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CLINICAL TRIAL REPORT

Baseline CD4$^+$/CD8$^+$ T_{EMRA} Levels and Early Response Predict Survival After Umbilical Cord-Derived Mesenchymal Stem Cells Infusion in Acute GVHD Patients: A Randomized Double-Blinded Placebo-Controlled Multicentre Clinical Trial

Sze-Piaw Chin[1,2], Sen Mui Tan[3], Kian Meng Chang[4], S Fadilah Abdul Wahid[5], Azizan Sharif[6], Nik Syazana Izyan Saffery[1], Sharifah Shahnaz Syed Abd Kadir[3], Kim Wah Ho[4], Kong Yong Then[1], Soon Keng Cheong[2]

[1]Cytopeutics Sdn Bhd, Cyberjaya, Selangor, Malaysia; [2]M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman - Kampus Sungai Long, Kajang, Selangor, Malaysia; [3]Department of Haematology, Hospital Ampang, Ampang, Selangor, Malaysia; [4]Haematology Unit, Sunway Medical Centre, Subang Jaya, Selangor, Malaysia; [5]Fakulti Perubatan, Universiti Kebangsaan Malaysia, Cheras, Selangor, Malaysia; [6]Haematology Unit, Hospital Sultanah Aminah, Johor Bahru, Johor, Malaysia

Correspondence: Sze-Piaw Chin, Cytopeutics Sdn Bhd, Cyberjaya, Selangor, Malaysia, Email chin.sze.piaw@gmail.com

Background: A major challenge after allogeneic haematopoietic stem cell transplantation for haematologic malignancies is the management of acute graft-versus-host disease (aGVHD), which remains associated with poor prognosis despite therapeutic advancements. We conducted a randomized, double-blinded, placebo-controlled Phase I/II clinical trial to assess the safety and efficacy of umbilical cord-derived mesenchymal stem cells (Cyto-MSC) as an upfront treatment in patients with grade II–IV aGVHD.

Methods: In this multicentre trial, 22 grade II–IV aGVHD patients were randomized to receive up to three infusions of Cyto-MSC (n = 14) or placebo (n = 8), alongside standard corticosteroid therapy. The primary endpoints were overall response (OR) at Day 28 and overall survival (OS) at 12 months. The secondary endpoints included correlation between responses at Day 28 with 12-month OS and exploratory analyses of immune cell subsets.

Results: No treatment-related adverse events were observed. There were no significant differences between Cyto-MSC and placebo in the OR at Day 28 and 12-month OS. Among patients with severe grade III–IV aGVHD who achieved OR by Day 28, those treated with Cyto-MSC had significantly improved 12-month OS compared to placebo (100% vs 50%, p=0.039). Furthermore, in patients with severe aGVHD and baseline CD4$^+$ T_{EMRA} >35% or CD8$^+$ T_{EMRA} >70%, the survival benefit was pronounced in the Cyto-MSC group (83.3% and 100%, respectively). In contrast, none of the placebo-treated patients with baseline CD4$^+$ T_{EMRA} <35% (p=0.007) or CD8$^+$ T_{EMRA} <70% (p=0.005) survived at 12 months. OS was significantly associated with OR at Day 28 (p<0.001), baseline CD4+ T_{EMRA} (p=0.004), and baseline CD8+ T_{EMRA} (p=0.004).

Conclusion: Patients with severe grade III–IV aGVHD, particularly those who respond early or have elevated baseline CD4$^+$ T_{EMRA} (>35%) or CD8$^+$ T_{EMRA} (>70%) levels, may have an overall survival advantage when treated with Cyto-MSC as an upfront therapy in combination with standard corticosteroids.

Keywords: acute graft-versus-host disease, mesenchymal stem cells, predictors, T_{EMRA} cells, CD4$^+$/CD8$^+$, upfront treatment

Introduction

The major hurdle that impedes the therapeutic success of allogeneic haematopoietic stem cell transplantation (HSCT) in treating haematological malignancies, immune disorders, and other diseases, lies in effectively managing treatment-

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related complications, particularly graft-versus-host-disease (GVHD). Approximately 5 in 10 HSCT recipients develop GVHD within 100 days post-transplantation, and the incidence may be higher with unmatched donors.[1] Acute GVHD (aGVHD), particularly grade III–IV, is associated with high morbidity and mortality.[2,3] A further challenge is distinguishing GVHD from endothelial injury syndromes such as transplant-associated thrombotic microangiopathy and sinusoidal obstruction syndrome/veno-occlusive disease, which share overlapping features but require different management.[4] Despite recent advances in prophylaxis and treatment strategies, the prognosis of aGVHD remains poor.

Mesenchymal stem cells (MSCs) have shown immunomodulatory properties in preclinical and early clinical studies, suggesting their potential as a therapeutic option for aGVHD.[5–10] In a single-arm, Phase II/III clinical trial involving steroid-refractory aGVHD patients, an overall response (OR) rate of 60% was observed 4 weeks after MSCs infusion, leading to the approval of Temcell (allogeneic human bone marrow-derived MSCs) for aGVHD treatment in Japan.[5] These findings align with the promising results from a single-arm, prospective study of remestemcel-L (allogeneic human bone marrow-derived MSCs), which reported a Day 28 OR of 65%, with an overall survival (OS) of 82% achieved by Day 100 among the responders.[6] In 2018, Bloor et al completed the first clinical trial using induced pluripotent stem cells-derived MSCs for steroid-resistant GVHD patients, which reported an OR rate of 73.3% at Day 28 and 86.7% survived at Day 100.[7]

Despite promising results from previous clinical trials, MSCs have primarily been used as second-line treatment beyond Day 14 or Day 28, following the failure of initial therapeutic interventions.[8–10] However, the established correlation between OR at Day 28 and OS highlights the critical importance of achieving early treatment responses.[11,12] These findings prompt investigation into the potential advantages of upfront MSCs administration to improve OR at Day 28 and subsequently enhance OS. Furthermore, the variability in clinical outcomes reflects the underlying biological heterogeneity among patients with aGVHD. The identification of predictive markers capable of stratifying patients based on treatment responsiveness is essential for optimizing outcomes and ensuring cost-effective care. This study reports the results of a randomized, double-blinded, placebo-controlled phase I/II trial evaluating the safety and efficacy of Cyto-MSC administered upfront in combination with corticosteroids in patients with grade II–IV aGVHD. Additionally, we assess the prognostic value of immune cell subsets in predicting clinical outcomes.

Materials and Methods

Study Design

This was a multicentre, randomized, double-blinded, placebo-controlled phase I/II clinical trial conducted across four hospitals in Malaysia: Hospital Ampang, Hospital Canselor Tuanku Muhriz UKM, Hospital Sultanah Aminah Johor Bahru, and Sunway Medical Centre. The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. The study received approval from institutional review boards at each participating centre, including the National Committee on Ethics of Cell Research and Therapy (NCERT) and the Medical Research and Ethics Committee (MREC) Malaysia (Reference: NMRR-17-114-36047). The study was also registered on ClinicalTrials.gov (Identifier: NCT03847844).

Participants

Eligible participants were at least 16 years old who developed grade II–IV aGVHD post allogeneic HSCT for malignant or non-malignant haematological disorders. Diagnosis and staging of aGVHD were performed according to the number and extent of organ involvement, using the 1994 Consensus Conference criteria.[13] Exclusion criteria included recent enrolment in another clinical trial, active infections, renal dysfunction, received immune-modulatory treatments in the past 12 months, progressive underlying disease or incomplete remission at transplant, HSCT for solid tumour disease, significant comorbidities or any contraindication to MSCs therapy.

The study aimed to enrol 40 patients (20 per group) to detect a 2.5-fold improvement in response rate with 80% power at a 0.05 significance level, assuming a 70% response rate with Cyto-MSC. A Data Safety Monitoring Board (DSMB) reviewed safety data biannually. Seven patients in the initial safety cohort received Cyto-MSC and were monitored for 6 months.

Following DSMB approval, 16 additional patients were enrolled and randomized (1:1) to Cyto-MSC or placebo in the second cohort. Data from both cohorts were pooled for the final analysis, in accordance with the study protocol.

Randomization and Blinding

Patients were randomly assigned to receive either Cyto-MSC or placebo using a centralized, computer-generated randomization schedule. Allocation concealment was ensured through sealed opaque envelopes prepared by an independent statistician. Blinding was maintained for all participants and study personnel, including investigators, coordinators, nursing staff, and outcome assessors. The investigational product (Cyto-MSC) and placebo were prepared by an independent, unblinded biotechnologist who was not involved in any aspect of patient care or outcome evaluation. After preparation, the treatment bottle, containing either Cyto-MSC or placebo, was wrapped to ensure blinding during infusion. Emergency unblinding was permitted only when clinically necessary for patient safety. Throughout the study, no instances of unblinding occurred, and all efficacy and safety assessments were performed by blinded assessors.

Study Interventions

Cyto-MSC is allogeneic mesenchymal stem cells derived from the umbilical cord of full-term, healthy babies with written parental consent. All cell processing was conducted in a certified Good Manufacturing Practice (GMP) contract laboratory of Cytopeutics, Cyberjaya, Malaysia. Cell isolation, culture, expansion, processing, and preparation followed a previously established protocol.[14] Prior to release, random vials from each Cyto-MSC production batch underwent quality control testing to confirm identity, purity, safety, biological potency, and stability. Post-thaw cell viability was also evaluated, with a threshold of >90% considered acceptable. The cryopreserved investigational product was transported in a cryoshipper to the medical centre on the treatment day. The final cell preparation, which included thawing, washing, and re-suspending the cells in 20 mL of normal saline, was performed in a clean treatment room. The cells were then re-suspended in a 400 mL normal saline bag for intravenous infusion over 2 hours. On Days 1 and 4, all patients received either Cyto-MSC (5×10^6 cells/kg) or placebo, regardless of their response. If a patient showed partial or no response by Day 7, a third infusion of Cyto-MSC or placebo was administered.

All patients received standard first-line corticosteroid therapy, with detailed information provided in the Supplementary Material. Briefly, the standard first-line treatment for aGVHD comprised intravenous methylprednisolone at a dose of 2 mg/kg/day, administered in conjunction with existing GVHD prophylaxis. Prophylactic regimens included a calcineurin inhibitor, either cyclosporine A or tacrolimus, maintained at therapeutic trough levels, with the addition of mycophenolate mofetil (MMF) as indicated by the conditioning protocol. Treatment modifications, including corticosteroid dose escalation and the introduction of MMF, were implemented based on clinical response. Escalation to second- and third-line therapies was guided by institutional protocols. Additionally, to avoid interference with the MSCs' efficacy, a 12-hour gap was maintained between corticosteroid administration and Cyto-MSC/placebo infusions, as suggested by previous study.[15]

Outcome Measures

Outcome measures included assessments of aGVHD grading for treatment response, immune cell subsets, and monitoring of adverse events (AE) and serious adverse events (SAE). Data were collected during follow-up visits up to 12 months. Response rates, including complete response (CR), partial response (PR), and OR (sum of PR and CR) were recorded. CR was defined as aGVHD resolution to grade 0, PR as at least one grade improvement, and no response (NR) as no improvement or disease progression. Primary endpoints were the rates of response at Day 28 and OS rate at 12 months. Secondary endpoints included the correlation between responses at Day 28 with 12-month OS and exploratory analyses of immune cell subsets to evaluate their potential as prognostic biomarkers.

Immune Cell Subsets Analysis

Peripheral blood (10–15 mL) was collected in EDTA tubes at baseline (Day 0), Day 14, Day 28, Month 2, and Month 3. Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque®-1077 (Sigma-Aldrich, Germany), cryopreserved, and stored in liquid nitrogen for subsequent flow cytometry analysis. Flow cytometry was performed to

characterize T-cell subsets (Panel 1) and regulatory T (Treg) cells (Panel 2). Panel 1 identified naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}) and terminally differentiated effector memory re-expressing CD45RA (T_{EMRA}) in $CD4^+$ and $CD8^+$ T cells, while Panel 2 assessed Cutaneous Lymphocyte Antigen (CLA) or α4β7 Treg. In cases with limited cell yield, only Panel 1 was conducted. Details on antibodies (BD Biosciences, CA, USA; Table S1) and flow cytometry protocol are provided in the Supplementary Materials. Briefly, the gating strategy involved identifying T cells as CD3+ leukocytes and subsequently separated into CD4+ and CD8+ subsets. Within each subset, cells were further classified according to CD45RA and CCR7 expression into T_N, T_{CM}, T_{EM}, and T_{EMRA}. T_{EMRA} cells were characterized by the phenotype $CCR7^-$CD45RA+ (Figure S1).

Statistical Analysis

All data were analyzed using SPSS version 12 (SPSS, Inc. Chicago, IL, USA) on a modified intent-to-treat population, defined as all enrolled patients with at least two evaluable datasets. Categorical data were compared using Chi-square or Fisher's exact tests, while continuous data were analyzed using the Independent *T*-test, Mann–Whitney *U*-test, or Wilcoxon signed-rank test, as appropriate. Survival outcomes were analyzed using the Kaplan-Meier method and the Log-rank (Mantel-Cox) test. Cut-off values for immune cell subsets were determined using receiver operating characteristics (ROC) curve analysis, with optimal thresholds determined by the Youden Index. Test performance was assessed by area under the curve (AUC) with 95% CI, and sensitivity and specificity were calculated at the selected cut-off. Analyses were performed using Stata version 14. Subgroup analyses were performed based on baseline GVHD grade, Day 28 OR, 12-month OS and baseline immune cell subset levels. Associations between survival outcomes and these variables among severe grade III–IV GVHD patients were evaluated using Kaplan–Meier survival analysis with Log rank tests. A p-value of <0.05 was considered statistically significant.

Results

Patient Characteristics

Between February 2018 and May 2023, 53 patients were screened, and 26 met the eligibility criteria. Of these, one patient withdrew consent prior to infusion, and two were reclassified as having chronic GVHD, resulting in 23 patients enrolled in the study (Figure 1). In the first cohort, seven patients received Cyto-MSC. However, one was excluded from the analysis due to incomplete data following a non-treatment-related SAE, leaving six evaluable patients. In the second cohort, 16 eligible patients were randomized (8 per group) to receive Cyto-MSC or placebo. Baseline demographic and characteristics of the 22 evaluable patients are presented in Table 1, with no significant differences between the treatment groups.

Safety Outcomes

A total of 55 infusions were administered across both cohorts in the study, with 35 infusions of Cyto-MSC and 20 infusions of placebo. No acute infusion-related toxicities or adverse events were reported during or immediately following the infusions. Twelve SAEs were documented, and all of which were considered unrelated to the investigational product or associated treatment procedures. Of these, two were non-fatal: one seizure and one case of broncho-pulmonary haemorrhage. Ten patient deaths occurred during the study: 6 of 15 (40%) in the Cyto-MSC group, including one patient from the first cohort with incomplete data, and 4 of 8 (50%) in the placebo group. All deaths in the placebo group were attributed to infections related to the underlying disease. In the Cyto-MSC group, causes of death included transplant-related complications (thrombotic microangiopathy, n=1; disseminated intravascular coagulation, n=1), infections (n=2), and progression of the underlying disease (n=2). Infection-related mortality was notably higher in the placebo group, with 4 of 8 patients (50%) affected, compared with 2 of 15 patients (13.3%) in the Cyto-MSC group (p=0.131).

Efficacy Outcomes: Treatment Responses at Day 28

In the Cyto-MSC group, 8 patients received two infusions, while 6 patients required three infusions. In the placebo group, 4 patients each received two or three infusions of normal saline. A higher proportion of patients in the placebo

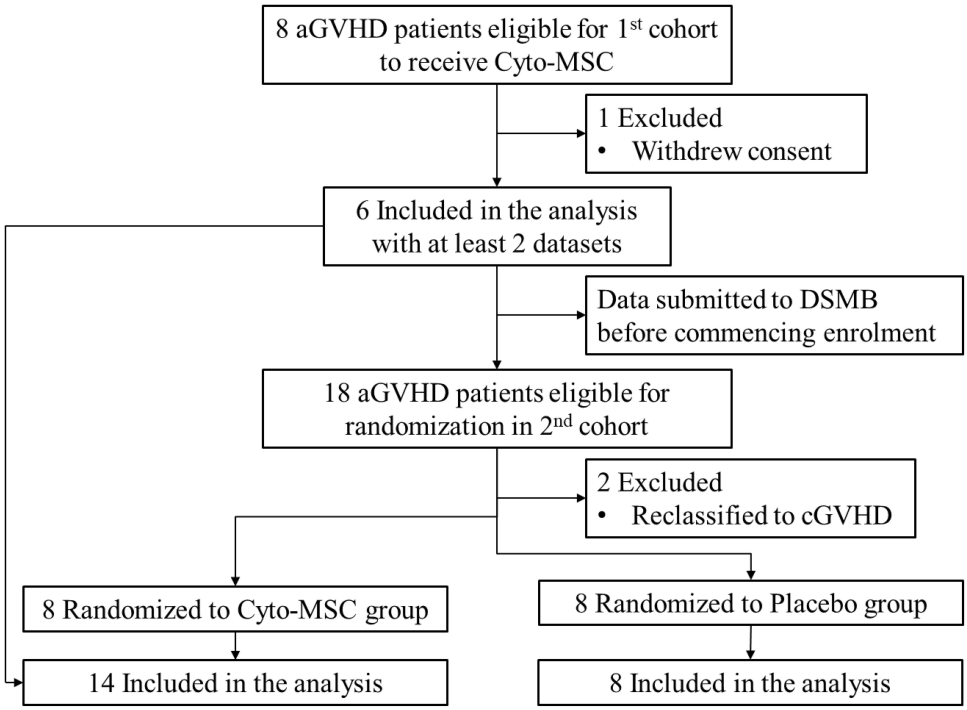

Figure 1 Patient disposition. A total of 26 patients were eligible and consented to participate, with a final recruited number of 14 in the Cyto-MSC group, including 6 patients in the first cohort, and 8 in the placebo group.
Abbreviations: aGVHD, Acute graft-versus-host disease; cGVHD, Chronic graft-versus-host disease; DSMB, Data Safety Monitoring Board.

group required a third infusion, due to lower CR rates by Day 7 (37.5% vs 64.3% in the Cyto-MSC group; p=0.378). At Day 28, the CR rate increased to 78.6% in the Cyto-MSC group, and 62.5% in the placebo group. Although the OR rate at Day 28 was higher in the Cyto-MSC group, the difference was not statistically significant (p=0.426; Figure 2). By Day 28, relapse and/or GVHD progression was significantly higher in the placebo group (37.5%) compared with no events in the Cyto-MSC group (p=0.032). In the subgroup of patients with grade III–IV aGVHD at randomization (10 in the Cyto-MSC group and 7 in the placebo group), the OR rate at Day 28 was 70% in the Cyto-MSC group compared with 57.1% in the placebo group (p=0.644).

Table 1 Baseline Demographics

	Cyto-MSC (n = 14)	Placebo (n = 8)	*p*-value
Age, years (mean ± SD)	33 ± 13	34 ± 14	0.866
Sex			0.183
Male	5 (35.7%)	6 (75.0%)	
Female	9 (64.3%)	2 (25.0%)	
Diagnosis			0.289
Acute Myeloid Leukaemia	5 (35.7%)	4 (50.0%)	
Acute Lymphoblastic Leukaemia	4 (28.6%)	2 (25.0%)	
Chronic Myeloid Leukaemia	1 (7.1%)	2 (25.0%)	
Chronic Lymphocytic Leukaemia	1 (7.1%)	0 (0%)	
Hodgkin Lymphoma	1 (7.1%)	0 (0%)	
Myelodysplastic syndrome	2 (14.3%)	0 (0%)	

(*Continued*)

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Table 1 (Continued).

	Cyto-MSC (n = 14)	Placebo (n = 8)	*p*-value
Donor			0.984
Matched related	10 (71.4%)	6 (75.0%)	
Matched unrelated	2 (14.3%)	1 (12.5%)	
Haploidentical related	2 (14.3%)	1 (12.5%)	
Remission status			0.365
Partial remission	0 (0%)	1 (12.5%)	
Complete remission	10 (71.4%)	5 (62.5%)	
Refractory/Relapsed	1 (7.1%)	1 (12.5%)	
Others	3 (21.4%)	1 (12.5%)	
Type of conditioning			0.604
Reduced intensity	5 (35.7%)	2 (25.0%)	
Full myeloid	9 (64.3%)	6 (75.0%)	
aGVHD grade at randomization			0.466
II	4 (28.6%)	1 (12.5%)	
III	9 (64.3%)	7 (87.5%)	
IV	1 (7.1%)	0 (0%)	

Efficacy Assessment: Overall Survival at 12 Months

Using Kaplan-Meier survival analysis, the 12-month cumulative survival probabilities were not significantly different between the Cyto-MSC and placebo groups (56.7% vs 37.5%; p=0.414; Figure 3). Among patients with grade III–IV aGVHD, the 12-month OS was 66.7% in the Cyto-MSC group and 28.6% in the placebo group, with no statistically significant difference observed (p=0.204).

Efficacy Assessment: Correlation Between OR at Day 28 and OS at 12 Months

Patients who achieved an OR by Day 28 demonstrated a 12-month OS of 75%, whereas none of the non-responders survived to 12 months. A significant survival benefit was observed for patients with an OR at Day 28 compared with non-responders, as demonstrated by the Log rank test (p<0.001; Table 2). Based on OR at Day 28, 75% of Cyto-MSC patients and 60% of placebo patients survived at 12 months, while none of the non-responders in either group survived. In

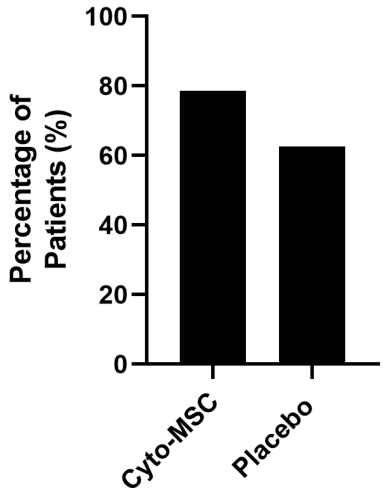

Figure 2 Percentage of patients in Cyto-MSC and placebo groups that achieved overall response (OR) at Day 28; Cyto-MSC (78.6%), Placebo (62.5%).

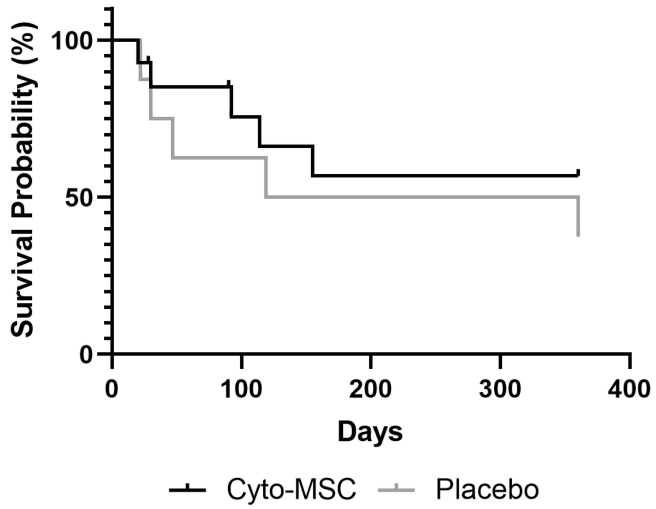

Figure 3 12-month cumulative survival probabilities between Cyto-MSC and placebo (56.7%; 95% CI, 27.5%-85.9% vs 37.5%; 95% CI, 4.0%-71.0%; *p*=0.414).

a further subgroup analysis of patients with grade III–IV aGVHD who achieved OR by Day 28, the Cyto-MSC group demonstrated significantly higher 12-month OS at 100%, compared to 50% in the placebo group (*p*=0.039). In contrast, non-responding grade III–IV patients in both groups had 0% survival at 12 months (Figure 4).

Efficacy Assessment: Immune Cell Subsets Analysis

Data from both of the cohorts were pooled together for exploratory immune cell subsets analysis, including 14 Cyto-MSC and 8 placebo patients. The immunological profiling of $CD4^+$ and $CD8^+$ T cell subsets was measured at multiple time-points to assess their prognostic value and temporal dynamics in relation to aGVHD clinical grading (Tables S2 and S3). In general, no significant differences were observed between groups in all of the immune cell subsets (T_N, T_{CM}, T_{EM}, T_{EMRA} and α4β7 Treg). ROC curve analysis identified optimal baseline cut-off values of CD4+ T_{EMRA} >35% and CD8+ T_{EMRA} >70% for predicting treatment outcomes. These thresholds yielded a sensitivity of 70.6% and specificity of 80.0% for CD4+ T_{EMRA}, and a sensitivity of 76.5% and specificity of 80.0% for CD8+ T_{EMRA} (Figure S2). ROC analyses for other T-cell subsets were also performed and are summarized in Table S4.

Efficacy Assessment: Correlation Between Baseline Levels of $CD4^+$/ $CD8^+$ T_{EMRA} and OS at 12 Months

Kaplan–Meier analysis was also performed to evaluate survival according to baseline CD4+ T_{EMRA} levels. Patients with baseline CD4+ T_{EMRA} proportions greater than 35% demonstrated a 12-month OS of 76.9%, compared with 22.2% among those with levels below 35%. The Log rank test indicated a significant survival advantage for patients with higher baseline CD4+ T_{EMRA} levels (*p*=0.004; Table 2). OS rates among patients with >35% $CD4^+$ T_{EMRA} were 83.3% in the

Table 2 Kaplan Meier Survival Analyses Comparing Subgroups Defined by Treatment Response or Baseline Immune Subset Levels

Subgroup Comparison	Survival Proportion	*p*-value
OR at Day 28 vs NR at Day 28	75.0% vs 0%	<0.001
Baseline CD4+ T_{EMRA} >35% vs <35%	76.9% vs 22.2%	0.004
Baseline CD8+ T_{EMRA} >70% vs <70%	71.4% vs 25.0%	0.004

Note: *P*-values were calculated using the Log rank test.
Abbreviations: NR, No response; OR, Overall response; T_{EMRA}, Terminally differentiated effector memory T cells re-expressing CD45RA.

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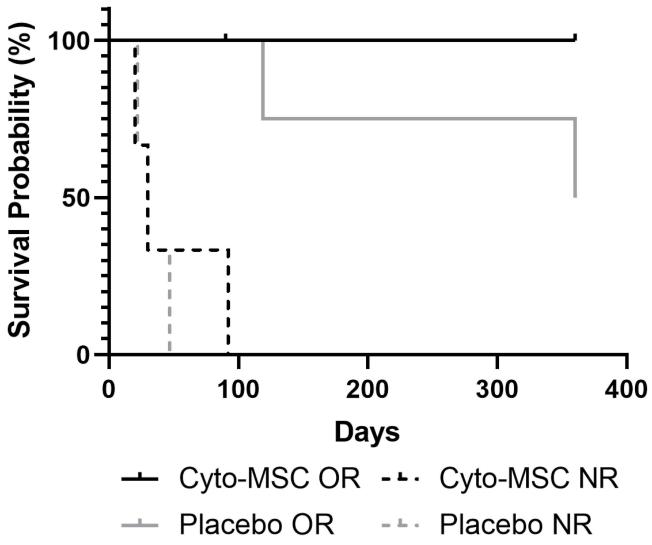

Figure 4 12-month overall survival based on response at Day 28 among grade III–IV patients. OS was significantly higher in the Cyto-MSC group compared with placebo (100% vs 50%; p=0.039). Cyto-MSC OR (100%), Cyto-MSC NR (0%), Placebo OR (50%; 95% CI, 1%-99%), Placebo NR (0%). No response (NR); Overall response (OR).

Cyto-MSC group and 60% in the placebo group, compared to 22.2% and 0%, respectively, in those with <35% $CD4^+$ T_{EMRA} (Table S5). Among the grade III–IV subgroup, those treated with Cyto-MSC and with >35% $CD4^+$ T_{EMRA} had significantly higher OS compared to placebo recipients with <35% $CD4^+$ T_{EMRA} (83.3% vs 0%; p=0.007; Figure 5).

Survival was also analyzed according to baseline CD8+ T_{EMRA} levels. Patients with CD8+ T_{EMRA} proportions greater than 70% demonstrated a 12-month OS of 71.4%, compared with 25% among those with levels below 70%. The Log rank test confirmed a significant survival advantage for patients with higher baseline CD8+ T_{EMRA} levels (p=0.004; Table 2). OS at 12 months was higher in patients with >70% $CD8^+$ T_{EMRA} with survival rates of 71.4% for Cyto-MSC-treated patients and 60% for those receiving placebo. In contrast, patients with <70% $CD8^+$ T_{EMRA} had markedly lower OS rates (30% and 0%, respectively; Table S5). Remarkably, OS was significantly higher in the subgroup grade III–IV Cyto-MSC patients with >70% $CD8^+$ T_{EMRA} levels than in placebo-treated patients with lower levels (100% vs 0%; p=0.005; Figure 6).

Discussion

Despite modern strategies, prognosis of aGVHD remains poor, representing a leading cause of morbidity and mortality following HSCT. High mortality, recurrence, and progression to chronic GVHD indicate a significant unmet need for innovative, effective treatment. Clinical studies of MSCs for aGVHD have reported the safety profile and promising efficacy results, including good response and tolerability.[5,6,16–19] These data have led to the approval of autologous bone marrow-derived MSCs (remestemcel-L and Temcell®) in Canada, New Zealand, and Japan for steroid-refractory aGVHD. In a retrospective study of Temcell®, a higher OR rate was observed in patients who received second-line Temcell® therapy within < 14 days following first-line steroid therapy than ≥ 14 days, underscoring the importance of early MSCs therapy in potentially improving response and reducing mortality in aGVHD management.[20]

This study provides insight into the safety, feasibility, and efficacy of allogeneic UC-MSCs (Cyto-MSC) as an upfront treatment in combination with corticosteroids for grade II–IV aGVHD. Following review of safety data from seven patients receiving Cyto-MSC in the first cohort, the DSMB recommended continuation to the second cohort. Consistent with prior MSCs trials, Cyto-MSC was well tolerated with no treatment-related serious adverse events. Importantly, no acute infusion reactions occurred, and adverse events were attributed to underlying disease or transplant complications, affirming the established safety profile of MSCs reported in systematic reviews and meta-analyses.[21,22]

Although the Day 28 OR rate with Cyto-MSC was not statistically significant compared to placebo (78.6% vs 62.5%), it was notably higher than rates reported in studies using MSCs as second-line therapy, which range between 56–65%.[20] Real-world data also support improved responses with early MSCs use (<14 days from first-line steroid therapy),

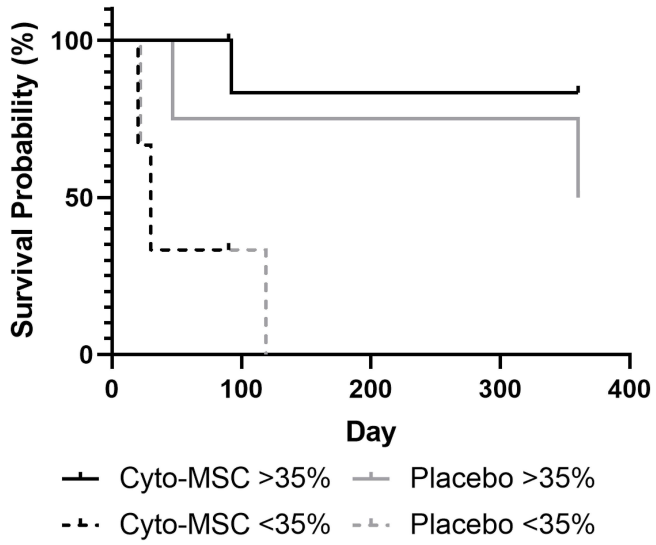

Figure 5 12-month overall survival based on baseline level of $CD4^+T_{EMRA}$ among grade III–IV aGVHD patients. Cyto-MSC–treated patients with >35% CD4+ T_{EMRA} showed significantly higher OS compared with placebo recipients with <35% CD4+ T_{EMRA} (83.3% vs 0%; p=0.007). $CD4^+$ T_{EMRA} >35%; Cyto-MSC (83.3%; 95% CI, 53.5%-100.0%), Placebo (50.0%; 95% CI, 1.0%-99.0%). $CD4^+$ T_{EMRA} <35%; Cyto-MSC (33.3%; 95% CI, 0%-86.6%), Placebo (0%). EMRA: Terminally differentiated effector memory re-expressing CD45RA.

suggesting that upfront administration alongside corticosteroids may offer a therapeutic advantage in aGVHD. Similarly, although not statistically significant, the 12-month OS in the Cyto-MSC group (56.7%) was higher than in the control group (37.5%) and exceeded survival rates reported for second-line MSCs therapies, which typically range from 44–50%.[5,17] Notably, a Japanese cellular therapy registry reported a 12-month OS of only 27% across patients receiving second-, third-, or fourth-line MSCs therapy, with a modest increase to 33% for those treated earlier with second-line MSCs.[16] Additionally, none of the Cyto-MSC-treated patients experienced relapse/progression compared to 37.5% in the placebo group. These findings further support the potential benefit of early, upfront MSCs administration in improving long-term outcomes, including survival and disease control in aGVHD.

Importantly, this trial demonstrated a correlation between Day 28 response and 12-month survival, reinforcing the clinical utility of Day 28 OR as a surrogate marker for treatment efficacy. This relationship has been well documented in earlier MSCs trials and remains a cornerstone for therapeutic decision-making in aGVHD.[6,19] Although the OR at Day 28 and 12-month OS did not differ significantly between groups, subgroup analyses revealed notable benefits in Cyto-MSC–treated patients with severe (grade III–IV) aGVHD. The rationale for focusing on this subgroup stems from clinical observations that patients with grade I–II aGVHD generally respond to standard first- or second-line immunosuppressive therapies. In contrast, grade III–IV aGVHD represents a more aggressive disease phenotype, often refractory to corticosteroids and associated with significantly worse outcomes.[2,3] Within this high-risk population, those who achieved OR by Day 28 had significantly improved 12-month OS with Cyto-MSC compared to placebo. This is especially compelling as patients with severe aGVHD, particularly when resistant to first-line treatment, carry an extremely poor prognosis, with historical one-year OS rates around 30%, even with therapeutic intervention.[23]

A critical strength of this study was the integration of immunophenotyping to identify predictors of response. Specifically, elevated baseline $CD4^+$ T_{EMRA} (>35%) and $CD8^+$ T_{EMRA} (>70%) levels were associated with superior 12-month survival, particularly in patients receiving Cyto-MSC. T_{EMRA} cells are a terminally differentiated subset of memory T cells characterized by high cytotoxic potential, expression of senescence-associated markers, and reduced proliferative capacity.[24–26] In the context of aGVHD, their role is multifaceted. CD8+ T_{EMRA} cells have been shown to exert immediate effector functions, including interferon gamma and granzyme B secretion, which may contribute to the rapid elimination of activated alloreactive T cells or modulate inflammation through cytotoxic regulatory mechanisms.[27,28] CD4+ T_{EMRA} cells, although less well-studied, have been reported to exhibit a mixed pro-inflammatory and immunomodulatory profile and may reflect immune exhaustion or adaptation after prolonged antigen exposure.[29]

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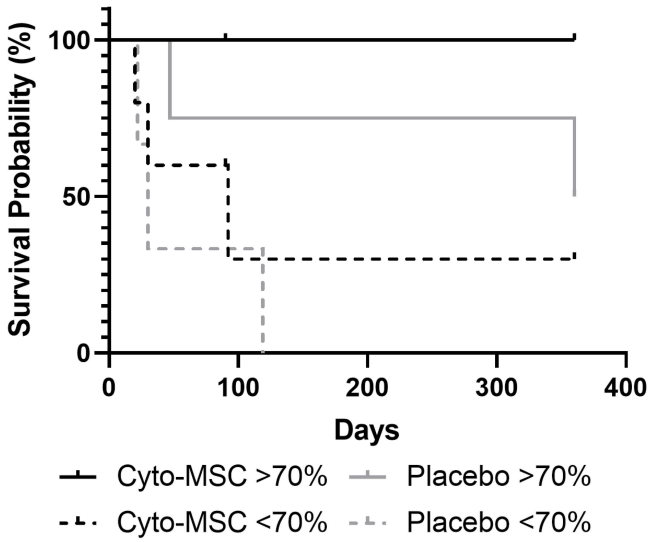

Figure 6 12-month overall survival based on baseline level of $CD8^+T_{EMRA}$ among grade III–IV aGVHD patients. OS was significantly higher in patients with >70% CD8+ T_{EMRA} treated with Cyto-MSC compared to placebo patients with lower levels (100% vs 0%; p=0.005). $CD8^+$ T_{EMRA} >70%; Cyto-MSC (100%), Placebo (50.0%; 95% CI, 1.0%-99.0%). $CD8^+$ T_{EMRA} <70%; Cyto-MSC (30.0%; 95% CI, 0.0%-76.8%), Placebo (0%). EMRA: Terminally differentiated effector memory re-expressing CD45RA.

Importantly, the expansion of T_{EMRA} cells at baseline may signify an immune system in a more differentiated and less reactive state, potentially limiting the proliferation of alloreactive naïve or central memory T cells that are primarily responsible for initiating and sustaining GVHD.[30] This immunological context may enhance responsiveness to MSCs, which exert anti-inflammatory and immunomodulatory effects, particularly through interactions with activated T cells and modulation of their cytokine milieu. In contrast, low levels of baseline T_{EMRA} cells, especially among placebo-treated patients, were associated with poor survival outcomes, suggesting that the absence of this subset may reflect a more naïve, hyperreactive immune phenotype prone to severe GVHD manifestations. These findings are consistent with prior studies demonstrating that the differentiation status of T cells, including the abundance of effector memory and T_{EMRA} subsets, correlates with GVHD risk, progression, and therapeutic outcomes.[31,32]

The ability to identify patients with elevated CD4+ and CD8+ T_{EMRA} levels as likely responders to MSCs therapy could allow for early risk stratification and informed therapeutic decision-making. Such profiling represents a promising step toward personalized medicine in aGVHD, enabling more targeted and cost-effective use of immunomodulatory therapies like Cyto-MSC. Several alternative immunomodulatory approaches have been explored, including extracorporeal photopheresis (ECP), which has demonstrated favourable outcomes in real-world GVHD cohorts with response rates comparable to those observed in our study.[33] In contrast to ECP, which is typically employed as a second-line therapy, Cyto-MSC was administered upfront in combination with corticosteroids. Early modulation of the immune response, supported by the observed association between early treatment response and improved survival, may therefore represent a therapeutic advantage of Cyto-MSC in high-risk aGVHD. Collectively, these approaches exemplify the evolving shift toward immunomodulatory therapies, within which Cyto-MSC emerges as a potential promising strategy.

Limitation

It is acknowledged that the small sample size is a limitation of the study, as it was originally designed to include 40 patients to achieve statistical significance in the clinical response rates between groups. This explains the observed lack of significant difference in the primary endpoints due to the inadequate study numbers. The recruitment rate was much slower than anticipated, especially during the COVID-19 pandemic, which limited the number of patients undergoing high-risk bone marrow transplants, resulting in a global decline in the incidence of aGVHD.[34] This challenge is consistent with larger, global multi-centre clinical studies, given the rarity and complexity of the disease. Despite the small sample size, significant differences in subgroup analyses of the secondary endpoints, particularly the immune cell subsets, were demonstrated. This

is of particular interest as it defines a specific patient subset that will likely benefit from the treatment. Nonetheless, the receptor repertoire and functional properties of T_{EMRA} cells remain insufficiently characterized in the context of transplantation and immune-mediated disorders and warrant further investigation. Another limitation is the absence of data on hematopoietic cell transplantation comorbidity index and cytomegalovirus serostatus, which limits our ability to adjust for comorbidity burden and underlying viral risk. Both are established determinants of post-transplant outcomes and should be incorporated into future trials to enable more comprehensive risk stratification and interpretation of outcomes. Further investigation with a larger sample size is also warranted to determine the definite relationship between baseline T_{EMRA} proportions and the independent effects of MSCs on treatment outcomes.

Conclusion

Cyto-MSC was shown to be safe and well tolerated when administered as upfront treatment alongside standard corticosteroids in patients with grade II–IV aGVHD. While primary outcomes did not reach statistical significance, survival benefits were observed, particularly in patients with severe (grade III–IV) disease who achieved an early treatment response. Notably, elevated baseline levels of $CD4^+$ T_{EMRA} (>35%) and $CD8^+$ T_{EMRA} (>70%) were associated with significantly improved 12-month survival among Cyto-MSC–treated patients. These findings highlight the potential utility of immunophenotyping to identify patients most likely to benefit from MSCs therapy. Incorporating baseline T_{EMRA} profiling into clinical decision-making may support a more personalized approach to aGVHD treatment, guiding early intervention strategies with Cyto-MSC to improve patient outcomes.

Abbreviations

aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; HSCT, haematopoietic stem cell transplantation; UC-MSCs, umbilical cord-derived mesenchymal stem cells; Cyto-MSC, allogeneic human umbilical cord-derived mesenchymal stem cells used in the study; NCERT, National Committee on Ethics of Cell Research and Therapy; MREC, Medical Research and Ethics Committee; DSMB, Data Safety Monitoring Board; GMP, Good Manufacturing Practice; MMF, mycophenolate mofetil; ECP, extracorporeal photopheresis; CR, complete response; PR, partial response; NR, no response; OR, overall response; OS, overall survival; PBMC, peripheral blood mononuclear cell; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; AE, adverse event; SAE, serious adverse event; T_N, Naïve T cell; T_{EM}, effector memory T cell; T_{EMRA}, terminally differentiated effector memory T cell re-expressing CD45RA; T_{CM}, central memory T cell; T_{reg}, regulatory T cell; CLA, cutaneous lymphocyte antigen.

Data Sharing Statement

Deidentified individual participant data and relevant study datasets generated and analyzed in this study will be made available from the corresponding author upon reasonable request, in accordance with institutional and ethical guidelines to protect patient privacy.

Ethics Approval and Informed Consent

The study received ethics approval from institutional review boards at each participating centre, including MREC, Malaysia (reference number: NMRR-17-114-36047). This study adhered to the principles outlined in the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All participants provided informed consent prior to their inclusion in the study.

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Disclosure

SPC advises Cytopeutics Sdn Bhd on regulatory, clinical, and research activities. In addition, he has a patent GVHD issued to Cytopeutics. SKC and KYT sit on Cytopeutics Sdn Bhd's medical advisory board. The authors report no other conflicts of interest in this work.

References

1. Jamil MO, Mineishi S. State-of-the-art acute and chronic GVHD treatment. *Int J Hematol*. 2015;101(5):452–466. doi:10.1007/s12185-015-1785-1
2. Malard F, Holler E, Sandmaier BM, Huang H, Mohty M. Acute graft-versus-host disease. *Nat Rev Dis Primers*. 2023;9(1):27. doi:10.1038/s41572-023-00438-1
3. Holtan SG, Yu J, Choe HK, et al. Disease progression, treatments, hospitalization, and clinical outcomes in acute GVHD: a multicenter chart review. *Bone Marrow Transplant*. 2022;57(10):1581–1585. doi:10.1038/s41409-022-01764-w
4. Evangelidis P, Gavriilaki E, Tsakiris DA. Thrombotic complications after hematopoietic stem cell transplantation and other cellular therapies. *Thrombosis Update*. 2024;16:100186. doi:10.1016/j.tru.2024.100186
5. Muroi K, Miyamura K, Okada M, et al. Bone marrow-derived mesenchymal stem cells (JR-031) for steroid-refractory grade III or IV acute graft-versus-host disease: a phase II/III study. *Int J Hematol*. 2016;103(2):243–250. doi:10.1007/s12185-015-1915-9
6. Kurtzberg J, Prockop S, Chaudhury S, et al. Study 275: updated expanded access program for remestemcel-L in steroid-refractory acute graft-versus-host disease in children. *Biol Blood Marrow Transplant*. 2020;26(5):855–864. doi:10.1016/j.bbmt.2020.01.026
7. Bloor AJC, Patel A, Griffin JE, et al. Production, safety and efficacy of iPSC-derived mesenchymal stromal cells in acute steroid-resistant graft versus host disease: a Phase I, multicenter, open-label, dose-escalation study. *Nat Med*. 2020;26(11):1720–1725. doi:10.1038/s41591-020-1050-x
8. von Bonin M, Stölzel F, Goedecke A, et al. Treatment of refractory acute GVHD with third-party MSC expanded in platelet lysate-containing medium. *Bone Marrow Transplant*. 2009;43(3):245–251. doi:10.1038/bmt.2008.316
9. Lucchini G, Introna M, Dander E, et al. Platelet-lysate-expanded mesenchymal stromal cells as a salvage therapy for severe resistant graft-versus-host disease in a pediatric population. *Biol Blood Marrow Transplant*. 2010;16(9):1293–1301. doi:10.1016/j.bbmt.2010.03.017
10. Muroi K, Miyamura K, Ohashi K, et al. Unrelated allogeneic bone marrow-derived mesenchymal stem cells for steroid-refractory acute graft-versus-host disease: a phase I/II study. *Int J Hematol*. 2013;98(2):206–213. doi:10.1007/s12185-013-1399-4
11. Levine JE, Logan B, Wu J, et al. Graft-versus-host disease treatment: predictors of survival. *Biol Blood Marrow Transplant*. 2010;16(12):1693–1699. doi:10.1016/j.bbmt.2010.05.019
12. Kadri N, Amu S, Iacobaeus E, Boberg E, Le Blanc K. Current perspectives on mesenchymal stromal cell therapy for graft versus host disease. *Cell Mol Immunol*. 2023;20(6):613–625. doi:10.1038/s41423-023-01022-z
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15(6):825–828.
14. Chin SP, Mohd-Shahrizal MY, Liyana MZ, Then KY, Cheong SK, Yong KW. High dose of intravenous allogeneic umbilical cord-derived mesenchymal stem cells (CLV-100) infusion displays better immunomodulatory effect among healthy volunteers: a Phase 1 clinical study. *Stem Cells Int*. 2020;2020:4–8. doi:10.1155/2020/8877003
15. Gan Y, Zhang T, Chen X, et al. Steroids enable mesenchymal stromal cells to promote CD8+ T cell proliferation via VEGF-C. *Adv Sci*. 2021;8(12):2003712. doi:10.1002/advs.202003712
16. Murata M, Terakura S, Wake A, et al. Off-the-shelf bone marrow-derived mesenchymal stem cell treatment for acute graft-versus-host disease: real-world evidence. *Bone Marrow Transplant*. 2021;56(10):2355–2366. doi:10.1038/s41409-021-01304-y
17. Introna M, Lucchini G, Dander E, et al. Treatment of graft versus host disease with mesenchymal stromal cells: a Phase I study on 40 adult and pediatric patients. *Biol Blood Marrow Transplant*. 2014;20(3):375–381. doi:10.1016/j.bbmt.2013.11.033
18. Kebriaei P, Hayes J, Daly A, et al. A Phase 3 randomized study of remestemcel-L versus placebo added to second-line therapy in patients with steroid-refractory acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2020;26(5):835–844. doi:10.1016/j.bbmt.2019.08.029
19. Kurtzberg J, Abdel-Azim H, Carpenter P, et al. A phase 3, single-arm, prospective study of remestemcel-L, ex vivo culture-expanded adult human mesenchymal stromal cells for the treatment of pediatric patients who failed to respond to steroid treatment for acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2020;26(5):845–854. doi:10.1016/j.bbmt.2020.01.018
20. Murata M, Teshima T. Treatment of steroid-refractory acute graft-versus-host disease using commercial mesenchymal stem cell products. *Front Immunol*. 2021;12(August):1–8. doi:10.3389/fimmu.2021.724380
21. 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(1):CD009768. doi:10.1002/14651858.CD009768.pub2
22. 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. doi:10.1371/journal.pone.0047559

23. Jamani K, Russell JA, Daly A, et al. Prognosis of grade 3-4 acute GVHD continues to be dismal. *Bone Marrow Transplant*. 2013;48 (10):1359–1361. doi:10.1038/bmt.2013.59
24. Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? *Nat Rev Immunol*. 2011;11 (4):289–295. doi:10.1038/nri2959
25. Pulko V, Davies JS, Martinez C, et al. Human memory T cells with a naive phenotype accumulate with aging and respond to persistent viruses. *Nat Immunol*. 2016;17(8):966–975. doi:10.1038/ni.3483.Human
26. Thome JJC, Farber DL. Emerging concepts in tissue-resident T cells: lessons from humans. *Trends Immunol*. 2015;36(7):428–435. doi:10.1177/0022146515594631.Marriage
27. Geginat J, Paroni M, Maglie S, et al. Plasticity of human CD4 T cell subsets. *Front Immunol*. 2014;5(DEC):1–10. doi:10.3389/fimmu.2014.00630
28. Klarenbeek PL, Remmerswaal EBM, ten Berge IJM, et al. Deep sequencing of antiviral T-cell responses to HCMV and EBV in humans reveals a stable repertoire that is maintained for many years. *PLoS Pathog*. 2012;8(9):e1002889. doi:10.1371/journal.ppat.1002889
29. Henson SM, Riddell NE, Akbar AN. Properties of end-stage human T cells defined by CD45RA re-expression. *Curr Opin Immunol*. 2012;24 (4):476–481. doi:10.1016/j.coi.2012.04.001
30. Koreth J, Matsuoka K, Kim HT, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. *N Engl J Med*. 2011;365(22):2055–2066. doi:10.1056/nejmoa1108188
31. Kennedy-Nasser AA, Ku S, Castillo-Caro P, et al. Ultra-low dose IL-2 for GVHD prophylaxis after allogeneic hematopoietic stem cell transplantation mediates expansion of regulatory T cells without diminishing antiviral and antileukemic activity. *Clin Cancer Res*. 2014;20 (8):2215–2225. doi:10.1158/1078-0432.CCR-13-3205.Ultra
32. Carlson MJ, West ML, Coghill JM, Panoskaltsis-Mortari A, Blazar BR, Serody JS. In vitro differentiated TH17 cells mediate lethal acute graft-versus-host disease with severe cutaneous and pulmonary pathologic manifestations. *Blood*. 2016;113(6):1365–1374. doi:10.1182/blood-2008-06-162420
33. Gavriilaki E, Papchianou E, Karavalakis G, et al. Safety and efficacy of extracorporeal photopheresis for acute and chronic graft-versus-host disease. *Pharmaceuticals*. 2024;17(10):1279. doi:10.3390/ph17101279
34. Canaani J, Beohou E, Labopin M, et al. Trends in patient outcome over the past two decades following allogeneic stem cell transplantation for acute myeloid leukaemia: an ALWP/EBMT analysis. *J Intern Med*. 2019;285(4):407–418. doi:10.1111/joim.12854