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# Third-party bone marrow–derived mesenchymal stromal cell infusion before liver transplantation: A randomized controlled trial

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## Abstract

Mesenchymal stromal cells (MSC) have emerged as a promising therapy to minimize the immunosuppressive regimen or induce tolerance in solid organ transplantation. In this randomized open-label phase Ib/IIa clinical trial, 20 liver transplant patients were randomly allocated (1:1) to receive a single pretransplant intravenous infusion of third-party bone marrow–derived MSC or standard of care alone. The primary endpoint was the safety profile of MSC administration during the 1-year follow-up. In all, 19 patients completed the study, and none of those who received MSC experienced infusion-related complications. The incidence of serious and non-serious adverse events was similar in the two groups. Circulating Treg/memory Treg and tolerant NK subset of CD56$^{bright}$ NK cells increased slightly over baseline, albeit not to a statistically significant extent, in MSC-treated patients but not in the control group. Graft function and survival, as well as histologic parameters and intragraft expression of tolerance-associated transcripts in 1-year protocol biopsies were similar in the two

**Abbreviations:** BM, bone marrow; CMV, cytomegalovirus; HAMP, Hepcidin Antimicrobial Peptide; MMF, mycophenolate mofetil; MSC, mesenchymal stromal cells; PBMC, peripheral blood mononuclear cells; rATG, rabbit anti-thymocyte globulin; TAC, tacrolimus; TFRC, Transferrin Receptor-1/CD71; Treg, regulatory T cells.

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groups. In conclusion, pretransplant MSC infusion in liver transplant recipients was safe and induced mild positive changes in immunoregulatory T and NK cells in the peripheral blood. This study opens the way for a trial on possible tolerogenic efficacy of MSC in liver transplantation. ClinicalTrials.gov identifier: NCT02260375.

**KEYWORDS**
clinical research/practice, liver transplantation/hepatology, stem cells, tolerance

## 1 | INTRODUCTION

Liver transplantation is currently the only therapeutic option for patients with end-stage liver failure.[1] Liver transplant recipients rely on life-long immunosuppressive agents to prevent episodes of acute rejection and graft loss, but are also exposed to drug-related complications resulting from non-specific inhibition of the host immune system, which enhances the risk of life-threatening infections and malignancies.[2,3] Moreover, off-target drug effects, including nephrotoxicity,[4] cardiovascular disease, and metabolic disorders,[2,5] can severely impact the long-term outcomes of liver transplantation, leading to reduced graft and patient survival. Even though the array of currently available drugs has proven successful in the prevention and treatment of acute graft rejection, similar outcomes have not been achieved in preventing chronic allograft dysfunction and extending long-term graft survival.[6] The induction of donor-specific immune tolerance would overcome these shortcomings, potentially making it possible to wean patients off immunosuppressive drugs while promoting indefinite graft survival.[7]

The liver is a spontaneously pro-tolerogenic organ due to its intrinsic immunoregulatory properties, which enables the use of milder immunosuppressive regimens compared to heart and kidney transplantation.[8] Nonetheless, results from clinical studies indicate that spontaneous operational tolerance after immunosuppressive drug withdrawal has been achieved successfully in only approximately 20%–30% of liver transplant recipients.[7] Therefore, innovative strategies to induce long-lasting donor-specific immunologic tolerance are urgently needed even for liver graft recipients.

Mesenchymal stromal cells (MSC) have emerged as one of the most promising candidates among immunomodulatory cell-based therapies in solid organ transplantation.[9] MSC are multipotent, non-hematopoietic cells that can be isolated from different accessible tissues, including bone marrow (BM), umbilical cord, and adipose tissue.[10-12] This heterogeneous cell population displays potent immunoregulatory properties that derive from their interaction with cells from both the innate and adaptive immune systems,[13,14] triggering natural and self-sustaining mechanisms of immunoregulation.[15] As demonstrated in experimental models of heart, kidney, liver, and islet transplantation,[16-19] MSC control the host-versus-graft immune response and induce immune tolerance by down-regulating the activity of antigen presenting cells,[17,20,21] inhibiting cytotoxic/memory T cell activation,[16,22] promoting the generation of regulatory T cells,[16,18,19,21-23] as well as suppressing B-cell activation and natural killer cell-cytotoxicity.[13,15]

Several clinical studies in renal transplant recipients have provided encouraging results regarding the effects of MSC-based cell therapy on the modulation of subclinical rejection,[24] the minimization of concomitant immunosuppressive drug regimens,[25,26] and the induction of a milieu that favors the development of graft tolerance.[27-30] Less encouraging results, however, have been achieved with MSC infusion in liver transplantation[31]; though safe, MSC treatment failed to promote a pro-tolerogenic milieu and immunosuppression discontinuation was unsuccessful. The timing of MSC administration and/or the immunosuppressive regimen adopted could have contributed to hindering the development of a pro-tolerogenic environment.

We designed a prospective randomized controlled trial to assess the safety and mechanistic immunoregulatory effects of a single intravenous infusion of third-party ex-vivo expanded BM-derived MSC in deceased-donor liver transplant recipients under conditioning with low-dose rabbit anti-thymocyte globulin (rATG) and low-dose maintenance immunosuppression.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design

This randomized, controlled, open-label, parallel-group, phase Ib/IIa clinical trial had two distinct phases, a safety/mechanistic phase and a long-term efficacy phase; here we report the results of the former (12-month follow-up). The patients screened for eligibility were adult (≥18 years old) men and women with end-stage liver failure undergoing deceased-donor liver transplantation for the first time, who were recruited from two liver transplant centers in Italy, the Azienda Socio Sanitaria Territoriale (ASST) Papa Giovanni XXIII, Bergamo and the Azienda Ospedaliero-Universitaria-Policlinico S. Orsola-Malpighi, Bologna. We excluded recipients if they had any clinically relevant conditions that could affect study participation and/or results, such as a recent history of malignancy (except for hepatocarcinoma falling within the Milan criteria), specific contraindications to MSC infusion, or if they were pregnant or breastfeeding.

The Aldo e Cele Daccò Clinical Research Center for Rare Diseases of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS coordinated and monitored the study, which was conducted in compliance

with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol (available as Data S1) was approved by national regulatory authorities and by the local ethics committees. Written informed consent was obtained from all participants before enrolment. The study was registered with ClinicalTrials.gov (identifier NCT02260375) and the EU Clinical Trials Register (EudraCT 2014-001531-37).

## 2.2 | Procedures

Eligible patients were allocated 1:1 to receive ex-vivo expanded BM-derived third-party MSC or standard of care alone. Open-label treatment allocation was carried out through a randomization sequence stratified by center, generated with SAS (version 9.2) by an investigator not directly involved in the trial.

Liver transplantation was performed according to standard practices of the center. Participants allocated to the treatment arm received intravenous third-party BM-derived MSC as a single infusion (1–2 × $10^6$ cells/kg) after pre-medication with chlorphenamine, immediately before the surgical procedure. The chosen dose is consistent with published experience with MSC in solid organ transplantation, GVHD, and autoimmune diseases.[24-28,32-36] The timing (few hours before transplantation) has been established based on our previous findings that, to avoid impairing cell viability, MSC should not be infused during RATG induction therapy and that, compared to posttransplant infusion, pretransplant MSC administration avoided the risk of intragraft inflammatory events and acute graft dysfunction while promoting immunomodulation and Treg expanding potential.[16,22,27,28] Third-party BM-derived MSC from healthy donors were isolated, expanded ex-vivo and fully characterized according to Good Manufacturing Practice standards at the cell factory Laboratorio di Terapia Cellulare G. Lanzani, ASST-Papa Giovanni XXIII, as previously described.[37] Detailed procedures and the relative release criteria are available as Supplementary Information.

All patients, independent of allocation, received induction immunosuppressive therapy with low-dose rATG (0.5 mg/kg/day) from day 0 up to day 7 posttransplantation and intravenous methylprednisolone from days 0, 1, and 2 posttransplantation (500, 250, and 125 mg, respectively). Thereafter, oral prednisone was administered and progressively tapered until discontinuation after day 7 posttransplant, wherever deemed clinically feasible. Maintenance immunosuppression included tacrolimus (TAC, dose adjusted to maintain trough blood concentration of 10–15 ng/ml during the first postoperative month, and of 5–10 ng/ml up to 12 months posttransplant) and oral mycophenolate mofetil (MMF, 750 mg BID from the day of transplant).

All patients received standard antibacterial prophylaxis, including trimethoprim/sulfamethoxazole or inhaled pentamidine up to 6 months posttransplant. Intravenous ganciclovir was administered from day 4 to day 14 posttransplant in case of a serological mismatch for CMV antibodies between the donor and the recipient ($R^-$, $D^+$).

A detailed description of all sampling and measurements performed is available as Supplementary Information.

## 2.3 | Outcomes

The primary outcome of the study was the safety of MSC infusion in liver transplant recipients. Short-term safety was assessed by continuous monitoring of vital signs (blood pressure, heart rate, oxygen saturation) during the infusion and at standard time intervals during the following 12 h. All infusion-related adverse events, including hypotension, tachycardia, tachypnea/dyspnea, hypoxemia, fever, and clinical findings consistent with transfusion incompatibility or hypersensitivity reactions (e.g., skin rash or shock) were managed accordingly and reported in the electronic case report form. Patients were periodically monitored for the occurrence of any adverse events, including infections and malignancies, during the 1-year follow-up. All patient- and investigator-reported adverse events were recorded up to study end. Adverse events were summarized according to the organ system classification and preferred terms of the Medical Dictionary for Regulatory Activities (MedDRA version 18.0).

Secondary outcomes included the percentage and counts of peripheral blood T-, NK- and B-cell subsets, and serum hepcidin-25 levels at different time points compared to baseline, as well as 1-year liver tissue whole-genome transcriptomic profiling and mRNA levels of TFRC and HAMP. As exploratory outcomes, we also evaluated tissue mRNA levels of FoxP3, graft and patient survival, as well as liver graft function parameters at 12-month posttransplantation.

## 2.4 | Statistical analyses

Continuous and categorical variables were reported as mean ± SD, median [IQR], or *n* (%), as appropriate. All outcomes recorded during the 1-year observation period or until study dropout were assessed using the intention-to-treat principle. The primary safety outcome was assessed by comparing the frequency of serious and non-serious adverse events between groups with Fisher's exact test. Within-group differences in continuous variables over time were assessed using repeated measure ANOVA or Friedman's test, as appropriate; follow-up tests were performed in case of significance. Differences in continuous variables between the two groups were analyzed with ANCOVA to account for baseline values. Statistical significance was assumed at a 5% level of probability (two-tailed). All analyses were performed with GraphPad Prism version 8 (GraphPad Software Inc) and R version 3.6.1 (R Foundation for Statistical Computing).

# 3 | RESULTS

## 3.1 | Baseline characteristics

Between November 2014 and April 2018, a total of 50 patients were screened for eligibility, 20 of whom were randomized to receive either standard of care alone or with pretransplant MSC infusion (reasons for exclusion are listed in Figure 1). All recipients were Caucasian and 65% were males, with a median age of 60.2

(IQR: 54.5–66.1) years and an average BMI of 24.4 ± 4.1 kg/m$^2$. The majority of donors were males (55.0%) with a median age of 62.5 (IQR: 45.3–74.8) years. Baseline characteristics, the cold and warm ischemia time, clinical data as well as induction immunosuppressive drugs were well balanced between the two groups (Table 1). All patients allocated to MSC received the target cell dose (range: 0.90–1.67 × 10$^6$ cells/kg) at 0.5–3.0 h before the start of the liver transplant procedure. Overall, 19 (95.0%) patients completed the 1-year study follow-up.

## 3.2 | Safety

None of the patients who received MSC had infusion-related adverse events. During the first 2 days posttransplantation, an increase in serum C-reactive protein was found, similar in patients given MSC and in controls (Figure 2A). Moreover, serum concentration of the proinflammatory cytokines IL-1β, IL-6, and TNF-α did not significantly change in MSC-treated patients during the first month posttransplant compared to pretransplant levels or to control patients (Figure 2B–D), excluding early inflammatory events possibly attributable to cell infusion. During the 1-year follow-up, a total of 217 adverse events were reported, of which 21 were serious. Ten patients (50.0%) experienced at least one SAE, six of whom were from the MSC group and four from the control group. Both serious and non-serious adverse events were balanced between groups (Table 2).

Overall, 14 patients (70.0%) experienced at least one infectious episode during follow-up, eight of whom were from the MSC group and six from the control group. The high frequency of infections was expected in this high-risk population, and no significant difference could be detected between the two allocation groups (80.0% vs 60.0%, $p$ = .63). Similarly, we did not observe any significant difference between treatments in terms of the frequency of patients experiencing at least one infectious episode when serious events (MSC 40.0% – control 10.0%, $p$ = .30) and non-serious events (MSC 80.0% – control 50.0%, $p$ = .35) were considered separately. CMV infection or reactivation were observed in four patients allocated to MSC and in three controls; however, none of the patients developed CMV disease. None of the patients enrolled in the study developed cancer during the 1-year follow-up.

## 3.3 | Phenotype of circulating leukocyte subsets

CD4$^+$ T cell counts and percentages over CD45$^+$ cells (Figure 3A,B) decreased substantially at 1–2 weeks posttransplant in both groups. However, the reduction of CD4$^+$ T cell counts reached statistical significance only in the control group, whereas CD4$^+$ T cell percentages were reduced significantly only in the MSC group. CD4$^+$ T cell counts and percentages recovered partially at 6 and 12 months after transplantation but CD4$^+$ T cell percentages remained significantly lower than pretransplant values in the MSC group. At 12 months of follow-up, baseline-adjusted CD4$^+$ T cell percentages were significantly lower in the MSC-treated than in control patients ($p$ = .036, Figure 3B).

The frequency of regulatory T cells (Treg, CD4$^+$CD25$^{high}$Foxp3$^+$CD127$^-$, Figure 3C) and of memory Treg (CD4$^+$CD25$^{high}$Foxp3$^+$CD127$^-$CD45RA$^-$CD45RO$^+$, Figure 3D) tended to increase in MSC-treated patients at 1–2 weeks posttransplant compared to controls, even though these differences did not reach statistical significance. When adjusted for baseline values, the frequency of both Treg and memory Treg at

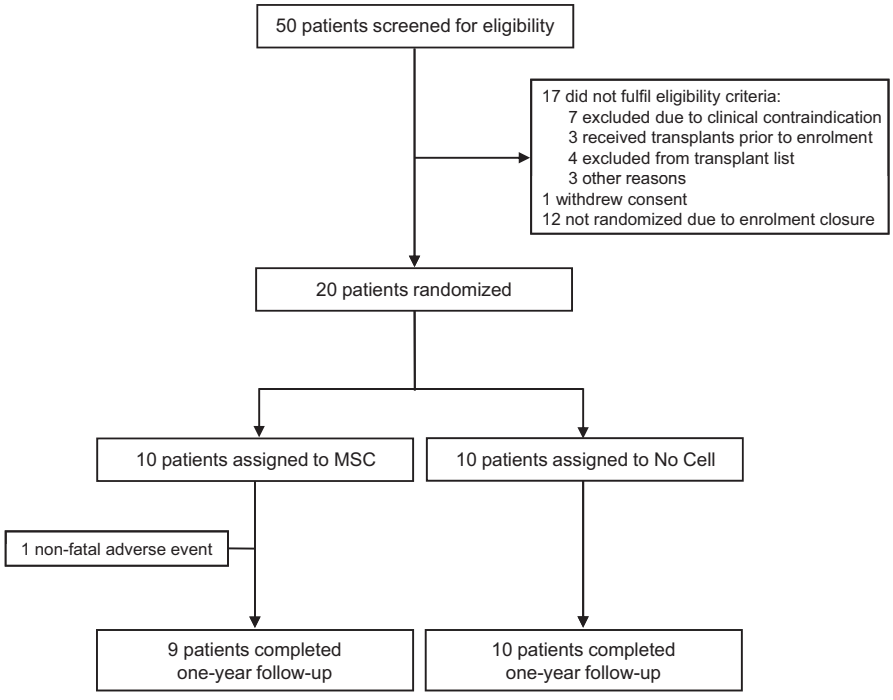


**FIGURE 1** CONSORT flow diagram

**TABLE 1** Baseline patient characteristics

| | MSC (*n* = 10) | Control (*n* = 10) | Overall (*n* = 20) |
|---|---|---|---|
| Recipient | | | |
| Sex (M, %) | 7 (70%) | 6 (60%) | 13 (65%) |
| Age (years) | 60.2 [57.8–65.9] | 60.5 [53.8–66.6] | 60.2 [54.5–66.1] |
| Weight (kg) | 72.0 ± 14.9 | 69.4 ± 15.6 | 70.7 ± 14.9 |
| BMI (kg/m$^2$) | 24.7 ± 4.6 | 24.0 ± 3.7 | 24.4 ± 4.1 |
| Liver disease (*n*, %) | | | |
| Alcoholic cirrhosis | 2 (20%) | 6 (60%) | 8 (40%) |
| Chronic hepatitis B | 2 (20%) | 1 (10%) | 3 (15%) |
| Chronic hepatitis C | 1 (10%) | 2 (20%) | 3 (15%) |
| Primary biliary cirrhosis | 1 (10%) | 0 (0%) | 1 (5%) |
| Other | 4 (40%) | 1 (10%) | 5 (25%) |
| Hepatocellular carcinoma (*n*, %) | 3 (30%) | 6 (60%) | 9 (45%) |
| Diabetes (*n*, %) | 5 (50%) | 6 (60%) | 11 (55%) |
| MELD Score[a] | 19 [11–27] | 17 [10–18] | 17 [10–22] |
| Viral serology (*n* pos, %) | | | |
| *HBsAg* | 2 (20%) | 1 (10%) | 3 (15%) |
| *HCVAb* | 1 (10%) | 2 (20%) | 3 (15%) |
| *HIVAb* | 0 (0%) | 0 (0%) | 0 (0%) |
| *Toxo IgG* | 7 (70%) | 6 (60%) | 13 (65%) |
| *EBV IgG* | 6 (60%) | 5 (50%) | 11 (55%) |
| Cold ischemia time (min) | 367 ± 90 | 350 ± 54 | 359 ± 73 |
| Warm ischemia time (min) | 49 [39–86] | 43 [34–47] | 44 [36–70] |
| Bilirubin Tot/Dir (mg/dl) | 4.4 ± 3.1 / 2.3 ± 2.0 | 2.3 ± 1.4 / 1.2 ± 0.9 | 3.3 ± 2.5 / 1.7 ± 1.6 |
| INR | 1.52 ± 0.42 | 1.39 ± 0.24 | 1.46 ± 0.34 |
| Albumin (g/dl) | 3.85 ± 0.35 | 3.68 ± 0.56 | 3.76 ± 0.46 |
| GOT (IU/L) | 42 [29–59] | 33 [28–45] | 37 [29–55] |
| GPT (IU/L) | 24 [21–58] | 23 [21–27] | 23 [21–32] |
| Alkaline phosphatase (IU/L) | 133 [99–197] | 145 [84–208] | 139 [98–199] |
| Gamma-GT (IU/L) | 56 [27–102] | 61 [31–102] | 58 [32–98] |
| Hemoglobin (g/dl) | 11.2 ± 1.9 | 11.6 ± 2.1 | 11.4 ± 2.0 |
| Platelets (10$^3$/μl) | 52 [36–166] | 67 [38–88] | 59 [37–101] |
| Creatinine (mg/dl) | 0.86 ± 0.29 | 0.82 ± 0.14 | 0.84 ± 0.22 |
| Cholesterol Tot/HDL (mg/dl) | 118 ± 55 / 38 ± 19 | 121 ± 44 / 39 ± 13 | 120 ± 48 / 38 ± 16 |
| Triglycerides (mg/dl) | 66 [51–149] | 57 [54–91] | 59 [54–122] |
| Donor | | | |
| Sex (M, %) | 5 (50%) | 6 (60%) | 11 (55%) |
| Age (years) | 64.4 ± 12.9 | 55.1 ± 21.2 | 59.8 ± 17.7 |
| Viral serology (*n* pos, %) | | | |
| *HBcAb* | 2 (20%) | 0 (0%) | 2 (10%) |
| *CMV IgG* | 7 (70%) | 6 (60%) | 13 (65%) |
| Induction therapy | | | |
| Total rATG dose (mg/kg) | 3.50 [2.52–3.79] | 3.50 [3.30–3.99] | 3.50 [3.29–3.85] |
| MSC dose (10$^6$ cells/kg) | 1.35 [0.90–1.67] | – | – |

*Note:* Baseline patient characteristics are reported as number (%), mean ± SD, and median [IQR], as appropriate.

Abbreviations: BMI, body mass index; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; MSC, mesenchymal stromal cells; rATG, rabbit anti-thymocyte globulins.

[a]Based on OPTN/UNOS modifications (https://optn.transplant.hrsa.gov/media/2371/liver_policynotice_20171221.pdf).

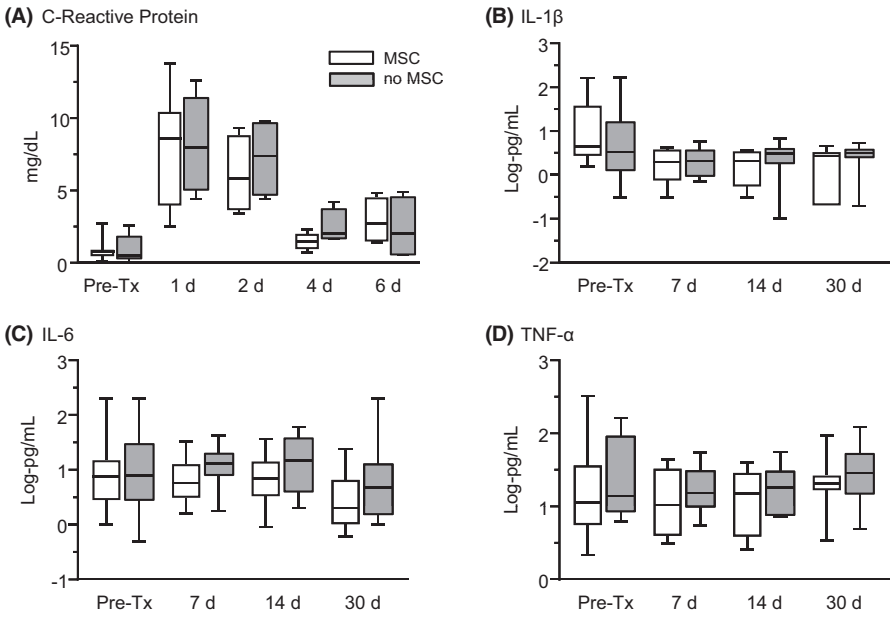


**FIGURE 2** Serum levels of C reactive protein and proinflammatory cytokines in patients allocated to MSC (white bar) and control (gray bar). Serum levels of (A) C-reactive protein during the first 6 days after transplant and serum levels of (B) IL-1β, (C) IL-6, and (D) TNF-α at pretransplant and 7, 14, and 30 days posttransplant. Plots display the median, 25th and 75th percentiles of distribution (boxes), and whiskers extend to the minimum and maximum values of the series

1–2 weeks posttransplant did not differ significantly between the two groups ($p$ = .196 and $p$ = .071, respectively). At 6 and 12 months posttransplant, the frequency of Treg and memory Treg was comparable between the two arms.

$CD8^+$ T cell counts did not undergo significant variations during follow-up in either group, whereas their percentage decreased at weeks 1–2 in both groups and then increased compared to pretransplant levels at 6 and 12 months posttransplant (Figure 4A,B). We also focused on $CD8^+$ T cell subsets and found that $CD8^+$ memory T cells ($CD45RA^-CD45RO^+$, Figure 4C) did not change appreciably during the follow-up in either of the two groups. A similar profile was observed for $CD8^+$ TEMRA ($CD45RA^+CD45RO^-CCR7^-$, Figure 4D). We observed an increasing trend in $CD8^+$ T cells with a naïve phenotype ($CD45RA^+CD45RO^-CCR7^+$, Figure 4E) early posttransplant in patients who received MSC. This cell type became the most abundant $CD8^+$ T cell subset at 1–2 weeks posttransplant in this patient group, but not in the control group. At 6 and 12 months of follow-up, naïve $CD8^+$ T cells decreased and percentages were similar between the two groups of patients.

In patients from whom donor and recipient peripheral blood viable cells were available at sufficient number (MSC-treated patients, $n$ = 3; controls, $n$ = 2), anti-donor cytotoxic activity of $CD8^+$ T cells was evaluated ex-vivo in a cell-mediated lympholysis (CML) assay. CML decreased to very low levels compared to baseline in both MSC-treated and control patients (Figure S1). Depletion of Tregs from posttransplant peripheral blood lymphocytes resulted in the increase in percentage donor cell lysis, more pronounced in MSC-treated patients than in the control (Figure S1).

No significant changes were observed in NK cell counts (Figure 5A) between the patient groups at any time point posttransplant. The frequency of cytotoxic ($CD3^-CD16^+CD56^{dim}CD11b^+CD27^-$, Figure 5B) NK cells over time decreased significantly at 1–2 weeks, 6- and 12-month posttransplant in the control group compared to baseline, whereas they did not change in MSC-treated patients, likely due to lower pretransplant values. A significant difference in cytotoxic NK cells between the two allocation arms was observed at 1–2 weeks posttransplant when pretransplant values were taken into account ($p$ = .023 by ANCOVA).

In MSC-treated patients and in the control group, the frequency of $CD56^{bright}$ NK cells ($CD56^{bright}CD16^{+/-}$ NK cells, Figure 5C) was similar, even though a non-significant trend toward an increase was noted in MSC-treated patients at 6- and 12-month follow-up, reflected by a similar trend of tolerant ($CD27^-CD56^{bright}CD16^{+/-}$, Figure 5D) but not regulatory ($CD27^+CD56^{bright}CD16^{+/-}$, Figure 5E) NK cell subtypes. There were no remarkable differences in percentages of $CD3^+CD56^+$ NKT cells during the follow-up, either within or between patient groups (Figure 5F).

B-cell counts in MSC-treated and control patients were similar before and after transplantation (Figure S2). No significant changes were observed during the follow-up between patient groups in the percentages of B-cell subpopulations defined based on CD38/IgD, CD27/IgD/IgM or CD38/CD24 phenotype classifications (Table S2).

### 3.4 | Graft survival, histology, and graft levels of tolerance-associated transcripts

One patient from the MSC group experienced primary graft dysfunction; liver biopsies were consistent with ischemia–reperfusion injury with biliary regression, in the absence of acute rejection. One month later, the patient received a second liver transplant, leading to permanent dropout from the study. All other participants completed the 12-month posttransplantation follow-up.

Liver graft function parameters showed a rapid trend toward normalization in the days after transplant (Table S3). Total bilirubin and INR were still normal or close to normality at 6 and 12 months in both groups. Serum albumin increased significantly over time in

**TABLE 2** Serious adverse events (SAEs) and non-serious adverse events (AEs) according to the MedDRA classification system

| | Events | | | Patients | | |
|---|---|---|---|---|---|---|
| **SAEs** | **Overall (*n* = 21)** | **MSC (*n* = 14)** | **Control (*n* = 7)** | **Overall (*n* = 20)** | **MSC (*n* = 10)** | **Control (*n* = 10)** |
| MedDRA System (SOC) | | | | | | |
| Gastrointestinal disorders | 2 (9.5%) | 2 (14.3%) | 0 (0.0%) | 2 (10.0%) | 2 (20.0%) | 0 (0.0%) |
| General disorders and administration site conditions | 1 (4.7%) | 1 (7.1%) | 0 (0.0%) | 1 (5.0%) | 1 (10.0%) | 0 (0.0%) |
| Hepatobiliary disorders | 4 (19.1%) | 1 (7.1%) | 3 (42.9%) | 3 (15.0%) | 1 (10.0%) | 2 (20.0%) |
| Infections and infestations | 6 (28.6%) | 5 (35.7%) | 1 (14.3%) | 5 (25.0%) | 4 (40.0%) | 1 (10.0%) |
| Injury, poisoning and procedural complications | 5 (23.8%) | 3 (21.4%) | 2 (28.6%) | 4 (20.0%) | 2 (20.0%) | 2 (20.0%) |
| Respiratory, thoracic and mediastinal disorders | 2 (9.5%) | 2 (14.3%) | 0 (0.0%) | 1 (5.0%) | 1 (10.0%) | 0 (0.0%) |
| Vascular disorders | 1 (4.7%) | 0 (0.0%) | 1 (14.3%) | 1 (5.0%) | 0 (0.0%) | 1 (10.0%) |
| | **Events** | | | **Patients** | | |
| **AEs** | **Overall (*n* = 196)** | **MSC (*n* = 104)** | **Control (*n* = 92)** | **Overall (*n* = 20)** | **MSC (*n* = 10)** | **Control (*n* = 10)** |
| MedDRA System (SOC) | | | | | | |
| Blood and lymphatic system disorders | 29 (14.8%) | 18 (17.3%) | 11 (12.0%) | 15 (75.0%) | 8 (80.0%) | 7 (70.0%) |
| Cardiac disorders | 2 (1.0%) | 0 (0.0%) | 2 (2.2%) | 2 (10.0%) | 0 (0.0%) | 2 (20.0%) |
| Endocrine disorders | 1 (0.5%) | 0 (0.0%) | 1 (1.1%) | 1 (5.0%) | 1 (10.0%) | 1 (10.0%) |
| Gastrointestinal disorders | 13 (6.6%) | 8 (7.7%) | 5 (5.4%) | 11 (55.0%) | 6 (60.0%) | 5 (50.0%) |
| General disorders and administration site conditions | 9 (4.6%) | 4 (3.8%) | 5 (5.4%) | 8 (4.0%) | 3 (30.0%) | 5 (50.0%) |
| Hepatobiliary disorders | 8 (4.1%) | 4 (3.8%) | 4 (4.3%) | 4 (20.0%) | 2 (20.0%) | 2 (20.0%) |
| Infections and infestations | 25 (12.8%) | 16 (15.4%) | 9 (9.8%) | 13 (65.0%) | 8 (80.0%) | 5 (50.0%) |
| Injury, poisoning and procedural complications | 21 (10.7%) | 13 (12.5%) | 8 (8.7%) | 13 (65.0%) | 8 (80.0%) | 5 (50.0%) |
| Investigations | 4 (2.0%) | 3 (2.9%) | 1 (1.1%) | 4 (20.0%) | 3 (30.0%) | 1 (10.0%) |
| Metabolism and nutrition disorders | 26 (13.3%) | 12 (11.5%) | 14 (15.2%) | 16 (80.0%) | 8 (80.0%) | 8 (80.0%) |
| Musculoskeletal and connective tissue disorders | 3 (1.5%) | 2 (1.9%) | 1 (1.1%) | 3 (15.0%) | 2 (20.0%) | 1 (10.0%) |
| Neoplasms benign, malignant and unspecified | 1 (0.5%) | 0 (0.0%) | 1 (1.1%) | 1 (5.0%) | 0 (0.0%) | 1 (10.0%) |
| Nervous system disorders | 11 (5.6%) | 4 (3.8%) | 7 (7.6%) | 9 (45.0%) | 4 (40.0%) | 5 (50.0%) |
| Psychiatric disorders | 6 (3.1%) | 2 (1.9%) | 4 (4.3%) | 6 (30.0%) | 2 (20.0%) | 4 (40.0%) |
| Renal and urinary disorders | 11 (5.6%) | 5 (4.8%) | 6 (6.5%) | 9 (45.0%) | 5 (50.0%) | 4 (40.0%) |
| Respiratory, thoracic and mediastinal disorders | 9 (4.6%) | 4 (3.8%) | 5 (5.4%) | 7 (35.0%) | 3 (30.0%) | 4 (40.0%) |
| Skin and subcutaneous tissue disorders | 7 (3.6%) | 5 (4.8%) | 2 (2.2%) | 6 (30.0%) | 4 (40.0%) | 2 (20.0%) |
| Vascular disorders | 10 (5.1%) | 4 (3.8%) | 6 (6.5%) | 10 (50.0%) | 4 (40.0%) | 6 (60.0%) |

*Note:* The number (%) of events over the total number of events and the number (%) of patients experiencing at least one event over the total number of patients are listed for each category.

the control group and exhibited a similar trend in the MSC group. No significant differences in these variables (Figure 6) and in liver enzymes (Table S3) were detected between the two groups at any time point. Similarly, tacrolimus trough levels did not differ significantly between the two groups during follow-up (Figure S3). Serum hepcidin-25 levels increased in both groups after transplantation, but no significant difference was observed between patient groups (Figure S4).

Eight patients in the MSC group and nine patients in the control group underwent 12-month protocol biopsies. Protocol biopsies were not performed in three patients due to study dropout ($n = 1$) and thrombocytopenia ($n = 2$). Graft biopsies revealed no sign of acute rejection or minimal changes according to the Banff criteria (MSC: one patient with RAI = 1; Control: two patients with RAI = 1 and one patient with RAI = 2) and a comparable degree of portal fibrosis between the two groups (Table 3). At genome-wide differential gene expression analysis, variation within samples, between sequencing replicates, was low with correlation coefficients ranging from 0.999 to 1. For this reason, sequencing replicates were collapsed together to obtain a higher depth. Principal component analysis and hierarchical clustering (Figure 7A,B) did not show clusters corresponding to the treatment groups. Differentially expressed gene analysis resulted in eight genes being significantly differentially expressed between treatment and control at an adjusted $p$ value of .05. These

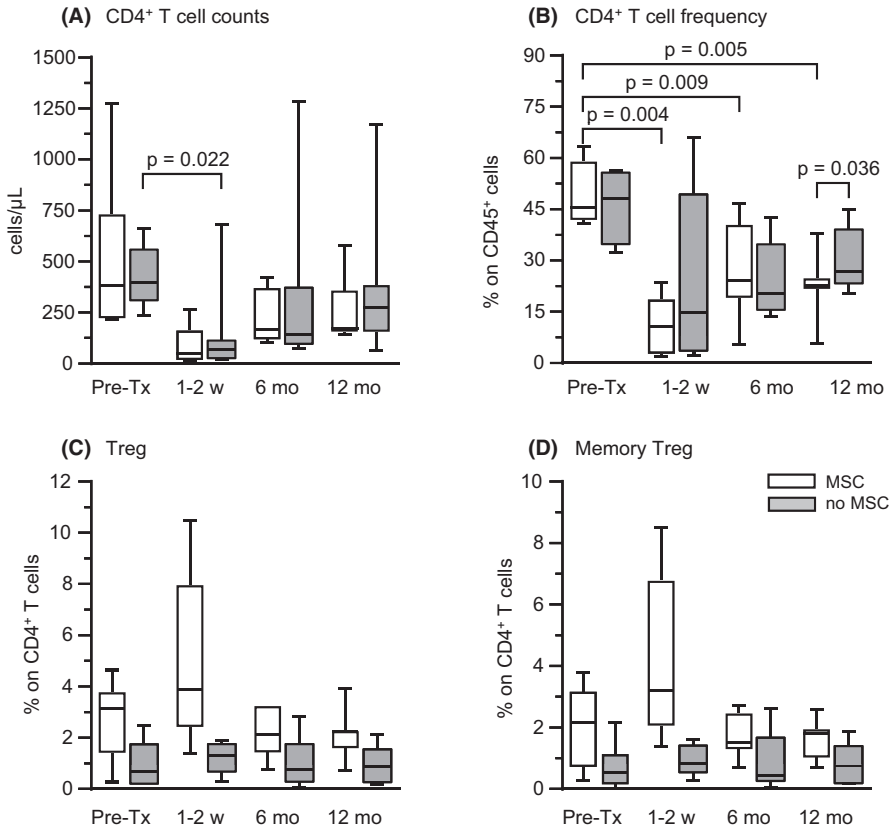


**FIGURE 3** Immunophenotype of peripheral $CD4^+$ T cell subsets over time in patients allocated to MSC (white bar) and control (gray bar). Counts (A) and frequency (B) of total $CD4^+$ T cells; frequency of total [$CD4^+CD25^{high}Foxp3^+CD127^-$] (C) and memory [$CD4^+CD25^{high}Foxp3^+CD127^-CD45RA^-CD45RO^+$] (D) regulatory T cells (Treg) among $CD4^+$ T cells. Plots display the median, 25th and 75th percentiles of distribution (boxes), and whiskers extend to the minimum and maximum values of the series

genes are Aldo-reductase Family 1 Member B10 (AKR1B10), Aldo-reductase Family 1 Member C2 (AKR1C2), Ficolin 2 (FCN2), Flavin Containing Dimethylaniline Monoxygenase 2 (FMO2), Solute Carrier Family 22, Member 10 (SLC22A10), Solute Carrier Family 3, Member 1 (SLC3A1), Wnt Family Member 5B (WNT5B), and Guanylate Cyclase 1 Soluble Subunit Beta 2 (GUCY1B2), most of which are protective genes against oxidative stress (Table S4). The liver tissue expression of the transcription factor FoxP3, as well as that of HAMP and TFRC—iron homeostasis regulators found to be selectively increased in operationally tolerant liver transplant recipients[38]—evaluated by quantitative real-time PCR, was comparable between the two groups at 12-month posttransplant (Figure 7C).

Additional biopsies were performed in all patients with any unexplained rise in liver cytolysis and/or cholestasis markers. Two of them had minimal histologic changes according to the Banff classification (RAI = 2), but the cause of cholestasis was ultimately found to be either mechanical or infectious. Accordingly, the immunosuppressive regimen was not modified significantly.

## 4 | DISCUSSION

In this open-label prospective randomized controlled trial in liver transplant recipients conditioned with low-dose rATG, we showed that a single pretransplant intravenous infusion of third-party ex-vivo-expanded bone marrow–derived MSC is safe and well tolerated.

Our study confirms and extends the preliminary results from a recent non-randomized clinical trial on the effects of intravenous bone marrow–derived MSC infusion in liver transplant recipients.[31] Although systemic MSC administration could potentially lead to acute infusional reactions, the available experience from trials has largely curtailed their clinical relevance.[25,26,29,32,39,40] Similarly, MSC embolization in the pulmonary circulation has been reported in animal models, but this process seems to have little impact on respiratory function in humans.[40] Owing to their immunoregulatory properties, MSC may theoretically increase the risk of infection and malignancy, especially in immunosuppressed patients. A single, early, small study in renal transplant recipients reported a 50% incidence of infections in six patients given MSC a few weeks posttransplantation,[24] but subsequent trials allayed this concern.[26,29,32,39] Similarly, malignant transformation or facilitation of tumor development was never reported in clinical trials with MSC.[41] In our study, none of the patients developed infusional reactions, proinflammatory cytokine release, or respiratory complications due to MSC infusion, and the incidence of infections was similar in the two allocation groups, with most events being non-serious in nature; also, none of the patients developed malignancies during the 1-year follow-up, confirming the short-term safety of this cell therapy in liver transplant recipients.

MSC display unique, potent, and undisputed immunoregulatory properties,[42] which include the ability to drive T cell differentiation toward natural immunosuppressive Tregs[14,43] and to engage recipient monocyte/phagocytes by releasing

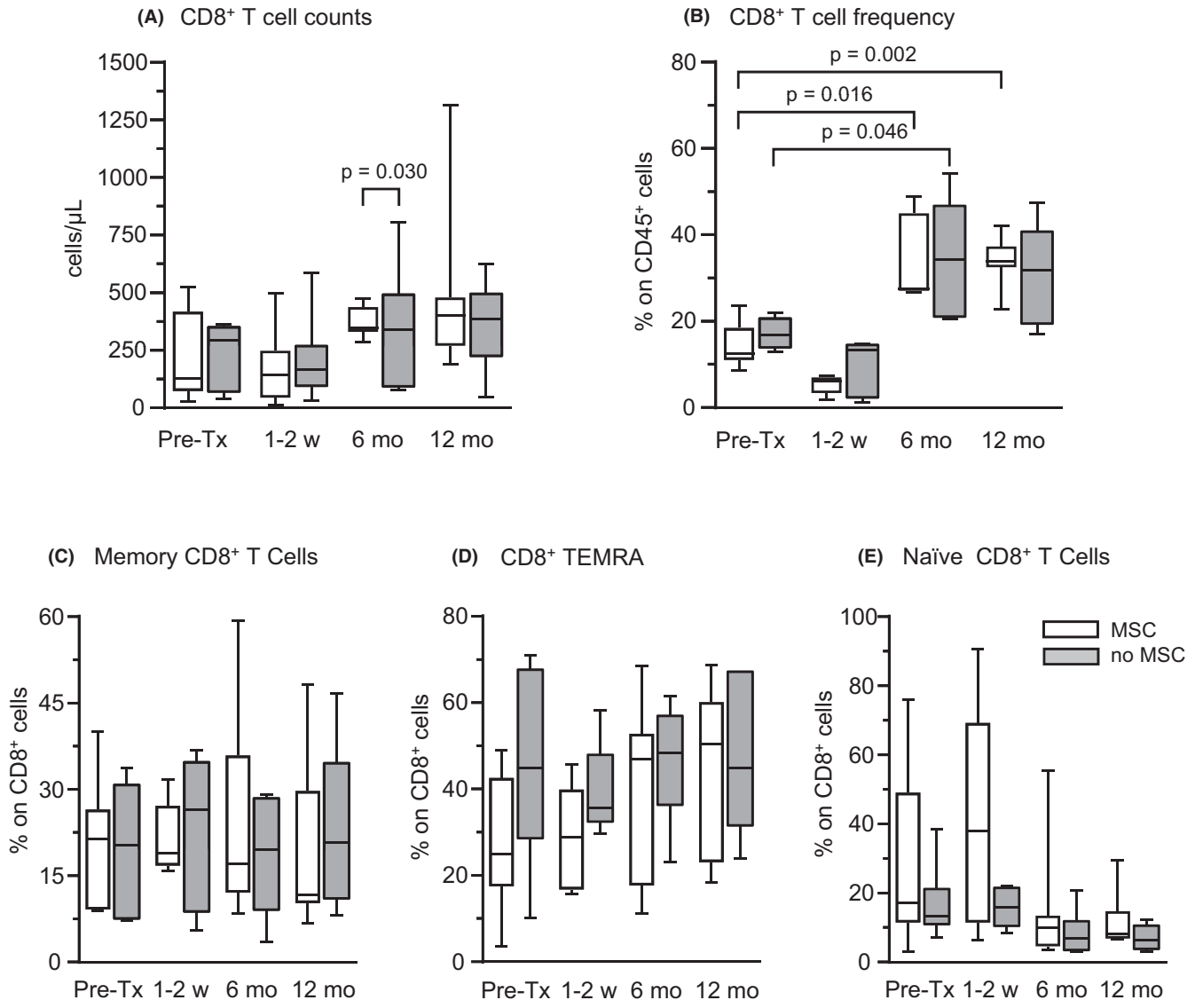


**FIGURE 4** Immunophenotype of peripheral $CD8^+$ T cell subsets over time in patients allocated to MSC (white bar) and control (gray bar). Counts (A) and frequency (B) of total $CD8^+$ T cells; frequency of memory [$CD45RA^-CD45RO^+$] (C), TEMRA [$CD45RA^+CD45RO^-CCR7^-$], (D) and naïve [$CD45RA^+CD45RO^-CCR7^+$] (E) among $CD8^+$ T cells. Plots display the median, 25th and 75th percentiles of distribution (boxes), and whiskers extend to the minimum and maximum values of the series

extracellular vesicles[44] or by modulating apoptosis,[45] conferring on these cells long-term immunosuppressive activity, eventually also through the induction of a Treg phenotype.[46,47] Preclinical studies in experimental models of organ transplantation have documented that autologous and allogeneic MSC have a comparable ability to induce Treg expansion and prolong graft survival,[18,23,48] suggesting that allogeneic MSC are as effective as autologous cells in promoting immune-regulation possibly because of their low immunogenicity, which would limit host immune reaction against cell alloantigens.[49] Similarly to what we[28,29] and others[50] have documented in living-related donor kidney transplant recipients given autologous BM-MSC, here we found in liver transplant recipients a trend toward an increase in the percentage of Tregs in the peripheral blood in the first 2 weeks after third-party allogeneic MSC infusion. This effect on Treg was not due to the concomitant induction and maintenance immunosuppressive drugs, since it was not observed in the control group of liver transplant recipients who did not receive MSC but were treated with the same pharmacologic immunosuppressive regimen. Notably, the tendency toward an increase in the Treg percentage in MSC-treated recipients occurred despite the marked reduction in the total $CD4^+$ T cell counts, due to the peripheral blood T cell depletion caused by peritransplant induction therapy with thymoglobulin. These findings suggest that in the early phase of homeostatic cell proliferation that follows thymoglobulin-induced T cell lymphopenia, MSC promoted Treg production or skewed emerging T cells toward a Treg phenotype. Both MSC-treated and control patients displayed anti-donor $CD8^+$ T cell hypo-responsiveness ex-vivo during the posttransplant period. With the limitation of the small sample size, in MSC-treated patients, the hyporesponsiveness was largely

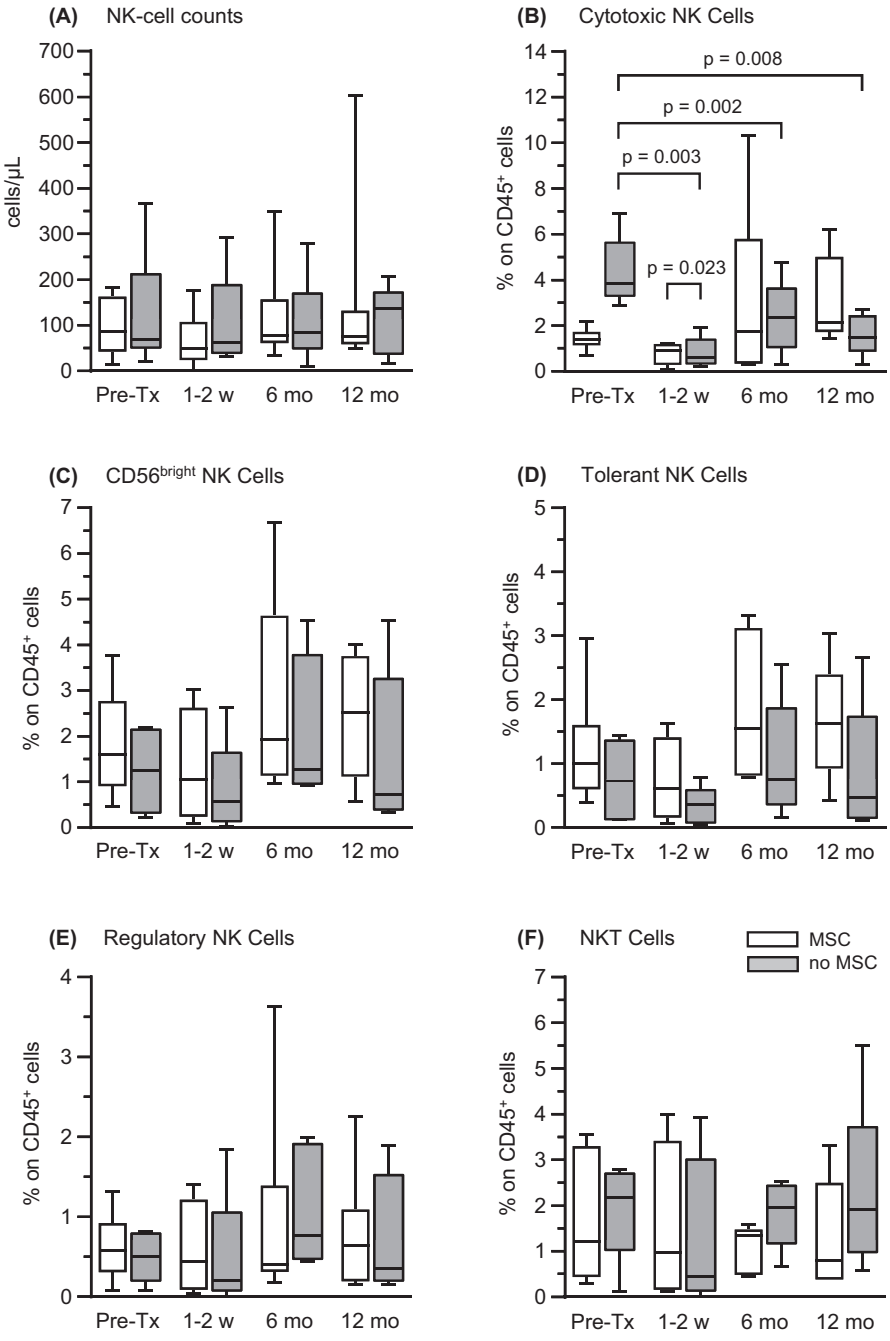


**FIGURE 5** Immunophenotype of peripheral NK cell subsets over time in patients allocated to MSC (white bar) and control (gray bar). Profile of NK cell counts (A), percentages of cytotoxic $CD3^{-}CD16^{+/-}CD56^{dim}CD11b^{+}CD27^{-}$ cytotoxic NK cells (B), of $CD3^{-}CD56^{bright}CD16^{+/-}$ NK cells (C) and of their subtypes: tolerant NK cells [$CD3^{-}CD56^{bright}CD16^{+/-}CD27^{-}$] (D), regulatory NK cells [$CD3^{-}CD56^{bright}CD16^{+/-}$ $CD27^{+}$] (E), and NKT cells [$CD3^{+}CD56^{+}$] (F) among $CD45^{+}$ cells. Plots display the median, 25th and 75th percentiles of distribution (boxes), and whiskers extend to the minimum and maximum values of the series

driven by Tregs, as documented by higher anti-donor $CD8^{+}$ T cell–mediated lysis in this group than in a control patient after in-vitro Treg depletion. These findings would possibly anticipate the development of a pro-tolerogenic environment in MSC transplant recipients and eventually immune tolerance in the future. Contrary to our results, others have shown that in the same setting of liver transplantation, third-party BM-derived MSC infusion did not influence Treg number, proliferation, or phenotype.[31] Both studies adopted a similar maintenance immunosuppressive regimen with tacrolimus, MMF, and steroids. Thus, this different outcome in Treg generation following MSC infusion could be attributed to the lack of induction therapy with the T cell depleting thymoglobulin in the latter, which precluded the activation of T cell homeostatic proliferation, a key process in the MSC-promoting Treg phenotype. Furthermore, there is a possibility that Detry et al[31] failed to modify the recipient immune status with MSC, since these cells were infused a few days post–liver transplantation and were probably mainly recruited into the organ that had recently been exposed to ischemia/reperfusion injury, instead of homing to lymphoid tissues, where they may promote immunomodulation. Indeed, experimental evidence has shown MSC homing to the site of tissue damage in models of stroke,[51] tumors,[52] myocardial ischemia,[53] acute renal failure,[54] and liver ischemia/reperfusion injury.[55] Moreover, in a mouse model of kidney transplantation, BM-MSC, given intravenously 2 days after surgery, mainly homed to the kidney graft and failed to induce Tregs.[16] On the contrary,

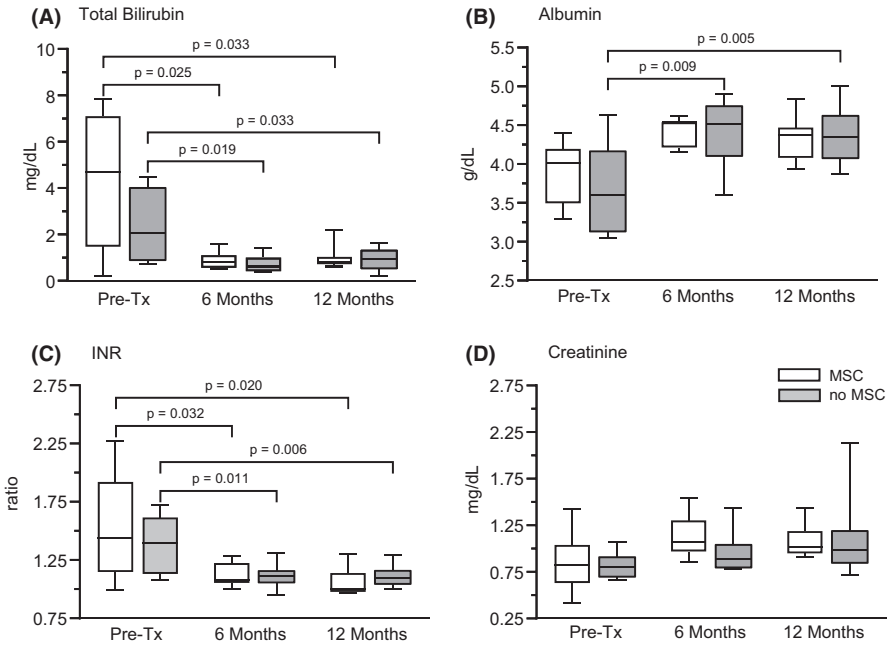


**FIGURE 6** Liver and renal function parameters during the follow-up in patients allocated to MSC (white bar) and control (gray bar). Total bilirubin (A), albumin (B), INR (C), and creatinine (D) in patients at baseline and at 6 and 12 months after transplant. Plots display the median, 25th and 75th percentiles of distribution (boxes), and whiskers extend to the minimum and maximum values of the series

**TABLE 3** Banff scores on 1-year protocol liver allograft biopsies

| | Portal inflammation | Bile duct inflammation/ damage | Venous endothelial inflammation | Rejection activity index (RAI) | Portal fibrosis | Central fibrosis |
|---|---|---|---|---|---|---|
| MSC | | | | | | |
| 001-01 | 0 | 0 | 0 | 0 | 1 | 0 |
| 002-01 | 0 | 0 | 0 | 0 | 0 | 0 |
| 003-01 | 0 | 0 | 0 | 0 | – | 0 |
| 006-01 | 0 | 0 | 0 | 0 | 1 | – |
| 008-01 | 0 | 0 | 0 | 0 | 1 | 0 |
| 004-02 | 0 | 0 | 0 | 0 | 0 | 0 |
| 006-02 | 0 | 0 | 0 | 0 | 1 | 0 |
| 007-02 | 1 | 0 | 0 | 1 | 1 | 0 |
| Controls | | | | | | |
| 009-01 | 0 | 0 | 0 | 0 | 0 | 0 |
| 010-01 | 0 | 0 | 0 | 0 | 2 | 0 |
| 005-01 | – | – | – | – | 2 | – |
| 007-01 | 1 | 1 | 0 | 2 | 0 | 0 |
| 004-01 | 0 | 0 | 0 | 0 | 2 | 1 |
| 001-02 | 0 | 0 | 0 | 0 | 1 | 1 |
| 005-02 | 0 | 1 | 0 | 1 | 1 | 0 |
| 008-02 | 0 | 0 | 0 | 0 | 0 | 0 |
| 011-02 | 1 | 0 | 0 | 1 | 0 | 0 |

when mice were given MSC the day before or the day of kidney transplantation, these cells mainly localized in the spleen and promoted the generation of Tregs,[18,22] a result consistent with our present finding in liver transplant patients given MSC up to 3 h before starting the surgical procedure.

We also analyzed the impact of MSC on the phenotype of circulating NK cells, a group of innate lymphocytes capable of targeting cell recognition and lysis without prior antigen priming.[56] While NK cells have classically been considered proinflammatory, contributing to graft rejection through proinflammatory cytokine release such as IFNγ, there is an increasing evidence that NK cell populations have an important immunoregulatory function.[57] This property has been attributed mainly to the CD56$^{bright}$ NK cell phenotype, the main producers of regulatory cytokines.[56] We found,

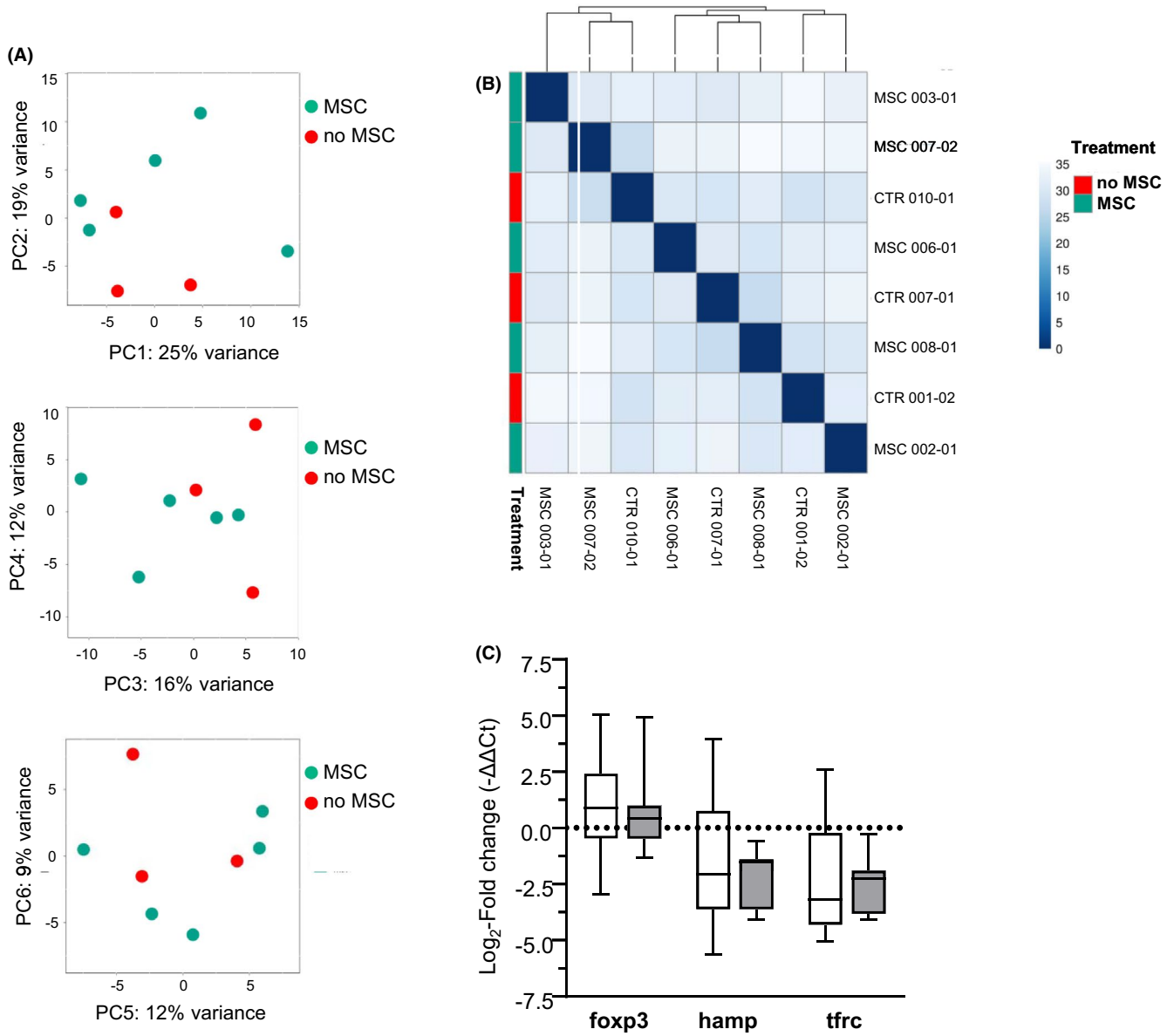


FIGURE 7 Transcriptomic profiling and expression of tolerance-related transcripts in liver tissue from protocol biopsies obtained at 12 months. Principal component analysis score plots of PC1 and PC2, PC3 and PC4, PC5 and PC6 (A), hierarchical clustering (B) and relative quantification ($Log_2$-Fold Change) of FoxP3, HAMP, and TFRC transcripts (C) in patients allocated to MSC (white bar) and control (gray bar) based on calibrator values. Plots in panel C display the median, 25th and 75th percentiles of distribution (boxes), and whiskers extend to the minimum and maximum values of the series

for the first time, that in liver transplant patients given MSC, the percentage of circulating $CD56^{bright}$ NK cells in total $CD45^{+}$ cells increased slightly compared to pretransplant/pre-infusion values, as well as to control liver transplant recipients at 6- and 12-month follow-up. This effect can be attributed to the MSC-induced increase in the tolerant $CD56^{bright}$ NK cell subset, the main liver-derived NK cell subpopulation.[58]

Together, our findings suggest that in the liver transplantation setting, MSC promote an immune-regulated environment, acting on multiple cell targets with potential regulatory properties in the host immune response. Nonetheless, the intragraft mRNA expression of the HAMP and TFRC genes, proposed as possible biomarkers of operational tolerance to liver transplantation,[38] was comparable in MSC-treated and control patients in a protocol biopsy performed 12-month posttransplant. We cannot rule out possible changes in the intragraft expression of these genes, specifically in the MSC-treated groups at time points later than 12 months, when the pro-tolerogenic milieu in the graft, if any, would be expected to be fully established and sustained.

We acknowledge that this preliminary safety work with BM-MSC in a small number of liver transplant recipients has many limitations. According to the approved study protocol, the primary aim of MSC safety was assessed only up to 1-year post–cell infusion and organ transplantation, precluding the collection of

long-term information, particularly on the risk of malignancies. However, a large amount of literature in the past two decades has reassuringly shown and confirmed the exceptional long-term safety of MSC-based therapy, even in immunosuppressed patients undergoing hematopoietic cell or solid organ transplantation.[29,40,41] Nonetheless, our liver transplant patients who received MSC are on active follow-up to monitor, beyond the first 12-month post-infusion, long-term adverse events that would possibly be attributable to cell therapy.

There is also in-vitro evidence that tacrolimus might decrease MSC immunosuppressive properties[59,60] and, conversely, that MSC might lower the immunosuppressive capacities of tacrolimus, exposing the patients to an increased risk of graft rejection. Although the triple therapy maintenance immunosuppressive regimen adopted in our two liver transplant centers included tacrolimus, no acute graft rejection occurred in the MSC-treated and control groups during the 12-month follow-up, indicating that in our setting, in-vivo, the drug and the infused cells did not affect each other's reciprocal immunosuppressive effects. In addition, the single MSC infusion did not affect tacrolimus pharmacokinetics, since blood tacrolimus trough levels were similar in the two study groups during the first month post–liver transplantation. Given the small sample size, it would be important to confirm these findings in future studies with larger cohorts.

The mild favorable effect of MSC that we observed on peripheral blood Treg expansion in cell-treated patients does not rule out the possibility that MSC have an important immunomodulatory effect in liver transplantation. It might just reflect heterogeneity in the level of MSC immunomodulatory function, since the number of cells infused was similar in all transplant patients. Indeed, it has been documented that MSC preparations display substantial donor-to-donor variability in their capability to dampen the alloimmune response in-vitro, despite common immunophenotype and tri-lineage differentiation potential.[61]

In conclusion, this prospective controlled trial confirmed, for the first time in a randomized design, the safety of third-party bone marrow-derived MSC in liver transplant patients receiving both induction and maintenance tacrolimus-based immunosuppression. These findings and the effect, albeit mild, of pretransplant MSC infusion on the regulatory cell profile will allow us to move to the second phase of the project with a pilot efficacy study to achieve operational tolerance after complete withdrawal of maintenance immunosuppressive therapy.

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## DISCLOSURE

The authors of this study have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

## DATA AVAILABILITY STATEMENT

Study data may be made available to interested researchers upon reasonable request.

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## REFERENCES

1. Adams DH, Sanchez-Fueyo A, Samuel D. From immunosuppression to tolerance. *J Hepatol*. 2015;62(1 Suppl):S170-185.
2. Watt KDS, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. *Am J Transplant*. 2010;10(6):1420-1427.
3. Watt KDS, Pedersen RA, Kremers WK, Heimbach JK, Sanchez W, Gores GJ. Long-term probability of and mortality from de novo malignancy after liver transplantation. *Gastroenterology*. 2009;137(6):2010-2017.
4. Levitsky J, O'Leary JG, Asrani S, et al. Protecting the kidney in liver transplant recipients: practice-based recommendations from the American Society of Transplantation Liver and Intestine Community of Practice. *Am J Transplant*. 2016;16(9):2532-2544.
5. VanWagner LB, Lapin B, Levitsky J, et al. High early cardiovascular mortality after liver transplantation. *Liver Transpl*. 2014;20(11):1306-1316.
6. Lodhi SA, Lamb KE, Meier-Kriesche HU. Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success. *Am J Transplant*. 2011;11(6):1226-1235.
7. Levitsky J, Feng S. Tolerance in clinical liver transplantation. *Hum Immunol*. 2018;79(5):283-287.
8. Sánchez-Fueyo A, Strom TB. Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. *Gastroenterology*. 2011;140(1):51-64.
9. Casiraghi F, Perico N, Remuzzi G. Mesenchymal stromal cells for tolerance induction in organ transplantation. *Hum Immunol*. 2018;79(5):304-313.
10. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-317.
11. Majore I, Moretti P, Stahl F, Hass R, Kasper C. Growth and differentiation properties of mesenchymal stromal cell populations derived from whole human umbilical cord. *Stem Cell Rev Rep*. 2011;7(1):17-31.
12. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13(12):4279-4295.
13. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. *Nat Rev Immunol*. 2012;12(5):383-396.
14. Casiraghi F, Perico N, Cortinovis M, Remuzzi G. Mesenchymal stromal cells in renal transplantation: opportunities and challenges. *Nat Rev Nephrol*. 2016;12(4):241-253.
15. Podestà MA, Remuzzi G, Casiraghi F. Mesenchymal stromal cells for transplant tolerance. *Front Immunol*. 2019;10:1287.
16. Casiraghi F, Azzollini N, Todeschini M, et al. Localization of mesenchymal stromal cells dictates their immune or proinflammatory effects in kidney transplantation. *Am J Transplant*. 2012;12(9):2373-2383.

17. Ge W, Jiang J, Baroja ML, et al. Infusion of mesenchymal stem cells and rapamycin synergize to attenuate alloimmune responses and promote cardiac allograft tolerance. *Am J Transplant*. 2009;9(8):1760-1772.
18. Casiraghi F, Azzollini N, Cassis P, et al. Pretransplant infusion of mesenchymal stem cells prolongs the survival of a semiallogeneic heart transplant through the generation of regulatory T cells. *J Immunol*. 2008;181(6):3933-3946.
19. Ge W, Jiang J, Arp J, Liu W, Garcia B, Wang H. Regulatory T cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. *Transplantation*. 2010;90(12):1312-1320.
20. Ko JH, Lee HJ, Jeong HJ, et al. Mesenchymal stem/stromal cells precondition lung monocytes/macrophages to produce tolerance against allo- and autoimmunity in the eye. *Proc Natl Acad Sci USA*. 2016;113(1):158-163.
21. Lohan P, Murphy N, Treacy O, et al. Third-party allogeneic mesenchymal stromal cells prevent rejection in a pre-sensitized high-risk model of corneal transplantation. *Front Immunol*. 2018;9:2666.
22. Casiraghi F, Todeschini M, Azzollini N, et al. Effect of timing and complement receptor antagonism on intragraft recruitment and protolerogenic effects of mesenchymal stromal cells in murine kidney transplantation. *Transplantation*. 2019;103(6):1121-1130.
23. Wang Y, Zhang A, Ye Z, Xie H, Zheng S. Bone marrow-derived mesenchymal stem cells inhibit acute rejection of rat liver allografts in association with regulatory T cell expansion. *Transplant Proc*. 2009;41(10):4352-4356.
24. Reinders MEJ, de Fijter JW, Roelofs H, et al. Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study. *Stem Cells Transl Med*. 2013;2(2):107-111.
25. Pan G-H, Chen Z, Xu LU, et al. Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: a prospective, non-randomized study. *Oncotarget*. 2016;7(11):12089-12101.
26. Tan J, Wu W, Xu X, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA*. 2012;307(11):1169-1177.
27. Perico N, Casiraghi F, Introna M, et al. Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility. *Clin J Am Soc Nephrol*. 2011;6(2):412-422.
28. Perico N, Casiraghi F, Gotti E, et al. Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation. *Transpl Int*. 2013;26(9):867-878.
29. Perico N, Casiraghi F, Todeschini M, et al. Long-term clinical and immunological profile of kidney transplant patients given mesenchymal stromal cell immunotherapy. *Front Immunol*. 2018;9:1359.
30. Casiraghi F, Perico N, Gotti E, et al. Kidney transplant tolerance associated with remote autologous mesenchymal stromal cell administration. *Stem Cells Transl Med*. 2020;9(4):427-432.
31. Detry O, Vandermeulen M, Delbouille M-H, et al. Infusion of mesenchymal stromal cells after deceased liver transplantation: a phase I-II, open-label, clinical study. *J Hepatol*. 2017;67(1):47-55.
32. Dreyer GJ, Groeneweg KE, Heidt S, et al. Human leukocyte antigen selected allogeneic mesenchymal stromal cell therapy in renal transplantation: the Neptune study, a phase I single-center study. *Am J Transplant*. Published online April 10, 2020. 2020;20(10):2905-2915.
33. Chambers DC, Enever D, Lawrence S, et al. Mesenchymal stromal cell therapy for chronic lung allograft dysfunction: results of a first-in-man study. *Stem Cells Transl Med*. 2017;6(4):1152-1157.
34. Keller CA, Gonwa TA, Hodge DO, Hei DJ, Centanni JM, Zubair AC. Feasibility, safety, and tolerance of mesenchymal stem cell therapy for obstructive chronic lung allograft dysfunction. *Stem Cells Transl Med*. 2018;7(2):161-167.
35. Fisher SA, Cutler A, Doree C, et al. Mesenchymal stromal cells as treatment or prophylaxis for acute or chronic graft-versus-host disease in haematopoietic stem cell transplant (HSCT) recipients with a haematological condition. *Cochrane Database Syst Rev*. 2019;1:CD009768.
36. Pistoia V, Raffaghello L. Mesenchymal stromal cells and autoimmunity. *Int Immunol*. 2017;29(2):49-58.
37. Capelli C, Domenghini M, Borleri G, et al. Human platelet lysate allows expansion and clinical grade production of mesenchymal stromal cells from small samples of bone marrow aspirates or marrow filter washouts. *Bone Marrow Transplant*. 2007;40(8):785-791.
38. Bohne F, Martínez-Llordella M, Lozano J-J, et al. Intra-graft expression of genes involved in iron homeostasis predicts the development of operational tolerance in human liver transplantation. *J Clin Invest*. 2012;122(1):368-382.
39. Erpicum P, Weekers L, Detry O, et al. Infusion of third-party mesenchymal stromal cells after kidney transplantation: a phase I-II, open-label, clinical study. *Kidney Int*. 2019;95(3):693-707.
40. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One*. 2012;7(10):e47559.
41. Casiraghi F, Remuzzi G, Abbate M, Perico N. Multipotent mesenchymal stromal cell therapy and risk of malignancies. *Stem Cell Rev Rep*. 2013;9(1):65-79.
42. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell*. 2013;13(4):392-402.
43. Najar M, Raicevic G, Fayyad-Kazan H, Bron D, Toungouz M, Lagneaux L. Mesenchymal stromal cells and immunomodulation: a gathering of regulatory immune cells. *Cytotherapy*. 2016;18(2):160-171.
44. Gonçalves FDC, Luk F, Korevaar SS, et al. Membrane particles generated from mesenchymal stromal cells modulate immune responses by selective targeting of pro-inflammatory monocytes. *Sci Rep*. 2017;7(1):12100.
45. Galleu A, Riffo-Vasquez Y, Trento C, et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. *Sci Transl Med*. 2017;9(416):eaam7828.
46. Melief SM, Schrama E, Brugman MH, et al. Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. *Stem Cells*. 2013;31(9):1980-1991.
47. de Witte SFH, Luk F, Sierra Parraga JM, et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. *Stem Cells*. 2018;36(4):602-615.
48. Kuo Y-R, Chen C-C, Shih H-S, et al. Prolongation of composite tissue allotransplant survival by treatment with bone marrow mesenchymal stem cells is correlated with T cell regulation in a swine hind-limb model. *Plast Reconstr Surg*. 2011;127(2):569-579.
49. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol*. 2014;32(3):252-260.
50. Mudrabettu C, Kumar V, Rakha A, et al. Safety and efficacy of autologous mesenchymal stromal cells transplantation in patients undergoing living donor kidney transplantation: a pilot study. *Nephrology*. 2015;20(1):25-33.
51. Li Y, Chen J, Wang L, Zhang L, Lu M, Chopp M. Intracerebral transplantation of bone marrow stromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Neurosci Lett*. 2001;316(2):67-70.
52. Spaeth E, Klopp A, Dembinski J, Andreeff M, Marini F. Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. *Gene Ther*. 2008;15(10):730-738.
53. Kawada H, Fujita J, Kinjo K, et al. Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. *Blood*. 2004;104(12):3581-3587.

54. Herrera MB, Bussolati B, Bruno S, et al. Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury. *Kidney Int*. 2007;72(4):430-441.
55. Zheng J, Li H, He L, et al. Preconditioning of umbilical cord-derived mesenchymal stem cells by rapamycin increases cell migration and ameliorates liver ischaemia/reperfusion injury in mice via the CXCR4/CXCL12 axis. *Cell Prolif*. 2019;52(2):e12546.
56. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol*. 2001;22(11):633-640.
57. Harmon C, Sanchez-Fueyo A, O'Farrelly C, Houlihan DD. Natural killer cells and liver transplantation: orchestrators of rejection or tolerance? *Am J Transplant*. 2016;16(3):751-757.
58. Wu Y, Tian Z, Wei H. Developmental and functional control of natural killer cells by cytokines. *Front Immunol*. 2017;8:930.
59. Buron F, Morelon E, Perrin H, Malcus C, Moulin FT, Héquet O. Mesenchymal stem cell and immunosuppressive drug interactions. *Transplantation*. 2009;87(12):1899-1900; discussion 1900–1901.
60. Buron F, Perrin H, Malcus C, et al. Human mesenchymal stem cells and immunosuppressive drug interactions in allogeneic responses: an in vitro study using human cells. *Transplant Proc*. 2009;41(8):3347-3352.
61. Ketterl N, Brachtl G, Schuh C, et al. A robust potency assay highlights significant donor variation of human mesenchymal stem/progenitor cell immune modulatory capacity and extended radioresistance. *Stem Cell Res Ther*. 2015;6:236.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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## APPENDIX 1

### MSC-LIVER Study Organization

*Coordinating Center*: Mario Negri Institute for Pharmacological Research IRCCS (Giuseppe Remuzzi, Norberto Perico, Federica Casiraghi).

*Clinical Centers*: ASST-PG23 Hospital Bergamo—Department of Organ Failure and Transplantation (Michele Colledan, Marco F. Zambelli, Stefania Camagni) and Gastroenterology, Hepatology and Transplantation, Department of Medicine (Stefano Fagiuoli, Giulia Magini, Anna Baldan, Maria Grazia Lucà, Loredana Rota). General Surgery and Transplant Unit, Department of Medical and Surgical Science, Azienda Ospedaliero-Universitaria-Policlinico S. Orsola-Malpighi Bologna (Antonio Daniele Pinna, Matteo Cescon, Valentina Bertuzzo, Lorenzo Maroni, Matteo Ravaioli, Mariacristina Morelli).

*Centralized Laboratories*: G. Lanzani Laboratory of Cell Therapy, ASST-PG23, Bergamo (Martino Introna, Josee Therese Golay, Chiara Capelli, Olga Pedrini). Emilia Romagna Cord Blood Bank—Immunohematology and Transfusion Medicine—Azienda Ospedaliero-Universitaria-Policlinico S. Orsola-Malpighi, Bologna, Italy (Marina Buzzi, Sabrina Manara, Barbara Morara). Laboratory of Immunology of Organ Transplantation (Federica Casiraghi, Marta Todeschini, Marilena Mister, Manuel Podestà, Pamela Y. R. Ordonez).

*Trial management*: Clinical Research Centre for Rare Diseases "Aldo and Cele Daccò" Ranica (BG). Monitoring, and Pharmacovigilance (Nadia Rubis, Olimpia Diadei, Alessandro Villa); Database and Data Validation (Davide Martinetti, Sergio Carminati); Randomization (Giovanni Antonio Giuliano); Statistical Analysis (Manuel Alfredo Podestà); Regulatory Affairs (Paola Boccardo, Sara Peracchi).