**Supplemental material**

**Supplemental methods**

**MSC production process**

The MSC production process was identical to the process conducted for the clinical trial by Le Blanc et al in 2008\(^2\): Bone-marrow mononuclear cells were separated by density gradient centrifugation as previously described\(^3,4\). Washed cells were resuspended in Dulbecco's modified Eagle's medium–low glucose (Life Technologies, Gaithersburg, MD, USA, or Paisley, UK) supplemented with 10% fetal bovine serum (National Veterinary Institute, Uppsala, Sweden, or HyClone, Logan, UT, USA) and plated at a density of 160 000 cells per cm\(^2\). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO\(_2\) in 175 cm\(^2\) flasks (Falcon, Franklin Lakes, New Jersey, USA, or Greiner Bio-One, Frickenhausen, Germany). When the cultures were near confluence (>80%), the cells were detached by treatment with trypsin and EDTA (Invitrogen, Grand Island, NY, USA, or Lonza Verviers, Verviers, Belgium) and replated at a density of 4000 cells per cm\(^2\). When 2×10\(^6\) cells or more were obtained, they were harvested and either cryopreserved in 10% dimethyl sulphoxide (Research Industries, Salt Lake City, UT, USA, or Leiden University Medical Centre Pharmacy, Netherlands) or washed repeatedly and resuspended to a final concentration of 2×10\(^6\) cells per mL in saline solution according to local guidelines.

Criteria for release of mesenchymal stem cells for clinical use included absence of visible clumps, spindle-shape morphology, absence of contamination by pathogens (as documented by aerobic and anaerobic cultures before release), viability greater than 95%, and immune phenotyping proving expression of CD73, CD90, and CD105 surface molecules (>90%) and absence of CD34, CD45, CD14, and CD3\(^5\).

**Peripheral blood mononuclear cell and plasma separation**

For plasma separation, the blood was collected in ethylene-diamine-tetraacetic acid (EDTA) tubes and kept on ice before centrifugation. The plasma was then frozen at -80°C until analysis. Blood was collected in heparinized tubes for peripheral blood mononuclear cell (PBMC) separation. PBMCs were isolated by centrifugation using Ficoll-Paque PLUS (GE Healthcare Bio-Sciences AB, GE Healthcare, Uppsala, Sweden) and stored in 10% EDTA diluted in human ab plasma in aliquots in liquid nitrogen. Absolute lymphocyte counts were obtained from the Department of Clinical Chemistry, Karolinska University Hospital, Stockholm and absolute cell numbers calculated by multiplying absolute lymphocyte counts with the percentages obtained from flow cytometry analysis.

### Supplemental table 1: Mesenchymal stromal cell donor and graft characteristics

| Donor characteristics |  |
|-----------------------|---|
| Number of donors      | 18 |
| Donor sex (male/female)| 10/8 |
| Donor age (median, range) | 29.5 (3-44)\(^#\) |
| Number of doses per donor (median, range) | 2.5 (1-12) |
| Number of donors per patient (median, range) | 3 (1-6) |

| Graft characteristics |  |
|-----------------------|---|
| Total number of grafts | 71 |
| MSC cell dose per graft (mean, range) | 2.03 (1.3-3) \times 10\(^6\)/kg |
| Unrelated donor (n, %)* | 58 (82) |
| First degree relative (n, %)* | 10 (14) |
| Other relatives (n, %)* | 3 (4) |

| Culture passage at MSC harvest |  |
|-------------------------------|---|
| Passage 2 (n, %) | 8 (11) |
| Passage 3 (n, %) | 63 (89) |

* No HLA matching was performed.
# Three children were among the MSC donors. All of them donated bone marrow to an HLA-identical sibling undergoing allogeneic haematopoietic stem cell transplantation. With their parents’ permission, a minor part of that donated bone marrow was used for MSC production for this clinical trial.
Flow Cytometry Analysis
PBMCs were gently thawed and washed twice with a complete media RPMI containing 10% FCS. Cells were stained for various surface markers for 20 minutes at 4°C with antibodies described in Suppl. Table 2. Aqua fluorescent reactive dye for viability analysis was purchased from Invitrogen (Carlsbad, CA, USA).

Soluble Biomarker Analysis
The following cytokines and chemokines were analysed using a custom Bio-Plex® x-Plex™ (Bio-Rad Laboratories AB, Solna, Sweden): Interleukin (IL)-1b, -2, -6, -7, -8, -10, -17a, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, C-X-C motif ligand (CXCL)-2, -9, -10, chemokine C-C motif ligand 2, basic fibroblast growth factor (b-FGF). B-cell activating factor (BAFF) was analysed using ELISA as per the manufacturer’s instructions.

Micro RNA (miRNA) Analysis
Circulating plasma miRNA were analysed in seven patients (patients 1, 2, 5-9) before and at two time-points (1-3 hrs and 24 hrs) after the first MSC infusion. Total RNA isolation and analysis were conducted at Exiqon Services (Vedbaek, Denmark). Briefly, total RNA for miRNA analysis was extracted from 200μl plasma using the miRCURY™ RNA isolation kit (Exiqon). RNA spike-ins were added to the samples before isolation in order to monitor RNA extraction efficiency. RNA was reverse transcribed using the mercury LNATM Universal RT miRNA PCR, Polyadenylation and cDNA synthesis kit (Exiqon) and analyzed in a LightCycler® 480 Real-Time PCR System (Roche Diagnostics Scandinavia AB, Bromma, Sweden) in 384 well plates using the protocol for mercury LNATM Universal RT miRNA PCR. All data were normalized to the average of assays detected in all samples (average assay Cq).

Linear mixed effects models
These models were applied for analysing absolute peripheral blood mononuclear cell subset counts. For analysing the differences between responders (R) and non-responders (NR), we used the following model: \( \text{Cell count} = A \times \text{Responder} (0 \ or \ 1) + B \times \text{Number of previous infusions} \). Long and short term changes were evaluated for R and NR separately. For analysing long term changes, we used the model \( \text{Cell count} = A \times \text{Number of previous infusions} \), and for short term changes, we used \( \text{Cell count} = A \times \text{Days since last infusion} + B \times \text{number of previous infusions} \). Subject was set as the random effect for all models. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. Analysis was performed using R statistical software\(^6\), with the lme4 package\(^7\).
### Supplemental Table 2: Flow cytometry antibody panels used in the study.

| Panel number | Used for infusion(s) | Antibody (concentration) |
|--------------|-----------------------|---------------------------|
|              |                       | PE | PE-Cy5 | PE-Cy7 | AF488 | BV421 | BV605 | PerCP-Cy5.5 | APC | APC-Cy7 | AF700 | AmCyan |
| 1            | 1, 3, 6, 9            | CD14 (1/400) | CD3 (1/100) | CD21 (1/100) | IgD (1/100) | CD5 (1/100) | CD27 (1/100) | CD19 (1/100) | CD38 (1/100) | BAFF-R (1/100) | Aqua live dead (1/1000) |
| 2            | 3                     | CD19 (1/100) | CD22 (1/100) | IgM (1/100) | IgD (1/100) | CD5 (1/100) | CD27 (1/100) | Ki67 (1/100) | CD38 (1/100) | Aqua live dead (1/1000) |
| 3            | 3                     | CXCR3 (1/100) | CD4 (1/100) | CD127 (1/100) | CD8 (1/100) | CD3 (1/100) | CD56 (1/100) | CD19 (1/100) | CD27 (1/100) | CD45RA (1/1000) | Aqua live dead (1/1000) |
| 4            | 6                     | CXCR3 (1/100) | CD4 (1/100) | CD127 (1/100) | CD8 (1/100) | CD3 (1/100) | CD56 (1/100) | PD1 (1/100) | CD27 (1/100) | CD45RA (1/1000) | Aqua live dead (1/1000) |
| 5            | 1, 9                  | CXCR3 (1/100) | CD4 (1/100) | CD62L (1/100) | CD8 (1/100) | CD3 (1/100) | CD56 (1/100) | PD1 (1/100) | CD27 (1/100) | CD45RA (1/1000) | Aqua live dead (1/1000) |
| 6            | 6                     | FoxP3 (1/50) | CD4 (1/100) | CD8 (1/100) | CD3 (1/100) | IL17 (1/100) | CD62L (1/100) | CD31 (1/100) | CD56 (1/100) | CD45RA (1/1000) | Aqua live dead (1/1000) |
| 7            | 1, 9                  | FoxP3 (1/50) | CD4 (1/100) | CD127 (1/100) | CD3 (1/100) | CCR7 (1/100) | CD62L (1/100) | CD31 (1/100) | CD56 (1/100) | CD27 (1/100) | Aqua live dead (1/1000) |
| 8            | 3                     | FoxP3 (1/50) | CD4 (1/100) | CD62L (1/100) | CD31 (1/100) | CD3 (1/100) | CD56 (1/100) | Ki67 (1/100) | CCR7 (1/100) | CD45RA (1/1000) | Aqua live dead (1/1000) |

PB: Pacific Blue, PE: Phycoerythrin, Cy: Cyanine dye, FITC: Fluorescein Isothiocyanate, BV: Brilliant Violet, PerCP: Peridinin-chlorophyll-protein complex, APC: Allophycocyanin, AF: Alexa Fluor, CD: Cluster of Differentiation, PD: programmed death, FoxP3: Forkhead Box P3, CCR: C-C chemokine Receptor, CXCR3: C-X-C motif chemokine Receptor 3
**Supplementary Table 3: Adverse events recorded before final evaluation**

| Adverse event*          | N  | Comments                                                                 |
|-------------------------|----|---------------------------------------------------------------------------|
| Grade 3 infection       | 5  | 2 pneumonias (1 Metapneumovirus, 1 with unknown microbiology), 1 bacterial keratitis, 1 soft tissue infection, 1 adenovirus colitis. |
| Skin dysplasia          | 1  | Melanocytic                                                              |
| Cervix dysplasia        | 1  |                                                                           |
| Recurrence of M-protein | 1  | After 7 infusions. The patient discontinued the study.                    |
| Increasing recipient chimerism | 1  | Patient with CLL. CD19 recipient chimerism increased after 3 infusions. The patient discontinued the study. |
| Death                   | 1  | Due to progressive cGvHD after 1 MSC infusion.                            |

*Adverse events recorded until final evaluation at 12 months after discontinuing MSC treatment. N: Number of events, CLL: Chronic Lymphocytic Leukaemia, cGvHD: chronic Graft versus Host Disease, MSC: Mesenchymal Stromal Cell
## Supplemental table 4: Histopathological evaluations of skin biopsies

| Responders | Patient | Location | Review | Location | Review | Change |
|------------|---------|----------|--------|----------|--------|--------|
| **Responders** | | | | | | |
| **5** | Abdomen | Lamellar str. corneum, normal thickness, basal KC with vacuolization, basal membrane thickening, sparse dermal lymphocytic infiltrate, also at the dermal/subcutaneous border, sclerosis. | Abdomen | Epidermis normal, sparse dermal lymphocytic infiltrate, sclerosis unchanged. | Epidermal changes improved |
| | | **Diagnosis:** Sclerotic GvHD with discrete acute changes in the epidermis | | **Diagnosis:** Sclerotic GvHD | |
| **7** | Left part of back | Epidermis 3-4 layers, vacuolated melanocytes, melanophages. | Right part of back | Epidermis 6 layers. | Epidermal changes completely regressed |
| | | **Diagnosis:** Acute changes in the epidermis grade 1-2 | | **Diagnosis:** Normal skin | |
| **8** | Right groin | Lamellar str.corneum, acanthosis (10 layers), dilated vessels, sclerosis superficial and deep, sparse infiltrate. | Location unknown | Normal epidermis, sclerosis only deep, less than in 1 (could be location dependent). | Epidermal changes improved |
| | | **Diagnosis:** Sclerotic GvHD with discrete lichenoid aspects within the epidermis | | **Diagnosis:** Sclerotic GvHD | |
| **9** | Right groin | Epidermis normal, vessels number increased, sclerosis, no infiltrate. | Right groin | Vacuolated melanocytes, sclerosis slightly increased. | No improvement, now some acute changes in the epidermis |
| | | **Diagnosis:** Sclerotic GvHD | | **Diagnosis:** Sclerotic GvHD with some acute changes in the epidermis | |
| **11** | Dorsal side of right thigh | Slight edema in papillary dermis, otherwise normal. | Back of right thigh | Slight edema in papillary dermis, otherwise normal. | No diagnostic changes |
| | | **Diagnosis:** Dermal edema | | **Diagnosis:** Dermal edema | |
| **Non-responders** | | | | | | |
| **10** | Right breast | Epidermis 7 layers, vacuolated KC within basal cell layer, single cell dyskeratosis basal and suprabasal, attached lymphocytes, dermal edema, sparse lymphocytes, sclerosis deep dermis (superficial changes could be drug-induced-multiforme-like pattern. | Left side of back | Epidermis normal, sclerosis superficial and deep (progression), dilated vessels. | Epidermal changes improved |
| | | **Diagnosis:** Sclerotic GvHD with multiforme –like changes in the epidermis, drug-induced or GvHD | | **Diagnosis:** Progression of Sclerotic GvHD | Sclerosis progressed |

GvHD: Graft versus Host Disease, KC: Keratinocytes
Supplemental table 5: Summary of immune phenotyping results.

| Subset                  | Long term values | 1 day after infusion | 7 days after infusion |
|-------------------------|------------------|----------------------|-----------------------|
|                         | R vs NR          | N pre inf R          | N pre inf NR          | Days after inf R | Days after inf NR | Days after inf R | Days after inf NR |
| Lymphocytes             | + 0.37 - 0.75 - 0.29 | + 0.0014 + 0.16      | + 0.0005 + 0.53      |
| CD3+ CD56-              | + 0.13 + 0.96 - 0.51 | + 0.013 + 0.21       | + 0.0012 + 0.54      |
| CD4+ CD8-               | + 0.26 - 0.63 - 0.25 | + 0.039 + 0.28       | + 0.0025 + 0.89      |
| CD4+ CD27+ CD45RA+      | + 0.0062 - 0.26 + 0.75 | + 0.011 + 0.53       | + 0.0021 - 0.26      |
| CD4+ CD27+ CD45RA-      | + 0.47 - 0.24 - 0.28 | + 0.041 + 0.23       | + 0.0012 + 0.82      |
| CD4+ CD27- CD45RA+      | - 0.65 + 0.36 - 0.13 | + 0.72 + 0.66        | - 0.93 + 0.94        |
| CD4+ CD27- CD45RA-      | + 0.63 + 0.51 - 0.26 | + 0.65 + 0.43        | + 0.89 + 0.63        |
| CD4+ FoxP3+             | + 0.29 - 0.46 + 0.17 | + 0.0042 + 0.19      | + 0.0057 - 0.83      |
| CD4- CD8+               | + 0.13 + 0.72 + 0.28 | + 0.062 + 0.43       | + 0.048 + 0.22       |
| CD8+ CD27+ CD45RA+      | + 0.17 + 0.92 + 0.03 | + 0.1 + 0.86        | + 0.0097 - 0.87      |
| CD8+ CD27+ CD45RA-      | - 0.67 + 0.29 + 0.47 | + 0.13 + 0.3         | + 0.052 + 0.64       |
| CD8+ CD27- CD45RA+      | + 0.16 + 0.82 + 0.49 | + 0.14 + 0.45        | + 0.26 + 0.94        |
| CD8+ CD27- CD45RA-      | + 0.17 + 0.44 + 0.87 | + 0.044 + 0.45       | + 0.26 + 0.34        |
| CD3- CD56+              | - 0.32 + 0.43 - 0.037 | + 0.25 + 0.93       | + 0.46 - 0.99        |
| CD56bright              | - 0.27 + 0.26 + 0.19 | + 0.13 - 0.7         | + 0.092 - 0.45       |
| CD56dim                 | - 0.32 + 0.45 - 0.033 | + 0.28 + 0.9        | + 0.5 + 0.95        |
| CD3+ CD56+              | - 0.39 + 0.84 - 0.22 | + 0.084 + 0.6        | + 0.4 + 0.46        |
| CD3+ CD56+ CD8+ CD4-    | + 0.37 + 0.77 + 0.56 | + 0.097 + 0.62      | + 0.24 + 0.13        |
| CD3+ CD56+ CD8- CD4+    | - 0.26 - 0.38 - 0.13 | + 0.21 + 0.84        | + 0.77 + 0.9        |
| CD3- CD19+              | + 0.17 - 0.31 + 0.14 | + 0.14 - 0.13        | + 0.012 - 0.34       |
| CD19+ CD27+ IgD-        | + 0.57 - 0.37 + 0.096 | + 0.2 - 0.15        | + 0.064 - 0.37       |
| CD19+ CD21hi CD27- IgD+ | + 0.048 - 0.32 - 0.73 | + 0.17 + 0.66       | + 0.011 + 0.5        |
| CD19+ CD5+              | - 0.72 + 0.75 + 0.13 | + 0.23 - 0.32        | + 0.066 - 0.37       |
| CD19+ IgD+ CD38low      | + 0.12 - 0.45 + 0.098 | + 0.13 - 0.12        | + 0.0093 - 0.36      |

Values displayed are p-values for the coefficients in the linear mixed effects models: 
- **Cell count** = \(A \times \text{Responder (0 or 1)} + B \times \text{Number of previous infusions}\) for long term changes and differences between responders and non-responders. 
- **Cell count** = \(A \times \text{Days since last infusion} + B \times \text{number of previous infusions}\) for evaluating short term changes. The random effect was set to patient identifier. Different shades of green correspond to different significance levels. +/− indicates a positive or negative coefficient. In R vs NR + indicates higher levels in responders. In Num pre inf + indicates a trend to increasing levels long term during the study. In Days after inf + indicates increasing levels short term since last infusion. R: Responder. NR: Non-Responder. pre: previous. inf: infusion.
### Supplemental figure 1: Response heatmap with final evaluation included

NIH organ score, NIH global score, range of motion (ROM) and body surface area (BSA) percentage involved with sclerosis. Time points are at study enrolment, end of MSC treatment and final evaluation, one year after end of treatment. Black boxes denote organs with response and red boxes denote organs with progression during MSC treatment. J: Joints, M: Muscles, F: Fascia, P: Prednisolone, Tac: Tacrolimus, MMF: Mycophenolate Mofetil, CNI: Calcineurin Inhibitors, ECP: Extracorporeal Photopheresis, MSC: Mesenchymal Stromal Cells

| Patient | Time point | Skin | Oral | Eyes | GI | Liver | Lungs | J/M/F | % BSA sclerosis | ROM | Global score | Follow up time (months) | Current immunosuppression |
|---------|------------|------|------|------|----|-------|-------|-------|-----------------|-----|--------------|--------------------------|--------------------------|
| 1       | Before MSC | 3    | 1    | 1    | 0  | 0     | 0     | 1     | 10              | 5   | 23           | 7                        | No immunosuppression     |
|         | End of treatment | 3   | 1    | 0    | 0  | 0     | 0     | 1     | 5              | 3   | 4           | 4                        |                          |
|         | 1 year after | 0   | 0    | 0    | 0  | 0     | 1     | 0     | 0              | 0   | 24           | 6                        |                          |
| 5       | Before MSC | 3    | 0    | 0    | 0  | 0     | 2     | 0     | 15              | 5   | 22           | 6                        | No immunosuppression     |
|         | End of treatment | 3   | 1    | 1    | 1  | 0     | 0     | 1     | 5              | 3   | 24           | 4                        |                          |
|         | 1 year after | 3   | 0    | 1    | 1  | 0     | 0     | 1     | 3              | 8   | 25           | 8                        |                          |
| 7       | Before MSC | 0    | 1    | 1    | 0  | 1     | 3     | 0     | 0              | 0   | 25           | 8                        | Lung transplant P+Tac+MMF|
|         | End of treatment | 0   | 2    | 0    | 0  | 0     | 1     | 0     | 0              | 0   | 25           | 6                        |                          |
|         | 1 year after | 0   | 2    | 1    | 0  | 0     | 1     | 0     | 0              | 0   | 25           | 7                        |                          |
| 8       | Before MSC | 3    | 0    | 2    | 0  | 0     | 0     | 3     | 15              | 10  | 13           | 8                        | No steroids, tapering CNI|
|         | End of treatment | 3   | 1    | 2    | 0  | 0     | 0     | 2     | 10             | 10  | 13           | 8                        |                          |
|         | 1 year after | 3   | 1    | 2    | 1  | 0     | 0     | 3     | 10             | 10  | 12           | 7                        |                          |
| 9       | Before MSC | 3    | 1    | 3    | 0  | 0     | 2     | 2     | 50              | 45  | 17           | 6                        | No steroids, tapering CNI|
|         | End of treatment | 3   | 1    | 1    | 1  | 0     | 2     | 2     | 45             | 45  | 17           | 6                        |                          |
|         | 1 year after | 3   | 1    | 2    | 1  | 0     | 2     | 2     | 40             | 40  | 17           | 6                        |                          |
| 11      | Before MSC | 3    | 2    | 2    | 0  | 0     | 0     | 2     | 15              | 15  | 14           | 6                        | Addition of ECP & Rituximab|
|         | End of treatment | 3   | 0    | 1    | 0  | 0     | 0     | 2     | 1              | 1   | 15           | 4                        |                          |
|         | 1 year after | 3   | 0    | 3    | 0  | 1     | 0     | 2     | 2.5            | 2.5 | 13           | 6                        |                          |
| 2       | Before MSC | 2    | 2    | 0    | 0  | 0     | 2     | 0     | 0              | 0   | 22           | 6                        | Increased                |
|         | End of treatment | 2   | 3    | 0    | 0  | 0     | 2     | 0     | 0              | 0   | 25           | 8                        |                          |
|         | 1 year after | 1   | 3    | 0    | 1  | 0     | 2     | 0     | 0              | 0   | 25           | 6                        |                          |
| 6       | Before MSC | 3    | 0    | 2    | 2  | 1     | 2     | 3     | 30              | 30  | 11           | 8                        | Increased                |
|         | End of treatment | 3   | 2    | 2    | 1  | 2     | 2     | 3     | 30             | 30  | 11           | 8                        |                          |
|         | 1 year after | 3   | 2    | 1    | 2  | 1     | 2     | 3     | 35             | 35  | 9            | 9                        |                          |
| 10      | Before MSC | 3    | 2    | 2    | 1  | 2     | 0     | 3     | 20              | 20  | 14           | 8                        | Died                     |
|         | End of treatment | 3   | 1    | 2    | 2  | 1     | 0     | 3     | 20             | 20  | 16           | 8                        |                          |

**Organ score**
0 | 1 | 2 | 3

**Global score**
0 | 2 | 4 | 6 | 8 | 10

**BSA % sclerosis**
0 | 10 | 20 | 30 | 40 | 50

**Range of motion (ROM)**
25 | 20 | 15 | 10 | 5

**Organ system with response**

**Organ system with progression**
### Treatment of Chronic GvHD with Mesenchymal Stromal Cells Induces Durable Responses; a Phase II Study

von Bahr L & Boberg E et al

#### Supplemental figure 2: Timeline of clinical events during and after the study.

The top row of each patient lists cGvHD treatments before and after the study. At the end of each timeline, current immunosuppressive therapy is listed in italic letters. The bottom row of each patient lists cGvHD status and clinical events. The heatmap coloured cells shows the global cGvHD grade at each study evaluation. Downwards pointing arrows depict cGvHD progression, new cGvHD treatments or death or relapse after inclusion.

* Exact date of cGvHD onset unknown – patient is from another part of Sweden. ** Likely cause of death was sudden cardiac event. *** Cause of death was progressive cGvHD. cGvHD: chronic Graft versus Host Disease, CyA: Cyclosporine A, P: Prednisolone, FMC: Fetal Membrane Cells, Tac: Tacrolimus, Sir: Sirolimus, Eve: Everolimus, Thal: Thalidomide, Ruxo: Ruxolitinib, Infl: Infliximab, MMF: Mycophenolate Mofetil, MTX: Methotrexate, Ima: Imatinib, ChK: Chlorokine, R: Rituximab, C: Cyclophosphamide.

| Patient | Events before study inclusion | Duration of study | Events after study completion | Duration of follow-up (months) | Follow-up until | Hematological relapse at last follow up | Alive at last follow up |
|---------|-------------------------------|-------------------|-------------------------------|-------------------------------|-----------------|---------------------------------------|------------------------|
| 1       | CyA<P, MMF, ECP, MTX          | MSC x 6           | MSC x 1                       | ▼ P and CyA tapered and withdrawn. | No cGvHD treatment | No cGvHD initiation | 99 | 2019 oct | No | Yes |
| 5       | CyA<0, MTX                    | MSC x 6           | MSC x 2                       | ▼ P and CyA tapered and withdrawn. | ▼ Ruxo for 3 months | No current treatment | 87 | 2019 oct | No | Yes |
| 7       | CyA<0, IF                      | MSC x 6           | MSC x 1                       | ▼ Several therapies. Finally lung transplantation. | P + Tac = MMF   | ▼ cGvHD brief recurrence | 80 | 2019 oct | No | Yes |
| 8       | CyA<P, Sir, MMF, Inf, R, Tac, ECP, Ima, chK, FMC | MSC x 6           | MSC x 3                       | Tapering to lost to CyA follow up | P + Tac + MMF | No | 34 | 2016 Feb | No | Yes |
| 9       | Tac<P, Thal, Ima/minib, ECP | MSC x 6           | MSC x 3                       | Entosipro briefly. ▼ Ruxo 1 year. Mada of cGvHD worse. | Tapering CyA  | Some remaining cGvHD | 72 | 2019 Nov | No | Yes |
| 11      | Tac<P, Ima/minib             | MSC x 6           | MSC x 3                       | ▼ Ruxo, ECP          | Ruxo + ECP      | No | 56 | 2019 oct | No | Yes |
| 2       | CyA<P, Sir, Ima, ECP         | MSC x 6           | MSC x 6                       | ▼ CyA, Eve, Thal, Ruxo (still treated) | Ruxo            | No | 98 | 2019 oct | No | Yes |
| 6       | CyA<0, MTX, R, ECP, MMF      | MSC x 6           | MSC x 3                       | ▼ Many therapies attempted | P + CyA + C    | Responding to Ruxo | 82 | 2019 oct | No | Yes |
| 10      | CyA+MMF, P, R, MTX           | MSC x 6           | ▼ Ruxolitinib                 | ▼ Died              | No | 22 | 2016 sep | No | No | No*** |
| 3       | Tac<P, R                     | MSC x 3           | relapse                      |             | Yes | 3 | 2012 July | No | Yes | No*** |
| 4       | CyA<0                        | MSC x 1           | relapse                      | ▼ Died              | No | 2 | 2013 mar | No | No | No*** |

Years from study inclusion: -8 -7 -6 -5 -4 -3 -2 -1 0 1/2 1 2 3 4 5 6 7 8
Supplemental figure 3: Basic B- and T-cell subsets. A and B: Representative plots of the flow cytometry analysis. C and D: Relative and absolute numbers of T-cells and B-cells were comparable between R and NR throughout the study. Error bars show mean +/- SEM.
Supplemental figure 4: Responders had higher proportion of CD4+ CCR7+ T-cells compared to non-responders. The absolute numbers of CD4+ CCR7+ T-cells were higher in R compared to NR. P-value for absolute numbers with t-test and for relative numbers with Wilcoxon’s rank sum test. * = p < 0.05, ** = p < 0.005
Supplemental figure 5: Activated naïve B-cells. A: Analysis was performed using IgD and CD38 to differentiate B-cells at different stages of development according to\(^1\). Representative plots and legend is shown. B: Relative numbers of B-cells at different development stages before first infusion. C: Relative and absolute numbers of activated naïve B-cells (B2) are higher in responders throughout the study. D: Absolute numbers of activated naïve B-cells increase 7 days after each MSC infusion. P-values for relative numbers are calculated using Wilcoxon’s rank sum test. P-values in D are derived from the mixed effects model and represent the significance of the factor Days since last infusion. The P-values displayed are the P-values for coefficient A. * = p < 0.05, ** = p < 0.005. Error bars show mean +/- SEM. MSC: Mesenchymal Stromal Cell, CG: Germinal Center
Supplemental figure 6: Proposed biomarkers of response to MSC therapy in cGvHD: Relative changes of CXCL9 and CXCL10 concentrations from before the first infusion to before the 6:th infusion (5 months after treatment start) are shown. The relative change in CXCL10 was completely differential between responders and non-responders, increasing in non-responders and decreasing in responders. MSC: Mesenchymal Stromal Cell, cGvHD: chronic Graft-versus-Host Disease, CXCL: Chemokine (C-X-C motif) ligand.
Supplemental figure 7: Treg subpopulation analysis revealed functional differences in Tregs between R and NR: A: representative plots of the flow cytometry analysis. Tregs were subdivided according to Sakaguchi et al\textsuperscript{8} using CD45RA and FoxP3. B: R had a higher proportion of naïve Tregs (CD45RA+ FoxP3lo) while NR had a higher proportion of non-Tregs (CD45RA- FoxP3lo) among total Tregs. Data from infusion 3. P-values obtained by Wilcoxon rank-sum test. * = $p < 0.05$. Error bars show mean +/- SEM. MSC: Mesenchymal Stromal Cell, Treg: regulatory T-cell, Breg: regulatory B-cell.
References
1. Peng Y, Chen X, Liu Q, et al. Alteration of naive and memory B-cell subset in chronic graft-versus-host disease patients after treatment with mesenchymal stromal cells. Stem Cells Transl Med. 2014;3(9):1023-1031.
2. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet. 2008;371(9624):1579-1586.
3. Haynesworth SE, Goshima J, Goldberg VM, Caplan AI. Characterization of cells with osteogenic potential from human marrow. Bone. 1992;13(1):81-88.
4. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol. 2003;57(1):11-20.
5. 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.
6. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2016.
7. Bates D, Maechler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015;67(1):1-48.
8. Miyara M, Yoshioka Y, Kitoh A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity. 2009;30(6):899-911.