ISSN: 2573-9565

Review Article

Journal of Clinical Review & Case Reports

Crohn's Disease: Hematopoietic and Mesenchymal Stem Cell Therapy as Potential Treatment

Brianna Weir and Vincent S Gallicchio*

Department of Biological Sciences, College of Sciences, Clemson University, Clemson, SC, USA

***Corresponding author**
Dr. Vincent Gallicchio, Department of Biological Sciences, College of Science, Clemson University, Clemson, SC, 29632, E-mail: vsgall@clemson.edu

Submitted: 28 Mar 2020; **Accepted**: 01 Apr 2020; **Published**: 08 Apr 2020

Abstract

Purpose: *The purpose of this work was to comprehensively review literature to determine the safety and efficacy of stem cells for the treatment of Crohn's disease and compare the effects of hematopoietic stem cells (HSCs) to that of mesenchymal stem cells (MSCs).*

Results: *The data search included 5 animal models and clinical trials found on PubMed. The 5 studies included for HSCs and the 15 trails for MSCs found them to be a safe and effective as a treatment option (allogeneic and autologous bone marrow and adipose derived) for CD except for one multicenter randomized trial for HSC therapy. Most trials investigate the impact of stem cells specifically on perianal fistula, a common occurrence in patients with CD.*

Conclusion: *While both HSCs and MSCs proved their safety and efficacy, MSC studies showed a greater therapeutic effect over HSCs. Adipose MSCs and Bone Marrow Derived MSCs reached similar clinical healing rates, with studies backing support for both sides. Across the board, autologous stem cell transplants proved to be safer and more effective compared to their allogeneic counterparts.*

Keywords: Crohn's Disease, Stem Cell Therapy, Mesenchymal Stem Cells, Hematopoietic Stem Cells, Fistula

Abbreviations

AD-MSC: Adipose Derived Mesenchymal Stem Cells
ASC: Adult Stem Cells
AHSCT: Autologous Hematopoietic Stem Cell Therapy
BM-MSCT: Bone Marrow Derived Mesenchymal Stem Cell Therapy
CCECAI: Canine Chronic Enderopathic Activity Index
CD: Crohn's disease
CDAI: Crohn's disease Activity Index
CIBDAI: Canine Inflammatory Bowel Disease Activity Index
HSC: Hematopoietic Stem Cells
HSCT: Hematopoietic Stem Cell Therapy
IBD: Inflammatory Bowel Disease
MSC: Mesenchymal Stem Cells
MSCT: Mesenchymal Stem Cell Therapy
PBSC: Peripheral Blood Stem Cells
TNF: Tumor Necrosis Factor
UC: Ulcerative Colitis

Introduction

Crohn's disease is a chronic and reoccurring inflammatory bowel disease (IBD). Inflammatory bowel disease encompasses two recurrent chronic diseases; Crohn's disease (CD) and ulcerative colitis (UC). The main element in CD is inflammation which is seen all the way through the intestinal wall, from the mucosa to the serosa. Inflammation often causes abscesses and fistula which 25-33% of patients have in the perianal region [1]. This complaint is the most common as it causes discomfort and often local rectal bleeding, decreasing the patient's quality of life. Gross rectal bleeding may also occur in CD, this dark blood indicates lower GI bleeding and is seen most in patients with isolated colonic disease. It can also cause the abdomen to become tender and distended/full which is palpable. Although this disease usually impacts the terminal ileum and right colon, it is a disease of the gastrointestinal tract so its effects can be seen from the mouth all the way down to the perianal area, which can be seen in the figure below [2].

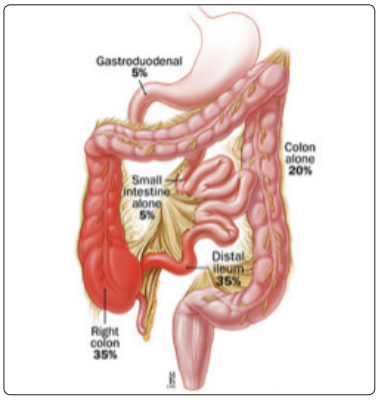

Figure 1: Anatony of the distribution of Crohn's disease [3]

One third of Crohn's patients experience micro and macroscopic lesions of the gastric antrum and the duodenum. These segmented lesions are a key-way CD is distinguished from UC. Lesions form CD are often described as having a cobblestone appearance due to the deep longitudinal and transverse ulcers with intervening mucosal edema. These edemas are the build-up of tissue fluid inside the tissue layers of the body. The culmination of mucosal edemas, ulcers, and inflammation causes the bowel and mesentery to thicken. A low-grade fever is also prevalent due to the inflammation associated with Crohn's, whereas a high-grade fever could be an indication of infection or an abscess. The most common symptom of CD is diarrhea due to decreased water absorption or increased electrolyte secretion. It can also be caused by bile acid due to a deceased/resected bowel or by small bowel bacterial overgrowth. Crohn's disease can also lead to malabsorption from the removal of parts of the intestine. This malabsorption, paired with decreased oral intake, also leads to weight loss which is seen in 10-20% of patients [3].

Crohn's disease is classified into 3 prevalent patterns; primarily inflammatory, fistulising, and stenosis (obstructing). The primarily inflammatory type of CD stays localized to the mucosa and submucosa of the gastrointestinal tract. This causes diarrhea and pain from partial obstructions/blockages. The fistulising CD is defined by the intra-abdominal fistula created in the bowel which opens to another bowel loop or proximal organ. Stenosis CD is caused by muscular hypertrophy that leads to collagen scarring in the small intestine.

Initially, CD was considered a rare condition in North America and Europe. However, a steady rise in cases was noted after WWII and has been increasing over the last century. It seems that as socioeconomic status improves, so does the incidence of IBD [4]. The rate of increase has recently stabilized and the annual incidence of CD in North America is now reported to be 3.1–20.2 per 100,000 with a prevalence of 201 per 100,000 population. CD is also increasing in Japan, Hong Kong, and Korea from 1980-2003 yet is still considered a rare disease in Africa and South America. CD has been known to be 2 to 4 times more prevalent in the Ashkenazi Jewish population. Recent studies have found that the frequency of the NOD2/CARD15 gene is a possible reason for this increase [5]. CD is equally prevalent among men and women. The diagnosis age for Crohn's disease tends to be in the late teen years and into the early 20's, although the median age of flare ups is 29.5-34.9 [6].

The cause of Crohn's disease is unknown but here are multiple factors researchers have identified which are associated with Crohn's disease. Environmental triggers, genetic predispositions, and immune system disturbances are the most prevalent potential causes of Crohn's disease.

One's diet, appendix history, and smoking tendencies are environmental factors that may have an impact on one's chance of developing CD. In recently diagnosed IBD patients, it was found that they consumed less dietary fiber, less raw fruits, and vegetables when compared with healthy individuals. In a Nurse's Health Study, long term dietary fiber intake was associated with a lower risk for CD. It was also seen that fiber intake from fruits had greater success in reducing one's risk than legumes, cereals, or whole grains did. The hypothesized impact that fiber has on CD is a protective effect since the fiber is metabolized by the intestinal bacteria and turned into short-chain fatty acids that help inhibit transcription of pro-inflammatory mediators, a key issue in CD. Another possible relationship with developing Crohn's disease is appendectomy, as people who have had appendectomies have a higher incidence rate of CD. However, it is argued that the inflammation which causes appendicitis is related to possible underlying CD. The most substantial evidence of environmental factors influencing Crohn's disease is the relationship between smoking and CD. People who smoke double their chance of CD. This includes early life exposure to smoking and second-hand smoking. Smoking is also associated with early onset of CD, more frequent need for immunosuppression, more surgical interventions and higher rates of postoperative disease recurrence [3]. In the cohort study published, patients were followed up for 12-18 months and a lower relapse risk was observed among those patients who stopped smoking for at least 6 months. In ex-smokers, the clinical course was similar to that seen in patients who have never smoked. Therefore, CD patients should be reminded of the disadvantages of smoking and the benefits, in regards to CD, of quitting smoking [7].

Research shows that there is a hereditary factor in CD as 15% of people have reported that they have a family history of Crohn's disease [8]. It is estimated that if both parents are impacted by CD then the chance of their children developing CD is 1 in 3 [9]. It is not yet used as a diagnostic tool since it is not known why many people carrying these genes for CD may never develop it and is limiting genetic testing to only identifying potential at risk individuals. The first gene found to be associated with CD is the NOD2/CARD15 gene, as 20% of IBD patients in North America and Europe may have a mutation in this gene. It was shown that a carrier of the heterozygous NOD2/CARD15 gene is 2 to 4 times more likely to develop CD and those with homozygous alleles are 20 to 40 times more likely to have CD. Further research has identified and confirmed 71 susceptibility loci for Crohn's disease on 17 chromosomes so far [10].

In a healthy human, the immune system usually attacks and kills foreign invaders, such as bacteria, viruses, fungi, and other microorganisms. However, in people with IBD, the immune system mounts an inappropriate response to the intestinal tract, resulting in inflammation.

The treatment for someone with Crohn's disease is based on the severity of their symptoms and the extent to which their GI tract is involved. These factors decide whether a patient will proceed with the typical "Step-Up" therapy where less aggressive drugs are used before stepping up to stronger drugs. Contrastly, the "Step Down" approach starts with stronger drugs and decreases the strength once remission is achieved as maintenance therapy. This choice is also based on the patient's tolerance to medications and the physician's preference [11]. There are five main types of medications used to treat CD: Aminosalicylates, Corticosteroids, Immunomodulators, Antibiotics, and Biological Therapies. Aminosalicylates are anti-inflammatories that contain 5-aminosalicylic acid (5-ASA). Corticosteroids control the body's ability to begin and maintain an inflammatory process as a way to keep it under control. These are only used to treat short term flare-ups since the long-term side effects include infection, bone loss, weight gain, cataracts, skin fragility, sleep disturbance, and mood swings. Immunomodulators adjust the immune system so that it cannot maintain ongoing inflammation. Antibiotics have been used to treat infections from abscesses and fistulas but not the underlying causes of CD. Lastly, biological

therapies are used for patients who are experiencing severe CD and work by targeting an inflammatory protein called tumor necrosis factor (TNF). Surgical treatment is also a common option as 70% of people with Crohn's disease require surgery after recurrent flare-ups [12]. Different types of surgical procedures are performed for Crohn's disease depending on the severity of illness and location of the disease in the intestines. In Figure 2 below, a systematic review of CD found the percentage of real-world treatment options being used. Thiopurines and medication-based treatment are currently the most practiced option [13].

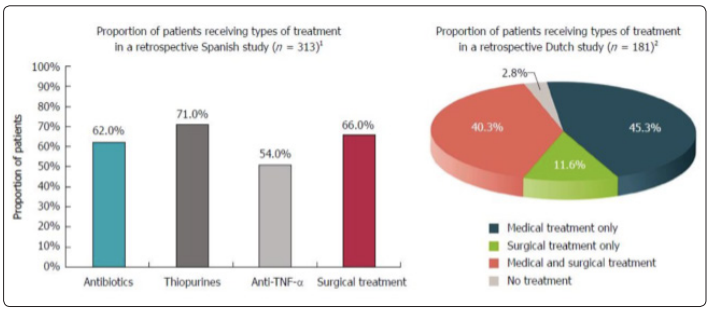

Figure 2: Treatment Patterns. Real-world treatment Patterns identified in two retrospective studies- one conducted in Spain and one in the Netherlands. [1] Retrospective multicenter study. [2] Retrospective single center study [13]

However, 30% of patients who have surgery for Crohn's disease experience recurrence of their symptoms within three years and up to 60% will have a recurrence within ten years. Providing it to be not an effective option for sustained clinical remission [14]. Additionally, around 25% of CD patients develop fistulas within 20 years of their diagnosis. The current clinical treatment options have had suboptimal results of fistula closure as there is a 90% recurrence rate when treated with antibiotics, 33% of patients do not respond to anti-TNF medications, and 10% of patients do not respond to the other existing medications offered [15].

Discussion

The four types of stem cells being researched are adult stem cells, embryonic stem cells, mesenchymal stem cells, and induced pluripotent stem cells. Adult stem cells (ASCs) can divide and self-renew indefinitely. They have been found in the brain, bone marrow, blood (hematopoietic), skin, adipose tissue, and the liver. Mesenchymal stem cells (MSCs) come from the connective tissue called the stroma which surrounds the body's organs and tissues. Induced pluripotent stem cells (iPSCs) are created in laboratories and have very similar properties to embryonic stem cells. All 4 types of stem cells can be autologous stem cells, previously harvested from the patient, or allogeneic stem cells, which are taken from a donor.

Animal Studies

Multiple animal studies have been conducted to research different types of stem cells impacts on different aspects of CD. One animal study performed in 2015, tested on dogs with IBD, found that a single intravenous infusion of allogeneic adipose-derived mesenchymal stem cells (AD-MSCs) was well tolerated by the dogs and appeared to produce clinical benefits when looking at their severe IBD [16]. By day 42 post treatment, 9 out of the 11 dogs reached the study's definition of clinical remission, which was reduction of initial CIBDAI and CCECAI by more than 75% [17]. Another animal study using allogenic AD-MSCs administered a single injection into knockout mice and found they suffered less frequently from diarrhea and increased weight gain compared to the control group of mice. The appearance of the intestine suggested that the allogeneic AD-MSC animals had milder colitis and maintained fecal material rigidity [18]. Another study used a rat model for local application of AD-MSCs which resulted in significantly higher fistula closure rate. AD-MSCs were used in this induced CD mice model where systemic infusion of AD-MSCs improved the clinical and histopathologic severity of colitis, reduced diarrhea and inflammation, and increased mice survival. This study found that AD-MSCs down-regulated Th1-driven inflammatory responses which subsequently decreased the production of inflammatory cytokines and chemokines [19]. In another study dogs with perianal fistulas were treated intralesionally with 2×107 human embryonic stem cell derived MSCT. All dogs showed progression toward clinical remission, except for one dog that relapsed by 6 months [20]. An ongoing animal trial's protocol has a primary endpoint that will measure the occurrence of adverse events related to acute infusion toxicity and its secondary endpoint is to measure long-term adverse events and efficacy. This protocol is the first study to investigate the safety and efficacy of amnion derived MSC use for CD treatment [21].

Hematopoietic Stem Cell Transplant

Hematopoietic Stem Cells are being researched for a viable treatment option for CD as they have shown the ability to migrate to damaged tissue. This function provides them with further properties to differentiate to epithelial or immune-modulatory cells to restore normal mucosal tissue and integrity. HSCs are multipotent cells that are blood-forming and can be isolated from bone marrow, umbilical cord, or peripheral blood. They have self-renewal capabilities, and the potential to differentiate into blood and immune cells. Autologous hematopoietic stem cell transplant aims to reset the patient's immune system through de *novo* regeneration of their T-cell reserves and repopulation of epithelial cells in the intestine with bone-marrow-derived cells [22]. This aims to help patients achieve clinical and endoscopic remission. Autologous HSCT is being pursued over allogeneic HSCT since allogeneic HSCT carries high morbidity and mortality rate when used for the treatment of IBD although it holds the potential to correct the patient's genetic predisposition.

Allogeneic HSCT

Due to the negative side effects of allogeneic stem cell transplant, there is not substantial research on its effects as the primary treatment for CD nor is it recommended for IBD/CD treatment besides in the specific cases mentioned below. It was first mentioned of being used for primary treatment in literature in a 2009 study when used to treat four of the nine patients participating in the study that were found to have matching mutations within the distinct IL-10 subunit protein encoding genes. Remission was achieved by one of the patients after allogeneic HSCT [23]. Before allogeneic HSCT was tested as primary treatment, Ditschkowski et al., published a study where 11 patients underwent allogeneic HSCT between 1994 and 2002 for acute and chronic myeloid leukemia and myelodysplastic syndrome that also had coexisting CD or UC. Ten patients are alive (one death due to pulmonary fungal infection not related to the HSCT) during the post-transplantation follow-up of 34 months. None of these patients showed IBD activity after their SCT [24]. Another study was done where allogeneic HSCT was used to treat 6 patients with leukemia and coexisting CD. One patient died of sepsis, the study did not specify if the death was related to the HSCT. Of the 5 remaining patients, 4 remained CD free when checked up

on during a range of 4.5 to 15.3 years after their transplants [25].

Autologous HSCT

Autologous HSCT has been a more explored route in regards to clinical trials. As seen in Table 1, CD has been subsequently treated when patients received autologous HSCT for other diseases [26]. These successful treatments of CD could have to do with pre-transplant diet/regimen required before the procedures which often include immunosuppressant therapy. As CD is an autoimmune disease, this could repress its symptoms while trying to treat the other disease in question.

Table 1. Studies where AHSCT was used to treat alternative diseases in patients with CD [26].

Study	Year	Target Disease for AHSCT	Length of CD	Sex, Age	Results
Drakos et al.	1993	Non-Hodgkin's lymphoma	22 years	Female, 41 y/o	6-months CD remission post-transplant
Castro et al.	1996	Breast Cancer	11 years	Female, N/A	2-years CD remission post-transplant
Kashyap et al.	1998	Non-Hodgkin's lymphoma	8 years	Male, 21 y/o	7-years CD remission post-transplant
Musso et al.	2000	Non-Hodgkin's lymphoma	10 years	Male, 30 y/o	Remission for both diseases for 3 years post-transplant
Soderholm et al.	2002	Myeloid leukemia	3 years	Female, 57 y/o	Clinical remission for both diseases 1,2,3, and 5 years post-transplant
Anumakonda et al.	2007	Non-Hodgkin's lymphoma	16 years	Female, 32 y/o	CD remission for 8 years followed by a release

In 2003, the Chicago group published the first series of CD patients subjected autologous HSCT as primary treatment for IBD where their first four patients entered clinical remission in terms of CDAI along with no diarrhea or abdominal pain. However, minor inflammation of the colon seen during colonoscopic evaluation occurred up to 1-year post-transplant [16]. A later publication by this same Chicago group showcased the results of a phase I autologous HSCT trial in 12 patients with CD and starting activity index (CDAI) scores of 250-450. Eleven out of the 12 patients presented sustained CD remission where their CDAI was less than 150 after a follow up time of 18.5 months (median). Only one patient was reported with a recurrence of active CD after 15 months [27]. A 2008 study using peripheral blood stem cells as autologous HSCT (vs CD34+ cells) also had clinical remission achieved in all patients, with a median CDAI of 91 and complete endoscopic remission achieved in two thirds of the patients as complete fistula closure was observed. Three fourths of the patients maintained both clinical and endoscopic remission after a follow-up at 16.5 months (median) even after withdrawal from maintenance drugs. This autologous HSCT was deemed safe as no deaths or life-threatening infection occurred during this 2008 trial [28]. Clerici conducted a study where 5 out of 6 patients maintained clinical and endoscopic remission without further treatment after 12 months. One relapse observed after 12 months which required urgent small bowel major resection followed by maintenance treatment with azathioprine but it was not stated whether this was an adverse event due to the SCT. At 12 months, the transplant had resulted in the normalization of all immunological parameters which were being measured (TLR-4, TNF-α, IL-10, T-reg cells) [29]. Hasslebaltt conducted a mono center phase I/II trial with autologous peripheral blood HSCT where mobilization chemotherapy caused temporary remission in some cases and five out of the twelve patients achieved clinical and endoscopic remission within 6 months after autologous peripheral blood HSCT. However, unlike the other studies conducted so far, relapses occurred in 7/9 patients during follow-ups. These relapses could be explained by 25% of patients undergoing a colectomy after the transplant and the persistence of the genetic predisposition in almost all of the patients [30]. One of the largest studies conducted for HSCT in CD found that 24 of 24 patients went into remission (defined as a CDAI of less than 150). The percentage of clinical relapse-free survival (defined as the percent free of restarting CD medical therapy after transplantation) is 91% at 1 year and 19% at 5 years. The percentage of patients in remission (CDAI < 150), steroid-free, or medication-free at any post-transplantation evaluation interval more than 5 years after transplantation has remained at or greater than 70%, 80%, and 60%, respectively [31]. Despite all of this positive data as the largest multicenter randomized trial published to date (the ASTIC Trial), it ultimately reported no benefit of HSCT over mobilization alone at 1 year. This trial had 45 patients enrolled from 11 European transplant units and defined the primary endpoint by being steroid-free clinical remission for 3 months with mucosal healing and absence of radiological evidence of active inflammation. At 1-year post HSCT, 3-month steroid-free clinical remission was seen in 13 of 34 patients with available data for the whole year and complete endoscopic healing was noted in 19 of 38 patients. Critics of the outcome believe that there was no benefit found from

this study because the predefined primary endpoint was the most stringent seen yet for a clinical trial of CD. When assessed using traditional endpoints like those seen in clinical trials of conventional CD treatment, autologous HSCT showed statistically significant clinical and endoscopic benefits. However, it was still found to be associated with a high chance of adverse events, infections being the most common (76 serious adverse events occurred in 23 of 40 patients) [32].

Mesenchymal Stem Cells

Mesenchymal Stem Cells (MSCs) can be derived from a number of different tissue types varying from bone marrow, skeletal muscle, bone, umbilical cord, and adipose tissue and can be administered locally or systemically. With regards to MSC research for CD treatment, bone-marrow and adipose tissue have been the most researched. MSCs exert many immunomodulatory effects by inhibiting the proliferation and function of Th1 and Th17 cells, which are pro-inflammatory. They also promote T-reg differentiation which controls the body's immune response to antigens and helps prevent autoimmune diseases. This could help in CD as MSCs home to sites of inflammation and tissue damage. They promote tissue regeneration through cell replacement where they encourage the regenerative capacity of *in situ* cells or through immunomodulatory mechanisms. Interferon-gamma rays were investigated as a means of licensing for MSCs and it was found that interferon-gamma ray licensing enhances MSCs' immunomodulatory properties. Interferon-gamma licensing of MSCs was also found to decrease cell death when preserved via cryopreservation and following the actual infusion [33]. Comparisons of MSC compared to HSC has found MSC to be significantly safer, as one review found HSCT to cause lupus erythematosus, BK virus infection, fever, chest pain, renal function impairment, and sometimes death [34]. Research on MSC-based therapies has shown to have durable efficacy with low recurrence so far. In one study, MSCT in fistulising CD was successful in healing between 60%-88% of participants compared to 50% with infliximab, a drug currently used for CD. During this trial, remission was seen at 24 weeks to 52 weeks [35]. The safety of MSC has also been researched in a groundbreaking study about the outcome in women with perianal fistula and CD treated with stem cell therapy who were pregnant. It was found that stem cell therapy does not affect the woman's ability to conceive, the course of pregnancy, pregnancy outcomes, or newborns' health [36]. Although more than 600 trials have been performed using MSCs in the treatment of IBD and CD, less than 20 of these were industry-sponsored phase III trials [37]. One systematic review identified other problems with MSCT concerning the price, two surgical interventions that are required, the time-consuming expansion of stem cells, and non-standardized dose of cells used in varying studies [38]. Another review found the need for control arms to be used in studies as only 3 of the 11 that met their criteria included control arms to provide a baseline when comparing results [39]. Comparisons have shown that the therapeutic effects seem to be similar when analyzing adipose derived MSCs and bone marrow derived MSCs in treating CD [40]. However, adipose derived MSCs are more easily isolated, a safer approach, and allows for larger amounts to be obtained compared with the bone marrow. Thus, more clinical trials should research using adipose-derived MSCs, as it seems to be the more practical option.

Allogeneic Bone-Marrow MSC

Allogeneic bone-marrow derived MSCs (allogeneic BM-MSCs) have been investigated in the treatment of IBD, including CD cases. A recent phase I study that intravenously injected allogeneic bone marrow tissue derived MSC (150–200 millions per patient) on patients suffering from CD or UC. Clinico-morphological remission was found in 40 patients during the follow up. Although it was ineffective as treatment for 10 patients with IBD, only 2 of those patients were suffering from CD [41]. A research group at Leiden University Medical Center performed a double-blind dose-finding study for allogeneic bone marrow derived MSCT in 21 patients with refractory perianal fistulising Crohn's disease. This study similarly found success as 13 out of 15 patients (87%) treated with allogeneic bone-marrow MSCs were available for long-term follow-up. No serious adverse events related to bone marrow MSC-therapy were found (2 non-MSCT related issues observed). The most success was found in cohort 2 as all fistulas were closed 4 years after bone-marrow MSC therapy was locally applied. Success was also found in cohort 1 and 3 as 63% and 43% of the fistulas were closed upon reevaluation [42]. A phase II pilot clinical study by Liang et al, treated 9 individuals suffering from CD with allogeneic BM-MSC transplantation. The participants received intravenous injection of 2×10^6 or 8×10^6 bone marrow MSC/kg based on their body weights. All patients were evaluated 28 days post-transplantation and all had significant reduction in CDAI. Clinical remission (reduction in CDAI $\geq$100) was observed in 3 of the 9 subjects but mild adverse events not related to the MSCT were reported in three of the individuals [43]. The most recent study on allogeneic bone-marrow MSCT in 2015 found that a dosage of 3×10^7 MSCs allogeneic bone marrow-derived MSC appeared to advance healing of refractory perianal fistulas in patients with CD. This was discovered through an early phase 2, double-blind, placebo-controlled, randomized study with local application of allogeneic bone marrow MSC did not show any adverse effects [44].

Autologous Bone-Marrow MSC

Autologous BM-MSCT has more research surrounding its use for CD than allogeneic BM-MSCT. Ciccocioppo et. al., documented the feasibility, safety and efficacy of these stem cells through local injection of 50 million per patient of autologous BM-MSC without addition of fibrin glue into the fistula of 12 Crohn's patients. This phase 1 study found observable rectal mucosal healing and 7 cases of complete fistula closure along with 3 cases of incomplete closure [45]. In one long term study, spanning from 2007 to 2014, researchers tracked CD fistulas after local injections of autologous BM-MSCT. They found that the percentage of patients who remained in remission (defined by a CDAI score below 150 points) fluctuated heavily at each annual evaluation during the further 5-year follow-ups, regardless of which therapy was undertaken. No matter how severe the fluctuation, the percent of participants in remission was close to 90% at the end of the 72 month data collection [46]. Another study administered intra-fistula autologous BM-MSC injections to 10 participants every 4 weeks and stem cell expansion was successful in all cases. Sustained, complete closure (seven cases) or incomplete closure (three cases) of fistula tract was observed in the 12 months after injections [47]. Both the long term study and this study recorded no adverse side effects. However, the first-in-human phase 1 safety clinical trial of metabolically fit autologous BM-MSCT, seven patients had serious adverse events of which five were hospitalizations for a CD flare. They still ruled BM-MSCT as a safe and feasible option at intravenous doses of up to 10 million cells/kg as only two of the serious adverse events were believed to be related to the BM-MSC infusion and there was no dose limiting toxicity seen [48]. In another phase 1 study, doses of 1–2 millions

of MSC/kg BM-MSC were given to 10 patients (dosage based on fistula size). While 6 patients responded to the BM-MSCT positively (partial or complete fistula closure), 3 patients required surgery after their CD worsened for reasons unrelated to their BM-MSCT [49].

Allogenic Adipose MSC

Allogenic adipose MSC has been researched as a treatment option for CD. In Park et al. 2015 pilot clinical trial a group of patients was delivered 1×10^7 cells/ml while group 2 was delivered 3×10^7 cells/ml three weeks later. No adverse events related to the treatment with allogeneic adipose-derived MSCs were found upon check-up and two patients in group 1 achieved complete closure of their fistula by month 4 and month 6. Success was also found in group 2 as one patient achieved complete closure at 8 weeks, fairly quickly after their treatment. The low occurrence was supported by the fact that closure was sustained up to month 8 in all three of those patients [50]. Panés performed a Phase 3 randomized, double-blind controlled study in which 49 hospitals in seven European countries participated and found success as 50% of participants with the allogeneic adipose derived MSC reached remission compared to 34% of the patients who received the placebo treatment [51]. A breakthrough for MSCT has been found in 2018, as the European Union approved its first MSC advanced therapy, Darvadstrocel, for the treatment of complex perianal fistulas [52].

Autologous Adipose MSC

Meta analysis of 13 studies performed in 2019 found that 63% (with a 95% confidence interval) of patients achieved clinical healing as a result of local therapy with autologous adipose MSCs. This same review found that clinical healing increased when MSCs were administered based on fistula size versus a fixed dose given to all patients (80% vs 55%) [52]. Another study found that freshly isolated autologous adipose MSCs with anti-TNF drugs have the most potential in treating CD. They found that the anti-TNF drugs greatly improve the proliferation and migration of the stem cells, increasing clinical success rate [30]. Lee et al. performed a phase I clinical trial which proved the efficacy and safety of autologous adipose MSC and allowed them to move onto a phase II, forty-three patient trial. This trial injected Adipose MSC amounts proportional to fistula size and the fistula tract was filled with ASCs along with fibrin glue after intralesional injection of Adipose MSCs. Complete fistula healing was observed in 27/33 patients (82%) by 8 weeks after autologous adipose MSC injection. Of 27 patients with fistula healing, 26 patients completed additional observation study for 1-year and 23 patients (88%) sustained complete closure [53].

Conclusion/Summary

Overall, HSCs and MSCs appear to offer a safe and effective treatment approach for aspects of CD, including inflammation and fistula closure. Based on subgroup analysis, autologous stem cells offer a safer treatment option, regardless of the tissue type. MSCs achieve better clinical results over HSCs when reviewing the data available. Adipose and bone marrow-derived MSCs had similar clinical results.

Even with varying types of stem cells proving clinical potential to treat CD, SCT has been associated with adverse events, leaving room for improvement in the design of the studies. There lies problems within the unknown optimal origin of tissue type, dosage, and method of administration [54]. Moving forward, a set definition for fistula healing and clinical remission must be set across the board, making comparison of data between studies clearer. More studies should focus on adipose MSCs versus bone marrow derived MSCs to attain a clear front runner for the best CD treatment, as there are studies which support both sides. Clinically, adipose derived MSCs can be obtained easier and in larger amounts which is an important quality to have if MSCT wants to become the go-to for CD treatment. One thing to further investigate when reviewing CD studies is whether the researchers took the patient's smoking history into account when evaluating results, as smoking is proven to lead to more surgical interventions and higher rates of postoperative disease recurrence. It would also be beneficial to consistently remind patients the harming impacts smoking has on their CD and recommend they quit to help reverse those negative effects. Finally, stem cell therapy should be considered as a regular treatment option for Crohn's disease, specifically perianal fissures, instead of a last resort.

References

1. Walfish AE, Companioni RA (2019) Crohn Disease. Merck Manual. doi: 10.3390/books978-3-03921-440-2
2. Crohn's Disease (2001) Hopkin's Medicine. Retrieved from https://www.hopkinsmedicine.org/gastroenterology_hepatology/_pdfs/small_large_intestine/crohns_disease.pdf
3. Gajendran M, Loganathan P, Catinella A, Hashash J (2018) A comprehensive review and update on Crohn's disease. Science Direct 64: 20-57.
4. Loftus EV (2016) Update on the Incidence and Prevalence of Inflammatory Bowel Disease in the United States. Gastroenterology and Hepatology 2016: 704-707.
5. Zhou Z, Lin X-yu, Akolkar PN, Gulwani-Akolkar B, Levine J, et al. (2002) Variation at NOD2/CARD15 in familial and sporadic cases of Crohn's disease in the Ashkenazi Jewish population. The American Journal of Gastroenterology 97: 3095-3101.
6. The Facts About Inflammatory Bowel Diseases (2014) Crohn's and Colitis Foundation of America. https://www.crohnscolitisfoundation.org
7. Underner M, Perriot J, Cosnes J, Beau P, Peiffer G, et al. (2016) Tabagisme, sevrage tabagique et maladie de Crohn. La Presse Médicale 45: 390-402.
8. AN Ananthakrishnan (2015) Epidemiology and risk factors for IBD. Nat Rev Gastroenterol Hepatol 2015: 205-217.
9. L Halme, P Paavola-Sakki, U Turunen, M Lappalainen, M Farkkila, et al. (2006) Family and twin studies in inflammatory bowel disease. World J Gastroenterol 12: 3668-3672.
10. Franke A McGovern DP Barrett JC, Kai Wang, Graham L Radford-Smith, et al. (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 42: 1118-1125.
11. Akobeng AK (2008) Crohn's disease: current treatment options. Archives of Disease in Childhood 93: 787-792.
12. Gajendran M, Loganathan P, Catinella A, Hashash J (2018) A comprehensive review and update on Crohn's disease. Science Direct 64: 45.
13. Panes J, Reinisch W, Rupniewska E, Khan S, Forns J, et al. (2018) Burden and outcomes for complex perianal fistulas in Crohn's disease: Systematic review. Baishideng Publishing Group 2018: 4821-4834.
14. Panes J (2016) Stem Cell Therapy for Perianal Fistulas in Crohn's Disease. Gastroenterology and Hepatology 2016: 637-640.
15. De Francesco F, Romano M, Zarantonello L, Ruffolo C, Nari D, et al. (2016) The role of adipose stem cells in inflammatory

bowel disease: From biology to novel therapeutic strategies. Cancer Biology and Therapy 2016: 889-898.
16. Pérez-Merino E, Usón-Casaús J, Zaragoza-Bayle C, Duque-Carrasco J, Mariñas-Pardo L, et al. (2015) Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Clinical and laboratory outcomes. The Veterinary Journal 206: 385-390.
17. Pérez-Merino, Usón-Casaús, Zaragoza-Bayle, Duque-Carrasco, Mariñas-Pardo, et al. (2015) Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Clinical and laboratory outcomes. The Veterinary Journal 206: 385-390.
18. Jung Kang, Kim K, Kim H, Jang Park, Song (2015) Human adipose-derived stem cells attenuate inflammatory bowel disease in IL-10 knockout mice. Science Direct 47: 86-93.
19. Ryska, Serclova, Mestak, Matuskova, Vesely, et al. (2017) Local application of adipose-derived mesenchymal stem cells supports the healing of fistula: prospective randomised study on rat model of fistulising Crohn's disease. Taylor Francis Online 2017: 543-550.
20. Hoffman A, Dow S (2016) Concise Review: Stem Cell Trials Using Companion Animal Disease Models. Stem Cell Journals 34: 1709-1729.
21. Otagiri, Ohnishi, Hayashi, Kumagai, M Ito, et al. (2018) Evaluation of amnion-derived mesenchymal stem cells for treatment-resistant moderate Crohn's disease: study protocol for a phase I/II, dual-centre, open-label, uncontrolled, dose–response trial. BMJ Open Gastroenterol 5: e000206.
22. Boltin D, Kamenetsky Z, Tsadok Perets, Snir Sapoznikov, Schmilovitz-Weiss, et al. (2017) Circulating Bone Marrow-Derived CD45-/CD34 /CD133 /VEGF Endothelial Progenitor Cells in Adults with Crohn's Disease. Dig Dis Sci 62: 633-638.
23. Erik-Oliver Glocker, Daniel Kotlarz, Kaan Boztug, E Michael Gertz, Alejandro A, et al. (2009) Schäffer, Inflammatory Bowel Disease and Mutations Affecting the Interleukin-10 Receptor. N Engl J Med 361: 2033-2045.
24. Markus Ditschkowski, Hermann Einsele, Rainer Schwerdtfeger, Donald Bunjes, Rudolf Trenschel, et al. (2003) Improvement of inflammatory bowel disease after allogeneic stem-cell transplantation. Transplantation 75: 1745-1747.
25. Cubero L, Sullivan KM, McDonald GB (1998) Course of Crohn's disease after allogeneic marrow transplantation. Gastroenterology 114: 433-440.
26. DiNicola CA, Zand A, Hommes DW (2017) Autologous hematopoietic stem cells for refractory Crohn's disease. Expert Opin Biol Ther 17: 555-564.
27. Craig RM, Oyama Y, Traynor AE (2005) Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. Gastroenterology 2005: 552-563.
28. Cassinotti, Annaloro, Ardizzone, Onida, Volpe, et al. (2007) Autologous haematopoietic stem cell transplantation without CD34 cell selection in refractory Crohn's disease. Gut 57: 211-217.
29. Clerici M, Cassinotti A, Onida F, Trabattoni D, Annaloro C, et al. (2011) Immunomodulatory effects of unselected haematopoietic stem cells autotransplantation in refractory Crohns disease. Digestive and Liver Disease 43: 946-952.
30. Hasselblatt P, Drognitz K, Potthoff K, Bertz H, Kruis W, et al. (2012) Remission of refractory Crohns disease by high-dose cyclophosphamide and autologous peripheral blood stem cell transplantation. Alimentary Pharmacology & Therapeutics 36: 725-735.
31. Burt RK, Craig RM, Milanetti F, Quigley K, Gozdziak P, et al. (2010) Autologous nonmyeloablative hematopoietic stem cell transplantation in patients with severe anti-TNF refractory Crohn disease: long-term follow-up. Blood 116: 6123-6132.
32. Lindsay JO, Allez M, Clark MM, Labopin M, Ricart E, et al. (2016) 991 Prediction of Clinical and Endoscopic Remission After Autologous Stem Cell Transplantation in Treatment Refractory Crohns Disease: Pooled Results From the ASTIC Trial. Gastroenterology 150: S203.
33. Carvalho AÉS, Sousa MRR, Alencar-Silva T, Carvalho JL, Saldanha-Araujo F (2019) Mesenchymal stem cells immunomodulation: The road to IFN-γ licensing and the path ahead. Cytokine & Growth Factor Reviews 47: 32-42.
34. Zhang XM, Zhang YJ, Wang W, Wei YQ, Deng HX (2017) Mesenchymal Stem Cells to Treat Crohns Disease with Fistula. Human Gene Therapy 28: 534-540.
35. Turse EP, Dailey FE, Naseer M, Partyka EK, Tahan V (2018) One more chance of fistula healing in inflammatory bowel disease: Stem cell therapy. World Journal of Clinical Cases 6: 493-500.
36. Sanz-Baro R, García-Arranz M, Guadalajara H, Quintana PDL, Herreros MD, et al. (2015) First-in-Human Case Study: Pregnancy in Women With Crohns Perianal Fistula Treated With Adipose-Derived Stem Cells: A Safety Study. STEM CELLS Translational Medicine 4: 598-602.
37. Wainstein C, Quera R, Kronberg U, Conejero A, López-Kostner F, et al. (2015) Mesenchymal stem cells and platelet-rich plasma in the treatment of patients with perineal Crohn's disease. International Journal of Colorectal Disease 31: 725-726.
38. Bor R, Fábián A, Farkas K, Molnár T, Szepes Z (2018) Human mesenchymal stem cell therapy in the management of luminal and perianal fistulizing Crohn's disease – review of pathomechanism and existing clinical data. Expert Opinion on Biological Therapy 18: 737-745.
39. Lightner AL, Wang Z, Zubair AC, Dozois EJ (2018) A Systematic Review and Meta-analysis of Mesenchymal Stem Cell Injections for the Treatment of Perianal Crohn's Disease. Diseases of the Colon & Rectum 61: 629-640.
40. Xie M, He X, Yang Z, Lan P, Lian L, et al. (2014) Comparison of Adipose-Derived and Bone-Marrow Mesenchymal Stem Cells in a Murine Model of Crohns Disease. Dig Dis Sci 62: 115-123.
41. L B Lazebnik, A G Konopliannikov, O V Kniazev, A I Parfenov, T M Tsaregorodtseva, et al. (2010) Use of allogeneic mesenchymal stem cells in the treatment of intestinal inflammatory diseases. Ter Arkh 82: 38-43.
42. Barnhoorn MC, Wasser MNJM, Roelofs H, Maljaars PWJ, Molendijk I, et al. (2019) Long-term Evaluation of Allogeneic Bone Marrow-derived Mesenchymal Stromal Cell Therapy for Crohn's Disease Perianal Fistulas. J Crohns Colitis 14: 64-70.
43. Turse EP, Dailey FE, Naseer M, Partyka EK, Bragg JD, et al. (2018) Stem cells for luminal, fistulizing, and perianal inflammatory bowel disease: a comprehensive updated review of the literature. Stem Cells and Cloning: Advances and Applications 11: 95-113.
44. Molendijk I, Bonsing BA, Roelofs H, Peeters KC, Wasser MN, et al. (2015) Allogeneic Bone Marrow–Derived Mesenchymal Stromal Cells Promote Healing of Refractory Perianal Fistulas in Patients With Crohn's Disease. Gastroenterology 149: 918-927.e6
45. Ciccocioppo R, Gallia A, Sgarella A, Kruzliak P, Gobbi PG,

et al. (2015) Long-Term Follow-Up of Crohn Disease Fistulas After Local Injections of Bone Marrow–Derived Mesenchymal Stem Cells. Mayo Clinic Proceedings 90: 747-755.
46. Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, et al. (2011) Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohns disease. Gut 60: 788-798.
47. Dhere T, Copland I, Garcia M, Chiang KY, Chinnadurai R, et al. (2016) The safety of autologous and metabolically fit bone marrow mesenchymal stromal cells in medically refractory Crohns disease - a phase 1 trial with three doses. Alimentary Pharmacology & Therapeutics 44: 471-481.
48. Voswinkel J, Francois S, Simon JM, Benderitter M, Gorin NC, et al. (2013) Use of Mesenchymal Stem Cells (MSC) in Chronic Inflammatory Fistulizing and Fibrotic Diseases: a Comprehensive Review. Clinical Reviews in Allergy & Immunology 45: 180-192.
49. Park KJ, Ryoo SB, Kim JS, Kim TI, Baik SH, et al. (2016) Allogeneic adipose-derived stem cells for the treatment of perianal fistula in Crohns disease: a pilot clinical trial. Colorectal Disease 18: 468-476.
50. Park KJ, Ryoo SB, Kim JS, Kim TI, Baik SH, et al. (2016) Allogeneic adipose-derived stem cells for the treatment of perianal fistula in Crohns disease: a pilot clinical trial. Colorectal Disease 18: 468-476.
51. Panés J, García-Olmo D, Assche GV, Colombel JF, Reinisch W, et al. (2016) Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohns disease: a phase 3 randomised, double-blind controlled trial. The Lancet 388: 1281-1290.
52. Lightner AL, Wang Z, Zubair AC, Dozois EJ (2018) A Systematic Review and Meta-analysis of Mesenchymal Stem Cell Injections for the Treatment of Perianal Crohn's Disease. Diseases of the Colon & Rectum 61: 629-640.
53. Lee WY, Park KJ, Cho YB, Yoon SN, Song KH, et al. (2013) Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. Stem Cells 31: 2575-2581.
54. Grégoire C, Lechanteur C, Briquet A, Baudoux É, Baron F, et al. (2016) Review article: mesenchymal stromal cell therapy for inflammatory bowel diseases. Alimentary Pharmacology & Therapeutics 45: 205-221.