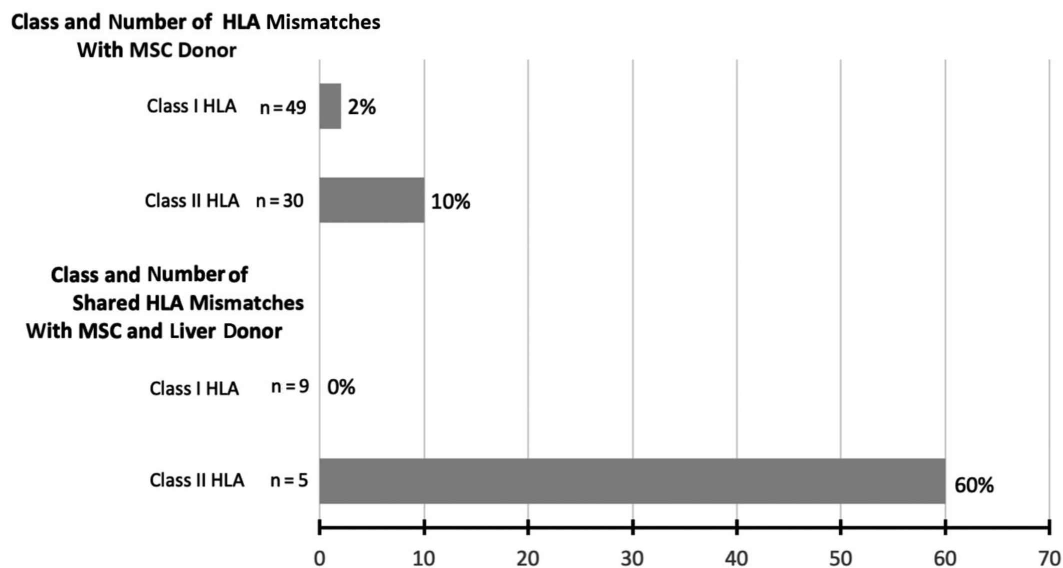

FIG. 2. Prevalence of locus-specific de novo $_{MSC}$DSA relative to the number of HLA mismatches with MSC donors (upper) and prevalence of locus-specific de novo $_{liver+MSC}$DSA relative to the number of shared HLA mismatches (lower).

was 2.2% (n = 5) and 7% (n = 9) in the control and MSC groups, respectively (P = 0.36). The prevalence of de novo $_{liver}$DSA for class II HLA mismatches was 6.9% (n = 2) and 29.6% (n = 8) in the control and MSC groups, respectively (P = 0.04; Figs. 1 and 3A and Table 3).

DE NOVO $_{MSC}$DSA

In the MSC group, 3 patients developed at least 1 de novo $_{MSC}$DSA. Patient 3 developed 1 class I $_{MSC}$DSA (B57:01), detected from month 1 (with MFI>5000) to the end of follow-up, and 1 class II $_{MSC}$DSA at month

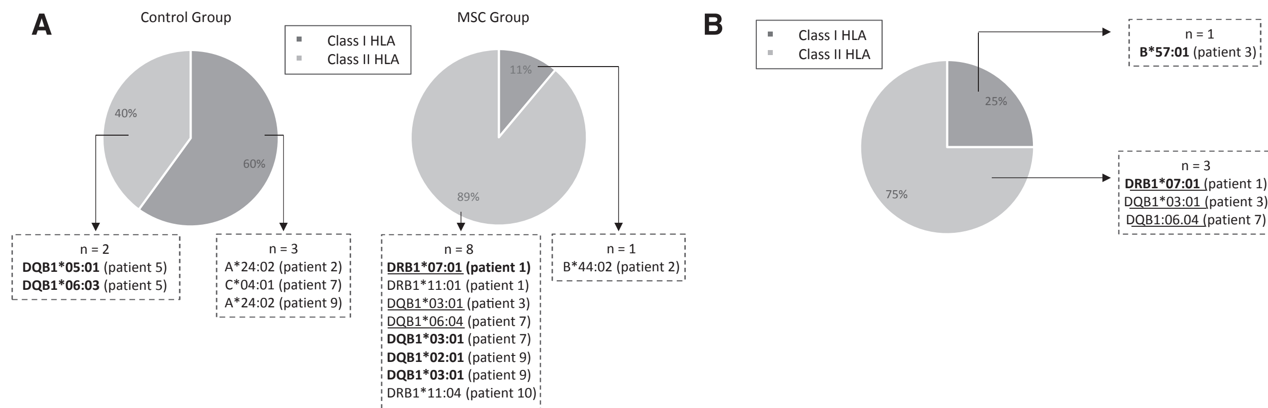

FIG. 3. Class and identity of de novo(A) $_{Liver}$DSA and (B) $_{MSC}$DSA. Underlined DSA in cases of $_{liver+MSC}$DSA. Bold DSA in cases of MFI >5000.

12. De novo $_{MSC}$DSA class II was detected in patient 7 and patient 1 (MFI >5000) at month 1 and month 6, respectively (Table 3). All of the de novo $_{MSC}$DSA class II were linked to a shared HLA mismatch between the liver and MSC donors (Table 3 and Fig. 3B). Considering the 5 HLA class II shared mismatches, 3 (60%) led to de novo $_{liver+MSC}$DSA detection in the MSC group (Figs. 2 and 3B and Table 3).

Discussion

We report the long-term results of the first clinical phase 1, prospective, controlled study aiming to evaluate the feasibility, safety, and tolerability of MSC infusion in 10 LT recipients. The 1-year results showed that a posttransplantation intravenous infusion of 1.5 to 3 × 10^6/kg MSC was well tolerated without evidence of pulmonary dysfunction or cytokine-release syndrome and without short-term toxicity.[9] These short-term safety results were recently confirmed in a study evaluating the effect of third-party MSC injection before LT.[20] After a median follow-up of 85 months, this study did not detect any toxicity attributed to this MSC infusion, particularly no increased rate of infection or cancer in LT recipients who received MSCs. If the short-term safety of MSC infusion has already been proven by numerous clinical studies providing early data, the present study adds new important information on the absence of the long-term deleterious clinical adverse effects of MSC infusion in this particular patient population receiving immunosuppressive drugs after LT.

In this small series, the potential advantages of MSC infusion were evaluated as secondary endpoints. With regard to the different studied parameters, a single allo-MSC posttransplant infusion did not appear to provide a clear clinical benefit to LT recipients in the long-term: there was no detected difference in liver graft survival or function. In addition, the overall rate of biliary complications was not lower in the patients treated with MSCs compared with the control group. There was no detected difference in fibrosis or Banff scores in the performed graft biopsies, and no patient from either group suffered from acute graft rejection requiring treatment. In some trials by Chinese groups, MSCs have been proposed as possibly playing a role in the management of the acute rejection of liver grafts,[21] in the prevention of antibody-mediated rejection after ABO-incompatible LT,[22] and in the management of ischemic-type biliary lesions.[23] In this study of 20 LTs, 1 recipient who had received MSCs developed NAS that ultimately required retransplantation after failure of surgical management. Biliary ASs were also detected in both groups and were not less frequent in the MSC patients. These issues deserve to be specifically analyzed in further studies on larger cohorts of patients.

This study also confirmed the preliminary data from our group[8] and others[2] on the potential immunogenicity of MSCs, an issue that might be particularly important in the field of organ transplantation. In LT, the impact of DSAs is not yet clearly understood. So far, donor and recipient HLA matching is not routinely recommended in deceased donor LT

because of the tolerogenic properties of the liver contributing to its resistance to antibody-mediated injuries.[24] Nevertheless, recent articles have shown that the appearance of de novo DSAs could be linked to an increased risk of rejection and lower graft and patient survival rates.[25,26]

Considering that MSC immunomodulatory properties may decrease immune responses against liver HLAs and the formation of de novo $_{liver}$DSA, the comparison of the prevalence of $_{liver}$DSA in the MSC and control groups is of particular importance. The appearance of $_{MSC}$DSA is also relevant to determine if MSCs promote an MSC-directed immune response in the host. In vitro, MSCs classically do not express class II HLA nor costimulatory molecules such as CD40, B7-1, or B7-2, but do express low levels of class I HLA. Because of these characteristics, MSCs were initially considered as minimally immunogenic and thus "immune privileged." However, the upregulation of both major histocompatibility complex (MHC)–I and MHC-II antigens on MSCs after interferon γ exposure, in addition to preclinical evidence of an immune response against MSCs, have brought into question that notion of low immunogenicity.[27,28] These concerns are particularly relevant in the field of solid organ transplantation. Indeed, in the case of a shared mismatch between third-party MSC donor and graft donor, MSCs could theoretically promote an immune response leading to the production of additional DSAs with their inherent risks to the graft.

There is in fact limited data on sensitization by MSCs. In 2019, Avivar-Valderas et al. reported that of 63 patients treated with allogenic adipose-derived MSCs used for perineal fistulas of Crohn's disease, 23 developed class I $_{MSC}$DSA 12 weeks after injection, and none against class II HLA, with no consequences on efficacy.[29] In an article reporting the use of allogeneic BM-MSCs for rheumatological diseases in 2 clinicals trials, de novo $_{MSC}$DSA could be detected only in 2 of 23 treated patients during the 2-year follow-up.[30] An article showing greater efficacy in allogeneic (versus autologous) MSCs for the treatment of nonischemic dilated cardiomyopathy reported $_{MSC}$DSA in only 1 patient without clinical impact.[31] This was approximately the same incidence as previous studies employing MSCs for cardiac diseases.[32,33] In 2019, our team published the results of a phase I/II trial reporting the use of third-party MSCs after KT in which MSCs were randomly assigned to KT recipients without HLA matching with kidney recipients or donors. De novo $_{MSC}$DSA class I with MFI >1500 were detected in only 1 patient. A total of 2 more de novo $_{MSC}$DSA (classes I and II) and 1 shared de novo kidney/MSC DSA (class II) with MFI <1500 were also detected.[8] However, one may question the clinical relevance of these $_{MSC}$DSA data given their low MFI values and the stability of the graft function during follow-up.[8]

Considering that MSCs could be immunogenic and cause sensitization against the graft, another team recently published a study in which a matching strategy protocol to prevent repeated mismatches between MSCs and kidney donors in 10 KT patients was used.[34] In this study, selected third-party BM MSCs were injected 6 months after KT. No de novo MSCs nor kidney DSAs were detected during the 6-month follow-up after MSC infusion.

In the present study, because all of the class II $_{MSC}$DSA were also $_{liver}$DSA because of a shared mismatch, it is difficult to differentiate sensitization caused by the liver graft or by the MSC HLA class II antigen recognition by the host immune system. Nevertheless, the high prevalence (60%) of HLA antibody detection in cases of shared mismatches in class II loci in our study might suggest that this combination could promote immunogenicity. In other reported studies evaluating this issue, most of the detected $_{MSC}$DSA after MSC infusion were against class I HLA. Nevertheless, it seems that in cases of shared class II HLA mismatches between MSCs and the graft, class II DSA could also be promoted. These observations could potentially urge caution by avoiding repeated mismatches between third-party MSCs and graft donors at least for HLA class II or by using autologous MSCs, especially for KT, but this needs to be investigated further with larger cohorts. These observations may lead to reconsidering the risk of development of $_{MSC}$DSA as well as the necessity of a matching strategy. However, the absence of impact on long-term allograft outcomes in this study is in line with previous data in nontransplant settings regarding the clinical significance of $_{MSC}$DSA.[16]

There are many shortcomings to this study. First, it is clear that this first study in 10 LT recipients does not prove the long-term safety of MSC infusion after transplantation. These results will have to be confirmed by further studies in larger groups of liver recipients, focusing particularly on the potential immunogenicity of MSCs. The absence of detectable effects of MSCs

might be attributed to an insufficient sample size, to the immunosuppressive regimen, or to an insufficient MSC dosing, which should possibly be increased or repeated. The timing (preoperative, intraoperative, or postoperative) and the infusion routes (peripheral vein, portal vein, or hepatic artery) of MSC infusion should also be evaluated. Different MSC sources (BM, fat tissue, liver) or donors (organ donor, organ recipient) should also be tested in further studies.

In conclusion, this first prospective clinical trial investigating the safety of injecting allogeneic MSCs after deceased donor LT did not demonstrate potential adverse effects, particularly no increased rate of opportunistic complications. Injecting allogeneic MSCs after deceased donor LT may promote $_{liver}$DSA class II emergence in LT recipients. This subject deserves further investigation. The potential benefits of MSC injections in the context of organ transplantation have yet to be demonstrated.

Acknowledgments: The authors value and thank all of the members of the liver transplantation program of the University of Liege Hospital (physicians, nurses, coordinators, etc.), who contributed extensively to this study, and the LTCG.

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