Stem cell therapy in inflammatory bowel disease: which, when and how?

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Purpose of review

Stem cell therapy has emerged as a promising therapeutic strategy for inflammatory bowel diseases (IBDs). Currently, stem cell therapy is not part of the standard of care and is usually only performed as a part of clinical trials. In this review, clinical results, proposed underlying mechanisms, and future research directions will be discussed.

Recent findings

Administration of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) has been evaluated for IBD treatment over the past years. MSC therapy is being explored as a treatment option for fistulizing Crohn's disease and for luminal Crohn's disease. It is shown to be well tolerated, but results on efficacy are inconsistent. HSC transplantation seems to be very effective, but serious adverse events are common. Therefore, future research should focus on improving efficacy of MSC therapy, and on improvement of safety of HSC therapy.

Summary

Both MSC and HSC therapy offer clinical potential, but currently are not routinely used for IBD treatment. MSC therapy seems well tolerated but results on efficacy are conflicting. HSC transplantation is shown to be effective but safety concerns remain. Nonetheless, for severe refractory IBD cases, stem cell therapy could well become the next-generation treatment option.

Keywords

hematopoietic stem cells, inflammatory bowel disease, mesenchymal stem cells

INTRODUCTION

Stem cell therapy is a field that has developed considerably in the past years. Stem cells have the capacity to generate different types of daughter cells, and thus have been proposed as a valuable tool for regeneration of damaged tissue. Hematopoietic stem cells (HSCs) are capable of regenerating immune cells [1], which creates the theoretical possibility to create a new immune system without autoimmunity in inflammatory bowel disease (IBD) patients [2[•]]. Mesenchymal stem cells (MSCs) can differentiate into different mesenchymal cell lines and also exert immunosuppressive functions, which might be beneficial in IBD [3[•]]. Several other adult stem cells have been explored in preclinical settings as well. In this article, the emphasis will be on HSCs and MSCs because those have been already used in clinical settings.

PATHOGENESIS OF INFLAMMATORY BOWEL DISEASES

The pathogenesis of IBD remains largely unclear even though considerable advances in this field have been made. IBD is thought to be caused by a dysregulated immune response against communal, nonpathogenic bowel antigens in a genetically susceptible individual. Both genetic and

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KEY POINTS

- Both HSCT and MSC therapy are currently being evaluated for IBD treatment in trial settings.
- HSCT offers the opportunity to generate a new immune system free of autoimmunity. Currently, the main limitation of HSCT is safety, but efficacy has been shown consistently.
- MSCs have tissue regenerative properties and exert immunosuppressive functions, theoretically making them a valuable tool for IBD treatment. MSC therapy is well tolerated, but results on efficacy have been inconsistent. Further research needs to be aimed at improving MSC efficacy by optimizing isolation, expansion and stimulation procedures of MSCs.

environmental factors play a role in IBD development, mediated by changes in innate and adaptive immune function, epithelial barrier function and microbiome composition [4–6]. Genome-wide association studies (GWASs) start to shed more light on these processes, and the application of high throughput methods to analyze the expression of many factors involved in IBD pathogenesis will further improve our knowledge about IBD.

Based on cytokine profiles observed in IBD patients, Crohn's disease is traditionally thought to be mainly mediated by a Th1-cell response, and ulcerative colitis by a Th2-like response [7,8]. Recently, it became clear that this separation is more nuanced. Crohn's disease seems to be mediated by a Th1 response combined with a Th17 response, both causing tissue injury. In ulcerative colitis the response is mediated by cytokines similar to those observed in a Th2 response, mediated by NK-cells causing direct and indirect tissue injury [8]. Evidence for the involvement of T-cells, NK-cells and dendritic cells in IBD pathophysiology has been confirmed in a large recent GWAS [9]. Other components of the adaptive immune response play a role in IBD pathogenesis as well. For example, bacterial recognition and antigen loading of bacterial fragments onto MHC molecules through the process of autophagy appear to be important [10]. In addition, increased expression of adhesion molecules, chemokines and mRNA involved in oxidative stress is observed in IBD patients [7], which indicates important roles for cell trafficking and responses to tissue injury in disease mechanisms.

HEMATOPOIETIC STEM CELL TRANSPLANTATION

Originally, hematopoietic stem cell transplantation (HSCT) was used as treatment for different

malignancies [1]. The idea of using HSCT for autoimmune conditions emerged after the observation of positive effects in animal models. This concept was supported by the observation that human autoimmune diseases improved in patients undergoing HSCT for other indications [2]. The HSCT procedure starts with mobilization of HSCs from the bone marrow into the peripheral blood and harvesting of those HSCs. Before the transplantation, the patient undergoes a preparative treatment that ablates the immune system, after which the HSCs are administered intravenously [1]. The rationale behind HSCT for autoimmune and chronic inflammatory diseases, including IBD, is that this procedure can 'reset' the immune system. The preparative chemotherapy eliminates the immune system, including autoreactive T-cells. By subsequently performing autologous HSCT, hematopoietic precursors will generate a new tolerant T-cell population, mediated by T-cell selection processes in the thymus. This concept is supported by two studies analyzing immune reconstitution after autologous HSCT in patients with multiple sclerosis [11] and systemic lupus erythematosus [12], which showed increased thymic output after HSCT with the development of a new and diverse T-cell repertoire. Allogeneic transplantation offers the additional benefit of removing the genetic susceptibility of the immune system, and evidence for an additional beneficial effect of graft-versus-autoimmunity has been described as well. Allogeneic HSCT, however, is associated with higher complication and mortality rates and is therefore being discouraged as treatment for autoimmune diseases in current guidelines [2]. In IBD patients with severe refractory disease that are not eligible for surgery, autologous HSCT can be considered, using a relatively low-dose chemotherapy (compared with the regimens used in cancer treatment), usually consisting of cyclophosphamide and anti thymocyte globulin antibodies.

Currently, the most extensive experience in HSCT for IBD has been published by Burt *et al.* [13]. Twenty-four Crohn's disease patients with severe disease refractory to conventional therapy, including anti-TNF α antibodies, received autologous HSCT. Of these, 91% stayed in remission for 1 year after HSCT, 57% for 3 years and 19% for 5 years [13]. Recently, another group reported similar outcomes in a cohort of 10 refractory Crohn's disease patients, with remission rates of 80% after 1 year, 40% after 3 years, and 30% after 5 years. Also, out of four patients that suffered from fistula, three experienced closure of the fistula tract [14]. In our experience, two refractory Crohn's disease patients underwent autologous HSCT. Both patients

achieved disease remission after the transplantation procedure. One patient restarted medication after 12 months, and no luminal relapse was observed during the follow-up period of 6 years. The other patient restarted medication after 6 months, relapsed only after 5 years, and achieved remission again after switching medication [15]. These results are promising, but for a definite assessment of the efficacy of HSCT for IBD, the results of a randomized controlled phase III trial are warranted. Currently, a multicenter, prospective, randomized phase III study is being performed that analyzes the effect of autologous HSCT vs. stem cell mobilization alone in refractory Crohn's disease patients (ASTIC-trial) (<http://www.nottingham.ac.uk/icr/astic>). All patients have been recruited and preliminary data were presented recently. A median fall in the Crohn's disease activity index of 162 was observed in the HSCT group, compared with 82 in the group receiving mobilization alone. Patients that received HSCT also considerably improved endoscopically, while no improvement was seen in the control group. Though the results are preliminary, autologous HSCT seems to be an effective treatment for refractory Crohn's disease. Serious adverse events in both groups were common though, raising concerns about safety [16[■]].

A recent analysis assessing safety of HSCT in 70 patients with autoimmune diseases in the United Kingdom showed an overall survival of 87% after 1 year and 65% after 5 years following allogeneic HSCT. For autologous HSCT the survival was 85% at 1 year and 78% after 5 years. Age was strongly correlated with survival, with the highest survival in the 18–39 year age group (5 year survival 95%). The underlying autoimmune disease affected the outcome as well. The most common cause of death was infection after both autologous and allogeneic HSCT [17]. Another analysis of 900 autoimmune patients undergoing HSCT showed a 5-year survival rate of 85%. Half of the deaths were related to transplantation, with the main cause of death being infection. Outcomes were interestingly not dependent on the type of conditioning regimen, but were strongly correlated with the transplantation center [18]. Because of the inherent risks associated with HSCT, this therapy is only considered in severe refractory cases, in which the potential benefit weighs out against the risks. Currently, all trials have focused on severe refractory Crohn's disease patients. No trials analyzing HSCT for ulcerative colitis patients have been published yet, though improvement of ulcerative colitis after both allogeneic and autologous HSCT has been described in ulcerative colitis patients undergoing HSCT for other indications [19,20] and in a case of

autoimmune anemia combined with ulcerative colitis [21].

To increase the applicability of HSCT for IBD, improvement of safety is a top priority. As mentioned above, infectious complications are the most common cause of treatment-related mortality due to prolonged lymphopenia after HSCT. To shorten the lymphopenic period after HSCT, different approaches have been sought to accelerate immune reconstitution after HSCT. In this context administration of keratinocyte growth factor, growth hormone and cytokines such as IL-2 and IL-7 has been tested, with the goal of stimulating thymic function and promoting lymphocyte survival [22[■],23[■]]. Also, adoptive transfer of specific T cells [22[■]], and ex-vivo expansion of hematopoietic precursor cells [24,25,26[■]] are being explored to improve safety. To improve efficacy, allogeneic HSCT might be considered because the genetic susceptibility of the immune system will be permanently removed. Currently, a phase I trial assessing toxicity and efficacy of allogeneic HSCT in Crohn's disease is being performed (<http://www.clinicaltrials.gov/ct2/show/NCT01288053>).

MESENCHYMAL STEM CELL THERAPY

MSCs, also called mesenchymal stromal cells, are a heterogeneous group of stromal cells that have the capability to differentiate into different mesenchymal cell types and also exert immunosuppressive functions. The combination of those two properties makes the application of MSCs for tissue regeneration in inflammation-induced tissue injury a promising approach [3[■]]. MSCs are isolated from stromal tissues based on their plastic adherence or using specific surface markers [27]. Originally, MSC research mainly focused on bone marrow-derived MSCs (bm-MSCs). Alternatively, adipose tissue-derived MSCs (ad-MSCs) can be isolated in high frequencies from liposuction aspirates. The immunophenotypes of ad-MSCs and bm-MSCs are more than 90% identical, and ad-MSCs have a similar or even enhanced immunosuppressive capacity compared with bm-MSCs. Since MSCs in liposuction aspirates are abundant, these cells can be used clinically without ex-vivo expansion, in contrast to bm-MSCs [28]. These properties mean that ad-MSCs can be a valuable, more accessible, and safe alternative to bm-MSCs.

In-vitro experiments have shown that MSCs are able to interfere with components of both innate and adaptive immune system. In the adaptive immune system MSCs interfere with complement, toll-like receptor signaling, macrophages, dendritic cells, neutrophils and NK cells. In the adaptive

immune system, MSCs inhibit T-cell function, shift T-cell balance and induce Treg cells. Inhibition of B-cells has also been suggested in different studies [29²²]. Before MSCs exert their immunosuppressive function they require priming by pro-inflammatory cytokines such as IFN γ , TNF α and IL-1 β . The immunosuppressive effect that MSCs exert is mainly mediated by soluble factors, but certain MSC-immune cell interactions are contact-dependent [29²²]. Depending on the environment, MSCs can also acquire pro-inflammatory properties [29²²,30²³]. This suggests that in the event of active infection, MSCs might adopt a pro-inflammatory phenotype, whereas in the case of an exaggerated inflammatory response they will adopt an immunosuppressive phenotype (Fig. 1). In different mouse models, a positive effect of MSCs on experimental colitis is shown as well. MSCs are infused either intravenously [31] or intraperitoneally [32,33] and improve experimental colitis [31–33]. Our group confirmed *in vivo* that IFN γ -stimulated MSCs have enhanced immunosuppressive functions by showing reduced weight loss and lower histology scores in two mouse models of colitis [33].

The first clinical successes with MSCs in inflammatory conditions were achieved in severe graft versus host disease (GvHD). Several encouraging results are obtained from clinical trials assessing allogeneic MSCs for the treatment [34] and prevention [35] of GvHD. Significant improvement of severe GvHD was observed after administration of

MSCs compared to a control population [34], and significantly fewer patients developed severe GvHD after HSCT if MSCs were co-infused simultaneously with the graft [35]. However, a randomized placebo-controlled trial using industrially manufactured allogeneic MSCs for GvHD treatment failed to show a durable complete response for ≥ 28 days [36].

In the IBD field MSCs have been tested in clinical trials for two indications, namely fistulizing disease and luminal disease (Table 1) [37,38,39²⁴, 40,41²⁵,42²⁶,43,44²⁷,45²⁸]. For luminal disease, the rationale for the use of MSCs lies mainly in their immunosuppressive capacity. In fistulizing disease, the differentiation potential of MSCs is thought to be of crucial importance as well to achieve fistula tract closure. Several phase I/II trials have been performed analyzing ad-MSCs for fistulizing disease, using autologous [37,38,39²⁴] or allogeneic [41²⁵] MSCs. One study evaluated the effect of bm-MSCs on fistulizing Crohn's disease [40]. The outcomes of these trials are promising and the procedure seems safe. A placebo-controlled trial evaluating the efficacy of ad-MSCs compared with fibrin glue for Crohn's disease related and unrelated fistula found 70% healing in the treatment group, which was four-fold higher than in the control group [38]. After 4 years of follow-up, however, only 33% remained healed in the treatment group vs. 15% in the control group [39²⁴]. Recently the results of the FATT1 trial were published, a phase III randomized controlled trial assessing the efficacy of autologous ad-MSCs for

FIGURE 1. The two faces of mesenchymal stem cells (MSCs). It is thought that under inflammatory conditions, MSCs can acquire an immune suppressive phenotype (left), suppressing among others T-cells, macrophages, dendritic cells, natural killer cells, and neutrophils. Under influence of an active infection, MSCs might acquire a phenotype that activates the immune response and support pathogen clearance (right).

Table 1. Published trials analyzing mesenchymal stem cell treatment for different fistulizing diseases and for luminal inflammatory bowel disease

	Indication	MSC source	Results
Fistulizing disease			
Garcia-Olmo <i>et al.</i> [37]	CD	ad-MSC (auto)	6/8 healed, 2/8 improvement (8 wk)
Garcia-Olmo <i>et al.</i> [38]	CD and non-CD	ad-MSC (auto)	71% ad-MSC ↔ 16% fibrin glue (8 wk)
Guadalajara [39 [■]]			33% ad-MSC ↔ 15% fibrin glue (4yr)
Ciccocioppo <i>et al.</i> [40]	CD	bm-MSC (auto)	7/10 healed, 3/10 improved (1 yr)
de la Portilla <i>et al.</i> [41 [■]]	CD	ad-MSC (allo)	14/20 closure (24 wk)
Herreros <i>et al.</i> [42 [■]]	Non-CD	ad-MSC (auto)	42% ad-MSC ↔ 39% fibrin glue (24 wk)
Luminal IBD			
Duijvestein <i>et al.</i> [43]	CD	bm-MSC (auto)	Improvement 3/10, no remission
Liang <i>et al.</i> [44 [■]]	CD and UC	bm/uc-MSC (allo)	Remission 5/8
Forbes <i>et al.</i> [45 [■]]	CD	bm-MSC	Improvement 12/14, remission 8/14

ad-MSC, adipose tissue derived MSC; allo, allogeneic; auto, autologous; bm-MSC, bone marrow derived MSC; CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis; uc-MSC, umbilical cord derived MSC; wk, week; yr, year.

perianal fistulas that were not related to Crohn's disease. Disappointingly, no significant difference between the different treatment groups was shown [42[■]]. To assess the efficacy of MSCs for Crohn's disease-related fistula, the ADMIRE-Crohn's disease study is currently being performed (<http://www.clinicaltrials.gov/ct2/show/NCT01541579>). Several trials for luminal Crohn's disease have been performed as well. A phase I trial we performed using autologous bm-MSCs for luminal Crohn's disease, designed primarily for safety and feasibility, showed improvement in three out of 10 patients, of which none achieved remission [43]. Another group treated four Crohn's disease and three ulcerative colitis patients with allogeneic MSCs. Five patients achieved remission, of which two stayed in remission for over 2 years [44[■]]. Recently, preliminary results of a phase II trial analyzing the use of allogeneic bm-MSCs for refractory luminal Crohn's disease were presented. Improvement was observed in 12 out of 14 included patients, remission in eight and endoscopic improvement in seven [45[■]]. These studies were not designed to assess efficacy, but did demonstrate safety of intravenous (i.v.) infusion of MSCs. This notion is supported by a recent meta-analysis analyzing safety of i.v. MSC infusion for many different indications. MSC infusion is associated with a transient fever, but not with acute infusion toxicity, organ system complications, infection, malignancies, or death [46[■]].

MSC therapy for IBD represents a promising strategy but results have been inconsistent. The comparison between different studies is challenging because different isolation, selection and expansion protocols are being utilized. The development of

uniform protocols is warranted in order to achieve reproducible and consistent results. To improve efficacy, robust priming of MSCs is probably of crucial importance. In the currently performed trials, no priming of MSCs has been performed. Therefore, determining the optimal protocols to prime MSCs before administration might improve the clinical results. Second, MSCs are a heterogeneous cell population and it has been described that different subsets of these cells have different functional capacities [27]. Identifying and isolating a subpopulation of MSCs with enhanced immunosuppressive properties might be a promising strategy to improve clinical efficacy. Lastly, notable differences in immunosuppressive capacity between MSCs from different donors have been found [47[■]]. Therefore, a careful selection process of the right donor might be favorable for outcomes too.

EMERGING STEM CELL THERAPIES

Several other stem cells have been explored in preclinical settings as well. Induced pluripotent stem cells (iPSCs) are pluripotent cells derived from terminally differentiated cells by dedifferentiation. The generation of iPSCs creates the possibility of generating tissue specific cells, but also HSCs or MSCs [48]. Intestinal stem cells also carry a strong therapeutic potential to enhance mucosal healing. After administration of ex-vivo expanded colonic stem cells, engraftment of the cells in, and healing of, colonic mucosa has been shown in mice [49[■]]. Lastly, specific cellular therapies are offering clinical potential as well. For example, administration of ex-vivo expanded Treg cells

has been shown beneficial in a GvHD mouse model [50].

CONCLUSION

Treatment of refractory IBD patients remains a challenge. HSCT and MSC therapy are both promising strategies to improve disease control in this patient group. HSCT is effective, but is also accompanied by a high complication rate. Improving safety will increase the applicability of this therapy in IBD patients. MSC therapy seems promising but results have been inconsistent. Optimization of this therapeutic strategy is therefore strongly warranted. For both therapies results of phase III studies for IBD are still lacking but are expected in the near future and will shed more light on the efficacy. Next to those two clinically investigated therapies, several other stem cell and cellular therapies are being explored in preclinical settings. Some of those have a promising clinical potential, but further research needs to be performed to explore safety and feasibility of those approaches.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 477).

1. Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med* 2006; 354:1813–1826.
 2. Snowden JA, Saccardi R, Allez M, *et al.* Haematopoietic SCT in severe ■ autoimmune diseases: updated guidelines of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2012; 47:770–790.
- The most recent guidelines on HSCT for autoimmune indications, reviewing the different considerations that need to be made regarding the different phases of the transplant procedure specific for different autoimmune indications.
3. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the ■ innate immune system. *Nat Rev Immunol* 2012; 12:383–396.
- Excellent review discussing the use of MSCs for inflammatory disorders in particular and the underlying mechanisms.
4. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet* 2012; 380:1590–1605.
 5. Ordas I, Eckmann L, Talamini M, *et al.* Ulcerative colitis. *Lancet* 2012; 380:1606–1619.
 6. Scharl M, Rogler G. Inflammatory bowel disease pathogenesis: what is new? *Curr Opin Gastroenterol* 2012; 28:301–309.
 7. Christophi GP, Rong R, Holtzapfel PG, *et al.* Immune markers and differential ■ signaling networks in ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2012; 18:2342–2356.
- This study identified important regulatory pathways in IBD pathogenesis by identifying gene expression of 70 genes involved in inflammation in 98 biopsy specimens.

8. Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 2011; 140:1756–1767.
 9. Jostins L, Ripke S, Weersma RK, *et al.* Host-microbe interactions have shaped ■ the genetic architecture of inflammatory bowel disease. *Nature* 2012; 491:119–124.
- Large GWAS analyzing 75 000 IBD cases and controls, thereby shedding more light on the pathogenesis of IBD. It is suggested that the interaction between immune system and microbes plays a pivotal role herein.
10. Cario E. Commensal-innate immune miscommunication in IBD pathogenesis. *Dig Dis* 2012; 30:334–340.
 11. Muraro PA, Douek DC, Packer A, *et al.* Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. *J Exp Med* 2005; 201:805–816.
 12. 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; 113:214–223.
 13. Burt RK, Craig RM, Milanetti F, *et al.* Autologous nonmyeloablative hematopoietic stem cell transplantation in patients with severe anti-TNF refractory Crohn disease: long-term follow-up. *Blood* 2010; 116:6123–6132.
 14. Cassinotti A, Onida F, Annaloro C, *et al.* P362. Autologous haematopoietic ■ stem cell transplantation without CD34+ cell selection for refractory Crohn's disease: the Milan experience after 5 years. *J Crohns Colitis* 2012; 6:S153–S154.
- Long-term follow-up of 10 severe Crohn's disease patients undergoing HSCT, with encouraging results.
15. 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; 5:543–549.
 16. Hawkey C, Allez M, Ardizzone S, *et al.* 9 Clinical and endoscopic improvement ■ following hematopoietic stem cell transplantation in the ASTIC trial. *J Crohn's Colitis* 2013; 7:S4.
- Preliminary results of the first phase III randomized controlled trial analyzing autologous HSCT for Crohn's disease.
17. Snowden JA, Pearce RM, Lee J, *et al.* Haematopoietic stem cell transplantation (HSCT) in severe autoimmune diseases: analysis of UK outcomes from the British Society of Blood and Marrow Transplantation (BSBMT) data registry 1997–2009. *Br J Haematol* 2012; 157:742–746.
 18. Farge D, Labopin M, Tyndall A, *et al.* Autologous hematopoietic stem cell transplantation for autoimmune diseases: an observational study on 12 years' experience from the European Group for Blood and Marrow Transplantation Working Party on Autoimmune Diseases. *Haematologica* 2010; 95:284–292.
 19. Ditschkowski M, Einsele H, Schwerdtfeger R, *et al.* Improvement of inflammatory bowel disease after allogeneic stem-cell transplantation. *Transplantation* 2003; 75:1745–1747.
 20. Marti JL, Mayordomo JL, Isla MD, *et al.* PBSC autotransplant for inflammatory bowel disease (IBD): a case of ulcerative colitis. *Bone Marrow Transplant* 2001; 28:109–110.
 21. Yu LZ, Qian S, Hong M, *et al.* A case of ulcerative colitis associated with autoimmune hemolytic anemia successfully treated by autologous hematopoietic stem cell transplantation. *Am J Gastroenterol* 2010; 105:2302–2304.
 22. Oevermann L, Lang P, Feuchtinger T, *et al.* Immune reconstitution and ■ strategies for rebuilding the immune system after haploidentical stem cell transplantation. *Ann N Y Acad Sci* 2012; 1266:161–170.
- Excellent review that summarizes the different approaches that can enhance immune reconstitution after allogeneic HSCT based on clinical studies.
23. Toubert A, Glaudy S, Douay C, Clave E. Thymus and immune reconstitution ■ after allogeneic hematopoietic stem cell transplantation in humans: never say never again. *Tissue Antigens* 2012; 79:83–89.
- Excellent review describing the background behind different strategies that might be explored to enhance T-cell reconstitution after HSCT, with a strong emphasis on the function of the thymus in this process.
24. Delaney C, Heimfeld S, Brashem-Stein C, *et al.* Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med* 2010; 16:232–236.
 25. Eyrich M, Schreiber SC, Wollny G, *et al.* Predifferentiated human committed T-lymphoid progenitors promote peripheral T-cell reconstitution after stem cell transplantation in immunodeficient mice. *Eur J Immunol* 2011; 41:3596–3603.
 26. de Lima M, McNiece I, Robinson SN, *et al.* Cord-blood engraftment with ■ ex vivo mesenchymal-cell coculture. *N Engl J Med* 2012; 367:2305–2315.
- Recent clinical study showing that ex-vivo expansion of cord blood cells upon coculture with MSC is feasible and has a positive effect on hematopoietic engraftment of these cells after transplantation.
27. Sivasubramanian K, Lehnen D, Ghazanfari R, *et al.* Phenotypic and functional heterogeneity of human bone marrow- and amnion-derived MSC subsets. *Ann N Y Acad Sci* 2012; 266:94–106.
 28. Bassi G, Pacelli L, Carusone R, *et al.* Adipose-derived stromal cells (ASCs). *Transfus Apher Sci* 2012; 47:193–198.

29. English K. Mechanisms of mesenchymal stromal cell immunomodulation. ■ ■ Immunol Cell Biol 2013; 91:19–26.
Excellent comprehensive review that summarizes the different effects MSCs exert on the immune system.
30. Dazzi F, Lopes L, Weng L. Mesenchymal stromal cells: a key player in 'innate ■ ■ tolerance'? Immunology 2012; 137:206–213.
Clarifying review regarding the different properties of MSCs in different environments and their possible physiologic role.
31. He XW, He XS, Lian L, *et al.* Systemic infusion of bone marrow-derived mesenchymal stem cells for treatment of experimental colitis in mice. Dig Dis Sci 2012; 57:3136–3144.
32. Castelo-Branco MT, Soares ID, Lopes DV, *et al.* Intraperitoneal but not intravenous cryopreserved mesenchymal stromal cells home to the inflamed colon and ameliorate experimental colitis. PLoS One 2012; 7:e33360.
33. Duijvestein M, Wildenberg ME, Welling MM, *et al.* Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. Stem Cells 2011; 29:1549–1558.
34. Le Blanc K, Frasson 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; 371:1579–1586.
35. Bernardo ME, Ball LM, Cometa AM, *et al.* Co-infusion of ex vivo-expanded, parental MSCs prevents life-threatening acute GVHD, but does not reduce the risk of graft failure in pediatric patients undergoing allogeneic umbilical cord blood transplantation. Bone Marrow Transplant 2011; 46:200–207.
36. Martin PJ, Uberti JP, Soiffer RJ, *et al.* Prochymal improves response rates in patients with steroid-refractory acute graft versus host disease (SR-GVHD) involving the liver and gut: results of a randomized, placebo-controlled, multicenter phase III trial in GVHD. Biol Blood Marrow Transplant 2010; 16:S169–S170.
37. Garcia-Olmo D, Garcia-Arnan M, Herreros D, *et al.* A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum 2005; 48:1416–1423.
38. Garcia-Olmo D, Herreros D, Pascual I, *et al.* Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. Dis Colon Rectum 2009; 52:79–86.
39. Guadalajara H, Herreros D, De-La-Quintana P, *et al.* Long-term follow-up of ■ ■ patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. Int J Colorectal Dis 2012; 27:595–600.
Long-term follow-up of a placebo-controlled study analyzing MSCs for fistula in Crohn's disease and non-Crohn's disease patients, showing sustained response in a subset of patients.
40. 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; 60:788–798.
41. de la Portilla F, Alba F, Garcia-Olmo D, *et al.* Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. Int J Colorectal Dis 2013; 28:313–323.
This study shows that allogeneic MSC therapy is well tolerated and seems beneficial for Crohn's disease fistula.
42. Herreros MD, Garcia-Arnan M, Guadalajara H, *et al.* Autologous expanded ■ ■ adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1 fistula Advanced Therapy Trial 1) and long-term evaluation. Dis Colon Rectum 2012; 55:762–772.
For non-Crohn's disease related fistula negative results were shown in this phase III randomized controlled trial assessing the efficacy of MSCs.
43. 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; 59:1662–1669.
44. Liang J, Zhang H, Wang D, *et al.* Allogeneic mesenchymal stem cell trans- ■ ■ plantation in seven patients with refractory inflammatory bowel disease. Gut 2012; 61:468–469.
The only study evaluating the response of ulcerative colitis after MSC therapy.
45. Forbes G, Sturm M, Leong R, *et al.* P590 Allogeneic mesenchymal stromal cells ■ ■ for biologic refractory luminal Crohn's disease. J Crohn's Colitis 2013; 7:S247.
First encouraging results of study evaluating allogeneic MSCs for luminal Crohn's disease.
46. Lalu MM, McIntyre L, Pugliese C, *et al.* Safety of cell therapy with mesench- ■ ■ ymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. PLoS One 2012; 7:e47559.
This meta-analysis shows that MSC therapy is well tolerated. No increased risk of death, infections or malignancy was observed.
47. Francois M, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression ■ ■ correlates with cytokine induction of indoleamine 2,3-dioxygenase and by-stander M2 macrophage differentiation. Mol Ther 2012; 20:187–195.
This study shows the central role of stimulation of MSCs with pro-inflammatory cytokines for their immunosuppressive function, and the role of IDO as mediator in this process.
48. Wu SM, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. Nat Cell Biol 2011; 13:497–505.
49. Yui S, Nakamura T, Sato T, *et al.* Functional engraftment of colon epithelium ■ ■ expanded in vitro from a single adult Lgr5(+) stem cell. Nat Med 2012; 18:618–623.
Landmark study showing the feasibility of the generation of colon epithelium from one single adult stem cell, and engraftment of these cells in colon epithelium in mice.
50. Hippen KL, Merkel SC, Schirm DK, *et al.* Massive ex vivo expansion of human natural regulatory T cells (Tregs) with minimal loss of in vivo functional activity. Sci Transl Med 2011; 3:83ra41.