Mesenchymal stem cells expanded *in vitro* with human serum for the treatment of acute and chronic graft-*versus*-host disease: results of a phase I/II clinical trial

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Supplementary Appendix

Methods

Bone marrow aspiration

MSC expansion and characterization

The procedure was performed under GMP conditions. Mononuclear cells from bone marow (BM) were isolated by a density-gradient centrifugation (Ficoll-Paque, GE Healthcare Bio-Sciences, AB, Uppsala, Sweden). Briefly, 2 volumes of BM were added to 1 volume of Ficoll and centrifuged at 800g for 20 min. Mononuclear cells were resuspended and plated in non-coated 175-cm^2 polystyrene culture flasks (Corning Costar, Celbio, Milan, Italy) in modified Eagle's medium-α (α -MEM) with 1% penicillin/streptomycin (Pen/Strep; Gibco) at a concentration of 160,000 cells/cm^2. The medium was supplemented with 10% autologous serum (AS) and 1 ng/mL basic fibroblast growth factor (bFGF, Sigma, St Louis, MO, USA). In cases of slow growth, 5% PL was added to the medium.

Cells were incubated at 37°C in an atmosphere with 90% humidity and 5% CO_2. The medium was completely replaced twice a week. The first passage was performed when cells reached a confluence of 80% (days 10-15). MSC were then replated at a concentration of 1,000 to 5,000 cells/cm^2 and passaged when 80% confluence was reached.[1]

Cells were characterized by flow cytometry and differentiation assays. For flow cytometry analysis, 200,000 MSCs were incubated with the following antibodies: CD34-allophycocyanin (APC), CD44-fluorescein isothiocyanate (FITC), CD45-peridinin chlorophyll protein (PerCP), CD73-phycoerytrin (PE) and CD90-FITC (BD, San Diego, CA, USA). A cytometer FACSCalibur was used and data were analyzed using the Paint-

Online Supplementary Table S1. Prior immunosuppressive treatment and time of response of patients receiving MSC.

ID / a-cGVHD	Prior immunosuppressive treatment	Concurrent
1 acute	Steroids, pentostatin, infliximab	Steroids
4 acute	Steroids, pentostatin	Steroids
9 acute	Steroids, pentostatin	Steroids
16 acute	Steroids, pentostatin	Steroids
22 acute	Steroids, pentostatin, mycophenolate	Steroids, mycophenolate
24 acute	Steroids, pentostatin, beclomethasone, high-dose prednisone	High-dose prednisone
25 acute	Beclomethasone, steroids	Beclomethasone, steroids
26 acute	Steroids, pentostatin	Steroids
27 acute	Steroids, rapamycin	Steroids, rapamycin
29 acute	Steroids, pentostatin	Steroids
2 chronic	Steroids, rapamycin, rituximab	Steroids, rapamycin
7 chronic	Steroids, mycophenolate, ciclosporin A, rapamycin, inolimomab	Steroids, mycophenolate, ciclosporin A, rapamycin
11 chronic	Steroids, tacrolimus	Steroids, tacrolimus
13 chronic	Steroids, rituximab	Steroids
14 chronic	Steroids, rapamycin, rituximab	Steroids, rapamycin
18 chronic	Steroids, rituximab	Steroids
20 chronic	Steroids, rituximab, rapamycin, PUVA	Steroids
21 chronic	Steroids, tacrolimus, mycophenolate	Tacrolimus, mycophenolate

A-Gate program (BD), as previously reported.[1,2]

Osteogenic, adipogeneic and chondrogeneic differentiation were performed as previously reported.[1-3] Autologous serum was obtained using autologous plasma, as described elsewhere.[3]

To obtain PL, 4-5 pooled platelets from the Blood Bank of the Clínica Universitaria de Navarra or from the University Hospital of Salamanca were frozen at -80°C and then thawed at 37°C. The platelets were used despite the ABO type, the volume of each pool was 300-400 mL. Samples were centrifuged at 900g for 30 min and the supernatant was used as a supplement. To avoid gel formation 10 UI of heparin per 5 mL of medium were added before use.[1,2]

Results

MSC expansion and donor safety

Overall, 28 expansions were performed. Donor data are shown in Table 1 available in the main text. Donors underwent iliac crest aspiration of 5-10 mL of BM until a total volume of 100 mL was obtained. Aspiration was performed under short propofol sedation under surgical conditions following strict aseptic measures. The entire procedure lasted less than 15 min from the beginning of anesthesia.

No severe adverse event was observed among healthy donors. Three of them reported local pain which responded to the usual painkillers. Although this procedure may cause some inconvenience to the donor no adverse events were reported after plasmapheresis. Eleven expansions were not administered in this clinical trial and the reasons are shown in Table 1 which is available in the main text. However, the cells could be used for other patients when the trial finished after informed consent was obtained from donors.

Patients

Online Supplementary Table S1 shows the prior immunosuppressive treatment and time of response. After MSC steroids, dose was progressively lowered.

References

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