How I treat refractory chronic graft-versus-host disease

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Why Does GVHD Occur?

- Donor immune cells contained in the allograft mount an attack against the recipient antigens
- Cells in the graft see recipient tissue as foreign
- Immunocompetent cells begin to attack host cells both normal and those damaged by illness or by the preconditioning

Causes and Risk Factors

- Incompatible HLA match
- Older age of recipient and/or donor
- Multiparous female donor to male recipient
- Stem cells from peripheral blood rather than bone marrow or UCB
- Ineffective GVHD prophylaxis
- Intense preconditioning
- CMV serostatus

Nassereddine. Anticancer Res. 2017;37:1547. Zeiser. NEJM. 2017;377:2167. Copelan. Blood Rev. 2019;34:34

Slide credit:

Non-HLA genetic factors in development GvHD

- Examples include polymorphisms in the genes encoding cytokines such as the tumor necrosis factors, the interleukins (IL-1, IL-6, and IL-10), interferon gamma (IFN-γ), and transforming growth factor-β3 (TGF-β3) and the expression of the killer cell immunoglobulin-like receptors (KIR).

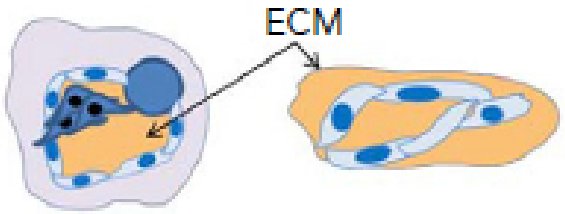
'Red Flag' Poor Prognostic Marker	Ongoing cGVHD Pathophysiology (histochemical patient/basic science data)	Additional Urgent Work-up
A *↓ Forced expiratory volume (FEV1) if FEV1/FVC <0.7 Hypoxia after 2 min. ambulation Dry cough, abnormal lung exam	Airway blockage and/or constriction  	High resolution expiratory chest CT Scan Diagnostic Bronchoscopy (rule out infection)
B *↑3x normal Total Bilirubin (T. bili) *↑3x normal Alkaline phosphatase (Alk Phos, AP)	Biliary Duct 'withering and drop-out' 	Serum PCR tests for viruses (CMV, HSV, adeno, EBV, Hepatitis virus if indicated) Consider liver biopsy to rule out drug toxicity or infection
C * >10% weight loss Acute diarrhea	Mouth ulcerations → precludes eating Esophageal strictures Lower gut GVHD	PCR of feces for enteroviruses Endoscopy
D ↓Platelets	Immune attack → Bone marrow, Thymus, Secondary lymph organs 	Plasma virus Blood culture Bone marrow biopsy

Figure 2. Assessment of worsening cGVHD reflective of cGVHD pathophysiology that requires urgent attention. (A) Decrease in FEV1 may reflect pathology of

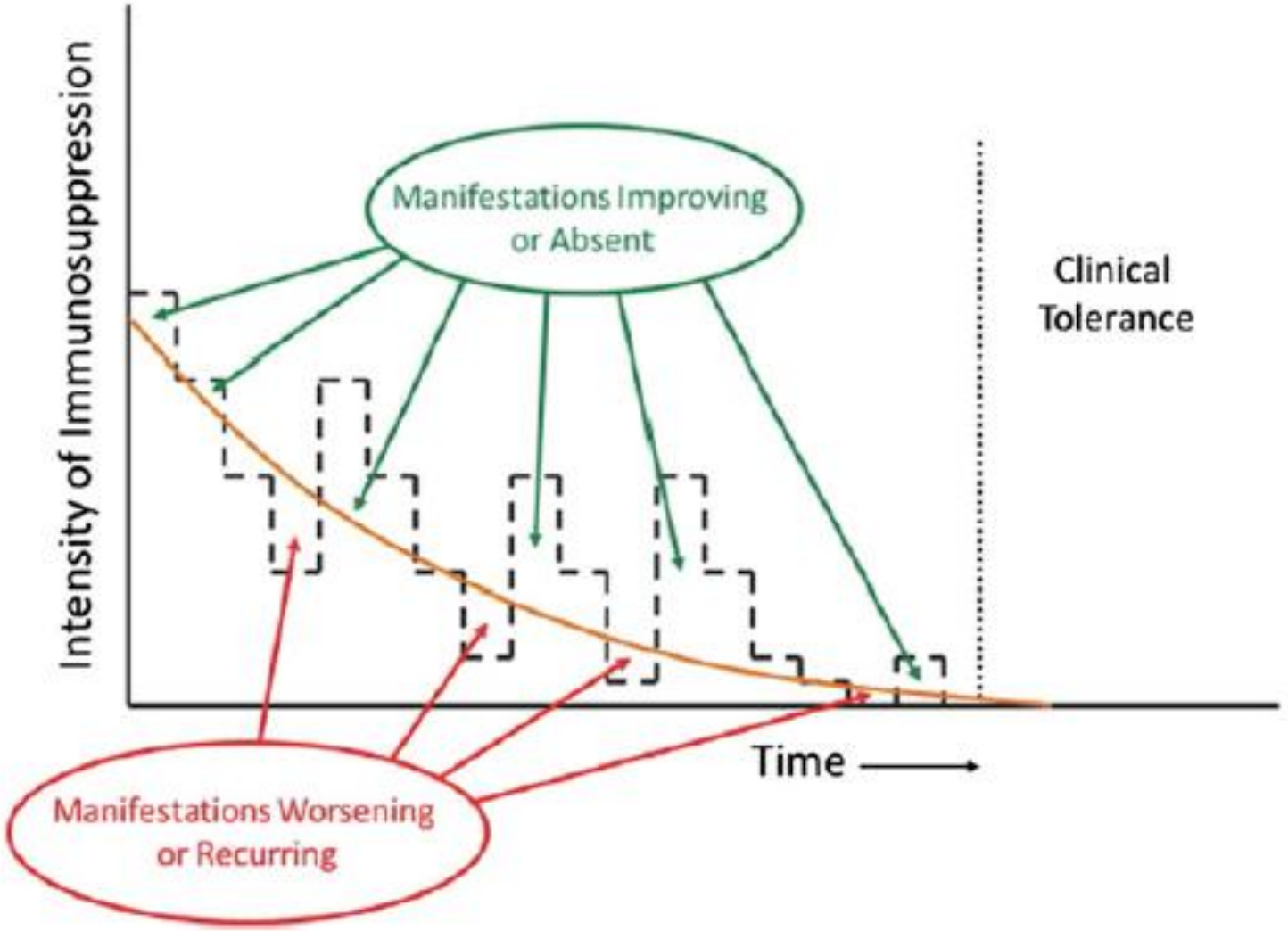
Introduction

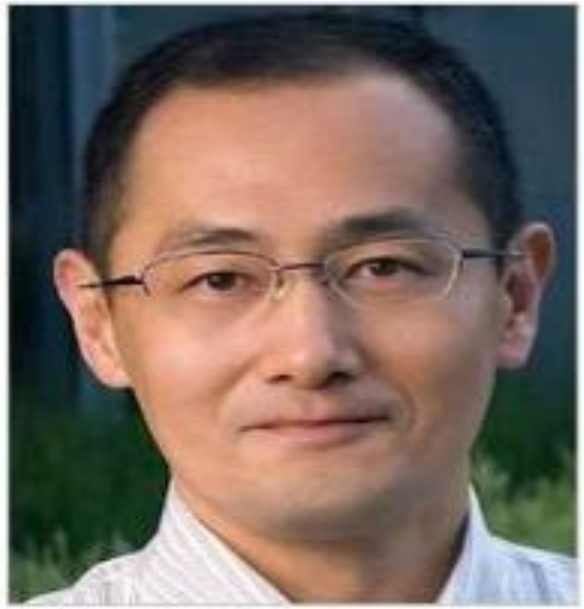
- With > 30 000 allogeneic worldwide each year 35% to 50% of recipients cGVHD.
- > 20% of patients with cGVHD achieve a durable partial (PR) or complete response (CR) .
- survive 1 year after initial therapy without additional systemic therapy.
- treatment refractory cGVHD is relatively common and patients likely will require ongoing therapy.

Goal of GVHD treatment

- long-term goal of GVHD treatment is the development of immunologic tolerance, indicated by successful withdrawal of all immunosuppressive treatment without recurrence or clinically significant exacerbation of disease manifestations.
- The current therapeutic approach functions primarily to prevent immune mediated damage, while awaiting the development of tolerance.

Continuous recalibration of immunosuppressive treatment in order to avoid over-treatment or under-treatment.





Shinya Yamanaka:	James A Thomson
OCT3/4	OCT3/4,
SOX2	SOX2
C-myc	NANOG
Klf4	LIN28

iPSCs were 1st produced in 2007 from human cells by Shinya Yamanka team at Kyoto University Japan, and by James Thomson's team at the University of Wisconsin-Madison. independently

Agents used for secondary treatment of chronic GVHD

Table 6. Agents used for secondary treatment of chronic GVHD*

Treatment	% Overall response*	Survival
ECP	65-70	70%-78% at 1 y
Rituximab	66-86	72% at 1 y
Imatinib	22-79	75%-84% at 1.5 y
Pentostatin	53-56	34%-60% at 1-3 y
Mesenchymal stem cells	50-74	78% at 2 y
Mycophenolate mofetil	26-64	67%-96% at 1 y
mTOR inhibitor	76	72% at 3 y
Interleukin-2	52	Not reported
Other therapies summarized in other reviews**		
Calcineurin inhibitor		
High-dose methylprednisolone		
Methotrexate		
Thalidomide		
Hydroxychloroquine		
Clofazimine		
Thoracoabdominal irradiation		
Alefacept		
Infliximab		
Etanercept[70]		

Table 1. Adverse reactions of commonly used therapies in refractory chronic GVHD[14]

Agent	Potential major adverse effects (with major study citations)	Common (>10%) generally less severe adverse effects
Bortezomib	Peripheral neuropathy, thrombocytopenia, malignancy relapse[106]	Herpes virus reactivation
ECP	Vascular access complications[107]	Thrombocytopenia
FAM	New FDA MedWatch warning; warning only applies to azithromycin use in prophylactic (not treatment) setting[108,109]	
Ibrutinib (Imbruvica R)	Pneumonia,[29] impaired platelet function	Fatigue, muscle pain, peripheral edema
Imatinib		Peripheral edema
Interleukin-2	Injection site induration, infections[36]	Constitutional flu-like symptoms
MMF (Cellcept)	Viral reactivation, hypertension, pneumonia, posttransplantation lymphoproliferative disease[110]	GI toxicity, neutropenia, leukopenia
Pamolidomide	Tremor, muscle cramps, peripheral neuropathy[111]	Skin rash
Rituximab (Rituxan R)	Infection, late neutropenia[38,39,112]	B lymphopenia
Ruxolitinib (Jakafi R)	Viral reactivation/infection, bacterial infections[35]	Cytopenias
Sirolimus (Rapamune)	TAM when used in combination with calcineurin inhibitors, renal insufficiency,[113] proteinuria	Peripheral edema, hyperlipidemia, cytopenias

This list of agents represents a fraction of agents being actively evaluated. Preferred use of any agent still requires validation via larger clinical trials.

ECP, extracorporeal photopheresis; FAM, fluticasone, azithromycin, and montelukast; FDA, US Food and Drug Administration; GI, gastrointestinal; MMF, mycophenolate mofetil; TAM, transplantation-associated microangiopathy.

Goal of GVHD treatment

- A classical therapeutic approach to the prevention and treatment of cGVHD has been broad immunosuppression, but more recently adjuvant immunotherapies have been tested.
- Immunomodulatory approaches with T cells, including chimeric antigen receptor (CAR) and regulatory T cells (Treg), with natural killer (NK) cells and innate lymphoid cells (ILCs), and finally with mesenchymal stromal cells (MSC) and extracellular vesicles thereof.

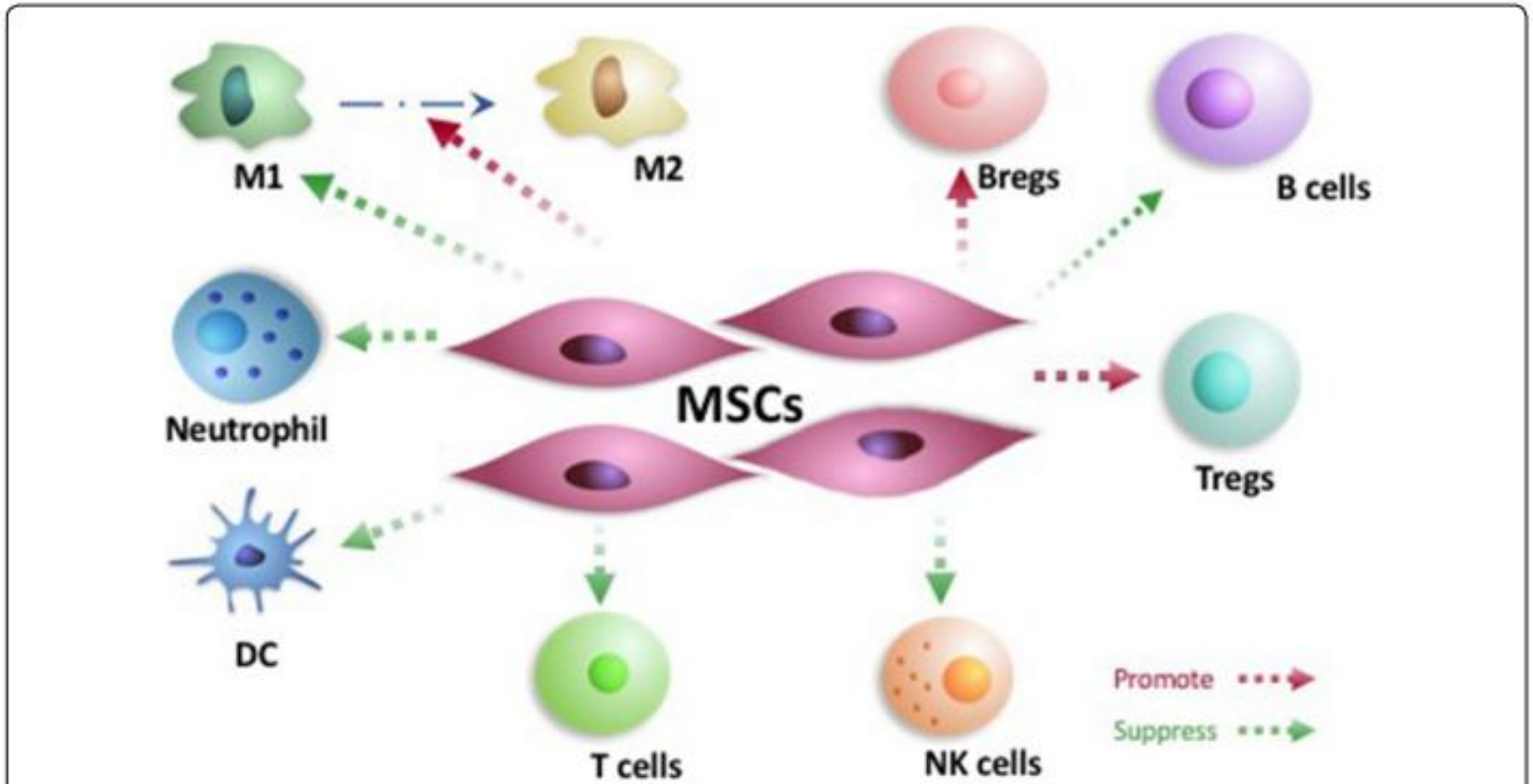


Fig. 1 Regulation of immune cells related with MSCs. MSCs interrelate with the regulation of immunoregulatory function of various cells. In terms of T cells, MSCs inhibit the proliferation and activation of T cell. Meanwhile, sums of data address that conventional T cells may transform to regulatory T cells (including CD4 + CD25 + FoxP3 + Treg, CD8 + CD28−Treg and IL-10 + Tr1) given the function of MSCs. Further, with direct contact between cells and transforming the phenotype of natural killer (NK) cells, MSCs has also been proven highly effective in inhibiting the proliferation, cytotoxic effect and the secretion of various cytokines of NK cells. For B cells, MSCs can render the cell cycle stagnant in the G0/G1 phase and trigger the inhibition of B cells proliferation. Moreover, recent studies indicated that MSCs can enlarge the proportion of regulatory B cells (Bregs), such as $CD5^{+}$ B cells, $CD19^{+}$ $CD24^{high}CD38^{high}$ B cells, and other Bregs secreting IL-10. Jiang also put forward that human MSCs, as the most efficient one among the antigen-presenting cells (APCs), can inhibit the transformation from monocyte into dendritic cells (DCs). In the meantime, MSCs can inhibit the function of M1 macrophage cells, and induce the transformation of M1 macrophage cells to M2 macrophage cells. Also, MSCs are associated with the suppression of neutrophils

mesenchymal stromal cells (MSCs)

- MSCs are fibroblast-like multipotent progenitor cells with immunosuppressive properties in vitro and in vivo.
- Indeed, overall response rates of MSC for aGVHD range from 30-80% .

- Due to many trial patients had been heavily pre- treated, and due to different application procedures.
- Identification of adequate biomarkers could help to personalize and adapt effective cGVHD therapy or even prevention.

MSC treatment

- MSC significant increases in naïve T cells, B cells, and Tregs 7 days after each infusion.
- Induction of CD5+ regulatory B cells with reduced inflammatory cytokine production by T cells.
- CXCL9 and CXCL10 chemokine levels were strongly elevated in responders as compared to non- responders.
- Protective against CD8+ endothelial-specific cytotoxic T cells.
- Rendering them as potential new biomarkers of MSC therapy outcome.

Factors determining survival rate

- There was a level of HLA-mismatch between donor and recipient , conditioning regimens , length and nature of immunosuppression , as well as the number of repeated MSC doses .
- MSCs may also work in patients with severe refractory cGVHD and still induce durable responses.

Human placental mesenchymal stromal cell-derived exosome-enriched extracellular vesicles for chronic cutaneous graft-versus-host disease

A case report

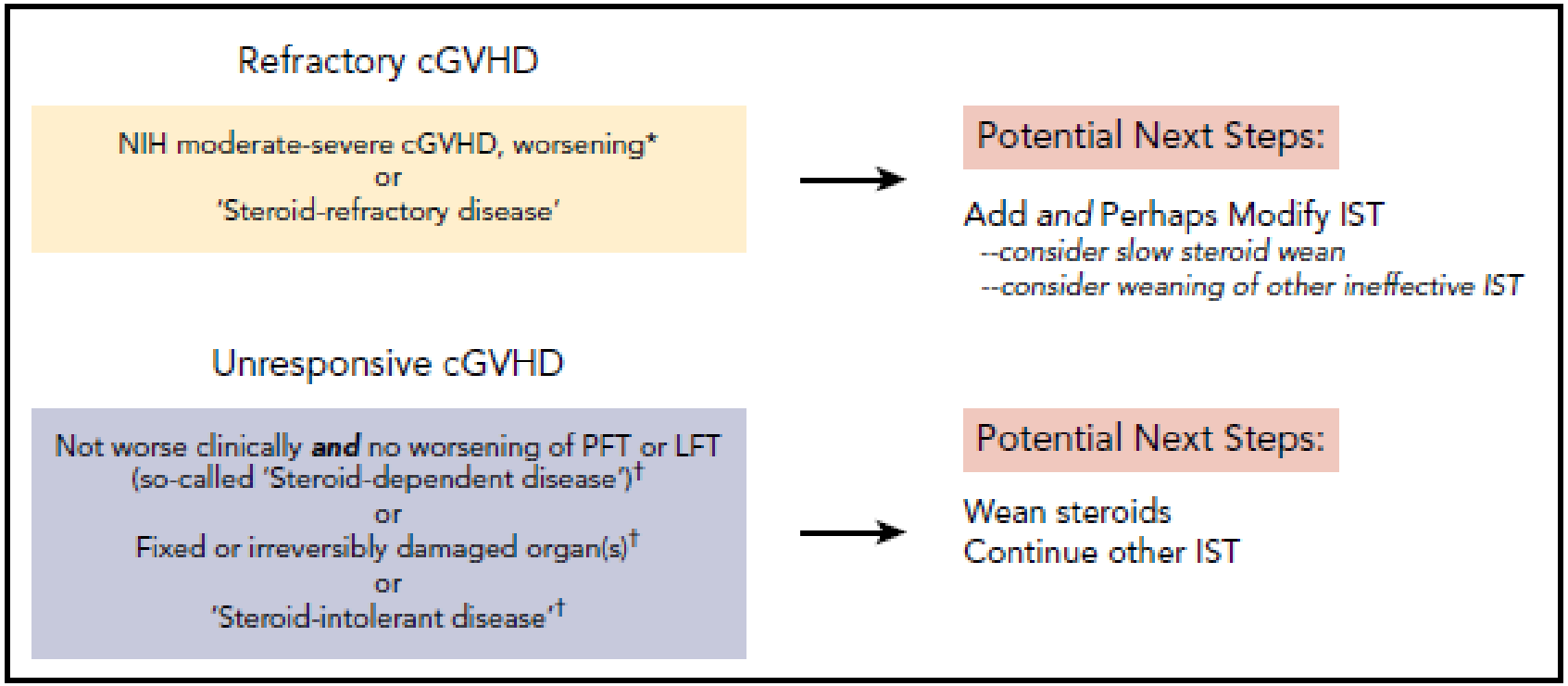


Figure 1. NIH global severity assessments to determine need for intervention in patients with ongoing cGVHD.[24,25] Our approach to patients seen in our multidisciplinary

Clinical application of MSCs in GVHD

- The first application of MSCs in GVHD was reported in 2004 and achieved a striking clinical response .
- The clinical application of MSCs has been a new research hotspot for worldwide GVHD treatment ever since.

MSC-EVs for treatment of cGvHD:

- MSC-EVs are immunologically active and induce elevated expression of anti-inflammatory IL10 and TGFb1, and reduced levels of pro-inflammatory IL1b, IL6, TNFA and IL12P40.

- Induce Tregs both in vitro and in vivo, and MSC-EV infusion has been shown to enhance the survival of allogeneic skin grafts .
- Immunosuppressive activities of MSC- EVs mediated by activation of MYD88-dependant signaling in monocytes to induce an anti-inflammatory M2-like phenotype via a TLR-dependent signaling pathway.

MSC-EVs for treatment of cGvHD:

- Activated monocytes then polarize activated Tconvs to Tregs, inducing Treg expansion and an attenuated activated immune system.
- MSC-EV have therapeutic treating pulmonary complications of cGVHD.
- During the course of MSC-EV therapy, pro- inflammatory cytokine response reduced (IL-1b, TNF-a and IFN-ɣ) clinical symptoms of GVHD improved, cutaneous and mucosal GVHD, which was stable 4 months following completion of therapy .

METHODS AND PATIENT

2.1 | Cell isolation

hPMSCs derived from human placenta (single donor) tissue were isolated and identified by the method has been described by Pelekanos et al.[12] Also, the donor's blood sample was negative for viral infections including mycoplasma, cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) evaluated by polymerase chain reaction (PCR). The cells exhibited surface expression of mesenchymal markers (CD73, CD 105, CD90 and CD 44; Figure S1) and were negative for haematopoietic markers (CD45, CD 34 and HLA-DR) identified as hPMSCs. Also, the cells at passages 2–3 were assessed for osteogenic, adipogenic and chondrogenic differentiation potentials.

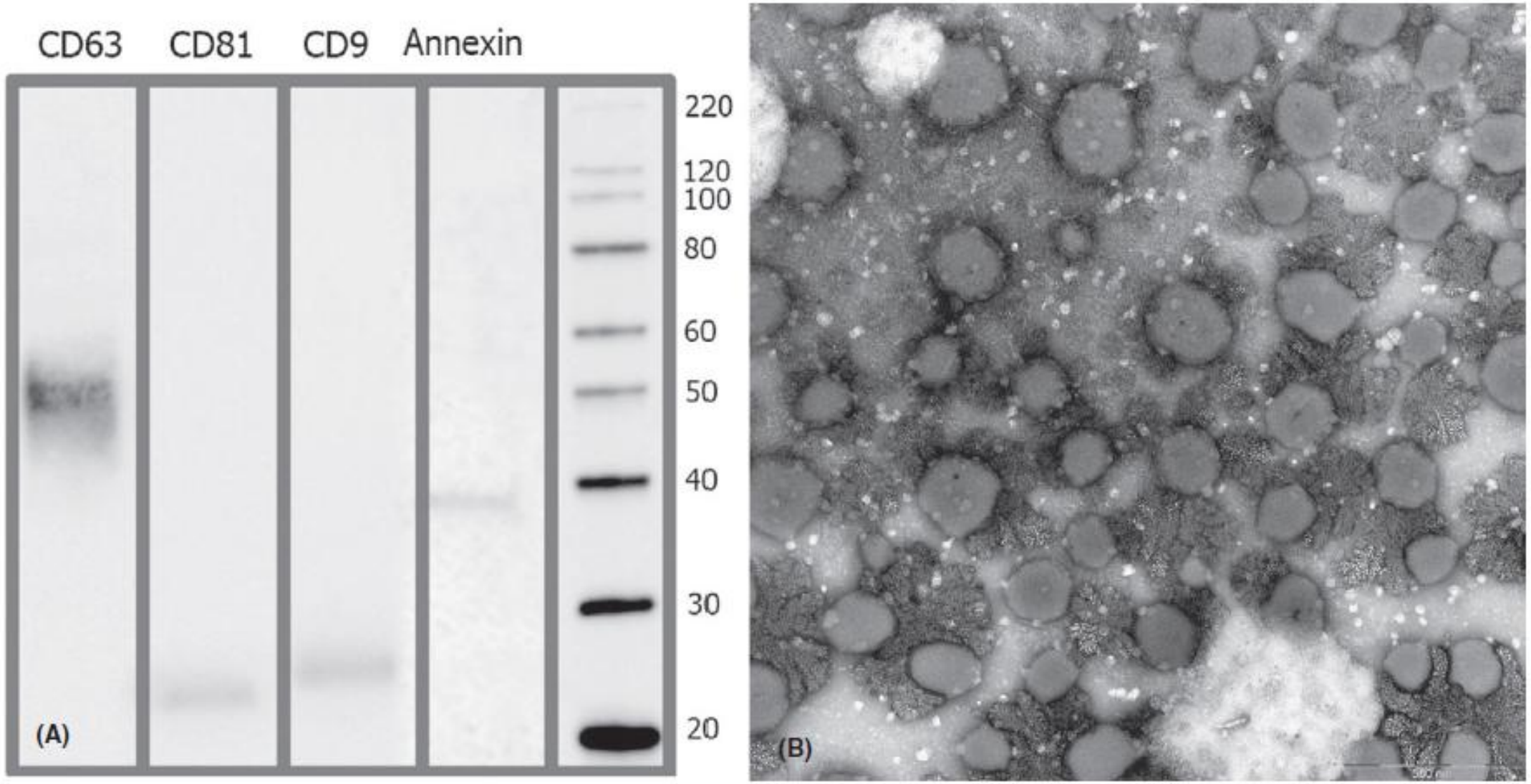


FIGURE 1 (A) Western blot analysis of purified extracellular vesicles. The lines are showing CD63, CD81, CD9 and annexin as markers for extracellular vesicles form the human placental mesenchymal stem cells. Numbers. (B) Transmission electron microscopy of purified exosomes-enriched extracellular vesicles

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SHORT COMMUNICATION

WILEY

Human placental mesenchymal stromal cell-derived exosome-enriched extracellular vesicles for chronic cutaneous graft-versus-host disease: A case report

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Keywords: cutaneous GVHD, exosome, graft-versus-host disease, human mesenchymal stromal cell

3 | CASE PRESENTATION

The patient was a 39-year-old Caucasian male diagnosed with AML type M4 5 years ago (April 2016). Following routine treatments and after reaching complete remission, he underwent PBSCT (one session) from an identical donor (brother). After the transplantation, he presented with acute gastrointestinal (GI) GVHD on a prophylaxis immune suppression regime, with his symptoms and signs brought under control through increasing corticosteroid and cyclosporin dosages. After a year, the cutaneous cGVHD started, which did not respond to extracorporeal photopheresis (12 sessions), tacrolimus, imatinib and high-dose corticosteroids. Also, during the last 18 months, the patient was receiving 5 mg per day of prednisolone and 50 mg and 25 mg per odds and even days (respectively) of cyclosporine for 18 months.

The frequency of organ involvement

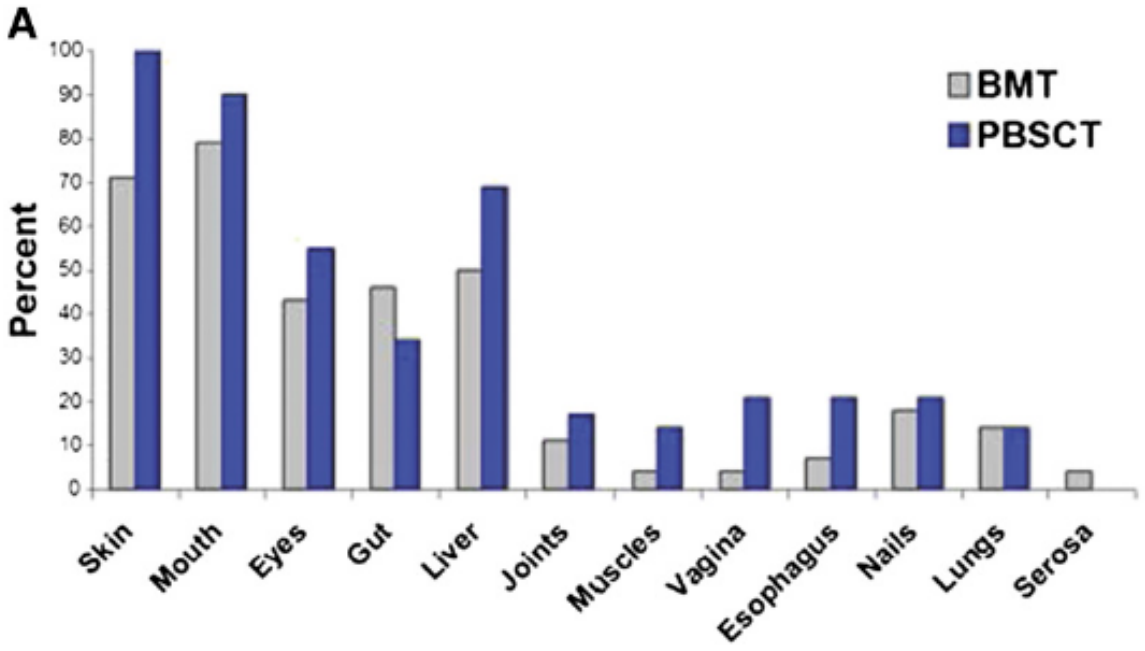
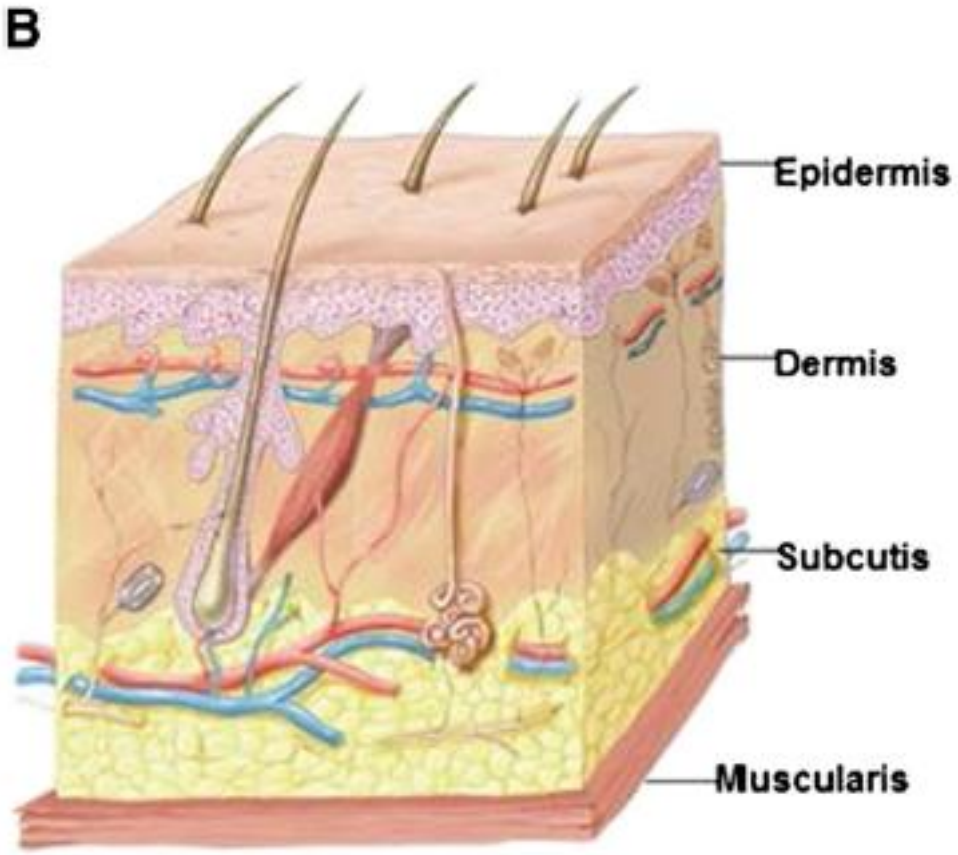


Figure 1. The frequency of involvement by chronic GVHD varies across organs and sites and is higher after HCT with mobilized blood cells as compared with marrow. (A) The most frequently involved organs and sites are the skin, mouth, eyes, gastrointestinal tract, and liver.[3] (B) Chronic GVHD can affect all layers of the skin. Photographs of each manifestation in italic may be found in the supplemental Data, available on the *Blood* Web site. Artwork by Delilah Cohn, MFA, CMI, used with permission.

Chronic GVHD can affect all layers of skin

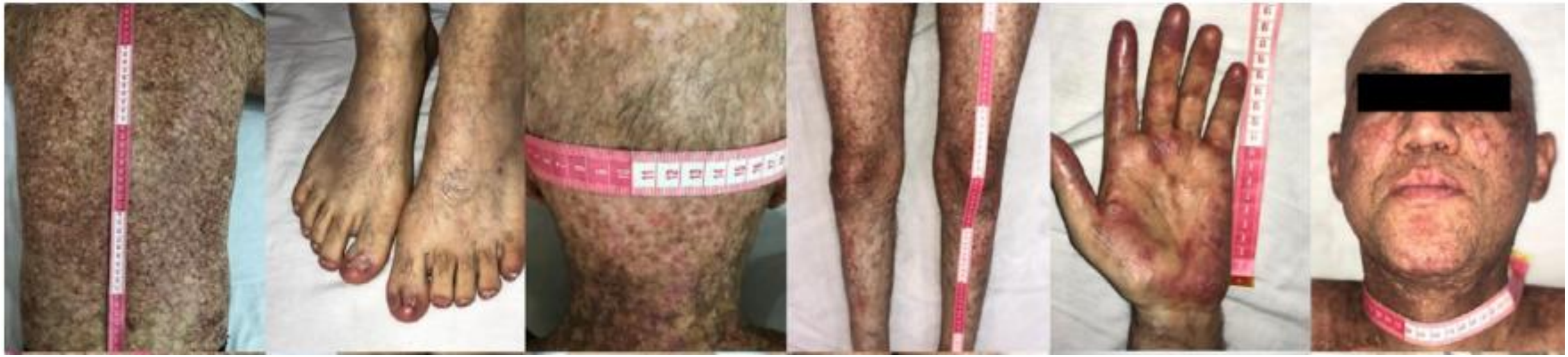


Manifestations

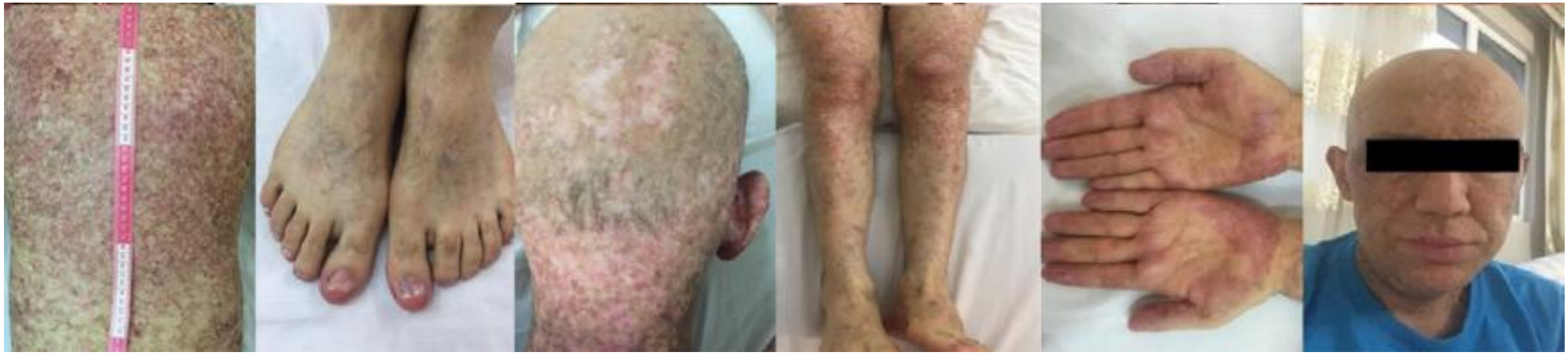
- *Lichen planus-like feature*
- *Lichen sclerosus-like*
- *Poikiloderma*
- *Keratosis pilaris*
- Depigmentation
- Alopecia

- Dermal sclerosis
- Edema (early fasciitis / early sclerosis)
- *Deep Sclerosis*
- Fasciitis
- Myositis

Cutaneous chronic graft-versus- host disease in the patient. pictures represent before the extracellular vesicles therapy



Cutaneous chronic graft-versus- host disease in the patient. pictures represent after 4 ,extracellular vesicles therapy



DISCUSSION

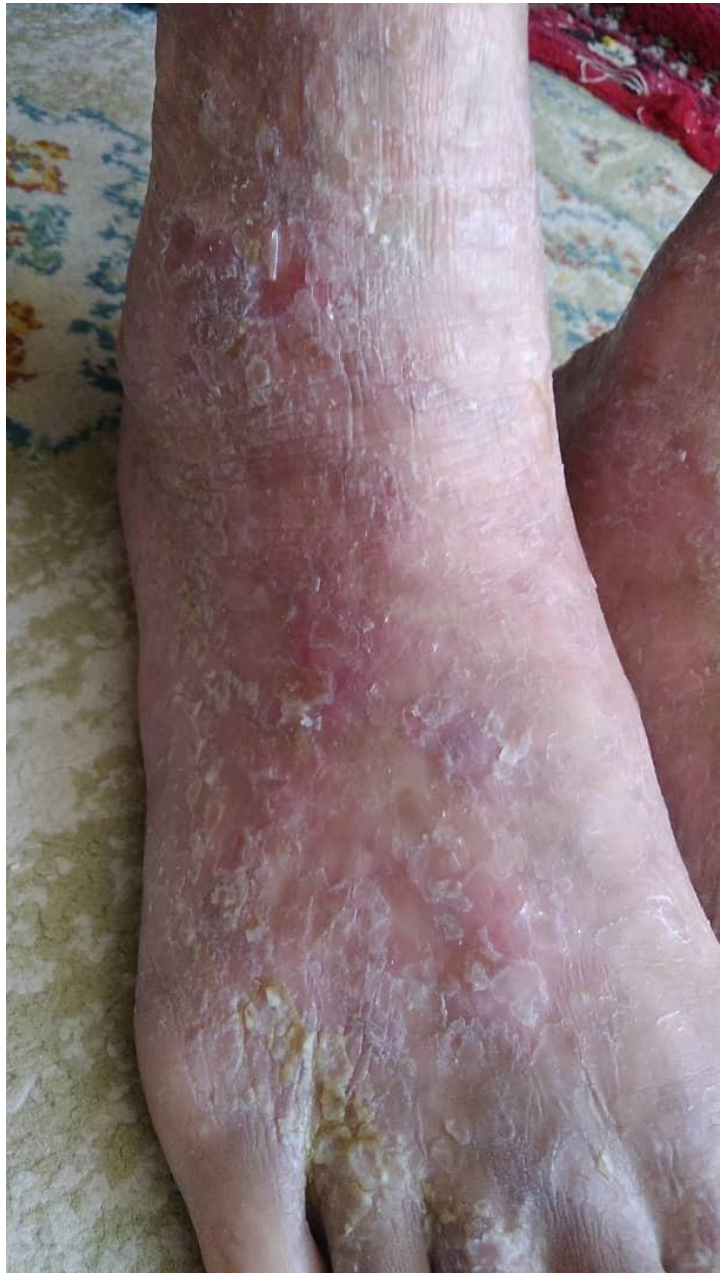
- It was shown that the mentioned treatment could decrease the signs and symptoms caused by cutaneous cGVHD, specifically hyperpigmentations and ulcers caused by skin dryness.
- Also, the cutaneous inflammation decreased significantly and was more evident than other manifestations due to the anti-inflammatory potential of the treatment.

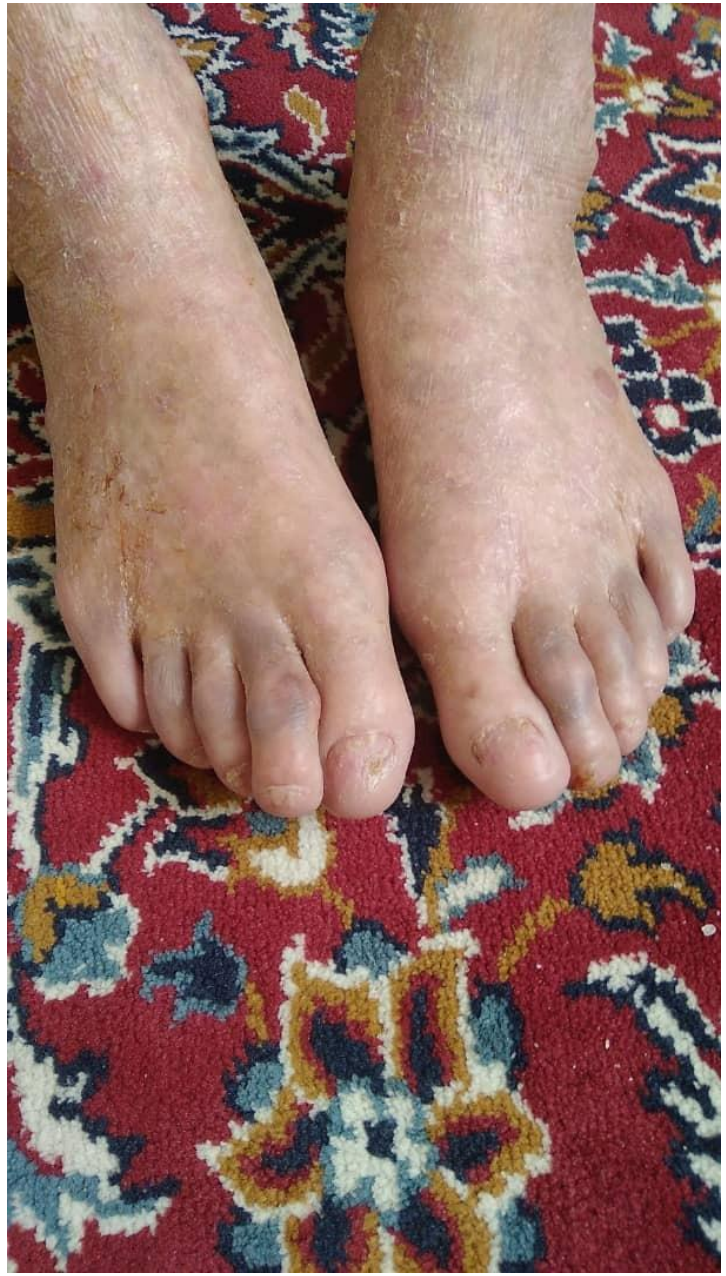
Laboratory variables before and 4 weeks after the last session of intervention

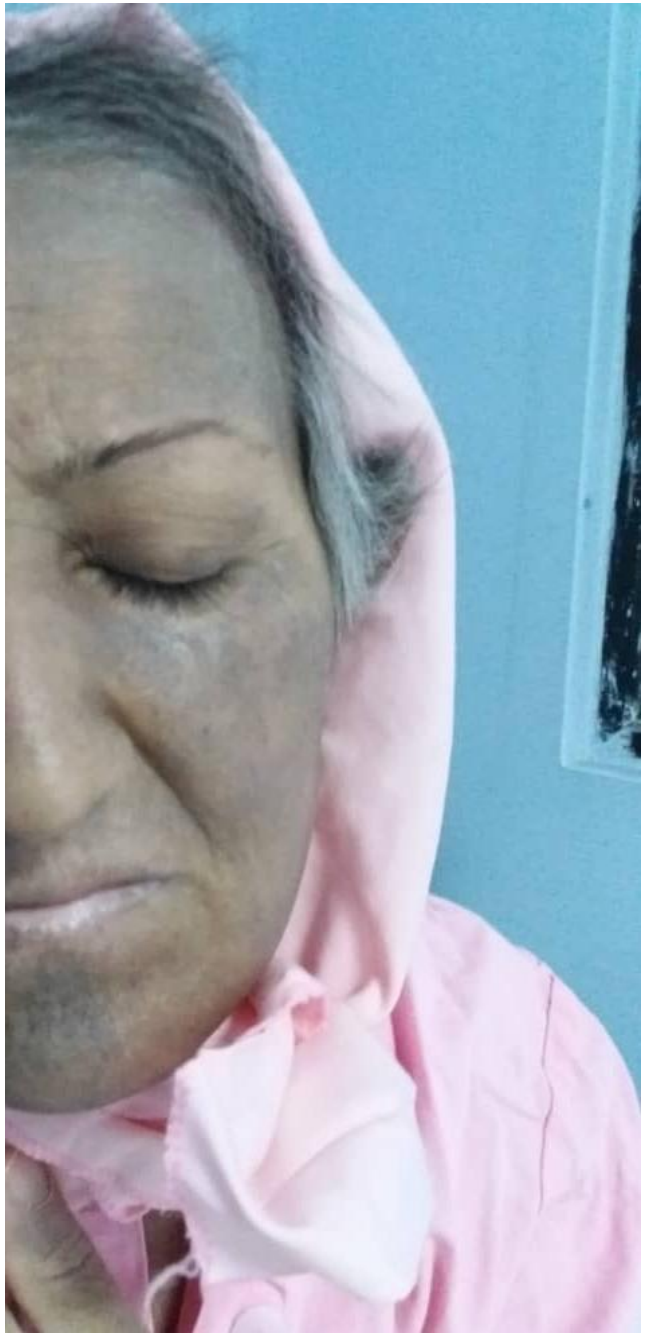
Variable	Before treatment	After the fourth intervention
White blood cells/mm^3	6830	7800
Neutrophils/mm^3	2049	3666
Lymphocytes/mm^3	3346	3588
Monocytes/mm^3	1229	390
Eosinophils/mm^3	204	2
Haemoglobin gr/dl	17.7	17.3
Haematocrit (%)	51.1	48.3
Platelet $\times 10^3/mm^3$	225	228
C-reactive protein	1+	Negative
Erythrocyte sedimentation rate (mm/h)	23	10
Creatinine mg/dl	0.8	0.9
Aspartate transaminase IU/L	59	57
Alkaline transaminase IU/L	50	59
Alkaline phosphatase U/L	333	365
Total bilirubin	0.8	0.8
Direct bilirubin	0.4	0.4
Lactate dehydrogenase U/ml	482	461

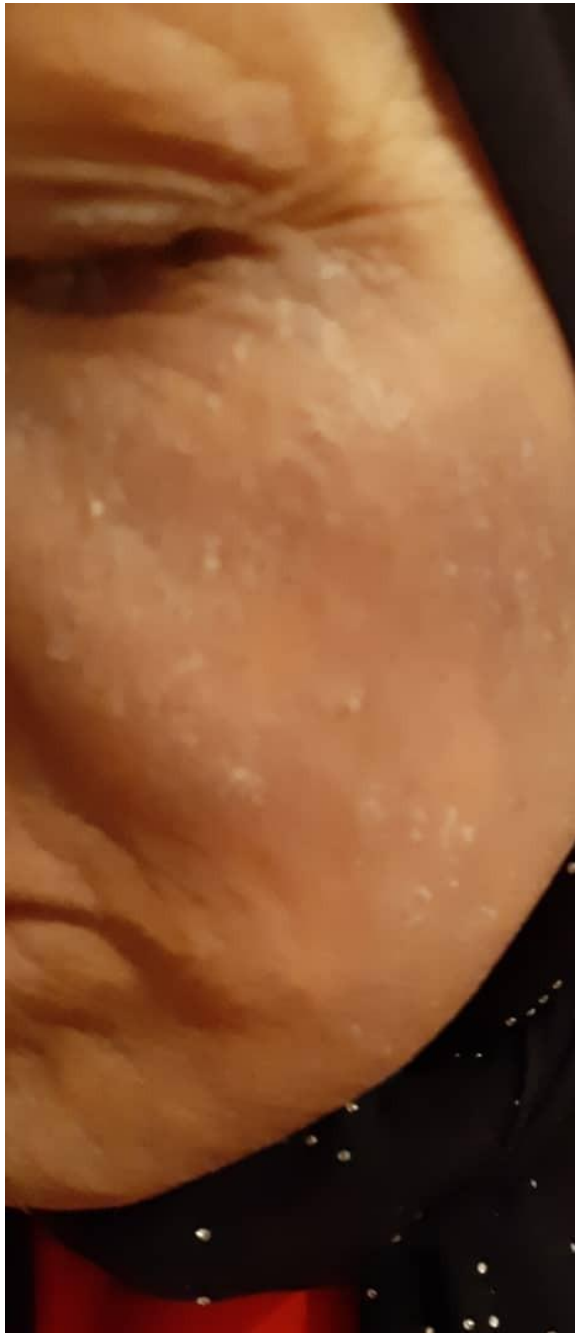
DISCUSSION

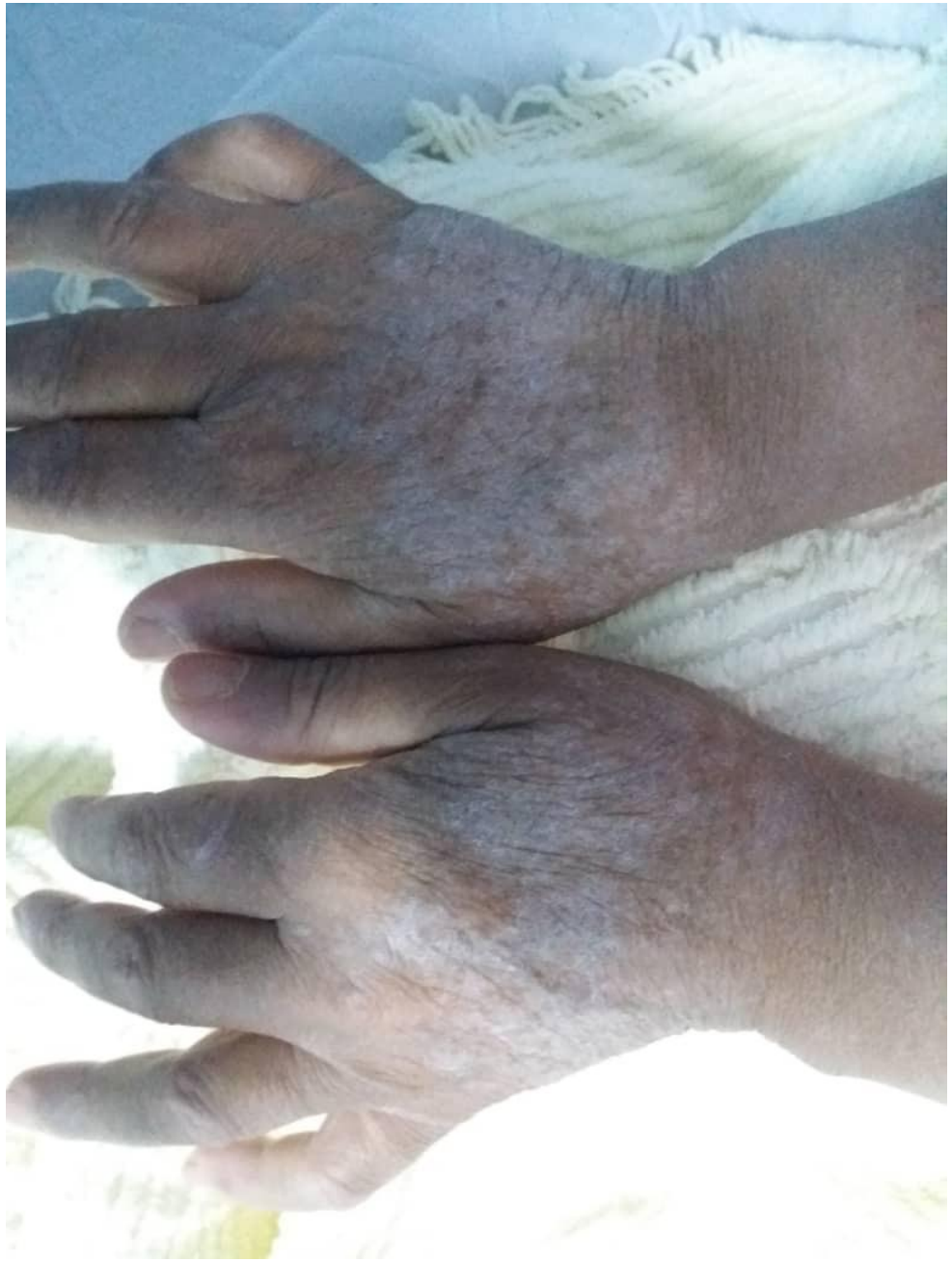
- As shown in Table , monocytes have been decreased from 18% to 5%, which is clinically significant.
- It has been shown that donor monocytes could be involved in the pathogenesis of GVHD.
- In patients diagnosed with GVHD, it has been shown that the intermediate CD14++ CD16+ monocytes could promote the induction of a subset of Th17 glucocorticoid resistance cells.
- Thus, it seems that our intervention was able to reduce this effect in our patient who did not respond to corticosteroid therapy.

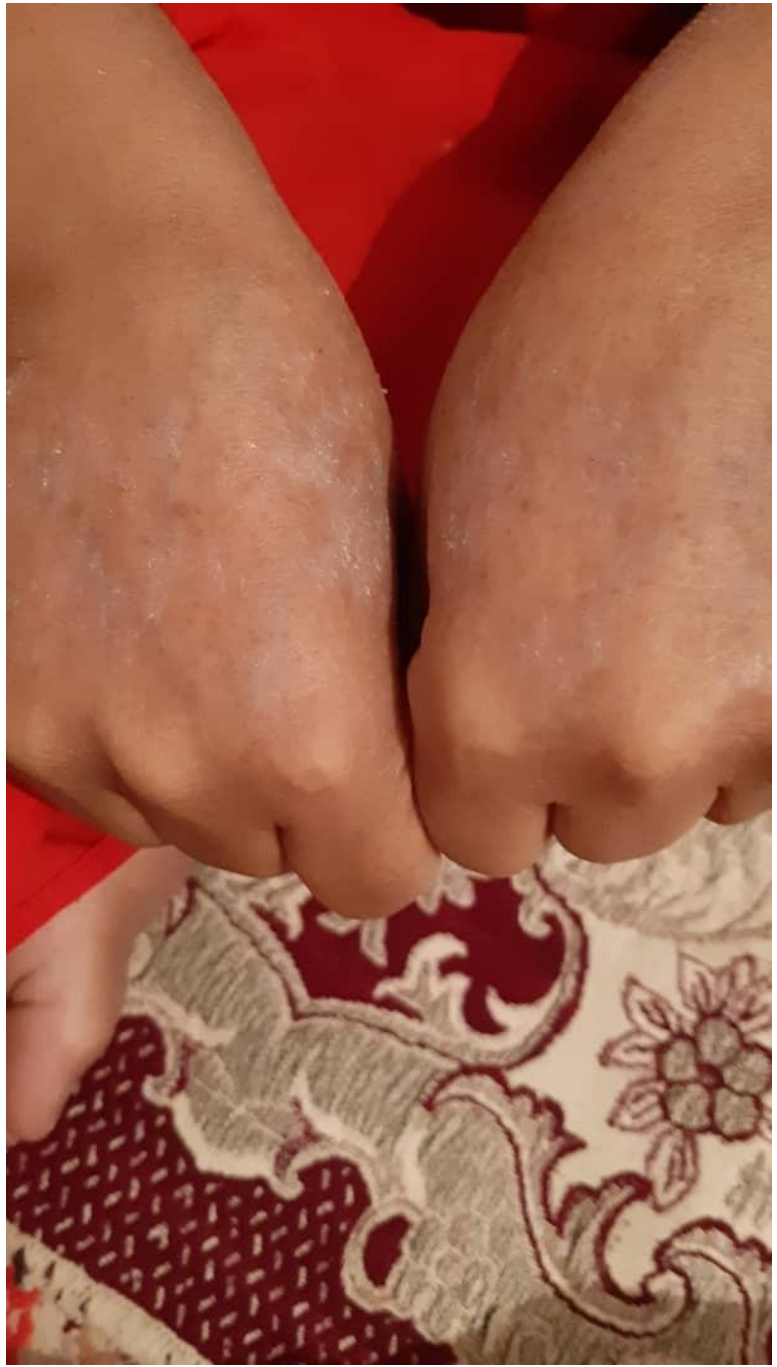












Prediction of the application of MSCs

- Quite a few data illustrated that the lymphocytes populations are expected to offer better treatment, especially T and NK cells.
- Further, patients with low levels of IL-6 and IL-22, T17 related cytokines before the therapy are likely to achieve complete remission or partial remission.
- Instead, patients expressed high levels of bilirubin before MSCs treatment tend to respond worse .
- In addition, a special attention from clinicians also should be paid to cell dose, patient age and type of organ involvement

MSC-derived extracellular vesicles:

- Secretome, including extracellular vesicles (EVs) , their therapeutic efficacy, ‘paracrine effect , results in Treg induction .
- Inhibitory effect on T-cell activation and differentiation, as well as reducing T-cell proliferation and IFN-g release .
- Main subcategories of EV, including microvesicles , microparticles and exosomes .
- Bioactive molecules including lipids, proteins, mRNA, tRNA, lncRNA, microRNA and mitochondrial DNA .
-

MSC-derived extracellular vesicles:

- Produced from cell supernatants and not the cells themselves, large scale production is more feasible .
- MSC-EV are less , adverse immune response compared to their parental cells, due to their lack of MHC class I/II molecules as a safer therapy , with no observed side effects .
- Nano-sized in nature, migrate through most physiological barriers, allowing effective concentrations to accumulate in target tissues.

MSC-derived extracellular vesicles:

- No standardization surrounding the optimal protocols for isolation of MSC-EV and identifying or characterizing MSC-EV phenotypes .

- Much work is required between researchers, clinicians and the regulatory authorities in order to stand arise all aspects relating to production of EV-based therapeutics prior to routine clinical application.

MSC-derived extracellular vesicles:

- As EVs are non-viable and non-replicating they may avoid the risk of unregulated cell growth, autoimmune disease and occlusion in the microvasculature , lack complex metabolic activity and as such, the risk of reprogramming by the environment is reduced.
- The nano-size of MSC-EV allows for sterilization by filtration, minimize the risk of biological contamination, the regulatory requirements for clinical grade production of EVs may not be as restrictive as for cellular therapy .
- MSC-EV from conventional MSC do not contain a nucleus or transgenic product they do not fall into a currently defined advanced therapy medicinal product (ATMP) category.

Conclusion:

- MSC source (e.g bone marrow, adipose tissue, synovial membrane, umbilical cord), EV production (eg. culture system, medium composition, cell-adherence support, bioreactors, stimulation), EV isolation (e.g. centrifugation techniques, size-based fractionation, ultrafiltration), quality controls, EV dosage and storage, and stability.

- Thus, the heterogeneity of MSCs used for EV production as well as of the isolated EVs requires extensive further consideration and will be the focus of researchers, clinicians and regulatory authorities prior to any approved industrial or clinical use of MSC-EV.