Allogenic stem cells for anal Crohn’s fistula: treating early improves the deep response rate

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Study protocol

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Abstract

Purpose:
The aim of this study was to evaluate the real-life clinical and radiological efficacy of darvadstrocel injection into complex perianal fistulas in Crohn's disease. Secondary endpoints were to assess symptomatic efficacy, outcomes and factors associated with complete combined clinical-radiological response (deep response).

Methods:
After marketing the product in France, all patients treated consecutively were included. A complete clinical response was defined by a complete closure of all external openings with no discharge on pressure. A partial response was defined by closure of ≥ 50% of external openings with no discharge on pressure. A complete radiological response (MRI), evaluated at least after six months of follow-up, was defined by a completely fibrotic sequel without abscess.

Results:
Forty-three patients were included (M/F: 22/21, median age 37 [26-45] years). The fistulas of all patients were already drained with seton(s) and were on biologic treatment. After a median follow-up of 383 [359-505] days, 28 (65%) patients showed a clinical response (22 complete and 6 partial). Only 16 (37%) achieved a deep response. The PDAI decreased significantly after treatment: 39 (91%) patients reported symptomatic improvement in terms of discharge, pain, and induration, and 28 (65%) no longer had any perineal symptoms. Only a short history of Crohn's disease < 3 years was significantly associated with deep response (OR 4.5 [1.0-19.1], p = 0.04).

Conclusion:
Darvadstrocel injection resulted in a clinical response for two thirds of patients and deep response for one third. A shorter duration of Crohn's disease was associated with deep response.

Introduction
Perianal fistulas worsen the prognosis of Crohn's disease (CD) [1], severely alter the quality of life of patients [2], and increase healthcare costs [3]. Biotherapies (in particular, infliximab) improve their therapeutic management [4], approximately doubling the healing rate over a placebo. However, the primary failure rate of fistula closure remains high (40%) [5] and recurrences are frequent, especially in complex fistulas [6]. After drainage, the simple removal of the seton appears to be less effective than surgical closure of the tract [7, 8], which is unfortunately associated with high failure rates. Stem cells represent a recent alternative due to their immunomodulatory, anti-inflammatory, regenerative, and reparative effects [9]. A double-blind, multicenter, randomized controlled trial evaluated the injection of allogeneic stem cells in 212 patients with complex Crohn's fistula who had failed classical strategies. The
cure rates at week 24 [10] and week 52 [11] were significantly higher than those for simple surgical closure of the internal opening (50 versus 34%, p = 0.024 and 56 versus 39%, p = 0.01 respectively). These results led to marketing authorization in Europe and their commercialization (darvadstrocel, Takeda company) in France since 2020. To date, evaluations of this treatment in real-life situations are still limited [12–14]. Our objective was to collect data from the initial experience in France in 2 tertiary centers with at least one year of clinical and radiological follow-up.

Materials And Methods

Patients selection

Under conditions established by the General Health Service, stem cells were allocated for patients with anal fistulas related to CD after failure of good surgical drainage and adequate treatment with biologics. Before deciding injection, previous drainage with a seton of all collections and a complete endoscopic response or moderate activity (CDAI < 220) were mandatory. Biologics and immunosuppressants were reviewed and adapted according to last ECCO guidelines and available staff. All the patients had pre-operative MRI and pre-operative endoscopy to guarantee the marketing authorization. Concerning the pelvic MRI, the acquisition protocol used high-resolution, T2-weighted, fat-saturation, fast-spin echo pulse sequences and T1-weighted, fat-saturation, fast-spin echo or three-dimensional gradient echo sequences before and after intravenous injection of gadolinium. MRI data collected were inflammation of the fistula tract, its total length and maximal depth, and the presence of collection. All MRIs were reviewed in each center by specialized radiologists. In Saint-Joseph Hospital, patient consent was obtained before data collection, which was performed prospectively in a REDCAP database (Research Electronic Data Capture, https://projectredcap.org/resources/citations/). At Rennes University Hospital, a prospective database for monitoring all patients with anoperineal Crohn’s disease has been in place since January 2005. The extraction of the specific data for this study from this database and patient consent were performed retrospectively. The data recorded were as follows: sex, height, weight, body mass index (BMI), age, luminal CD phenotype at diagnosis according to the Montreal classification [15], extra-intestinal manifestations, smoking habits, past and actual treatments (steroids, immunosuppressants, and anti-TNFα) and past surgical history (anal fistula surgery, ileal and/or colonic surgery, and stoma). The characteristics of the fistula were assessed based on the data of the exploration under anesthesia performed for all the patients as recommended by the ADMIRE study 2 to 3 weeks before injection and of the last MRI. Anatomical classification of perianal CD according to the Cardiff-Hughes classification was used to classify the tracts (F0, F1, F2) [16]. Before injection, all patients had an F2 fistula according to this classification and a complex one according to the classification of the AGA with of course no abscess [17]. Fistulas were considered multibranched when there was a main tract and at least one secondary tract such as horseshoe or suprapelvator extension. In addition, CD activity was assessed with the Harvey-Bradshaw Index (HBI) [18] and perianal CD activity was assessed with the Perianal Disease Activity Index (PDAI) [19].

Procedure & Follow-up
Stem cell injections were prepared and the cells injected as previously described (10). Surgical procedures were performed under anesthesia after antibiotic prophylaxis (metronidazole). Once the setons were removed and the fistula tract(s) curetted, the internal openings were closed by direct suturing with absorbable sutures. In addition, patients with a large internal opening underwent a minimal flap advancement. According to the manufacturer’s recommendations, darvadstrocel was injected around the internal openings and along the fistula tract(s) (24 ml). The procedure was completed by gentle massaging of the tract(s) [20]. Follow-up and events were recorded over at least one year (3, 6, and 12 months and last visit) complemented by an MRI evaluation at least six months after the procedure. Therapeutic procedures after stem cell injection were recorded, including medical treatments modification (optimization, switch of immunosuppressant/biological therapy) and surgeries (fistula drainage, intestinal resection).

Endpoints

The primary endpoints were clinical and radiological response.

The clinical response was defined as follows:

- Complete: complete closure with invisible external openings with no discharge at pressure
- Partial: complete closure of $\geq 50\%$ of the external openings with no discharge at pressure from any of the external openings
- Failure: closure of < 50% of the external openings ± the persistence of discharge at pressure from one or all of the external openings ± the occurrence of a new tract or abscess.

The radiological response was defined as follows:

- Complete: fibrous sequelae with no T2 hypersignal or enhancement after the injection of gadolinium into the tract(s), and no abscesses
- Partial: decrease of the inflammatory signal (T2 hypersignal and enhancement after the injection of gadolinium) ± the length ± the largest diameter of the tracts, with no abscesses
- Failure: absence of radiological modification or occurrence of a new fistula tract ± abscesses

A complete combined clinical-radiological response (deep response) was defined as the association of a complete clinical response with a complete radiological response.

The secondary endpoints were:

- Symptomatic efficacy, assessed with the PDAI
- Outcomes
- Factors associated with deep response

**Statistical analysis**

Data are expressed as medians and percentiles (interquartile range: 25% and 75%) or as numbers and percentages of the cohort. The nonparametric Wilcoxon signed-rank test was used for continuous variables and nonparametric Pearson or Fisher exact tests for categorical variables. The judgement criterion was deep response that combined an asymptomatic status plus clinical closure of the external openings combined with MRI healing of the tract (no inflammation and/or disappearance of the tract). Asymptomatic response was defined by the complete relief of discomfort and discharge and a stepwise regression analyses was performed to identify factors associated with deep response. The results are expressed as odds ratios (ORs) with 95% confidence intervals [CIs]. Statistical analyses were performed using JMP Pro 13.0.0 software (SAS institute).

**Ethical considerations**

The study protocol was approved by the “Comité de Protection des Personnes” GH Paris Saint-Joseph (IRB 00012157) and registered in clinicaltrial.gov (NCT05177003). The database Fondamentum is secured (nominative access code) with a CNIL (Commission nationale de l’informatique et des libertés) declaration (CNIL n°1412467).

**Results**

**Study population and procedure**

Forty-three patients injected consecutively were included in this study. The main characteristics of the population study are summarized in Table 1. The median follow-up after stem cell injection was 383 [359–505] days. No patient was lost to follow up and MRI was performed at referral and after injection for all 43 (100%) patients. Closure of the internal opening was performed using simple stitches for 33 (77%) patients and a rectal flap for 8 (19%). The internal opening was not closed for 2 (5%) patients because of a rectal stricture.
Table 1
Study population characteristics at baseline

<table>
<thead>
<tr>
<th>Study population characteristics</th>
<th>Global population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global population</strong></td>
<td><strong>N = 43</strong></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>22/21</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37 [26–45]</td>
</tr>
<tr>
<td>BMI</td>
<td>24 [21–28]</td>
</tr>
<tr>
<td>Obesity</td>
<td>08 (19)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>• Smoker</td>
<td>12 (28)</td>
</tr>
<tr>
<td>• Former smoker</td>
<td>05 (12)</td>
</tr>
<tr>
<td>• Non-smoker</td>
<td>26 (60)</td>
</tr>
<tr>
<td>Characteristics of luminal CD</td>
<td>94 [36–170]</td>
</tr>
<tr>
<td>Duration of CD (months)</td>
<td></td>
</tr>
<tr>
<td>Montreal Classification</td>
<td></td>
</tr>
<tr>
<td>A1 &lt; 16</td>
<td>07 (16)</td>
</tr>
<tr>
<td>A2 &lt; 40</td>
<td>32 (74)</td>
</tr>
<tr>
<td>A3 ≥ 40</td>
<td>04 (09)</td>
</tr>
<tr>
<td>L1</td>
<td>08 (19)</td>
</tr>
<tr>
<td>L2</td>
<td>13 (30)</td>
</tr>
<tr>
<td>L3</td>
<td>20 (47)</td>
</tr>
<tr>
<td>B2 / B3</td>
<td>08 (19) / 08 (19)</td>
</tr>
<tr>
<td>Extra-intestinal manifestations</td>
<td>07 (16)</td>
</tr>
<tr>
<td>Luminal response at referral</td>
<td>36 (84)</td>
</tr>
<tr>
<td>Harvey Bradshaw score at referral</td>
<td>01 [1–2]</td>
</tr>
<tr>
<td>Prior treatments before drainage</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
</tr>
</tbody>
</table>

Clinical, radiological and symptomatic response

The clinical, symptomatic and MRI assessments during visits at referral and follow-up are shown in Table 2. By clinical assessment, 22 (51%) showed complete closure of all their external openings, 6 (14%) showed at least 50% closure of their tracts, and 6 (14%) showed remaining inflammatory external openings. Sixteen (37%) patients were in deep response (Fig. 1). At the last outpatient visit, 39 (91%) patients declared symptomatic improvement and 28 (65%) were completely asymptomatic with no pain, no discharge, and no induration. The PDAI score decreased for all but seven patients (84%). None of the patients in clinical ± radiological partial response had any therapeutic modification because they were asymptomatic. However, all the patients with clinical ± radiological failure had an optimization or even a
change in the biologic drug and 8 (19%) patients among them required a new surgical drainage. No patient during the follow-up had luminal surgery.

Table 2

Characteristics of anal fistulas at referral and improvement at follow-up (n (%) or median [IQR 25–75])

<table>
<thead>
<tr>
<th>Follow-up (months)</th>
<th>Referral</th>
<th>[1–3]</th>
<th>[3–6]</th>
<th>[9–12+]</th>
<th>Last visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>43</td>
<td>42</td>
<td>42</td>
<td>41</td>
<td>43</td>
</tr>
</tbody>
</table>

**Clinical assessment**

<table>
<thead>
<tr>
<th></th>
<th>Referral</th>
<th>[1–3]</th>
<th>[3–6]</th>
<th>[9–12+]</th>
<th>Last visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDAI</td>
<td>06 [5–8]</td>
<td>03 [1–4]*</td>
<td>03 [0–6]*</td>
<td>02 [0–4]*</td>
<td>00 [0–4]*</td>
</tr>
<tr>
<td>Anal pain/discomfort</td>
<td>27 (63)</td>
<td>10 (24)</td>
<td>11 (26)</td>
<td>07 (17)</td>
<td>06 (14)</td>
</tr>
<tr>
<td>Discharge</td>
<td>37 (86)</td>
<td>25 (60)</td>
<td>22 (52)</td>
<td>14 (34)</td>
<td>16 (37)</td>
</tr>
<tr>
<td>F0*</td>
<td>00 (00)</td>
<td>14 (34)</td>
<td>15 (36)</td>
<td>19 (46)</td>
<td>22 (51)</td>
</tr>
<tr>
<td>F1*</td>
<td>00 (00)</td>
<td>01 (02)</td>
<td>02 (05)</td>
<td>03 (07)</td>
<td>03 (07)</td>
</tr>
<tr>
<td>F2*</td>
<td>43 (100)</td>
<td>27 (64)</td>
<td>25 (59)</td>
<td>19 (46)</td>
<td>18 (42)</td>
</tr>
<tr>
<td>Anal stricture</td>
<td>08 (19)</td>
<td>05 (12)</td>
<td>03 (07)</td>
<td>01 (02)</td>
<td>00 (00)</td>
</tr>
<tr>
<td>Anal ulcerations</td>
<td>01 (02)</td>
<td>00 (00)</td>
<td>01 (02)</td>
<td>00 (00)</td>
<td>00 (00)</td>
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</tbody>
</table>

**MRI assessment**

<table>
<thead>
<tr>
<th></th>
<th>Referral</th>
<th>[1–3]</th>
<th>[3–6]</th>
<th>[9–12+]</th>
<th>Last visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fistula healing</td>
<td>00 (00)</td>
<td>16 (37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main tract length (mm)</td>
<td>53 [35–100]*</td>
<td>20 [00–50]*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal tract depth (mm)</td>
<td>06 [03–09]*</td>
<td>03 [00–07]*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory tract and masses</td>
<td>39 (91)</td>
<td>15 (35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Branched tracts</td>
<td>30 (70)</td>
<td>14 (33)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IQR: I.Q.R. for interquartile range of 25–75%, PDAI: Perineal Disease Activity Index, F0: no fistula tract, F1: low fistula tract, F2: high and/or complex fistula tract according to Cardiff-Hughes classification (16)

*p < 0.001

**Outcomes**

No severe adverse events were reported after surgery during the year of follow-up apart from mild to moderate pain following the injection, the duration of which did not exceed the first week. No reaction of local rejection was observed.
Factors associated with combined complete clinical-radiological response (deep response)

In univariate analysis, a shorter duration of CD history at referral (history < 3 years 7/11 versus 9/32, p = 0.03) was significantly predictive of deep response. All other factors studied including the study population and fistula characteristics described in Table 1 were not associated with this deep response, in particularly the Montreal Inflammatory status (B1) (12/27 versus 04/16, p = 0.19), a shorter duration of anal fistula at referral (history < 3 years 8/14 versus 8/29, p = 0.06), and the complex status of the fistula tract (12/26 versus 4/17, p = 0.13). By multivariate analysis, the short duration of CD history (CD duration < 3 years; OR 4.5 [1.0-19.1], p = 0.04) remains significant and was associated with a better deep response rate.

Discussion

Algorithms have recently improved the management of perineal Crohn's fistulas, but the place of stem cells is still a subject of debate [21]. After one year of follow-up after treatment with allogeneic stem cells, our study reports a partial response for two thirds and a complete response for one half of patients, respectively. The PDAI decreased markedly after treatment and 91% of the patients reported a significant improvement in discharge, pain, and induration. Although inflammatory tracts improved in two thirds of the cases by MRI, deep response was observed in only one third of patients. Our results are similar to those of certain previous studies [12, 13] but less favorable than those for others [14]. These differences could be explained by a more severe fistula profile (multi-operated, "old", multibranched, associated with anal stricture, in failure of a previous obturation technique in 1/3 of cases) and stronger criteria to analyze the response. In the present study, complete clinical response required complete disappearance of all external openings and not only the absence of discharge on pressure. Similarly, complete response on MRI was defined by fibrotic sequelae without inflammatory enhancement after gadolinium injection [22, 23] and not just an abscess of less than 20 mm. All these criteria are more selective than that used in the ADMIRE study [10]. Indeed, deep complete clinical-radiological response meets treat-to-target goals [24]. This has been shown to be associated with a more durable clinical response and a lower relapse rate [25]. The positive results in terms of clinical and symptomatic response were obtained in the vast majority of cases within 6 months after injection, but 17% of patients responded beyond 6 months of the injection despite the absence of modification of their biologic treatment (Table 1). This result raises the question of the duration of action of stem cells in vivo and the possibility and timing of a new injection to improve the response rate.

In our study, a duration of CD progression < 3 years was associated with deep response (OR: 4.5). We observed a similar trend with a shorter duration of Crohn's fistula but it did not reach significance. These results should encourage consideration of this treatment earlier in the natural history of the disease before the occurrence of irreversible fibrosis which limits the action of stem cells. This result is all the more important as the marketing authorization of the product is broad and raises the question of the
place of stem cells in our therapeutic algorithm. Offering this treatment at an early inflammatory stage of the disease, even before a first classic surgical obturation technique fails, makes sense since it is a local immunomodulatory treatment. That being said, the distribution of this therapy is still limited to expert centers because of its cost and the organizational difficulties of the injection linked to the short duration of stem cell viability.

No adverse events were noted in the present study. However, the limited number of patients and the duration of follow-up did not allow safety profile assessment on the long term. Other limitations of our study were the absence of a comparator group, the absence of evaluation of the quality of life and the absence of a validated radiological score.

Conclusion

Based on stricter clinical and radiological evaluation criteria than those of ADMIRE, stem cells injection in our study showed clinical response in about two thirds of patients and deep response in about one third of patients, respectively. However, more than 90% of patients experienced significant symptomatic improvement in pain and discharge. These preliminary results suggest an anti-inflammatory and immunomodulatory effect of the stem cells rather than a reparative and “closure” effect. A short duration of the Crohn's disease was significantly associated with a better deep remission. This result asks the question of the efficacy of the stem cells on old and fibrous fistulas that are less sensitive to their anti-inflammatory process and leads us to consider this treatment earlier in our therapeutic algorithm.

Abbreviations

BMI = Body Mass Index, CD = Crohn's disease, TNFα = Tumour Necrosis Factor α, AZA = azathioprine, MTX = methotrexate, 6MP = 6 mercaptopurine. L1 = ileal, L2 = colonic, L3 = ileo-colonic, F0 = no fistula tract, F1 = low fistula tract, F2 = high and/or complex fistula tract, IQR= I.Q.R. = interquartile range: 25% and 75%, PDAI = Perineal Disease Activity Index

Declarations

Author's Contribution:

Nadia Fathallah and Laurent Siproudhis wrote the manuscript.

Mélissa Akaffou, Mohamed Amine Haouari, Amandine Landemaine, Elise Pommaret, Lucas Spindler, Charlène Brochard, Guillaume Bouguen and Vincent de Parades reviewed the manuscript.

Competing interest:

- NF received research grants from Abbvie, Amgen, Tillots, Sandoz, Takeda, served as a consultant for Takeda and received consulting and speaking fees from Abbvie, Tillots and Takeda (not related to this
study).

- LS received research grants from Takeda (not related to this study), AbbVie, Janssen, consulting and speaking fees from Takeda, AbbVie, Amgen, Janssen and Pfizer.

- EP received speaking fees from Takeda

- MA, AD, LS and MAH have no financial or proprietary interests in any material discussed in this article.

- CB received research grants from Takeda (not related to this study), AbbVie and Janssen, and speaking fees from Takeda

- GB received research grants from Takeda (not related to this study), AbbVie, Janssen, consulting fees from Takeda and speaking fees from AbbVie, Amgen, Janssen, Pfizer.

- VdP received research grants from Abbvie, Amgen, Tillots, Sandoz, Takeda, served as a consultant for Takeda and received consulting and speaking fees from Abbvie, Tillots and Takeda (not related to this study).

**Funding:**

None

**Ethics approval and Informed Consent:**

The study protocol was approved by the “Comité de Protection des Personnes” GH Paris Saint-Joseph (IRB 00012157) and registered in clinicaltrial.gov (NCT05177003). The database Fondamentum is secured (nominative access code) with a CNIL (Commission nationale de l'informatique et des libertés) declaration (CNIL n°1412467).

Informed consent was obtained from all individual participants included in the study.

**Acknowledgments:**

We would like to thank Lila Hafit for her help with the scheduling of patient visits and follow-up.

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**Figures**

Clinical, radiological and symptomatic response after stem cell injection in our series

<table>
<thead>
<tr>
<th>Clinical, radiological and symptomatic response</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Diagram showing clinical, radiological and symptomatic response" /></td>
</tr>
</tbody>
</table>

The clinical response was defined as follows:
- Complete: complete closure with invisible external openings with no discharge of pressure
- Partial: complete closure of > 50% of the external openings with no discharge at pressure from any of the external openings
- Failure: closure of < 50% of the external openings or the persistence of discharge at pressure from one or all of the external openings or the occurrence of a new tract or abscess.

The radiological response was defined as follows:
- Complete: fibrous sequela with no T2 hyperintensity or enhancement after the injection of gadolinium into the tract(s), and no abscesses
- Partial: decrease of the inflammatory signal (T2 hyperintensity and enhancement after the injection of gadolinium) by the length is the largest diameter of the tracts, with no abscesses
- Failure: absence of radiological modification or occurrence of a new fistula tract or abscesses

**Figure 1**

Clinical, radiological and symptomatic response after stem cell injection in our series