ORIGINAL CONTRIBUTION

Durable Response in Patients With Refractory Fistulizing Perianal Crohn's Disease Using Autologous Mesenchymal Stem Cells on a Dissolvable Matrix: Results from the Phase I Stem Cell on Matrix Plug Trial

Eric J. Dozois, M.D.[1] • Amy L. Lightner, M.D.[1] • Allan B. Dietz, Ph.D.[2]
Joel G. Fletcher, M.D.[3] • Yong S. Lee, Ph.D.[3] • Jessica J. Friton, M.A.[4]
William A. Faubion, M.D.[4]

1 Department of Colon and Rectal Surgery, Mayo Clinic, Rochester, Minnesota
2 Department of Laboratory Medicine, Mayo Clinic, Rochester, Minnesota
3 Department of Radiology, Mayo Clinic, Rochester, Minnesota
4 Department of Gastroenterology, Mayo Clinic, Rochester Minnesota

BACKGROUND: Refractory perianal Crohn's disease remains notoriously difficult to treat. We developed a novel technology using a commercially available bioabsorbable fistula plug to deliver autologous adipose-derived mesenchymal stem cells.

OBJECTIVE: This study aimed to assess therapeutic safety and feasibility in the completed STOMP (stem cells on matrix plugs) phase 1 clinical trial.

DESIGN: Prospective single-arm phase I clinical trial.

SETTING: Tertiary academic medical center.

PATIENTS: Adults (aged 18–65 y) with complex single-tract Crohn's disease perianal fistula who have failed conventional therapy were included in this study.

INTERVENTION: Autologous adipose-derived mesenchymal stem cells were isolated, ex vivo culture expanded, and seeded onto a commercially available bioabsorbable fistula plug. Six weeks later, patients returned to the operating room for removal of the seton and placement of the stem cell-loaded plug.

MAIN OUTCOME MEASURES: Patients were followed up for a total of 8 visits through 12 months. Safety was the primary end point; clinical healing and MRI response were secondary end points.

RESULTS: Twenty patients (12 females; mean age 36 y) were treated with the stem cell–loaded plug. Of the 20 patients enrolled, 3 were not included in the 12-month analysis because of study withdrawal. Through 12 months, no patient experienced a serious adverse event related to the stem cell–loaded plug. Four patients experienced 7 serious adverse events and 12 patients experienced 22 adverse events. Complete clinical healing occurred in 14 of 18 patients at 6 months and 13 of 17 patients at 12 months. MRI response was observed in 12 of 18 patients at 6 months.

LIMITATIONS: The main limitations were the small sample size and restrictive inclusion criteria.

CONCLUSIONS: A stem cell–loaded plug can safely and effectively deliver cell-based therapy for patients with single-tract fistulizing perianal Crohn's disease. See **Video Abstract** at http://links.lww.com/DCR/C70.

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Correspondence: Eric J. Dozois, M.D., Department of Colon and Rectal Surgery, Mayo Clinic, 200 1st St SW, Rochester, MN 55905. E-mail: dozois.eric@mayo.edu

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RESPUESTA DURADERA OBSERVADA EN PACIENTES CON ENFERMEDAD DE CROHN PERIANAL FISTULIZANTE REFRACTARIA MEDIANTE EL USO DE CÉLULAS MADRE MESENQUIMALES AUTÓLOGAS EN UNA MATRIZ DISOLUBLE: RESULTADOS DEL ENSAYO DE FASE I STEM CELL ON MATRIX PLUG

ANTECEDENTES: La enfermedad de Crohn perianal refractaria sigue siendo notoriamente difícil de tratar. Desarrollamos una tecnología novedosa utilizando un tapón de fístula bioabsorbible disponible comercialmente para administrar células madre mesenquimales derivadas de tejido adiposo autólogo.

OBJETIVO: Evaluar la seguridad y viabilidad terapéutica en el ensayo finalizado STOMP.

DISEÑO: Ensayo clínico prospectivo de fase I de un solo brazo.

AJUSTE: Centro médico académico terciario.

PACIENTES: Adultos (18-65) con fístula perianal compleja de la enfermedad de Crohn de un solo tracto que han fracasado con la terapia convencional.

INTERVENCIÓN: Se aislaron células madre mesenquimales derivadas de tejido adiposo autólogo, se expandieron en cultivo ex vivo y se sembraron en un tapón de fístula bioabsorbible disponible comercialmente. Seis semanas después, los pacientes regresaron al quirófano para retirar el setón y colocar el tapón cargado de células madre.

PRINCIPALES MEDIDAS DE RESULTADO: Los pacientes fueron seguidos durante un total de 8 visitas durante 12 meses. La seguridad fue el criterio principal de valoración; la curación clínica y la respuesta a la resonancia magnética fueron criterios de valoración secundarios.

RESULTADOS: Veinte pacientes (12 mujeres, edad media 36 años) fueron tratados con el tapón cargado de células madre. De los 20 pacientes inscritos, tres no se incluyeron en el análisis de 12 meses porque se retiraron del estudio. A lo largo de 12 meses, ningún paciente experimentó un evento adverso grave relacionado con el tapón cargado de células madre. Cuatro pacientes experimentaron 7 eventos adversos graves y 12 pacientes experimentaron 22 eventos adversos. La curación clínica completa ocurrió en 14 de 18 pacientes a los 6 meses y en 13 de 17 pacientes a los 12 meses. La respuesta a la resonancia magnética se observó en 12 de 18 pacientes a los 6 meses.

LIMITACIONES: Las principales limitaciones son el tamaño pequeño de la muestra y los criterios de inclusión restrictivos.

CONCLUSIONES: Un tapón cargado de células madre se puede administrar de manera segura y efectiva, una terapia basada en células para pacientes con enfermedad de Crohn perianal fistulizante de un solo tracto. Consule **Video Resumen** en http://links.lww.com/DCR/C70. *(Traducción— Dr. Yesenia Rojas-Khalil)*

KEY WORDS: Crohn's disease; Matrix; Mesenchymal stem cell; Perianal fistula.

Crohn's disease (CD) is a chronic inflammatory disease of the GI tract of unknown cause, which continues to increase in incidence.[1,2] Up to 26% of patients with CD will develop perianal fistulizing disease during their disease course.[1] CD involving the perianal region is notoriously difficult to treat and is a devastating and disabling condition with a significant negative impact on quality of life.[2] Despite advancements in immunotherapy, up to two-thirds of patients will have recurrent disease,[3] and 20% require proctectomy with a permanent colostomy to relieve the morbidity associated with poorly controlled perianal disease.[4,5]

Mesenchymal stem cells (MSCs) have demonstrated potential as a novel and effective therapeutic strategy for the management of perianal CD. Since the initial case report of MSCs healing a refractory rectovaginal fistula in 2003,[6] 11 phase I,[7–11] phase II,[10,12,13] and phase III[14] trials have been performed to study the safety and efficacy of MSCs for perianal CD. Significant heterogeneity in protocols using allogeneic[8,11,13,14] or autologous MSCs,[6,7,9,10,12,15,16] derived from both bone marrow (BM)[11,15] and adipose tissue,[6,8–10,14] administered at various doses, with[6,8,9] or without[11,14] scaffolding, make it difficult to directly compare treatment efficacy among protocols. Efficacy, however, has been reported at encouraging rates ranging from 50% in the large phase III randomized controlled trial to 88% in a phase II trial.[14] Although these rates are encouraging, there are substantial differences in disease status and outcome measures making direct comparisons of technologies difficult. Fortunately, all trials have confirmed safety with relatively few adverse events (AEs) or serious adverse events (SAEs) related to the delivery of MSCs, and negligible risk of incontinence associated with these cell-based interventions for perianal CD.

We have developed a novel approach to therapy by seeding autologous adipose-derived MSCs (AD-MSCs) on a bioabsorbable scaffold (MSC-MATRIX) to provide an optimized cell-based treatment approach for patients who had refractory perianal CD. Our rationale was to provide a sustained local exposure of MSCs along the entire fistula tract by delivering them on an implantable matrix. To assess the safety of this potential future therapeutic approach in patients with poorly controlled perianal disease, we designed a prospective, single-arm, phase I trial. The primary aims of this study were to determine the feasibility and safety of using an autologous AD-MSC-MATRIX for the treatment of refractory single-tract

perianal fistulas. The secondary aims were to assess fistula response both clinically and radiographically. Early results of 12 patients at 6 months demonstrated no product-related SAEs and complete healing in 83%.[8] We herein report all 20 enrolled patients through 12 months of the now completed phase I STOMP trial.

MATERIALS AND METHODS

Patient Selection

Following Investigational New Drug authorization (IND #15356) by the Food and Drug Administration, the institutional review board (IRB#12-009716) approval was obtained at Mayo Clinic, Rochester, MN, for a phase I safety trial of AD-MSCs on a MATRIX (Gore Bio-A Fistula Plug; W.L. Gore, Flagstaff, AZ) for the treatment of perianal CD. Inclusion criteria included adults (aged >18 y) with medically and surgically refractory single perianal fistula tract in the setting of CD. Refractory to medical therapy was defined as the patient having failed standard medical therapy for perianal CD including anti–tumor necrosis factor agents. Refractory to surgical therapy was defined as failure to heal after placement and removal of a seton or after attempts at other more invasive surgical procedures (Table 1). Exclusion criteria comprised patients with cryptoglandular disease, branching multitract perianal disease, rectovaginal or perineal body fistulas, presence of hepatitis B and C, HIV, pregnant or lactating, exposure to another investigational drug within 30 days, history of malignancy including melanoma (with the exception of localized skin cancers), and inability to complete an MRI. Six-month clinical and MRI response data have been published previously on 12 patients,[8] with all subsequent data and interpretations/reports unique to the current study.

Study Activity

Patients underwent a baseline pelvic MRI with fistula protocol, following study consent. Then, patients were taken to the operating room for abdominal wall adipose tissue harvest and an examination under anesthesia (EUA). Harvest of adipose tissue was performed by making a 2-cm incision in the left lower abdomen and extracting 1 to 2 g of subcutaneous adipose tissue that was sent to the Mayo Clinic Immune Progenitor and Cell Therapeutics laboratory for expansion, testing, and adherence onto the MATRIX. The MATRIX is shaped as a plug with 6 tailorable tubes to fill the defect and a disk to occlude the internal opening. The MATRIX material is a copolymer of glycolide and trimethylene carbonate formed into a web. Characterization of the properties of this material across various product forms

TABLE 1. Patient demographics and previous medical and surgical management

Study ID no.	*Sex*	*Perianal disease duration (y)*	*Previous medical management*	*Previous surgical management*	*Age at enrollment (y)*
01	F	13	IFX, ADA, 6-MP, steroids	Seton; fistulotomy	20
02	M	4	IFX, ADA, AZA	Seton; fistulotomy	58
03	F	2	IFX, ADA	Seton, drainage	40
04	M	6	IFX, ADA	Seton	18
05	M	4	6-MP, ADA	Seton; fecal diversion	24
06	F	7	IFX, CZP, steroids	Seton, fistulotomy	26
07	F	2	IFX, ADA, AZA, steroids	Seton	34
08	F	6	IFX + 6MP	Seton, drainage	50
09	M	17	ADA, AZA	Seton, abscess drainage	31
10	M	10	IFX, AZA, steroids	Seton	56
11	F	3	IFX, MTX, ADA	Seton, drainage	21
12	M	4	ADA	Seton; abscess drainage; fistulotomy	42
13	M	1	IFX, ADA, AZA, Vedo	Seton	36
14	F	2	6MP, ADA, Vedo	Seton; abscess drainage; fecal diversion	24
15	F	1	IFX, AZA, ADA, Vedo	Seton	33
16	F	1.5	AZA, ADA	Seton	36
17	F	9	IFX, CZP, MXT	Seton, porcine plug, abscess drainage	41
18	F	1.5	ADA, AZA, Vedo	Seton; abscess drainage; fecal diversion, fistulotomy, LIFT	49
19	M	4	ADA,	MAF, seton	40
20	F	11	AZA, ADA, IFX, Vedo + MXT	MAF, seton	51

ADA = adalimumab; AZA = azathioprine; CZP = certolizumab; IFX = infliximab; LIFT = ligation of interphincteric fistula tract; MAF = mucosal advancement flap; 6-MP = 6-mercaptopurine; MXT = methotrexate; Vedo = vedolizumab.

and clinical applications has been reported.[17] The EUA consisted of examining fistula anatomy, curettage cleansing of the fistula tract, seton placement drain and control of any ongoing sepsis. Patients returned to the operating room 6 weeks later for a EUA with placement of MATRIX. The MATRIX was delivered equivalently to the manufacturer's direction for the MATRIX. Essentially, the 6 tubes of the MSC-MATRIX were trimmed as needed to allow for a snug fit with the fistula tract's diameter. The disk of the MSC-MATRIX was sutured to the rectal wall at the internal opening in a 6-point manner using absorbable 3.0 PDS suture. Patients were seen in the clinic on day 1, 2 weeks, 1 month, 2 months, 3 months, 6 months, and 12 months. Pelvic MRI was prospectively planned on all patients at 2 weeks, 2 months, and 6 months and if clinically indicated at 12 months. Patients were allowed to undergo rescreening for retreatment with a second MSC-MATRIX (same inclusion criteria) if there was a failure of fistula clinical closure at the 6-month evaluation.

Evaluation of Response to Treatment

The primary end point of this study was to determine the feasibility and safety of using an autologous AD-MSC-MATRIX for the treatment of refractory CD perianal fistulas. AEs were defined as worsening (change in severity or frequency) of CD present at the time of the study, intercurrent illnesses, abnormal laboratory values, or clinically significant abnormalities in physical examination, vital signs, weight, fistula drainage, and SAEs defined as events that range from hospitalization to life-threatening. Recording of SAEs and AEs began at time of consent and until participation in the study ended.

To better attribute cause and effect for SAEs and AEs as they related to the MSC-MATRIX, we categorized events as related to the patients underlying CD, related to the MSC-MATRIX, and related to the surgical procedure itself. Events were attributed to CD when events were consistent with activity of their disease and remote from the site of the study intervention. Events attributed to the surgical procedure were those occurring in the first 30 days after surgery. Events attributed to the MSC-MATRIX were those events thought to be directly related to either the MSCs embedded in the matrix or the matrix itself.

In this study, the secondary efficacy end point (fistula response) was assessed in 2 ways: 1) clinically and 2) by MRI. A clinically healed outcome was defined as absence of drainage on clinical examination when the tract and site of external opening were palpated. MRI response was defined by a decrease in the diameter and length of the T2-weighted hyperintense fistula tract on T2-weighted fast spin-echo images (expressed as percentage change from baseline), without development of abscess or additional ramifications of the treated fistula, and without increase in the Van Assche MRI perianal fistula severity score.[8,18] A decrease in the Van Assche score was not required for treatment response, as marked reductions in fistula size can be observed without changes in the Van Assche score. However, any increase in the Van Assche score was considered failure of response, as an increase in fistula ramifications or abscess would increase score components. All clinical examinations were performed by the treating gastroenterologist or colon and rectal surgeon in their outpatient clinic, and all study MRIs were read by a single GI radiologist, who was not blinded to the date of imaging examinations.

Statistical Analysis

Significance of radiologic changes in length and diameter of perianal fistula and Van Assche score was determined using a paired *T* test and reported as a 1-tail *p* value.

RESULTS

Feasibility

Twenty of 38 screened patients were enrolled and treated after obtaining consent. Twelve were female, and the median age was 36 years (range, 18–58 y). All had persistent refractory perianal CD with a median duration of disease for 4 years and a median number of 5 previous EUAs. All patients had a seton before placement of MSC-MATRIX. All patients had been exposed to biologic therapy for treatment of their CD and remained on biologic therapy throughout their trial participation. All patients had persistent fistulizing disease despite prior medical and surgical interventions (Table 1). Of the 20 patients enrolled, 2 were not included in the analysis at 6 months (MSC-MATRIX dislodged 10 d after surgery, n = 1; patient withdrew because of noncompliance, n = 1). A third patient died from reasons unrelated to the study protocol or interventions and was not included in the 12-month analysis.

All 20 patients had successful subcutaneous adipose tissue harvest. There was maintenance of sterility and expansion of the MSCs after adipose tissue harvest in all cases. All patients had successful adherence of MSCs to the MATRIX, with each MATRIX having delivered approximately 20 million AD-MSCs (Fig. 1). All patients had successful MSC-MATRIX surgical placement.

Safety

No SAEs were determined to be directly related to the MSC-MATRIX. Seven SAEs occurred in 4 patients (Table 2). Twenty-two AEs occurred in 12 patients (Table 3). Two patients developed a perianal abscess that required drainage procedures and 1 patient required widening of the external opening to facilitate drainage. There were 2 nonserious AEs as a result of seroma formation at the adipose tissue collection site. Of the 22 nonserious AEs, 10 were

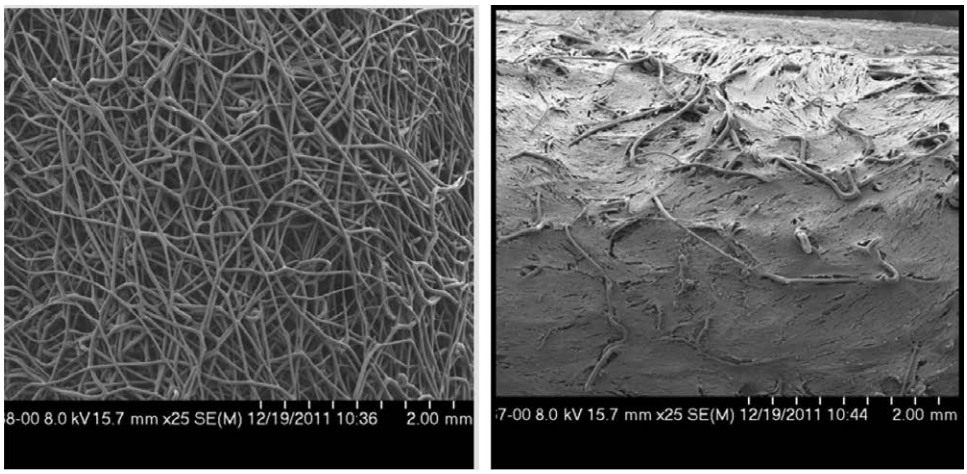

FIGURE 1. MSC adhering to matrix, without cells (left) and after cell adherence (right). MSC = mesenchymal stem cells.

Table 2. Serious adverse events

Study ID no.	*Event*	*Relatedness to Crohn's disease*	*Following MSC-MATRIX placement (d)*
14	Ischioanal abscess	Yes	Before placement
14	Pain: refractory perianal	Yes	Before placement
16	Perianal abscess	No	15
12	Fistula tract debridement	No	38
14	Perianal abscess	Yes	95
14	Elective stoma takedown	Yes	240
18	Death (unrelated to study intervention)	No	248

MSC-MATRIX = mesenchymal stem cell on a bioabsorbable scaffold.

related to underlying CD and 12 were unrelated to underlying CD or the study interventions. One patient died during the study period, but the death was not associated with any trial interventions.

Clinical Efficacy

At 6 months postintervention, 14 of 18 patients (78%) had complete clinical healing (Table 4). At 12 months postintervention, 13 of 17 patients (76%) had complete clinical healing and 4 had no response. Of the 4 patients with no response, 2 developed perianal abscess recurrence requiring drainage and seton placement, one of which had a fistula tract with a 90-degree angle making MSC-MATRIX placement technically difficult, and 2 experienced persistent drainage from the treated fistula, one of which had a large diameter internal opening and tract.

At 12 months, of the 6 perianal abscess events that happened after plug placement, 2 of those patients were considered failures at 12 months because of persistent drainage (with setons still in place), and 3 of those patients with interim abscess recurrence ultimately healed at 1 year. One of the patients withdrew before the 6-month time point.

No patients experienced incontinence of stool. Other than a single patient switching from infliximab to adalimumab therapy, no patients underwent a change in primary anti-Crohn's therapy throughout the 12 months. Two patients received antibiotics (<30 d after surgery) at the discretion of the clinical team for signs of early infection in the treated fistula.

Efficacy as Assessed by MRI

Six months after MSC-MATRIX placement, a pelvic MRI was performed. None of the 18 patients had an increase in the Van Assche score, 2 had small collections observed on their baseline examination (12 and 3 mm in longest linear dimension), and 13 had a decrease in diameter and length. MRI criteria for treatment response were demonstrated in 12 of 18 patients (67.0%; [Table 4]). The large majority of patients with MRI response had clinical healing (10/12; 83.0%), whereas 4 of 14 patients (29.0%) with clinical healing still had persistent fistulas on MRI despite clinical closure. Overall, there was a significant decrease in the diameter ($p = 0.0001$) and length ($p = 0.03$) of the fistulas that were treated. The mean change in the length of the treated fistula tract was –13.2 and in the diameter of the treated fistula tract was –4.2 mm in responding patients. In 6 treatment failures, the mean change in the length (–8.3 mm) and diameter of the fistula tracts still decreased (–0.7 mm). Overall, there was a significant decrease in the length of T2-weighted hyperintensity within the fistula tract (median decrease, 20.2%; range, 5.1.0% to 100.0%; $p = 0.007$), with

TABLE 3. Adverse events

Study ID no.	*Event*	*Relatedness Crohn's disease*	*Following MSC-MATRIX placement (d)*
01	Gluteal abscess	Yes	Before placement
12	Perianal abscess	Yes	Before placement
04	Stitch seroma[a]	No	Before placement
03	Stitch seroma[a]	No	Before placement
04	Hives post-IFX infusion	No	32
10	Perianal abscess	Yes	35
11	Perianal abscess	Yes	57
17	Ovarian serous cystadenoma	No	81
10	New branching tract-off treated tract	Yes	85
02	Perianal abscess	Yes	85
14	Perianal pain	Yes	138
03	Viral illness	No	156
15	Pneumonia	No	165
13	Perianal abscess	Yes	168
13	New complex fistula tracts	Yes	168
04	IFX infiltration	No	199
03	MRSA on nose	No	217
03	Chronic cough	No	232
14	Nonhealing chronic wound	Yes	364
06	Dermatitis	No	364
06	Urinary tract infection	No	364
06	Gastritis	No	364

IFX = infliximab; MRSA = methicillin resistant staphylococcus aureus; MSC-MATRIX = mesenchymal stem cell on a bioabsorbable scaffold.
[a]Unrelated to MSC-MATRIX placement, but was related to study procedure for fat biopsy collection.

two-thirds of patients demonstrating a drop in signal within the fistula. Similarly, Van Assche perianal severity scores also decreased significantly (median 13 to median 9; $p < 0.001$), without worsening in any of the patients.

Pelvic MRI was performed in 11 patients at 12 months after MSC-MATRIX placement at the discretion and management preferences of the clinical team. Five patients did not undergo imaging at 12 months because of lack of clinical indication (the 6 mo research MRI demonstrated MRI response and clinical healing was sustained at 12 mo). None of the 11 patients had an increase in the Van Assche score, 1 continued to have a small collection, and 5 had a decrease in diameter and length. MRI criteria for treatment response were demonstrated in 4 of these patients, with 3 of these fistulas also demonstrating clinical healing. In this population, 5 of 11 fistulas demonstrated decreased length and diameter, 7 of 11 had T2 signal decrease, and 5 of 11 had decreased gadolinium enhancement (Table 4). The 1 patient who was retreated after 6 months (subject #17) had a pelvic MRI 6 months after retreatment and demonstrated MRI response without clinical closure.

DISCUSSION

This prospective, single-arm phase I trial aimed primarily to assess the feasibility and safety of using an autologous AD-MSC-MATRIX for the treatment of patients with refractory perianal fistulas secondary to CD. The secondary aims were to assess fistula response both clinically and radiographically. Using this novel intervention, we found a high level of safety, 100% feasibility, and a durable clinical response demonstrated by complete clinical healing in 76% of patients at 12 months.

MSCs are an emerging therapy for the treatment of perianal CD, a common phenotype of CD that is notoriously difficult to treat. Several phase I,[7–11] phase II,[10,12,13] and phase III[14] trials have now been conducted that have highlighted the efficacy and safety of MSCs for perianal CD. Overall, clinical trial results are promising, reporting no increased risk of incontinence, and potential for improved treatment efficacy over conventional medical and surgical approaches.

Because the MSC-MATRIX used in our study is a novel approach, we have no benchmarks to compare our results. If we compare our results to the most common use of MSCs to treat perianal Crohn's fistulas (injection of MSCs and suture closure of the internal opening), we find a similar feasibility and adverse event profile. When we compare our efficacy results to other data published using MSCs, we find our results commensurate with the 50% to 80% healing rate reported. Our 76% healing rate at 12 months is highly significant given that our cohort had highly refractory fistulas and had disease for a mean of >5 years. Less frequently reported in the literature is the durability of MSC therapy, with many studies reporting 6-month outcomes. We were encouraged to see that most patients that achieved initial healing remained healed at 12 months follow-up.

Despite our encouraging results and those of other investigators, there remain several unanswered questions regarding cell-based therapy for perianal CD. First, there

TABLE 4. Clinical and radiographic outcomes

				6-mo MRI characteristics				12-mo MRI characteristics					
Subject ID no.#	*Sex*	*Age*	*Baseline fistula type/assessment*	*Clinical outcome (healed vs failed)*	*Fistula size (no change vs smaller[a])*	*T2-signal hyperintensity (no change vs decrease)*	*Gadolinium enhancement (no change, decrease, increase)*	*Radiographic response (none vs response)*	*Clinical outcome (healed vs failed)*	*Fistula size (no change vs smaller[a])*	*T2-signal hyperintensity (no change vs decrease)*	*Gadolinium enhancement (no change, decrease, increase)*	*Radiographic response (none vs response)*
1	F	20	Transsphincteric w/ puborectalis/levator extensions	Healed	Smaller	Decreased	Decreased	Response	Healed	12-mo MRI not performed			
2	M	58	Suprasphincteric	Failure	No change	Decreased	Decreased	None	Failure	No change	Decreased	Decreased	None
3	F	40	Intersphincteric	Healed	Smaller	Decreased	Decreased	Response	Healed	12-mo MRI not performed			
4	M	18	Intersphincteric	Healed	Smaller	Decreased	Decreased	Response	Healed	No change	Decreased	Increased	None
5	M	24	Transsphincteric w/ puborectalis/levator extension	Healed	Smaller	Decreased	Decreased	Response	Healed	12-mo MRI not performed			
6	F	26	Transsphincteric	Healed	Smaller	Decreased	Increased	Response	Healed	No change	No change	Increased	None
7	F	34	Transsphincteric	Healed	Smaller	Decreased	Decreased	Response	Healed	12-mo MRI not performed			
8	F	50	Transsphincteric	Failure	N/A[b]	N/A[b]	N/A[b]	None	Failure	Smaller	Increased	Increased	Response
9	M	31	Transsphincteric	Healed	No change	No change	Decreased	None	Healed	Smaller	Decreased	Decreased	Response
10	M	56	Intersphincteric	Healed	Smaller	No change	Increased	None	Healed	Smaller	Decreased	Increased	None
11	F	21	Transsphincteric	Healed	Smaller	Decreased	Decreased	Response	Healed	No change	Increased	Decreased	None
12	M	42	Transsphincteric	Healed	No change	No change	No change	None	Healed	Smaller	Decreased	Increased	Response
13	M	36		Withdrawal					Withdrawal				
14	F	24	Suprasphincteric	Failure	Smaller	No change	Increased	Response	Failure	No change	No change	Decreased	None
15	F	33	Trans/anol vaginal	Healed	Smaller	Decreased	Decreased	Response	Healed	12-mo MRI not performed			
16	F	36	Transsphincteric	Healed	No change	Decreased	Increased	None	Healed	No Change	Decreased	Decreased	None
17	F	41	Transsphincteric	Failure	Smaller	Decreased	N/A[b]	Response	Failure[c]	Smaller[c]	No change[c]	N/A[b, c]	Response[c]
18	F	49	Intersphincteric	Healed	Smaller	N/A[b]	N/A[b]	Response	Withdrawal				
19	M	40	Suprasphincteric	MSC-MATRIX fell out 10 d after placement					N/A				
20	F	51	Extrasphincteric anorectal	Healed	Smaller	Decreased	N/A[b]	Response	Healed	Smaller	Decreased	N/A[b]	Response

MSC-MATRIX = mesenchymal stem cell on a bioabsorbable scaffold; N/A, not available.
[a]Smaller indicates decrease in diameter and length.
[b]Unable to calculate.
[c]Retreatment after 6-mo failure.

is a limited understanding regarding the mechanism of healing with MSCs. The recent success of MSCs in treating severe inflammatory disorders, such as graft-versus-host disease,[19] systemic lupus erythematosus,[20] multiple sclerosis,[21] and CD,[9] has highlighted the therapeutic benefit of the immunomodulatory characteristics of MSCs.[22,23] These immunomodulatory properties are executed by MSCs' migration to sites of active inflammation or tissue injury, secretion of anti-inflammatory molecules like interleukin-10, hepatocyte growth factor, transforming growth factor β 1,[24] and paracrine signaling to nearby cells to maintain the local anti-inflammatory environment.[25,26] It is in this way that MSCs can upregulate a $CD4^+$ T-cell subset of regulatory T cells.[27,28] Further investigation is needed to better understand the function of MSCs and how they are interacting with nearby cells in the local peri-fistula environment to achieve healing. Second, there has been limited investigation regarding potential alloimmunity after delivery of allogeneic cells. MSCs are thought to be immunoprivileged because of their lack of major histocompatibility complex class II expression.[29] However, many studies have still used AD-MSCs, given the concern for alloimmunity after the delivery of allogeneic MSCs.[30–32] The literature that exists on alloimmunity after MSC delivery is controversial; yet, the largest trial to date of MSC in fistula reported an anti-HLA antibody rate to be 35%.[29,30,33] The clinical significance of anti-HLA antibodies, particularly on retreatment, is uncertain. Third, although we understand the importance of closing the internal opening of the fistula tract to achieve healing, less is understood about the potential benefit of a mechanical matrix to deliver MSCs. Surgeons have attempted fistula closure with mechanical Tisseel fibrin glue and plug devices with limited success.[34–36] However, we sought to develop the concept of cells on a matrix both for the mechanical purposes of the sealing the fistula tract, and also to potentially prolong the local exposure of MSCs. Future investigation may highlight the multifactorial added utility of a matrix for optimal delivery of MSCs to fistula(s) and improved healing rates.

Fortunately, there have been no reported SAEs or AEs related to MSCs among the numerous phase I, phase II, and phase III studies now performed. In the largest clinical trial to date of the direct injection of allogeneic MSCs in over 200 patients, the patient dropout rate because of AE was less than 6%, and this rate was the same in both the control and treatment arms. The near-identical rates of AEs in both the treatment and control arms underscore the safety of the MSCs themselves.[14] Similarly, in our trial, no SAEs related to the MSC-MATRIX occurred. The most common AEs in our series were consistent with previous reports and included perianal abscess and pain.

Little is also known about the use of imaging as a response parameter in stem cell treatments of perianal fistula. Panes et al[14] defined combined remission as complete closure of external openings without development of abscesses >2 cm at pelvic MRI. We defined MRI response as a decrease in the size of the fistula (diameter and length) without development or persistence of abscess (of any size), or additional ramifications of the treated fistula, so it should be realized that we used stringent imaging response criteria. None of our patients had an increase in the Van Assche score. Additionally, most patients had decreases in the size of T2 hyperintense tract, indicating that these changes are likely good response parameters when treated fistulas are followed by imaging. Interestingly, the magnetic resonance novel index for fistula imaging in Crohn's disease scale was recently created and demonstrated moderate to substantial intra- and interobserver variability for hyperintensity of the fistula tract on T2-weighted images as well as the length of the fistula tract.[37] Our findings suggest the magnetic resonance novel index for fistula imaging in Crohn's disease scale might be used to assess response in the future, in addition to (or in lieu of) our response definitions.

The main limitations of this study are the small sample size and restrictive inclusion criteria. Most of the 18 screen failures were because of unsuitable fistula anatomy: 8 were too complex (branched, intersphincteric, and/or horseshoe) and 8 too simple (suitable for fistulotomy). Because of the fistula plug design, we could only include patients with single-tract perianal fistulas. We were able to convert some patients with branching disease to a single-tract architecture by performing a fistulotomy on side branches of the main tract that were outside the sphincter complex. Moreover, how fistula size, architecture, and duration of having the fistula before intervention impacts outcomes were not evaluated in this phase I study. Additionally, the GI radiologist evaluating MRI examinations was not blinded to the date of imaging examinations, potentially affecting image analysis. However, this methodology was intentionally chosen before image interpretation to assist with the identification of MRI-responsive features and early identification of potential complications given the small numbers of patients in this phase I study.

Despite these limitations, our data, which show a high and sustained clinical efficacy using a matrix plus cells approach to patients with refractory perianal CD, is very encouraging for a group of patients with so few treatment options and significant morbidity from active disease. No current therapy, medical or surgical, has demonstrated such a high clinical healing rate in this patient population. Some patients in our trial had the active perianal disease for years and found resolution with the MSC-MATRIX intervention. Given this, we are encouraged to further study how cells on a bioabsorbable matrix that can be surgically implanted can benefit larger groups of patients with perianal CD.

CONCLUSION

We performed this phase I clinical trial to study a novel treatment strategy for perianal CD using a commercially available bioabsorbable fistula plug coated with AD-MSCs. After 12 months of follow-up, we found a consistently high safety profile and sustained efficacy in clinical and MRI healing in those treated. As a result, a phase II trial is warranted to further study efficacy of the MSC-MATRIX as a potential new therapeutic option for patients with perianal CD who have failed conventional therapy.

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