Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial

*Julián Panés, Damián García-Olmo, Gert Van Assche, Jean Frederic Colombel, Walter Reinisch, Daniel C Baumgart, Axel Dignass, Maria Nachury, Marc Ferrante, Lili Kazemi-Shirazi, Jean C Grimaud, Fernando de la Portilla, Eran Goldin, Marie Paule Richard, Anne Leselbaum, Silvio Danese, for the ADMIRE CD Study Group Collaborators**

Summary

Background **Complex perianal fistulas in Crohn's disease are challenging to treat. Allogeneic, expanded, adipose-derived stem cells (Cx601) are a promising new therapeutic approach. We aimed to assess the safety and efficacy of Cx601 for treatment-refractory complex perianal fistulas in patients with Crohn's disease.**

Methods **We did this randomised, double-blind, parallel-group, placebo-controlled study at 49 hospitals in seven European countries and Israel from July 6, 2012, to July 27, 2015. Adult patients (≥18 years) with Crohn's disease and treatment-refractory, draining complex perianal fistulas were randomly assigned (1:1) using a pre-established randomisation list to a single intralesional injection of 120 million Cx601 cells or 24 mL saline solution (placebo), with stratification according to concomitant baseline treatment. Treatment was administered by an unmasked surgeon, with a masked gastroenterologist and radiologist assessing the therapeutic effect. The primary endpoint was combined remission at week 24 (ie, clinical assessment of closure of all treated external openings that were draining at baseline, and absence of collections >2 cm of the treated perianal fistulas confirmed by masked central MRI). Efficacy was assessed in the intention-to-treat (ITT) and modified ITT populations; safety was assessed in the safety population. This study is registered with ClinicalTrials.gov, number NCT01541579.**

Findings **212 patients were randomly assigned: 107 to Cx601 and 105 to placebo. A significantly greater proportion of patients treated with Cx601 versus placebo achieved combined remission in the ITT (53 of 107 [50%] *vs* 36 of 105 [34%]; difference 15·2%, 97·5% CI 0·2–30·3; p=0·024) and modified ITT populations (53 of 103 [51%] *vs* 36 of 101 [36%]; 15·8%, 0·5–31·2; p=0·021). 18 (17%) of 103 patients in the Cx601 group versus 30 (29%) of 103 in the placebo group experienced treatment-related adverse events, the most common of which were anal abscess (six in the Cx601 group *vs* nine in the placebo group) and proctalgia (five *vs* nine).**

Interpretation **Cx601 is an effective and safe treatment for complex perianal fistulas in patients with Crohn's disease who did not respond to conventional or biological treatments, or both.**

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*Members listed at end of paper

Department of Gastroenterology, Hospital Clínic, IDIBAPS, Centro Investigación Biomédica en Red Enfermedades Hepáticas y Digestivas, Barcelona, Spain (Prof J Panés MD); **Department of Surgery, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain** (D García-Olmo MD); **Department of Gastroenterology and Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium** (Prof G Van Assche MD, Prof M Ferrante MD); **Department of Gastroenterology, Icahn School of Medicine at Mount Sinai, New York, NY, USA** (Prof J F Colombel MD); **Department of Internal Medicine III, Division of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria** (Prof W Reinisch MD, Prof L Kazemi-Shirazi MD); **Department of Internal Medicine, Division of Gastroenterology and Hepatology, McMaster University, Hamilton, ON, Canada** (Prof W Reinisch); **Department of Gastroenterology and Hepatology, Charité Medical School-Humboldt-University of Berlin, Berlin, Germany** (Prof D C Baumgart MD); **Department of Medicine Klinik I, Agaplesion Markus Krankenhaus, Frankfurt, Germany** (Prof A Dignass MD); **Department of Gastroenterology and**

Introduction

Crohn's disease is a chronic inflammatory bowel disease characterised by transmural inflammation and fistula formation.[1] The prevalence of Crohn's disease varies geographically, with the highest figures reported in the USA, Canada, and Europe, where prevalence rates above 300 per 100 000 people have been described.[2]

Perianal fistulas are a common complication of Crohn's disease and are estimated to affect up to 28% of patients in the first two decades after diagnosis,[3,4] particularly those with colonic disease and rectal involvement.[5] They severely impair patients' quality of life and cause substantial morbidity.[6] About 70–80% of perianal fistulas are complex,[4,7] and these are challenging to treat since they are particularly refractory to conventional medical treatment strategies (ie, antibiotics and immunomodulators) and anti-tumour necrosis factor (anti-TNF) treatments.[8–12] Furthermore, 60–70% of patients relapse after stopping treatment,[13–17] and only a few patients achieve long-term remission.[18] So far, the only approved drug that has shown efficacy in a randomised clinical trial setting is the anti-TNF drug infliximab.[8,12] Failure of or intolerability to medical treatment can ultimately result in debilitating surgical approaches, such as diverting stoma or proctectomy.[19] Therefore, there remains an unmet need for alternative treatments for perianal fistulising Crohn's disease.

Although the exact pathogenesis of perianal fistulas is largely unknown, they are thought to arise from an epithelial defect that might be caused by ongoing inflammation.[6] Adipose-derived mesenchymal stem cells are a promising new approach for the treatment of such fistulas because of their anti-inflammatory and immunomodulatory potential.[20–22] Initial proof of concept was achieved in an open-label phase 1/2a clinical study[23] of allogeneic, expanded adipose-derived stem cells (Cx601) in 24 patients with Crohn's disease and complex perianal fistulas,[23] with 56% of patients showing complete closure

Hepatology, Centre Hospitalier Universitaire Lille, Lille, France (M Nachury MD)**; Department of Hepato-Gastroenterology, Hôpital Nord, Marseille, France** (Prof J C Grimaud MD)**; Department of Surgery, Unit of Coloproctology, University Virgen del Rocio Hospital, Centro Superior de Investigaciones, University of Seville, Seville, Spain** (Prof F de la Portilla MD)**; Digestive Diseases Institute, Shaare Zedek Medical Center, Jerusalem, Israel** (Prof E Goldin MD)**; TiGenix, Parque Tecnológico de Madrid, Madrid, Spain** (M P Richard MD, A Leselbaum MD)**; and Humanitas University, IBD Center, Department of Gastroenterology, Instituto Clinico Humanitas, Rozzano, Milan, Italy** (Prof S Danese MD)

Correspondence to: Prof Julián Panés, Department of Gastroenterology, Hospital Clínic, 08036 Barcelona, Spain **jpanes@clinic.ub.es**

Research in context

Evidence before this study

We searched PubMed from Jan 1, 1980, to Dec 31, 2015, for publications on the treatment of complex perianal fistulas in patients with Crohn's disease using the following search terms: "perianal", "fistul*", "Crohn's disease", and "treatment". This search yielded 639 results, of which 52 were clinical studies. These studies showed that: (1) existing pharmacological treatments for complex perianal fistulas have low efficacy in inducing fistula healing (antibiotics 21–48%; thiopurines 20–40%; anti-tumour necrosis factor [TNF] treatment 23% complete responders [36% of 64% of patients who responded to induction treatment]); (2) the only approved drug that has shown efficacy in a randomised clinical trial is the anti-TNF drug infliximab; and (3) few treatment options exist for drug-treatment-refractory patients, and repeated surgical options are associated with substantial morbidity (eg, incontinence) and with a significant risk of permanent stoma. These findings emphasised the need for novel treatment options for treatment-refractory complex perianal fistulas in patients with Crohn's disease. Available data suggest that Crohn's-disease-associated fistulas originate from an epithelial defect that might be caused by ongoing inflammation. Since adipose-derived mesenchymal stem cells have anti-inflammatory and immunomodulatory potential, they seem to be suitable candidates to treat this disorder. Initial clinical results suggested they might have therapeutic potential in this setting.

Added value of this study

This study is, to our knowledge, the first randomised, placebo-controlled study of adipose-derived mesenchymal stem cells (Cx601) for the treatment of complex treatment-refractory perianal fistulas in patients with Crohn's disease. Our findings suggest that local treatment with Cx601 added on to established treatments for Crohn's disease might open new therapeutic options for refractory perianal disease. In this study, we assessed therapeutic effect using an innovative and distinctive primary endpoint combining both clinical assessment of fistula closure and MRI. 50% of patients treated with Cx601, compared with 34% of the placebo group, achieved combined remission 24 weeks after treatment, and the stem-cell treatment was well tolerated.

Implications of all the available evidence

Our findings suggest that Cx601 might offer patients with Crohn's disease who have treatment-refractory complex perianal fistulas a novel and minimally invasive closure alternative to avoid the need for systemic immunosuppression or surgery.

of the external opening and the absence of collections measured by MRI of the treated fistula 24 weeks after treatment. We therefore undertook a placebo-controlled study to assess the safety and efficacy of Cx601 added on to current medical treatment for treatment-refractory complex perianal fistulas in patients with Crohn's disease.

Methods

Study design and participants

We did this phase 3, randomised, double-blind, parallel-group, placebo-controlled study at 49 hospitals in seven European countries and Israel from July 6, 2012, to July 27, 2015. The study design is shown in the appendix (p 5). We enrolled adults aged 18 years or older who had non-active or mildly active luminal Crohn's disease for at least 6 months, defined by a Crohn's Disease Activity Index (CDAI) of 220 or less,[24] and had complex perianal fistulas, defined as one or more of the following: high inter-sphincteric, high trans-sphincteric, extra-sphincteric, or supra-sphincteric origin; at least two external openings; or associated collections. The fistulas had to have a maximum of two internal and three external openings, and had to have been draining for at least 6 weeks before inclusion.

See Online for appendix

We excluded patients if they had rectovaginal fistulas; rectal or anal stenosis; or active severe proctitis, defined as the presence of superficial or deep ulcers, since surgical closure of the internal orifice can be compromised in the presence of an inflamed friable rectal mucosa.[25] We also excluded patients with diverting stomas, or an abscess or collections larger than 2 cm that were not properly drained at the fistula preparation visit (see later). Eligible patients had to be refractory to at least one of the following treatments: the antibiotics ciprofloxacin or metronidazole (refractory defined as no response after 1 month), the immunomodulators azathioprine, 6-mercaptopurine, or methotrexate (refractory defined as no response after 3 months), or induction or maintenance anti-TNF treatments. Patients refractory only to antibiotics were to represent less than 25% of the total population. We also excluded patients who had not received previous treatment for perianal fistulising Crohn's disease, including antibiotics, and those who underwent previous surgery for the active fistula other than drainage or seton placement. Patients were not eligible if they had received corticosteroids within the previous 4 weeks.

We did the study in accordance with the 2008 Declaration of Helsinki and all relevant international, national, and local rules and regulations. The protocol was approved by the local ethics committee of participating centres. All patients gave written informed consent before enrolment.

Randomisation and masking

Patients were randomly assigned (1:1) by a centrally located computer-generated randomisation list to Cx601 or placebo after the fistula preparation visit at least

2 weeks before investigational product administration (appendix p 5). Patients were stratified based on concomitant treatment at randomisation (anti-TNF, immunomodulator, both, or neither). Treatments were assigned using a pre-established randomisation list generated by the Department of Biostatistics, Linical (Madrid, Spain).

Masking of treatments was not possible because the cell suspension was clearly different to saline solution (ie, placebo). The double-blind study design was maintained by the treatment being administered by an unmasked surgeon, and a masked gastroenterologist and radiologist both assessing the therapeutic effect. The radiologists who centrally read MRI scans were provided with figures to identify the treated fistulas, but were masked to patient data, order of examinations, and treatment received. Surgeons were not permitted to share information about the treatment used in the surgical procedure with the gastroenterologist and were not allowed to participate in any clinical assessment of the fistula during the study.

Procedures

A pelvic MRI scan was done at screening to guide the surgical procedures and to assess the presence of abscesses by a central reader masked to treatment and scans sequence. Additionally, patients underwent a fistula preparation visit, including examination under anaesthesia, fistula curettage, and seton placement as clinically indicated at least 2 weeks before investigational product administration (appendix p 5). These procedures were done to ensure homogeneity of the baseline characteristics of the study population. If a seton was placed, it had to be withdrawn immediately before investigational product administration.

At the treatment administration visit, patients first had the seton or setons removed, if present. Second, closure of the internal opening was done using polyglactin absorbable 2/0 stitches and was confirmed by pressured injection of 10 mL physiological saline solution through the external opening. Subsequently, patients in the Cx601 group received a single injection of 120 million Cx601 cells distributed into the tissue adjacent to all fistula tracts and internal openings. This dose was selected to be able to treat up to three fistula tracts per patient, since a greater fistula healing rate was reported in the phase 1/2a study with 40 million versus 20 million cells per tract.[23] Isolation and expansion of Cx601 were done as previously described.[23] Briefly, Cx601 cells were isolated from the stromal vascular fraction of human lipoaspirates. The lipoaspirates were extracted by liposuction from healthy adult donors after obtaining their informed consent and in line with the principles in Directive 2006/17/EC and Directive 2006/86/EC of the European Parliament. Cells were subsequently expanded according to classic cell culture practices with culture media containing 10% fetal bovine serum of certified origin and quality and kept cryopreserved until use. Cells from a single donor were used in each Cx601 dose. Before administration, 120 million cells were formulated in 24 mL of culture medium and shipped as four vials of 6 mL to the hospital for use by the surgeon on the day they were received. The formulated product can be stored between 15°C and 25°C for a maximum of 48 h. Patients in the placebo group received an identical volume of saline solution (ie, 24 mL). Study treatments were administered with a fine 20 G, long needle. First, half of the dose was injected via the anal canal into the tissue surrounding the sutured internal opening or openings, then the other half was injected through the external opening or openings into the fistula walls (no deeper than 2 mm) all along the fistula tract or tracts, making several micro-blebs. Surgeons were provided with a training CD-ROM and a surgical protocol reference guide. A trained technician was also present for the first patients treated at each site to ensure the correct administration of the study treatment and to complete the pre-established surgical checklist.

After investigational product administration, patients could be treated with antibiotics for no more than 4 weeks. Immunomodulators and anti-TNF drugs were maintained at stable doses throughout the study. Initiation or dose increases of these drugs were not allowed. A steroid course was permitted to treat occurrences of luminal disease during the study, with a starting dose of 40 mg tapered over a maximum of 12 weeks.

Fistula closure was clinically assessed at weeks 6, 12, 18, and 24 by the masked investigator who examined for the presence of spontaneous drainage and drainage after gentle finger compression at the treated external openings, as previously reported,[12] and was radiologically assessed by masked, centrally read pelvic MRI scans at week 24. Treatment-emergent adverse events (TEAEs) were assessed at all study visits and were coded according to the Medical Dictionary for Regulatory Activities version 17.0. Severity of perianal Crohn's disease was assessed by masked investigators at baseline and all study visits with the Perianal Disease Activity Index (PDAI).[26] Quality of life, measured with the Inflammatory Bowel Disease Questionnaire (IBDQ),[27] and CDAI[24] were assessed at baseline and week 24.

After a protocol amendment during the study on May 17, 2013, a subset of patients was screened for anti-HLA antibodies with Luminex (Austin, TX, USA) technology at baseline and week 12 (LABScreen Mixed; One Lambda, Canoga Park, CA, USA). A positive cutoff was defined as a mean fluorescence intensity up to 800 arbitrary units. HLA-positive sera were confirmed using LABScreen Single Antigen (One Lambda). We used HLA fusion 3.0 software (One Lambda) on the LABScan100 flow cytometer (Luminex) to interpret the results. The cutoff for a positive result on positive sera was defined as standardised fluorescence intensity up to 20 000 arbitrary units.

Outcomes

The primary endpoint was combined remission at week 24, defined as the clinical assessment of closure of all treated external openings that were draining at baseline, and the absence of collections larger than 2 cm of the treated perianal fistulas in at least two of three dimensions, confirmed by masked central MRI (BioClinica, Munich, Germany). Clinical assessment of closure was defined as the absence of draining despite gentle finger compression.[12]

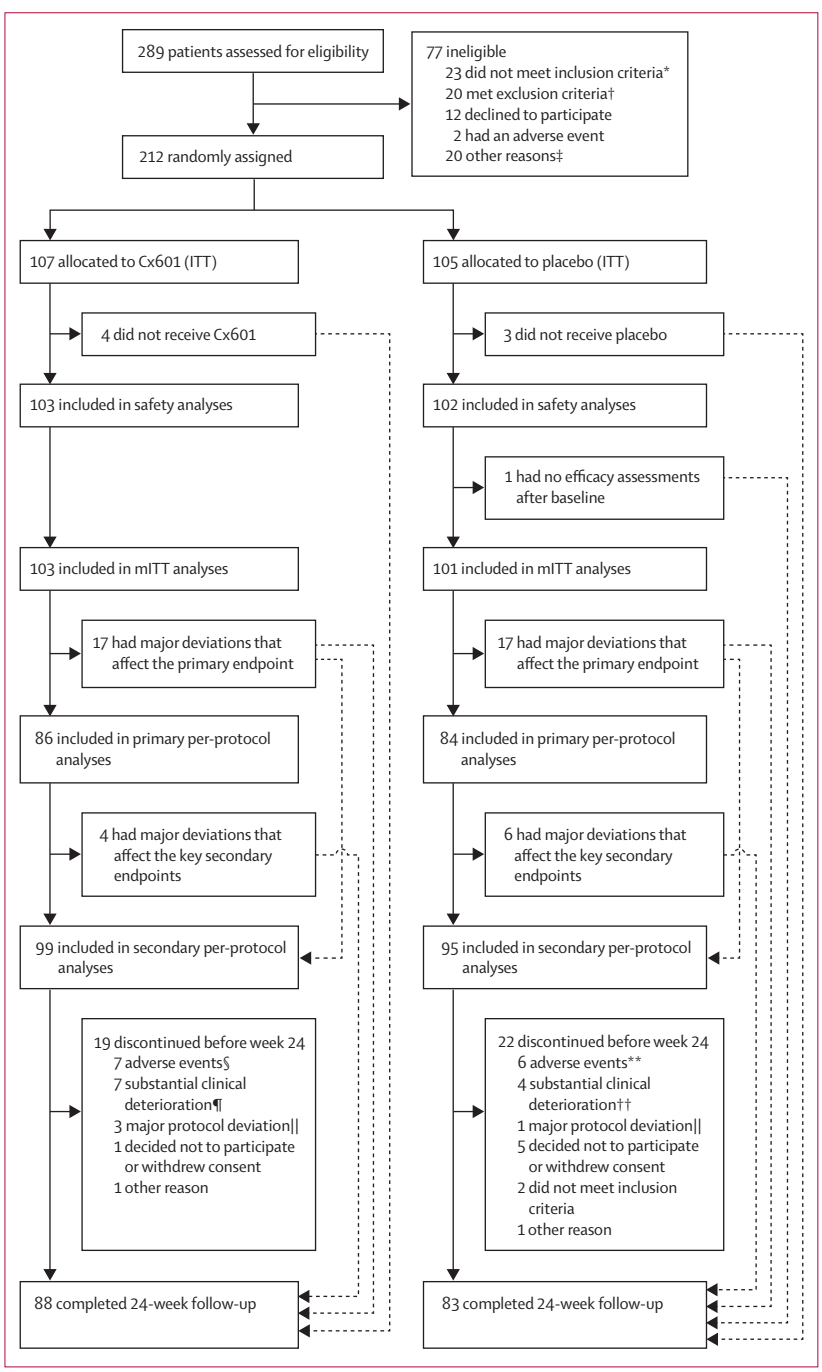

Figure 1: **Trial profile**

Cx601=allogeneic, expanded, adipose-derived stem cells. ITT=intention to treat. mITT=modified intention to treat. *19 did not have complex perianal fistulas, two had a positive pregnancy test, one did not provide signed informed consent, and one did not have non-active or mildly active luminal Crohn's disease. †Five had abscess or collection >2 cm, three had surgery for fistulas other than drainage or seton, two had dominant luminal active Crohn's disease needing immediate treatment, two had more than two internal openings, one had concomitant rectovaginal fistula, one had rectal or anal stenosis or active proctitis, one had diverting stomas, one had ongoing steroid treatment or steroids in past 4 weeks, one had renal impairment, one had hepatic impairment, one had a history or presence of malignant tumour, and one had allergies or hypersensitivities to antibiotics. ‡Two had closed fistulas, four investigator's or surgeon's decision, one had surgical procedures for other reasons than fistula, one fistula was not healing or patient had worsening of fistula symptoms, in one no fistula tract was found, in one patient 12 mL could not be administered into the tract, in one the primary opening could not be identified, one was deemed ineligible by the surgeon, one was unable to fit into the MRI machine, one had non-permitted drug treatment, one had low-grade dysplasia in colon, one had received Cx601 previously, and four were recorded as screen failures. §One each of deep vein thrombosis, Crohn's flare, intestinal obstruction, and Crohn's disease, and three with anal abscess. ¶Three with serious treatment-emergent adverse events of abscess, of which two needed a new surgery; one with treatment-emergent adverse event of abscess needing surgery; and three with fistulas not healing, of which one needed new surgery. ||Worsening of Crohn's disease needing a change in treatment. **One with a fistula, one with proctalgia, and four with anal abscess. ††Two with fistulas, one with abscess on a new fistula needing new surgery, and one with abscess needing a new course of antibiotics.

There were two key secondary efficacy endpoints: clinical remission, defined as closure of all treated external openings that were draining at baseline despite gentle finger compression, and response, defined as closure of at least 50% of all treated external openings that were draining at baseline, by week 24. At this timepoint, we also assessed the secondary endpoints of PDAI, CDAI, IBDQ, time to combined remission, relapse and time to relapse, and van Assche score. The safety endpoint was adverse events, including TEAEs, TEAEs related to study treatment, serious TEAEs, serious TEAEs related to study treatment, TEAEs leading to study withdrawal, procedure-emergent non-TEAEs, and deaths.

Statistical analysis

The planned sample size to be screened was 278 patients to randomly assign at least 208 patients (104 to each group). The sample size was sufficient to detect a minimum 25% difference in the percentage of patients with combined remission between Cx601 and placebo (anticipated minimum combined remission rates were 50% for Cx601 and 25% for placebo)[8,12,23,28] with a two-sided type I alpha error level of 0·025, 80% power, and allowing for 20% of patients to discontinue the study.

Efficacy analyses were done in the intention-to-treat (ITT) population, which included all randomly assigned patients, and the modified ITT (mITT) population, which included all randomly assigned patients who received study treatment and had at least one efficacy assessment after baseline. We analysed efficacy in the mITT population since its definition more closely resembles the ITT population in other

randomised clinical trials and provides a more reliable estimate of treatment effects. The primary endpoint was also analysed in the per-protocol population, which included all randomised and treated patients who had both an MRI after baseline and clinical fistula assessment, with no major protocol deviations that affected the primary endpoint. The two key secondary efficacy endpoints (clinical remission and response) were also analysed in the secondary per-protocol population, defined as all randomised and treated patients who had at least one clinical fistula assessment after baseline, with no major protocol deviations that affected these secondary endpoints. TEAEs were analysed in the safety population, defined as all patients who received study treatment.

We analysed the primary endpoint using a stratified Cochran-Mantel-Haenszel test, adjusting for randomisation strata (ie, Crohn's disease treatments at randomisation), with a two-sided type I error level of 0·025. Treatment differences were expressed with 97·5% CIs. The primary analysis was done in the ITT population. A non-response or non-remission was imputed if an MRI scan or clinical assessment was not done after baseline by week 24 and if a rescue event took place before week 24. A rescue event was defined as antibiotic treatment for more than 4 weeks; corticoids at 40 mg prednisone equivalent for at least 12 weeks; new anti-TNF compared with baseline treatment for at least 8 weeks; new immunomodulator compared with baseline treatment for at least 12 weeks; or a surgical intervention for the treated fistula. The effects of rescue events and missing data conventions on efficacy were explored in supportive and sensitivity analyses of the primary endpoint.

To address the issue of multiplicity, we grouped the two key secondary endpoints (clinical remission and response by week 24) into a short-term group with a gatekeeping method by Hochberg's testing procedure to control the overall type I error, with the primary efficacy endpoint acting as the gatekeeper. We assessed statistical significance with a two-sided type I error level of 0·05. No statistical adjustment for multiplicity was made for non-key secondary endpoints. Percentages and treatment differences were expressed with 95% CIs calculated with a Wald's asymptotic method. Time to clinical remission and response were analysed with Kaplan-Meier estimates, supplemented with hazard ratios (HRs) from a stratified Cox-proportional model. Cox regression was done with adjustment for the randomisation stratum. For patients without an event (clinical remission, clinical response, or relapse), we applied censoring at the date of the last visit at which the patient was observed. Safety outcomes were presented with descriptive statistics. We did the statistical analyses in SAS (version 9.1.3 or later).

This study is registered with ClinicalTrials.gov, number NCT01541579.

Role of the funding source

Employees of the funder had a role in study design, data collection, data analysis, and data interpretation. Employees of the funder had a role in the writing of this report. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

	Cx601 (n=107)	Placebo (n=105)
Age (years)	39·0 (13·1)	37·6 (13·1)
Sex		
Male	60 (56%)	56 (53%)
Female	47 (44%)	49 (47%)
Ethnic origin		
Caucasian	100 (93%)	96 (91%)
Black	4 (4%)	1 (1%)
Other	0	1 (1%)
Missing	3 (3%)	7 (7%)
Weight (kg)	73·9 (15·0)	71·3 (14·9)
Crohn's disease duration (years)	12·1 (10·0)	11·3 (8·9)
Crohn's disease treatment in past 6 months		
Antibiotics	82 (77%)	74 (70%)
Immunomodulators	89 (83%)	77 (73%)
Anti-TNF	83 (78%)	84 (80%)
Concomitant Crohn's disease treatments (stratification factor)		
Anti-TNF	37 (35%)	33 (31%)
Immunomodulators	16 (15%)	22 (21%)
Anti-TNF and immunomodulators	28 (26%)	31 (30%)
Neither	26 (24%)	19 (18%)
Other concomitant Crohn's disease treatments (safety population)		
Antibiotics	56/103 (54%)	41/102 (39%)
Glucocorticoids	6/103 (5%)	7/102 (6%)
None	43/103 (41%)	57/102 (55%)
Perianal Crohn's Disease Activity Index score*	6·8 (2·5)	6·6 (2·9)
Fistula internal openings (safety population)		
0	0/103	1/102 (1%)
1	82/103 (80%)	90/102 (88%)
2	21/103 (20%)	11/102 (11%)
Fistula external openings (safety population)		
1	58/103 (56%)	73/102 (72%)
2	37/103 (36%)	25/102 (25%)
>2	8/103 (8%)	4/102 (4%)
Crohn's Disease Activity Index score†	88·7 (48·8)	94·2 (58·7)
Inflammatory Bowel Disease Questionnaire score‡	174·1 (31·2)	169·1 (36·7)
C-reactive protein (nmol/L)	81·9 (123·8)	64·8 (102·9)
Haemoglobin (g/L)	134 (13)	135 (13)

Data are mean (SD) or number (%). Percentages might not always add up to exactly 100% as a result of rounding. Cx601=allogeneic, expanded, adipose-derived stem cells. TNF=tumour necrosis factor. *Scores range from 0 to 20; higher scores suggest more severe disease. †Scores range from 0 to 600; higher scores suggest more severe disease. ‡Scores range from 32 to 224; higher scores suggest better quality of life.

Table 1: **Patient characteristics in the intention-to-treat population**

Results

289 patients were screened, 212 of whom were randomly assigned: 107 to Cx601 and 105 to placebo (figure 1). The baseline characteristics of the two groups in the ITT population were similar (table 1). Most patients had received at least one treatment for Crohn's disease in the past 6 months. 48 (45%) of 107 patients in the Cx601 group compared with 31 (30%) of 105 in the placebo group had more than one tract fistula. The baseline characteristics of patients in the mITT population were similar to those in the ITT population (appendix p 1). 201 (95%) of 212 randomly assigned patients had a seton placed during the preparation visit: 105 (98%) of 107 patients in the Cx601 group and 96 (91%) of 105 patients in the placebo group. 171 (81%) of 212 randomly assigned patients completed the 24-week follow-up (figure 1). During the study, one patient in the Cx601 group and four patients in the placebo group received steroids for flare up of Crohn's disease (all were less than 40 mg prednisone equivalent for less than 12 weeks). No patient received antibiotics or anti-TNF treatment as a rescue event in the study, whereas one patient received a rescue immunomodulator.

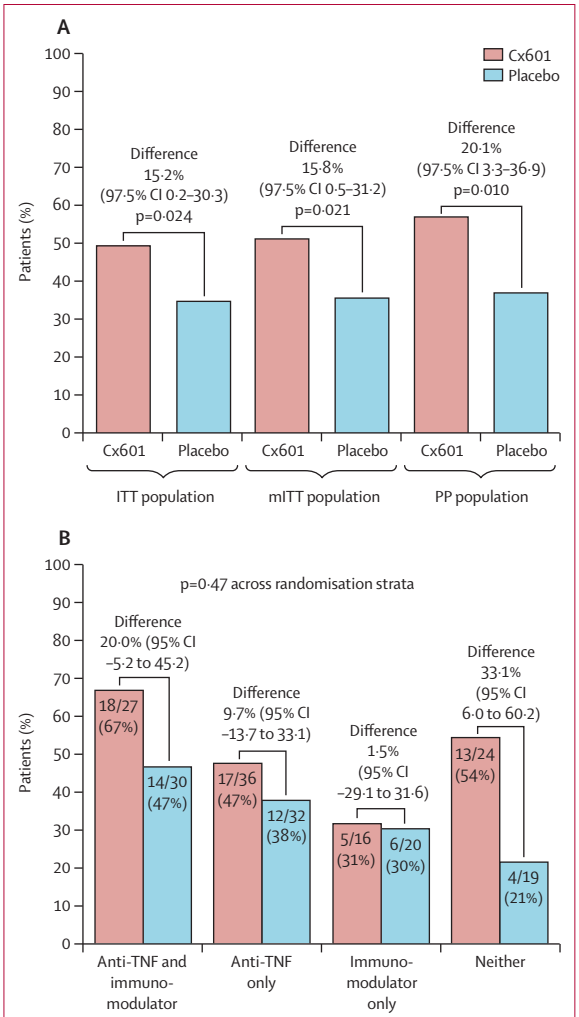

Figure 2: **Primary endpoint**
Combined remission at week 24 in (A) ITT, mITT, and PP populations; and (B) according to randomisation stratification factors (ie, Crohn's disease treatments being received at the time of randomisation) in the mITT population. Cx601=allogeneic, expanded, adipose-derived stem cells. mITT=modified intention to treat. ITT=intention to treat. PP=per protocol. TNF=tumour necrosis factor.

A significantly greater proportion of patients in the Cx601 group than the placebo group achieved the primary endpoint of combined remission at week 24 in the ITT population (53 of 107 [50%] *vs* 36 of 105 [34%], respectively; difference 15·2%, 97·5% CI 0·2–30·3; p=0·024) and the mITT population (53 of 103 [51%] *vs* 36 of 101 [36%]; 15·8%, 0·5–31·2; p=0·021; figure 2A). These results were confirmed in the per-protocol population (figure 2A) and in additional supportive and sensitivity analyses (appendix p 2). In the mITT population, the effect of Cx601 on combined remission was proportionally greater than placebo in the four randomisation strata, with the difference between groups being greatest in patients receiving neither (difference 33·1%, 95% CI 6·0 to 60·2) or both anti-TNF and immunomodulator treatments (20·0%, −5·2 to 45·2) at randomisation (figure 2B); however, the difference in the treatment effect between the four stratification groups was not significant (p=0·47).

Table 2 summarises the key secondary efficacy endpoint data. In the ITT population, 57 (53%) of 107 patients in the Cx601 group versus 43 (41%) of 105 in the placebo group achieved clinical remission (difference 12·3%, 95% CI −1·0 to 25·7; p=0·064) and 71 (66%) in the Cx601 group versus 56 (53%) in the placebo group had a response (13·0%, −0·1 to 26·1; p=0·054) by week 24. We noted similar results for these endpoints in the mITT and secondary per-protocol populations (table 2).

In both the ITT and mITT populations, the median time to clinical remission was shorter with Cx601 (6·7 weeks, 95% CI 6·4–11·9) than with placebo (14·6 weeks, 11·9–22·9; HR 0·57, 95% CI 0·41–0·79), as was the median time to response (Cx601 6·3 weeks, 6·0–6·6 *vs* placebo 11·7 weeks, 6·7–12·9; HR 0·59, 95% CI 0·43–0·81). Kaplan-Meier plots of the time to clinical remission and response in the ITT and mITT populations are shown in the appendix (pp 6–9).

The improvement in PDAI with Cx601 in the mITT population was significantly greater than with placebo at week 6 (change from baseline treatment difference −1·0, 95% CI −1·7 to −0·3), week 12 (−1·2, −2·0 to −0·4), and week 18 (−1·2, −2·0 to −0·3), but not at week 24 (−0·8, −1·8 to 0·2; appendix p 3). However, the mean total PDAI score at week 24 with Cx601 (4·4 [SD 3·6]) was close to the threshold for inactive perianal disease (PDAI <4), at which patients do not need medical or surgical treatment.[29] For total and subdomain IBDQ and CDAI scores, time to combined remission, relapse and time to relapse, and van Assche score, we noted no significant differences between treatment groups (appendix pp 3–4).

68 (66%) of 103 patients in the Cx601 group and 66 (65%) of 102 in the placebo group experienced TEAEs (table 3).

	Intention-to-treat population				Modified intention-to-treat population				Secondary per-protocol population			
	Cx601 (n=107)	Placebo (n=105)	Difference (95% CI)*	p value	Cx601 (n=103)	Placebo (n=101)	Difference (95% CI)*	p value	Cx601 (n=99)	Placebo (n=95)	Difference (95% CI)*	p value
Clinical remission by week 24	57 (53%)	43 (41%)	12·3% (−1·0 to 25·7)	0·064	57 (55%)	43 (43%)	12·8% (−0·8 to 26·4)	0·057	56 (57%)	41 (43%)	13·4% (−0·5 to 27·4)	0·052
Response by week 24	71 (66%)	56 (53%)	13·0% (−0·1 to 26·1)	0·054	71 (69%)	56 (55%)	13·5% (0·3 to 26·7)	0·045	69 (70%)	53 (56%)	13·9% (0·4 to 27·4)	0·041

Data are number (%), unless otherwise specified. Cx601=allogeneic, expanded, adipose-derived stem cells. *Difference in percentages.

Table 2: **Key secondary efficacy endpoints**

The most commonly reported TEAEs were proctalgia, anal abscess, and nasopharyngitis. 18 (17%) of 103 patients in the Cx601 group versus 30 (29%) of 103 in the placebo group experienced treatment-related adverse events, the most common of which were anal abscess and proctalgia. Most TEAEs were mild or moderate in intensity. Five (5%) of 103 patients in the Cx601 group and six (6%) of 102 in the placebo group withdrew from the study because of TEAEs. 18 (17%) of patients in the Cx601 group versus 14 (14%) in the placebo group experienced serious TEAEs, the most common of which was anal abscess (Cx601 9% *vs* placebo 7%). Five (5%) of patients in the Cx601 group versus seven (7%) in the placebo group experienced serious treatment-related adverse events. Five patients (5%) in each group had a serious treatment-related adverse event of anal abscess. No deaths occurred during the study.

Blood samples from 63 Cx601-treated and 60 placebo-treated patients were analysed for the presence of donor-specific antibodies at baseline and week 12. Ten (16%) patients in the Cx601 group and nine (15%) in the placebo group had pre-existing IgG HLA class I antibodies at baseline. At week 12, 18 (34%) of 53 Cx601-treated patients and none of the placebo-treated patients who tested negative at baseline generated anti-HLA class I antibodies. There were no immune reactions or TEAEs associated with the development of donor-specific antibodies, and no association between positivity for donor-specific antibodies and therapeutic response.

	Cx601 (n=103)	Placebo (n=102)
Overall	68 (66%)	66 (65%)
TEAEs leading to study withdrawal	5 (5%)	6 (6%)
TEAEs in ≥5·0% of patients*		
Proctalgia	13 (13%)	11 (11%)
Anal abscess	12 (12%)	13 (13%)
Nasopharyngitis	10 (10%)	5 (5%)
Diarrhoea	7 (7%)	3 (3%)
Abdominal pain	4 (4%)	6 (6%)
Fistula†	3 (3%)	6 (6%)
Treatment-related adverse events	18 (17%)	30 (29%)
Treatment-related adverse events in ≥2·0% of patients*		
Anal abscess	6 (6%)	9 (9%)
Proctalgia	5 (5%)	9 (9%)
Procedural pain	1 (1%)	2 (2%)
Fistula discharge‡	1 (1%)	2 (2%)
Induration	0	2 (2%)
Serious TEAEs§	18 (17%)	14 (14%)
Serious TEAEs in ≥2·0% of patients*		
Anal abscess	9 (9%)	7 (7%)
Serious treatment-related adverse events	5 (5%)	7 (7%)
Anal abscess	5 (5%)	5 (5%)
Proctalgia	0	1 (1%)
Anal inflammation	0	1 (1%)
Liver abscess	0	1 (1%)

Cx601=allogeneic, expanded, adipose-derived stem cells. TEAE=treatment-emergent adverse event (MedDRA, version 17.0). *In either treatment group. †New fistula, reopening of closed fistula. ‡Fistula discharge in a closed fistula. §Defined as any adverse event that at any dose resulted in death, was life-threatening, caused permanent incapacity or disability, resulted in hospital admission or prolonged a hospital stay, was a medically significant event, or was a suspected transmission of an infectious drug.

Table 3: **Treatment-emergent adverse events up to week 24 in the safety population**

Discussion

Our findings show that in a difficult-to-treat study population of patients who had Crohn's disease with complex perianal fistulas and had not responded to conventional or biological treatment, 50% of patients treated with an injection of Cx601 alone or added on to current medical treatment achieved combined remission at week 24 compared with 34% of those who received placebo. This result was consistent across all statistical populations, despite more patients in the Cx601 group than the placebo group having more than one tract fistula. Our findings reflect the comparison of Cx601 added on to current standard of care versus standard of care alone. Patients were stratified according to their concomitant treatment at randomisation (anti-TNF or immunomodulator, both, or neither). Continued use of these drugs is likely to have also had a beneficial effect, although the stratification of patients ensured that this effect was similar in both treatment groups.

Our results are in agreement with previous findings from a phase 1 study[30] using injection of a variable

number of autologous adipose-derived stem cells according to fistula size (3×10^7–40×10^7 cells), which showed complete closure of the external orifice 8 weeks after the injection in three of nine patients, and partial closure in five of nine patients, and a phase 2 trial[31] with the same product showing complete closure at week 8 in 27 (64%) of 42 patients.

The primary endpoint in our study of combined remission is more stringent than that used in other clinical studies of medical treatments for perianal fistulas in Crohn's disease, which have typically assessed response or complete response (ie, ≥50% or 100% reduction in the number of draining fistulas, respectively).[8,12] Our study is, to our knowledge, the first large-scale, randomised placebo-controlled clinical trial to use clinical assessment of fistula closure and MRI assessment of absence of abscesses as recommended in the European Crohn's and Colitis Organisation guidelines.[32] In a phase 2 study of the efficacy of local injection of 1×10^7, 3×10^7, or 9×10^7 allogeneic bone-marrow-derived mesenchymal stem cells that used the same outcome as in our study, a response was noted at week 12 in seven of 15 patients treated with stem cells compared with two of six receiving placebo.[33]

The secondary efficacy analyses reinforce the clinical benefit of Cx601. With Cx601, the time to clinical remission and response was rapid, occurring in about half of the time of that in the placebo group. However, the difference in clinical remission rates between the Cx601 and placebo groups was not significant, which might be in part related to a substantially higher placebo effect (43%) than expected and reported in previous studies (13–19%).[8,12,28] Fistula curettage, surgical drainage, and internal orifice closure might have increased the placebo response (as well as the response to Cx601). Furthermore, concomitant immunomodulator or anti-TNF treatment, or both, are likely to have had a beneficial effect in both treatment groups. Whereas beneficial improvements in PDAI scores were noted in the Cx601 group compared with the placebo group, there were no differences in IBDQ and CDAI scores between treatment groups, probably because of the low CDAI score (an inclusion criterion) and high IBDQ score at baseline. Furthermore, these instruments have been validated for the overall evaluation of Crohn's disease, and not to assess the severity of complex perianal fistulas.

The safety data show that Cx601 was well tolerated in the study population, which is in agreement with the results of the previous phase 1/2a study[23] and other studies of autologous adipose-derived or allogeneic bone marrow-derived mesenchymal stem cells.[30,31,33] Treatment-related adverse events and treatment-related serious adverse events occurred slightly more frequently in the placebo group and so might be related to the natural course of disease or surgical study procedures. Donor-specific antibodies had no clinical relevance in terms of affecting the efficacy of Cx601 or in provoking TEAEs. We did not identify any evidence of any immune reaction or specific TEAE associated with antibody development. The favourable safety profile might represent an advantage over anti-TNF treatments, which are associated with several serious safety concerns, such as an increased risk of infections, including tuberculosis, and loss of response associated with immunogenicity to the drug.[34,35]

The results of this study are encouraging, in view of the need for effective and well-tolerated new treatment options for patients with Crohn's disease and complex perianal fistulas. This study also has major implications for the care of patients with Crohn's disease with treatment-refractory complex perianal fistulas, because many of them must currently undergo repeated surgery with the risk of damaging sphincteric muscles, resulting in faecal incontinence, and 12–38% of patients with perianal fistulising disease and repeated treatment failure need proctectomy.[36] By contrast, the administration of a single injection of Cx601 is minimally invasive and can be done in an outpatient setting by an experienced surgeon.

Limitations of this trial were the exclusion of younger patients and patients with more than two internal and three external openings, as well as those with other types of treatment-refractory fistulas (eg, rectovaginal or abdominal) and those with previous surgery other than drainage and seton placement. Furthermore, whether TEAEs were related to Cx601 or to the associated preparation procedures was not established. The study results are provided up to week 24, with long-term follow-up of the patients to 2 years underway. Future studies should assess the safety and efficacy of repeat Cx601 doses. The properties of mesenchymal stem cells from different tissues (eg, placenta or bone marrow) are not the same, and so the results obtained with the preparation of adipose-derived stem cells used in this study cannot be extrapolated to mesenchymal stem cells obtained from other sources.

Although the mechanism of action of Cx601 has not been elucidated in human studies, findings from preclinical investigations have shown that expanded allogeneic adipose-derived mesenchymal stem cells exert an immunomodulatory action in the presence of inflammatory mediators (notably interferon γ), mediated via the induction of indoleamine 2,3-dioxygenase and subsequent degradation of tryptophan to kynurenine. The enzymatic activity of indoleamine 2,3-dioxygenase results in inhibition of T-lymphocyte function, and proliferation of and an increase in the number of regulatory T cells. Additionally, production of pro-inflammatory cytokines (eg, interferon γ and TNF α) is reduced, whereas production of anti-inflammatory cytokines (eg, interleukin 10) is increased.[20,21] Also, mesenchymal stem cells inhibit Th17 cell differentiation and can exert anti-inflammatory effects by inducing a regulatory T-cell phenotype in these cells.[37]

In conclusion, Cx601 is an effective and safe treatment for complex perianal fistulas in patients with Crohn's disease who did not respond to conventional or biological treatments, or both.

Contributors

JP, GVA, JFC, WR, DCB, and SD designed the study, interpreted the results, and drafted the manuscript. DG-O designed the surgical protocol, designed the study, interpreted the results, and reviewed the manuscript. AD, MN, MF, LK-S, JCG, FdlP, and EG acquired data and critically reviewed the manuscript. MPR and AL analysed data, interpreted the results, and drafted the manuscript. All authors read and approved the final version of the manuscript.

ADMIRE CD Study Group

Steering Committee: Austria Walter Reinisch (Medical University of Vienna, Vienna); *Belgium* Gert Van Assche (University Hospitals Leuven, KU Leuven, Leuven); *Italy* Silvio Danese (Istituto Clinico Humanitas, Milan); *Spain* Julián Panés (Hospital Clínic, IDIBAPS, Centro de Investigación biomédica en Red en Enfermedades Hepáticas y Figestivas, Barcelona), Daniel C Baumgart (Hospital Universitario Fundación Jiménez Díaz, Madrid); *USA* Jean F Colombel (Icahn School of Medicine at Mount Sinai, New York, NY).
Global Surgical Coordinator Damián García-Olmo (Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain).
Collaborators: Austria Anton Stift (Medical University of Vienna, Vienna); Jörg Tschmelitsch, Karl Mrak (St Veit/Glan Hospital, St Veit an der Glan); Herbert Tilg (University Clinic Innsbruck, Innsbruck); *Belgium* André D'Hoore (University Hospitals Leuven, Leuven); Danny De Looze (Gent University Hospital); Filip Baert, Paul Pattyn (Hospital Hartziekenhuis, Roeselare); *France* Philippe Zerbib (Centre Hospitalier Universitaire Lille, Lille); Frank Zerbib (Centre Hospitalier Universitaire Bordeaux); Stéphanie Viennot (Centre Hospitalier Universitaire Caen, Caen); Jean-Louis Dupas, Charles Sabbagh (Centre Hospitalier Universitaire Amiens, Amiens); Pierre-Charles Orsoni (Centre Hospitalier Universitaire Marseille, Marseille); Xavier Hebuterne (Centre Hospitalier Universitaire Nice, Nice); Matthieu Allez (Hospital Saint-Louis, Paris); Yves Panis (Hospital Beaujon, Paris); *Germany* Thomas Schulz (Markus Hospital, Frankfurt); Max Reinshagen (Klinikum Braunschweig, Braunschweig); Roland Scherer, Andreas Sturm (Deutsches Rotes Kreuz-Kliniken Westend Schwesternschaft, Berlin); Wolfgang Kruis (Kalk Hospital, Cologne); *Israel* Daniel-Simon Duek, Matti Waterman (Rambam Health Care Campus, Haifa); Ron Shapiro, Adi Lahat-Zok (Sheba Medical Center, Tel Hashomer); Sigal Fishman, Hagit Tulchinsky (Tel Aviv Sourasky Medical Center); Yair Edden (Shaare Zedek Medical Center, Jerusalem); *Italy* Antonino Spinelli (Istituto Clinico Humanitas, Milan); Vito Annese (University Careggi Hospital, Florence); Anna Kohn (San Camillo Hospital, Rome); Alfredo Papa, Luigi Sofo (Catholic University of the Sacred Heart, Rome); Imerio Angriman (Padova Hospital, Padua); Gabriele Riegler, Francesco Selvaggi (Second University of Naples, Naples); *Netherlands* Bas Oldenburg (University Medical Center Utrecht, Utrecht); Ad A van Bodegraven, Wilhelmus J H J Meijerink (VU University Medical Center, Amsterdam); Gijs van den Brink, Adrianus Wilhelmus Bemelman (Academic Medical Center, Amsterdam); Lennard Gilissen, Gust Van Montfort (Catharina Hospital, Eindhoven); *Spain* Raúl Almenara (Hospital Clinic Barcelona, Barcelona); María Dolores Martín Arranz, María Isabel Prieto Niego, Mariano García-Arranz (La Paz University Hospital, Madrid); Javier Pérez Gisbert, Mónica Sánchez Gallardo (Hospital La Princesa, Madrid); Belén Beltrán Niclós, Rosana Palasí (La Fe University Hospital, Valencia); Carlos Taxonera Samsó (San Carlos Hospital Clinic, Madrid); Jose Manuel Herrera Justiniano (Hospital Virgen del Rocio, Seville); Ricardo Rada Morgades (Hospital Juan Ramón Jiménez, Huelva); Gonzalo Gómez Gómez (University Hospital 12 October, Madrid); Daniel Carpio López, Erika Barreiro (Montecelo Hospital, Pontevedra); Xavier Cortés Rizo (Sagunto Hospital, Valencia); Antonio López-Sanromán (Hospital Ramón y Cajal, Madrid); Joaquín Hinojosa de Val, Amparo Solana (Hospital Manises, Valencia); F Xavier González Argenté (Hospital Son Espases, Palma de Mallorca); Carlos Pastor, Hector Guadalajara Labajo (Fundación Jiménez Díaz, Madrid).

Declaration of interests

JP has received personal fees from TiGenix, AbbVie, Boehringer Ingelheim, Galapagos, Pfizer, Janssen, and Takeda. DG-O has received personal fees from TiGenix, and has a patent "Identification and isolation of multipotent cells from non-osteochondral mesenchymal tissue" (10157355957US), pending to TiGenix, and a patent "Use of adipose tissue derived stromal stem cells in treating fistula" (US11/167061), pending to TiGenix. GVA has received personal fees from TiGenix, MSD, Janssen, and Takeda; and grants and personal fees from AbbVie. JFC has received grants and personal fees from AbbVie, Janssen, and Takeda; personal fees from Amgen, Boehringer Ingelheim, Celgene, Celltrion, Enterome, Ferring, Genentech, Medimmune, Merck, Pfizer, Protagonist, Second Genome, Seres, Shire, Theradiag, and PPM Services; and stock options from Genfit and Intestinal Biotech Development. WR has received personal fees from TiGenix; has served as a speaker for Abbott Laboratories, AbbVie, Aesca, Aptalis, Centocor, Celltrion, Danone Austria, Elan, Falk Pharma, Ferring, Immundiagnostik, Mitsubishi Tanabe Pharma Corporation, MSD, Otsuka, PDL, Pharmacosmos, Schering-Plough, Shire, Takeda, Therakos, Vifor, and Yakult; has served as a consultant for Abbott Laboratories, AbbVie, Aesca, Amgen, AM Pharma, Astellas, AstraZeneca, Avaxia, BioClinica, Biogen IDEC, Boehringer Ingelheim, Bristol-Myers Squibb, Cellerix, Chemocentryx, Celgene, Centocor, Celltrion, Covance, Danone Austria, Elan, Falk Pharma, Ferring, Galapagos, Genentech, Gilead, Grünenthal, ICON, Index Pharma, Inova, Janssen, Johnson & Johnson, Kyowa Hakko Kirin Pharma, Lipid Therapeutics, MedImmune, Millennium, Mitsubishi Tanabe Pharma Corporation, MSD, Nestlé, Novartis, Ocera, Otsuka, PDL, Pharmacosmos, Pfizer, Procter & Gamble, Prometheus, Robarts Clinical Trial, Schering-Plough, Second Genome, Setpointmedical, Takeda, Therakos, TiGenix, UCB, Vifor, Zyngenia, and 4SC; has served as an advisory board member for Abbott Laboratories, AbbVie, Aesca, Amgen, AM Pharma, Astellas, AstraZeneca, Avaxia, Biogen IDEC, Boehringer Ingelheim, Bristol-Myers Squibb, Cellerix, Chemocentryx, Celgene, Centocor, Celltrion, Danone Austria, Elan, Ferring, Galapagos, Genentech, Grünenthal, Inova, Janssen, Johnson & Johnson, Kyowa Hakko Kirin Pharma, Lipid Therapeutics, MedImmune, Millennium, Mitsubishi Tanabe Pharma Corporation, MSD, Nestlé, Novartis, Ocera, Otsuka, PDL, Pharmacosmos, Pfizer, Procter & Gamble, Prometheus, Schering-Plough, Second Genome, Setpointmedical, Takeda, Therakos, TiGenix, UCB, Zyngenia, and 4SC; and has received research funding from Abbott Laboratories, AbbVie, Aesca, Centocor, Falk Pharma, Immundiagnsotik, and MSD. DCB reports unrestricted research grants from Shire and Hitachi; personal fees and non-financial support from AbbVie, Merck (MSD), Takeda, Ferring, Recordati, Genentech (Roche Group), Janssen, and Dr Falk; personal fees from Biogen, Foreward Pharma, and Tigenix; and non-financial support from Nestlé. All of his activities and contracts conformi with the "FSA-Kodex Fachkreise" (voluntary self-monitoring code for expert consultants to the pharmaceutical industry), have been checked by the legal department of Charité Universitätsmedizin Berlin, and have been approved by the directorate of the Faculty of Medicine Charité Universitätsmedizin Berlin. AD has received grants and non-financial support from TiGenix; and personal fees and non-financial support from AbbVie, Dr Falk, Ferring, MSD, Takeda, Pharmacosmos, Mundipharma, Vifor, Hospira, Hexal, Allergosan, Janssen, Otsuka, and TiGenix. MN has received personal fees and non-financial support from AbbVie, MSD, and Takeda; and personal fees from Boehringer Ingelheim. MF has received non-financial support from TiGenix; grants, personal fees, and non-financial support from Takeda; and personal fees and non-financial support from MSD, Janssen, AbbVie, Chiesi, Tillotts, Ferring, Falk, Mitsubishi, Zeria, and Boehringer Ingelheim. LK-S has received non-financial support from and been a principal investigator for a study sponsored by TiGenix; has been a principal investigator for a study sponsored by SigmaTau and Sanofi; has received personal fees from MSD, AbbVie, Ferring, MerckSerono/Dr Falk, Chiesi, Novartis, Roche, Abbott, and Phadia Austria/Thermo Fisher Scientific; and has received non-financial support from Mylan, Abbott, MSD, Gilead, MerckSerono/Dr Falk, and Novartis. MPR and AL have received personal fees from TiGenix. SD has received personal fees from AbbVie, MSD, Takeda, Janssen, Mundipharma, Hospira, and Pfizer. JCG, FdlP, and EG declare no competing interests.

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