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INFLAMMATORY BOWEL DISEASE (S HANAUER, SECTION EDITOR)

Stem Cell Therapies for Inflammatory Bowel Disease

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Abstract

Purpose of Review Stem cell therapies have demonstrated safety and efficacy in the treatment of perianal Crohn's disease as compared to conventional therapy. Thus, an understanding of their place in the treatment algorithm for inflammatory bowel disease has become imperative as we move into an era of regenerative medicine.

Recent Findings There have now been over a dozen clinical trials highlighting stem cells as a useful therapeutic in Crohn's disease. Due to the success in the local treatment for perianal Crohn's disease, investigation is continuing in the space of targeted systemic delivery for the treatment of luminal disease.

Summary As we increase the number of patients treated in clinical trials, it is imperative to define the optimal cell donor, optimize treatment dosing and retreatment protocols, and understand methods for safely targeting and treating intraluminal disease.

Keywords Mesenchymal stem cells · Stem cells · Luminal Crohn's disease · Perianal Crohn's disease · Regenerative medicine

Introduction

Crohn's disease is a chronic inflammatory disease of the gastrointestinal tract of unknown etiology, which continues to increase in incidence for unknown reasons [1, 2]. The transmural nature of the inflammation can cause fistulas of which perianal fistulizing disease is the most common phenotype occurring in up to 20% of CD patients [1]. Up to 10% of women develop a rectovaginal fistula [2, 3], a particularly devastating and disabling condition with a significant negative impact on quality of life [4]. Perianal and rectovaginal fistulas are notoriously difficult to treat with patients reporting ongoing symptoms of perianal pain, incontinence, stool and air per vagina, dyspareunia, and significantly diminished quality of life [2, 5]. Desperate to alleviate symptoms, patients cycle through numerous systemic immunosuppressives and operative interventions, at risk for serious opportunistic infection and incontinence, respectively. They suffer significant morbidity and want relief, but there are significant limitations to our current standards of care.

Unfortunately, perianal CD is notoriously difficult to cure with 37% of patients experiencing refractory disease [6] despite the majority of patients being placed on biologic therapy and 90% undergoing surgical intervention [1]. The most well-studied immunosuppressive for the treatment of fistulizing CD is infliximab. Unfortunately, while up to 55% of patients achieve closure of their fistula tracts at 3 months, only 36% of patients remained in remission at 1 year [7, 8]. The large number of surgical options including fistulotomy, fistula plugs and glue, anal/rectal advancement flaps, or tissue interposition grafts highlights that no particular intervention is effective. A superficial fistulotomy, while most effective, is rarely performed due to the risk for incontinence and a potential non-healing wound [2, 5]. An anocutaneous flap or rectal advancement flap is possible with healing rates of up to 70%, but rarely used due to anal canal and rectal inflammation [9–11] which significantly impairs healing [12]. Ultimately, despite the numerous available medical therapies and surgical techniques, up to 20% undergo a proctectomy, desperate to alleviate their symptoms and regain their quality of life [13, 14].

This lack of efficacy and risk of side effects from immunosuppressive agents [15–17] and incontinence with surgical interventions [18] has driven investigators to seek alternative therapies. The field of regenerative medicine is expanding at an exponential rate and the use of stem cell–based therapy is

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being applied to a vast array of disease states. Recently, success of mesenchymal stem cells (MSCs) specifically in treating severe inflammatory disorders such as graft-versus-host disease (GvHD) [19], systemic lupus erythematosus [20], myocardial infarction [21], multiple sclerosis [22], and Crohn's disease (CD) [23] has highlighted the therapeutic benefit of the immunomodulatory characteristics of MSCs [24, 25]. The important immunomodulatory property specific to CD is the ability of MSCs to upregulate a $CD4^+$ T cell subset of regulatory T cells (Tregs), a cell type known to be deficient in CD [26, 27]. It has been well established that the depletion of Treg cells and imbalance of Treg to T effector cells plays a key role in the pathogenesis of CD [28, 29]. Therefore, MSCs' ability to upregulate Treg cells, migrate to sites of inflammation, and dampen immune responses underscores the escalating interest in using MSCs to treat CD.

Clinical Trials to Date

Since the first report of successful healing of a rectovaginal fistula following injection mesenchymal stem cells (MSCs) in 2003 [30], several phase I [23, 31–34], phase II [33, 35, 36], and phase III [37•] trials, including over 300 patients, have demonstrated safety and efficacy of this emerging therapy for perianal CD. Trials to date have used a combination of allogeneic [32, 34, 36, 37•] and autologous MSCs [23, 30, 31, 33, 35, 38, 39] derived from both the bone marrow [34, 38] and adipose tissue [23, 30, 32, 33, 37•], administered at various doses, given as a singular or repeat injection, and delivered with [23, 30, 32] and without [34, 37•] scaffolding—all encouraging with regard to both safety and efficacy (Table 1).

Outstanding Questions

While safety and efficacy have now been well established in the aforementioned trials to date (Table 1), there remain a number of outstanding questions.

Who Is the Optimal Allogeneic Stem Cell Donor?

Interestingly, studies suggest significant donor-to-donor variability in MSC viability, cytokine secretion, Treg induction, and differentiation. This donor-to-donor variability has the potential to introduce significant heterogeneity into study efficacy when autologous cells are utilized since each patients' cells may exhibit different functions. And, donor-to-donor variability could affect results from trials utilizing allogeneic cells from different donors or pooled donors. It, therefore, becomes imperative to research which donors provide the optimal cell type for clinical trial utilization.

In vitro work has shown that older age significantly impacts proliferation and viability of MSCs [40], and that female sex improves the anti-inflammatory properties of BM-MSCs, thereby altering their therapeutic effects [41]. Of particular interest is the recent data demonstrating that MSCs harvested from patients with CD exhibit reduced immunosuppressive capabilities when compared to MSCs from healthy donors [42]. If CD patients have impaired MSCs, then autologous MSCs in the setting of perianal CD may be less effective than allogeneic cells from healthy donors. Further research will help define the optimal patient donor for allogeneic cell expansion and utilization in clinical trials.

Should We Use Allogeneic or Autologous Cells?

Clinical trials in perianal CD have utilized both allogeneic [32, 34, 36, 37•] MSCs or autologous MSCs [23, 30, 31, 33, 35, 38, 39], but these cell types were never compared in a trial for superior efficacy. Some authors support the use of autologous MSCs given the concern for alloimmunity following the delivery of allogeneic MSCs [43–45]. However, a substantial body of evidence reporting the immunotolerance of MSCs underscoring their ability to be used safely without the generation of an immune response following delivery [43, 46]. Without an alloimmune response, allogeneic MSCs are better suited for clinical trials since they provide an "off the shelf" product at the point of patient care without added delay mandated in autologous MSC harvest and expansion. In addition, they do not require the complex institutional infrastructure needed for autologous cellular harvest from each treated patient, but can be purchased as a pharmaceutical product. In addition to these important logistical considerations, there is substantial evidence that not all donor MSCs are equivalent with regard to immunosuppressive function. Thus, utilizing an optimized allogeneic product would introduce less study heterogeneity and may have the potential to optimize outcomes.

What Is the Optimal Dose and Frequency of Cell Delivery?

Unfortunately, studies performed to date have utilized varying doses of cells and redosing protocols for cell delivery. Molendijk et al. [34] randomized patients to a single injection of 1×10^7 MSCs, 3×10^7 MSCs, or 9×10^7 MSCs, and found that 3×10^7 MSCs provided the best rates of healing rather than the maximal dose. Another study by Cho et al. [31] tested a variety of MSC dosage's in relation to the length of the fistula tract (3×10^7 cells per 1 cm length of tract), but no comparisons in efficacy were made. Panes et al. used a fixed dose of MSCs (12×10^7), but in some patients, this dose was split between two fistula tracts versus one dominant tract [37•]. Interestingly, none of these studies that used variable dosing noted increased healing rates with increased quantities

Table 1 Clinical trials

Name of Study	Type of study	Location	# of patients with CD	Intervention	Type and source of stem cells	Outcome	Results	Use of MRI	adverse events
García-Olmo et al 2003 [30]	Case report	Spain	1	Local injection of stem cells	Autologous, Adipose tissue	Complete epithelialization of external opening	Fistula healed in 1 week, No recurrence till 3 months post treatment	No	None
García-Olmo et al 2005 [23]	Phase I, open label, single arm	Spain	4	Local injection of 3×10^6 million MSC	Autologous, Adipose tissue	Complete epithelialization of external opening	3 of 4 rectovaginal or perianal fistula (75%) at 8 weeks	No	None
García-Olmo et al 2009 [33]	Phase IIb, open label, double arm, random-ized	Spain	14	Local injection of 2×10^6 MSC plus fibrin glue as compared to fibrin glue alone; second dose of 4×10^6 MSC if fistula healing was not seen at 8 weeks	Autologous, Adipose tissue	Complete epithelialization of external opening	5 of 7 fistulas (71%) in MSC versus 1 of 7 fistulas (14%) healed in fibrin glue alone at 8 weeks	No	15 non serious AE; 4 serious AE, 1 related to MSCs (perinala abscess)
Cho et al 2013 [31]	Phase I, open label, single arm	Korea	10	1×10^7,2×10^7,4×10^7, cells/mL based on the size of the fistula (total of 3–40 $\times 10^7$cells)	Autologous, Adipose tissue	Complete epithelialization of external opening	3 of 10 patients (30%) had complete healing at 8 weeks post treatment; sustained at 8 months	No	13 AE were reported in seven patients (70%); 3 SAE in 2 patients (20%, one related with seton placement
Lee et al 2013 [39]	Phase II, open label, single arm	Korea	33	3×10^7 or 6×10^7 cells per 1 cm of fistula length; average number of 15.8×10^7 cells), followed by a second injection of 1.5 times more cells (average number of 19.1×10^7cells) if fistula closure was not complete at 8 weeks	Autologous, Adipose tissue	Complete epithelialization of external opening	27 of 33 patients (82%) had complete healing at 8 weeks; 88% sustained closure at 1 year	No	28 AE, all unrelated to MSC; 1 SAE unrelated to MSC
Cho et al 2015 [35]	Phase II exten-sion of Lee phase II	Korea	24	9–42 $\times 10^7$ cells based on length of fistula tract	Autologous, Adipose tissue	Complete epithelialization of external opening	20 of 24 patients (83%) had sustained closure at 2 years	No	53 AE, all unrelated to MSC
Ciccocioppo et al 2011[38]	Open label, single arm	Italy	10	1.5 to 3×10^7 MSC every 4 weeks until an improvement was obtained or when autologous MSCs were no longer available (2–5 injections)	Autologous, Adipose	No drainage on clinical exam as well as healed on MRI	6 of 9 patients (67%) with complete closure at 8 weeks; all sustained closure at 1 year	Yes	No adverse events
de la Portilla et al 2013 [36]	Phase I/IIa open label, single arm	Spain	24	Local injection of 2×10^6 MSCs; second injection of 4×10^6 if unhealed at 14 weeks	Allogeneic, Adipose tissue	Absence of drainage and complete epithelization, plus absence of collections measured by MRI	5 out of 18 fistulas (28%) closed at 24 weeks post treatment. 7 out of 18 patients (47%) had closure of external openings at 24 weeks post treatment.	Yes	four SAE (three anal abscesses and one uterine leimyoma), so the group concluded the treatment had an acceptable safety profile.
Panes J et al 2016 [37•]	Phase III, RCT	Europe/Israel	212	Local injection of stem cells	Allogeneic, Adipose tissue	Absence of drainage and <2 cm fluid collection on MRI	50% ($n = 53$ of 107) healed in the MSC group compared with	Yes	Overall, 68 (66%) in treatment, 66 in placebo (65%); SAE in 18 (17%)

Table 1 (continued)

Name of Study	Type of study	Location	# of patients with CD	Intervention	Type and source of stem cells	Outcome	Results	Use of MRI	adverse events
							34% ($n = 36$ of 105, $p = 0.024$) at 24 weeks		and 14 (14%), majority anal abscess
Molenkijk et al 2015 [34]	Open label, 4 arms	Netherlands	21	N = 5 in 10^7 MSC dose (G1) N = 5 in 3×10^7 MSC dose (G2) $N = 5$ in 9×10^7 MSC dose (G3) $N = 6$ in placebo (G4)	Allogeneic, Adipose	Absence of drainage and <2 cm fluid collection	12-week fistula healing: G1:2/5 G2:4/5 G3:1/5 G4:2/6	Yes	50 AE, most common was common cold, 4 abscesses
Dietz et al 2017 [32]	Phase I, open label, single arm	USA	12	20 million cells on a GORE Bio A Plug	Autologous Adipose tissue on Matrix	Absence of drainage and improvement in Van Assche score on MRI	10 of 12 patients with healing at 6 months (83%)	Yes	No adverse events

of cells. However, results from Garcia-Olmo et al. [33] and Ciccocioppo et al. [38] have suggested that repeat injections at 8 to 12 weeks may increase healing rates.

Is Healing Sustainable?

Durability of fistula healing is an important measure of success as approximately 70% of CD patients relapse on discontinuation of treatment [47–49]. With infliximab, the only approved medical therapeutic for perianal CD, only 23% of patients had sustained closure of their perianal fistulas at 1 year. [8] The only phase III to date using MSCs had a primary endpoint of 24 weeks, at which time the primary endpoint of combined clinical and radiographic remission was significantly higher in the treatment group than in the control group (51 versus 36%; $p = 0.021$). A more recent update of the sustainability to 1 year also found significantly improved rates of healing as compared to placebo in the MSC delivery arm (56 versus 39%; $p = 0.01$). These results highlight that MSC therapeutics may offer a sustained treatment response for perianal disease [50••].

Utilization for Luminal Disease

While direct local injection of MSCs has clearly proven safe and effective for perianal Crohn's disease, there is a significant desire to determine optimal methods for the use of cell-based therapies to treat luminal disease, both Crohn's and ulcerative colitis. When considering cellular delivery for intestinal disease, there are several options for MSC delivery. MSCs could be delivered via intraperitoneal injection, submucosal endoscopic delivery, venous delivery, or targeted intra-arterial delivery in interventional radiology. Because intravenous delivery results in pulmonary trapping following MSC delivery [51], direct targeted arterial delivery may prove most useful in utilizing MSC's paracrine effects to recruit additional cells to the area of inflammation. However, further research is needed in this area since one randomized trial of 82 Crohn's patients received four doses of umbilical cord–derived MSCs intravenously and the treatment arm ($n = 41$) had decreased Crohn's disease activity index score, Harvey-Bradshaw score, and corticosteroid use at 12 months follow-up without any pulmonary events [52]. And, another phase II study of 16 medically refractory Crohn's disease patients, who received MSC intravenously, and were found to have a reduction in Crohn's disease activity index and endoscopic severity also had no adverse events or pulmonary events [53••].

The primary limitation in utilizing an intra-arterial delivery method of MSCs is the risk of an embolic event resulting in intestinal ischemia. However, reports from the interventional radiology literature describe a < 1% overall incidence of vascular complications following selective intra-arterial infusion [54]. This provides some reassurance that intra-arterial delivery

is likely safe and may prove highly effective. However, this technique for the treatment of inflammatory bowel disease remains largely unstudied to date. In CD, there is one reported case of a patient receiving 10^5/kg of MSCs followed by a second injection 4 weeks later of 10^6/kg of MSCs into the ileocolic via interventional radiology to treat terminal ileal disease. Not only were there no adverse events but the patient also tolerated the procedure well with reported clinical improvement [55]. In UC, a much larger trial performed by Hu et al. investigated the safety and efficacy of intra-arterial delivery of MSCs for colonic mucosal healing in the setting of ulcerative colitis. Seventy patients were safely treated with an intra-arterial delivery of 1.5×10^7 MSCs in 10 mL to the SMA. Overall, there was significant mucosal healing seen in treated patients [56].

Logistical Barriers to Implementation

As an expanding number of reported clinical trials are demonstrating clinical success, the next hurdle is how to translate and implement these regenerative products into clinical practice or patient service lines. While stem cell delivery for perianal fistula has been successful, it is expensive and has yet to become commercially available or covered by insurance. In addition, delivery of cells is challenging as the shelf life is 24–48 h and many institutions may not have the infrastructure to receive cells. When utilizing autologous cells or allogeneic cells shipped frozen, a good manufacturing practice (GMP) grade lab is required at the institution for cell thawing or cell manufacturing. GMP grade facilities are costly and are not widespread across hospitals. Therefore, most patients in the near future will likely be treated in the context of a clinical trial, offered at a limited number of institutions with the required infrastructure and funding. Therefore, significant ongoing research is being performed in developing alternative acellular regenerative products, with similar function to MSCs, that can be manufactured at a lower cost, with a longer shelf life, and can be delivered without the need for a complex infrastructure on the receiving end. While we continue to explore alternative regenerative therapies, it will be important that the personnel delivering cellular therapeutics become well versed in regenerative therapy and the needs for their institutions' infrastructure to create service lines to treat a greater number of patients.

Conclusions

It is an exciting time as we enter an era of regenerative-based therapy for the treatment of inflammatory bowel disease, offering novel treatment approaches without the risk of systemic immunosuppression or surgical complications. It remains imperative, however, to continually ask how to better optimize ongoing clinical trials. Going forward, it will be important to define the optimal stem cell donor in the setting of allogeneic therapy, understand whether allogeneic product results in a clinically significant alloimmunity preventing re-treatment, and determine optimal dosing and redosing protocols. Answers to these important questions will allow us to continue to make strides within regenerative medicine, apply our findings to systemic treatment for luminal disease, and optimize patient outcomes.

Compliance with Ethical Standards

Conflict of Interest Amy Lightner reports working as a consultant for Takeda.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Schwartz DA, Loftus EV Jr, Tremaine WJ, Panaccione R, Harmsen WS, Zinsmeister AR, et al. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. Gastroenterology. 2002;122(4):875–80.
2. Hannaway CD, Hull TL. Current considerations in the management of rectovaginal fistula from Crohn's disease. Color Dis. 2008;10(8): 747–55 discussion 755-746.
3. Radcliffe AG, Ritchie JK, Hawley PR, Lennard-Jones JE, Northover JM. Anovaginal and rectovaginal fistulas in Crohn's disease. Dis Colon Rectum. 1988;31(2):94–9.
4. Sands BE, Blank MA, Patel K, van Deventer SJ, Study AI. Long-term treatment of rectovaginal fistulas in Crohn's disease: response to infliximab in the ACCENT II study. Clin Gastroenterol Hepatol. 2004;2(10):912–20.
5. Andreani SM, Dang HH, Grondona P, Khan AZ, Edwards DP. Rectovaginal fistula in Crohn's disease. Dis Colon Rectum. 2007;50(12):2215–22.
6. Molendijk I, Nuij VJ, van der Meulen-de Jong AE, van der Woude CJ. Disappointing durable remission rates in complex Crohn's disease fistula. Inflamm Bowel Dis. 2014;20(11):2022–8.
7. Present DH, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med. 1999;340(18):1398–405.
8. Sands BE, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med. 2004;350(9):876–85.
9. Hesterberg R, Schmidt WU, Muller F, Roher HD. Treatment of anovaginal fistulas with an anocutaneous flap in patients with Crohn's disease. Int J Color Dis. 1993;8(1):51–4.
10. Hull TL, Fazio VW. Surgical approaches to low anovaginal fistula in Crohn's disease. Am J Surg. 1997;173(2):95–8.
11. Kodner IJ, Mazor A, Shemesh EI, Fry RD, Fleshman JW, Birnbaum EH. Endorectal advancement flap repair of rectovaginal and other complicated anorectal fistulas. Surgery. 1993;114(4):682–9 discussion 689-690.

12. Sonoda T, Hull T, Piedmonte MR, Fazio VW. Outcomes of primary repair of anorectal and rectovaginal fistulas using the endorectal advancement flap. Dis Colon Rectum. 2002;45(12):1622–8.
13. Steele SR, Kumar R, Feingold DL, Rafferty JL, Buie WD, Standards Practice Task Force of the American Society of Colon and Rectal Surgeons. Practice parameters for the management of perianal abscess and fistula-in-ano. Dis Colon Rectum. 2011;54(12):1465–74.
14. Wolff BG, Culp CE, Beart RW Jr, Ilstrup DM, Ready RL. Anorectal Crohn's disease. A long-term perspective. Dis Colon Rectum. 1985;28(10):709–11.
15. West RL, van der Woude CJ, Hansen BE, et al. Clinical and endosonographic effect of ciprofloxacin on the treatment of perianal fistulae in Crohn's disease with infliximab: a double-blind placebo-controlled study. Aliment Pharmacol Ther. 2004;20(11–12):1329–36.
16. Pearson DC, May GR, Fick GH, Sutherland LR. Azathioprine and 6-mercaptopurine in Crohn disease: a meta-analysis. Ann Intern Med. 1995;123(2):132–42.
17. Ford AC, Sandborn WJ, Khan KJ, Hanauer SB, Talley NJ, Moayyedi P. Efficacy of biological therapies in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol. 2011;106(4):644–59.
18. Soltani A, Kaiser AM. Endorectal advancement flap for cryptoglandular or Crohn's fistula-in-ano. Dis Colon Rectum. 2010;53(4):486–95.
19. Le Blanc K, Frassoni 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(9624):1579–86.
20. Sun L, Wang D, Liang J, Zhang H, Feng X, Wang H, et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis Rheum. 2010;62(8):2467–75.
21. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009;5(1):54–63.
22. Yamout B, Hourani R, Salti H, Barada W, el-Hajj T, al-Kutoubi A, et al. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. J Neuroimmunol. 2010;227(1–2):185–9.
23. Garcia-Olmo D, Garcia-Arranz M, Herreros D, Pascual I, Peiro C, Rodriguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum. 2005;48(7):1416–23.
24. Gharibi T, Ahmadi M, Seyfizadeh N, Jadidi-Niaragh F, Yousefi M. Immunomodulatory characteristics of mesenchymal stem cells and their role in the treatment of multiple sclerosis. Cell Immunol. 2015;293(2):113–21.
25. Kimbrel EA, Kouris NA, Yavanian GJ, Chu J, Qin Y, Chan A, et al. Mesenchymal stem cell population derived from human pluripotent stem cells displays potent immunomodulatory and therapeutic properties. Stem Cells Dev. 2014;23(14):1611–24.
26. English K. Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol. 2013;91(1):19–26.
27. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells. 2004;22(7):1330–7.
28. Mayne CG, Williams CB. Induced and natural regulatory T cells in the development of inflammatory bowel disease. Inflamm Bowel Dis. 2013;19(8):1772–88.
29. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol. 2005;6(4):345–52.
30. Garcia-Olmo D, Garcia-Arranz M, Garcia LG, et al. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. Int J Color Dis. 2003;18(5):451–4.
31. Cho YB, Lee WY, Park KJ, Kim M, Yoo HW, Yu CS. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. Cell Transplant. 2013;22(2):279–85.
32. Dietz AB, Dozois EJ, Fletcher JG, Butler GW, Radel D, Lightner AL, et al. Autologous mesenchymal stem cells, applied in a bioabsorbable matrix, for treatment of perianal fistulas in patients with Crohn's disease. Gastroenterology. 2017;153(1):59–62 **e52**.
33. Garcia-Olmo D, Herreros D, Pascual I, Pascual JA, del-Valle E, Zorrilla J, et al. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. Dis Colon Rectum. 2009;52(1):79–86.
34. Molendijk I, Bonsing BA, Roelofs H, Peeters KCMJ, Wasser MNJM, Dijkstra G, et al. Allogeneic bone marrow-derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. Gastroenterology. 2015;149(4):918–927 e916.
35. Cho YB, Park KJ, Yoon SN, Song KH, Kim DS, Jung SH, et al. Long-term results of adipose-derived stem cell therapy for the treatment of Crohn's fistula. Stem Cells Transl Med. 2015;4(5):532–7.
36. de la Portilla F, Alba F, Garcia-Olmo D, Herrerias JM, Gonzalez FX, Galindo A. 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 Color Dis. 2013;28(3):313–23.
37.• Panes J, Garcia-Olmo D, Van Assche G, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet*. 2016;388(10051):1281–90 **First phase III trial utilizing MSCs for the treament of perianal Crohn's disease which showed that the treatment arm had improved healing compared to the control.**
38. Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut. 2011;60(6):788–98.
39. Lee WY, Park KJ, Cho YB, Yoon SN, Song KH, Kim DS, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. Stem Cells. 2013;31(11):2575–81.
40. Lo Surdo JL, Millis BA, Bauer SR. Automated microscopy as a quantitative method to measure differences in adipogenic differentiation in preparations of human mesenchymal stromal cells. *Cytotherapy*. 2013;15(12):1527–40.
41. Sammour I, Somashekar S, Huang J, Batlahally S, Breton M, Valasaki K, et al. The effect of gender on mesenchymal stem cell (MSC) efficacy in neonatal hyperoxia-induced lung injury. PLoS One. 2016;11(10):e0164269.
42. Serena C, Keiran N, Madeira A, Maymó-Masip E, Ejarque M, Terrón-Puig M, et al. Crohn's disease disturbs the immune properties of human adipose-derived stem cells related to inflammasome activation. Stem Cell Rep. 2017;9(4):1109–23.
43. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol. 2014;32(3):252–60.
44. Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA. 2012;308(22):2369–79.
45. Pezzanite LM, Fortier LA, Antczak DF, Cassano JM, Brosnahan MM, Miller D, et al. Equine allogeneic bone marrow-derived mesenchymal stromal cells elicit antibody responses in vivo. Stem Cell Res Ther. 2015;6:54.
46. Trounson A, McDonald C. Stem cell therapies in clinical trials: progress and challenges. Cell Stem Cell. 2015;17(1):11–22.
47. Brandt LJ, Bernstein LH, Boley SJ, Frank MS. Metronidazole therapy for perineal Crohn's disease: a follow-up study. Gastroenterology. 1982;83(2):383–7.

48. Goldstein ES, Marion JF, Present DH. 6-Mercaptopurine is effective in Crohn's disease without concomitant steroids. Inflamm Bowel Dis. 2004;10(2):79–84.
49. Pearson DC, May GR, Fick GH, Sutherland LR. Azathioprine and 6-mercaptopurine in Crohn disease. A meta-analysis. *Ann Intern Med*. 1995;123(2):132–42.
50.•• Panes J, Garcia-Olmo D, Van Assche G, et al. Long-term efficacy and safety of stem cell therapy (cx601) for complex perianal fistulas in patients with Crohn's disease. *Gastroenterology*. 2018;154(5):1334–1342 e1334 **Reported one year outcomes of the first phase III trials utilizing MSCs for the treatment of perinal Crohn's disease, demonstrating the lasting effects of using cellular therapy.**
51. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev. 2009;18(5):683–92.
52. Zhang J, Lv S, Liu X, Song B, Shi L. Umbilical cord mesenchymal stem cell treatment for Crohn's disease: a randomized controlled clinical trial. Gut Liver. 2018;12(1):73–8.
53.•• Forbes GM, Sturm MJ, Leong RW, et al. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. *Clin Gastroenterol Hepatol*. 2014;12(1):64–71 **A phase II study which shows systemic delivery of MSCs may prove effective for the treatment of medically refractory Crohn's luminal disease. THere were no adverse events related to the MSCs themselves.**
54. Hessel SJ, Adams DF, Abrams HL. Complications of angiography. Radiology. 1981;138(2):273–81.
55. Dinesen M, Lundmark M, Albrechtsen M. Complete genome sequences of two isolates of Kalanchoe latent virus. Arch Virol. 2009;154(7):1173–5.
56. Hu J, Zhao G, Zhang L, Qiao C, di A, Gao H, et al. Safety and therapeutic effect of mesenchymal stem cell infusion on moderate to severe ulcerative colitis. Exp Ther Med. 2016;12(5):2983–9.