

Stem cell-based therapies in inflammatory bowel disease: promises and pitfalls

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Abstract: Inflammatory bowel disease (IBD) is a chronic, often relapsing, condition that deeply impacts the quality of life for many patients. Although there have been significant advances in medical treatments, a large proportion of patients become refractory to available therapeutic options. Stem-cell therapy through hematopoietic stem cells (HSCs) or mesenchymal stem (stromal) cells (MSCs) is a promising therapeutic option for severe refractory cases especially when surgery is not feasible. In HSC transplantation, the objective is to destroy the 'autoreactive' immune cells responsible for disease chronicity, and to re-establish gut tolerance to gut microbes. In perianal Crohn's disease (CD), the objective is to deposit MSCs locally in fistulizing tracts to down-regulate the local immune response and induce wound healing. Results from upcoming and ongoing clinical trials will set the path of these novel therapeutic options that have the capability to successfully treat severe refractory Crohn's patients.

Keywords: Crohn's disease, hematopoietic stem cells, inflammatory bowel disease, mesenchymal stem cells, stem-cell therapy

Introduction

Inflammatory bowel diseases (IBDs) can be categorized as ulcerative colitis (UC) or Crohn's disease (CD) and may affect any location throughout the gastrointestinal tract. Unfortunately, incidence and prevalence of IBD is ever-increasing for both pediatric and adult populations, signaling its emergence as a global disease especially in Western countries [Gazouli *et al.* 2014]. Every 1 in 200 Americans bears a diagnosis [Molodecky *et al.* 2012]. Symptoms of active disease include abdominal pain, diarrhea, unintended weight loss, and most commonly fatigue [Hendrickson *et al.* 2002; Bielefeldt *et al.* 2009]. Quality of life is impacted and patients become more susceptible to develop colorectal cancer [Beaugerie and Itzkowitz, 2015]. Seen also are high rates of surgeries, hospitalizations, and drug adverse side-effects [Ramirez and Fleshner, 2006]. Resection rate for CD is at 29% and colectomy rate in UC is lower at 12%; both rates measured within 7 years of initial diagnosis [Vester-Andersen *et al.* 2014].

IBD is a destructive and debilitating lifetime condition that has a significant impact on quality of

life [Mitchell *et al.* 1988]. Disease relapse affects many aspects of patients' lives. Homeostatic mental wellbeing and physical health are at risk, not to mention the profound impact IBD has on personal relationships and work productivity [Zand *et al.* 2015]. In a patient survey involving over 5600 responses, three quarters of the members reported that IBD symptoms affect their ability to enjoy leisure activities, and around 69% of the same members report symptoms that affect their ability to be productive at work [Ghosh and Mitchell, 2007; Zand *et al.* 2015]. The ultimate goal in treating IBD is to achieve deep remission (symptom control and endoscopic healing of mucosal lesions [Rogler *et al.* 2013]), and reduce long-term disability while maintaining a normal quality of life [Hommes *et al.* 2012].

The current treatment for IBD is centered upon symptom control in a stepwise approach. This method begins with medications: 5-aminosalicylic acid (5-ASA) agents and antibiotics. Subsequently followed by corticosteroids, immunomodulators, and biologics. When all else fails, the final option tends to be surgery [Thomas and Lodhia, 2014].

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Half of CD patients require surgical resection at some point during the disease course [Peyrin-Biroulet and Lemann, 2011]. However, some CD patients refuse surgery or are not eligible candidates given the large extent of small bowel disease. The high risk of developing short-bowel syndrome is a factor to consider. A particular subset of patients exists who are refractory to all current medical therapies and cannot undergo surgery [Hwang and Varma, 2008]. Stem-cell therapy is a promising alternative to treat ongoing tissue damage by resetting the underlying disease process, through alteration of the mucosal immune response [Heslop *et al.* 2015]. Existing and ongoing studies show promising yet inconclusive results. The outcomes obtained from past and current clinical trials have potential to add a new branch of disease management for patients with IBD, significantly improving the quality of life for those who need it the most.

Etiopathogenesis of IBD

While there have been significant advances into the pathogenetic insight of IBD, the exact etiology is still unknown. Genome-wide association studies (GWASs) helped to identify genetic risk loci, where 28 markers are shared between UC and CD [Franke *et al.* 2010; Anderson *et al.* 2011]. The anti-inflammatory cytokine interleukin (IL)-10 locus was initially associated with UC [Franke *et al.* 2008] later to be associated also in CD [Franke *et al.* 2010]. Deficiency in IL-10 and its receptor leads to severe early-onset colitis [Shah *et al.* 2012]. The manifestation of IBD often involves genetic and nongenetic cues; orchestration of complex genetic [Franke *et al.* 2010; Fiocchi, 2012], environmental [Cosnes *et al.* 2011], and microbial [Chassaing and Darfeuille-Michaud, 2011] factors. A genetically susceptible host can develop a dysregulated immune response to commensal bacteria and luminal antigens [Lanzoni *et al.* 2008]. Environmental stimuli can also trigger a change in the innate and adaptive immune function in epithelial barrier function and microbiome composition leading to an active disease state [Baumgart and Sandborn, 2012; Ordas *et al.* 2012; Scharl and Rogler, 2012].

Although the etiology is not fully elucidated, IBD is nonetheless classified as an autoimmune disease. Autoimmune activation is seen with circulating antibodies against epithelial barrier function and commensal enteric bacterial population involvement [Broberger and Perlmann, 1959;

Wen and Fiocchi, 2004]. In both UC and CD, antibodies are present against a range of autoantigens including lymphocyte antigens [Korsmeyer *et al.* 1975]. Two commonly studied autoantibodies in autoimmune diseases are: atypical perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) [Das and Biancone, 2008]. A study by Duerr and colleagues showed P-ANCA are present in 60–75% of UC patients with primary sclerosing cholangitis [Duerr *et al.* 1991]. More recent studies show autoimmunity against human tropomyosin isoform 5 (hTM5) as a critical epithelial autoantigen in UC, which can induce both cellular and humoral immune responses [Geng *et al.* 2001; Das *et al.* 2008; Ebert *et al.* 2009]. These hTM5-specific immunoglobulin G (IgG) autoantibodies show a direct pathogenic effect on the destruction of colonic epithelial cells.

Although current research has reached significant advancements in understanding the underlying course of action in the development of IBD, the exact mechanisms are still vague and unclear. This results in a difficult treatment course for IBD patients. Until the exact pathways and mechanisms are understood, current medical treatment for IBD is aimed at symptom control, often by the way of immunosuppression.

Stem-cell therapy

Stem cells are defined as undifferentiated cells having the capacity to differentiate into tissue or organ-specific cells, and are characterized as either embryonic or somatic (adult) stem cells. Stem cells differ depending on origin and function [Smith *et al.* 2006]. Stem-cell therapy can either be autologous (isolated from the same individual) or allogeneic (isolated from a donor, ideally human leukocyte antigen [HLA]-matched). There has been inconsistent yet promising results from clinical trials in the past few decades, using stem-cell therapy through hematopoietic stem cells or mesenchymal stem cells [Reya *et al.* 2001]. Transplant data from 2013 show that over 320,000 patients have received either autologous or allogeneic stem-cell transplants for various indications [Pasquini and Zhu, 2015].

Hematopoietic stem-cell therapy

Hematopoietic stem cells are multipotent cells that have a self-renewal property, and are capable

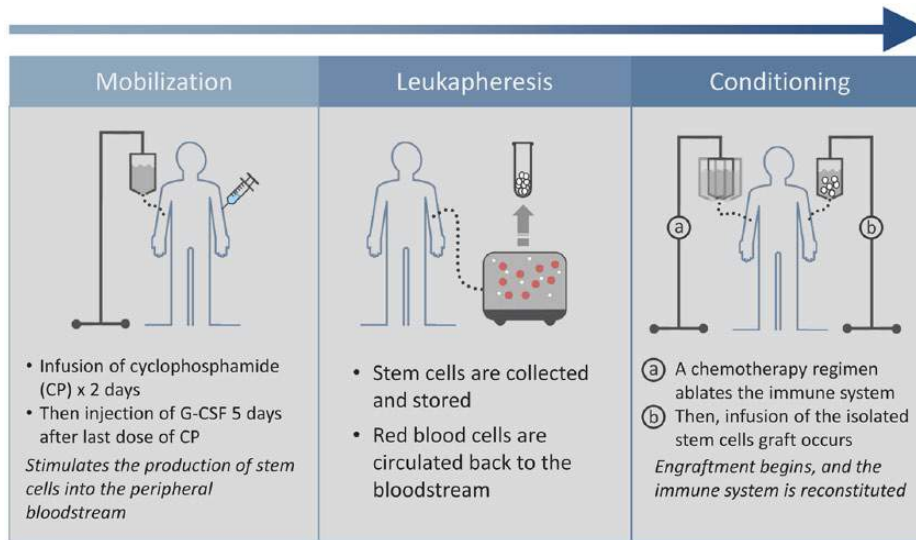


Figure 1. Autologous stem-cell transplantation.

of differentiating into blood and immune cells. These stem cells can be isolated from the bone marrow (BM), umbilical cord blood, or more commonly peripheral blood [Da Silva Meirelles *et al.* 2006]. They are progenitors of both myeloid (monocytes, erythrocytes, macrophages, neutrophils, and dendritic cells) and lymphoid (T cells, B cells, and natural killer cells) lineages [Pittenger *et al.* 1999]. HSCs have the capability to directly migrate to a damaged tissue or differentiate to epithelial or immunomodulatory cells in order to restore normal mucosal tissue [Kavanagh and Kalia, 2011].

The HSC multistep procedure begins with a pre-transplant screening process that involves (but is not limited to) history and examination, blood tests, serologies, colonoscopy, small bowel magnetic resonance imaging (MRI), or pelvic MRI (only if perianal disease), and BM aspiration. The subsequent transplant procedure involves three main steps:

- (1) Hematopoietic stem cell transplantation (HSCT) begins with mobilization of stem cells from the patient (autologous [Figure 1]) or an HLA-matched donor (allogeneic) which begins with infusion of cyclophosphamide (CP) for 2 consecutive days to remove lymphocyte cells. Then 5 days following the last administration of CP, involves subcutaneous injection of granulocyte colony-stimulating factor (G-CSF) to stimulate BM to produce stem cells

that are subsequently released to the bloodstream [Sallerfors and Olofsson, 1992]. Administration of G-CSF continues for 4–5 days and ends the day before leukapheresis. Prophylactic antibiotics are recommended.

- (2) The second step, leukapheresis, aims to collect the $CD34^+$ cells from the peripheral blood or BM up to a target number of $3-8 \times 10^6$. Collected cells are then cryopreserved until infusion.
- (3) The final transplant step is conditioning with transplantation. This step involves total body immune ablation through chemotherapy to eliminate autoreactive cells. Intravenous CP is administered for 4 consecutive days; with infusion of rabbit antithymocyte globulin (rbATG) 2 days after the first dose of CP. Methylprednisolone is also administered to improve tolerability. Transplantation of the isolated cells begins 5 days after the first administration of CP and 24 hours following the end of rbATG and methylprednisolone. The stem cells are then transplanted through a central venous catheter to the patient in varying dosages. Careful isolation is essential to reduce the risk of transplant morbidities and mortality. Engraftment and reconstitution of the immune system last about 2–4 weeks. Postengraftment is the final phase of HSCT, where follow up is essential, especially in allogeneic HSCT, when risk of chronic graft-versus-host disease (GvHD) can occur. In GvHD the

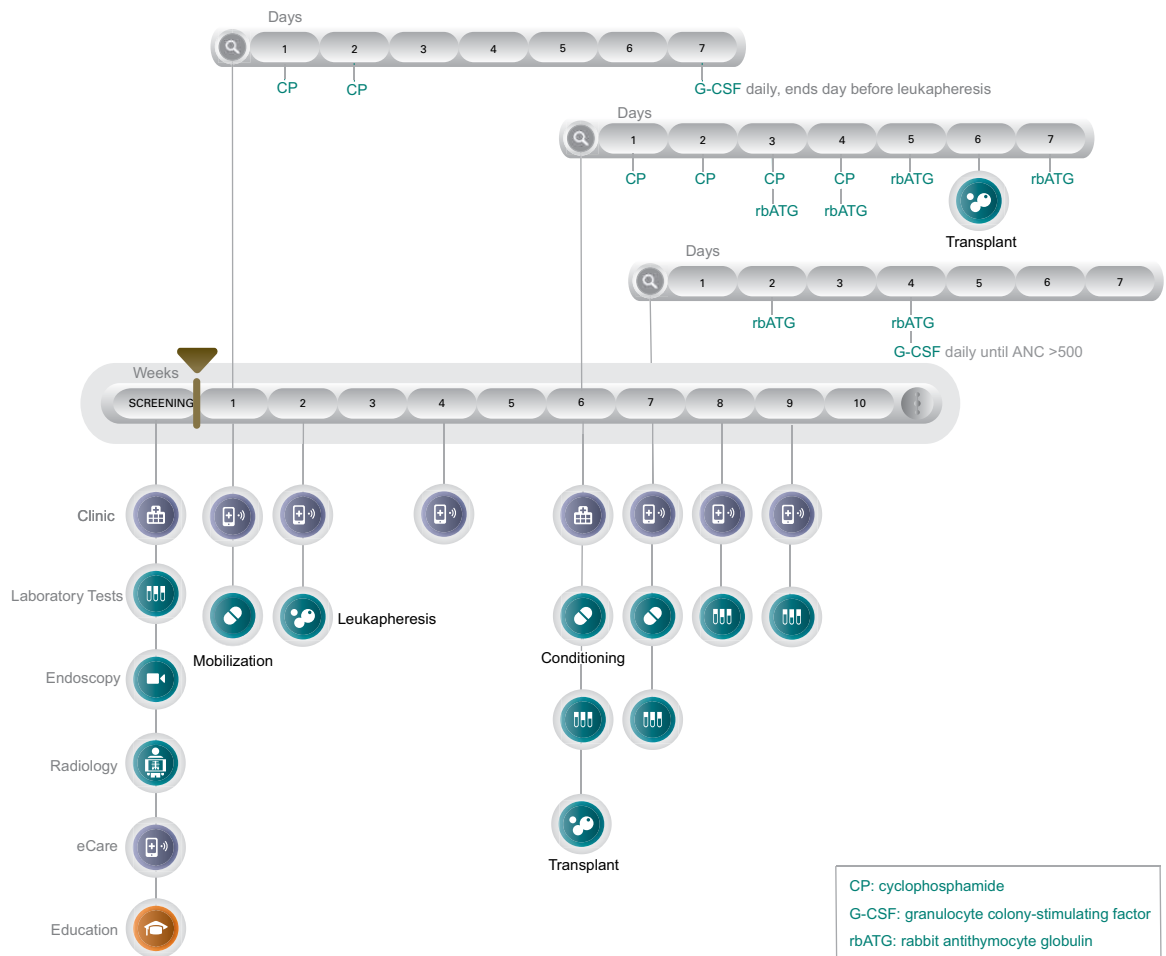


Figure 2. HSCT Care Pathway™. ANC, absolute neutrophil count.

donor effector cells are activated and attack the recipient’s immune system [Lee *et al.* 2003]. Mild forms of chronic GvHD can be successfully treated with steroids or immunosuppressive medications (e.g. azathioprine) [Iwasaki, 2004]. Patients follow up with their physicians 1, 2, 4, 6, 12, and 24 months after discharge.

The protocol for stem-cell transplantation is very precise and can be confusing for the patient to follow and understand without a guideline and proper education. Implemented and designed by UCLA Center for IBD, Los Angeles, USA, is an individualized scenario where each step of the transplant process is laid out in a detailed care pathway accessible to the patient and provider. The following HSCT Care Pathway [Figure 2] begins with a screening period. During this time, indication for transplant is established and

a multidisciplinary care team is coordinated including GI, hematology, and oncology. When not hospitalized, patients capture their symptoms through a mobile application (eCare). All screening examinations and tests are performed prior to mobilization at week 1. Mobilization (week 1) and leukapheresis (week 2) can be carried out in an outpatient setting. Hospitalization occurs at week 6, initiating the conditioning phase, and infusion of CD34+ stem cells. The patient is followed closely for about 4–5 weeks post-infusion to monitor for side-effects, toxicity, and infections. When absolute neutrophil count (ANC) reaches >500 the patient is discharged and then is scheduled for follow-up visits. The HSCT Care Pathway is customizable beyond standard protocol procedures, and can be reset or extended.

Modeling of an individualized and customizable scenario is clearly laid out in a visual timeline that

is available to both the patient and provider on the web or a mobile platform. Each circle is an active icon where the patient can click through to pull information to see results of lab tests, schedule a clinic visit, or capture their symptoms.

Historically, animal models provided first insight into HSCT showing it could be an efficacious mode of treatment for autoimmune diseases [Bekkum, 2000; Van Bekkum, 2000; Ikehara, 2001]. Initially, HSCT was used to treat malignant hematological indications in humans [Yin and Jowitt, 1992; Bierman *et al.* 2003]. In clinical practice, several case studies reported improvement of autoimmune disease activity following HSCT for concomitant aplastic anemia or malignancy [Van Deen, 2012]. In a case where two patients with severe aplastic anemia underwent allogeneic BM transplant (BMT), following transplantation their case of severe rheumatoid arthritis had become quiescent in junction with resolution of the aplastic anemia indication. Reported remission lasted for 13 years following transplantation [Lowenthal *et al.* 1993]. Expanding further, experimental results and phase I–II trials show that high-dose chemotherapy with autologous HSCT can successfully treat severe cases of various autoimmune diseases [Gratwohl *et al.* 2005]. Historically, autoimmune diseases treated with HSCT include but are not limited to multiple sclerosis [Burt *et al.* 1995], lupus, and rheumatoid arthritis [Jacobs *et al.* 1986]. The central aim of HSCT effectiveness is to ‘reset’ the immune system by eliminating self-reactive T-lymphocytes and memory cells that contribute as the effectors of a dysregulated immune system [Martinez-Montiel Mdel *et al.* 2014]. Retrospective analyses and prospective studies show feasibility and safety of auto- and allo-HSCT for autoimmune diseases [Daikeler *et al.* 2009; Farge *et al.* 2010].

In the largest cohort study, the outcomes of 900 patients from 1996–2007 were measured for autologous HSCT transplants of various indications [Farge *et al.* 2010]. The survival rate of autoimmune HSCT transplants was 85% at 5 years with 43% progression-free survival. Around 30% of patients showed a complete response, despite full immune reconstitution. Analysis showed the type of autoimmune disease was most relevant to the outcome, rather than the transplant technique. Around half of the deaths (often due to infection) were treatment related and appeared to be strongly correlated with the transplant center and not the

conditioning regimen [Farge *et al.* 2010]. The following prospective study involved a high rate of complications. It involved 26 refractory CD patients who underwent an auto-HSCT. During the mobilization phase, 16 cases of febrile neutropenia, 1 case of bacteremia, and 2 cases related to septic shock were seen. A total of 21 patients then entered the conditioning phase. Infectious complications continued, and noninfectious complications occurred in 6 patients: 1 who suffered an rbATG drug reaction, 12 mucositis events, and 2 hemorrhagic complications. In addition, 1 patient suffered from a cytomegalovirus leading to death by multiorgan failure [Jauregui-Amezaga *et al.* 2014]. Initially, IBD patients underwent HSCT for other indications (such as leukemia and non-Hodgkin’s lymphoma [NHL]). The first case of a CD patient undergoing an autologous BMT for NHL occurred in 1993, resulting in a 6-month remissive state [Drakos *et al.* 1993]. Other study results varied but were promising, showing a remissive state for the primary indication along with clinical and endoscopic improvement for UC or CD [Yin *et al.* 1992; Drakos *et al.* 1993; Castro *et al.* 1996; Kashyap, 1998; Lopez-Cubero *et al.* 1998]. By complete immune ablation and reconstitution with hematopoietic stem cells, the body is able to generate naïve lymphoid and myeloid cells [Swenson and Theise, 2010], thus reducing T-cell activity against mucosal self-antigens and inflammation. The inflammatory process is controlled, but the underlying genetic predispositions are not modified with an autologous transplant. Small studies have been performed showing auto-HSCT’s ability to induce a remissive state. A 2010 study by Burt and colleagues included 24 CD patients where remission rates (defined as supplementary medication was not initiated) were at 91%, 57%, and 19% at years 1, 3, and 5 respectively [Burt *et al.* 2010]. Another study showed all patients entered clinical remission at a 3-month follow up following transplantation; then at a 16.5-month follow up, 75% of patients remained in clinical and endoscopic remission. However, it is important to note the small sample size only included four CD patients [Cassinotti *et al.* 2008].

Although data are limited, there are a few case reports of UC patients undergoing stem-cell transplantation for other indications. One patient was showed to have prolonged clinical remission after high-dose chemotherapy and peripheral-blood stem-cell (PBSC) transplantation for high-risk breast cancer. Following transplant, the patient reported no symptoms and was off

IBD-related medication for 2 years [Castro *et al.* 1996]. Another case of a 57-year-old woman with distal UC undergoing auto-HSCT for breast cancer was asymptomatic and discontinued IBD maintenance therapy 6 months after transplant. However, it is important to note that the patient did not have any IBD-related symptoms before the transplant and there was no colonoscopy to confirm disease remission at 6 months. At a 25 month follow up, the patient developed rectal bleeding and became asymptomatic only after restarting maintenance mesalamine therapy [Marti *et al.* 2001]. Stem-cell transplants in UC show a favorable but short-lived response. There is a randomized, double-blind, placebo-controlled phase II study of MultiStem® Cell Therapy (Athersys Inc., USA) for moderate-to-severe UC. The study showed good tolerability but failed to show efficacy over 8 weeks [Lehmann, 2014]. MultiStem is a manufactured biologic product isolated from human BM. The single intravenous (IV) administration did not show statistically significant improvement.

Methods in HSCT for IBD still vary, and the protocols are ever changing. For the most part, studies select CD34+ PBSCs to use for transplant. The reason is that CD34+ is a hematopoietic progenitor cell antigen that functions as a cell-cell adhesion factor [Satterthwaite *et al.* 1992; Simmons *et al.* 1992]. When attempting to isolate HSCs, effector cells are present in the blood sample. Theoretically when selecting for CD34+ in the sample, the stem cells are selected and not the effector cells [Larocca *et al.* 2006]. Two studies carried out by Cassinotti and colleagues evaluated the safety and efficacy of unselected CD34+ PBSCs for auto-HSCT [Cassinotti *et al.* 2008; Clerici *et al.* 2011; Cassinotti *et al.* 2012]. For both studies, no improvement was seen following mobilization but the procedure was well tolerated. In the 2007 study with four refractory CD patients, preliminary results showed 75% of patients remained in clinical and endoscopic remission at a 16.5-month follow up, with full fistula closure observed for all patients. In a study 4 years later, following transplantation all patients ($n = 6$) remained in remission for 1–3 months without any immunosuppressive treatment. Late outcomes at 1-year follow up showed five out of six of the patients displayed full response and maintained both clinical and endoscopic remission without further treatment [Clerici *et al.* 2011]. The protocol to extract unselected CD34+ PBSCs is less technical and expensive to perform.

This offers a potential opportunity for midsized transplant centers to provide HSCT for their refractory patients. However, the studies mentioned above involved a limited sample size. There is a need for long-term follow up and randomized control studies with a larger cohort of patients.

A study carried out by Oyama and colleagues showed improvement with hematopoietic autologous peripheral blood (PB) HSCT with low-dose CP and G-CSF [Oyama *et al.* 2005]. At an 18.5 month follow up, 11 out of 12 patients were off all immunosuppressive drugs and had achieved clinical remission. It is important to note this study measured clinical outcomes and did not report on any endoscopic findings. A monocenter phase I and II trial carried out by Hasselblatt and colleagues measured the outcomes of 12 patients with refractory CD undergoing autologous PB HSCT with immunoablation conditioning [Hasselblatt *et al.* 2012]. Mobilization was carried out with CP and G-CSF, harvesting CD30+-selected PBSCs. Around 56% of the patients showed clinical and endoscopic improvement in response to the mobilization procedure. Continuing with nine patients entering the conditioning phase, five patients remained in a remissive state at a 6-month follow up. One of these patients showed complete remission of pyoderma gangrenosum extraintestinal manifestation, and another patient had a complete fistula closure. However, seven of the nine patients had a relapse of disease at a 3-year follow-up. It is interesting to note that mucosal healing achieved in 56% of patients in this study is noticeably higher than previous results of phase II trials involving TNF- α inhibitors as the mode of therapy with no transplant [Hanauer *et al.* 2002].

The ASTIC study, currently the only multicenter randomized phase III interventional study, carried out by the European Group for Blood and Marrow Transplantation (EBMT) aimed to test the potential clinical benefits of high-dose immunoablation of early *versus* delayed (59 weeks) auto-HSCT. All 45 patients were mobilized prior to randomization. The early group received immediate HSCT 4 weeks post-leukapheresis, while the control delay group waited a year for transplantation and continued conventional therapy. A stringent primary endpoint for full clinical remission was defined by a Crohn's Disease Activity Index (CDAI) score <150, no use of corticosteroid, immunosuppressive, or biologic drugs for the last 3 months. Lastly, no endoscopic or

radiological evidence of active disease along any part of the GI. Secondary endpoints were the individual components of the primary composite outcome along with labs, quality of life (QoL), and imaging. Results from this long-term study have just been released, where only 2 out of 23 patients from the early treatment group achieved full clinical and endoscopic remission compared to 1 out of 22 from the controlled delay group [Hawkey *et al.* 2015]. Showing there is no statistical difference in sustained disease remission between the HSCT-treated group *versus* conventional therapy controls. The trial also stated all patients experienced a non-serious adverse complication, with infections as the most common. One patient in the early treatment group died 20 days after the conditioning phase, due to sinusoidal obstructive syndrome.

The authors concluded the study against widespread use of HSCT for patients with refractory CD; A stark contrast from the preliminary results, showing promising durable benefit 1 year after transplant [Hawkey, 2014]. The authors did address the stringent endpoint to justify treatment toxicity. However, before dismissing the widespread use of HSCT for refractory CD patients it is important to dissect the suboptimal end points used in the study design [Hommes and Lacey, 2016]. If a patient did not meet any composite criteria, the transplant was classified as a failed treatment, including use of corticosteroid or immunosuppressive drugs. A maintenance immunosuppressive regimen could have had a positive impact on the rates of remission. Studies have shown induction should include maintenance therapy in order to prevent relapse of the disease [Burt *et al.* 2010]. A different conclusion can be reached if the endpoints were less demanding in addition to including maintenance therapy after transplant for this difficult-to-treat refractory patient population. Allogeneic HSCT has the ability to genetically correct the underlying disease with a healthy donor [Lopez-Cubero *et al.* 1998]. However, it is associated with a higher risk of complication and mortality rate when compared to auto-HSCT. There is also a high risk of a noninfectious adoptive autoimmunity transfer of IBD, leading to a phenotypic manifestation in patients receiving allogeneic BMT. A newly diagnosed fulminant Crohn's colitis occurred in a patient with NHL following nonmyeloablative stem-cell transplantation [Sonwalkar *et al.* 2003]. There are also two cases of patients developing UC after BMT [Spiers, 1984; Baron *et al.* 1998].

Learning from these unfortunate cases, it is important to fully screen donors for known genetic risk factors and avoid mismatches for HLA class genes.

Although the limited number of phase I–III trials show promising results, the occurrence of relapse makes it difficult to categorize HSCT as a complete curative treatment. When remission rates drop from 91% at 1 year to 19% at 5 years, long-term outcome measurements are necessary [Burt *et al.* 2010]. It is also interesting to note that most studies did not initiate any form of combination therapy post-transplant. Future studies could look into the safety and efficacy of combination therapy following HSCT. Expert transplant centers should collaborate to continue biobanking and grow the registry of both successful and unsuccessful HSCT cases. Having more information about individual cases will provide new insights to treatment mechanisms leading to improvements in protocol. A risk–benefit analysis must be fully discussed with the patient before initiating treatment.

Mesenchymal cell transplants

Mesenchymal stem cells (MSCs) are multipotent cells capable of differentiating into several types of cells and are present in all tissues of the body [Da Silva Meirelles *et al.* 2006]. Friedenstein and colleagues first described the mesenchymal stem-cell concept in 1974 when it was isolated from BM [Friedenstein *et al.* 1974]. The cells can also be successfully isolated from umbilical cord blood or adipose tissue for clinical application [Zuk *et al.* 2002; Sensebe *et al.* 2010]. Adipose-derived stem cells are easily extracted in large quantities with reduced morbidity and discomfort for the patient.

A study by Melief and colleagues confirmed that BM and adipose-derived MSCs had similar immune-modulating functions for multilineage differentiation, while adipose tissue had more potent modulating property over BM due to the increased level of cytokine secretion [Melief *et al.* 2013]. The International Society for Cellular Therapy has set specific defining characteristics for a stem cell to be labeled as mesenchymal: (1) it must have the ability to adhere to plastic under standard culture conditions; (2) it must express CD105, CD73, and CD90 (but not express CD45, CD34, CD14, CD11b, CD79 α , CD19, or HLA-class II molecules); (3) cells must have

multipotent *in vitro* differentiation potential to osteoblasts, adipocytes and chondroblasts [Dominici *et al.* 2006].

In addition to differentiation, and more relevant to clinical practice, MSCs have immunomodulating capabilities to downregulate mucosal immune reactivity and promote tissue healing [Bartholomew *et al.* 2002; Krampera *et al.* 2003; Stappenbeck and Miyoshi, 2009]. Additional studies show that MSCs have the ability to inhibit T-cell proliferation *in vitro* [Bartholomew *et al.* 2002; Le Blanc *et al.* 2003a], and inhibit lymphocyte proliferation by activating a programmed cell death pathway [Augello *et al.* 2005]. The specific immunomodulating mechanisms of MSCs are still unclear, but evidence shows that the influence of the target cell occurs within close proximity and is not solely dependent on cell-to-cell contact [Di Nicola *et al.* 2002; Ankrum *et al.* 2014]. Promising results demonstrate the functional ability of the potential of MSCs to influence the immune response and dampen inflammation, affirming their clinical practicality for therapeutic transplantation in autoimmune diseases.

Administration of MSCs over HSCT is advantageous in regards to their low immunogenicity property, eliminating the need for chemotherapy, which is necessary in HSCT. The thought of low immunogenicity of MSCs stem from the fact that they express low levels of major histocompatibility complex (MHC) class I, no MHC class II, and no costimulatory molecules that would activate T cells [Le Blanc *et al.* 2003b; Ankrum *et al.* 2014]. MHC molecules are found at the cell surface and function to display intracellular proteins to cytotoxic T cells, thus triggering an immune response against the nonself antigen [Janeway *et al.* 2001]. MHCs are also synonymous with HLAs, which are alloantigens that are surface proteins responsible for immune system regulation [Bjorkman *et al.* 1987]. Low immunogenicity would give allogeneic MSCs the ability to evade the immune system and allow their usage across MHC barriers [Schu *et al.* 2012]. Due to the nonimmunogenic properties and ease of expansion, unmatched allogeneic MSCs are advantageous in that they can be mass manufactured making it easier for midsized transplant centers to provide their patient population with the possibility of transplantation. However, *in vitro* work by Nauta and colleagues explains that MSCs are not intrinsically immunoprivileged. Under varying conditions, allogeneic MSCs are capable of stimulating a memory T-cell

response that can result in graft rejection [Nauta *et al.* 2006].

Today, MSCs have been used to treat CD in systemic (intra-arterial or intravenous) transplantation for active luminal disease or local administration for fistulizing disease. There are only a few studies reporting mesenchymal stem cell transplantation (MSCT) for CD and UC [Lazebnik *et al.* 2012a; Lazebnik *et al.* 2012b; Liang *et al.* 2012]. At least 25% of CD patients develop debilitating perianal fistulas within 20 years of diagnosis [Hellers *et al.* 1980; Schwartz *et al.* 2002], with complex fistulas present 78% of the time, and are discernibly more difficult to treat over simple fistulas [Molendijk *et al.* 2014]. Treatment for fistulas has shifted from a surgically-based approach to a more immunosuppressant and biologic step-wise approach. Unfortunately despite the best medical advances, durable fistula closure rates remain low at 37% [Molendijk *et al.* 2014].

In regards to local administration, currently accepted forms of treatment for MSCs in IBD are indicated for the fistulizing phenotype in CD. Two studies in 2013 aimed to heal fistulizing CD, one study with autologous MSCs and the other with allogeneic adipose MSCs. For autologous treatment, results showed complete fistula healing in 82% (27 out of 33) patients 8 weeks following final injection [Lee *et al.* 2013]. The allogeneic treatment demonstrated full closure in 30% of the patients, with the remaining patients showing a reduction of draining from fistula tracts [De La Portilla *et al.* 2013]. Local administration of autologous MSCs was shown to be well tolerated and feasible in a study that included 10 CD patients with actively-draining complex perianal and enterocutaneous fistulas. Following *ex vivo* isolation and expansion, all patients showed either complete or partial fistula closure without any adverse effects. All patients showed a reduction in disease activity, most often after the second administration [Ciccocioppo *et al.* 2011]. However, drawbacks of auto-MSCT are seen with the efficacy of harvesting time, which requires several weeks for *ex vivo* expansion [Brandenberger, 2011]. In comparison, allogeneic transplants seem to be more convenient in terms of time and feasible due to MSCs low immunogenicity characteristic from healthy donors. In the most recent study carried out by Molendijk and colleagues involved the first placebo controlled, double-blinded, randomized,

dose-escalating clinical trial to date [Molendijk *et al.* 2015]. This study included 21 CD patients undergoing local administration of allogeneic BM MSCs for perianal fistulas. Throughout the 24-week trial, no serious adverse events occurred. Also, the dosage of 3×10^7 MSCs showed most efficacious (when compared to 1×10^7 and 9×10^7 MSCs) with a 85.7% successful fistula healing rate at week 12; only 33% showed healing for the placebo group.

In addition to local injections of MSCs, there have been significant advances in systemic infusions of MSCs for luminal CD. Unique to MSCs, an ‘off-the-shelf’ product (Prochymal) has been developed by a third-party laboratory Osiris Therapeutics Inc. (USA) These cells are isolated from the BM of healthy donors and expanded *ex vivo*. The MSCs purified by Osiris are cultured and packaged. Remarkably, up to 10,000 dosages can be obtained from a single donor [Rattue, 2012]. Several phase I–II studies included the Prochymal MSC product for transplant. The first human trial of systemic MSCs was carried out by Onken and colleagues and evaluated nine patients with active CD, who previously failed immunosuppressants and a course of steroids [Onken *et al.* 2006]. Patients were randomized to intravenously receive Prochymal allogeneic MSCs in varying doses. A total of 3 of 10 patients achieved clinical remission by day 14 following transplantation. No serious adverse events occurred during treatment in all groups, and infusions were well tolerated. Duijvestein and colleagues continued to show that autologous BM MSCT appeared to be well tolerated and feasible in the treatment of refractory luminal CD [Duijvestein *et al.* 2010]. Additionally, no serious adverse events occurred during harvesting and IV administration.

A phase II study, carried out by Forbes and colleagues measured clinical response at 42 days after a 4-week course of allogeneic infusions of MSCs [Forbes *et al.* 2014]. Among the 15 patients, a reduction in the CDAI score was seen along with 8 patients entering clinical remission. Currently, a large (330 enrolled patients) multicenter, randomized, double-blind, phase III trial is underway with an expected completion in late 2018 [Raina, 2007]. The purpose of the study is to evaluate the safety and efficacy of Prochymal MSCs at different IV doses for moderate-to-severe CD. Two preliminary analyses have been performed thus far. Of the 207 patients, 148 have successfully reached the 28-day primary endpoint assessment

of an absolute CDAI score <150 , signifying a clinical remissive state [Meldrum, 2014].

Notwithstanding the promising improvement of clinical outcomes, it is necessary to consider the current hazard and safety before enrolling a patient for MSCT. A few studies isolated various modes of MHCs to verify there is no risk for chromosomal aberration development after long-term culturing or induction of tumors during *in vitro* and *in vivo* experiments [Bernardo *et al.* 2007; Macias *et al.* 2010; Tarte *et al.* 2010]. Culture standards need to be perfected, and all possible hazards in the cell sample need to be eliminated before administration. Several phase I–III trials were carried out with historical or concomitant immunomodulating therapy. A study by Duijvestein and colleagues investigated the effect of immunosuppressive drugs (i.e., azathioprine, methotrexate, 6-MP, and anti-TNF α) on the MSC phenotype, survival, and differentiation or immunosuppressive capacity. Their results confirmed mesenchymal stromal cell function is not affected by common drugs used to treat refractory IBD patients [Duijvestein *et al.* 2011]. Moreover, 6-MP and anti-TNF α antibodies enhanced the inhibitory effect, suggesting clinical safety and usefulness of combination therapy.

As discussed in existing literature, there is a complex variety of pathways and mechanisms through which MSCs respond to damaged inflamed tissue in the gut. Future studies should focus on the exact mechanism in which MSCs operate, providing vital information to improve current therapeutic strategies. It is also valuable to look into initiating combination therapy following transplantation to improve remission rates [Duijvestein *et al.* 2011]. Continuing exploration of other stem-cell sources in preclinical animal models is essential. In addition to an understanding of basic science, the protocols for isolation and administration should improve as we move forward towards stem-cell therapies for refractory CD patients and other autoimmune diseases.

Induced pluripotent stem cells

It is possible that mature specialized cells can be reprogrammed to become pluripotent [Gurdon, 1962]. Induced pluripotent stem cells (iPSCs) have this capability and hold great potential for regenerative medicine. Induction to an embryonic-like cell stage is initiated by the introduction of specific transcription factors (Oct4, Sox2, Klf4,

and c-Myc) [Takahashi and Yamanaka, 2006]. The vast capacity to differentiate and infinite ability to propagate are ideal for replacing damaged and diseased tissue. An innovative protocol has been published for the direct generation of intestinal tissue from human embryonic stem cells (ESCs) and iPSCs *in vitro*, by manipulating growth factors [Spence *et al.* 2011]. Also, use of iPSCs eliminates the ethical issue that exists with the use of ESCs.

It is known that regulatory T cells (Tregs) can be used to affect autoimmunity and organ rejection; however, human therapeutic options for Tregs are limited due to the challenging nature of isolating an ideal amount of functional Tregs. A study by Haque and colleagues provided an approach to generate functional Tregs, induced by iPSCs [Haque *et al.* 2012]. Results from this study provide a novel therapeutic approach to treat autoimmune diseases from Tregs, maintaining immunological tolerance and suppressing autoreactive cells. Adoptive cell transfer (ACT) of Tregs showed encouraging results in experimental settings for autoimmune diseases such as rheumatoid arthritis [Wright *et al.* 2009] and systemic lupus [Scalapino and Daikh, 2009]. Conversely, due to its similarity with ESCs there is still a significant risk of teratoma formation [Knoepfler, 2009].

Animal and human models exploring iPSCs in IBD have not yet been explored, and there is limited literature on case studies for iPSCs used to treat autoimmune diseases. Further development of novel therapeutic strategies should involve extensive research and assessment of safety and should eliminate risk factors associated with the transplant before it is introduced in the clinical environment.

Conclusion

The goal in treating IBD remains the same: to achieve deep remission and halt any ongoing disease progression. Hematopoietic and mesenchymal stem cells have been shown to be a potential alternative therapy for disease control in refractory CD. Continued in-depth investigation is warranted to fully understand the complex cellular mechanisms. The information from current ongoing phase III clinical trials will provide a valuable roadmap for the future of stem-cell therapy. Cellular therapy must not be limited to HSCs or MSCs, other cellular therapies should continue to be explored in a preclinical setting.

Future investigation must focus on perfecting safety and feasibility, with the goal of improving quality of life for the patient.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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