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Autologous Microfragmented Adipose Tissue Injection in Refractory Complex Crohn's Perianal Fistulas: Long-Term Results at 6.7Years Mean Follow-up

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Background: Nowadays, there is a clear need tor new viable therapeutic options to face complex perianal Crohn's disease (PCD). Results of our previous pilot study demonstrated the efficacy and safety of locai injection of autologous microfragmented adipose tissue (MFat) in this setting. This study aims to evaluate the long-term tollow-up results in the same cohort of patients.

Methods: Data on clinica! and radiologica! remission and surgical recurrence rates were prospectively collected on the 15 patients with complex fistulizing PCD refractory to combined bio-surgical therapy, originally treated with locai MFat injection, with a mean 6.7 years tollow-up.

Results: In our previous study, at 24-week tollow-up, combined remission was reported in 66.7% of patients, while clinica! remission was achieved in 93% of cases. At a 6.7-year tollow-up, 9 of the 10 healed patients maintained remission. The patient with recurrence was successfully reoperated. Three out of 5 patients who failed primary combined remission were retreated, with 2 obtaining combined remission and 1 failing. One patient refused any subsequent treatment due to good quality of lite. The last patient presented delayed healing at a 1-year follow-up. Overall success rate alter rescue therapy at the final tollow-up reached 86.6%. Safety was maintained throughout ali follow-up periods.

Conclusions: This is the longest tollow-up published trial on MFat injection tor PCD. Our results show that patients who achieved closure in the first 24 weeks sustained response at long-term evaluation. In addition, there may be a rationale in repeating treatment as rescue therapy in not responding to patients.

Lay Summary

Perianal Crohn's disease remains highly debilitating. New regenerative medicine treatments have shown conflicting results tor patients resistant to conventional therapies. Our study with the combination of microfragmented adipose tissue injection and surgical drainage of sepsis, confirms the long-term safety and efficacy of this novel therapy.

Key Words: perianal Crohn's disease, Lipogems, autologous microfragmented adipose tissue transplant, complex perianal fistulas, fistulectomy

Introduction

Perianal Crohn's Disease (PCD) is a disabling condition burdened by significant morbidity, crushing impact on patients' quality of life, and significantly high direct and indirect costs. Although the therapeutic armamentarium has considerably grown in the last decades, complex fistulizing disease stili represents a challenge for gastroenterologists and surgeons. [1]

Nowadays, although there is no treatment definitely proved to be the gold standard, bio-surgical approach represents the main trend emerging from the literature as the most accepted therapeutic strategy in severe PCD. Indeed, since the introduction of anti-TNF-alpha agents changed the treatment algorithm from simple contrai of sepsis to complete closure of fistulas, systemic, or locai administration of biologica! therapy combined with surgical sepsis contrai is considered the first-line treatment for complex PCD, as recommended in many recent international guidelines.[2,3] However, probably due to the wide heterogeneity of clinica! pictures and to the lack of standardized treatment algorithms, data from literature show success rates widely ranging from 0% to 99%.[4,5] In addition, over 60% of healed patients present with relapse after 1 year.[6,7]

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Key Messages

- What is already known? Local injection of autologous microfragmented adipose tissue (MFat) combined with surgical drainage of sepsis is a valid therapeutic option for multi-resistant complex perianal Crohn's disease.
- What is new here? Our results from the longest follow-up ever-published study on this feature (mean 6.7 years) confirm the long-term efficacy in terms of combined remission.
- How can this study help patient care? This innovative technique provides a 1-step, non-expanded, ready-to-use, safe, low-cost, viable, and effective MFat with a minimal manipulation, compliant with the regulatory panorama in daily clinical practice.

Finally, the treatment algorithm for patients with unhealed fistulas after a combined bio-surgical therapy is not well defined yet, but repair surgery, such as endorectal advancement flap and biological plug placement, could be a valuable rescue surgical option in patients who obtained mucosal healing of the rectum.

However, despite optimal surgical and medical management, not more than 50%-60% of our patients achieve actual remission.[8,9] Even worse, it has to be highlighted that the risk of permanent stoma is still reported as high as 50%, while it rises up to 90% in patients with active proctitis.[10-12]

Based on these assumptions, there is a clear need for innovative and advanced therapeutic options, in order to offer a rescue treatment to the patients refractory to bio-surgical approach and/or primary/rescue surgical repair.[13]

In the last 2 decades, a bunch of studies have demonstrated the potential of regenerative medicine treatments using mesenchymal stem cells (MSCs) due to their well-known ability to down-regulate the immune reactivity of the mucosa and promote tissue healing. However, the routine clinical utilization of such a strategy is strongly limited by the high costs and the long-time interval required for ex-vivo Good Manufacturing Practice (GMP) cell expansion and the complex restrictions related to their extensive manipulation.[14]

Lipogems is a commercially available system able to overcome the complexity of current GMP requirements since it intraoperatively provides injectable autologous microfragmented adipose tissue (MFat), preserving the stromal vascular niche rich in activated cellular elements with MSCs and pericytes characteristics. Several studies[15-18] show that pericytes, cells located around blood vessels, express the same surface markers as MSCs and, upon vascular injury, they are activated becoming functional MSCs. These cells do not differentiate into other cell types, but secrete bioactive molecules with immunomodulatory properties,[19] preventing autoimmune responses, and promoting a regenerative microenvironment. Once tissue repair is completed, MSCs revert to their pericyte phenotype, stabilizing the surrounding environment.[20,21] Their primary function, then, is not transdifferentiation, but facilitating the tissue's natural self-healing processes. Several studies have already proven the ability of MFat in terms of controlling inflammation, immunomodulation, and fibrosis, as well as promoting damage repair and tissue regeneration, both *in vitro* and in *in vivo* and *ex vivo* models in different clinical settings,[22-25] including perianal fistulizing cryptoglandular disease.[26]

On this basis, in 2016 we performed a clinical study on 15 patients with complex multi-resistant PCD who were treated by combined surgical drainage of sepsis and injection of MFat; our results showed a 67% of combined remission and a 93% of clinical remission at 24-week follow-up.[27]

One of the concerns regarding the use of regenerative medicine in PCD disease is represented by the sustainability of the results obtained in the short-term period. To the best of our knowledge, there are only a few studies reporting outcome results at long-term follow-up.[28-33]

Once assessed the feasibility and safety of the technique, we needed to assess the sustainability of the encouraging outcomes initially found at 24 weeks. Therefore, the goal of the present study was to analyze the fate of the 15 originally treated patients at long-term follow-up, assessing the maintenance rate of combined remission and the need for subsequent surgical, medical, or regenerative treatment in patients with recurrence. Finally, we evaluated the success rate of retreatment with surgery or MFat re-injection in patients who had failed healing at 24-week follow-up.

Materials and Methods

Study Design and Population

This study is a longitudinal clinical trial and represents the final follow-up of our previous nonprofit prospective pilot study with 24 weeks of follow-up (ClinicalTrials.gov number: NCT01541579), in which 15 adult patients with PCD and biological treatment-refractory, draining, complex perianal fistulas were treated with MFat.[27] In the first protocol, all patients were assessed by means of clinical examination at baseline and at 2, 4, 8, 12, and 24 weeks after treatment, while magnetic resonance imaging (MRI) evaluation was performed at baseline and 24 weeks of follow-up. Final outcomes were determined at a 24-week follow-up assessing success rate, defined as combined clinical and radiological remission.[27] The study was approved by the Ethics Committee of IRCCS S. Orsola-Malpighi Hospital (prot Lipogems-crohn2, n° 4/2016/U/Sper, January 19, 2016), and all the procedures mentioned in this study were carried out in accordance with the ethical standards and with the Helsinki Declaration of 1975, as revised in 2000. Patients were informed about risks and benefits and expressed and signed their informed consent.

In the present study, each patient was evaluated at least 6.5 years after treatment administration.

We reviewed the corresponding medical records of all patients from the final visit onwards of the previous prospective pilot study. Patients were then invited to attend a final follow-up visit, where a detailed clinical history was taken. Investigators collected information on medical treatments received by the patients since the last visit of the previous study (specifically, whether they had received biological therapy, antibiotics, immunosuppressants, and other treatments taken to treat perianal fistulas and Crohn's disease), and on surgical procedures due to PCD. Finally, clinical outcome of the treated fistulas was assessed. All patients subsequently underwent pelvic MRI performed by the same experienced radiologist (AC).

The primary endpoint of this study was the long-term efficacy of the treatment in terms of combined remission ("healing"), defined as the coexistence of closure of all treated external openings that were draining at baseline clinically

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assessed (absence of drainage upon gentle finger compression) and absence of residual collections > 3 mm of the treated perianal fistulas assessed by pelvic MRI.[34]

"Clinical remission" is defined as the absence of drainage upon gentle finger compression of the external openings present at baseline and presence of residual collections > 3 mm at the pelvic MRI.

"Recurrence" was defined as the occurrence of a new fistula or reactivation of the original one in healed patients.

Patients with persistent failure or recurrence who underwent reoperation represent in this study the "rescue group." Outcomes of reoperated patients were assessed by clinical and radiological measurements.

Adverse events since the final visit of the preceding study were reported according to standard regulatory definitions (serious or not, severity, causality).

Surgical Procedures in the "Rescue Group"

MFat preparation

The Lipogems processing kit (Lipogems International Spa, Milan, Italy) was used to harvest, process, and inject the autologous tissue under sterile conditions according to the manufacturer's Instruction[27]during the same surgical procedure.

Adipose tissue was harvested either from the inferior abdomen or from the inner thigh (with the patient placed in a lithotomy position), depending on the macroscopic availability of adipose tissue. Before the harvesting, the donor site was injected with 150-250 cc of modified Klein Solution (500 cc saline, 1 cc epinephrine 1/1000 IU) using a disposable 17G blunt cannula connected to a 60-cc luer-lock syringe. The fat was then harvested (50-100 cc) using a 13G blunt cannula connected to a 20 mL VacLok syringe. This lipoaspirate was processed in the dedicated adipose tissue–processing device, rigorously avoiding the presence of air, first by pushing it through a cluster reduction filter and then by mechanical emulsification through shaking the lipoaspirate in the completely closed system with 5 stainless-steel beads. This separated and washed away oil and blood using the gravity counterflow of saline solution. Adipocyte clusters collected at the top of the adipose tissue–processing device underwent a second adipose cluster reduction by being passed through a size reduction filter. The resulting MFat was then collected into 1 mL syringes for subsequent use.

Cone-like fistulectomy and MFat injection

Examination under anesthesia was performed in order to identify all the fistula tracts and abscesses; eventual purulent material was drained and the fistula tracts curetted. Necrotic and inflamed tissues were excised using a "cone-like" fistulectomy at each fistula tract.[35] After local surgical drainage of the perianal disease, 15-20 mL of MFat was injected using a 21G needle circumferentially into the submucosa surrounding the internal fistula orifice and in the perianal tissue along the residual fistula tract (Figure 1).[27]

Endoanal mucosal flap

After drainage of sepsis, in patients presenting with mucosal healing, endoanal mucosal flap was performed by closing the internal orifice of the fistula at the muscular plane by interrupted 2-0 PDS sutures placement; adrenaline 1:100 000 was then injected in the sub mucosa proximally to the internal orifice and a U-shaped mucosal flap was lifted, dragged down to cover the internal orifice, and sutured tension-free to the muscular plane with interrupted Vycril 3-0 sutures.

Statistical Analysis

Statistical analysis was performed using R Statistical Software (version 4.0.0; R Foundation for Statistical Computing) and GraphPad Prism software (v 6.0; GraphPad Software Inc.). The Shapiro–Wilk normality test was used to evaluate the normal distribution of the sample. Continuous variables were expressed as the mean ± standard deviation (SD) and medians and first and third quartiles [Q1-Q3]. The between-group differences for continuous variables were evaluated and were evaluated with the unpaired Student *t*

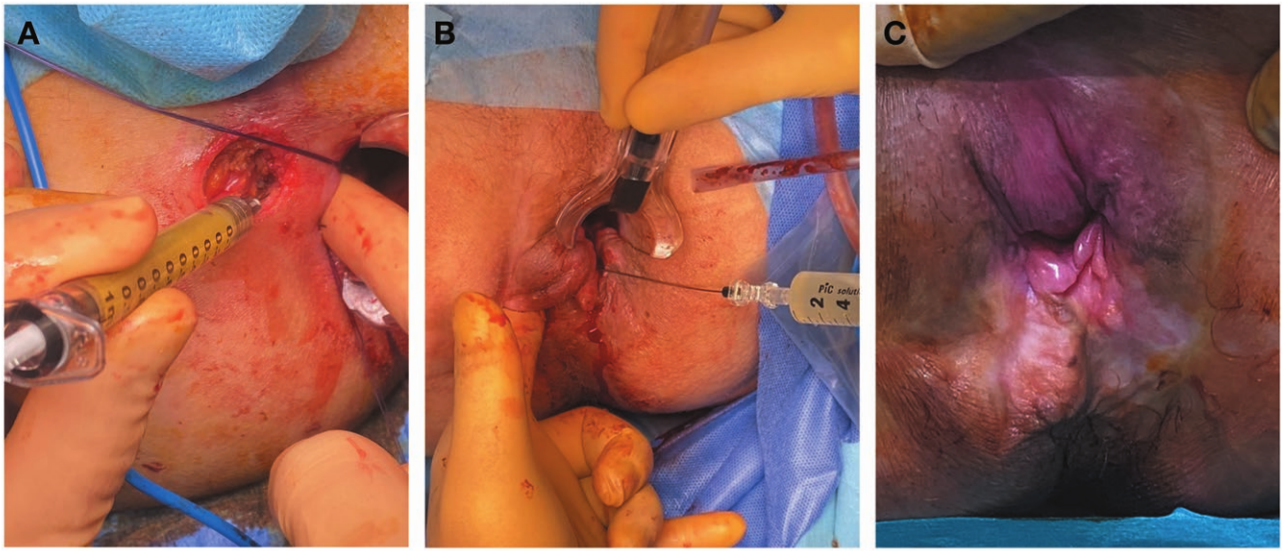

Figure 1. Digital photographs of surgical procedure: "cone-like" fistulectomy and microfragmented adipose tissue injection in the perianal tissue along the residual fistula tract (A) and circumferentially into the submucosa surrounding the internal fistula orifice (B); clinical examination in a combined remission patient (C).

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test or Mann–Whitney test, according to the characteristics of the data distribution. Categorical variables are expressed in numbers of cases and frequencies; their differences were tested using the chi-square test or Fisher's exact test. For all analyses, the significance level was set at a *P* value lower than .05.

Results

Follow-up length ranges from 6.5 to 7.0 years, with a mean of 6.7 years (SD: 0.22 years). The baseline demographic and clinical characteristics of patients at the time of index surgery have been described in the previous article,[27] while those related to the follow-up period are reported in Table 1.

As previously described at 24-week follow-up, 10 out of 15 patients (66.6%) presented combined remission, while a significantly greater percentage of patients (93.3%) achieved clinical remission. Neither intraoperative nor post-operative major complications or adverse events occurred.

At the final follow-up, 9 out of 10 patients (90%) who reached combined remission at 24 weeks of follow-up maintained clinically and radiologically assessed healing (Figures 2 and 3). In 6 of them, stable clinical and endoscopic remission was observed with no need of biological therapy, while in 3, biological therapy was restarted for endoscopic mild/moderate proctitis.

The remaining patient was submitted 2 years after Lipogems treatment to re-do ileocolic anastomosis for anastomotic recurrence. Three years later, the patient presented with a recurrent fistula at 1 of the 2 orifices of the previously healed horse-shoe fistula and needed reoperation. A mucous endoanal flap was performed taking advantage of mucosal healing, so obtaining a new combined remission was assessed at 6 months after surgery (Figure 3).

One out of the 5 not-responders patients at 24-week follow-up, a 39-year-old male, with luminal disease in stable clinical and endoscopic remission (no biological therapy), presented with clinically healed fistula 6 months later at an outpatient follow-up visit; closure of fistula was then confirmed at pelvic MRI. The remaining 4 non-responder patients still presented with persistence of active fistulous tracts or abscess at 3-year follow-up (Figure 3).

Among them, 1 patient, with substenotic terminal ileitis treated with mesalazine and antibiotics, refused reoperation due to paucity of symptoms and satisfactory quality of life. The remaining 3 patients underwent reoperation ("rescue group"). Respectively, the first patient, a 39-year-old male, with luminal disease in stable clinical and endoscopic remission (no biological therapy) was submitted 3 years after failure to mucosal advancement flap and reached combined remission, confirmed at 6-month post-operative MRI. The second patient, a 65-year-old female, with asymptomatic mild thickening of terminal ileum in treatment with mesalazine, was reoperated at 3.6 years by means of MFat injection and mucous endoanal flap with failure characterized by the persistence of a small residual track. She is now waiting for re-suture of the flap (Figure 3).

Finally, one patient, a 54-year-old female, with terminal ileitis and associate mild proctitis in treatment with biologics, unsuccessfully underwent reoperation with MFat injection at 4-year follow-up. However, she finally obtained combined remission after a third procedure 2 years later when, in addition

Table 1. Patients' demographics at baseline.

Group	Failure at final FU	Healing at final FU	Overall	*P* value
No. of patients	2	13	15	
Age at FU (years)	51.2 ± 18.8 51.2 [44.5-57.8]	45.4 ± 11.3 40.6 [37.3-53.1]	46.2 ± 11.8 40.6 [37.6-55.1]	.542
FU (years)	6.5 ± 0.0 6.5 [6.5-6.5]	6.7 ± 0.2 6.6 [6.5-7.0]	6.7 ± 0.2 6.6 [6.5-6.8]	.104
FU at new surgery (years)	4.8 ± 2.4 4.8 [4.0-5.7]	5.7 ± 2.0 6.5 [6.5-6.6]	5.6 ± 2.0 6.5 [4.8-6.6]	.384
Gender				.919
Female	1 (50.0)	7 (53.8)	8 (53.3)	
Male	1 (50.0)	6 (46.2)	7 (46.7)	

Data are reported as mean ± SD and median [Q1-Q3] or number of patients (percentage).
Abbreviations: FU, follow-up; No.: number; Q1, first quartile; Q3, third quartile; SD, standard deviation.

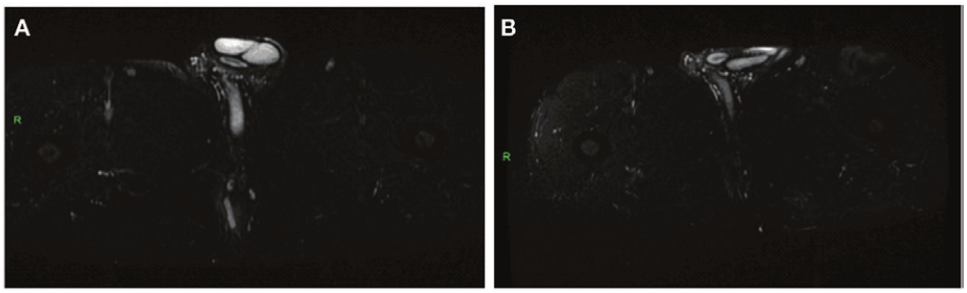

Figure 2. Clinical case example of a combined remission patient. Pre-operative (A) and 6.5 years after surgery (B) magnetic resonance imaging.

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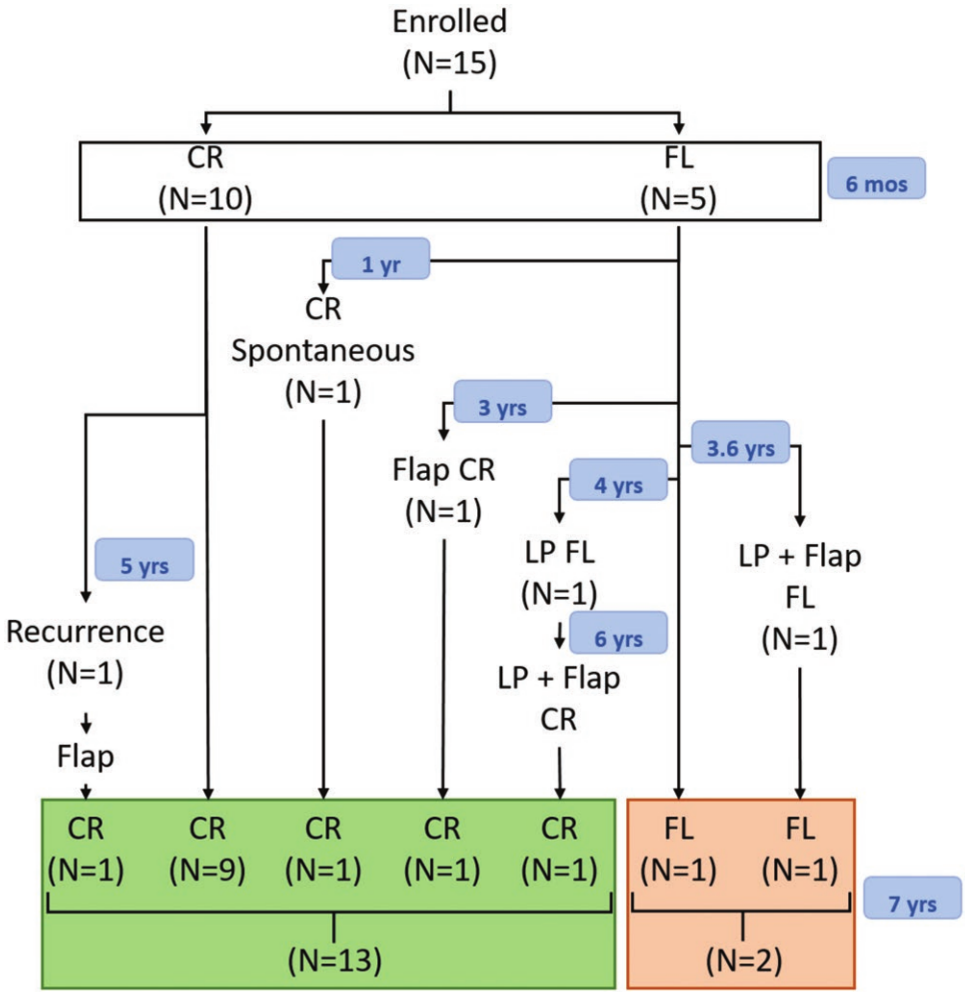

Figure 3. Fate of the patients. CR, combined remission; FL, failure; yrs, years of follow-up; mos, months of follow-up; N, number.

to MFat re-injection, a mucous flap was performed thanks to mucosal healing (Figure 3).

Overall success rate after rescue therapy at the final follow-up (re-injection ± flap) reached 86.6%.

The evaluation of risk factors for failure was conducted considering all surgeries with MFat ($N = 18$) as single independent events, each one performed in patients with different clinical features potentially influencing the outcome (Table 2).

No statistically significant differences in failure rates were observed in terms of the presence of stenosis, proctitis, luminal active disease, associated biological therapy, and site of liposuction at the time of each surgery (Table 2).

Discussion

While up to 60% of patients with complex PCD can achieve fistula healing with an optimized combination of surgical drainage and biological therapy and/or endoanal mucosal flaps, the remaining 40% of patients are obliged to live with their exhausting condition, which implies a significantly impaired quality of life, or having to face a proctectomy with definitive oostomy.[10-12]

These patients have been recently identified as class 2b, 2c, and 3 in a new classification system based on disease severity and outcomes, simultaneously synchronizing patient's and expert's goals.[36] To date, the rescue treatment algorithm to reach in these cases the aim of complete fistula healing or, at least, improvement of quality of life and prevention of transition to more advanced classes,[37] is not yet well defined. Regenerative medicine can serve as a crucial resource in this context. Indeed, an increasing number of clinical trials have recently shown very encouraging results on the safety and efficacy of local treatment using adipose-derived MSCs (ADSCs).[38] The rationale for this innovative therapeutic strategy is represented by the potential of these cells to accelerate tissue healing mainly through stimulation of local cells via a paracrine mechanism and anti-inflammatory and/or immunomodulatory activity, thus creating a suitable microenvironment for tissue regeneration.[39]

Our Group has recently reported the results of a single local administration of MFat processed with the 1-step, minimally invasive, non-expanded, ready-to-use, and enzyme-free Lipogems technology.[27] Among 15 patients with persistent complex fistulizing PCD after bio-surgical approach and subsequent surgical rescue repair, 10 patients (66.7%) had combined clinical and radiological remission, 4 patients (26.6%) showed only clinical remission, and 1 patient (6.7%) failed at 24 weeks of follow-up. These results are consistent with others reported in many trials.[30-33] In particular, in a recent meta-analysis on efficacy and safety of MSCs for complex

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Table 2. Risk factors for failure.

	Failure ($N = 6$)	Healing ($N = 12$)	Overall ($N = 18$)	P value
Stenosis				.245
No	4 (66.7)	11 (91.7)	15 (83.3)	
Yes	2 (33.3)	1 (8.3)	3 (16.7)	
Proctitis				.569
No	4 (66.7)	10 (83.3)	14 (77.8)	
Yes	2 (33.3)	2 (16.7)	4 (22.2)	
Luminal activity				.141
No	2 (33.3)	9 (75.0)	11 (61.1)	
Yes	4 (66.7)	3 (25.0)	7 (38.9)	
Post-operative biologics				.245
No	6 (100.0)	8 (66.7)	14 (77.8)	
Yes	0 (0.0)	4 (33.3)	4 (22.2)	
Site of liposuction				.515
Inferior abdomen	6 (100.0)	9 (75.0)	15 (83.3)	
Inner thigh	0 (0.0)	3 (25.0)	3 (16.7)	

Data are reported as number of patients (percentage).
Abbreviations: FU, follow-up; N, number.

fistulizing PCD, authors included only 7 RCTs with an at least acceptable level of evidence out of 1080 studies found in literature. However, despite the high risk of bias described, even in these 7 included trials, the clinical (61.4%) and combined remission (49.3%) rates reported appear to be encouraging.[26,40] Actually, several limitations affect the reliability of current literature, including lack of standardization in inclusion criteria, patient stratification and definition of healing, together with absence of control groups, small sample sizes, and high dropout rates. Other confounding factors are related to the variability of associated surgical procedures and techniques for cell harvesting, processing, and injection[32,36,41-45]

Finally, most studies report the success of regenerative medicine treatments by simply analyzing short-term healing rates, without addressing risk, prevention, and management of long-term recurrence.[40] Given that recurrence is a very common issue in patients with complex PCD with important implications for the patient's quality of life and healthcare costs, a long-term evaluation of efficacy of these regenerative treatments is apposite.[38,40]

Cho et al. reported long-term results of a previous phase II study with fistula size-related doses of ADSCs combined with fibrin glue. Authors retrospectively found a complete healing rate of 81% and 75% at 12 and 24 months of follow-up, respectively. In the sub-analysis of sustained response, 20 out of 24 patients (83.3%) healed at 2-month follow-up, maintained complete closure at 24 months after surgery with recurrence in only 4 patients.[46]

This persistent efficacy has not been confirmed by other similar studies. Park et al.[47] reported the results of an open-label study characterized by injection of increasing doses of allogeneic ADSCs together with fibrin glue in 6 patients with complex PCD. Authors described a complete clinical fistula healing in 50% of patients at 8 months after surgery. Ciccocioppo et al.[48] presented the results of monthly (median 4 doses) local administration of bone–marrow-derived MSCs in 10 patients with refractory complex PCD, with a clinical healing rate of 70% at 1-year follow-up.

However, during subsequent follow-up, the efficacy of MSCs treatment declined and the probability of fistula relapse-free survival (defined as percentage of patients not presenting clinical evidence of fistula) was 50% at 2 years and 37% at 4 years of follow-up.[49]

Lee et al.,[50] in a phase II study, treated 43 patients with ADSCs and fibrin glue injection, repeating injection in not-healed patients at 8 weeks after surgery. They found complete fistula healing in 27 out of 33 patients (82%) at 16-week follow-up and 23 out of 26 patients, who completed an additional observational study (88%, but 53% of all enrolled patients), had a sustained complete closure with a recurrence rate of 11.5% at 1-year follow-up.

A phase II clinical trial conducted by a Spanish Group on efficacy of ADSCs on complex PCD fistulas reported a healing rate of 71%[45]; however, the long-term retrospective follow-up study showed that only 7 out of 12 initial responders maintained complete closure.[41]

Efficacy and safety of Darvadstrocel (an "off-the-shelf" expanded allogeneic ADSCs drug) for treating complex PCD fistulas were extensively evaluated in 3 consecutive multicenter clinical trials. In the first double-blind RCT ADMIRE-CD[29] on 212 refractory complex PCD patients, a clinical remission was reported in 64%, 80%, and 56% of Darvadstrocel-treated patients, and in 47%, 47%, and 40% of control arm patients at 24, 52, and 104 weeks of follow-up, respectively.[30] Subsequently, in the retrospective study INSPECT[31] on the same cohort of patients, the long-term efficacy, evaluated only in terms of clinical remission for up to 156 weeks on 43 Darvadstrocel-treated patients and 46 control patients, resulted maintained in 53.5% and 45.7%, respectively. In the third observational retrospective study PRIME,[42] a combined clinical and radiological response was observed at 6 months of follow-up in 63.1% of Darvadstrocel-treated patients. However, authors themselves highlighted that not all clinically healed patients had radiological closure and *vice versa*. This latter unexpected result might be the consequent expression of a potential bias in radiological healing definition, since "the persistence of a residual collection up to 2 cm" probably not always reflect a safe closure of the fistula tract.

A further limitation of the ADMIRE-CD and INSPECT studies is the loss to follow-up of a significant number of patients, which reduces the reliability of the results.[29-31]

Finally, the retrospective evaluation of a pilot study on 10 patients with refractory complex PCD fistulas, treated with autologous adipose-derived stromal vascular fraction combined with microfat, in which a combined remission rate of 60% had been reported at 1-year follow-up, showed a sustained combined remission in 7 patients (70%) at 3 years of follow-up. In particular, among those healed, 1 patient relapsed, while 2 patients with primary failure achieved delayed combined remission.[51]

Based on these assumptions, data reported in our study could clarify some concerns about the real potential of regenerative therapy in complex fistulazing PCD. Indeed, our results represent the very first evidence of a long-lasting effect of the MFat innovative procedure as the more than satisfactory success rate reported at 1-year follow-up is maintained in terms of combined remission in almost all patients at a minimum of 6.5 years after surgery, with a very low recurrence rate. One of

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the reasons for this might be found in the proven long-lasting antinflammatory activity of MFat, whose "stability" might be associated with its internal structural characteristics. Indeed, MFat appears to be resistant to environmentally poor tissue culture conditions (serum free), maintaining the long-term capability to release cytokines[39,52]

In addition to the specific action of the transplanted tissue, other peculiar features of our study design likely contributed to achieving quite reliable results. One of these factors is the surgical strategy. In fact, even though some authors still argue that surgical management "is not recommended for complex fistulae…because of the high risk of anal incontinence,"[46] national and international guidelines ratify that combination of surgical drainage of sepsis with subsequent biological therapy significantly enhances healing rate.[2] However, none of them clearly define what the gold-standard surgical technique should be so in clinical practice and protocols authors utilize different interventions even when supported by very low levels of evidence.[34,53] We strongly believe that the deep sanitization of perianal spaces, by means of "cone-like" partial fistulectomy, completely excising all primary and secondary tracts together with chronic granulation tissues and abscesses, most enhances the effectiveness of any subsequent strategy, reducing at the same time the risk of recurrence without jeopardizing sphincter function, especially when performed by experienced surgeons.

Another pivotal factor in our protocol is the definition of healing. The current literature is limited by a lack of standardized outcome measures. As highlighted above, the weakness of exclusively clinical criteria as "complete closure of external opening without any sign of drainage and inflammation," has already been demonstrated in different studies[13,54] as the most probable reason for high recurrence rates.[40,46]

This aspect is further substantiated by the results of our protocol which showed the early clinical reactivation of treated fistula in patient with only clinical remission. Even though the reliability and responsiveness of radiographic endpoints for PCD trials require further investigation (Hurt, ma), the combination of cross-sectional imaging to the physical examination, especially with the strict parameters we applied in the protocol (residual collection not greater than 3 mm), appears to be at present the most reliable definition of success.[34,55]

Another interesting aspect that emerges when analyzing our results is the potential benefit of re-injection of MFat in patients who failed the primary treatment, which has been already investigated for ADSCs with different algorithms, obtaining good results.[56]

However, the success rate reported in our study after retreatment with MFat injection appears to be primarily linked to a simultaneous mucosal flap placement. Based on our experience, the achievement of mucosal healing with a clear macroscopic amelioration of the tissues surrounding the internal orifice represents one of the beneficial effects of local injection of biologic agents, even when the treatment does not lead to the closure of target fistulae.[35] Thanks to this local recover of damaged and inflamed tissues, failed patients become suitable for direct reparative surgery. Similarly, in the present study, 3 originally failed patients, showing at follow-up with local mucosal healing, were successfully treated with *U*-shaped or vertical mucosal flap (alone or combined with MFat re-injection). The potential of the combination of MFat injection and surgical repair in terms of improving outcomes is evident, and there are some preliminary results in the literature that strengthen this hypothesis.[57,58] It is then advisable to explore with further RCTs the potential benefits of this combined strategy in patients with less aggressive disease characteristics (ie, class 2a sec. Gedolf).

As it is a follow-up evaluation of a pilot protocol, our study presents some limitations such as the retrospective design, the small number of patients, the lack of a control group, the procedures performed by the same surgeon. In particular, this follow-up study was mainly designed as a proof of concept and safety evaluation,[40] and the sample size of 15 patients was considered adequate for this type of investigation.[27] In addition, in the pilot study were enrolled only patients refractory to combined bio-surgical treatment and subsequent reparative surgery/ies,[37] so that a comparison with long-term results of surgical treatment does not appear appropriate.

Conclusions

The therapeutic goal in complex fistulizing PCD is to completely close the fistula without recurrence, or at least to improve patients' quality of life as much as possible.[36,59] Unfortunately, literature data show that Crohn's fistula healing is still hard to achieve, and fistulae often recur after the initial response to current treatments. The main problem is probably represented by the lack of a standardized treatment algorithm.

The local injection of autologous MFat combined with surgical drainage of perianal sepsis represents a simple, feasible, 1-step, safe, and effective innovative therapeutic approach for the treatment of multi-resistant complex fistulizing PCD.[60]

Finally, the particular significance of this study, based on the longest follow-up ever-published data (mean 6.7 years) in this field, to the best of our knowledge, lies in the preliminary validation of the long-lasting efficacy of this treatment that could potentially represent a new chance of rescue therapy for patients with no other viable treatment options left.

Based on these assumptions, the ongoing multicenter randomized placebo-controlled trial (NCT03555773) has been designed to confirm these preliminary results and better define the role of the procedure eventually widening the range of indications and changing its position in the treatment algorithm.

Further research is finally required, such as dose-finding studies, evaluation of scheduled repeated treatments, combination with other procedures, and obviously high-level RCTs to allow confirmation of clinical and radiological efficacy.[48,61]

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Authors Contributions

A.C., C.I., P.G., G.P., equally contributed to the work. S.L. contributed to the study concept and design, surgical

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procedure, acquisition of data, statistical analysis, data interpretation, manuscript writing. A.C. contributed to the study concept and design, MRI examination, data interpretation, and manuscript writing. P.G. and F.R. contributed to the study concept and design and critical revision of the manuscript. C.I. and L.G. contributed to surgical procedure, acquisition of data, and manuscript writing. R.V. and G.S. contributed to surgical procedure and manuscript writing. A.M. contributed to the data collection, data analyses, and manuscript writing. G.P. contributed to the surgical procedure, study supervision, and critical revision of the manuscript. N.K.D. contributed to the revision of the manuscript.

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Conflicts of Interest

All the authors have no conflict of interest, except Alessandra Menon who is consultant for Lipogems Internationa SpA and Adler Ortho SpA. The study was approved by the Ethics Committee of IRCCS S. Orsola-Malpighi Hospital (prot Lipogems-crohn2, n° 4/2016/U/Sper, January 19, 2016).

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