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Skeletal Muscle–Derived Cell Implantation for the Treatment of Fecal Incontinence: A Randomized, Placebo-Controlled Study

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BACKGROUND AND AIMS: **Fecal incontinence (FI) improvement following injection of autologous skeletal muscle–derived cells has been previously suggested. This study aimed to test the efficacy and safety of said cells through a multicenter, placebo-controlled study, to determine an appropriate cell dose, and to delineate the target patient population that can most benefit from cell therapy.**

METHODS: **Patients experiencing FI for at least 6 months were randomized to receive a cell-free medium or low or high dose of cells. All patients received pelvic floor electrical stimulation before and after treatment. Incontinence episode frequency (IEF), FI quality of life, FI burden assessed on a visual analog scale, Wexner score, and parameters reflecting anorectal physiological function were all assessed for up to 12 months.**

RESULTS: **Cell therapy improved IEF, FI quality of life, and FI burden, reaching a preset level of statistical significance in IEF change compared with the control treatment. Post hoc exploratory analyses indicated that patients with limited FI duration and high IEF at baseline are most responsive to cells. Effects prevailed or increased in the high cell count group from 6 to 12 months but plateaued or diminished in the low cell count and control groups. Most physiological parameters remained unaltered. No unexpected adverse events were observed.**

CONCLUSIONS: **Injection of a high dose of autologous skeletal muscle–derived cells followed by electrical stimulation significantly improved FI, particularly in patients with limited FI duration and high IEF at baseline, and could become a valuable tool for treatment of FI, subject to confirmatory phase 3 trial(s). (ClinicalTrialRegister.eu; EudraCT Number: 2010-021463-32).**

Keywords: Fecal incontinence; Cell Therapy; Skeletal Muscle–Derived Cells; Myogenic Progenitor Cells; Regenerative Medicine.

Abbreviations used in this paper: **aSMDC, autologous skeletal muscle-derived cell; CI, confidence interval; CTR, control; EAS, external anal sphincter; HCC, high cell count; IEF, incontinence episode frequency; IMP, investigational medicinal product; ITT, intention to treat; LCC, low cell count; PFES, pelvic floor electrical stimulation; QoL, quality of life; TPP1, target population 1; TPP2, target population 2; V, visit; VAS, visual analog scale.**

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Fecal incontinence (FI) is a major burden diminishing quality of life (QoL) for those affected, particularly elderly people and women experiencing anal sphincter disruption. Recently a FI prevalence of 16.1% was reported, whereby 3.3% fulfilled the Rome IV criteria,[1] indicating that 1 in 30 adults is eventually affected. Treatment options include conservative measures such as dietary management and biofeedback therapy, as well as surgical approaches, which in the long term often are associated with complications and the recurrence of FI symptoms.[2] A different approach is the injection of autologous skeletal muscle–derived cells (aSMDCs) into the external anal sphincter (EAS) as a regenerative treatment. The rationale is that aSMDCs injected into or near skeletal muscle fibers will fuse to either form new muscle fibers or fuse with existing muscle fibers, thereby strengthening and ideally repairing the EAS. Long-term efficacy is based on stem cells allowing to repopulate the stem cell niche. Notably, aSMDCs express stem cell markers and exhibit differentiation potential.[3,4] Studies on aSMDCs for FI treatment showed significant improvement in Wexner score and FI-QoL up to 5 years in women with obstetric anal sphincter damage.[5–7] Studies including women and men with FI caused by muscle damage, muscle atrophy, or both resulted in a significant decline of FI symptom following cell injection.[3,8] No significant adverse events (AEs) were associated with this, indicating that the treatment is safe and can thus be considered as a future first-line therapy for the treatment of FI.[9] This present phase 2 clinical study aimed to (1) evaluate safety and efficacy of aSMDC injection in a double-blind randomized placebo-controlled trial; (2) conduct a priori statistical comparison of change in weekly incontinence frequency from baseline to 6 months posttreatment in cell vs placebo-treated patients; (3) determine the suitable cell dose for injection, as previous studies found no diminished effects when applying reduced cell numbers[3]; and (4) analyze secondary endpoints among treatment groups (ie, improvements in Wexner score, visual analog scale [VAS], FI-QoL, etc.) and subgroups to delineate the most appropriate target population.

What You Need to Know

Background: Skeletal muscle–derived cell therapy has been previously suggested for fecal incontinence treatment. However, placebo-controlled clinical trials are rare and are necessary to draw efficacy conclusions.

Findings: Treatment of patients experiencing chronic fecal incontinence with skeletal muscle–derived cells led to significant reduction in incontinence symptoms compared with control treated patients.

Implications for patient care: Subject to confirmatory phase 3 clinical trial(s), skeletal muscle–derived cell therapy could become a valuable treatment option for fecal incontinence.

Materials and Methods

Patients

Male and female patients at least 18 years of age were eligible for this study if they had diagnosed external anal sphincter weakness or damage confirmed through anorectal examination (see Supplementary Methods) and medical history assessment (eg, history of sphincter injury during childbirth), and if they had experienced FI for at least 6 months (Wexner score > 9). Patients had to have experienced at least 3 weekly incontinence episodes (incontinence episode frequency [IEF]) prior to their screening visit assessed via a 2-week incontinence diary. All inclusion and exclusion criteria are provided in Supplementary Table 1.

Treatment Protocol

Eligible patients were randomized to receive either cell therapy with a low dose (low cell count [LCC]: $5 \pm 1 \times 10^6$) or a high dose (high cell count [HCC], $50 \pm 10 \times 10^6$) of cells or were subjected to a control (CTR) treatment with an injection of cell-free medium (CTR). Pelvic floor electrical stimulation therapy is considered the gold standard for conservative treatment of FI, as it is known to stimulate muscle growth[10,11] and induces the production of endocrine signals in targeted muscles.[12] Thereby secreted myokines are hypothesized to modulate aSMDC behavior and improve cell engraftment.[13] Thus, concomitant electrical stimulation (pelvic floor electrical stimulation [PFES]) was included for all study groups. Pre- and posttreatment FI symptoms was recorded by a patient diary. For details on patient diaries, PFES, muscle cell isolation, culture and cell injection see the Supplementary Methods.

Study Endpoints

The primary endpoint was defined as the change in IEF from baseline (visit 0 [V0]) to the 6-month post-injection visit (V4 in Figure 1) between treatment arms (CTR, LCC, HCC). Secondary endpoints were changes in VAS, Wexner score, FI-QoL, responder rate (ie, 50% reduction in IEF compared with baseline), and anorectal physiology up to 12 months posttreatment.

Safety Assessment

Adverse events (AEs) and serious AEs were recorded; physical examinations and standard tests of hematology, blood chemistry, and urinalysis were conducted; and any

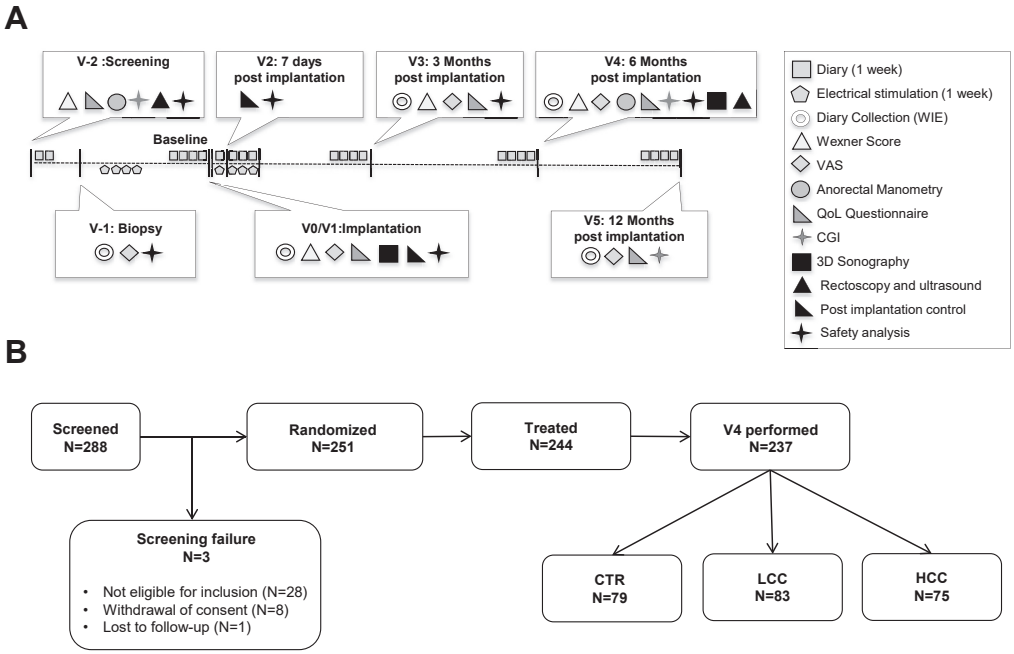

Figure 1. (*A*) Study scheme depicting the timeline of visits and procedures occurring. (*B*) Patient flow from screening to primary endpoint visit depicted. 3D, 3-dimensional; CGI, Clinical Global Impression Index; WIE, weekly incontinence episode.

concomitant medication was registered. The study was overseen by an independent data safety monitoring board. For the definition of AEs, see the Supplementary Methods.

Statistics

A relative effect size of 0.5 for the difference in change of IEF between treatment arms was assumed. Aiming for an alpha level of 2.5% and a power of 80%, at a predicted dropout rate of about 15%–20%, 84 patients per treatment arm were necessary to demonstrate a difference between of treatment arms. This estimate yielded a total of 252 patients to be randomized in a 1:1:1 ratio for the 3 treatment arms. For primary endpoint analysis, treatment arms were compared using a 1-sided Wilcoxon rank sum test considering a P value $<.025$ as significant. Secondary endpoints of continuous variables and post hoc subgroups were compared between study arms by 2-sided unpaired t test (if the compared study arms were normally distributed) or 2-sided Wilcoxon rank sum test (if the compared study arms were not normally distributed), considering a P value $<.05$ as significant. Noncontinuous variables were compared between study arms using a chi-square or Fisher test. A posteriori effect sizes (Cohen's d) were calculated by G*Power 3.1.9.4 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). All authors had access to study data and reviewed and approved the final article.

Results

Patient Demographics

Of the 288 patients screened, 251 were randomized, of whom 244 received CTR or cell (LCC or HCC) treatment. A total of 218 women and 19 men completed the study for at least 6-month follow-up (96%) (Figure 1). Median age of the participants was 63 (interquartile range, 53.8–70) years, experiencing FI for a median period of 5.3 (interquartile range, 2.7–9.9) years. Baseline demographics and clinical characteristics of patients available for primary endpoint analysis are summarized in Table 1. No significant difference in IEF was found between treatment groups at baseline.

Efficacy Outcomes

Incontinence Episode Frequency. Primary endpoint analysis showed a mean change by −3.2 (95% confidence interval [CI], −4.4 to −2.0) in the CTR group and by −4.3 (95% CI, −6.1 to −2.5) and −4.8 (95% CI, −6.4 to −3.2) in the LCC and HCC groups, respectively (Figure 2*A*). Statistical comparison between the HCC and CTR groups indicated a statistically significant difference ($P = .0175$, Cohen's $d = 0.26$). Complying with hierarchical test setup, a comparison between the LCC and CTR groups was conducted but did not achieve significance ($P = .1116$).

Subgroup-Specific Impact. To establish a patient group specifically responsive to cell treatment rather than to CTR treatment, we conducted explorative post hoc analyses. Patients with long FI duration showed less improvement after cell treatments, and thus patients were divided according to an arbitrary chosen beak point for FI duration of 10 years. A possible reason for a declining treatment effect by duration of FI is that for injected cells to restore EAS function, they must be injected into or near existing muscle tissue. This is supported by findings that aSMDCs have limited migratory capacity following implantation,[14,15] as well as by similar

Table 1. Baseline Demographic and Clinical Characteristics of Patients Enrolled in the Study as Assigned to the 3 Treatment Arms

	Placebo (n = 79)	Low Cell Count (n = 83)	High Cell Count (n = 75)	Total (N = 237)
Age, y	59.4 ± 14.8, 63.0 (52.7–71.0)	59.6 ± 13.2, 61.0 (54.0–69.0)	61.4 ± 13.7, 64.0 (54.0–70.5)	60.1 ± 13.9, 63.0 (53.8–70.0)
Sex				
Male	6 (7.6)	8 (9.6)	5 (6.7)	19 (8.0)
Female	73 (92.4)	75 (90.4)	70 (93.3)	218 (92.0)
BMI, kg/m^2	24.7 ± 4.6, 24.2 (20.8–27.5)	25.9 ± 4.4, 25.4 (22.7–28.3)	26.0 ± 4.7, 25.2 (22.7–28.2)	25.6 ± 4.6, 25.1 (22.2–27.9)
Time since first diagnosis, y	7.9 ± 8.6 5.5 (2.5–9.1)	6.7 ± 5.8 4.9 (2.9–8.8)	7.8 ± 7.1 5.3 (2.7–11.1)	7.5 ± 7.2 5.3 (2.7–9.9)
History of FI				
Muscle damage	28 (35.4)	28 (33.7)	23 (30.7)	79 (33.3)
Atrophy	40 (50.6)	40 (48.2)	41 (54.7)	121 (51.1)
Both	11 (13.9)	15 (18.1)	11 (14.7)	37 (15.6)
Baseline IEF	9.4 ± 7.1	10.8 ± 7.9	11.7 ± 10.1	10.6 ± 8.7

Values are mean ± SD, median (interquartile range), or n (%).
BMI, body mass index; FI, fecal incontinence; IEF, incontinence episode frequency.

findings in a rat preclinical model.[16] We assume that muscle regeneration may have been impaired in patients whose muscle tissue was scarce due to persistent scar formation of damaged EAS or because of time-dependent sarcopenia. Both conditions correlate with FI duration prior to the study. A reanalysis of the treatment effect in only those patients experiencing FI for ≤10 years (76% of the intention-to-treat [ITT] set; target population 1 [TPP1]), revealed the decline of IEF in the LCC (mean −4.9; 95% CI, −7.0 to −2.8) and HCC (mean −5.3; 95% CI, −7.3 to −3.3) groups significantly (LCC vs CTR: $P < .05$, $d = 0.27$; HCC vs CTR: $P < .05$, $d = 0.37$) exceeded that in the CTR group (mean −3.0; 95% CI, −4.4 to −1.6), suggesting that cell injection was superior to the CTR treatment in TPP1 than in the ITT patient group (Figure 2*B*).

Another factor diminishing the apparent impact of cell treatment was that IEF also declined in the CTR group. To capture treatment effects, the type of incontinence episodes (traces, little, or more) used for symptom assessment is important. Analyzing data of the ≤10 years of FI patients at 12-month follow-up, we saw the lowest 50% responder rate within each treatment arm in the episodes type "traces" (CTR: 22.2%, LCC: 37.1%, and HCC: 32.1%) as compared with episode types "a little" (CTR: 29.4%, LCC: 38.7%, and HCC: 55.6%) and "more" (CTR: 42.1%, LCC: 45.8%, and HCC: 33.3%). Accordingly, and in agreement with Wexner et al,[17] only TPP1 patients with >2 weekly incontinence episodes of more than traces were analyzed and traces were excluded from the IEF change calculation (target patient population TPP2). In TPP2 patients, the decline in IEF following HCC treatment (mean −6.8; 95% CI, −3.8 to −9.8) surpassed that of the CTR treatment (mean −3.8; 95% CI, −1.0 to −6.6) significantly (HCC vs CTR: $P < .05$, $d = 0.48$) (Figure 2C) at an even higher effect size than in the ITT population and TTP1. In line with the ITT set, no significant difference between the LCC (mean −5.4; 95% CI, −2.4 to −8.4) and CTR treatment was found.

Secondary Endpoints

IEF Time Course Up to 12 Months. Patients willing to participate beyond the V4 primary endpoint were included in 12-month posttreatment analysis up to V5. The 12-month (V5) data revealed a trend of difference between HCC- and CTR-treated patients in the ITT population and significant differences in TPP1 when comparing HCC vs CTR groups ($P < .015$, $d = 0.52$) and LCC vs CTR ($P = .043$, $d = 0.39$) and TPP2 when comparing HCC vs CTR groups ($P = .027$, $d = 0.48$) (Figures 2*A*', 2*B*', 2*C*')

Changes in IEF from baseline were also assessed at 1, 3, and 12 months postimplantation, to study treatment dynamics. In the ITT population and specified subgroups, the mean change in IEF of the CTR treatment diminished over time, whereas it remained constant or even increased in HCC-treated patients, most pronouncedly in the TPP2 subgroup. A plot showing the entire time course of mean changes in IEF is demonstrated in Figure 3. Descriptive statistics are summarized in Supplementary Table 2.

Responder Rates. In agreement with others,[18] we considered a reduction of IEF by ≥50% to be a clinically relevant improvement and classified patients showing at least this extent of reduction as responders. Accordingly, we evaluated our data regarding the proportion of

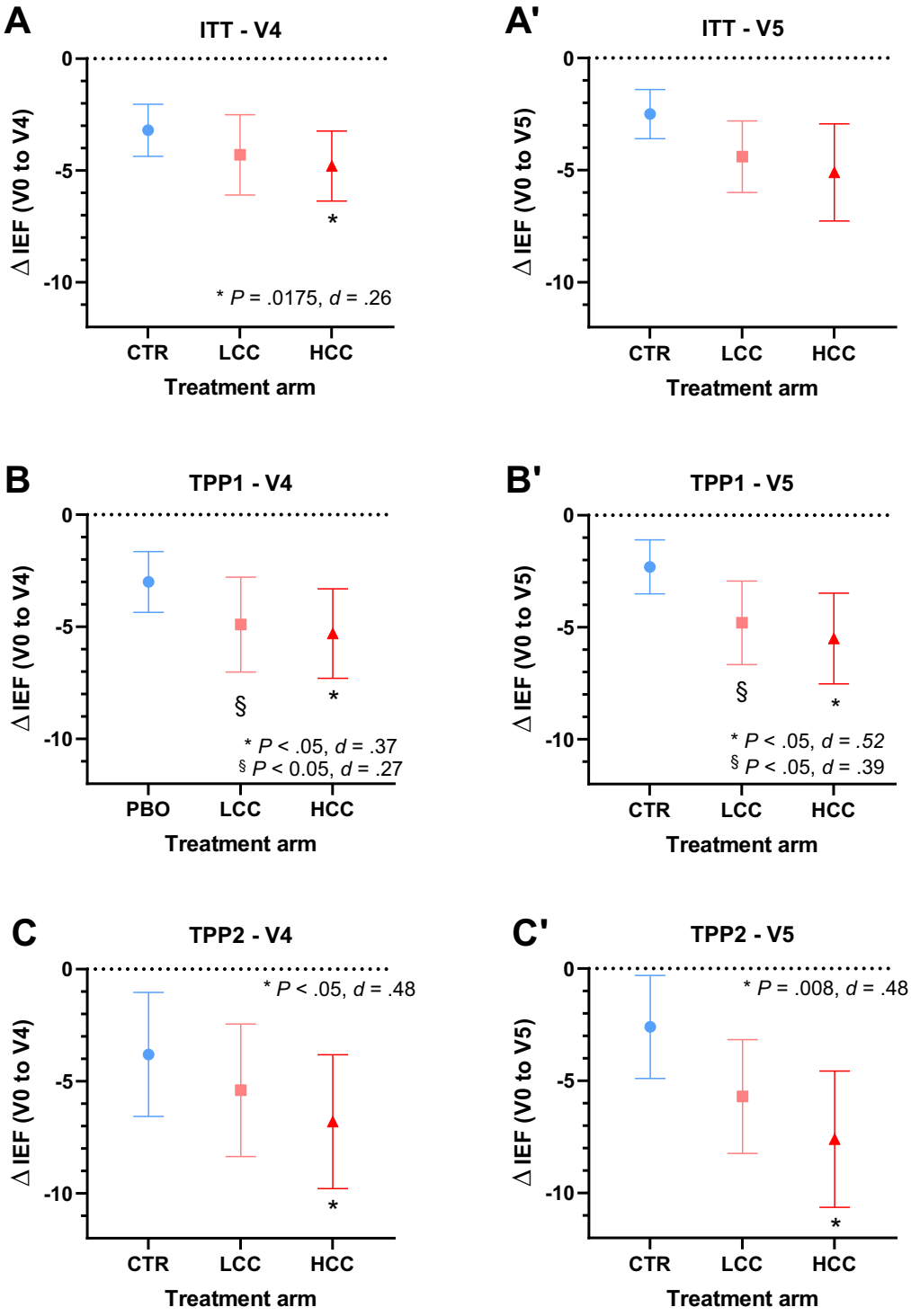

Figure 2. Absolute change in weekly IEF from baseline (V0) to (A–C) 6 months (V4) and (A'–C') 12 months (V5) in the ITT group and subgroups (TPP1, TPP2). TPP1 comprised patients of the ITT group that had duration of FI of ≤10 years. TPP2 includes only patients of ≤10 years' FI duration and >2 baseline IEF (excluding traces), wherein traces were not calculated for IEF changes. Data presented as mean ± 95% CI. Significant P values and corresponding effect sizes (Cohen's d) are depicted. Last-observation-carried-forward imputation for both V4 and V5 data was performed.

responders in each treatment arm, including data from 1 and 3 months postinjection, and revisiting subgroups as specified previously. In all groups and subpopulations defined, the proportion of responders continuously increased up to the 6-month follow-up, with the cell groups exceeding the CTR group in all cases, and with HCC exceeding LCC in all cases (Figure 4). Between 6 and 12 months, the responder proportion of the CTR and LCC groups either plateaued or even declined, whereas it continued to increase up 54.1 in the HCC group of the ITT population. The HCC group of TPP2 reached the highest responder rate of 72.2% at 12 months post-treatment. Although not statistically significant between groups in the ITT population, a trend toward a higher response rate was observed in favor of the HCC group at each time point. In accordance with the efficacy analysis on IEF reduction, the most pronounced differences between treatment arms were observed in subgroups, yielding significant improvements in HCC-treated ($P = .018$) TPP1 patients, as well as in HCC ($P = .008$) and LCC-treated ($P = .028$) patients of TPP2 each compared with CTR-treated patients. Descriptive analyses of the

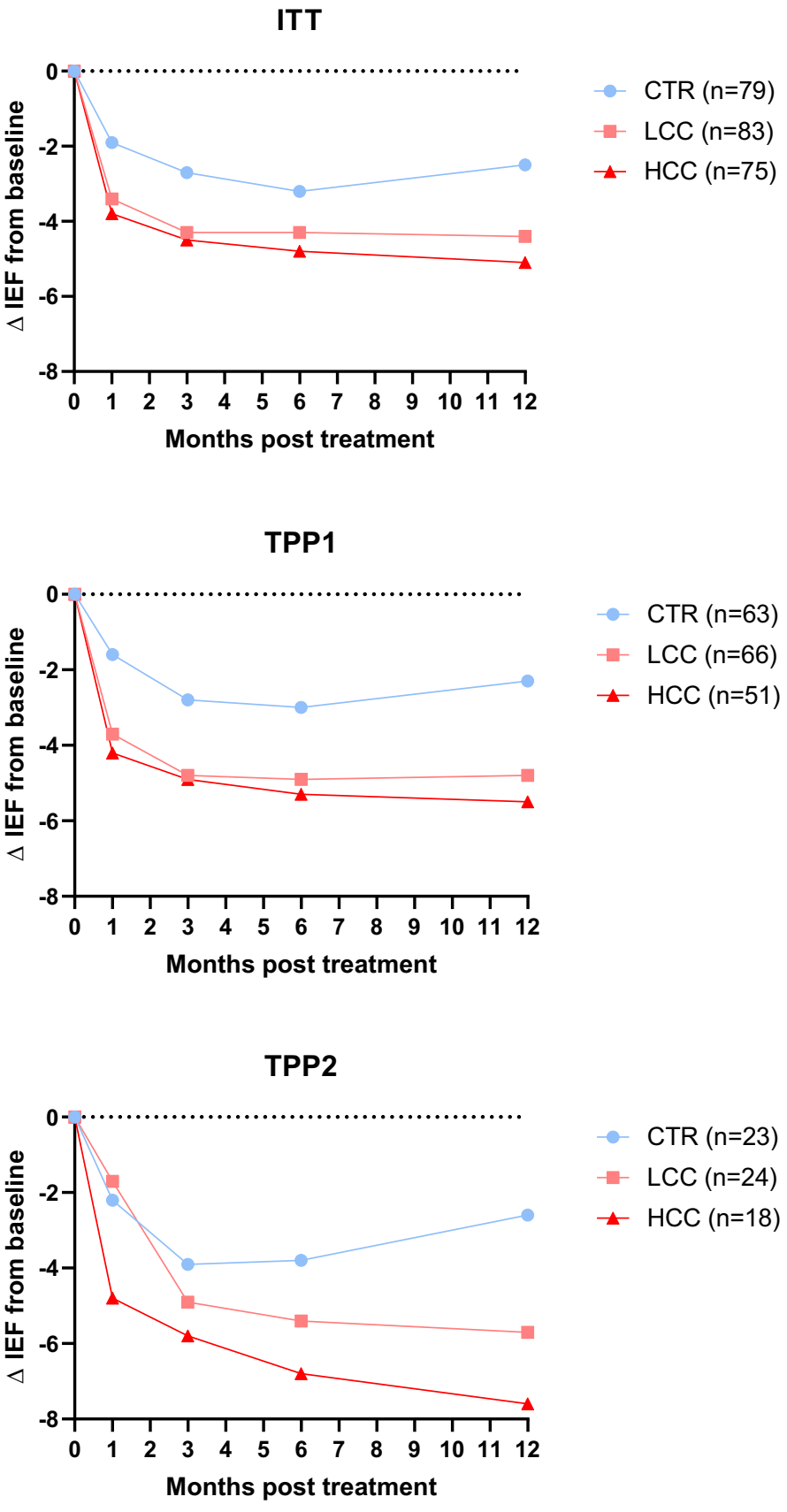

Figure 3. Time course of absolute change in weekly IEF from baseline (V0) to visit at 1, 3, 6, and 12 months posttreatment in ITT population and subgroups (TPP1, TPP2). Data are visualized as mean values. Last-observation-carried-forward imputation was conducted for 6- and 12-month data.

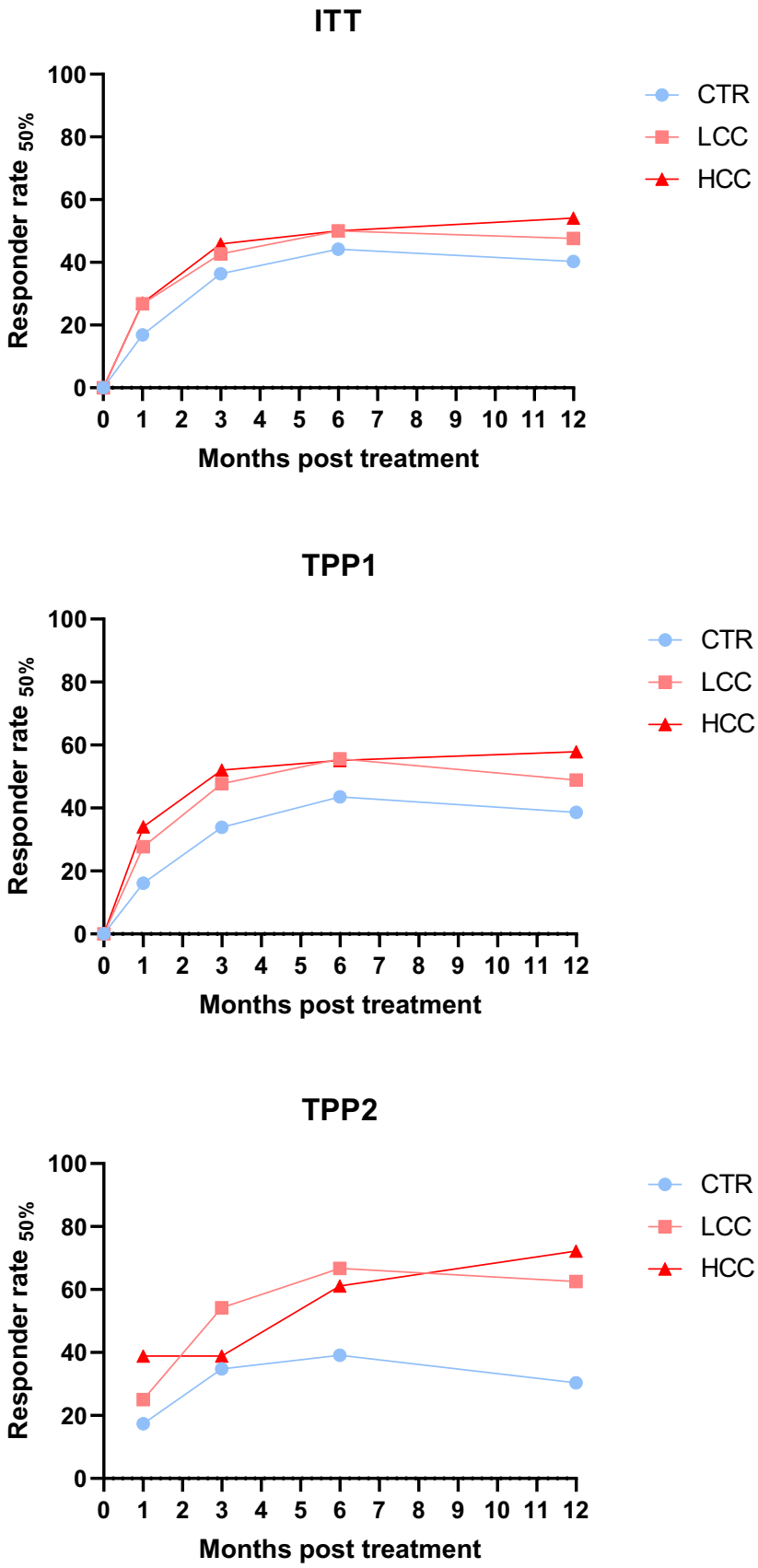

Figure 4. Time course of the ITT, TPP1, and TPP2 patient proportions having at least 50% reduction in IEF from baseline to 1-, 3-, 6-, and 12-month posttreatment study visits.

responder rates are summarized in Supplementary Table 3.

Further Endpoints. The VAS, used to assess everyday burden of FI for patients, showed a consistent decrease over the first 3 months in all treatment arms of ITT and subgroup patients but leveled off or even increased at 6 and 12 months in the CTR and LCC groups (Figure 5). In contrast, it continued to decline in the HCC group, resulting in significant improvements compared with the CTR group in TPP1 subgroup patients at 6 months ($P = .033$) and TPP2 subgroup patients at 6 months ($P = .006$) and 12 months ($P = .016$) postimplantation. Interestingly, TPP2 subgroup patients perceived significantly higher reduction in VAS following HCC compared with LCC implantation at 12 months posttreatment ($P = .039$) (Supplementary Table 4).

Wexner score analysis (Supplementary Table 7) and FI-QoL assessment (Supplementary Table 6 and Supplementary Figure 3) demonstrated significant treatment effects in TPP2 population. Anorectal physiology did not reveal any significant differences between study arms (Supplementary Figures 1 and 2).

Safety Outcomes

A total of 288 patients were included in the study. Of these, 251 underwent biopsy. Within this population, of 32 patients in total, 48 AEs (CTR: 18, LCC: 18, HCC: 12) related to study treatments (biopsy, PFES, investigational medicinal product [IMP], implantation) were recorded. Among these 48 AEs, 2 (both in the HCC group) were classified as serious AEs (pyelonephritis related to PFES, postoperative wound infection related to biopsy). In relation to study procedures: 1 AE was related to IMP, 3 AEs were related to biopsy, 25 AEs were related to implantation, 4 AEs were related to PFES, 1 AE was related to IMP or PFES, 8 AEs were related to implantation or PFES, 3 AEs were related to implantation or IMP or PFES, and 1 AE was related to IMP or implantation. A summary of treatment-emergent AEs with an incidence of at least 5% is given in Supplementary Table 5.

Discussion

In line with suggestions of several previous investigations,[3,5,7,8] the current study confirmed that aSMDC injection causes beneficial alterations in FI symptoms. Observed alterations were clearly meaningful and statistically significant compared between HCC and placebo CTR groups for the primary endpoint (IEF change from baseline to 6 months posttreatment). Also, placebo group patients significantly improved in IEF throughout the study duration, in line with placebo effects reported by Boyer et al.[7] Regenerative processes may have been triggered by the minor tissue injury ensuing from needle incision[19] in combination with the electric stimulation performed. Indeed, electrical stimulation may as such have had a beneficial impact of IEF,[20] possibly through stimulating myokine-regulated hypertrophy and angiogenesis,[21] and we believe that electrical stimulation must be included as an integral part of the treatment when ultimately a medicinal product is conceived. Thus, the CTR treatment applied here must be considered an "active control" and not a mere placebo. However, because the electrical stimulation treatment was applied to all patient groups, any improvement beyond that achieved by electrical stimulation will clearly document the benefits of the cell injection. In agreement with this notion, cell treatment exerted more pronounced beneficial effects in the ITT population not only regarding IEF, but also in terms of responder rate and other parameters considered clinically meaningful. To unravel which patients could profit most from cell therapy and thus represent the putative target population, we conducted explorative post hoc analyses driven by our hypotheses as to the mechanistic basis of the therapeutic efficacy of cell injection. In a subgroup analysis, an arbitrary limit for the duration of FI was set at 10 years. One hypothesized mode of action of aSMDCs for muscle regeneration in, eg, FI, is that intramuscular-injected myogenic progenitor cells fuse with existing myofibers to increase muscle functionality.[22,23] Increased fat and or fibrotic tissue occurrence within the EAS muscle might be detrimental for aSMDC efficacy, and because it is known that increasing age and skeletal muscle immobility (eg, owing to dysfunction/weakness) can cause time-dependent fibrosis (ie, loss of muscle tissue and conversion/infiltration of connective tissue).[24–26] According to this hypothesis, increased FI duration is detrimental to the efficacy of our proposed treatment.

In addition, we omitted patients with IEF 2 or less (excluding traces) at baseline, as benefits resulting from restoration of EAS function should be more pronounced when being previously more affected. In fact, in both selected subpopulations, IEF changes turned out to significantly exceed those in CTR patients. The best effects were observed when traces were excluded. This did not only apply to IEF, as a similar pattern of more pronounced responses in subgroups was also noted for the response rate and changes in VAS and Wexner scores. We also investigated how the type of incontinence episode might have affected the results. We hypothesize that incontinence episodes classified as "traces" should be excluded from analyses of therapies aiming to restore EAS function, as traces could be caused by an internal anal sphincter defect. Indeed, we saw major reductions in IEF of episodes classified as "more" and "a little" and much less reduction in episodes deemed traces. HCC almost uniformly produced better results than LCC. While improvements seen in CTR subjects and with LCC mostly leveled off or even declined between 6 and 12 months, they tended to persist and, in most cases, increased in the HCC group. In cell therapy, the optimal dose is more dependent on the capacity of cell acceptance and survival in the target tissue than on the number of injected cells.[27–29] However, considering that limited muscle tissue is available for interaction and fusion, chances for an interaction will increase upon

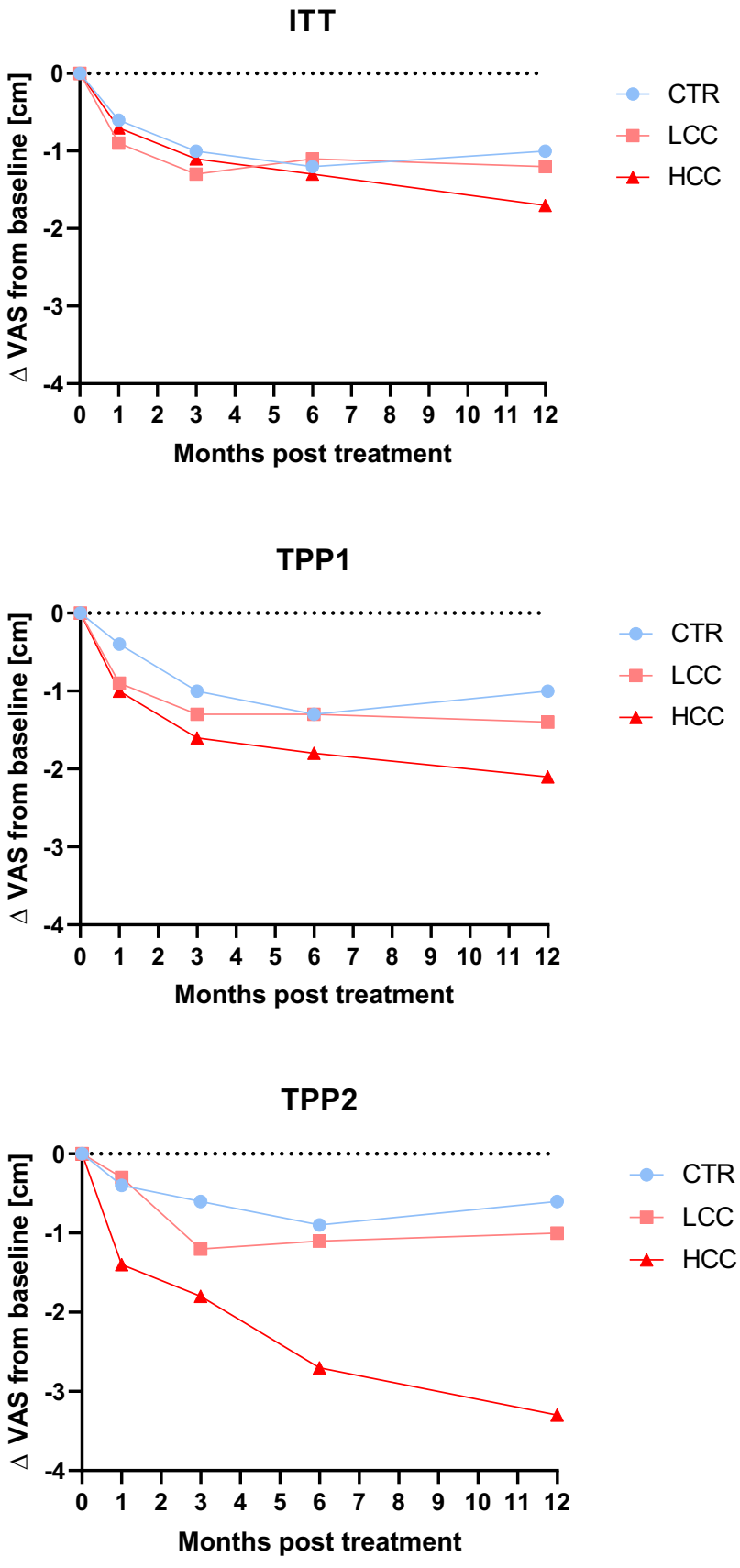

Figure 5. Time course of the mean VAS over each period by treatment arm in ITT, TPP1, and TPP2 patients at 1-, 3-, 6-, and 12-month posttreatment study visits. Data are visualized as mean. Last-observation-carried-forward imputation was performed for 6- and 12-month posttreatment study visit data.

injection of a higher cell dose. The present data favor a threshold cell dose as opposed to a dose-response relationship. Compared with previous clinical trials using muscle-derived cells for treatment of FI, the applied HCC cell dose of 40–60 $\times$ 10^6 is lower ($80 \pm 30 \times 10^3$,$121 \pm 12 \times 10^6$, $249 \pm 68 \times 10^6$ and $100 \pm 20 \times 10^6$).[3,5,7,8] However, as the cell number applied here could be produced from all patients with acceptable quality, this prompts us to continue to utilize HCC in future clinical studies and settles the dose-finding aspect of our trial.

The absence of serious cell injection–related adverse events that might have raised safety concerns is fully congruent with preceding studies.[3,5–7]

In conclusion, the HCC dose caused significant and persistent improvements at 6 months postinjection compared with CTR treatment, continuing up to at least 12 months. Effects even increased in subpopulations, demonstrating significant improvements in responder rate, Wexner score, FI-QoL, and VAS. These beneficial alterations were accompanied by a trend for improvement of maximal squeeze pressure in HCC and LCC compared with CTR treatment, most pronounced within subpopulations.

Our main findings encompass the proof of aSMDC efficacy and identification of a suitable target group and adequate cell numbers. Further patient selection criteria might be baseline manometric values and morphology of the EAS and internal anal sphincter; however, this was not within the scope of the present study. Bearing all discussed adjustments in mind, the present treatment approach has good prospects of becoming a valuable tool for treatment of a FI subject following confirmatory phase 3 trial(s) required for market approval. Future developments may become more personalized, taking the individual morphology of EAS and muscle volume into account and adjusting cell dose accordingly, providing an individualized and optimized patient outcome.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2022.07.039.

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Study Approval
The authors acknowledge the support of the International Clinical Trials Association (Dijon, France) as well as its national subcontractors for data management, statistical analysis, and operative clinical trial management. In addition, they thank Gerhard Krumschnabel for manuscript writing assistance. National authorities in Austria (BASG), Germany (PEI), Czech Republic (SUKL), the United Kingdom (MHRA), Bulgaria (Bulgarian Drug Agency), Sweden (MPA), Switzerland (Swiss Medic), and Slovenia (JAZMP) authorized the clinical trial, which was registered within the European Union Clinical Trial Register (EudraCT Number: 2010-021463-32). The study protocol was approved by at least 1 ethics committee per participating country, namely the Ethikkomission der Medizinischen Universität Graz (23-099 ex 10/11) in Austria, the Medizinische Ethik-Komission II der Medizinischen Fakultät Mannheim in Germany, the Regional Ethics Committee Stockholm in Sweden, the Central Ethics Committee of the Ministry of Health of the Czech Republic in the Czech Republic, the National Research Ethics Service NRES Committee London – Chelsea in the United Kingdom, the Republic of Slovenia National Medical Ethics Committee in Slovenia, the Комисията По етика за многоцентрови изпитвания in Bulgaria, the Comité de Protection des Personnes – CPP Sud Méditerranée IV in France, and the Kantonale Ethikkomission Zürich in Switzerland.

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Conflicts of Interest
These authors disclose the following: Melanie Amort, Rainer Marksteiner, and Marco Thurner are employees of the former Innovacell Biotechnologie AG, which recently merged with Innovacell AG. The remaining authors disclose no conflicts.

Supplementary Methods

Patient Diary

Patients kept an incontinence diary recording the date and time of each bowel movement, daily episodes of incontinence to stool, and urgency. In addition, they marked the severity of the perceived fecal incontinence (FI) burden on a visual analog scale and rated the amount of stool lost as "traces," "a little," or "more." Following screening, patients were required to complete an incontinence diary for 2 weeks before a muscle biopsy sample was taken from the musculus pectoralis major. Subsequently, a 4-week period of electrical stimulation treatment was initiated. After another 4 weeks of keeping a diary, patients received a cell or control injection, and underwent a postimplantation control on the following day. Patients received yet another 4-week period of electrical stimulation treatment paralleled by diary keeping. At 3, 5, and 12 months postinjection, patients attended control visits, which were all preceded by 4 weeks of diary completion (for details, see study protocol). Patients were reported a daily score on how FI affected their day, utilizing a visual analog scale, and kept track of FI episodes, classified into 3 categories: "traces," "a little" and "more." Further parameters were outlined in the scheme and included assessment of responder rates, anorectal manometry, and ultrasound measurements, and a FI quality-of-life (QoL) questionnaire.

Diagnostic Procedures

Anal endoscopy, ultrasound examination, and anorectal manometry measurement were performed according to standards set in each of the participating centers.

Anal Endosonography. Anal endosonography was performed in all patients. Investigators were experienced and trained in the technique. A B-K Medical Scanner Type Flexfocus, fitted with an 8838 high resolution endoanal probe was used (B-K Medical, Herlev, Denmark). Patients were examined in the supine position using standard technique. A 3-dimensional dataset that encompassed the entire anal canal length was obtained and discussed among participating investigators.

Anal Manometry. Parameters assessed included length of the anal canal, resting pressure, and maximal squeeze pressure. Balloon expulsion tests were used to measure the filling volume until the first sensation was reached, the volume for the desire to defecate, the volume of urgency for defecation, and the maximal tolerable volume. A Medspira mCompass Anorectal Manometry System (Medspira, Minneapolis, MN) was used to assess this data.

Pelvic Floor Electrical Stimulation

Electrical stimulation was self-administered by the patient in accordance with the manufacturer's instructions, using the CE-marked electrical stimulation device "contic" (tic Medizintechnik, Dorsten, Germany) provided by the sponsor for the course of the study. The device was delivered together with the current version of its operation manual and equipment as required for FI treatment (eg, rectal electrode, carrying case). Patients were instructed by authorized and trained hospital staff in the correct usage of the device. Two 4-week sessions of pelvic floor electrical stimulation were to be performed and were recorded by the device. The first session started 2 weeks after the biopsy visit (ie, 2 weeks after visit 1 [V1]) and the second started immediately after cell implantation (ie, 4 weeks after V0). The exact overall treatment regime is outlined in Figure 1.

The electrical stimulation sessions were recorded on the device, and the patients on average used the device for 24 days in all treatment groups after biopsy. Directly after the first electrical stimulation session, the patients completed a diary for 4 weeks, which was defined as baseline diary. Applying this procedure any impact of the electrical stimulation could be excluded from the efficacy evaluation.

Cell Preparation and Culture

A skeletal muscle biopsy was taken from the musculus pectoralis major of a given patient and mechanically separated into the muscle tissue and connective tissue. The muscle tissue was disintegrated, and muscle cells were then expanded for approximately 4 weeks in culture medium supplemented with fetal calf serum and basic fibroblast growth factor. Identity and purity of the harvested cells was assessed by flow cytometry using anti-CD56, anti-CD34, anti-CD90, anti-CD105, and anti-CD73 antibodies, the use of which is well described in the literature [4]. CD56 is a marker protein that is expressed on cultivated satellite cells [5]. Innovacell's experimental medicinal product must consist of 80 $\pm$ 20% CD56-positive autologous skeletal muscle-derived cells (aSMDCs). After adjusting cell counts and subsequent resuspension in 3 mL cell transportation medium (Ringer's lactate solution containing 2% human serum albumin and 5% dimethyl sulfoxide) to derive the final formulation of ICEF15, the investigational medicinal product were frozen in glass vials. Frozen glass vials were stored at $<-130^{\circ}C$ in the vapor phase of liquid nitrogen until use. ICEF15 must only be thawed immediately before injection in the patients' target tissues. Accordingly, cells were thawed by a standardized procedure via adding 3 mL Ringer's lactate solution at room temperature to give a total of 6 mL reconstituted ICEF15 ready for injection. The vitality of aSMDCs in reconstituted ICEF15 was validated and was found to be 90 $\pm$ 10%.

Patient Preparation and Myoblast Injection

aSMDC implantation was conducted in anaesthetized patients, as described previously.[1] Patients were placed

in a supine position and aSMDCs were injected under direct ultrasound control using a specifically designed injection device (U.S. Patent No. 9,333,307). Frozen aSMDCs were thawed at the implantation site, and each patient received an aliquot of their own cells diluted to the appropriate cell concentration with Ringer's lactate. The resulting total volume of 6 mL was administered in 12 depots (12 × 0.5 mL), each of which was extended in a circular array directly into the external anal sphincter. Injection of cells into the longitudinal muscle, internal anal sphincter, or subepithelium was avoided. All patients were hospitalized for 1 day for the procedure.

Safety Assessment

An adverse event (AE) was defined as any untoward medical occurrence in a patient or clinical study subject who had been administered a study medication (cells or control) and that did not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease whether considered related to the investigational medicinal product or not. AEs can be drug reactions, accidents, illnesses with onset during the study, exacerbation (increase in severity or frequency of existing symptoms or appearance of a new symptom) of preexisting illnesses, new illness or change in existing illness requiring new drug therapy, and any clinically significant changes from baseline detected concerning physical examination, vital signs, electrocardiography, or laboratory values.

Dose Selection

For the administration of study medication into the external anal sphincter, a 21G puncture needle was inserted into the external anal sphincter layer at a depth of 4.5 cm, and the medication was administered while retracting the needle by 3 cm. Skuk et al[2] examined the conditions for higher survival of transplanted LacZ-transfected myoblasts prepared from allogeneic monkeys into the muscles of cynomolgus monkeys and found that a dose of 10^5 to 10^6 cells per cm depth was sufficient to reach a plateau in engraftment. Applying these results to the present study in which 12 doses were applied at a depth of each 3 cm, a range of about 4×10^6 to 4×10^7 cells would be sufficient to reach the plateau of engraftable cells. Thus, low cell count (LCC) and high cell count (HCC) doses of 4–6 × 10^5 and 4–6 × 10^7 cells were selected for the present study.

Supplementary Results

QoL Assessment

FI-QoL scores were improved in all groups at the 6-month follow-up with significantly greater improvement in total scores ($P = .023$) as well as coping/behavior ($P = .016$) and embarrassment ($P = .010$) subscale scores in HCC compared with control subjects within the target population 2 (TPP2) population (Figure 6 and Supplementary Table 6). Total score differed significantly between HCC and control subjects in the TPP2 population in 12-month follow-up data ($P = .012$) (data not shown).

Anorectal Physiology

Manometric and sonographic assessment in alterations of the length of the anal canal and resting pressure indicated no significant changes from baseline to 6 months within cell groups. Within the control groups, a marked reduction in resting pressure was detected from baseline to 6 months follow-up, reaching a significant difference between the control and LCC group ($P = .009$ in intention-to-treat set). Within the TPP2 population, a significant increase in maximal squeeze pressure was observed following LCC implantation; however, no significant difference between the treatment groups could be observed (Supplementary Figure 1). Further, we noted significant enhancements in volumes to reach first sensation, desire to defecate, urgency for defecation, and maximal tolerable volume in all study arms of intention-to-treat set but no difference between the cell count and control groups (Supplementary Figure 2).

Wexner Score

Wexner scores were improving in all treatment groups at 3- and 6-month follow-up, reaching significance between HCC and control subjects in TPP2 population at 3 months ($P = .024$) and 6 months ($P = .037$) postimplantation (Supplementary Table 7).

Subgroup-Specific Analyses

The main subgroups investigated in our post hoc analyses are shown and discussed in the main text. In addition, other approaches were applied to isolate the most responsive patient population for the cell injection treatment.

Exclusion of Incontinence Episodes Classified as Traces. For this approach, we adopted the criteria for assessment of incontinence episode frequency and inclusion of patients from a comparable study investigating the efficacy of a sacral nerve stimulation device.[3] As a result, we excluded FI episodes categorized as traces, as these are often thought to be caused by dysfunction of the internal anal sphincter muscle. In addition, we included only patients with >2 incontinence episodes on average per week of more than staining.

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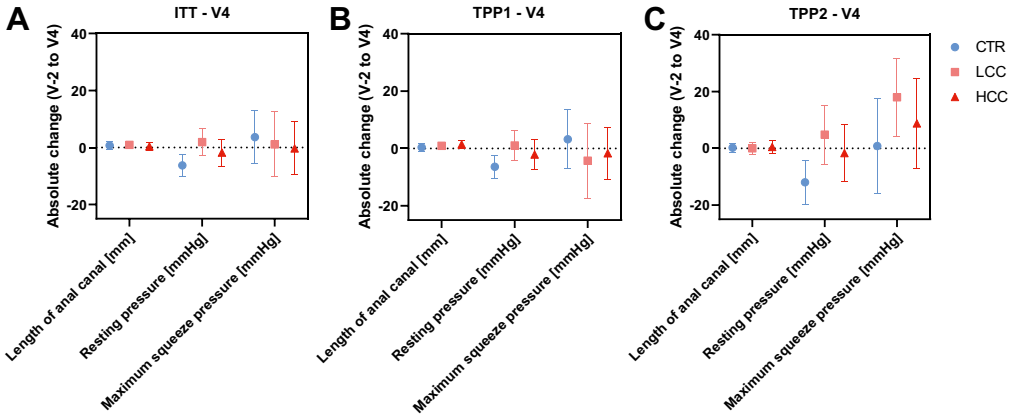
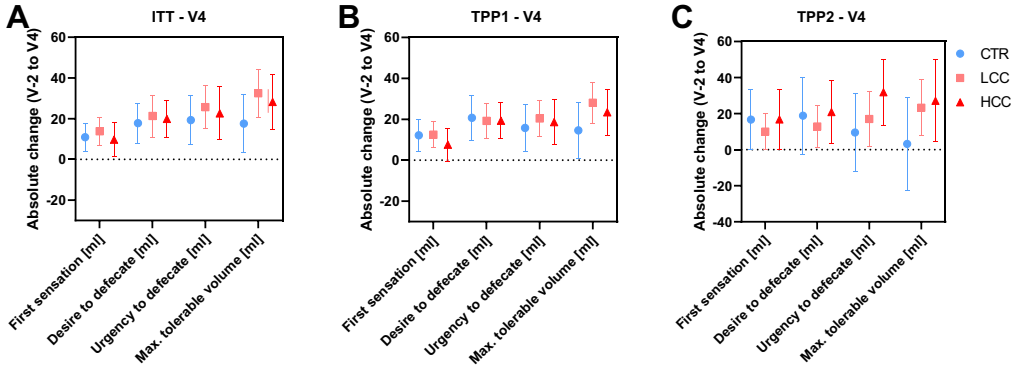
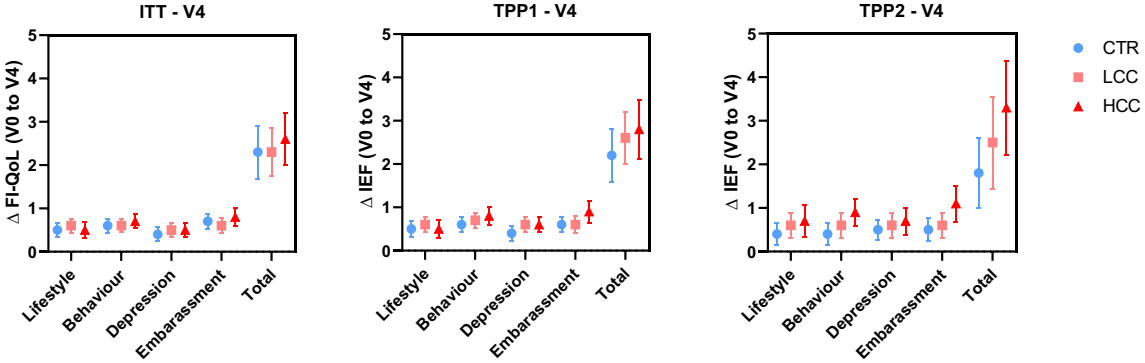


Supplementary Figure 1. Mean absolute change from baseline to 6 months in (*A*) length of anal canal, (*B*) resting pressure, and (*C*) maximal squeeze pressure in control (CTR), low cell count (LCC), and high cell count (HCC) groups. Values are mean and 95% confidence interval. ITT, intention to treat; TPP1, target population 1; TPP2, target population 2.



Supplementary Figure 2. Mean absolute change from baseline to 6 months in (*A*) volume for first sensation reached, (*B*) volume for desire to defecate, (*C*) volume for urgency for defecation, and (*D*) maximal tolerable volume in control (CTR), low cell count (LCC), and high cell count (HCC) groups. Values are mean and 95% confidence interval. ITT, intention to treat; TPP1, target population 1; TPP2, target population 2.



Supplementary Figure 3. Absolute change in fecal incontinence quality-of-life subtotal and total scores by treatment arm in the intention-to-treat (ITT), target population 1 (TPP1), and TPP2 patients from baseline to 6 months posttreatment study visit. Data visualized as mean and 95% confidence interval. CTR, control; HCC, high cell count; LCC, low cell count.

Supplementary Table 1. Criteria for Patient Inclusion and Exclusion

Principal Inclusion Criteria
1. Patients of ≥18 years of age
2. Patients experiencing fecal incontinence for more than 6 months, which is confirmed at screening by relevant medical history and anorectal examination
3. Patients with Wexner score >9
4. Patients with no indications against a surgery under anesthesia
5. Patients willing and able to comply with the study procedures
6. Patients who are mentally competent and able to understand all study requirements
7. Patients must agree to read and sign the informed consent form prior to any study-related procedures
8. Female patients of childbearing potential willing to use acceptable methods of contraception (birth control pills, barriers, or abstinence)
Interim Inclusion Criterion
Patients with a minimum of 3 incontinence episodes per week measured at visit 1 (diary distributed at screening visit)
Principal Exclusion Criteria
1. Patients with pathological findings (excluding sphincter damage) based on rectoscopy and ultrasound at the screening visit
2. Patients who have undergone any anorectal surgery within the last 6 months prior to screening visit
3. Patients with maximal 1 overlap repair in total
4. Patients with more than 2 anorectal surgical procedures (but maximal 1 overlap repair in total), such as the following: Primary repair after delivery and 1 overlap repair later on Implantation and explantation of a permanent neurostimulation system
5. Patients with overlap repair and associated early atrophy of external anal sphincter
6. Patients with a history of artificial anal sphincter surgery
7. Patients with trans- or perianal injection of any bulking products
8. Patients with a malignant disease not in remission for 5 years or more
9. Patients who had undergone radiation therapy
10. Patients who had undergone chemotherapy
11. Patients with compromised immune system and/or rheumatic disease
12. Patients under immunosuppressive therapy
13. Patients with a diagnosis of chronic inflammatory bowel disease
14. Patients with recurrent anal fistula disease
15. Patients with chronic diarrhea
16. Patients experiencing a disease that has not resolved within a time frame prior to screening as follows: fever and/or diarrhea of unknown reasons (4 weeks), HAV (4 months), toxoplasmosis (6 months), osteomyelitis, Q fever, rheumatic fever, tuberculosis, or *Salmonella* infections (2 years), and malaria (4 years)
17. Patients who, according to the clinical judgment of the investigator, are not suitable for inclusion due to acute anal sphincter injury including obstetric and other trauma, acute disc prolapse, or neurological diseases (spinal cord injury, multiple sclerosis, Parkinson's disease, stroke, etc.)
18. Patients with uncontrolled diabetes mellitus type 1 or 2, or experiencing diabetic peripheral neuropathic pain
19. Patients diagnosed with human immunodeficiency virus, acute or chronic viral hepatitis HCV, acute or chronic viral hepatitis HBV, active syphilis, HTLV (tested upon risk assessment by investigator)

Supplementary Table 1. Continued

20. Patients diagnosed with any kind of skeletal muscle disease and/or neuronal disorders
21. Patients with known hypersensitivity to any component of the product (autologous cells, ringer's lactate, human serum albumin, DMSO, bovine proteins, fibroblast growth factor)
22. Patients with clinically relevant abnormal laboratory values, any persistent chronic bacterial infections as well as local infections as indicated by a high level of the C-reactive protein of >35 mg/L and confirmed by bacteriological analysis, or with any bleeding disorder
23. Patients who, according to the clinical judgment of the investigator, are not suitable for this study
24. Patients who are currently participating or have participated in another clinical trial (testing a medical device or drug) within 30 days prior to the study begin or have previously participated in the current clinical study
25. Patients who are pregnant, lactating, or intending pregnancy in the near future, and those of childbearing potential who are not willing to use acceptable methods of contraception (birth control pills, barriers, or abstinence) or who have a positive pregnancy test (only to be performed in women of childbearing potential)
26. Patients dependent from the sponsor, CRO, or the investigator (eg, employees, relatives, etc.)
27. Patients deprived of their liberty by a judicial or administrative decision, patients admitted to a hospital, social institution or who are under a measure of legal protection, patients hospitalized without consent or who are in an emergency situation
28. Patients with severe myocardial disorders, irregular pulse, or a pacemaker
29. Patients with implantations of metal components in the electrical stimulation treatment area

Interim Exclusion Criterion

Patients with persistent bacterial infections confirmed by clinical signs and positive results in bacteriological testing at visit 1 (*Salmonella* [typhus], *Francisella tularensis* [tularemia], *Mycobacterium leprae* [leprosy], *Brucella*, *Rickettsia*)

CRO, contract research organization; DMSO, dimethyl sulfoxide; HAV, hepatitis A virus; HBV, hepatitis B virus, HCV, hepatitis C virus; HTLV, human T-lymphotropic virus.

Supplementary Table 2. Changes in IEF From Baseline Up to 12 Months Posttreatment

	CTR			LCC			HCC		
Months Posttreatment	Mean	SD	n	Mean	SD	n	Mean	SD	n
ITT									
1	–1.9	3.8	79	–3.4	6.0	83	–3.8	6.9	75
3	–2.7	4.8	79	–4.3	7.3	83	–4.5	6.9	75
6	–3.2	5.2	79	–4.3	8.2	83	–4.8	6.8	75
12	–2.5	4.9	79	–4.4	7.3	83	–5.1	9.4	75
TPP1									
1	–1.6	3.8	63	–3.7	6.3	66	–4.2	7.5	51
3	–2.8	4.4	63	–4.8	7.6	66	–4.9	7.0	51
6	–3.0	5.4	63	–4.9	8.6	66	–5.3	7.1	51
12	–2.3	4.8	63	–4.8	7.6	66	–5.5	7.2	51
TPP2									
1	–2.2	3.3	23	–1.7	5.6	24	–4.8	7.4	18
3	–3.9	7.5	23	–4.9	6.6	24	–5.8	6.0	18
6	–3.8	6.4	23	–5.4	7.0	24	–6.8	6.0	18
12	–2.6	5.3	23	–5.7	6.0	24	–7.6	6.1	18

Data are summarized by mean change in IEF from baseline to 1, 3, 6, and 12 months in patients of the ITT set, patients with duration of FI ≤10 years since diagnosis (TPP1), and patients with duration of FI ≤10 years since diagnosis having more than 2 weekly IEF at baseline (excluding traces), whereby traces were not calculated when calculating IEF changes (TPP2). Last-observation-carried-forward imputation was applied for posttreatment visits V4 (6 months) and V5 (12 months).

CTR, control; FI, fecal incontinence; HCC, high cell count; IEF, incontinence episode frequency; ITT, intention to treat; LCC, low cell count; TPP1, target population 1; TPP2, target population 2.

Supplementary Table 3. Comparison of 50% Response Rate Over Time

	CTR		LCC		HCC	
Months Posttreatment	Responder Rate (%)	n	Responder Rate (%)	n	Responder Rate (%)	n
ITT						
1	16.9	79	26.8	83	27.0	75
3	36.4	79	42.7	83	45.9	75
6	44.2	79	50.0	83	50.0	75
12	40.3	79	47.6	83	54.1	75
TPP1						
1	16.1	79	27.7	83	34.0	75
3	33.9	79	47.7	83	52.0	75
6	43.5	79	55.6	83	55.1	75
12	38.6	79	48.9	83	57.9	75
TPP2						
1	17.4	23	25.0	24	38.9	18
3	34.8	23	54.2	24	38.9	18
6	39.1	23	66.7	24	61.1	18
12	30.4	23	62.5	24	72.2	18

Data are summarized by responder rate of total patients by treatment groups (CTR, LCC, and HCC) at 1, 3, 6, and 12 months in patients of the ITT set, patients of the low duration of FI ($\leq$10 years since diagnosis, TPP1), patients with low duration and IEF at baseline $>$2 (excluding traces), whereby traces were not considered as incontinence episode for calculations (TPP2). Last-observation-carried-forward imputation was performed for 6- and 12-month data calculation.
CTR, control; FI, fecal incontinence; HCC, high cell count; IEF, incontinence episode frequency; ITT, intention to treat; LCC, low cell count; TPP1, target population 1; TPP2, target population 2.

Supplementary Table 4. Change in Visual Analog Scale Over Time

	CTR			LCC			HCC		
Months Posttreatment	Mean	SD	n	Mean	SD	n	Mean	SD	n
ITT									
1	–0.6	1.2	76	–0.9	1.8	76	–0.7	1.7	63
3	–1.0	1.7	79	–1.3	2.1	83	–1.1	2.1	75
6	–1.2	2	74	–1.1	2.3	76	–1.3	2.2	64
12	–1.0	1.9	63	–1.2	2.6	67	–1.7	2.6	54
TPP1									
1	–0.4	1.1	61	–0.9	1.8	60	–1.0	1.8	43
3	–1.0	1.8	60	–1.3	2.3	60	–1.6	2.2	42
6	–1.3	2.1	60	–1.3	2.4	61	–1.8	2.2	44
12	–1.0	1.9	52	–1.4	2.8	55	–2.1	2.8	36
TPP2									
1	–0.4	1.1	22	–0.3	1.6	20	–1.4	1.8	15
3	–0.6	1.4	22	–1.2	2.5	20	–1.8	2.1	15
6	–0.9	1.7	22	–1.1	2.6	21	–2.7	2.1	16
12	–0.6	1.7	21	–1	3.1	19	–3.3	2.7	13

Time course of absolute changes in mean visual analog scale (calculated over each diary period) from baseline to each posttreatment visit up to 12 months posttreatment in the ITT patient set, in patients with $\leq$10 years of FI (TPP1), and in those with $\leq$10 years of FI and more than 2 IEF at baseline (excluding traces) (TPP2).
CTR, control; FI, fecal incontinence; HCC, high cell count; IEF, incontinence episode frequency; ITT, intention to treat; LCC, low cell count; TPP1, target population 1; TPP2, target population 2.

Supplementary Table 5. Most Frequent TEAEs (Incidence >5%) by System Organ Class and Preferred Term (SS, n = 288)

	PBO (n = 81)	LCC (n = 87)	HCC (n = 76)	Total (N = 288)[a]
At least 1 TEAE	44 (54.3)	53 (60.9)	43 (56.6)	140 (48.6)
System organ class[b]				
Infections and infestations	26 (32.1)	27 (31.0)	18 (23.7)	71 (24.7)
[Nasopharyngitis]	10 (12.3)	10 (11.5)	10 (13.2)	30 (10.4)
[Urinary tract infection]	5 (6.2)	1 (1.1)	2 (2.6)	8 (2.8)
Gastrointestinal disorders	13 (16.0)	20 (23.0)	17 (22.4)	50 (17.4)
[Diarrhea]	5 (6.2)	7 (8.0)	5 (6.6)	17 (5.9)
[Constipation]	2 (2.5)	5 (5.7)	3 (3.9)	10 (3.5)
Musculoskeletal and connective tissue disorders	8 (9.9)	8 (9.2)	10 (13.2)	26 (9.0)
Nervous system disorders	9 (11.1)	3 (3.4)	8 (10.5)	20 (6.9)
[Headache]	5 (6.2)	2 (2.3)	7 (9.2)	14 (4.9)
Injury, poisoning, and procedural complications	5 (6.2)	5 (5.7)	8 (10.5)	18 (6.3)
Vascular disorders	3 (3.7)	4 (4.6)	6 (7.9)	13 (4.5)
Investigations	2 (2.5)	6 (6.9)	4 (5.3)	12 (4.2)
Surgical and medical procedures	3 (3.7)	4 (4.6)	5 (6.6)	12 (4.2)
Psychiatric disorders	3 (3.7)	5 (5.7)	3 (3.9)	11 (3.8)
Respiratory, thoracic, and mediastinal disorders	4 (4.9)	5 (5.7)	1 (1.3)	10 (3.5)
General disorders and administration site conditions	1 (1.2)	6 (6.9)	2 (2.6)	9 (3.1)

Values are n (%). Preferred terms are listed within brackets.
HCC, high cell count; LCC, low cell count; PBO, placebo; TEAE, treatment-emergent adverse event.
[a]44 patients did not receive any treatment, 37 of whom were not randomized.
[b]For patient count per system organ class and preferred term, patients were counted only once, even if they experienced multiple medical events in the same system organ class and preferred term.

Supplementary Table 6. Fecal Incontinence Quality-of-Life Scores

	CTR			LCC			HCC		
Months Posttreatment	Mean	SD	n	Mean	SD	n	Mean	SD	n
ITT									
12	2.3	2.7	77	2.3	2.5	79	2.6	2.6	74
TPP1									
12	2.2	2.4	62	2.6	2.4	63	2.8	2.4	50
TPP2									
12	1.8	1.8	22	2.5	2.5	24	3.3	2.1	17

Absolute change in fecal incontinence quality-of-life total scores from baseline (V0) to 6 months posttreatment (V4) by treatment arm in the ITT, TPP1, and TPP2 patient populations.
CTR, control; HCC, high cell count; ITT, intention to treat; LCC, low cell count; TPP1, target population 1; TPP2, target population 2.

Supplementary Table 7. Change in Wexner Scores

	CTR			LCC			HCC		
Months Posttreatment	Mean	SD	n	Mean	SD	n	Mean	SD	n
ITT									
3	–4.1	5.3	79	–4.7	5.7	83	–4.9	5.1	75
6	–5.3	6.0	79	–4.6	5.7	82	–5.4	5.5	74
TPP1									
3	–4.2	4.8	63	–5.3	5.4	66	–5.1	5.0	51
6	–5.2	5.8	63	–5.1	5.6	65	–6.0	5.3	50
TPP2									
3	–2.0	3.4	23	–5.2	6.0	24	–4.9	4.4	18
6	–2.8	4.3	23	–4.7	6.4	24	–5.9	4.9	17

Absolute change in Wexner scores from baseline (V0) to 3 months (V3) and 6 months (V4) posttreatment by treatment arm (CTR, LCC, HCC) in the ITT, TPP1, and TPP2 patient populations. Last-observation-carried-forward imputation was performed.
CTR, control; HCC, high cell count; ITT, intention to treat; LCC, low cell count; TPP1, target population 1; TPP2, target population 2.