La transplantation de cellules souches comme thérapie de sauvetage au cours de la maladie de crohn réfractaire : Une revue systématique de la littérature

Stem cell transplantation as rescue therapy for refractory crohn's disease: a sytematic review

Labidi Asma, Serghini Meriem, Ben Mustapha Nadia, Fekih Monia, Boubaker Jalel, Filali Azza

Service de Gastro-entérologie A. Hôpital la Rabta. Tunis. Université de Tunis El Manar

RÉSUMÉ

Prérequis: La maladie de Crohn est une affection chronique caractérisée par une succession de poussées entrecoupées de périodes de rémission. C'est une maladie à forte composante immunologique qui représente la cible des thérapies conventionnelles comme les immunosuppresseurs et les biothérapies. Toutefois, plusieurs patients restent intolérants ou réfractaires à ces thérapies.

Objectif: L'objectif de cette revue est de déteminer les effets de la transplantation des cellules souches chez les patients porteurs d'une maladie de Crohn réfractaire.

Méthodes: Une revue systématique des études observationnelles, essais cliniques et cas cliniques à propos de l'efficacité et l'innocuité de la transplantation des cellules souches chez les patients porteurs de maladie de Crohn réfractaire.

Résultats: La transplantation de cellules souches hématopoiétiques semble être efficace dans le maintien d'une rémission clinique et endoscopique chez les patients porteurs d'une maladie de Crohn réfractaire ou en cas d'intolérance aux thérapies actuelles. Toutefois, elle a été associée à une morbidité et une mortalité élevées. La transplantation de cellules souches mésenchymateuses pourrait induire une rémission chez les patients ayant une maladie de Crohn fistulisante réfractaire sans entrainer des effets indésirables sévères. Son impact sur la maladie de Crohn luminale est encore controversé.

Conclusion: La transplantation de cellules souches semble être prometteuse chez les patients ayant une une maladie de Crohn réfractaire. Toutefois, devant la morbidité et la mortalité élevées liées à la chimiothérapie, la transplantation de cellules souches hématopoïétiques ne devrait être appliquée qu'en dernier recours afin de contrôler cette affection. L'efficacité de la transplantation des cellules souches mésenchymateuses sur la maladie de Crohn fistulisante reste à prouver.

Mots-clés

Refractory Crohn's disease; hematopoietic stem cells; mesenchymal stem cells

SUMMARY

Background: Crohn's disease is a chronic relapsing- remitting affection. It has a strong immunologic component which represent the target of standard therapies including immunosppressants and biological therapies. However, many patients remain refracory or intolerant to these therapies.

Aim: The aim of this review is to determine the effects of stem cell transplantation in patients with refractory Crohn's disease.

Methods: Systematic review of observational studies, clinical trials and case reports that focused on the effectiveness and safety of stem cell transplantation in patients with refractory Crohn's disease.

Results: Hematopoietic stem cell transplantation seems to be efficient in maintaining clinical and endoscopic remission in patients with Crohn's disease refractory or intolerant to current therapies. However, it has been associated to high morbidity and mortality due to chemotherapy. Mesenchymal stem cell transplantation could induce remission in patients with fistulising refractory Crohns disease with no severe side effects. Its impact on luminal Crohns disease is still controversial.

Conclusion: Stem cell transplantation seems to hold promising in patients with refractory Crohn's disease. However, because of the high morbidity and mortality related to chemotherapy, hematopoietic stem cell transplantation should be used as last resort to control this disease. Effectiveness of mesenchymal stem cell transplantation in luminal Crohn's disease has yet to be proven.

Key-words

maladie de Crohn réfractaire; cellules souches hématopoiétiques; cellules souches mésenchymateuse

Crohn's disease (CD) is a chronic relapsing inflammmatory condition resulting from the interactions of genetic, immunologic, microbial and environmental factors that may affect any section of the digestive tract. This condition is characterized by aggressive acquired (T cell) immune responses to a subset of commensal enteric bacteria in genetically susceptible hosts to the detriment of regulatory T cell functions. In fact, the expression of most T-helper lymphocyte (TH1) and TH17-related proinflammatory cytokines and chemokines (IL-12, IL-23 and IL-27) is upregulated in Crohn's disease. This has led to the implement of immune based therapies aiming at restoring the immune balance, such as immunosuppressants and biological therapies. However, a significant percentage of patients remain refractory or intolerant to these therapies and require repeated surgery to manage disease complications. Stem cell transplantation has been of some value in other diseases characterized by a loss of immune tolerance and or/a T-helper 1-predominant immune response. Stem cell transplantation has been proposed as a rescue therapy in severe forms of refractory Crohn's disease. The aim of this review is to determine the value of stem cell transplantaion in patients with refractory Crohn's disease.

METHODS

PubMed and Cochrane data base was searched for original articles and systematic reviews published in English or French-language journals with the following keywords alone or in combination: "refractory Crohn's disease" "hematopoietic stem cell transplantation" "mesenchymal stem cell transplantation" "autologous transplantation " "allogenic transplantaton" .

Definition of a stem cell:

A stem cell is characterized by two properties:

-Self-renewal: The ability of a cell to proliferate while it maintains its undifferentiated state.

- Potency: The ability to differentiate into specialized cell types (1).

Stem cells exist in different tissues including namely bone marrow, fat, periosteum, hair follicles, subcutaneous tissues, muscle, placenta, umbilical cord blood, liver, lung, and spleen (3). The bone marrow contains at least two types of stem cells. One population consists of CD34 positive hematopoietic stem cells giving rise to all blood cells including myeloid and lymphoid lineages. A second population is known as mesenchymal stromal cells which provide support for the growth and differenciation of hematopoietic progenitor cells in bone marrow microenvironments. The latter population could also be isolated from a variety of the aforementioned tissues.

Haematopoietic stem cell transplantation:

The effectiveness of HSCT in the treatment of Crohn's disease (CD) was suggested by the growing experience with HSCT in autoimmune diseases and the improvement of the clinical course of disease in patients with CD that received transplantation for other indications. Mid to long term clinical remission after autologous and allogenic HSCT was reported in patients with CD following transplantation mostly for hematological malignancies such as non-Hodgkin lymphoma (5), Hodgkin's disease (6) and acute myeloid leukemia (7). Treatment-free remission of CD was maintained 3 to 7 years after HSCT.

The first reports of autologous HSCT specifically given for the treatment of Crohn's disease were published in 2003 and concerned five patients with severe disease activity refractory to conventional treatment with anti-TNF antibody(13).

Principles:

Haematopoietic stem cell transplantation (HSCT) is a procedure where haematopoietic stem cell of any source are given to a recipient with intention of replacing the haematopoietic system in total or in part (4).

Haematopoietic stem cells can be obtained from two sources:

- The transplant recipient in the context of autologous haematopoietic stem cell transplantation.
- A donor in the context of allogenic haematopoietic stem cell transplantation.

Allogenic transplantation could be efficient in patients with Crohn's disease by replacing the autoreactive circulating leukocytes bearing genetic information involved in the predisposition to Crohn's disease. Autologous transplantation might also be of benefit as it allows the removal from the body of committed lymphocyte clones which could restore the patient to the status quo of being predisposed to Crohn's disease.(8) Another purpose of autologous transplantation is to allow the patient to be given high doses of chemotherapy which could be efficient in patients with CD and would otherwise be too toxic to tolerate because the marrow would be severely damaged.

The transplantation process is based on the steps below:

a) Stem cell mobilisation: The aim of this step is to move out stem cells from the bone marrow to the peripheral bloodstream. It is achieved by administrating chemotherapy with cyclophosphamide around 2 g/m2 followed by granulocyte-colony stimulating factor (G-CSF, 5 ug/kg/day) until an enriched target CD34+cell count is achieved when selected CD34+ HSCT is considered.

b) Stem cell collection: Stem cells are harvested from peripheral blood by leukapheresis thanks to an automated cell separator. This process may take approximately 4-6 hours to complete each day. Repeated collections on subsequent days may be needed to collect enough stem cells for the transplant.

c) Stem cell storage: Stem cells are cryopreserved in liquid nitrogen until transplantation.

d) Conditioning chemotherapy: The conditioning regimen consists of high dose of cyclophosphamide (50 mg/kg BW/day) during 4 days followed by transplantation. Antithymocyte globulin could be associated as it may directly target the T-cell pool of the recipient, and may therefore decrease the disease relapse probability and contributes to eliminating long-lived immune cells from their bone marrow niche.(17) This pre-transplantation phase is intended to ablate immune cells and in particular autoreactive T cells(9) since cyclophosphamide has been shown to efficiently induce apoptosis of T lymphocytes(10).

e) Stem cell transplantation: Stem cells are infused intravenously through a central catheter. Infusion times range from 3 minutes to 5 hours depending upon the volume of cells to be infused. This step is considered to change the natural course of autoimmune diseases by'resetting' the immune system and allowing the outgrowth of self-tolerant T-cell clones and increased numbers of activated regulatory T cells (11).

Patients are hospitalised during the entire conditioning therapy and up to haematological recovery defined as white blood cell count (WBC) of more than 19×109/L for at least three consecutive days. They receive supportive care which consists mainly in antiemetic therapy, parenteral nutrition irradiated haemocomponent transfusions and antimicrobial prophylaxis.

Evidence for the effectiveness of stem cell transplantation in Crohn's disease:

Results from several monocenter pilot studies suggest that immunoablation followed by autoHSCT is efficient to induce remission of refractory CD, and even has the potential to induce medication-free remission for several years(12).

In a monocentre phase I/II trial, P. Hasselblatt et al reported clinical and endoscopic remission in 7/12 patients with refractory Crohn's disease after mobilisation phase. AutoPBSCT was performed in nine patients of which five patients achieved a clinical and endoscopic remission within 6 months after autoPBSCT. However, relapses occurred in 7/9 patients during follow-up. but disease activity could be controlled by low-dose corticosteroids and conventional immunosuppressive therapy (9). Similar findings were reported by Cassinotti A, et al in a phase I/II trial that was conducted over 4 patients with Crohn's disease refractory or intolerant to immunosuppressive drugs including infliximab. Clinical and endoscopic remission was maintained in 3 of 4 patients, up to 16.5 months after autologous stem cell transplantation (14). More recently, in another trial including 24 patients with refractory Crohn's disease, long term (more than 5 years) medication-free clinical and endoscopic remission was obtained in up to 60% of patients after autologous hematopoietic stem cell transplantation (15).

To evaluate the effects of stem cell transplantation in the complex pathogenetic model of CD, Clerici et al investigated,through a phase I/II trial, the possible effects of autologous HSCT on balance between pro-inflammatory and regulatory immune responses in seven patients with Crohn's disease refractory or intolerant to conventionanl therapies. Overall, regulatory T cells increased, whereas TLR4-expressing cells, TNFa , all higher in patients than healthy controls, decreased significantly after autoHSCT (11).

In some papers, mobilization chemotherapy without haematopoietic stem cell transplantation has been suggested to be sufficient to induce clinical and endoscopic remission in patients with refractory Crohn's disease. However, experiences from several trials indicate that severe relapses including fistulising disease may occur, which may even preclude patients from further transplantation. This may be probably explained by the persistence of the genetic predisposition (NOD2 mutations) in these patients. Definitive evidence regarding the efficacy of mobilisation therapy will be provided by the prospective ASTIC trial. The purpose of this trial is to evaluate safety of autoHSCT and to determine whether there is a potential clinical benefit of hematopoietic stem cell mobilisation followed by high dose immuno-ablation and autologous stem cell transplantation versus hematopoietic stem cell mobilisation only.

Safety:

Hence HSCT may be effective in patients with refractory CD, it is also associated with a large morbidity and mortality. In patients who underwent autoHSCT for autoimmune diseases, mortality has been reported to range between 1% and 10% (16). Early toxicity is related to direct organ damage either from immunosuppressive agents which are used at high doses or from infections and hemorrhages following bone marrow aplasia after conditioning chemotherapy.

Although acute or chronic graft versus host disease is more common after allogenic hematopoietic stem cell transplantation, it also occurs in patients undergoing autoHSCT. It is initiated by auto-effector T cells that recognize self–major histocompatibility complex (MHC) class II antigens(19). In most trials, leukapheresis procedure occurred without adverse events. However, during mobilization and conditioning phases, there have been some adverse events of variable seriousness: diarrhea, anorexia, nausea, vomiting, neutropenic fever, urinary infections, renal failure, vaginal bleeding and urinary retention namely after cyclophosphamide infusion. Thus far, to our knowledge, no deaths occurred during transplantation protocol for CD. Further detailed findings about procedure safety will be provided by the end of ASTIC trial.

Late toxicity is related to malignancy development due to the chemotherapy exposure (18). This highlights the importance of the supportive care during transplantation protocol. It consists mainly in irradiated haemocomponent transfusions and antimicrobial prophylaxis. Broad-spectrum antibiotics and antifungals are usually given in case of neutropenic fever.

Mesenchymal stem cell transplantation:

As it has been already mentioned, mesenchymal stem cells are widely distributed in vivo. They have the potential to generate a wide variety of mesenchymal cell lineages, including bone, cartilage, fat, muscle,tendon, stroma and cardiomyocytes(20). MSCs are rare in the bone marrow, representing 1 in 10.000 nucleated cells. MSCs are easily isolated as they adhere to plastic and are capable of substantial proliferation and expansion in culture.(21)

Immunomodulatory properties of mesenchymal stem cells and their therapeutic implications:

Mesenchymal stem cells have been suggested to influence both adaptative and innate immune functions. Overall, the effect of MSCs on the immune system is generally suppressive in nature.

Effect of MSCs on the innate immune system:

MSCs exert significant effect on cells of the innate immune system including monocytes, monocyte-derived dendritic cells (DCs), macrophages, natural killer (NK) cells, and neutrophils. Indeed, MSCs have been observed to inhibit maturation, activation and cytotoxicic functions of the innate immune system cells (28). Moreover, they significantly reduce secretion of the pro-inflammatory cytokines [eg. tumor necrosis factor (TNF)- and IL-1β], and increase the production of the anti-inflammatory cytokine (IL-10).

Effect of MSCs on the adaptative immune system:

MSCs are thought to suppress the activity of CD8+ cytotoxic T lymphocytes either directly or indirectly by increasing the relative proportion of CD4+ T helper 2(TH2) lymphocytes and CD4+ regulatory T lymphocytes. Thus, an increase in interleukin IL-4 production by CD4+ T H2 lymphocytes and a reduction in interferon (IFN)-γ

production by CD8+ T lymphocytes, favoring an overall anti-inflammatory state and suppression of T lymphocyte mediated immunity (22) The effect of MSCs on B lymphocytes is yet controversial. However, the majority of in vitro cell culture studies established that MSCs are able to suppress B lymphocyte either directly by secreting paracrine molecules or indirectly through their suppressive effect on T lymphocytes. (30)

Therapeutic applications of MSCs properties:
MSC therapy is subdivided into locals versus systemic and allogenic versus autologous. However, whatever they are administrated, they exert overall the same effects. In fact, in addition to its net effect on the immune system, MSCs when stimulated by injury and inflammation, release an abundant supply of growth factors and cytokines which exert a trophic effect on tissue, stimulate neoangiogenesis, limit cellular apoptosis, recruit immune and other cell types to the site of injury, and reduce fibrosis and maladaptive scarring that may develop following organ injury (27).
When systematically infused, MSCs are able to migrate to injured, inflamed tissues and exert therapeutic effects thanks to their homing capability (23).
By dint of the aforementioned properties, MSCs have been used for a variety of therapeutic applications (24). Among these applications, MSCs have been used to reduce clinical symptoms of osteogenesis imperfecta (25) and to treat large bone defects, in regenerative treatments to enhance repair of pancreatic islets , and in infarcted myocardium (26). They also have been applied as immunomodulatory treatment of autoimmune diseases, including Crohn's disease, multiple sclerosis , and rheumatoid arthritis.

Mesenchymal stem cell transplantation for CD patients:
Several studies have already suggested the effectiveness of MSCs transplantation in patients with fistulising refractory Crohn's disease. However, findings with regard of refractory luminal CD are yet controversial. In the first human trial of systemic MSCs in CD, Onken et al., treated 10 patients who had failed previous treatment with steroids and immunosuppressants and had active disease. Patients received allogenic human bone marrow–derived MSCs as intravenous infusions. All evaluable patients had a significant decrease in CDAI score from baseline. Response was noted in 33% of patients and all responders had previously failed infliximab therapy.
Another phase I study of autologous bone marrow–derived MSCs was conducted over 10 patients with luminal refractory CD. No clear signal of efficacy was observed. Remission was not achieved in any patient, and 3patients had a reduction of at least 70 points in CDAI, but the disease worsened significantly in 4 patients requiring surgery or rescue medication within 14 weeks after cell treatment (31).
Results concerning Crohn's disease fistula are, however, more encouraging. Thus, several studies have showed effectiveness of MSCs transplantation in healing fistulae. In a phase I/II clinical trial, forty-three patients with fistulae were treated with adipose tissue-derived stem cells. Fistula tract was filled with MSCs in combination with fibrin glue after intralesional injection of MSCs. Fistula healing (defined as complete closure of external opening without any sign of drainage and inflammation) was observed in 27 patients (82%) by 8 weeks after ASC injection. Among these patients, 88% sustained complete closure for 1 year (32).
In another trial, Ciccocioppo et al, investigated the feasibility and efficacy of serial intrafistular injections of autologous bone marrow-derived mesenchymal stromal cells (MSCs) in the treatment of fistulising Crohn's disease. Among 12 consecutive outpatients with fistulising refractory CD, ten patients received intrafistular MSC injections scheduled every 4 weeks, and were monitored by surgical, MRI and endoscopic evaluation for 12 months afterwards. Sustained complete closure (70%) of fistula tracks with a concomitant decrease of Crohn's disease and perianal disease activity indexes, and rectal mucosal healing were induced by treatment (33).

Safety:
The lack or paucity of major histocompatibility complex (MHC) class II in MSCs and other immunoactive co-stimulatory molecules renders these cells "immunoprivileged," allowing them to evade detection and elimination by foreign immune systems.
Because of their immunomodulatory effects, MSCs may theoreticaly, increase risk of infections and malignancy. However, most published current clinical trials suggest that the administration of MSCs is safe.
In a Meta-Analysis published in 2012 by the Canadian Critical Care Trials Group (34), safety of cell therapy with mesenchymal stromal cells was evaluated through eight randomized controlled trials including patients with several clinical conditions of ischemic stroke, Crohn's disease, cardiomyopathy, myocardial infarction, graft versus host disease, and healthy volunteers. There were no associations between MSC treatment and the development of acute infusional toxicity, organ system complications, infection, death, or malignancy.
There was, however, a significant association between MSC administration and transient fever which could be related to acute inflammatory reactions to particular preparations of MSCs (35).

CONCLUSION

Crohn's disease still represents a significant public health burden since a high number of patients remain refractory to approved therapies. Moreover, inability to provide a surgical solution to fistulizing manifestations, and the recurrent need for surgeries remain challenges requiring novel therapies in this disorder.
Hematopoietic stem cell transplantation has proven its efficacy in several trials as rescue therapy in patients with Crohn's disease refractory or intolerant to conventional therapies including biotherapy. However, on account of the significant associated morbidity and mortality which is related to chemotherapy, HSCT should be used as last resort in an attempt to control debilitating disease.
Mesenchymal stem cell transplantation is suggested to be efficient in patients with refractory Crohn's disease despite the controversial findings already provided with regard to luminal refractory Crohn's disease. The big advantage of the use of MSCs is the fact that this treatment does not involve conditioning chemotherapy.
Overall, further larger scale controlled clinical trials should be undertaken to further define long-term safety and efficacy of hematopoietic and mesenchymal stem cell transplantation , to better investigate the pathogenetic aspects of the reported clinical effects and to establish the predictors of an optimal response for better recruitment.

References

1. Prockop DJ. Marrow stromal cells as stem cells for non hematopoietic tissues. Science 1997;276:71-4.
2. Marmont AM. Stem cell transplantation for autoimmune disordesrs. Coincidental autoimmune disease in patients transplanted for conventional indications. Best Pract Res Clin Haematol 2004;17:223-32.
3. In 't Anker PS, Noort WA, Scherjon SA, et al. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. Haematologica 2003;88:845–52.
4. Ljungman P, Urbano-Ispizua A, Cavazzana-Calvo M, et al. Allogenic and autologous transplantation for haematological diseases, solid tumors and immune disorders: Definitions and current practice in Europe. Bone Marrow transplantat 2006 ; 37:439-49.
5. Musso M, Porretto F, Crescimanno A, Bondi F, Polizzi V, ScaloneR. Crohn's disease complicated by relapsed extranodal Hodgkin's lymphoma: prolonged complete remission after unmanipulated PBPC autotransplant. Bone Marrow Transplant 2000;26:921-3.
6. Kashyap A, Forman SJ. Autologous bone marrow transplantationfor non-Hodgkin's lymphoma resulting in long-term remission of coincidental Crohn's disease.Br J Haematol 1998;103:651-2.
7. Soderholm JD, Malm C, Juliusson G, Sjodahl R. Long-term endoscopic remission of Crohn disease after autologous stem cell transplantation for acute myeloid leukaemia. Scand JGastroenterol 2002;37:613-6.
8. Hawkey CJ. Stem cell transplantation for Crohn's disease. Best Pract Res Cl Haematol 2004;17: 317-325
9. Hasselblatt P, Drognitz K, Potthoff K et al. Remission of refractory Crohn's disease by high-dose cyclophosphamide and autologous peripheral blood stem cell transplantation. Aliment Pharmacol Ther. 2012;36:725-35.
10. Strauss G, Osen W, Debatin KM. Induction of apoptosis and modulation of activation and effector function in T cells by immunosuppressive drugs. Clin Exp Immunol 2002; 128: 255-66.
11. Clerici M, Cassinotti A, Onida F, et al. Immunomodulatory effects of unselected haematopoietic stem cells autotransplantation in refractory Crohn's disease. Dig Liver Dis 2011; 43: 946-52.
12. Hommes DW, Duijvestein M, Zelinkova Z, et al. Long-term follow-up of autologous hematopoietic stem cell transplantation for severe refractory Crohn's disease. J Crohns Colitis 2011;5: 543-9.
13. Burt RK, Traynor A, Oyama Y, Craig R. High-dose immune suppression and autologous hematopoietic stem cell transplantation in refractory Crohn's disease. Blood 2003;101:2064-6.
14. Cassinotti A, Annaloro C, Ardizzone S,et al. Autologous haematopoietic stem cell transplantation without CD34+ cell selection in refractory Crohn's disease.Gut. 2008 ;57:211-7
15. Burt RK, Craig RM, Milanetti F, et al. Autologous non myeloablative hematopoietic stem cell transplantation in patients with severe anti-TNF refractory Crohn disease: long-term follow-up Blood 2010;116:6123-32.
16. van Laar JM, Farge D, Sont JK, et al. The ASTIS trial: autologous stem cell transplantation versus iv pulse cyclophsophamide in poor prognosis systemic sclerosis, first results. Ann Rheum Dis 2012; 71: 151.
17. Alexander T, Thiel A, Rosen O, et al. Depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory SLE induces long-term remission through de novo generation of a juvenile and tolerant immune system. Blood 2009; 113:214-23.
18. Duijvestein M, van den Brink GR, Hommes DW. Stem cells as potential novel therapeutic strategy for inflammatory bowel disease. J Crohns Colitis 2008;2:99-106.
19. Miura Y, Thoburn CJ, Bright EC, et al. Characterization of the T-cell repertoire in autologous graft versus-host disease (GVHD): evidence for the involvement of antigen-driven T-cell response in the development of autologous GVHD. Blood 2001;98:868-76.
20. Kawada H, Fujita J, Kinjo K, et al. Non hematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 2004;104:3581-7.
21. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284: 143-7.
22. Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 2005;106:1755-61.
23. Chavakis E, Urbich C, Dimmeler S. Homing and engraftment of progenitor cells: a prerequisite for cell therapy. J Mol Cell Cardiol 2008;45:514-22
24. J. Tolar, K. Le Blanc, A. Keating, and B. R. Blazar, "Concise review: hitting the right spot with mesenchymal stromal cells. Stem Cells 2010; 28:1446-55.
25. Horwitz EM, Prockop DJ, Fitzpatrick LA , et al. Transplant ability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfect. Nat Med 1999;5:309-13.
26. Kawada H, Fujita J, Kinjo K et al., "Non hematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 2004;104: 3581-7.
27. Crisostomo PR, Markel TA, Wang Y, Meldrum DR. Surgically relevant aspects of stem cell paracrine effects. Surgery 2008; 143:577.
28. Spaggiari GM, Capobianco A, Abdelrazik H, et al. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 2008; 111:1327.
29. Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. Blood 2009; 113:6576.
30. Gerdoni E, Gallo B, Casazza S, et al. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. Ann Neurol 2007; 61:219.
31. Duijvestein M, Vos AC, Roelofs H, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut 2010;59:1662-9.
32. Yong Lee W, Joo Park K, Beom Cho Y et al. Autologous Adipose Tissue-derived Stem Cells Treatment Demonstrated Favorable and Sustainable Therapeutic Effect for Crohn's Fistula. Stem Cells. 2013 doi: 10.1002/stem.1357
33. Ciccocioppo R, Bernardo ME, Sgarella A et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut. 2011 ;60:788-98.
34. Lalu MM, McIntyre L, Pugliese C, et al. Safety of Cell Therapy with Mesenchymal Stromal Cells (safe cells): A Systematic Review and Meta-Analysis of Clinical Trials. PLoS ONE 2012;7: e47559.
35. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. Anesth Analg 2009;108: 759-69