Clinical Study Protocol

NCT Number: NCT03279081

Title: A Phase-III, Randomized, Double-blind, Parallel-group, Placebo-controlled, International, Multicentre Study to Assess Efficacy and Safety of Cx601, Adult Allogeneic Expanded Adipose-derived Stem Cells (eASC) for the Treatment of Complex Perianal Fistula(s) in Patients With Crohn's Disease Over a Period of 24 Weeks and a Follow-up Period up to 52 Weeks

Study Number: Cx601-0303

Document Version and Date: Version 3.0, 28-October-2019

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

Title Page

Protocol No.	Cx601-0303
Eudract No	2017-000725-12
Title	A phase III, randomized, double blind, parallel group, placebo controlled, international, multicentre study to assess efficacy and safety of Cx601, adult allogeneic expanded adipose-derived stem cells (eASC), for the treatment of complex perianal fistula(s) in patients with Crohn's disease over a period of 24 weeks and a follow-up period up to 52 weeks. ADMIRE-CD II study.
Study Sponsor	TIGENIX, S.A.U., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited C/ Marconi, 1 Parque Tecnológico de Madrid 28760, Tres Cantos, Madrid Spain Please note: TIGENIX S.A.U., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, may be referred to in this protocol as 'Tigenix', 'sponsor', or 'Takeda'.

[redacted] MD PhD, [redacted], Clinical Science

[redacted]

Tel [redacted]

Date	Protocol Amendment 03, 28 October 2019

Clinical Trial Protocol Approval

A phase III, randomized, double blind, parallel group, placebo controlled, international, multicentre study to assess efficacy and safety of Cx601, adult allogeneic expanded adipose-derived stem cells (eASC), for the treatment of complex perianal fistula(s) in patients with Crohn's disease over a period of 24 weeks and a follow-up period up to 52 weeks. ADMIRE-CD II study.

Electronic Signatures may be found on the last page of this document.

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[redacted], Clinical Science

DATE SIGNATURE

[redacted], PhD
Statistical and Quantitative Sciences

DATE SIGNATURE

Summary

Type of application	Phase III clinical trial of an advanced therapy investigational medicinal product (ATIMP)
Sponsor identification	TIGENIX, S.A.U., (a wholly owned subsidiary of Takeda) C/ Marconi, 1 Parque Tecnológico de Madrid 28760, Tres Cantos, Madrid
Title	A phase III, randomized, double blind, parallel group, placebo controlled, international, multicentre study to assess efficacy and safety of Cx601, adult allogeneic expanded adipose-derived stem cells (eASC), for the treatment of complex perianal fistula(s) in patients with Crohn's disease over a period of 24 weeks and a follow-up period up to 52 weeks. ADMIRE-CD II study.
Code	Cx601-0303
EudraCT No	2017-000725-12
Coordinator/Principal investigator	The North American Study Coordinator is [redacted], La Jolla, California, USA The North American Study Surgeon Coordinator is [redacted], Weston, Florida, USA The European and Israel Study Coordinator is [redacted], Barcelona, Spain. The European and Israel Study Surgeon Coordinator is [redacted], Madrid, Spain. National/regional coordinators could be designated by the Sponsor.
Centres	The study will be conducted in approximately 150 centres globally.
Study monitoring	PAREXEL
Medicinal product	Cx601, a suspension of adult allogeneic expanded adipose-derived stem cells (eASC), administered at a single dose of 120 million (M) cells (24 mL) locally injected into the tissue around the internal openings and into the walls of the fistula tracts.
Control	Placebo (saline solution)
Phase	III
Objectives	To evaluate the efficacy and safety of Cx601 compared to placebo for the treatment of complex perianal fistula(s) in subjects with

Crohn's disease at Week 24 with a follow-up period up to 52 weeks.

Design

Phase III, randomized, double-blind, parallel-group, placebo-controlled, global, multicentre study.

The primary efficacy analysis will be conducted at the Week 24 timepoint. The study will continue until Week 52.

The study will follow an add-on design; subjects receiving any ongoing concomitant medical treatment at stable doses at the time of Screening for Crohn's disease (CD) will be allowed to continue it throughout the study.

Study treatments will be allocated, in a 1:1 ratio, by central randomization through interactive web response system (IWRS). Eligible subjects will be stratified to one of the following 6 combinations based on:

1. *Concomitant treatment:*

- Current use of concomitant immunosuppressant (IS) or monoclonal antibodies (mAbs) (ie, anti-tumor necrosis factor [anti-TNF] or anti-integrin [ie, vedolizumab] or anti-interleukin [IL]12/23 [ie, ustekinumab]) as a single agent.
- Current use of concomitant IS or mAbs (.e, anti-TNF or anti-integrin [ie, vedolizumab] or anti-IL12/23 [ie, ustekinumab]) as combination treatment.
- No ongoing concomitant IS or mAbs at time of randomization.

2. *External opening(s):*

- 1 versus >1

Study population will consist of subjects whose perianal fistulas were previously treated and have shown an inadequate response, a loss of response, intolerance to IS or mAbs; with complex perianal fistula(s) draining at Screening visit despite previous standard medical treatment, with up to 2 internal openings and a maximum of 3 external openings based on clinical assessment; a central reading of a locally performed contrast enhanced (gadolinium) pelvic magnetic resonance imaging (MRI) will be performed to confirm location of the fistula and potential associated perianal abscess(es). In addition, clinically controlled, non-active or mildly active CD, during the last six months prior to Screening visit will be confirmed with:

1. A PRO-2[31] score less than 14 at Screening.

   The investigator will instruct the subject to complete (at the beginning of the screening period) daily the intensity of

abdominal pain (from 0 to 3) and the number of liquid stools per day during a complete week by using the PRO-2 diary (which will be provided as a separate document). PRO-2 scores will be calculated by the investigators based on subject's diary and according to the document provided in APPENDIX 1, AND

2. A colonoscopy documenting the absence of ulcers larger than 0.5 cm in the colonic mucosa:
    - If colonoscopy data are not available within 6 months before Screening:
        - A Simple Endoscopic Score for CD (SES-CD) ≤6 with absence of rectal ulcers larger than 0.5 cm must be documented in a colonoscopy performed at Screening before randomization.
    - If colonoscopy data are available within 6 months before Screening:
        - The absence of ulcers larger than 0.5 cm in the colonic mucosa must be documented; otherwise, a new colonoscopy at Screening before randomization will be mandatory.

      AND
        - Improvement or no worsening in abdominal pain and/or diarrhea, sustained for 1 week or more, since the last colonoscopy was performed in the clinical records until Screening visit,

      AND
        - No hemoglobin decrease greater than 2.0 g/dL or an unexplained rising C-reactive protein (CRP) greater than 5.0 mg/L to a concentration above the referenced upper limit of normal (ULN) (unless the rise is due to a known process other than luminal CD), since the last colonoscopy was performed as compared to results during the Screening visit,

      AND
        - No initiation or intensification of treatment with corticosteroids, IS, or mAbs dose regimen since the last endoscopy up to Screening visit.

Baseline homogeneity and optimal preparation of fistula(s) tract(s) for the local administration of Cx601 or placebo will be guaranteed by means of an examination under anaesthesia (EUA), fistula curettage and seton placement for all subjects, done at least 2 weeks and a maximum of 3 weeks before the day of study treatment administration and immediately before randomization. Mandatory antibiotics coverage will be administered during at least 7 days following the fistula curettage (ciprofloxacin and/or metronidazole are recommended unless documented previous intolerance or contraindication to both).

Seton(s) placed will be removed on the administration day, just before the administration of the study treatment.

Training on the surgical procedure and study treatment administration and technical support and assistance will be implemented for all participating sites by means of an unblinded sponsor representative available during the surgical procedure of the treatment administration to the first subject at each site (and some of the subsequent treatment administrations if required) to monitor the compliance to the Surgery Procedure Manual, especially the proper study treatment administration and to provide assistance to the surgeon's questions if needed.

Central reading of local pelvic MRIs will be performed. All local MRIs will be assessed centrally by the clinical research organization (CRO) MRI central lab in a treatment-blinded approach for eligibility and both treatment- and sequence-blinded for efficacy assessments at Week 24 and treatment-blinded at Week 52.

Disease or disorder under study

Complex perianal fistula(s) in subjects with otherwise clinically well controlled, non active or mildly active luminal CD.

Determination of sample size

The primary endpoint of the study is the proportion of subjects with combined remission at Week 24. The following assumptions are made in the sample size calculations:

- True combined remission rates at Week 24 of 42.2% and 30% in the Cx601 and placebo groups, respectively (based on estimates from a similar population in the Phase 3 ADMIRE-CD study).
- The family-wise Type-1 error rate will be controlled at 0.05 for the analyses of the primary and key secondary efficacy endpoints at Week 24.

Based on the above assumptions, a total sample size of 554 subjects (277 per treatment arm) will provide at least 85% power for the primary efficacy endpoint analysis at Week 24.

Inclusion criteria

All subjects must comply with ALL of the following inclusion

criteria:

(1) Signed informed consent.

(2) Subjects of either gender ≥18 years and ≤75 years of age.

(3) Subjects with CD diagnosed at least 6 months before Screening visit in accordance with accepted clinical, endoscopic, histological, and/or radiological criteria.

(4) Presence of complex perianal fistula(s) with a maximum of 2 internal openings and a maximum of 3 external openings based on clinical assessment; a central reading of locally performed contrast enhanced (gadolinium) pelvic MRI will be performed to confirm location of the fistula and potential associated perianal abscess(es). Fistula(s) must have been draining for at least 6 weeks before Screening visit. Actively draining simple subcutaneous fistula(s), at the time of Screening visit, are not allowed in this study. A complex perianal fistula is defined as a fistula that meets one or more of the following criteria:

- High inter-sphincteric, high trans-sphincteric, extra-sphincteric or supra-sphincteric.
- Presence of ≥2 external openings.
- Associated perianal abscess(es). Note: Abscesses that are larger than 2 cm in at least 2 dimensions on MRI must be confirmed to have been drained adequately by the surgeon during the preparation curettage in order to be eligible.

(5) Clinically controlled, nonactive or mildly active CD during the last 6 months before Screening visit with:

1. A PRO-2 score <14 at Screening, AND
2. A colonoscopy documenting the absence of ulcers larger than 0.5 cm in the colonic mucosa:
    - If colonoscopy data are not available within 6 months before Screening:
        - An SES-CD ≤6 with absence of rectal ulcers larger than 0.5 cm must be documented in a colonoscopy performed at Screening before randomization.
    - If colonoscopy data are available within 6 months before Screening,
        - The absence of ulcers larger than 0.5 cm in the colonic mucosa must be documented; otherwise a new colonoscopy at Screening before randomization will be mandatory,

    AND

        - Improvement or no worsening in abdominal

pain and/or diarrhea, sustained for 1 week or more, since the last colonoscopy was performed in the clinical records until Screening visit,

AND

- No hemoglobin decrease greater than 2.0 g/dL or an unexplained rising CRP greater than 5.0 mg/L to a concentration above the referenced ULN (unless the rise is due to a known process other than luminal CD) since the last colonoscopy was performed as compared to results during the Screening visit,

AND

- No initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs dose regimen since the last endoscopy up to Screening visit.

(6) Subjects whose perianal fistulas were previously treated and have shown an inadequate response (absence of closure of part or all fistula tracts, or new fistula during induction treatment) or a loss of response (fistula relapse during maintenance treatment after initial fistula closure) while they were receiving either an immunosuppressive agent or TNFα antagonist or vedolizumab or ustekinumab, or having documented intolerance (occurrence, at any time, of an unacceptable level of treatment-related side effects that makes necessary treatment discontinuation) to any of these treatments administered at least at approved or recommended doses during the minimum period mentioned:

- Immunosuppressive agents: at least 3 months treatment with azathioprine (2-3 mg/kg/day), 6-mercaptopurine (1-1.5 mg/kg/day), or subcutaneous/intramuscular methotrexate (25 mg/week) before Screening for the study.
- TNFα antagonists:
  - Infliximab[35]: at least 14 weeks treatment at the approved doses for induction and/or maintenance in CD before screening for the study. For induction: 1 intravenous dose of 5 mg/kg followed by the same dose 2 and 6 weeks after. For maintenance: 5-10 mg/kg intravenously every 8 weeks, or more frequently.
  - Adalimumab[36]: at least 14 weeks treatment at the approved doses for induction and/or maintenance in

CD before screening for the study. For induction: 1 subcutaneous dose of 160 mg, followed by 80 mg 2 weeks after. For maintenance: 40 mg subcutaneously every other week, or weekly.

- Certolizumab[27, 37] pegol: at least 14 weeks treatment at the approved doses for induction and/or maintenance in CD before screening for the study. For induction: 1 subcutaneous dose of 400 mg, followed by the same dose 2 and 4 weeks after. For maintenance: 400 mg subcutaneously every 2 to 4 weeks.

- Anti-integrin: at least 14 weeks treatment of the approved dose for induction and/or maintenance in CD before screening for the study. For induction: vedolizumab 300[32, 43] mg. For maintenance: vedolizumab 300 mg every 4 to 8 weeks.
- Anti-IL-12/23: at least 16 weeks treatment of the approved dose in CD before screening for the study. For induction: ustekinumab[38], approximately 6 mg/kg intravenously initially then followed by 90 mg subcutaneously every 8 weeks.

(7) Women of childbearing potential (WCBP) must have negative serum pregnancy test at Screening (sensitive to 25 IU human chorionic gonadotropin [hCG]). Both WCBP or male subjects participating in this study, with a WCBP as partner, must agree to use an adequate method of contraception during the entire duration of the study. An adequate method of contraception is defined as complete, non-periodic sexual abstinence (refraining from heterosexual intercourse), single-barrier method, vasectomy, adequate hormonal contraception (to have started at least 7 days before Screening visit), or an intra-uterine device (to have been in place for at least 2 months before Screening visit).

A WCBP, for the purposes of this study, is a sexually mature female who is not surgically sterile by means of a prior hysterectomy, bilateral oophorectomy, or bilateral tubal ligation, and who has not been naturally postmenopausal for at least the last 12 consecutive months (ie, has had menses at any time in the preceding 12 consecutive months).

Sexual abstinence for the purposes of this study, is considered a highly effective method of contraception only if defined as refraining from heterosexual intercourse during the entire period of the study duration.

Exclusion criteria

A subject will not be included in the study if he/she meets ANY of the following criteria:

(1) Concomitant rectovaginal or rectovesical fistula(s).
(2) Subject naïve to prior specific medical treatment for complex perianal fistula(s) including IS or anti-TNFs.
(3) Presence of a perianal collection >2 cm in at least 2 dimensions on the central reading MRI at Screening visit that was not adequately drained as confirmed by the surgeon during the preparation procedure.
(4) Severe rectal and/or anal stenosis and/or severe proctitis (defined as the presence of large [>0.5 cm diameter] ulcers in the rectum) that make it impossible to follow the Surgery Procedure Manual.
(5) Subject with diverting stomas.
(6) Active, uncontrolled infection requiring parenteral antibiotics.
(7) Subject with ongoing systemic or rectal steroids for CD in the last 2 weeks before the Preparation visit.
(8) Subjects with major alteration on any of the following laboratory tests or increased risk for the surgical procedure:
    a. Serum creatinine levels >1.5 times the ULN.
    b. Total bilirubin >1.5 times the ULN (unless predominantly non-conjugated due to documented history of Gilbert's syndrome).
    c. AST/ALT >3 times the ULN.
    d. Hemoglobin <10.0 g/dL.
    e. Platelets <75.0 x 10^9/L.
    f. Albuminemia <3.0 g/dL.
(9) Suspected or documented infectious enterocolitis within 2 weeks prior to Screening visit.
(10) Any prior invasive malignancy diagnosed within the last 5 years before the Screening visit. Subjects with basal cell carcinoma of the skin completely resected outside the perineal region can be included.
(11) Current or recent (within 6 months before the Screening visit) history of severe, progressive, and/or uncontrolled hepatic, haematological, gastrointestinal (other than CD), renal, endocrine, pulmonary, cardiac, neurological, or psychiatric disease that may result in subjects increased risk from study participation and/or lack of compliance with study procedures.
(12) Subjects with primary sclerosing cholangitis.
(13) Subjects with known chronically active hepatopathy of any origin, including cirrhosis and subjects with persistent positive HBV surface antigen (HBsAg) and quantitative HBV polymerase chain reaction (PCR), or positive serology for HCV and quantitative HCV PCR within 6 months before the Screening visit.
(14) Congenital or acquired immunodeficiencies, including

subjects known to be HIV carriers.

(15) Known allergies or hypersensitivity to penicillin or aminoglycosides; DMEM (Dulbecco Modified Eagle's Medium); bovine serum; local anaesthetics or gadolinium (MRI contrast).

(16) Contraindication to MRI scan (eg, due to the presence of pacemaker, hip replacement, or severe claustrophobia).

(17) Severe trauma within 6 months before the Screening visit.

(18) Pregnant or breastfeeding women.

(19) Subjects who do not wish to or cannot comply with study procedures.

(20) Subjects currently receiving, or having received any investigational drug within 3 months before the Screening visit.

(21) Subjects previously treated with Cx601 or other allogeneic stem cell therapy cannot be enrolled into this clinical study.

(22) Any major surgery of the gastrointestinal (GI) tract (including 1 or more segments of the colon or terminal ileum) within 6 months before screening or any minor surgery of the GI tract within 3 months before screening.

(23) Subjects who had local perianal surgery other than drainage for the fistula within 6 months before the Screening visit, or those who may need surgery in the perianal region for reasons other than fistulas at the time of inclusion in the study.

(24) Contraindication to the anaesthetic procedure.

Duration of treatment

A single dose of 120 million (M) cells Cx601 or matching placebo will be injected after curettage at Visit 0, the study treatment administration visit.

Endpoints

Primary endpoint

Proportion of subjects who achieve combined remission at Week 24 after investigational medicinal product (IMP) administration, where combined remission is defined as:

a) The closure of all treated external openings that were draining at baseline despite gentle finger compression, AND
b) Absence of collection(s) >2 cm (in at least 2 dimensions) of the treated perianal fistula(s) confirmed by blinded central MRI assessment.

Secondary endpoints

Key Secondary Efficacy Endpoints

- Proportion of subjects who achieve clinical remission at Week 24 after IMP administration where clinical remission is defined as the closure of all treated external fistula openings that were draining at baseline despite gentle finger compression.
- Time to clinical remission (weeks), assessed at Week 24, defined as the time from treatment start to first visit with closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical remission by Week 24 will be censored at that visit.

Other Secondary Efficacy Endpoints

- Proportion of subjects who achieve clinical response at Week 24 after IMP administration where clinical response is defined as closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression.
- Time to clinical response (weeks), assessed at Week 24, defined as the time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical response by Week 24 will be censored at that visit.
- Proportion of subjects who achieve combined remission at Week 52 after IMP administration, where combined remission is defined as:
  a) Closure of all treated external openings that were draining at baseline despite gentle finger compression, AND
  b) Absence of collection(s) >2cm (in at least 2 dimensions) confirmed by blinded central MRI assessment.

- Proportion of subjects who achieve clinical remission at Week 52 after IMP administration, where clinical remission is defined as closure of all treated external openings that were draining at baseline, despite gentle finger compression.
- Proportion of subjects who achieve clinical response at Week 52 after IMP administration, where clinical response defined as closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression.
- Time to clinical remission (weeks), assessed at Week 52, defined as the time from treatment start to first visit with closure of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical remission by Week 52 will be censored at that visit.
- Time to clinical response (weeks), assessed at Week 52, defined as the time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical response by Week 52 will be censored at that visit.
- Proportion of subjects with relapse from Week 24 combined remission response, where relapse is defined as
    a) Reopening of any of the treated external openings with active drainage as clinically assessed in subjects who were in combined remission,

       OR

    b) The development of a perianal fluid collection(s) >2 cm (in at least 2 dimensions) of the treated perianal fistula(s) confirmed by blinded central MRI assessment.

Safety Endpoints

- Incidence of treatment-emergent adverse events (TEAEs).
- Incidence of treatment-emergent serious adverse events (SAEs).
- Incidence of adverse events of special interest (AESIs).
- Vital signs.
- Laboratory parameters.

Exploratory Endpoints

- Change from baseline in total Perianal Disease Activity Index (PDAI) score at Weeks 6, 12, 18, 24, 36, and 52.
- Change from baseline in total PRO-2 score (defined as average daily stool frequency and average daily abdominal pain) at

Weeks 6, 12, 18, 24, 36, and 52.

- Change from baseline in blinded central MRI Van Assche score at Weeks 24 and 52.
- Change from baseline in modified Van Assche blinded central MRI score at Weeks 24 and 52.
- Change from baseline in the Magnetic Resonance Novel Index for Fistula Imaging for Crohn's Disease (MAGNIFI-CD) score at Weeks 24 and 52.
- Change from baseline at Weeks 24 and 52 in blinded central MRI analysis of hyperenhancement in T1 sequence, and hyperintensity in T2 sequence.
- Change from baseline in electronic patient-reported outcomes (PRO) listed below at Weeks 12, 24, and 52:
    a) Visual Analogue Scale (VAS) from 0 to 10 for perianal pain while standing, sitting, and defecating along last 2 weeks before the visit.
    b) Crohn's Disease Activity Index (CDAI) items score.
    c) Number of pads used per day along last 2 weeks before each visit.
    d) Work Productivity and Activity Impairment (WPAI) questionnaire.
    e) European Quality of Life-5 Dimensions (EQ-5D)[44]
    f) 36-Item Short Form Health Survey (SF36)
    g) Health Resources Utilization (HRU)
- Immunogenicity responses as measured by donor-specific antibody (DSA) levels.
- Change from baseline in cytokines, immune and other inflammation associated markers at Weeks 6, 12, 24, and 52.
- Change from baseline in the microbiome diversity at Week 6.

Statistical Method

Primary Efficacy Analysis

The primary efficacy analysis comparing Cx601 and placebo with respect to the primary endpoint (proportion of subjects with combined remission rate at Week 24) will be performed using a stratified Cochran-Mantel-Haenszel test, adjusting for the randomization strata. Point estimates of the treatment difference (Cx601 – placebo) in the combined remission rate along with 95% confidence interval will be provided.

The primary efficacy analysis will be performed on an intention-to-treat (ITT) population defined as all randomized subjects regardless

of being treated or not.

Handling of missing data and treatment failure in the primary efficacy analysis

A subject will be classified as a non-responder at Week 24 (single imputation) in any of the following situations:

a) Missing data, including no MRI or no clinical assessment at Week 24, evaluation of Week 24 combined remission is not possible; OR
b) Treatment failure is documented for a subject if they require rescue medication or procedure as defined in Section 3.2.4.3.

Other sensitivity analyses to assess the impact of missing data and the analysis populations on the analysis of the primary endpoint will be detailed in the statistical analysis plan (SAP).

Analysis of Secondary Endpoints

The key secondary endpoint of clinical remission at Week 24 will be analyzed using the same methodology used for the primary efficacy endpoint, including the handling of missing data and treatment failures.

The key secondary endpoint of time to clinical remission, assessed at Week 24, will be analyzed using the stratified log-rank test for comparing Cx601 and placebo. The Cox proportional hazards model will be used to obtain estimates of the hazard ratio and the associated CIs. Kaplan-Meier curves will be presented along with median survival times.

Multiplicity Adjustment

To address multiplicity, a gate-keeping approach will be used. The primary efficacy endpoint, the proportion of subjects with combined remission at Week 24, will be tested first. If the primary efficacy analysis is statistically significant, the Hochberg procedure will then be used to test the pool of the following 2 key secondary endpoints at Week 24:

- Proportion of subjects with clinical remission at Week 24
- Time to clinical remission (weeks)

Other secondary efficacy analysis

All binary endpoints will be analyzed using the same methodology as used for the primary efficacy endpoint described above, including the handling of missing data and treatment failures.

Time-to-event endpoints will be analyzed using the same methodology as used for the key secondary endpoint of time to

clinical remission, as described above.

Categorical data will be summarized with absolute and relative frequencies (percentages) and continuous data will be summarized by the number of subjects, mean, standard deviation, minimum, first quartile, median, third quartile, and maximum.

Additional details will be provided in the SAP.

Analysis of Exploratory Endpoints

All continuous endpoints will be analyzed using a mixed model for repeated measures (MMRM) with treatment, stratum, and visit (if data collected at multiple post-treatment visits) as fixed factors with the inclusion of the time by treatment interaction (if appropriate); if available, the baseline value will be used as covariate. The treatment comparisons and corresponding 95% CIs will be presented for each scheduled visit.

All binary endpoints will be analyzed using the same methodology as used for the primary efficacy endpoint described above, including the handling of missing data and treatment failures.

Antidonor antibody kinetics will be summarized by treatment group.

Interim Analysis

There is no interim analysis planned for this study.

Planned Study periods (for the whole study)

The total duration of the study will be approximately 63 months.

Planned start date (first subject enrolled on study): 3Q 2017.

Planned enrollment period: approximately 51 months.

Planned end-of-study date (clinical cut off): 12 months after the last subject is randomized.

Planned schedule of assessments

	Screening Period (maximum of 5 weeks)	**Preparation to V0 (≥2 weeks to ≤3 weeks)**	**From Study Treatment Day (D0) to Week 24**					**Follow-Up period up to Week 52**			
VISITS **Week (W)**	**Screening**[(1) (§§§)]	**Preparation**[(1)]	**Visit0**[(1)]	**Visit1 (W6)**	**Visit2 (W12)**	**Visit3 (W18)**	**Visit4 (W24)**	**Visit5 (W36)**	**Visit6 (W52)**	**Unscheduled Phone-call follow-up**[(2)]	**Early Termination (ET)**
Study days (D) (allowed deviation days in brackets)			**D0**[(18)]	**D42 (± 8)**	**D84 (± 8)**	**D126 (± 8)**	**D168 (± 8)**	**D252 (± 15)**	**D364 (± 15)**		**(± 15)**
Informed consent	X	-	-	-	-	-	-	-	-	-	-
Optional informed consent for exploratory biomarkers	X										
Inclusion and exclusion criteria check	X	X[(3)]	-	-	-	-	-	-	-	-	-
Colonoscopy to assess SES-CD score and document absence of colonic ulcers >0.5 cm (if no previous endoscopy done within 6 months of Screening visit[†††])	X[†††]		-	-	-	-	-	-	-	-	-
Prior CD history and perianal disease records	X	-	-	-	-	-	-	-	-	-	-
Medical history*	X	-	-	-	-	-	-	-	-	-	-
Physical examination[§]	X	X	X	X	X	X	X	X	X	NA	X[(17)]
Vital signs[†]	X	-	X	X	X	X	X	X	X	NA	X[(17)]
Pregnancy test in WOCBP[(4)]	X[(4)]	-	X[(4)]	-	X[(4)]	-	X[(4)]	-	X[(4)]	NA	X[(4) (17)]
Central Laboratory tests	X	-	X	-	X	-	X	-	X	NA	X[(17)]
Fistula clinical assessment	X[(5)]	-	X	X	X	X	X	X	X	NA	X[(17)]
Local pelvic MRI & central blinded MRI reading[††]	X	-	-	-	-	-	X	-	X	NA	X[(17)]

	Screening Period (maximum of 5 weeks)	**Preparation to V0 (≥2 weeks to ≤3 weeks)**	**From Study Treatment Day (D0) to Week 24**					**Follow-Up period up to Week 52**			
VISITS **Week (W)**	**Screening**[1] (§§§)	**Preparation**[1]	**Visit0**[1]	**Visit1 (W6)**	**Visit2 (W12)**	**Visit3 (W18)**	**Visit4 (W24)**	**Visit5 (W36)**	**Visit6 (W52)**	**Unscheduled Phone-call follow-up**[2]	**Early Termination (ET)**
Study days (D) (allowed deviation days in brackets)			**D0**[18]	**D42 (± 8)**	**D84 (± 8)**	**D126 (± 8)**	**D168 (± 8)**	**D252 (± 15)**	**D364 (± 15)**		**(± 15)**
PDAI & PRO-2, scores	X		X	X	X	X	X	X	X	X[16]	X[17]
Randomization	-	X[6]	-	-	-	-	-	-	-	-	-
Fistula preparation[7]	-	X	-	-	-	-	-	-	-	-	-
Treatment administration	-	-	X	-	-	-	-	-	-	-	-
ePROs: VAS, CDAI symptom diary, number of pads per day§§	X	X	X	-	X	-	X	-	X	X[16]	X[17]
ePROs: Work Productivity and Activity Impairment (WPAI),EQ-5D, SF-36 and Health Resources Utilization (HRU) §§	X	-	X	-	X	-	X	-	X	X[16]	-
Whole blood sample for PBMC[8]		X	X	X		X	X	X			X
Plasma sample for DAS and biomarkers[9]		X	X	X	X		X		X		X
Whole blood PaxGene sample[10]		X	X	X		X	X	X			X
Adverse events/ SAEs	X	X	X	X	X	X	X	X	X	X	X
Prior/Concomitant treatments[11]	X	X	X	X	X	X	X	X	X	X	X
Fistula curettage sample[12]		X	X								
Fistula exudate[13]		X	X								
Fistula swab for microbiome analysis[14]		X	X								
Fecal sample for microbiome	X			X							

	Screening Period (maximum of 5 weeks)	**Preparation to V0 (≥2 weeks to ≤3 weeks)**	**From Study Treatment Day (D0) to Week 24**					**Follow-Up period up to Week 52**			
VISITS **Week (W)**	**Screening** (1) (§§§)	**Preparation** (1)	**Visit0**(1)	**Visit1 (W6)**	**Visit2 (W12)**	**Visit3 (W18)**	**Visit4 (W24)**	**Visit5 (W36)**	**Visit6 (W52)**	**Unscheduled Phone-call follow-up**(2)	**Early Termination (ET)**
Study days (D) (allowed deviation days in brackets)			**D0**(18)	**D42 (± 8)**	**D84 (± 8)**	**D126 (± 8)**	**D168 (± 8)**	**D252 (± 15)**	**D364 (± 15)**		**(± 15)**
analysis (15)											

* Including smoking history (and current status), transplantation, blood transfusion, any prior pregnancy.

§ Including weight and height. Height to be done only at Screening visit.

† Temperature, heart rate and blood pressure.

†† Pelvic MRI performed locally (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location). A quality copy will be sent to the Central Imaging Lab within 24h from acquisition for immediate blinded central MRI reading as detailed in the specific manual. Blinded central MRI results (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location) will be communicated to the investigator and the surgeon prior to Preparation visit. A turnaround of 5 days for Central Reading is needed (considering adequate images have been sent). At V4 and V6 or Early termination (if applicable), copies of the MRIs performed locally will be sent to the Central Imaging Lab for central MRI reading, blinded to treatment and sequence at W24 and blinded to treatment at W52. Results at Weeks 24 and 52 will include assessment of collections >3 cm in three axes and directly related to the fistula tracts treated, and any new tracts that might appear. MRIs will also be assessed for Van Assche score, hyperenhancement in T1 sequence, and hyperintensity in T2 sequence. MRI at Week 24 might be repeated if images do not have adequate quality to assess the primary endpoint.

††† If colonoscopy data are not available within 6 months prior to Screening, a Simple Endoscopic Score for CD (SES-CD) less or equal to 6 with absence of rectal ulcers larger than 0.5 cm must be documented in a colonoscopy performed at Screening before randomization. If colonoscopy data are available within 6 months prior to Screening, the following must be documented; otherwise a new colonoscopy (as above) will be mandatory: the absence of ulcers larger than 0.5 cm in the colonic mucosa AND the improvement or no worsening in abdominal pain and/or in the diarrhea, sustained for one week or more, AND no hemoglobin decrease greater than 2.0 g/dL or an unexplained rising CRP, greater than 5.0 mg/L to a concentration above the referenced ULN (unless the rise is due to a known process other than luminal Crohn's Disease) since the last colonoscopy was performed in the clinical records until Screening visit AND no initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs dose regimen since the last endoscopy up to Screening visit.

§§ Number of pads used per day, CDAI Symptom diary and Pain Intensity Visual Analog Scale (VAS), standing, sitting and defecating. To be completed as home daily assessments 2 weeks prior to each site visit, starting at the preparation visit). At screening visit the device for electronic capture will be provided to the subject. WPAI, SF36, EQ-5D, HRU are to be reviewed in clinic by the Investigator during subject's visit.

§§§ For those subjects needing a re-screening due to an out-of-window Preparation Visit, the following procedures will need to be repeated and Preparation Visit re-scheduled based on protocol timelines: Vital signs, Physical examination, Central laboratory, Pregnancy test, Fistula clinical assessment, PRO-2 and Inclusion/Exclusion criteria check.

(1) From Screening visit to Preparation Visit there will be a maximum of 5 weeks. From Preparation Visit to Treatment Administration Visit (V0) there will be a minimum of 2 weeks and maximum of 3 weeks (necessary to have the treatment ready for administration).

(2) Unscheduled phone-calls are proposed for safety follow-up in case the subject could not attend any interim visits (W6, W12, W18, W36) or for any contact requested by the subject between scheduled visits.

(3) Inclusion / Exclusion criteria to be reconfirmed before randomization.

(4) For WOCBP, a serum pregnancy test must be performed at the Screening visit, thereafter urine pregnancy tests at every other visit.

(5) Fistula must have been draining for at least 6 weeks prior to Screening visit.

(6) Randomization must be requested after the preparation procedure allowing a minimum of 2 weeks and a maximum of 3 before treatment administration.

(7) Fistula preparation consists of Examination Under Anesthesia (EUA), curettage and seton placement(s).

(8) Whole blood sample for PBMC will be obtained at Preparation Visit, Visit 0, Visit 1, Visit 3, Visit 4, and Visit 5. The samples at Preparation Visit and at Visit 0 should be collected before the curettage/seton removal procedure. At ET visit, this sample is optional.

(9) A plasma sample will be taken across the visits for DSA testing and for assessment of soluble factors. The samples at the Preparation Visit and on Visit 0 should be collected before the curettage/seton removal procedure. At the ET visit, this sample is optional.

(10) A whole blood sample will be collected in a PaxGene tube across all visits, except at W12 and W52. At the ET, this sample is optional.

(11) Previous treatment within 2 years prior to Screening Visit (see Section 3.3.1.1 “Screening Visit” for more information).

(12) Curettage material (optional sample) will be obtained during Preparation Visit and Visit 0, the second curettage, before product administration. The processing of the curettage will be described in the lab manual.

(13) The discharge/suppuration will be collected as an optional sample, before the curettage process. Additional details will be provided in the lab manual.

(14) Before the curettage process during the Preparation Visit and Visit 0, an optional swab will be taken from fistula tract for microbiome analysis.

(15) Microbiome collection (optional). An in house microbiome collection kit will be provided to the subject who accept to participate in this research. Aditional details will be provided in the Laboratory Manual.

(16) Review electronic Patient Reported Outcomes since last scheduled visit.

(17) To be repeated unless results are available within 2 weeks +/- 1 week previous to the date of study withdrawal.

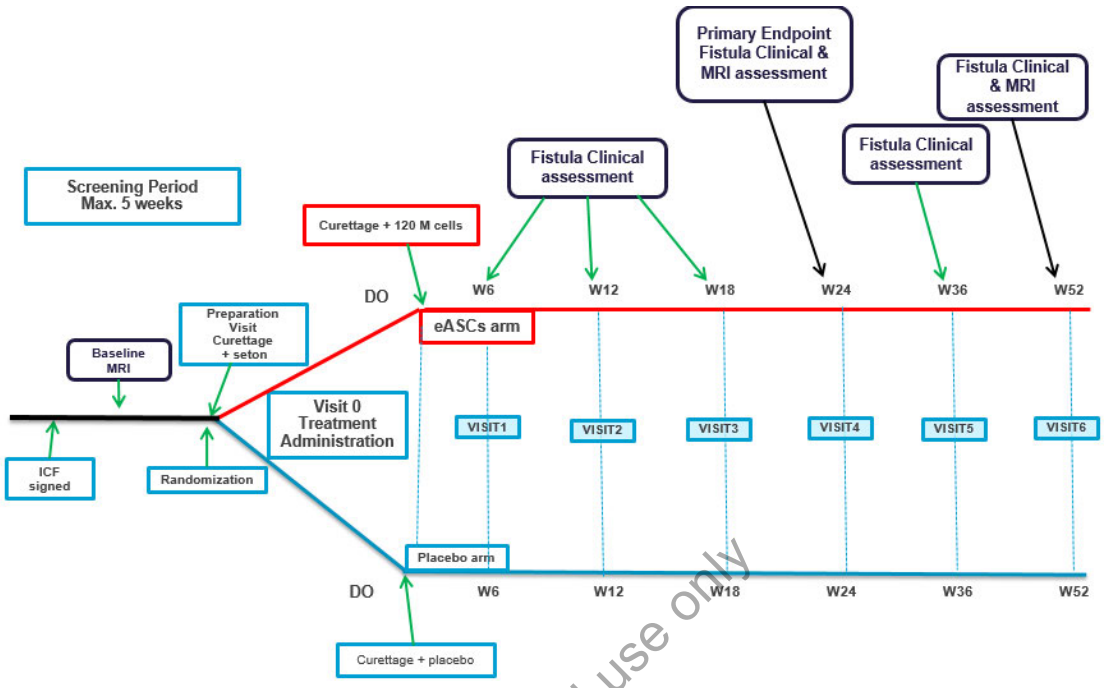
(18) All procedures should be performed before treatment administration. If there is any problem administering the IMP at the treatment administration visit, the visit should be rescheduled in at least 2 weeks. It is not necessary to repeat the preparation visit, the setons will be maintained until the rescheduled treatment visit and will be withdrawn just before the injection of the IMP. All V0 procedures are to be repeated.

Central laboratory tests:

Hematology: hemoglobin, hematocrit, erythrocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelet count

Biochemistry: C-reactive Protein (CRP), urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total bilirubin is above the ULN), potassium, sodium and chloride.

Planned visit schema



eASCs: expanded adipose-derived stem cells; MRI: magnetic resonance imaging.

Protocol Amendment 02 Summary of Changes

This document describes the changes in reference to the protocol incorporating Amendment 02. The primary purpose of this amendment is to update the protocol regarding increased sample size, modified endpoints, and statistical analyses. Minor grammatical, editorial, formatting, and administrative changes not affecting the conduct of the study are included for clarification and administrative purposes only. For specific descriptions of text changes and where the changes are located, see APPENDIX 4.

Changes in Amendment 02

1. Sentence added to state when marketing approval for Cx601 was received.
2. Editorial change to the secondary objectives.
3. Editorial change to exploratory objective and addition of an exploratory objective related to microbiome diversity.
4. Wording updated to reflect the planned increase in the study sample size from 326 subjects (specified in Version 3.0 of the protocol) to 554 subjects.
5. Wording added to clarify study design.
6. Addition of text for consistency with the summary section.
7. Editorial update to inclusion criterion.
8. Assessments added to Screening visit.
9. Assessment added for collection of microbiome sample.
10. Inclusion of new exploratory assessments.
11. Language added on hypersensitivity management.
12. Editorial changes to the description of the primary endpoint.
13. Editorial changes to the description of the secondary efficacy endpoints and safety endpoints. In addition, one of the key secondary endpoints was changed from ‘clinical response’ to ‘time to clinical remission’.
14. Editorial changes to the description of the exploratory endpoints and addition of exploratory endpoints.
15. Consistency update to treatment administration.
16. Text added to provide instruction on where information on study medication storage conditions can be found.
17. Inclusion of text to clarify where information on the IMP can be found.
18. Text added on steps to maintain study blind.
19. The serious adverse events list was updated to reflect the addition of the Takeda Medically Significant AE List as a new appendix.
20. A new section defining adverse events of special interest has been added to reflect editorial changes to safety endpoints.
21. Consistency change to list of AEs exempt from reporting
22. The SAE reporting process has been clarified and updated, including the recipient of the SAE form.
23. Addition of adverse event of special interest reporting requirements.
24. Update to pregnancy reporting requirements.
25. The statistical analytical plans section was updated to reflect revised current statistical plans.

26. Editorial changes and addition of the per-protocol set
27. Editorial changes and addition of the per-protocol set and removal of safety analysis set. Analysis for exploratory efficacy variables updated to ITT from mITT.
28. Editorial changes to the description of the primary efficacy analysis.
29. Editorial changes to the description of the secondary efficacy analysis. In addition, section updated to align with the change to one of the two key secondary endpoints.
30. The subgroup analysis section was updated to add a subgroup analysis for each of the two stratification factors, concomitant treatment (4 levels), and Region with USA and Non-USA as the subgroups of interest. In addition, levels of colonoscopy status at baseline were further clarified.
31. Editorial change to the description of exploratory efficacy analyses.
32. Editorial changes were made to the description of the safety analysis section.
33. Text to describe the sample size rationale for the planned increase in the total sample size to 554 subjects from the originally planned sample-size of 326 patients was added.
34. Editorial changes to the section describing the handling of dropouts or missing data were made for improved clarity. Language that was not applicable to the section was removed.
35. Editorial change to data quality assurance section.
36. Update to publication policy.
37. Editorial change to anal clock in Appendix 2.
38. Editorial change to schedule of assessment footnote.
39. The schedule of assessments table was updated to include optional samples of fistula curettage, fistula exudate, fistula swab for microbiome analysis and fecal sample for microbiome analysis.
40. Update to schedule of events footnote.

Table of Contents

Contacts

A separate contact information list will be provided to each site.

Takeda Development Center sponsored investigators per individual country requirements will be provided with emergency medical contact information cards to be carried by each subject.

General advice on protocol procedures should be obtained through the monitor assigned to the study site.

Contact Type/Role	United States/Canada Contact	European/Rest of World Contact
Serious adverse event and pregnancy reporting	Fax: +1-224-554-1052 Email: PVSafetyAmericas@tpna.com	Fax: +1-224-554-1052 Email: eupv@tgrd.com
Responsible Medical Officer (carries overall responsibility for the conduct of the study)	[redacted], MD PhD [redacted], Clinical Science Tel: [redacted]	[redacted], MD PhD [redacted], Clinical Science Tel: [redacted]

List of Abbreviations and Definition of Terms

Abbreviation	Description
3D	3 Dimensions
5-ASA	5- Aminosalicylic Acid
6-MP	6 Mercaptopurine
ADMIRE-CD	Adipose Derived Mesenchymal stem cells for Induction of REmission in perianal fistulising Crohn's Disease study
AE	Adverse Event
AGA	American Gastroenterological Association
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ASC	Adipose-derived Stem Cells
ATIMP	Advanced Therapy Investigational Medicinal Product
AUS	Anorectal Ultrasound
Bdir	Direct bilirubin
BT	Total bilirubin
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CI	Confidence Interval
CPK	Creatine PhosphoKinase
CRO	Contract Research Organization
CRP	C-Reactive Protein
CSR	Clinical Study Report
dL	deciliter
DMEM	Dulbecco Modified Eagle's Medium
DSA	Donor-specific antibodies
eASC	expanded Adipose-derived Stem Cells
EC	Ethics Committee(s)

Abbreviation	Description
ECCO	European Crohn's and Colitis Organization
eCRF	Electronic Case Report Form
EQ-5D	European Quality of Life-5 Dimensions
EMA	European Medicine Agency
ePRO	electronic Patient Reported Outcome
ET	Early Termination Visit
EU	European Union
EUA	Examination Under Anaesthesia
FDA	Food & Drug Administration
GCP	Good Clinical Practice
hCG	human Chorionic Gonadotropin
HBV, HCV	Hepatitis B Virus, Hepatitis C Virus
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
HRU	Health Resources Utilization
ICF	Informed Consent Form
IDO	Indoleamine 2,3-dioxygenase
IEC	Independent Ethics Committee
IFN	Interferon
IL	Interleukin
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IS	Immunosuppressant
ITT	Intention-To-Treat
IU	International units
IWRS	Interactive Web Response System
L	Liters
LMWH	Low Molecular Weight Heparin
M	Million
mAb(s)	Monoclonal antibody(ies)

Abbreviation	Description
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCS	Master Cell Stock
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified ITT
mL	Mililiters
MRI	Magnetic Resonance Imaging
NTEAE	Non Treatment Emergent Adverse Event
PCR	Polymerase Chain Reaction
PDAI	Perianal Disease Activity Index
PENTE	Procedure Emergent – Non Treatment Emergent adverse event
PP	Per Protocol
PRO-2	Patient reported outcomes measure derived from CDAI
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SES CD	Simple Endoscopic Score for Crohn's Disease
SF36	36-Item Short Form Health Survey
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Event
TE(S)AE	Treatment Emergent (serious) Adverse Event
TNF	Tumor Necrosis Factor
TF	Treatment failure
TTF	Time-to-treatment failure
ULN	Upper Limit of Normality
USA	United States of America
VAS	Visual Analogue Scale
WCBP	Women of ChildBearing Potential
WHO	World Health Organisation
WPAI	Work Productivity and Activity Impairment questionnaire

1 Introduction

1.1 Crohn's Disease and Fistulising Crohn's Disease

Crohn's disease is an idiopathic, chronic, transmural inflammatory process of the bowel that often leads to fibrosis and obstructive symptoms and can affect any part of the gastrointestinal tract from the mouth to the anus, believed to be the result of an imbalance between pro-inflammatory and anti-inflammatory mediators[1]. This transmural inflammation results in thickening of the bowel wall and narrowing of the lumen, and as Crohn's disease progresses, it is complicated by obstruction or deep ulceration leading to fistulization by way of the sinus tracts penetrating the serosa, microperforation, abscess formation, adhesions, and malabsorption[2].

The specific pathogenesis of Crohn's disease perianal fistulas is unknown, but two mechanisms have been proposed: (a) fistulas may begin as deep penetrating ulcers in the anus or rectum[3] and then extend over time as faeces are forced into the ulcer with the pressure of defecation; and (b) fistulas may also arise as a result of an infection or abscess of the anal glands that exist at the base of the anal crypts, which penetrate into the inter-sphincteric space and can easily ramify from this location[4].

The fistula tracts can communicate between intestinal segments, or between an intestinal segment and other organs (bladder, vagina), adjacent tissue or the skin. Fistulas are classified as internal when they communicate with adjacent organs (e.g., entero-enteric and rectovaginal fistulas) and external when they communicate with the dermal surface (i.e., enterocutaneous, peristomal and perianal fistulas).

Following the commonly used Parks classification based on the anatomical characteristics of perianal fistulas[5], from the inter-sphincteric space, a fistula can then tract downwards to the skin (superficial fistula and inter-sphinteric fistula), extend to the external anal sphincter (trans-sphincteric fistula), track upwards into the inter-sphincteric space (supra-sphincteric fistula), or extend out of the external sphincters and penetrate the elevator muscle into the rectum (extra-sphinteric fistula).

The risk of developing Crohn's disease perianal fistulas increases when the disease involves the distal bowel. Only 12% of patients with isolated ileal disease develop perianal fistulas, compared with 92% of patients with rectal involvement[6]. The 20 years-cumulative risk of perianal fistulas in patients with Crohn's disease ranges from 26% to 28%[6, 7]. Perianal disease is associated with high morbidity and, typically, with local pain and discharge; it therefore has a very negative impact on the quality of life of the affected patients.

1.2 Diagnosis of Perianal Fistulas

The starting point for management of perianal fistulas is a complete and accurate diagnosis of the lesions, which requires careful exploration of the anal and perianal region. An inadequate examination that fails to detect hidden lesions (abscesses or fistula branches) may result in perianal disease becoming persistent or recurrent.

There is consensus among the American Gastroenterological Association (AGA)[8, 9] and European Crohn's & Colitis Organization (ECCO)[10] working groups concerning the need to

complement the study of perianal disease with other diagnostic tools such as magnetic resonance imaging (MRI).

Magnetic Resonance Imaging

Pelvic MRI should be the initial procedure according to the ECCO consensus statement[11] because it is accurate, reproducible and non-invasive, although it is not needed routinely in simple fistulas. MRI has an accuracy of between 76% and 100% for diagnosis and classification of perianal fistulas[12, 13]. Imaging techniques in general and MRI in particular is essential to provide the surgeon with a virtual view that allows treating all lesions during the surgical procedure as well as to select the best surgical approach to avoid, if possible, more radical or extensive procedures which may increase the risk of functional impairment.

Examination Under Anaesthesia

In general surgeon practice, examination under anaesthesia (EUA) is considered the gold standard only in the hands of an experienced surgeon[11]. EUA has an accuracy of 90% for diagnosis and classification of fistulas and abscesses[13]. With this technique, it is possible to perform concomitant surgery of the lesions: incision and drainage of abscesses with seton placement, and other techniques to treat fistulas.

Anorectal ultrasound

Anorectal ultrasound (AUS) requires expertise, but can be equivalent to pelvic MRI in completing examination under anaesthesia if rectal stenosis has been excluded[11]. It offers a diagnostic accuracy of 56% to 100%, especially when performed by experts in conjunction with hydrogen peroxide enhancement. Associated findings do influence the surgical approach in 10%-15% of cases[13, 14]. Occasional pain caused by lesions of stenosis makes anorectal ultrasound difficult, but this can also be used to guide medico-surgical treatment of perianal fistulas in Crohn's disease, resulting in a high response rate[15].

The combination of either of these imaging techniques (MRI or AUS) with EUA yields a diagnostic accuracy of 100% for perianal disease.

1.3 Perianal Fistulas Classification Scores

Classification according to anatomical location (Parks' classification)

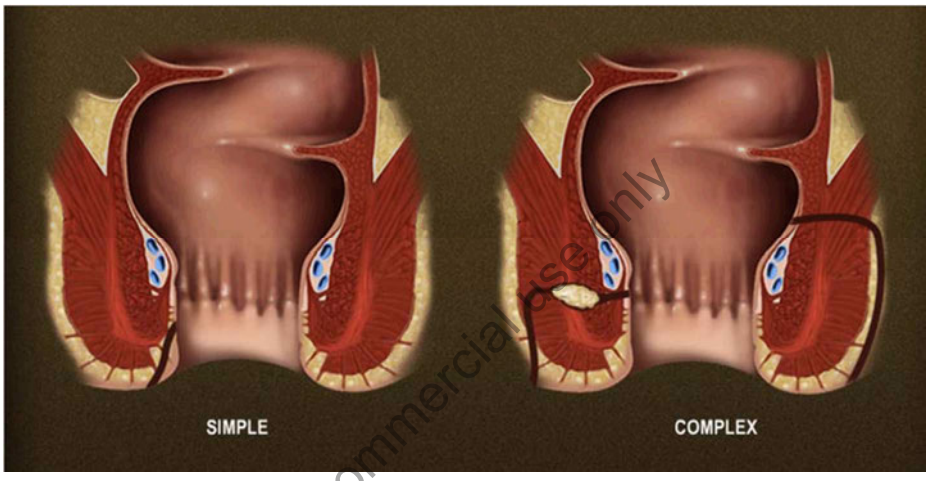
The 5 types of perianal fistula previously described following Parks' classification[5] are commonly used but do not include reference or a classification method reflecting the presence of abscesses and/or connection with other organs such as the vagina or bladder, even though such information is important for determining medical and surgical management of the disease.

Classification according to complexity

The AGA technical review proposes a simpler classification with just two categories: simple and complex fistulas[8,9].

- **Simple fistulas** are low (superficial, low inter-sphincteric or low trans-sphincteric), have a single external opening and are not associated with perianal abscess, connection to the vagina or bladder, rectal stenosis or macroscopic proctitis.
- **Complex fistulas** are high (high inter-sphincteric, high trans-sphincteric, supra-sphincteric or extra-sphincteric), can have several external openings and be associated with perianal abscess, connection to the vagina or bladder, rectal stenosis or macroscopic proctitis.

This classification has greater clinical relevance: simple fistulas respond better to treatment whereas complex ones have lower cure rates with medical treatment and an aggressive surgical procedure will often lead to incontinence.



Type of fistula according to complexity (image extracted from Griggs & Schwartz 2007) [16]

1.4 Treatment of Fistulising Crohn's Disease

Crohn's disease cannot be cured by current medical or surgical treatment[1]. The aim of therapy is to alleviate symptoms and treat complications of the disease in order to improve the patients' quality of life. More recently, aggressive treatments such as immunomodulators and biologics have aimed to modify the natural course of the Crohn´s disease[34].

Given that perianal disease is a prognostic factor of severe Crohn´s disease[28], and has a strong negative impact on quality of life, it requires special attention and should be treated. The spontaneous cure rate for perianal fistulas ranges from 6% to 13% in the placebo arm of three controlled studies[17, 19, 25]

Simple perianal fistulas

The ECCO consensus statement[11] recommends to assess if simple perianal fistula are symptomatic. If not, nothing has to be done. Noncutting seton or fistulotomy are only recommended when simple fistulas are symptomatic. Antibiotics, metronidazole (750–1500 mg/day [26]) or ciprofloxacin[29] (1000 mg/day) should be added.

The presence of a perianal abscess must be ruled out and if present should be drained as a matter of urgency.

Complex perianal fistulas

The ECCO consensus statement[11] recommends the seton placement for complex fistulas. The timing of removal depends of subsequent therapy. Active luminal Crohn's disease, if present, should be treated in conjunction with appropriate surgical management of fistulas.

The ECCO consensus statement[11] recommends antibiotics and azathioprine/mercaptopurine as the first choice of therapy for complex perianal fistula(s) in combination with surgical therapy, in spite of a lack of clinical trials. Infliximab or adalimumab are used as a second line medical treatment.

In the AGA technical review[8, 9], infliximab is recommended for treatment of complex perianal disease along with azathioprine or mercaptopurine and antibiotics for the induction phase. Maintenance with azathioprine or mercaptopurine is recommended, in some cases in association with infliximab.

Surgical treatment of complex perianal disease aims to control sepsis through abscess drainage and intervention in the fistulous tracts, including placement of noncutting setons[20]. Fistulectomy or fistulotomy are now rarely indicated in complex perianal fistulas in view of the high rate of incontinence associated with the procedure. In severe cases with high fistulas, endorectal flaps are useful. In patients with severe refractory disease, diverting colostomy or ileostomy or even proctectomy might be necessary. Other procedures such as those involving fibrin glues have been shown to be useful, but only in small uncontrolled series.

Complete fistula closure is usually clinically defined as absence of spontaneous suppuration or suppuration on applying gentle pressure[18]. The fistula drainage assessment is widely used for assessing treatment outcomes in clinical trials with the primary endpoint commonly defined as improvement or remission determined with this assessment measure which classifies fistulas as open (i.e., purulent material is expelled with gentle pressure) or closed[18].

Key point for achieving long-term closure of the fistulas is closure of the internal opening, thus avoiding septic material to enter the fistulous tract. Perianal abscesses (defined as T2 hyperintense cavities or cavities with a T2 hyperintense rim) visualized by pelvic MRI may reflect the presence of an abscess in a fistula tract, which means a dilation of the fistula tract caused by inflammatory material. Evidence of fluid perianal abscesses in the tract could be a sign of incomplete and non-permanent closure of the fistula. However, the implications on treatment outcome of inflammation seen as T2-hyperintense lesions in MRI remain unsure. It is uncertain whether the patients' condition will deteriorate with the formation of new abscesses and complex fistulas. No excessive development of new abscesses was observed in patients treated with infliximab and unconfirmed MRI[21] closure of the external opening. So, it seems reasonable to adhere to a clinical measure of closure, along with a MRI combined endpoint.

Cell therapy can be a simple, minimally invasive outpatient alternative that, based on the available preclinical and clinical data, would have significant benefits over current clinical management of CD patients with previously treated complex perianal fistula(s).

2 Rationale and Objectives

2.1 Treatment Rationale

The ultimate therapeutic goal in perianal Crohn's disease is a complete and sustained closure of the fistulas without development of abscesses and thereby avoiding the need for surgical interventions, and improving the patients' quality of life[1]. In a high number of patients, complete closure cannot be achieved despite medical treatment (including infliximab) and surgery in accordance with clinical practice.

Cell therapy based on stem cell technologies is rapidly being introduced in a variety of areas of medicine, particularly since the introduction of adult stem cells[22], avoiding ethical concerns, relative to embryonic stem cells. As adult adipose stem cells may be obtained in a technically simple way from subdermal adipose tissue, these cells represent adequate candidates for the treatment of autoimmune and inflammatory pathologies. Human lipoaspirates include a population of stem cells of mesenchymal origin with multilineage capacity: adipose derived mesenchymal stem cells (ASC). ASC obtained from human adipose tissue constitute an easily accessible and abundant source of stem cells for several applications as cell based medicinal products.

An extensive program of non-clinical pharmacology, biodistribution and safety studies has been conducted with eASC, Cx601 at TiGenix (now Takeda). Data from these studies indicate that Cx601 exhibits immunomodulatory functions. One of the key roles demonstrated for Cx601 has been the capacity to impair the proliferation of activated lymphocytes.

Specifically, co-culture of activated peripheral blood mononuclear cells (PBMC) with eASC results in dose-dependent reductions in PBMC proliferation and suggests that both cell-cell contact and soluble factors are involved in the mechanism of immunosuppression. Additionally, in the context of inflammation and tissue repair, co-culture of eASC and PBMCs during 120 hours reduced production of inflammatory cytokines interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). Stimulation by activated PBMCs which produce IFN-γ results in upregulation of eASC indoleamil 2-3 digoxigenase (IDO) and lead to the accumulation of the tryptophan metabolite, kynurenine, in vitro. These findings appear relevant to the pathophysiological environment of perianal fistula in CD, where both the role of the helper compartment of the T cells in the induction and maintenance of the intestinal inflammatory reaction in CD has been suggested in both animal models and clinical studies, and the production of IFN-γ by T-helper (Th1) cells is widely reported. Indeed, in a study where T-cells derived from the perianal fistulas of 9 patients with CD were characterised, accumulation of Th1, Th17, and Th17/Th1 cells in the fistula as compared to peripheral blood was apparent.

The anti-inflammatory activity of eASC has also been confirmed in colitic mouse model, where a single intraperitoneal treatment with the product was associated with reduced concentrations of inflammatory cytokines and up-regulation of the anti-inflammatory cytokine, IL-10, in comparison to untreated colitic mice. In addition, mice were less diseased and recovered more rapidly from the colitis.

The concerted action of local inflammation by pro-inflammatory cytokines and resultant release of immunosuppressive factors by MSC has been demonstrated to lead to an accumulation of

immune cells in close proximity to MSC, fabricating an environment in which the effects of locally acting factors produced by MSC are amplified leading to potent immunosuppression.

Biodistribution studies have demonstrated that after intrarectal injection of eASC, cells only distribute locally, with positive signals detected in the rectum and jejunum. In addition, a relatively short persistence (approximately 14 days) is reported and toxicology data for the intrarectal route shows the product is well tolerated at doses up to 10^6 cells per rat. When considered together, data indicate that eASC are likely to act locally in intralesional administration for treatment of perianal fistula, with no distribution expected and a possibility of mild host-mediated local reactions at the site of administration demonstrating a good tolerability.

Based on the therapeutic potential of eASC, TiGenix (now Takeda) developed the treatment for complex anal fistulas based on the local administration of the product.

As commented, the clinical investigation of the product (and the mechanism of action behind this local use) has initially been focused within the scope of such a local administration for the treatment of complex anal fistula. In autologous setting, eASC have been proven to be efficacious in healing of complex perianal fistulas in patients with Crohn´s disease. The clinical development of Cx601 has started to use such cells administered allogeneically to stimulate the healing of fistulas in Crohn's disease patients, which will bring some additional advantages such as the avoidance of the liposuction of each patient, and standardization of the investigational product, amongst others.

A first phase II exploratory trial (Cx601-0101) in Europe with allogeneic eASC administered locally for the treatment of complex perianal fistula revealed no significant clinical safety concerns at 6 months after the initial administration of eASC. Fifteen out of the 24 subjects who received initial treatment with 20 million eASC, received a second administration of 40 million cells, due to incomplete closure of the fistula at Week 12. After 24 weeks follow-up, 56.3% (ITT) of subjects had the external opening closed, 69.2% (ITT) of subjects had reduction in the number of initially draining tracts and 40.0% (ITT) of subjects had the fistulous disease closed (reepithelization, absence of suppuration and lack of collections >2 cm) (data on file).

A subsequent randomized phase III pivotal trial was initiated in Europe for the treatment of complex perianal fistula(s) in subjects with Crohn's disease. The pivotal, randomized, double-blind, placebo-controlled study, Cx601-0302, ADMIRE-CD study, conducted in a population of subjects aged 18 years or over, with Crohn's disease that was non-active or mildly active clinically (as defined by a CDAI ≤220 points) at baseline and with complex perianal fistulas that had shown an inadequate response to at least one conventional or biologic therapy.

In this study, 212 subjects were randomized on a 1:1 ratio to Cx601 or matching placebo (saline solution). Cx601 was administered as a single intra-lesional dose of 120 million cells and it demonstrated efficacy across a range of endpoints.

At Week 24 more subjects in the Cx601 treated group than in the placebo group had achieved complete closure of their fistula(s), as reported by Panés *et al*.[45] :

- Cx601 was associated with a statistically significant increase (p=0.024) in the percentage of subjects with combined remission at Week 24 (49.5%) compared to placebo (34.3%) with a difference in response rates between treatment groups of 15.2% (97.5% confidence interval: 0.2, 30.3) in the ITT population. Similar observations were

made in the modified ITT (mITT), per protocol and safety (*post-hoc analysis*) populations. Further confirmation of the robustness of the results was provided by 4 sensitivity analyses that assessed the effects of imputing conventions for missing data[39, 40] and rescue therapy (imputed as non-response) on the treatment effect and which also showed statistically significant increases in proportions of subjects with combined remission with Cx601 compared to placebo. *Post-hoc* analyses showed that the chance of combined remission was increased by 42% with Cx601 compared to placebo (relative risk 1.42; 97.5% CI 0.98 - 2.06).

- Increases in the percentages of subjects with clinical remission and response at Week 24 (key secondary endpoints) in the ITT population with Cx601 (53.3% and 66.4%, respectively) compared to placebo (41.0% and 53.3%, respectively) with treatment differences of 12.3% [p=0.064] and 13.0% [p=0.054], respectively in the ITT population. *Post-hoc* analyses showed the chance of clinical remission or Response was increased by 27% with Cx601 compared to placebo (relative risk 1.27, 95% CI 0.96, 1.68) and 23% (relative risk 1.23, 95% CI 0.98, 1.54), respectively. Similar observations were made in the mITT, per protocol and safety (*post-hoc analysis*) populations.
- Cx601 reduced the median time to clinical remission from 14.6 weeks in the placebo group to 6.7 weeks in the Cx601 group. Similarly, the median time to Response was also reduced by Cx601 from 11.7 weeks in the placebo group to 6.3 weeks in the Cx601 group.
- The percentage of subjects with Relapse by Week 24 was numerically lower for the Cx601 group than for the placebo group (38.0% and 50.0%, respectively). The median time to Relapse was similar for the Cx601 and placebo groups (19.1 and 18.0 weeks). Similar observations were made in the mITT and safety (*post-hoc analysis*) populations.
- Cx601 reduced the activity of the subjects' perianal disease (as assessed by the PDAI total score) such that it was close to inactive. The mean PDAI total score was reduced from 6.8 at baseline to 4.4 at Week 24 following Cx601 administration (a score of ≤4 indicates perianal disease which does not require medical or surgical treatment). With placebo, the mean PDAI total score reduced from 6.7 at baseline to 5.1 at Week 24.

The study also shown evidence of the persistence of efficacy of Cx601 up to Week 52, as:

- Cx601 was associated with increases in the percentages of subjects with combined remission (54.2%), clinical remission (57.0%) and Response (63.6%) at Week 52 compared to placebo (37.1%, 40.0% and 53.3%, respectively) with differences (95% CI) in response rates between treatment groups of 17.1% [3.9, 30.3; p=0.012], 17.0% [3.8, 30.3; p=0.016] and 10.2% [-3.0, 23.4; p=0.145], respectively. Similar observations were made in the mITT and safety (*post-hoc analysis*) populations. *Post-hoc* analyses showed the chance of combined remission, clinical remission or Response at Week 52 to be increased by 41% with Cx601 compared to placebo (relative risk 1.41, 95% CI 1.04, 1.92), 39% (relative risk 1.39, 95% CI 1.04, 1.86) and 17% (relative risk 1.17, 95% CI 0.93, 1.48), respectively.

- The percentage of subjects in the Cx601 group who had combined remission at Week 24 and then relapsed prior to Week 52 was lower in the Cx601 group than in the placebo group (25.0% and 44.1%, respectively). The difference in Relapse rate between the Cx601 and placebo groups was -19.1% (95% CI -39.5, 1.3). The chance of a subject with combined remission at Week 24 not relapsing by Week 52 was approximately twice as high with Cx601 than placebo (hazard ratio 2.12; 95% CI 1.00, 4.47).

This efficacy data was initially submitted to the European Medicine Agency (EMA) in March 2016 to support Marketing Approval Authorization (MAA) to demonstrate that Cx601 treatment effectively induces fistula closure, an effect that is maintained for up to 52 weeks, in adult subjects with complex perianal fistulas that have shown an inadequate response to at least one conventional or biologic therapy. Marketing approval was received from the EMA in March 2018.

In addition, Cx601 was well tolerated and safe at the intended dose of 120 million cells in this study.

Subjects were required to undergo EUA, fistula curettage, and, if clinically indicated, seton placement 2 to 3 weeks prior to administration of IMP. AEs collected during this time were therefore additionally collected. Non-treatment-emergent procedure-emergent AEs occurred in a small proportion of subjects and included procedural pain, nausea, vomiting, pyrexia, proctalgia, diarrhoea, nasopharyngitis, and hypotension.

Similar proportions of subjects experienced treatment-emergent AEs (TEAEs) in the Cx601 and placebo groups up to Week 52 visit (74.6%). The proportion of subjects who discontinued due to TEAEs was low overall (8.8%) and similar in both treatment groups up to Week 52 visit. However, subjects in the placebo group experienced a higher number (n=275) of individual TEAEs compared with the Cx601 group (n=250). The majority of TEAEs were mild or moderate in intensity; severe TEAEs (overall 10.7%) were reported for similar proportions of subjects in both Cx601 and placebo groups up to Week 52 visit. The majority of TEAEs (overall 68.3%) were considered to be not related to study treatment up to Week 52 visit. However, it is noteworthy that proportionally more subjects in the placebo group (n=49) reported treatment-related TEAEs than the Cx601 group (n=27) up to Week 52 visit.

TESAEs up to Week 52 visit were reported in a slightly higher proportion of subjects in the Cx601 group (24.3%) than the placebo group (20.6%). The most common TESAE was anal abscess, which was reported in slightly more subjects in the Cx601 group than the placebo group (13.6% versus 7.8%). The proportions of subjects with TESAEs of anal abscess considered related to study treatment or reported as severe were similar in the Cx601 (n= 9 and 4, respectively) and placebo (n=12 and 5, respectively) groups. There were no clinically important trends of untoward study treatment-related effects on hematology or clinical chemistry, physical examination, or vital signs.

In conclusion, this treatment would prevent one of the main causes of anal incontinence, would diminish recurrence of the fistula disease and would reduce drastically the significant disorders provoked by the standard fistula surgery in the subjects. Indeed, the procedure does not require hospital stay and avoids the work leave of classic procedures.

Completed and ongoing Cx601 studies are outlined in the table below.

Study	Study Design	Intervention (Dose/Route)	Population	Important study endpoints
Completed Studies				
Cx601-0101	Open-label, multicentre, phase I/II study in the EU (Spain)	20M (single dose) or 20M + 40M eASC/ Local injection Treatment of one fistula	Subjects with fistulizing perianal Crohn's disease; 24 included subjects	Primary: Safety parameters; Secondary: Closure of treated fistula (clinical & MRI) at 12 and 24 weeks, reduction in number draining tracts at 12 and 24 weeks
Cx601-0302 (ADMIRE-CD)	Randomized, double-blind, placebo-controlled, multicentre, phase III study in the EU (7 countries) + Israel	120 M eASC (single dose)/ Local injection Treatment of all draining fistulas	Subjects with fistulizing perianal Crohn's refractory to antibiotics or immunosuppressants, or anti-TNF therapy; planned inclusions n=278 screened subjects; n=208 randomized subjects for primary objective	Primary: Combined Remission: clinical closure at 24 weeks of all treated external openings that were draining at baseline and absence of collections >2 cm confirmed by blinded central MRI Key Secondary: Clinical Remission by W24 Response by W24

In summary, available preclinical and clinical results on cellular therapy with autologous and allogeneic eASC have shown that this is a safe treatment for fistulas that may overcome some of the problems encountered with surgery and systemic anti-TNFs (the latter in case of concomitant Crohn's disease), currently used for the management of perianal fistulas. The treatment of complex perianal fistulas by local application of allogeneic eASC intends to improve significantly the local conditions with very few inconveniences (ambulatory procedure) and minimal risk of possible complications (anal incontinence). Therefore, this is a new therapeutic resource that has initially demonstrated in the European phase III study Cx601-0302 to be safe and efficacious in this setting. Further confirmation of these efficacy results in the current global study is needed to help improve the quality of life of the patients in this highly debilitating chronic condition.

2.2 Study Dose Rationale

The proposal for a single dose of 120 million cells is based on previous non-clinical and clinical data, published literature, and the feedback received for the previous developed autologous version of Cx601 (Cx401) during the Food & Drug Administration (FDA) meetings (2006, 2008) and the European Medicine Agency (EMA) Scientific Advice (2011).

In the phase I/II study, one fistula tract (out of up to 3 tracts) was treated using either 20 million eASC or, if not effective, a dose of 40 million eASC 12 weeks after the first administration. To administer the 40 million dose the fistula had to undergo again full curettage and debridement whereby any partially closed fistula was re-opened returning the fistula to baseline conditions.

Main efficacy findings at Week 24 in the phase I/II study Cx601-0101 and the phase III study Cx601-0302, as above mentioned, support the single dose of 120 million cells as outlined in this phase III study.

Safety data supporting dose proposal:

- The safety of large doses of eASC was investigated in toxicology studies in animals with doses up to 10 million cells and follow-up ranging from 5-14 days to 3-6 months post administration. In these studies, the overall health status and survival of the animals was evaluated and revealed no concerns for safety, even though most of the studies were carried out in immunocompromised animals. Overall survival of the animals was around 90-95%.
- The good overall safety profile was also confirmed in the phase I/IIa and phase III clinical studies with local administration of allogeneic eASC.

In summary, the dosing for this study is derived from non-clinical data and clinical data previously reported by other investigators, and obtained scientific advice from the regulatory authorities. Based on the totality of the collected data, a dose of 120 million eASC as has been used in the completed pivotal EU phase III study is proposed to be used in this study.

2.3 Objectives

The study objective is to evaluate the efficacy and safety of Cx601 compared to placebo for the treatment of complex perianal fistula(s) patients with Crohn's disease (CD) at Week 24 with a follow-up period up to 52 weeks.

2.3.1 Primary Objective

To evaluate the combined remission of complex perianal fistula(s), defined as the clinical assessment at Week 24 of closure of all treated external openings that were draining at baseline despite gentle finger compression, and absence of collections >2 cm (in at least 2 dimensions) confirmed by blinded central MRI assessment at Week 24.

2.3.2 Secondary Objectives

- To evaluate the efficacy of Cx601 as compared to placebo in clinical remission at Week 24 and in time to clinical remission (weeks).
- To evaluate the efficacy and safety of Cx601 as compared to placebo in other clinical and time-to-event related endpoints at Weeks 24 and 52.

2.3.3 Exploratory Objectives

- To evaluate the efficacy of Cx601 as compared to placebo as measured by exploratory endpoints related to patient-reported outcomes, radiological measurements (MRI), cytokine, immune and inflammation-associated markers.
- To characterize microbiome diversity.

- To characterize the immunogenicity of Cx601 (donor-specific antibodies [DSA]) and the impact of immunogenicity on safety and clinical response.

3 Investigational Plan

3.1 Overall Study Design and Plan: Description

This will be a phase III, randomized, double-blind, parallel-group, placebo-controlled, global and multicentre study to assess the efficacy and safety at 24 weeks and with a follow-up period up to 52 weeks after the administration of a new therapy with eASC (Cx601) for the treatment of complex perianal fistulas in subjects with CD.

The study will follow an add-on design: subjects receiving any ongoing concomitant medical treatment for CD at stable doses at the time of Screening visit, will be allowed to continue it throughout the study. A total of approximately 740 subjects are planned to be screened in order to randomise 554 subjects in a 1:1 ratio to receive either a local injection of Cx601 (120 million cells) or matching placebo.

Eligible subjects will be stratified to one of the following 6 combinations based on:

1. *Concomitant treatment:*

- Current use of concomitant immunosuppressant (IS) or monoclonal antibodies (mAbs) (i.e., anti-tumor necrosis factor (anti-TNF) or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab) as single agent
- Current use of concomitant IS or mAbs (i.e., anti-TNF or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab) as combination treatment
- No ongoing concomitant IS or mAbs treatment at time of randomization

2. *External opening(s):*

- 1 versus >1

A 5-week screening period is scheduled to determine subject eligibility for inclusion in the study. Baseline homogeneity and optimal preparation of fistula(s) tract(s) for the local administration of Cx601 or placebo will be guaranteed by means of an examination under anaesthesia (EUA), fistula curettage and seton placement for all subjects, done at least 2 weeks and a maximum of 3 before the day of study treatment administration and immediately before randomization. Mandatory antibiotics coverage will be administered during at least 7 days following the fistula curettage (ciprofloxacin and/or metronidazole are recommended unless documented previous intolerance or contraindication to both).

Seton(s) placed will be removed on the administration day, just before the administration of the study treatment.

Training on the surgical procedure and study treatment administration and technical support and assistance will be implemented for all participating sites by means of an unblinded sponsor representative available during the surgical procedure of treatment administration to first subject at each site and some of the subsequent treatment administrations if required. They will also assess the quality and integrity of the administration procedure at each site. Central reading of

local pelvic MRIs will be performed. All local MRIs will be assessed centrally by the clinical research organization (CRO) MRI central lab in a treatment-blinded approach for eligibility and both treatment- and sequence-blinded for efficacy assessments at Week 24 and treatment-blinded for efficacy at Week 52. Study population will consist of subjects with complex perianal fistula(s) draining at Screening visit despite previous standard medical treatment, with up to 2 internal openings and a maximum of 3 external openings based on clinical assessment; a central reading of a locally performed contrast enhanced (gadolinium) pelvic MRI will be performed to confirm location of the fistula and potential associated perianal abscess(es). In addition, clinically controlled, non active or mildly active CD during the last 6 months prior to Screening visit will be confirmed with:

1. A PRO-2 score less than 14 at Screening.
   The investigator will instruct the subject to complete (at the beginning of the screening period) daily the intensity of abdominal pain (from 0 to 3) and the number of liquid stools per day during a complete week by using the PRO-2 diary (which will be provided as a separate document). PRO-2 scores will be calculated by the investigators based on subject's diary and according to the document provided in APPENDIX 1, AND
2. A colonoscopy documenting the absence of ulcers larger than 0.5 cm in the colonic mucosa:
   - If colonoscopy data are not available within 6 months prior to Screening:
     - a Simple Endoscopic Score for CD (SES-CD) ≤ 6 with absence of rectal ulcers larger than 0.5 cm must be documented in a colonoscopy performed at Screening before randomization.
   - If colonoscopy data are available within 6 months prior to Screening, the following must be documented, otherwise a new colonoscopy (as above) will be mandatory:
     - the absence of ulcers larger than 0.5 cm in the colonic mucosa.

   AND

     - the improvement or no worsening in abdominal pain and/or in the diarrhea, sustained for one week or more, since the last colonoscopy was performed in the clinical records until Screening visit.

   AND

     - no hemoglobin decrease greater than 2.0 g/dL or an unexplained rising C-reactive protein (CRP), greater than 5.0 mg/L to a concentration above the referenced ULN (unless the rise is due to a known process other than luminal Crohn's Disease), since the last colonoscopy was performed as compared to results during the Screening visit.

   AND

     - no initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs dose regimen since the last endoscopy up to Screening visit.

The primary efficacy analysis will be performed at Week 24. The double-blind design will be maintained up to Week 52 (both subject and investigator) by a site specific blinding plan for study treatment administration, blinded clinical evaluations of the fistula(s) by the investigator, and blinded centralized readings of local MRI assessments.

Fistula closure will be clinically evaluated at Weeks 6, 12, 18, 24, 36 and 52 after initial administration of study treatment, and local MRIs will be performed at Week 24 and Week 52. Central reading of all Screening MRIs will be blinded to treatment group allocation. Central reading of the local pelvic MRIs will be performed in a treatment- and sequence-blinded approach for efficacy assessments at Week 24 and treatment-blinded for Week 52, as MRI is part of the composite endpoint, combined remission.

3.2 Selection of Study Population

3.2.1 Inclusion Criteria

All subjects must comply with ALL of the following inclusion criteria:

(1) Signed informed consent.

(2) Subjects of either gender ≥18 years and ≤75 years of age.

(3) Subjects with CD diagnosed at least 6 months prior to Screening visit in accordance with accepted clinical, endoscopic, histological and/or radiological criteria.

(4) Presence of complex perianal fistula(s) with a maximum of 2 internal openings and a maximum of 3 external openings based on clinical assessment; a central reading of a locally performed contrast enhanced (gadolinium) pelvic MRI will be performed to confirm location of the fistula and potential associated perianal abscess(es). Fistula(s) must have been draining for at least 6 weeks prior to Screening visit. Actively draining simple subcutaneous fistula(s), at the time of Screening visit, are not allowed in this study. A complex perianal fistula is defined as a fistula that meets one or more of the following criteria:

   a. High inter-sphincteric, high trans-sphincteric, extra-sphincteric or supra-sphincteric.

   b. Presence of ≥2 external openings.

   c. Associated perianal abscess(es). Note: Abscesses that are larger than 2 cm in at least 2 dimensions on MRI must be confirmed to have been drained adequately by the surgeon during the preparation curettage in order to be eligible.

(5) Clinically controlled, non active or mildly active CD, during the last six months prior to Screening visit with:

   1. a PRO-2 score <14 at Screening, AND

   2. a colonoscopy documenting the absence of ulcers larger than 0.5 cm in the colonic mucosa:

      - If colonoscopy data are not available within 6 months prior to Screening:

- a SES-CD ≤6 with absence of rectal ulcers larger than 0.5 cm must be documented in a colonoscopy performed at Screening before randomization.

- If colonoscopy data are available within 6 months prior to Screening, the following must be documented, otherwise a new colonoscopy (as above) will be mandatory:
  - the absence of ulcers larger than 0.5 cm in the colonic mucosa.

AND

- the improvement or no worsening in abdominal pain and/or in the diarrhea, sustained for one week or more, since the last colonoscopy was performed in the clinical records until Screening visit.

AND

- no hemoglobin decrease ≥2.0 g/dL or an unexplained rising C-reactive protein (CRP), >5.0 mg/L to a concentration above the referenced ULN (unless the rise is due to a known process other than luminal Crohn's Disease), since the last colonoscopy was performed as compared to results during the Screening visit.

AND

- no initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs dose regimen since the last endoscopy up to Screening visit.

(6) Subjects whose perianal fistulas were previously treated and have shown an **inadequate response** (absence of closure of part or all fistula tracts, or new fistula during induction treatment) or **a loss of response** (fistula relapse during maintenance treatment after initial fistula closure) while they were receiving either an immunosuppressive agent or TNF-α antagonist or vedolizumab or ustekinumab, or having **documented intolerance** (occurrence, at any time, of an unacceptable level of treatment-related side effects that makes necessary treatment discontinuation) to any of these treatments administered at least at approved or recommended doses during the minimum period mentioned:

- Immunosuppressive agents: at least 3 months treatment with azathioprine (2-3 mg/kg/day), 6-mercaptopurine (1-1.5 mg/kg/day), or subcutaneous/intramuscular methotrexate (25 mg/week) prior to Screening for the study.
- TNFα antagonists:
  - Infliximab[35]: at least 14 weeks treatment at the approved doses for induction and/or maintenance in Crohn´s disease prior to screening for the study. For induction: 1 intravenous dose of 5 mg/kg followed by the same dose 2 and 6 weeks after. For maintenance: 5-10 mg/kg intravenously every 8 weeks, or more frequently.
  - Adalimumab[36]: at least 14 weeks treatment at the approved doses for induction and/or maintenance in Crohn's disease prior to screening for the study. For induction: 1 subcutaneous dose of 160 mg, followed by 80 mg 2 weeks after. For maintenance: 40 mg subcutaneously every other week, or weekly.

- Certolizumab[27, 37] pegol: at least 14 weeks treatment at the approved doses for induction and/or maintenance in Crohn´s disease prior to screening for the study. For induction: 1 subcutaneous dose of 400 mg, followed by the same dose 2 and 4 weeks after. For maintenance: 400 mg subcutaneously every 2 to 4 weeks.

- Anti-integrin: at least 14 weeks treatment of the approved dose for induction and/or maintenance in Crohn´s disease prior to screening for the study. For induction: Vedolizumab 300[32, 43] mg. For maintenance: Vedolizumab 300 mg every 4 to 8 weeks.
- Anti-IL-12/23: at least 16 weeks treatment of the approved dose in Crohn´s disease prior to screening for the study. For induction: Ustekinumab[38], approximately 6 mg/kg intravenously initially then followed by 90 mg subcutaneously every 8 weeks.

(7) Women of childbearing potential (WCBP) must have negative serum pregnancy test at Screening (sensitive to 25 IU human chorionic gonadotropin [hCG]). Both WCBP or male subjects participating in this study, with a WCBP as partner, must agree to use an adequate method of contraception during the entire duration of the study. An adequate method of contraception is defined as complete, non-periodic sexual abstinence (refraining from heterosexual intercourse), single-barrier method, vasectomy, adequate hormonal contraception (to have started at least 7 days prior to Screening visit), or an intra-uterine device (to have been in place for at least 2 months prior to Screening visit).

A WCBP, for the purposes of this study, is a sexually mature female; who is not surgically sterile by means of a prior hysterectomy, bilateral oophorectomy, or bilateral tubal ligation; and has not been naturally postmenopausal for at least the last 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

Sexual abstinence for the purposes of this study, is considered a highly effective method of contraception only if defined as refraining from heterosexual intercourse during the entire period of the study duration.

3.2.2 Exclusion Criteria

A subject will not be included in the study if he/she meets ANY of the following criteria:

(1) Concomitant rectovaginal or rectovesical fistula(s).
(2) Subject naïve to prior specific medical treatment for complex perianal fistula(s) including IS or anti-TNFs.
(3) Presence of a perianal collection >2 cm in at least two dimensions on the central reading MRI at Screening visit that was not adequately drained as confirmed by the surgeon during the preparation procedure (week -3 to day 0).
(4) Severe rectal and/or anal stenosis and/or severe proctitis (defined as the presence of large [>0.5 cm] ulcers in the rectum) that make impossible to follow the Surgery Procedure Manual.
(5) Subject with diverting stomas.
(6) Active, uncontrolled infection requiring parenteral antibiotics.
(7) Subject with ongoing systemic or rectal steroids for CD in the last 2 weeks prior to Preparation visit.
(8) Subjects with major alteration on any of the following laboratory tests or increased risk for the surgical procedure:

a. Serum creatinine levels >1.5 times the ULN
b. Total bilirubin >1.5 times the ULN (unless predominantly non-conjugated due to documented history of Gilbert's syndrome)
c. AST/ALT >3.0 times the ULN
d. Hemoglobin <10.0 g/dL
e. Platelets <75.0 x 10^9/L
f. Albuminemia <3.0 g/dL

(9) Suspected or documented infectious enterocolitis within 2 weeks prior to Screening visit.

(10) Any prior invasive malignancy diagnosed within the last 5 years prior to Screening visit. Subjects with basal-cell carcinoma of the skin completely resected outside the perineal region can be included.

(11) Current or recent (within 6 months prior to the Screening visit) history of severe, progressive, and/or uncontrolled hepatic, haematological, gastrointestinal (other than CD), renal, endocrine, pulmonary, cardiac, neurological or psychiatric disease that may result in subjects increased risk from study participation and/or lack of compliance with study procedures.

(12) Subjects with primary sclerosing cholangitis.

(13) Subjects with known chronically active hepatopathy of any origin, including cirrhosis and subjects with persistent positive HBV surface antigen (HBsAg) and quantitative HBV polymerase chain reaction (PCR), or positive serology for HCV and quantitative HCV PCR within 6 months prior to screening.

(14) Congenital or acquired immunodeficiencies, including subjects known to be HIV carriers

(15) Known allergies or hypersensitivity to penicillin or aminoglycosides; DMEM; bovine serum; local anaesthetics or gadolinium (MRI contrast).

(16) Contraindication to MRI scan (e.g., due to the presence of pacemaker, hip replacement or severe claustrophobia).

(17) Severe trauma within 6 months prior to Screening visit.

(18) Pregnant or breastfeeding women.

(19) Subjects who do not wish to or cannot comply with study procedures.

(20) Subjects currently receiving, or having received any investigational drug within 3 months prior to Screening visit.

(21) Subjects previously treated with Cx601 or other allogeneic stem-cell therapy cannot be enrolled into this clinical study.

(22) Any major surgery of the GI tract (including one or more segments of the colon or terminal ileum) within 6 months prior to screening or any minor surgery of the GI tract within 3 months prior to screening.

(23) Subjects who had local perianal surgery other than drainage for the fistula within 6 months prior to the Screening visit, or those who may need surgery in the perianal region for reasons other than fistulas at the time of inclusion in the study.

(24) Contraindication to the anaesthetic procedure.

3.2.3 Potential Re-Screening

For any subject not meeting eligibility criteria, it may be possible to re-screen the subject at a later date upon Sponsor's approval.

For those subjects needing a re-screening due to an out-of-window Preparation Visit, and upon Sponsor´s approval, the following procedures will need to be repeated and Preparation Visit re-scheduled based on protocol timelines:

- Vital signs
- Physical examination
- Central laboratory
- Pregnancy test
- Fistula clinical assessment
- PRO-2
- Inclusion/exclusión criteria check

3.2.4 Prior and Concomitant Therapy

3.2.4.1 Prior Medication

Prior medication is received prior to Treatment administration visit.

As defined in Inclusion criteria#6, all subjects participating in this study must have a complex perianal fistula(s) that either have shown:

- **Previous or current inadequate response** to heal the fistula(s) (defined as absence of closure of part or all fistula tracts, or new fistula during induction treatment), **loss of response** (fistula relapse during maintenance treatment after initial fistula closure) **or having documented intolerance** (occurrence, at any time, of an unacceptable level of treatment-related side effects that makes necessary treatment discontinuation) to any of the following treatments administered at least at approved or recommended doses during the minimum period mentioned:
    - Immunosuppressive agents: at least 3 months treatment with azathioprine (2-3 mg/kg/day), 6-mercaptopurine (1-1.5 mg/kg/day), or subcutaneous/intramuscular methotrexate (25 mg/week) prior to Screening for the study.
    - TNFα antagonists:
        - Infliximab[35]: at least 14 weeks treatment at the approved doses for induction and/or maintenance in Crohn´s disease prior to Screening for the study. For induction: 1 intravenous dose of 5 mg/kg followed by the same dose 2 and 6 weeks after. For maintenance: 5-10 mg/kg intravenously every 8 weeks, or more frequently.
        - Adalimumab[36]: at least 14 weeks treatment at the approved doses for induction and/or maintenance in Crohn´s disease prior to Screening for the study. For induction: 1 subcutaneous dose of 160 mg, followed by 80 mg 2 weeks after. For maintenance: 40 mg subcutaneously every other week, or weekly.
        - Certolizumab[27, 37] pegol: at least 14 weeks treatment at the approved doses for induction and/or maintenance prior to Screening for the study. For induction:

1 subcutaneous dose of 400 mg, followed by the same dose 2 and 4 weeks after. For maintenance: 400 mg subcutaneously every 2 to 4 weeks.

- Anti-integrin: at least 14 weeks treatment of the approved dose for induction and/or maintenance in Crohn's disease prior to Screening for the study. For induction: vedolizumab 300[32, 43] mg. For maintenance: vedolizumab 300 mg every 4 to 8 weeks.
- Anti-IL-12/23: at least 16 weeks treatment of the approved dose in Crohn's disease prior to Screening for the study. For induction: ustekinumab[38] approximately 6 mg/kg intravenously initially then followed by 90 mg subcutaneously every 8 weeks.

All prior treatments including previous immunosuppressive agents (i.e., azathioprine, mercaptopurine, or methotrexate), TNFα antagonist, vedolizumab or ustekinumab will be documented in the eCRF, as indicated, including: induction or maintenance, and if any, reasons for inadequate response, loss of response of perianal fistulas, or intolerance to each of these previous specific treatments.

- Prior medication **not allowed**:
  - Systemic and/or local (applied to perineal or perianal regions) steroids for CD must be tapered (if needed) and discontinued at least 2 weeks prior to the preparation visit.
  - Oral anticoagulation or anti-aggregation medications (with the exception of low doses of acetylsalicylic acid as prophilaxis for cardiac events) are not allowed from the Screening visit and up to the first visit after treatment administration (Week 6). If anticoagulation/anti-aggregation is mandatory, the subject must be switched to appropriate doses of low-molecular weight heparin (LMWH) as per standard practice during the Screening visit.
  - Use of any investigational therapy is not allowed within 3 months prior to the Screening visit.

3.2.4.2 Concomitant Medication

Concomitant medications are all medications taken on or after the date of Treatment administration (Visit 0), including those started before but which are ongoing the day of Visit 0.

Concomitant Medication Allowed

- The study follows an add-on design, thus, any ongoing treatments [i.e., immunosuppressants (IS) and/or biologics: anti-TNF, anti-integrin or anti-IL 12/23] for Crohn´s disease at Screening visit shall continue at unchanged doses, throughout the study, except for complications derived from their use, in which case a decrease in dose or suspension of the drug will be allowed. Concomitant ongoing treatment at randomization will be balanced between both treatment arms with a stratified allocation (see Section 3.5.3) according to single therapy or combination therapy of IS and biologics or none.
- The use of 5ASA. Dose changes will be allowed only to decrease the initial dose.
- Medications taken by the subject for any other prior/emergent disease(s)/conditions, other than systemic steroids will be allowed specifically in the circumstances specified below:
  - Inhalatory steroids or steroid-containing topical creams (exclusively for use

anywhere outside the perianal or perineal region) or drops (for ophthalmic/ear use) are allowed anytime during the study.

- Hydrocortisone, at replacement doses for adrenal insufficiency treatment and/or single intravenous dose of up to 300 mg acutely (or up to 60 mg of prednisolone) for the treatment of severe allergic reactions or life-threatening shock, are allowed anytime during the study.
- Orally administered, non-systemically acting, steroids as budesonide at 6 mg/daily for CD flare prevention.

- Medication taken for any disease or condition other than CD prior to trial entry will be allowed to continue during the entire experimental period of the study as deemed adequate by investigator and/or subject's primary care physician.
- In addition, investigator will dispense any medication needed to treat any emergent AE or any other new condition/disease related or not to CD diagnosis occurring during the study.
- Antibiotic prophylaxis starting immediately following fistula curettage in the preparation visit will be given to all subjects during 7 days. Recommendation is broad-spectrum coverage including anaerobes (i.e. *B.Fragilis*) with ciprofloxacin and/or metronidazole, unless documented intolerance or contraindication, in which case antibiotics will be switched accordingly to another with similar spectrum coverage. Subjects receiving metronidazole will be instructed to avoid any alcohol consumption during the same period and up to 48 hours after the last dose.
- Short course antibiotics (up to two weeks) for emerging suspected/documented infections different from perianal disease will be accepted without interfering with efficacy evaluation. Antibiotic prophylaxis should be avoided whenever is possible.
- Low-molecular weight heparin (LMWH).

Concomitant Medication **Not Allowed**

- Oral anticoagulation is not allowed from the Screening visit and up to the first visit after treatment administration (Week 6), during this period if needed subject must be on LMWH treatment.
- Use of systemic corticoids.
- Antibiotic prophylaxis in the absence of suspected/documented infection after Treatment administration visit (Visit 0).
- Extended antibiotic treatment beyond two weeks, unless documented infection and clinically indicated.
- Investigational products.

3.2.4.3 Rescue Medication and Procedures

Special circumstances where concomitant medication and other medical interventions will affect the evaluability of the subjects: rescue medication for CD, antibiotics, surgery and other situations leading to treatment failure (TF) or non-response imputation.

- Switch to or addition of any new immunosuppressant or mAb, not ongoing at Screening.

- Increase in dose or frequency of any prior ongoing immunosuppressant or mAb.
- Systemic or rectal steroids for a CD flare.
- Prolonged use of systemic antibiotics (more than two weeks) to treat perianal disease or any other suspected/documented infection after the treatment administration visit (V0).
- Subjects starting any investigational drugs for CD or any other local investigational treatments in the perianal region while participating in the study.
- Any new surgical procedure required in the perianal region for the fistula(s) or draining of collections or established abscess(es) or any ostomy required due to luminal CD flare.

Subjects requiring any of these rescue medications or interventions during the study will not be withdrawn from the study and will attend further visits for safety follow up until Week 52.

The criteria about rescue medication or interventions are applicable from the time it occur(s) until the remaining follow up throughout the study up to Week 52.

3.3 Efficacy and Safety Assessments / Variables

3.3.1 Efficacy and Safety Measurements Assessed

3.3.1.1 Study Procedures

As described in the previous study schedule of assessments, there are 7 visits scheduled up to Week 24, 2 visits scheduled for the follow-up period up to 52 weeks, and an Early Termination Visit, in addition to, eventually, unscheduled phone-call follow-up.

3.3.1.1.1 Screening Visit

From Screening visit to Preparation Visit there will be a maximum of 5 weeks.

- Informed consent: a written informed consent will be obtained from the subject before any study procedure is performed.

  A separate informed consent pertaining to the voluntary exploratory endpoints (obtaining tissue/fluids from the fistula curettage procedure and microbiome sampling) must be obtained prior to any assessment procedures and is included as part of the main ICF (where applicable). The provision of this consent is optional and independent of consent to the other aspects of the study.

- Medical history assessment will include smoking history and current status (number of pack-years), number of pregnancies, if applicable, transplantations, and blood transfusion(s).
- History of Crohn's disease and perianal fistula(s) will be recorded (date of diagnosis, perianal fistula(s) [start and stop dates of previous perianal fistulas, start date of perianal fistula(s) ongoing at Screening visit], number and type of previous surgical procedures including seton placement.
- Physical examination covering all body systems and including weight and height.

- Vital signs: systolic and diastolic blood pressure [mmHg], heart rate [beats/min], and body temperature [ºC].
- Serum pregnancy test, for females of childbearing potential.
- Hematology: hemoglobin, hematocrit, erythrocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelet count.
- Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total bilirubin is above the ULN), potassium, sodium, chloride.
- Blood sample for soluble factors studies.
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification). Fistula must have been draining for at least 6 weeks prior to Screening visit. The clinical assessment will consist of a physical examination of the fistula(s) by a blinded investigator to evaluate the presence of drainage spontaneously or after gentle finger compression through the external openings. The tracts and external openings must be clearly identified in the eCRF in order to ensure the same tracts are assessed during the study period.
- A detailed diagram will be included to locate and describe the external openings and draining status on every visit, by the blinded team (APPENDIX 2).
- Pelvic MRI performed locally (number of fistulas, location, type, collection(s) measured in 3 dimensions, if any, with fistula location). A quality copy will be sent within 24 hours from acquisition to the Central Imaging Lab for immediate blinded central MRI reading as detailed in the specific manual (i.e., the Image Acquisition Guidelines; as per these guidelines, turnaround is 5 days assuming images are adequately acquired according to image acquisition guidelines). Blinded central MRI results (number of fistulas, location, type, collection[s] measured in 3 dimensions, if any, with fistula location) will be communicated to the investigator and the surgeon prior to Preparation visit to guide the surgeon through the preparation process.
- PDAI[24,41]/PRO-2 (Perianal Disease Activity Index and Patients Reported Outcomes from CDAI) scores will be assessed and a diary to fill the information of the PRO-2 scale will be given to the subject (provided as a separate document). PRO-2 score will be calculated by the investigators before randomization based on subject's diary and according to the document provided in the protocol (APPENDIX 1).

  PRO-2 includes only 2 predictor variables, i.e. the number of liquid stools and the abdominal pain according to the same scoring system. PRO-2 combines the average daily liquid or soft stools frequency and the average daily abdominal pain severity. PRO-2 has not been validated so far although it is consensual that these are the most predictive variables of the CD activity. Its correspondence to the CDAI score[23, 24] is as follows[32]:

  - Remission: CDAI <150 = PRO-2 <8

  - CDAI <220 points = PRO-2 <14
  - CDAI 100 points drop = delta 8 points drop in PRO-2
  - CDAI 70 points drop = delta 5 points drop in PRO-2
- A device to collect electronically Patient Reported Outcomes (ePROs) will be provided to the subject:
  - VAS for pain while standing, sitting, and defecating; number of pads along last 2 weeks and CDAI symptom diary are to be entered in the handheld device by the subject daily at home during the 2 weeks prior to the visit (starting right after the Screening visit).
  - Work Productivity and Activity Impairment questionnaire (WPAI), EuroQoL 5 Dimensions (EQ-5D), SF36 and Health Resources Utilization (HRU) will be assessed and data will be entered in the handheld device during the subject's clinic visit.
- Review and record previous medication within 2 years before the Screening visit day, medication related to the treatment of Crohn's disease, the treatment of perianal fistulas, and anal abscesses. The following information regarding previous medication will also need to be reviewed and recorded: inadequate response, loss of response or intolerance to immunosuppressive agents (azathioprine, mercaptopurine, methotrexate), TNF antagonists, vedolizumab or uztekinumab. Within the last 6 months before the Screening visit, all blood products, steroids, and intravenous medications will need to be recorded; all other prior medications administered within 1 month prior to the screening visit should also be recorded.
- Check and record if endoscopy (colonoscopy) has been performed within 6 months prior to this Screening visit and if is possible to document the following, otherwise a new colonoscopy needs to be planned:
  - the absence of any colonic large ulcers larger than 0.5 cm.
  - the improvement or no worsening in abdominal pain and/or in the diarrhea (based on the average daily liquid or soft stools frequency and the average daily abdominal pain severity), sustained for one week or more, since the last colonoscopy was performed until the Screening visit.
  - No hemoglobin decrease greater than 2.0 g/dL or an unexplained rising C-reactive protein (CRP), greater than 5.0 mg/L to a concentration above the referenced ULN (unless the rise is due to a known process other than luminal Crohn's Disease), since the last colonoscopy was performed as compared to results during the Screening visit.
  - that there were no initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs dose regimen since the last endoscopy up to Screening visit.

  If an adequate colonoscopy was not performed within 6 months prior to the Screening Visit or the information requested as above is not adequately documented, then it has to be planned and performed to calculate the SES-CD score to be not more

than 6 and exclude severe proctitis (defined as any rectal ulcers larger than 0.5 cm) to check elegibility before next visit (preparation visit).

- Review and record concomitant medication including all medications taken by the subject at the time of the Screening visit.
- Schedule Preparation visit, transfer the Screening local MRI to the surgeon prior to Preparation visit and check that the results of the central blinded MRI are available to be communicated and review by the surgeon prior/during the Preparation visit.
- Assess and record AE at the time of Screening visit after the ICF has been signed by the subject.
- For microbiome collection (optional), kits for at home fecal sample collection will be provided to the subject. Additional details will be provided in the Laboratory Manual.

3.3.1.1.2 Preparation Visit

Remember: A minimum of 2 weeks and a maximum of 3 weeks are required between the preparation visit and the study Treatment Administration Visit/Visit 0 (necessary to have the treatment ready for administration).

Note: if an adequate endoscopy was not performed within the last 6 months or report was not available, a colonoscopy should have been scheduled and performed to check subject's eligibility before this visit.

- Surgeon should have received the Screening local pelvic MRI and the blinded central MRI review results prior to the Preparation visit.
- Prior to fistula preparation (to be performed by the blinded team):
    - Physical examination covering all body systems and including weight.
    - Assess and record Adverse Events.
    - Review and record concomitant medication (including any blood transfusion).
    - Assess and record all electronic Patient Reported Outcomes .
- Fistula preparation, consisting on EUA, curettage and seton placement for ALL subjects by the surgeon according to the Surgery Procedure Manual (provided as a separate document). This must be done at least 2 weeks and a maximum of 3 before the study treatment administration day.
    - Collection of curettage material (optional), details will be provided in the Laboratory Manual.
    - Microbiome collection (optional) from the fistula, details will be provided in the Laboratory Manual.
- Mandatory antibiotics coverage will be administered during at least 7 days following the fistula curettage (ciprofloxacin and/or metronidazole are recommended unless documented previous intolerance).

- Extra whole blood sample for cell responses and immunological tests:
    - Presence/absence of anti-donor antibodies.
    - Cell responses: study of cellular and soluble factors.
- Review of inclusion and exclusion criteria prior to randomization, including applicable central laboratory results and central MRI reading confirmation.
- Randomization of the subjects will be requested after the preparation procedure and after reviewing all the exclusion and inclusion criteria, allowing a minimum of 2 weeks and a maximum of 3 weeks before treatment administration.
- Schedule study Treatment Administration/Visit 0 (Day 0).

Randomization

Randomization of the subjects will be requested after the preparation procedure and the review of all the exclusion and inclusion criteria have been completed. The randomization will be performed through an IWRS procedure, which will also trigger the commencement of the IMP preparation process.

3.3.1.1.3 Study Treatment Administration/Visit 0 (Day 0)

The study treatment administration visit will take place in a minimum of 2 weeks and a maximum of 3 weeks from the preparation visit.

1. Prior to study treatment administration (to be performed by the blinded team):

- Physical examination including weight.
- Vital signs: temperature, heart rate and blood pressure.
- Urine pregnancy test, for women of childbearing potential (WCBP).
- Central Laboratory tests
    - Hematology: hemoglobin, hematocrit, erythrocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelet count.
    - Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total bilirubin is above the ULN), potassium, sodium, chloride.
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification).
- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit.
- Patient Reported Outcomes:

  - VAS for pain while standing, sitting, and defecating; number of pads along last 2 weeks and CDAI symptom diary. These will be entered in the handheld device daily by the subject at home during the 2 weeks prior to the visit.
  - WPAI, EQ-5D, SF36 and HRU will be assessed and data will be entered in the handheld device during the subject's clinic visit.

- Extra whole blood sample for cell responses and immunological tests should be taken before initiation of the study treatment procedures as in Section 3.3.1.1.3:
  - Presence/absence of anti-donor antibodies.
  - Cell responses: study of cellular and soluble factors.
- Assess and record Adverse Events.
- Review and record concomitant medication, taken since last visit (including any blood transfusion).
- Schedule Visit 1/ W6 and remind subject to complete at home the ePRO questionnaires (VAS, number of pads used and CDAI symptom diary) starting 2 weeks prior to the next visit.

2. Study treatment administration:

- All setons must be withdrawn; fistula(s) curettage should be done, placing a stitch on each internal opening according to the Surgery Procedure Manual (provided as a separate document). Subjects will be observed after their surgical procedure until full recovery, with special attention to signs and symptoms of potential allergic reactions.
- Collection of curettage material (optional) before treatment administration, details will be provided in the Laboratory Manual.
- Microbiome collection (optional) from the fistula, details will be provided in the Laboratory Manual.
- Similar specific training material will be prepared and implemented to standardize the procedures at all sites. Please refer to the Surgery Procedure Manual. More information is included in section 3.5.

  An unblinded sponsor representative will attend the first treatment administration, during the surgical procedure, at each site (and some of the subsequent if needed upon request) to monitor the compliance to the Surgery Procedure Manual, especially the proper study treatment administration and to provide assistance to the surgeon's questions if needed.

  Note that if there is any problem administering the IMP during Visit 0, the visit will need to be rescheduled. It will not be necessary to repeat the preparation visit, the setons will be maintained until the rescheduled treatment visit and will be withdrawn just before the injection of the IMP. The timeframe for the new V0 should be minimum of 2 weeks to a maximum of 3 weeks from the date of the original V0. All V0 procedures will be repeated during the rescheduled visit.

3.3.1.1.4 Week 6 Follow-up/Visit 1 (Day 42 ±8)

- Physical examination including weight.
- Vital signs: temperature, heart rate, and blood pressure.
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification).
- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit
- Extra whole blood sample for cell responses and immunological tests:
    - Presence/absence of anti-donor antibodies.
    - Cell responses and soluble factors.
- Assess and record Adverse Events.
- Review and record concomitant medications taken since last visit (including any blood transfusions).
- For microbiome collection (optional), kits for at home fecal sample collection will be provided to the subject. Additional details will be provided in the Laboratory Manual.
- Schedule Visit 2/W12 and remind subject to complete at home the ePRO questionnaires (VAS, number of pads used and CDAI symptom diary) starting 2 weeks before next visit.

3.3.1.1.5 Week 12 Follow-up/Visit 2 (Day 84 ±8)

- Physical examination including weight
- Vital signs: temperature, heart rate, and blood pressure
- Urine Pregnancy test, for WCBP
- Central Laboratory tests
    - Hematology: hemoglobin, hematocrit, erythrocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelet count.
    - Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total is above the ULN), potassium, sodium, chloride.
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification).

- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit.
- Patient Reported Outcomes:
    - VAS for pain while standing, sitting, and defecating; number of pads along last 2 weeks and CDAI symptom diary. These will be entered in the handheld device daily by the subject at home during the 2 weeks prior to the visit.
    - WPAI, EQ-5D, SF36 and HRU will be assessed and data will be entered in the handheld device during the subject's clinic visit.
- Extra whole blood sample for soluble factors
- Assess and record Adverse Events
- Review and record concomitant medications taken since last visit (including any blood transfusions)
- Schedule Visit 3/ W18

3.3.1.1.6 Week 18 Follow-up Visit/Visit 3 (Day 126 ±8)

- Physical examination including weight
- Vital signs: temperature, heart rate, and blood pressure
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification)
- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit
- Assess and record Adverse Events
- Review and record concomitant medication, taken since last visit (including any blood transfusion)
- Schedule Visit 4/W24 and remind subject to complete at home the ePRO questionnaires (VAS, number of pads used and CDAI symptom diary) starting 2 weeks before next visit

3.3.1.1.7 Week 24 Follow-up Visit/Visit 4 (Day 168 ±8)

- Physical examination including weight
- Vital signs: temperature, heart rate, and blood pressure
- Urine Pregnancy test, for WCBP
- Central laboratory tests
    - Hematology: hemoglobin, hematocrit, erythrocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), leukocytes,

lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelet count.

- Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total is above the ULN), potassium, sodium, chloride.

- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification).
- Pelvic MRI performed locally (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location). A quality copy will be sent to the Central Imaging Lab for blinded central MRI reading (blinded to sequence and treatment) as detailed in the specific manual (Image Acquisition Guidelines). Results at Week 24 will include assessment of collections >3mm in three axes and directly related to the fistula tracts treated, and any new tracts that might appear. MRIs will also be assessed for Van Assche score, hyperenhancement in T1 sequence, and hyperintensity in T2 sequence.
- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit.
- Patient Reported Outcomes:
  - VAS for pain while standing, sitting, and defecating; number of pads along last 2 weeks and CDAI symptom diary. These will be entered in the handheld device daily by the subject at home during the 2 weeks prior to the visit.
  - WPAI, EQ-5D, SF36 and HRU will be assessed and data will be entered in the handheld device during the subject's clinic visit.
- Extra whole blood sample for cell responses and immunological tests:
  - presence/absence of anti-donor antibodies
  - Cell responses and soluble factors
- Assess and record Adverse Events
- Review and record concomitant medication, taken since last visit (including any blood transfusion)
- Schedule Visit 5/W36

3.3.1.1.8 Week 36 Follow-up Visit/Visit 5 (Day 252 ±15)

- Physical examination including weight
- Vital signs: temperature, heart rate, and blood pressure
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification)

- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit
- Assess and record Adverse Events
- Review and record concomitant medications taken since last visit (including any blood transfusions)
- Schedule Visit 6/W52 and remind subject to complete at home the ePRO questionnaires (VAS, number of pads used and CDAI symptom diary) starting 2 weeks before next visit.

3.3.1.1.9 Week 52 Follow-up Visit/Visit 6 (Day 364 ±15)

- Physical examination including weight
- Vital signs: temperature, heart rate, and blood pressure
- Urine Pregnancy test, for WCBP
- Central laboratory tests
  - Hematology: hemoglobin, hematocrit, erythrocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelet count
  - Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total is above the ULN), potassium, sodium, chloride
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification)
- Pelvic MRI performed locally (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location). A quality copy will be sent to the Central Imaging Lab for blinded central MRI reading (blinded to treatment) as detailed in the specific manual (Image Acquisition Guidelines). Results at Week 52 will include assessment of collections >3 mm in three axes and directly related to the fistula tracts treated, and any new tracts that might appear. MRIs will also be assessed hyperenhancement in T1 sequence, and hyperintensity in T2 sequence
- Extra plasma sample for immunological tests:
  - presence/absence of anti-donor antibodies and soluble factors
- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit
- Patient Reported Outcomes:
  - VAS for pain while standing, sitting, and defecating; number of pads along last 2 weeks and CDAI symptom diary. These will be entered in the handheld device by the subject daily at home during the 2 weeks prior to the visit.
  - WPAI, EQ-5D, SF36 and HRU will be assessed and data will be entered in the handheld device during the subject's clinic visit.

- Assess and record Adverse Events
- Review and record concomitant medications taken since last visit (including any blood transfusions) and confirm subject's status

3.3.1.1.10 Early Termination Visit (±15 days of early termination):

Subjects must remain in the study and not withdrawn unless subject´s decision. All efforts should be made to keep subjects in the study instead of being withdrawn.

- Physical examination including weight.
- Vital signs: temperature, heart rate, and blood pressure.
- Urine Pregnancy test, for WCBP.
- Central laboratory tests:
    - Hematology: hemoglobin, hematocrit, erythrocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelet count.
    - Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total is above the ULN), potassium, sodium, chloride.
- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification).
- Pelvic MRI performed locally (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location). A quality copy will be sent to the Central Imaging Lab for blinded central MRI reading (blinded to sequence and treatment) as detailed in the specific manual (Image Acquisition Guidelines). Results will include assessment of collections >3mm in three axes and directly related to the fistula tracts treated, and any new tracts that might appear. MRIs will also be assessed for hyperenhancement in T1 sequence, and hyperintensity in T2 sequence, and Van Assche score will be calculated.
- PDAI and PRO-2 scores will be assessed and data will be entered in clinic during the visit.
- Patient Reported Outcomes:
    - VAS for pain while standing, sitting, and defecating; number of pads along last 2 weeks and CDAI symptom diary. These will be entered in the handheld device by the subject daily at home during the 2 weeks prior to the visit.
- Extra whole blood sample for cell responses and immunological tests (to be done only if ET visit occurs prior to W24 [Visit 4], if ET visit occurs after W24 visit was completed, only a plasma sample for immunological tests has to be collected):
    - presence/absence of anti-donor antibodies

- Cell responses and soluble factors

- Assess and record Adverse Events
- Review and record concomitant medications taken since last visit (including any blood transfusions)

3.3.1.1.11 Unscheduled Telephone Follow-up Calls:

Unscheduled telephone-calls are proposed for safety follow-up in case the subject cannot attend to any interim visits (W6, W12, W18, W36) or for any contact requested by the subject between scheduled visits. The following information needs to be recorded:

- Unscheduled phone-call follow-up date and reason
- Review electronic Patient Reported Outcome questionnaires since last visit
- Assess and record Adverse Events
- Review and record concomitant medication, taken since last visit (including any blood transfusion)

3.3.2 Efficacy and Safety Endpoints

3.3.2.1 Primary Endpoint

Proportion of subjects who achieve combined remission at Week 24 after IMP administration, where combined remission is defined as:

a) The closure of all treated external openings that were draining at baseline despite gentle finger compression
AND
b) Absence of collection(s) >2 cm (in at least 2 dimensions) of the treated perianal fistula(s) confirmed by blinded central MRI assessment.

3.3.2.2 Secondary Endpoints

Key Secondary Efficacy

- Proportion of subjects who achieve clinical remission at Week 24 after IMP administration, where clinical remission is defined as the closure of all treated external openings that were draining at baseline despite gentle finger compression.
- Time to clinical remission (weeks) assessed at Week 24, defined as the time from treatment start to first visit with closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical remission by Week 24 will be censored at that visit.

Other Secondary Efficacy Endpoints

- Proportion of subjects who achieve clinical response at Week 24 after IMP administration, where clinical response is defined as closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression.
- Time to clinical response (weeks) assessed at Week 24, defined as the time from treatment start to first visit with closure of at least 50% of all treated external openings that were

draining at baseline, despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical response by Week 24 will be censored at that visit.

- Proportion of subjects who achieve combined remission at Week 52 after IMP administration, where combined remission is defined as:
    a) The closure of all treated external openings that were draining at baseline despite gentle finger compression,
    AND
    b) Absence of collection(s) >2 cm (in at least 2 dimensions) confirmed by blinded central MRI assessment.
- Proportion of subjects who achieve clinical remission at Week 52 after IMP administration, where clinical remission is defined as closure of all treated external openings that were draining at baseline despite gentle finger compression.
- Proportion of subjects who achieve clinical response at Week 52 after IMP administration, where clinical response is defined as closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression.
- Time to clinical remission (weeks) assessed at Week 52, defined as the time from treatment start to first visit with closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical remission by Week 52 will be censored at that visit.
- Time to clinical response (weeks) assessed at Week 52, defined as the time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical response by Week 52 will be censored at that visit.
- Proportion of subjects with a relapse from Week 24 combined remission response, where a relapse is defined as:
    a) Reopening of any of the treated fistula(s) external openings with active drainage as clinically assessed in subjects who were in combined remission,
    OR
    b) The development of a perianal fluid collection >2 cm of the treated perianal fistulas confirmed by centrally read magnetic resonance imaging (MRI) assessment.

Safety Endpoints

- Incidence of treatment-emergent AEs (TEAEs)
- Incidence of treatment-emergent SAEs.
- Incidence of adverse events of special interest (AESIs).
- Vital signs.
- Laboratory parameters.

3.3.3 Exploratory Endpoints

- Change from baseline in total Perianal Disease Activity Index (PDAI) score at Weeks 6, 12, 18, 24, 36, and 52.
- Change from baseline in total PRO-2 score (defined as average daily stool frequency and average daily abdominal pain) at Weeks 6, 12, 18, 24, 36, and 52.

- Change from baseline in blinded central MRI Van Assche score at Weeks 24 and 52.
- Change from baseline in modified Van Assche blinded central MRI score at Weeks 24 and 52.
- Change from baseline in the Magnetic Resonance Novel Index for Fistula Imaging for Crohn's disease (MAGNIFI-CD) score at Weeks 24 and 52.
- Change from baseline at Weeks 24 and 52 in blinded central MRI analysis of hyperenhancement in T1 sequence, and hyperintensity in T2 sequence.
- Change from baseline in electronic Patient Reported Outcomes (PRO) listed below at Weeks 12, 24, and 52:
    a) Visual Analogue Scale (VAS) from 0 to 10 for perianal pain while standing, sitting, and defecating along last 2 weeks prior to the visit,
    b) CDAI items score,
    c) Number of pads used per day along last 2 weeks prior to each visit,
    d) Work Productivity and Activity Impairment Questionnaire (WPAI),
    e) EQ-5D[44],
    f) SF36,
    g) Health Resources Utilization (HRU).
- Immunogenicity responses as measured by donor-specific antibody (DSA) levels.
- Change from baseline in cytokines, immune- and other inflammation-associated markers at Weeks 6, 12, 24, and 52.
- Change from baseline in the microbiome diversity at Week 6.

3.4 Study Discontinuations

3.4.1 Discontinuation of Individual Subjects

3.4.1.1 Reasons for Withdrawal

Participation in the study is strictly voluntary. A subject has the right to withdraw from the study at any time for any reason. Subjects who withdraw their consent are considered withdrawn from the study. The investigator has the right to terminate participation of any subject at any time if it is deemed in the subject's best interest. The reason and circumstances for premature discontinuation will be documented in the subject's CRF.

Reasons of withdrawal include, but are not limited, to the following:

- Subject's decision; withdrawal of subject consent to participate in the study;
- Physician's decision based on subject's well-being;
- Participation in a new, interventional, clinical trial for CD and/or for perianal disease;
- Death.

For any consenting subject discontinuing the study, the investigator should plan ahead to:

Ask subject to take part, as far as possible, in the last medical visit in order to examine the subject's health conditions and perform the required blood sampling for the clinical blood tests, the serum pregnancy test (if it is required), the physical examination including the fistula clinical assessment, the pelvic MRI, the PDAI/ PRO-2 scores, AE assessment and concomitant medication recording.

Complete the eCRF, indicating in the Study Completion Visit, the date of termination and the unique reason for discontinuing the study, with all data not corresponding to a formal visit filled in the Early Termination visit.

In consenting subjects who do not come back for the scheduled study visits, documented efforts should be performed to convince them to continue attending study visits and, if unsuccessful, at least the exact reason(s) should be obtained for their discontinuation and any adverse event associated to it and its date of occurrence (at least month and year) should be recorded.

3.4.1.2 Handling of Withdrawals

Randomized subjects who withdraw their consent or are removed should undergo the Early Termination visit, if agreed by the subject (unless some specific procedure, i.e., pelvic MRI, assessments were conducted within 2 weeks [+/- 1 week window] prior to withdrawal). These data will be dated and registered in the eCRF.

3.4.1.3 Replacement of Withdrawals

No randomized subjects will be replaced, regardless of reason.

3.4.2 Discontinuation of Entire Study

If the study is prematurely terminated or suspended for any reason, the investigator has to inform the subjects and to ensure appropriate follow-up for them. The sponsor must promptly inform the investigators/institutions, and the regulatory authorities of the termination or suspension and the reason(s) for the termination or suspension. The Independent Ethics Committee (IEC) / Institutional Review Board (IRB) must also be informed promptly and provided with the reason(s) for the termination or suspension by the sponsor, or by the investigator/institution, as specified by the applicable regulatory requirement(s).

Reasons for terminating the study may include, but are not limited to the following:

- Incidence or severity of AE indicating a potential health hazard to subjects
- Subject enrolment is unsatisfactory
- Investigator does not adhere to protocol or applicable regulatory guidelines in conducting the study
- Ethical or medical reasons

3.5 Treatments

3.5.1 Treatments Administered

Cx601 is a 24 mL suspension of human expanded adipose-derived stem cells (eASC) of allogeneic origin in aseptic buffered human albumin solution presented in disposable vials with no preservative agents. The cells will be given at a dose of 120 million cells (5 million cells / mL) locally injected into the tissue around the internal openings and into the walls of the fistula tracts.

Placebo (saline solution) will be locally injected into the tissue around the internal openings and into the walls of the fistula tracts at the same quantity (volume: 24 mL) and following the same procedure described for the eASC. Throughout this protocol the treatment administrated in the study meaning Cx601 or matched-placebo will be referred to as IMP.

3.5.2 Identity of Investigational Product(s)

3.5.2.1 Dosage Form, Dose, and Administration Route

Investigational ATMP

Cx601 is a 24 mL suspension of human expanded adipose-derived stem cells (eASC) of allogeneic origin in aseptic buffered human albumin solution presented in disposable vials with no preservative agents. Cells are obtained through lipoaspiration from healthy individuals and expanded *ex vivo*. Cx601 for clinical use is supplied as a sterile, clear, white to yellowish suspension for local injection, provided in 4x6 mL vials (suspension of 5 million eASC per mL of Dulbecco Modified Eagle's Medium [DMEM] with human serum albumin). See the label on the investigational product vial for expiration date and time.

eASC will be re-suspended manually, moving them in a gentle manner, avoiding bubble formation, until a homogeneous suspension with no visible lumps is obtained. Once cells are re-suspended, the resulting cell suspension should be used immediately to prevent re-sedimentation of the cells. The cell suspension will be aspirated then into syringe using a conventional needle no thinner than 22G. The needle used to aspirate cells will be replaced by a longer needle, again no thinner than 22G.

A longer needle is needed in order to reach the internal opening(s) (e.g., 88 mm long) (needles used for spinal anesthesia around 90 mm long are adequate for such a purpose). The needle will be entered through the anus, and the contents of 2 vials of Cx601 will be injected into the tissue surrounding the internal opening(s), making several small blebs.

A needle will then be entered through the external opening(s), and the contents of the other 2 vials of Cx601 will be injected superficially along the walls of the fistula tracts to be treated, making several small blebs. It is important not to inject Cx601 into the lumen of the fistula tract(s) in order to avoid the leakage of cells. Specific training material will be prepared to homogenise all sites, and the eCRF will include a checklist of the fistula preparation and treatment administration procedures. In order to provide further assistance to the sites a sponsor representative specifically trained and experienced in administration protocol might be available at site. The Surgical Procedure Manual is provided as an independent document.

Placebo

The study placebo will consist of 24 mL of a matching saline solution for local injection in the fistula and will follow the same administration schema described for Cx601.

3.5.2.2 Packaging and Labelling

Packaging and labelling of the study drugs will be performed by the sponsor or its designee according to Good Manufacturing Practices (GMP) principles and local regulation. Products' labelling will report the following information:

- Name and address and contact phone of the Sponsor and Manufacturer
- The reference of the trial: code
- Pharmaceutical dosage form and administration route
- The number and units of the dosage form, batch number and expiry date and time
- The statement “clinical research sample”
- The assigned subject number
- Storage conditions
- The statement “do not irradiate”
- The investigator name and the site number
- The statement “allogeneic use”
- Use according the instruction manual included

Container

The product, Cx601, (cell suspension) is supplied in duly labelled glass vials, tightly closed with rubber stoppers and sealed with an aluminium cap.

The packaging material is made up by:

- Immediate package: type 1 glass sterile vials with sterile rubber stopper and aluminium seal
- Labels of white polyethylene printed in black ink by thermal transfer printer

Secondary packaging: cardboard box with corporate design containing inside the vials duly labelled.

“IMP Handling Instructions” is a printed document in which the product characteristics, indication, and method for use are described, is also enclosed with each product batch.

The placebo (saline solution) will be supplied in the same packaging as Cx601 for study purposes.

3.5.2.3 Storage and Disposition of Study Drug

Cx601 product, and matching placebo, will be shipped under temperature controlled conditions, using appropriate transport for biological samples. Shipping material is also duly labelled and has an attached package content list and instructions for use.

Specific instructions will be provided within a separate study manual. The study medication must be stored under the storage conditions specified in the manual.

3.5.2.4 Handling of Study Medication

Study medication will be shipped by specialised couriers to the hospital pharmacy or the corresponding operating room where the study treatment administration will be performed, according to local practice and/or regulations. See the label on the investigational product vial for expiration date and time.

The Drug Administrator or Designee will maintain adequate records of the receipt and disposition of study drug shipment to the site. All used and unused vials of study drug must be recorded and tracked until data is monitored. Vials then should be destroyed locally and destruction will be documented as appropriate.

Study Drug administration must be performed by authorised personnel with appropriate protocol training.

A specific Surgery Procedure Manual will be provided to the site as a separate document, ensuring appropriate training.

3.5.3 Method of Assigning Subjects to Treatment Groups

Treatments will be allocated by central randomization through interactive web recognition system (IWRS) and subjects will be stratified using the following criteria:

- Current immunosuppressant or biologics as single agent or as a combination or no ongoing concomitant treatment at Screening visit
- External opening(s) (1 or >1)

3.5.4 Blinding

Treatments will be prepared for administration close to the surgery room but if possible in a separate room and the administration of the treatment will be partially masked. The cell suspension will be aspirated into a syringe to unable identification of the IMP. There will be a specific blinding plan at every site, agreed and signed by the sponsor and the corresponding site personnel involved before any inclusion at that site.

However, in addition, the double-blind design will be preserved by having one investigator (administrator surgeon) who will administer the treatment and another investigator (efficacy evaluator) who will evaluate the fistula in a blinded fashion.

Members of the unblinded surgical team (i.e. assistant to surgeon) are not allowed to share any information about the potentially suspected study treatment used in the surgery procedure with the blinded investigators team (efficacy evaluators), but they will clearly identify the treated fistula(s) in order to allow the evaluator to assess the efficacy during the study visits.

Additionally, surgeons who administer the study treatment and any ancillary personnel involved in study treatment administration will not be allowed to participate in any clinical assessment of the fistula(s) during the study.

The emergency unblinding is available 7 days a week, 24 hours through the IWRS. Details are described in the IWRS manual.

The primary outcome of this study is determined based on the results of the efficacy analyses at Week 24. To evaluate long-term safety and efficacy of Cx601 compared to placebo, the study will continue in a blind fashion up to Week 52. Therefore, individuals unblinded to subject-level treatment allocation at Week 24 readout will not be directly involved in study conduct after they are unblinded. In order to be able to minimize bias for the treatment comparison of efficacy and safety at Week 52, study conduct during this period will be handled by a separate team who will remain blinded to subject level treatment allocation at all times during the conduct of the study.

3.5.5 Treatment Adherence

Treatment adherence will be ensured because treatment will be injected by clinical staff at Visit 0 (Day 0).

3.5.6 Drug Accountability

The drug accountability will be performed at the site. All used vials (eASC and placebo) will be stored at site until the local monitor has performed the corresponding documented reconciliation and drug accountability. Any dosage deviation should be clearly notified. The corresponding destruction will be documented as per local procedures and regulations (e.g. destruction certificate issued and filed in the corresponding study files).

3.6 Study Design, Including the Choice of Control Groups

Dose identification for cell therapy in clinical studies does not follow the same models used for traditional drugs. The models used to classically determine the dose are based on the Phase I studies, in which different aspects are assessed and taken into consideration such as the metabolic routes up to the excretion of the product. A cell therapy medicinal product consists of cells and not of isolated molecules. Therefore, cell products are not subject to commonly defined laws in pharmacokinetic and pharmacodynamic of traditional pharmacology products. For example, the absence of metabolism by hepatic CYPs is rather obvious. For these reasons, the decision related to a dose administration in cell therapy, even though based on scientific driven clinical data, does not follow the traditional diagram.

The safety and feasibility of local eASC administration has been demonstrated in previous phase I/II and III studies in which allogeneic eASC were injected in a dosage of 20-40 million cells, and a single dose of 120 million cells, respectively (see section 6.1).

The dose schedule consists of a single local administration of 120 million cells. No conventional dose finding studies have been performed and the rationale behind a single local administration is described in section 6.1.

Following the recommendations of the EMA guideline CPMP/EWP/2284/99, Rev.1, on the development of new medicinal products for the treatment of Crohn's disease[30], and the ICH E10, Step 4 July 2000 on choice of control group and related issues in clinical trials, this study will allow ongoing treatments continuation in an add-on design (i.e., immunosuppressants and/or biologics: TNF antagonists, anti-integrin or anti-IL12/23), thus the inclusion of a placebo comparator group is in accordance both with the Committee for Medicinal Products for Human

Use (CHMP, formerly CPMP) and the International Conference on Harmonization, and specifically for the treatment of Crohn's disease (CPMP/EWP/2284/99 Rev.1), which states: "*for an add-on indication, placebo is an acceptable comparator*". The placebo control group was considered acceptable in this situation by the CHMP at the Protocol Assistance meeting (meeting on 03 March 2011).

Similar training of the surgical procedure and study treatment administration will be implemented for all sites, with baseline homogeneity guaranteed by means of an exploration under anaesthesia, fistula curettage and seton placement for all the subjects, done at least 2 weeks before the administration day, and antibiotics prescribed during 7 days following fistula preparation (ciprofloxacin and/or metronidazole are recommended). Seton(s) will be withdrawn on the administration day, just before the administration of the study treatment. Similar training will be implemented for all sites, with specific training material (e.g., Surgical Procedure Manual) prepared to homogenise all of them.

4 Adverse Events

The development of the IMP, among others, is under the EU "Detailed guidelines on good clinical practice (GCP) specific to advanced therapy medicinal products (ATMP)" and it implies that relationship to procedure administration is to be also assessed for any adverse event reported among other obligations for specific reporting related to IMP administration and use.

Other guidances have being considered in the development of the study protocol; among other, US guidances ("Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) from Adipose Tissue: Regulatory Considerations"; "Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products, as an example").

All definitions listed below follow ICH E2A and apply for either IMP, its administration or procedure related to IMP preparations.

4.1 Definitions

4.1.1 Adverse Event Definition

An adverse event is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of an Investigational Medical Product (IMP), whether or not considered related to the IMP.

- Medical disorders, including concomitant diseases present at the time of signing the informed consent are only considered AEs if they worsen after this time. All baseline conditions should be recorded as part of Medical History.
- Changes in laboratory parameters (biochemistry, haematology), as well as abnormal results of other tests (worsening results), that are detected after the administration of study medication and that the investigator considers to be clinically relevant should be recorded as AEs or serious adverse events (SAEs), provided that the definitions given in this section and in the Section 4.1.4 ("Serious Adverse Event") are met, respectively. In contrast, clinically significant changes in laboratory parameters or other tests that are associated to the disease under study will not be rated as AEs or SAEs, unless the investigator judges them to be more serious than expected based on the subject condition.
- Fluctuations or re-occurrences of the disease under study (Crohn's disease), that are considered normal for the subject and are recorded in the Medical History, need not be reported as an AE. However, if the condition were to deteriorate (worsening) during the study this would then be recorded as an AE.
- For the purpose of this study, drainage of the treated fistula and abscesses, defining the primary endpoint, will not be captured as AEs unless there is evidence suggesting a causal relationship between the IMP or the administration procedure.

4.1.2 Adverse Reaction (AR)

All untoward and unintended responses to an IMP related to any dose administered. All AEs judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means that a relationship between the IMP and the adverse event cannot be ruled out. In this study, AEs for which no investigator assessment is available (missing or unknown) are considered as adverse reactions.

4.1.3 Unexpected Adverse Reaction (uAR)

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved IMP or the SmPC/ Product Information for a marketed drug).

Reports which add significant information on specificity or severity of a known, already documented Serious Adverse Reaction (SAR) constitute unexpected events. In the same way, when the outcome of the AR is not consistent with the applicable product information, this AR should be considered unexpected.

4.1.4 Serious Adverse Events

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital abnormality/birth defect, or
- Is a medically significant event, including any event or synonym described in the Takeda Medically Significant AE List (APPENDIX 3), or requires intervention to prevent at least one of the outcomes listed above, or
- Is a suspected transmission of an infectious agent.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situation, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. These events should also usually be considered serious. Examples of such adverse events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency (addiction) or drug abuse.

The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Hospital admission means that the subject has stayed at least 24 hours at the hospital or an emergency department for observation and/or treatment that could not have been made/administered at the physician's office or in an outpatient setting.

Prolongation of hospitalization is defined as any extension of an in-subject hospitalization beyond the stay required in relation to the original reason for the initial admission, as determined by the investigator or treating physician. Complications occurring during hospitalization are AEs. If a complication extends a hospitalization or meets any other seriousness criteria, the event will be considered a SAE.

The following reasons for hospitalization or prolongation of hospitalization are not considered AEs, and therefore not SAEs:

- Hospitalization for administration of IMPs. Exception: Hospitalization or prolonged hospitalization for a complication of study treatment administration will be reported as a SAE
- Hospitalization to perform an elective treatment of a condition prior to subject entry into the study without worsening from its pre-existing condition
- Hospitalization for diagnostic investigations (e.g., scans, endoscopy, sampling for laboratory tests, etc) that are not related to an adverse event
- Hospitalization for standard monitoring of a pre-existing disease or medical condition that is not worsen (e.g. hospitalization for coronary angiography in a subject with stable angina pectoris)
- Hospitalization for <24h (attendance at the Emergencies)
- Other reasons may include: hospitalizations for elective cosmetic surgery; due to social reasons or due to convenience reasons

4.1.5 Serious Unexpected Adverse Reaction (SUSAR)

All Suspected Adverse Reactions which occur in the trial and that are both unexpected and serious.

4.1.6 Adverse Events of Special Interest

An AE of special interest (serious or non-serious) is one of scientific and medical concern specific to the compound or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them and would be described in protocols and instructions provided for investigators as to how and when they should be reported to the sponsor.

AEs of special interest will include:

- Tumorigenicity
- Ectopic tissue formation
- Hypersensitivity
- Transmission of infectious agents
- Immunogenicity/alloimmune reactions
- Medication errors

4.2 Adverse Event Severity Assessment

Seriousness and Severity should not be confounded.

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache).

This is not the same as "serious," which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning (see Section 4.1.4). Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Severity of the adverse events will be recorded at the time they occur and will be rated according to the following criteria:

- Mild (asymptomatic), an event easily tolerated by the subject, causing minimal discomfort that does not prevent the subject from fulfilling daily activities.
- Moderate, symptomatic, but does not significantly interfere with function.
- Severe, causes a significant interference with function.

Death itself is not an adverse event, but rather the outcome of a previously reported event, which should be described using medical terminology. Only in those cases where either no cause for death is known (e.g. sudden death, Death NOS) the event "death" should be reported.

The terms death/death of unknown cause and sudden death are clearly distinct and must not be used interchangeably.

4.3 Adverse Event Relatedness Assessment (Causality Assessment)

The investigator must establish, based on his clinical judgment and the information on IMP provided by the sponsor the causal relationship between the investigational product and/or the surgical procedure and the occurrence of the AE/SAE.

The expression: "reasonable causal relationship" means to convey in general that there is evidence or argument to suggest a causal relationship. In this study, AEs that are considered as related to IMP, including for which no investigator assessment is available (missing or unknown) are considered as adverse reactions. For AEs considered as not related to study treatment it is assumed that there is no reasonable causal relationship.

Detailed considerations to be taken into account on causality assessment include:

- associative connections (time or place: plausibility),
- pharmacological explanations,
- previous knowledge of the drug,
- presence of characteristic clinical or pathological phenomena,
- exclusion of other causes and/or absence of alternative explanations.

Other causes, such as: the natural history of other underlying diseases, namely the disease under study, concomitant treatments, other risk factors like surgical procedures, and time relationship of the event to the IMP need to be taken into account for the assessment.

Causal relationship to the IMP will be classified:

- RELATED NO: if the following circumstances apply,
  - Not related: if there is no reasonable temporal association between event or laboratory tests abnormality onset and administration of the IMP or that can be reasonably explained by other factors, including underlying disease, complications, or concomitant medications. Clearly due to extraneous causes.
  - Unlikely: event or laboratory test abnormality, with a time to IMP administration that makes a relationship improbable (but not impossible). Diseases or other drugs provide plausible explanations.
- RELATED YES: if the following circumstances apply,
  - Possible: Event or laboratory test abnormality, with reasonable time relationship to drug administration. Could also be explained by disease or other drugs. Information on drug withdrawal is lacking or unclear.
  - Probable: Event or laboratory test abnormality, with reasonable time relationship to drug administration. Unlikely to be attributed to disease or other drugs. Response to withdrawal clinically reasonable. Rechallenge not necessary.
  - Definitive: Event or laboratory test abnormality, with plausible time relationship to drug administration. Cannot be explained by disease or other drugs. Response to withdrawal plausible (pharmacologically, pathologically). Event definitive pharmacologically or phenomenologically (an objective and specific medical disorder or a recognised pharmacological phenomenon). Rechallenge (if necessary).

Causal relationship to the IMP administration process will be classified:

- RELATED NO: if the following circumstances apply,
  - Not related: if there is no reasonable temporal association between event and IMP administration process or that can be reasonably explained by other factors, including underlying disease, complications, or concomitant medications. Clearly due to extraneous causes.
  - Unlikely: event with a time to IMP administration process that makes a relationship improbable (but not impossible). Diseases or other drugs or administration processes provide plausible explanations.
- RELATED YES: if the following circumstances apply,
  - Possible: Event with reasonable time relationship to IMP administration process. Could also be explained by disease or other drugs or administration processes. Information on drug withdrawal (not re-administered) is lacking or unclear.
  - Probable: Event with reasonable time relationship to IMP administration process. Unlikely to be attributed to disease or other drugs or

administration processes. Response to withdrawal (not re-administered) clinically reasonable.

- Definitive: Event with plausible time relationship to IMP administration process. Cannot be explained by disease or other drugs or processes. Response to withdrawal plausible (not re-administration). Event definitive phenomenologically (an objective and specific medical disorder).

The relatedness of the event will be performed:

- to the surgical procedure for events occurring before treatment administration and up to two weeks after the procedure has been performed.
- to the surgical procedure and to the study drug for all events occurring after the treatment administration(AES and SAEs).

All unexpected SAEs related to the IMP will be considered for regulatory reporting purpose (SUSARs).

4.4 Adverse Event Collection Period

All AEs that occur from signature of the informed consent will be recorded, regardless of the intensity, seriousness or relationship to study drug until the final Week 52 assessment.

- Pre-existing conditions will be collected on the baseline "Medical History" e-CRF module and will include, among others, active (symptomatic) diseases, diseases under treatment, chronic diseases and long term effects of past events present at the time of baseline assessment.
- Events that firstly occur within signature of the Informed Consent and administration of the study treatment [Non-Treatment Emergent Adverse Events (NTEAEs)] will be recorded. Any worsening of Medical History events along study treatment period will be subsequently recorded as AEs/SAEs as detailed in Section 4.5.
- Any AE that is ongoing at the last visit of the subject, either completed or withdrawn (as defined per protocol), should be followed up until the AE is resolved or stabilized or at least up to 30 days after the last protocol scheduled visit or last IMP dose administration (whatever is applicable).
- For extended follow-up incidental AEs/SAEs recording, applicable measures will be detailed.

All AEs elicited by the investigator during the defined AE collection period must be recorded in the CRF as per detailed instructions in the corresponding section. When an AE meets the criteria of seriousness (SAE), it must also be recorded on the SAE form/eCRF.

4.5 Adverse Event Reporting

4.5.1 Recording of Adverse Events

All adverse events occurring during the trial must be documented in the eCRF. This applies not only to those AEs supposedly related to the IMPs, but also to the surgical procedure or to any

undesired experience, whether or not a causal relationship is suspected. Whenever possible, attempts should be made by the Investigator to provide a specific "Diagnosis" or "Syndrome" in addition to a description of the reported signs and symptoms. AEs should be reported as separate and individual events.

All AEs and SAEs will be followed until they are resolved or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to a medical specialist Follow-up can be waivered in specific cases after consultation with the Sponsor. This permission must be documented per case and retained in the Sponsor File.

All AEs must be reported regardless of whether or not they are considered related to IMP administration or surgical procedure, with the exception of:

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline "Medical History" eCRF module.
- Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents.
- Medical or surgical procedures (e.g. endoscopy, appendectomy); however, the condition leading to these procedures, rather than the procedure itself, should be considered as AE.
- The expected fluctuations of any disease(s) pre-existent, ongoing, or detected at study start (e.g. worsening of the luminal Crohn´s disease).
- For the purpose of this study, drainage of the treated fistula and abscesses, defining the primary endpoint, will not be captured as AEs unless there is evidence suggesting a causal relationship between the IMP or the administration procedure. If the event meets the seriousness criteria such as hospitalization, the event will be reported as an SAE.

4.5.2 Serious Adverse Event Reporting

All SAEs will be reported by the investigator to the sponsor or its representative within 24 hours of the investigator becoming aware of the event, using the appropriate SAE form/pregnancy form/eCRF, as applicable.

The SAE information, duly completed, should be made available within 24 hours via SAE form/eCRF.

Initial minimum information for reporting an AE should include the following:

- AE and onset date
- Subject ID, sex, and age (or date of birth)
- Information on treatment received for unexpected and reportable SUSARs
- Name and address of the reporting physician
- Causal relationship to the study treatment or surgical procedure

The complete SAE form/eCRF containing all information will be made available to the contact listed in the contact list table at the start of the protocol within 24 hours. If the SAE is still active at the time of reporting or further information is obtained after initial communication, this information must be updated accordingly.

The Sponsor is the last responsible for appropriate qualification and reporting of safety information to the competent authorities, to the ECs/IRBs and to the investigators.

When a SAE is both unexpected and related to the IMPs ['Suspected Unexpected Serious Adverse Reaction' (SUSAR)] will require expedited reporting to the competent authorities and ECs/IRBs:

- Fatal or life threatening SUSARs will be reported within 7 days of the sponsor awareness of the event. Important additional information should be submitted within the following 8 days. All follow-up information later received will be reported within 15 days.
- All other SUSARs will be reported within 15 days of the sponsor becoming aware of the event.

In order to comply with Good Clinical Practice (GCP) requirements, all information provided to the investigator will be kept in the Investigator File at the site.

When a SAE is both unexpected and attributable to the investigational product, the sponsor will prepare a "Dear Investigator Letter", that will be sent to all investigators, where applicable.

4.5.3 Adverse Event of Special Interest Reporting

If the AE of special interest/abnormality, which occurs during the study, is considered to be clinically significant based on the criteria below, it should be recorded in an AE of Special Interest Name Form or an SAE Form if the AESI meets the seriousness criteria. The SAE Form should be completed and reported to the sponsor or its representative within 24 hours of the investigator becoming aware of the event.

The investigator should submit the original copy of the AE of Special Interest Name Form or the SAE Form to the sponsor.

Special interest AE/abnormality criteria include:

- Immunogenicity/alloimmune reactions.
- Hypersensitivity.
- Ectopic tissue formation.
- Medication errors.
- Tumorigenicity.
- Transmission of infectious agents.

AEs of special interest must be recorded as AEs in the eCRF. An evaluation form along with all other required documentation must be submitted to the sponsor.

4.5.4 Pregnancy reporting

Pregnancies of a female subject or the female partner of a male subject, occurring at least, while the subject is on protocol treatment or along the follow-up period, will be notified to the

investigator and immediately communicated to the Sponsor (within 24 hours) using the Pregnancy Form/eCRF.

All pregnancies that were initiated during the subject´s participation in the trial (or up to 30 days after last study visit) which come to the knowledge of the investigator, after the closure of the clinical data base. A pregnancy as such is not an AE *per se* but it is important enough to be reported in 24 hours like SAEs to the pharmacovigilance unit to be recorded in the safety database.

The pregnant subject or his consenting female partner will be followed until the end of the pregnancy and the outcome of the pregnancy will be notified to the Sponsor within 5 days using a Pregnancy form.

Any complication during the pregnancy, spontaneous or therapeutic abortion, stillbirth, neonatal death, birth defect/congenital anomaly, other serious infant condition must be reported and followed up as an SAE.

In the case of a live "normal" birth, the Sponsor should be informed as soon as the information is available. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs.

In addition, any infant death after 30 days that the Investigator suspects to be related to the *in utero* exposure to the investigational medicinal product(s) should also be reported.

The infant should be followed until 3 months after delivery to detect any complication or developmental impairment.

The Investigator is encouraged to provide outcome information of the pregnancy of the female partner of a male subject, if this information is available to the Investigator and the female partner gives her permission.

5 Protocol Deviations and Protocol Amendments

5.1 Protocol Deviations

Generally, as a Sponsor policy, no waivers for protocol deviation occurrence will be granted under any circumstances. Before the database lock, major protocol deviations will be taken into account for the efficacy analyses in the Per Protocol analysis set. Protocol deviations will be classified as major or minor. The following protocol deviations will be considered to be major:

(1) Non compliance with any of the inclusion/exclusion criterion, either identified before or after enrolment into the study,

(2) Any protocol deviation that may result in the non-evaluability of the subject for the primary endpoint,

(3) Any other deviation that may result in an increased risk or a potential harm to the subject well-being or any situation not compliant with Good Clinical Practices (GCP).

Any protocol deviation must be recorded and documented in the eCRF. Once the study has begun, any modification of the major vs. minor protocol deviation definitions will be specified in the SAP. This will be done before database lock.

5.2 Procedure for Protocol Amendments

Amendments that can result in substantial changes in the original protocol must be submitted and approved by the Institutional Review Board(s) (IRB)/ Ethics Committee(s) (EC) and competent authorities such as national regulatory agencies or any other required committees according to applicable local regulations.

All protocol amendments will be reported to the IRBs/EC involved in the trial and competent authorities before they are implemented, unless immediate action is required to prevent harm to the subjects.

6 Statistical Methods and Determination of Sample Size

6.1 Statistical and Analytical Plans

A statistical analysis plan (SAP) will be prepared and finalized before database unblinding. This document will provide further details regarding the derivation of analysis variables and analysis methodology to address all study objectives.

A targeted data review will be conducted before database lock. This review will assess the accuracy and completeness of the study database, subject evaluability, and appropriateness of the planned statistical methods.

6.1.1 Data Sets Analyzed

The following analysis sets will be defined:

- Intention to Treat (ITT): Includes all randomized subjects regardless of being treated or not or having any post-baseline measurements or not, according to their randomised treatment.
- Modified Intention to Treat (mITT): Includes all randomized subjects who have received the study treatment, according to the randomised treatment and for whom one post-baseline efficacy value is present, independently of the degree of adherence to the protocol.
- Per protocol (PP): Includes all randomized and treated subjects, according to the actual treatment they received and who adhered to the protocol with no major deviations, including at least one post-treatment efficacy evaluation available.
- Safety Analysis Dataset: Includes all randomized subjects who have received the study treatment, according to the actual treatment they received.

The main population for the primary and secondary efficacy analysis will be the intention to treat (ITT) analysis set.

6.1.2 Demographic and Other Baseline Characteristics

Baseline characteristics will be summarized descriptively for the ITT, mITT, safety, and PP analysis sets.

Categorical data will be summarized with absolute and relative frequencies (percentages), and continuous data will be summarized with the number of subjects, mean, median, standard deviation, the range (minimum and maximum) and the first and third quartiles.

6.1.3 Efficacy Analyses

The analyses the primary and key secondary endpoints will be performed on ITT, mITT, and PP analysis sets, with the ITT as the primary analysis set.

All exploratory variables will be analyzed on the ITT analysis set.

Sensitivity analyses for the primary and the key secondary endpoints with respect to missing values and the use of rescue medication or surgery will be detailed in the SAP.

Efficacy analyses will be performed with subjects grouped according to the randomized treatment assigned. Statistical hypothesis testing will be 2-sided, and significance will be assessed at the 5% level, unless otherwise noted.

6.1.3.1 Primary Efficacy Analysis

The primary efficacy analysis comparing Cx601 and placebo with respect to the primary endpoint (proportion of subjects with combined remission rate at Week 24) will be performed using a stratified Cochran-Mantel-Haenszel test, adjusting for the randomization strata. The point estimate of the treatment difference (Cx601 – placebo) in the combined remission rates along with the 95% CI will be provided.

The primary efficacy analysis will be performed on an intention-to-treat (ITT) population that includes all randomized subjects regardless of what treatment (if any) they received.

Handling of missing data and treatment failure:

A subject will be classified as a nonresponder at Week 24 (single imputation) in any of the following situations:

a) Missing data, including no MRI or no clinical assessment at Week 24, evaluation of Week 24 combined remission is not possible; OR
b) Treatment failure is documented for a subject if they require rescue medication or procedure as defined in Section 3.2.4.3.

Other sensitivity analyses to assess the impact of missing data and analysis populations on the analysis of the primary endpoint will be detailed in the SAP.

6.1.3.2 Secondary Efficacy Analyses

The key secondary endpoint of clinical remission at Week 24 will be analyzed with the same methodology that was used for the primary efficacy endpoint outlined above, including the handling of missing data and treatment failures.

The key secondary endpoint of time to clinical remission, assessed at Week 24, will be analyzed using the stratified log-rank test for comparing CX601 and placebo. The Cox proportional hazards model will be used to obtain estimates of the hazard ratio and the associated CIs. Kaplan-Meier curves will be presented along with median survival times.

Multiplicity adjustment: To address multiplicity, a gate-keeping approach will be used. First the primary efficacy endpoint, proportion of subjects with combined remission at Week 24, will be tested. If the primary efficacy analysis statistically significant, the Hochberg procedure will then be used to test the pool of the following 2 key secondary endpoints.

- Proportion of subjects with clinical remission at Week 24
- Time to clinical remission (weeks) assessed at Week 24

Additional details on the multiplicity adjustment will be provided in the SAP.

Other secondary efficacy analyses: All the binary endpoints will be analyzed using the same methodology as used for the primary efficacy endpoint described above, including the handling of missing data and treatment failures.

Time-to-event endpoints will be analyzed using the same methodology as used for the key secondary endpoint (time to clinical remission) as described above.

Categorical data will be summarized with absolute and relative frequencies (percentages) and continuous data will be summarized by the number of subjects, mean, standard deviation, minimum, first quartile, median, third quartile, and maximum.

Additional details will be provided in the SAP.

6.1.3.3 Subgroup Efficacy Analyses

Subgroup analyses for the primary and key secondary efficacy endpoints, using the ITT analysis set, will performed for the following subgroups (details will be provided in the SAP):

- Gender
- Race
- Age group
- Donor
- Region (Europe + Israel, North America)
- Region (USA, Non-USA)
- Smoking status (current/former/never)
- Colonoscopy status at baseline (full colonoscopy performed at baseline vs colonoscopy performed prior to baseline)
- Prior treatment with either immunosuppressants or biologics (i.e.: anti-TNF or anti-integrin or anti-IL12/23) or both
- Concomitant treatment (3 levels):
  - Concomitant immunosuppressant (IS) or monoclonal antibodies (mAbs) (i.e., anti-tumor necrosis factor (anti-TNF) or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab) as single agent
  - Concomitant IS or mAbs (i.e., anti-TNF or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab)) as combination treatment,
  - No ongoing concomitant IS or mAbs treatment at time of randomization
- External opening(s) (1 or > 1)
- Concomitant treatment (4 levels):
  - Concomitant immunosuppressant (IS) as single agent
  - Monoclonal antibodies (mAbs) (i.e., anti-tumor necrosis factor (anti-TNF) or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab) as single agent
  - Concomitant IS or mAbs (i.e., anti-TNF or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab)) as combination treatment,
  - No ongoing concomitant IS or mAbs treatment at time of randomization
- By level of combination of the following stratification factor (at randomization):
  - Current concomitant immunosuppressant or biologics as single agent or as a combination or no ongoing concomitant treatment at Screening visit
  - External opening(s) (1 or >1)

6.1.4 Exploratory Efficacy Analyses

The following exploratory endpoints will be analyzed:

- Change from baseline in total Perianal Disease Activity Index (PDAI) score at Week 6, 12, 18, 24, 36, and 52.
- Change from baseline in total PRO-2 score (defined as average daily stool frequency and average daily abdominal pain) at Week 6, 12, 18, 24, 36, and 52.
- Change from baseline in blinded central MRI Van Assche score at Weeks 24 and 52.
- Change from baseline in modified Van Assche blinded central MRI score at Weeks 24 and 52.
- Change from baseline in the Magnetic Resonance Novel Index for Fistula Imaging for Crohn's Disease (MAGNIFI-CD) score at Weeks 24 and 52.
- Change from baseline at Weeks 24 and 52 in blinded central MRI analysis of hyperenhancement in T1 sequence, and hyperintensity in T2 sequence.
- Change from baseline in electronic Patient Reported Outcomes (PRO) listed below, all measured through Weeks 12, 24 and 52:
    - Visual Analogue Scale (VAS) from 0 to 10 for perianal pain while standing, sitting, and defecating along last 2 weeks prior to the visit,
    - CDAI items score,
    - Number of pads used per day along last 2 weeks prior to each visit,
    - Work Productivity and Activity Impairment Questionnaire (WPAI),
    - EQ-5D[44],
    - SF36,
    - Health Resources Utilization (HRU)
- Immunogenicity responses as measured by donor-specific antibody (DSA) levels
- Change from baseline in cytokines, immune- and other inflammation-associated markers at Weeks 6, 12, 24, and 52.
- Change from baseline in the microbiome diversity at Week 6.

All continuous endpoints will be analyzed using a mixed model for repeated measures (MMRM) with treatment, stratum, and visit (if data collected at multiple post-treatment visits) as fixed factors with the inclusion of the time by treatment interaction (if appropriate); if available, the baseline value will be used as covariate. The treatment comparisons and corresponding 95% Cis will be presented for each scheduled visit.

All binary endpoints will be analyzed using the same methodology as used for the primary efficacy endpoint described above, including the handling of missing data and treatment failures.

Antidonor antibody kinetics will be summarized by treatment group. The results will be reported in a separate report.

6.1.5 Safety Analyses

The primary analysis set for all safety analyses will be the Safety analysis set. Analyses will be performed with subjects grouped according to the actual treatment received.

Detailed evaluation and summaries of safety endpoints will include:

- Incidence of treatment-emergent adverse events (TEAEs)
- Incidence of TEAEs related to study treatment
- Incidence of treatment-emergent serious adverse events (TESAEs)
- Incidence of AESIs
- Incidence of TESAEs related to study treatment
- Incidence of TEAEs and TESAEs according to their intensity/severity
- Incidence of TEAEs or TESAEs leading to study withdrawal
- Incidence of NTEAEs, whether or not related to surgical procedure(s), during fistula(s) preparation visit up to the to study treatment administration (if related, then, procedure-emergent non–treatment-emergent adverse event)
- Incidence of serious NTEAEs and serious TEAEs related to the procedure or treatment administration
- Incidence of TEAEs, whether or not related to the surgical procedure(s), following treatment administration and within up to two weeks since the last procedure
- Incidence of deaths (Fatal SAEs)
- Incidence of clinical findings on physical examination, vital signs, or laboratory tests
- Immunological humoral response by safety

Safety data (including vital signs and laboratory tests) will be summarized using descriptive statistics. The number of subjects experiencing each AE category will be summarized by system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) terminology.

Safety analysis will be performed on the Safety analysis set. Categorical data will be summarized with absolute and relative frequencies (percentages), and continuous data will be summarized with the number of subjects, mean, standard deviation, minimum, the first quartile, median, the third quartile, and maximum.

Non–treatment-emergent adverse events (serious and non-serious events that occur during the Screening period) will be collected and reported separately.

6.2 Interim Analysis

No interim analysis is planned for this study.

6.3 Determination of Sample Size

The primary endpoint of the study is the proportion of subjects with combined remission at Week 24. The following assumptions are made in the sample size calculations:

- True combined remission rates at Week 24 of 42.2% and 30% in the CX601 and placebo groups, respectively (based on estimates from a similar population in the Phase 3 ADMIRE-CD study).
- The family-wise Type-1 error rate will be controlled at 0.05 for the analyses of the primary and key secondary efficacy endpoints at Week 24.

Based on the above assumptions, a total sample size of 554 subjects (277 per treatment arm) will provide at least 85% power for the primary efficacy endpoint analysis at Week 24.

6.4 Handling of Dropouts or Missing Data

The following approach for imputation of missing data will be used in the Week 24 primary efficacy analysis:

A subject will be classified as a non-responder at Week 24 (single imputation) in any of the following situations:

a) Missing data, including no MRI or no clinical assessment at Week 24, evaluation of Week 24 combined remission is not possible; OR
b) Treatment failure is documented for a subject if they require rescue medication or procedure as defined in Section 3.2.4.3.

Other sensitivity analyses to assess the impact of missing data and the analysis populations on the analysis of the primary endpoint will be detailed in the SAP. The analysis of the secondary and other efficacy responder rate endpoints will use a similar approach as described above for handling of dropouts or missing data. Under this approach, a subject will be classified as a non-responder at a given visit in any of the following situations:

a) Missing data at the visit of interest; OR
b) Treatment failure is documented for a subject before the visit of interest if they require rescue medication or procedure as defined in Section 3.2.4.3.

7 Ethics

7.1 Independent Ethics Committee (IEC)/Institutional Review Board (IRB)

The protocol and consent form will be reviewed and approved by the IEC/IRB of each participating centre or by a Central IRB prior to study initiation. Each IEC/IRB is authorized by the respective authorities, whose composition is available to them and is not shown here. Each investigator will also be responsible for complying with ethical standards for clinical trials, as per local laws and regulations, during the study.

7.2 Ethical Conduct of the Study

This study will be performed in strict compliance with the Declaration of Helsinki (18th World Medical Assembly, 1964) and its last revision (Brazil, October 2013), ICH GCP, US Code of Federal Regulations (CFR), and local regulations.

7.3 Subject Information and Consent

Before being enrolled in the clinical study, subjects must consent to participate after the nature, scope and possible consequences of the clinical study have been explained in a form understandable to them. The investigator will provide the subject with an information form on the product and the study characteristics that should be read to and/or discussed with the subject in an understandable way. In this document, the subjects willing to consent to participate in this study will be informed of the nature, extent, design and conduct of the study and their consent will be obtained in writing prior to inclusion to the study schedule. Subjects will be given the opportunity to ask questions and will be informed of their right to withdraw from the study at any time, for any reason.

After reading the informed consent document, the subject must give consent in writing. The subject's consent must be confirmed at the time of consent by the personally dated signature of the subject and by the personally dated signature of the study team person conducting the informed consent discussions.

The original signed consent document will be retained by the principal investigator and a copy of the signed consent document will be given to the subject. The principal investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained. The investigator will not include in the study any subject without previously obtaining written consent from him/her.

7.4 Confidentiality

All study participants expressly agree not to disclose the identity of the subjects treated and to abide by the applicable regulations on confidentiality and data protection as regards data and information to which they have access. All the data related to the subjects will be collected and processed according to such applicable regulations.

All information obtained as a result of this study will be considered confidential until the sponsor gives formal approval for publication of results. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study

outcomes. The investigator may only inform on the study conduct and results to the sponsor or its representatives, IEC/IRB, and regulatory authorities, as applicable.

By conducting this study the centres and their investigators affirms to the sponsor that all study results and information furnished by the sponsor will be maintained in strict confidentiality. This confidential information will be the property of the sponsor and must not be disclosed by the centres and their investigators to third parties without prior written consent from the sponsor, and must only be used by the centres and their investigators for the study purposes.

8 Source Documents and Case Report Form Completion

8.1 Source Documents

According to the guidelines on Good Clinical Practice, the Monitors Team must check the electronic Case Report Form (eCRF) entries against the source documents, except for the pre-identified source data directly recorded in the electronic Case Report Form. The Informed Consent Form will include a statement by which the subject allows the Sponsor's duly authorised personnel, the IEC, and the regulatory authorities to have direct access to source data which supports the data on the Case Report Forms (e.g., subject's medical file, appointment books, original laboratory records, etc.) and allows the Sponsor's duly authorized personnel to access the surgery room during treatment administration to guarantee that study procedures are being done as per protocol. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information. The process for source data verification will be documented in the monitoring plan (separate document).

8.2 Case Report Forms

The investigator must keep a written or electronic file for each subject that participates in the clinical trial. In that file, the subjects' demographic and medical data will be kept, specially: name, date of birth, gender, medical history, diseases and concomitant drug, physical examination and clinical signs, observed adverse events, etc. It must be possible, in any moment, to identify the subject with his personal file. The period that the subject participates in the clinical trial must be clearly specified. All documentation will be transcribed to an electronic CRF. Any created documentation, specially the files that would be created by the technicians, must be filed. This includes the results of laboratory tests, etc. These documents must be identified with the subject initials, subject number, date of making and study code, in order to specify clearly the subject to whom this document belongs, and to identify the participants. The main objective is to get the most complete files of each candidate.

Any result obtained during the study development will be reported in the subject's electronic case report form.

The investigator will guarantee that all the documents sent to either the sponsor or PAREXEL, including the electronic case report form and other type of documents, do not contain any mention to the name of the subject. It is the investigator's duty to guarantee adequate filing and storage of the study documentation after the end of the study as specified in the Guidelines for Good Clinical Practice. All the original files must be kept following the hospital rules, research institutes or local regulation. All the electronic case report forms must be entirely completed. Any correction or amendment will be corrected by the investigator and the previous value recorded in the audit trail of the electronic data capture system along with the reason and date of the change. The electronic Case Report Form is considered an official document, and it must be available for the Health Authorities.

8.3 Monitoring

PAREXEL Blinded representatives of the Sponsor (will remain blinded to treatment throughout the study until database lock at Week 52) must be allowed to visit all study site locations

periodically and be permitted direct access to source data and any relevant documents needed to verify their accuracy, assess the data quality and study integrity. They will review study records on site and will compare them with source documents. They will also discuss the conduct of the study with the Investigator, and will verify that the facilities remain acceptable.

Additionally, PAREXEL unblinded sponsor representatives will attend the first treatment administration, during the surgical procedure, at each site (and some of the subsequent if needed upon request) to monitor the compliance to the Surgery Procedure Manual, especially the proper study treatment administration and to provide assistance to the surgeon's questions if needed.

8.4 Data Quality Assurance

For the purpose of ensuring compliance with the protocol, Good Clinical Practice and applicable regulatory requirements, the Investigator must permit audits by the Sponsor and inspection by applicable regulatory authorities.

The Investigator agrees to allow the auditors/inspectors to have direct access to his/her study records for review, being understood that these personnel is bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

As soon as the Investigator is notified of a future inspection by the authorities, he will inform the Sponsor and authorise the Sponsor to participate at this inspection.

The confidentiality of the data verified and the anonymity of the subjects should be respected during these inspections.

The study will be conducted with an electronic case report form (eCRF) with a qualified single data entry performed by the investigators with controlled and restricted access. Additional information on the data management will be provided in a specific document (e.g. Data Handling Manual). A full data validation plan will be performed and described in this Data Handling Manual which should be approved before the data entry process start.

The database quality will be assessed before the database lock; and only when the SAP is approved and the subject evaluability (i.e., protocol deviations) are determined.

9 Publication Policy and Study Documentation

9.1 Publication Policy

The investigator is obliged to provide the sponsor with complete test results and all data derived by the investigator from the study. During and after the study, only the sponsor may make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of the sponsor.

The sponsor may publish any data and information from the study (including data and information generated by the investigator) without the consent of the investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the study site agreement. In the event of any discrepancy between the protocol and the study site agreement, the study site agreement will prevail.

9.2 Study Documentation

The investigator must keep in his/her study file for the period of time indicated by the applicable regulations all the essential documents required to comply with GCP.

All records must be stored in a secure facility protected from fire, flood and unauthorised access, where they may be readily accessed in the event of an audit or inspection.

The sponsor, must keep the study master file as long as required by the applicable regulations.

The master file must include all essential documents required for the conduct of a clinical trial as per GCP.

9.3 Traceability and Retention of Data

The sponsor, the Hospital, the Institution and the Principal Investigator are each responsible for their respective duties and obligations in ensuring the traceability of the Product in accordance with applicable legislation or regulations.

The sponsor shall ensure compliance with the applicable legislation in relation to the donation and the donors of human cells (including consent, eligibility of donors, data protection and confidentiality, selection, evaluation and procurement).

10 Data Management Procedures

10.1 Review and Confirmation of Electronic Case Report Forms

Electronic CRFs will be filled directly by the investigators or specifically delegated dedicated site staff and reviewed and signed electronically by the investigators.

Data on all eCRFs and other external data will be reviewed and checked for omissions, apparent errors, and values requiring further clarification using computerized and manual procedures. Only authorized personnel will make corrections to the eCRF and all corrections will be documented in an audit trail.

10.2 Database Production and Verification

10.2.1.1 Data Validation Plan

A validation plan is set-up according to the protocol requirements, which describes the validation rules to be applied. Actions that should be taken in case of data abnormalities are detailed. In case of missing values, out of range values, data inconsistencies or values that fail logical checks, correction forms (queries) are edited and transmitted to the investigator for clarification.

10.2.1.2 Database

A database will be created in order to collect all clinical and other data from the clinical trial. The Data Management will take place at PAREXEL.

10.2.1.3 Database Access

Access to the eCRF database different than read-only will be restricted to the investigators, data managers and clinical monitors. Any entry in the database will be traceable through its identification and date. Audit trail of data changes will be assured.

10.2.1.4 Quality and Consistency Controls

Investigators or designees will enter the information required by the protocol onto the eCRFs. Each investigative site will be visited frequently by CRO monitors for source data verification as documented in the monitoring plan to review the eCRFs for completeness and accuracy. The CRO representative will highlight any discrepancies found and ensure that appropriate site personnel address the discrepancies. When a discrepancy results in corrected eCRF data, the correction will be reviewed again by data management for completeness and accuracy.

10.2.1.5 Data Queries

Data query forms will be generated to the investigator in order to clarify the data inconsistencies through the source data verification.

Only then and after all detected errors, inconsistencies or doubts cleared, will the database declared a clean file and protected accordingly.

10.2.1.6 Clean File

A clean file will be created, registered and locked once all applicable forms are completed, fully validated by source data verification and all previously issued queries have been resolved. At this stage, further changes or editing will be disallowed. The database lock report will be generated. System reports could be prepared on regular basis, as needed, to provide overview over the study progress in terms of data validation.

10.2.1.7 Data Coding

Coding will be performed according to standard dictionaries by the Clinical Coding group coders. Coding reports will be shared with the medical monitor on a regular basis to allow for medical review. Coder and medical monitors will collaborate closely to obtain a final code, if necessary, manual queries will be raised to clarify any potential issues to enable proper coding. The following dictionaries will be used:

- Medical History: MedDRA
- Associated pathologies: MedDRA
- Concomitant treatments: World Health Organisation (WHO) DRUG
- Adverse Event: MedDRA
- Laboratory tests and procedures: MedDRA

All case report forms and data checking records will be retained as permanent records of the study.

10.2.1.8 Site Data Verification

Periodic monitoring visits will be made for source data verification, and to check compliance with the protocol, GCP and applicable regulatory requirements. The monitoring activities will be performed by PAREXEL, under the supervision of the sponsor. Quality control steps will be taken for the analytical protocol, results, and report.

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APPENDIX 1

Patient Reported Outcome 2 (PRO-2) scoring adapted from Khanna et al. (2015)[31]

(b) Patient Reported Outcome 2 (PRO2)

VARIABLE	DAY							7 DAY AVERAGE	WEIGHTING FACTOR	TOTAL
	1	2	3	4	5	6	7			
Number of liquid or very soft stools									x 2 =	
Abdominal pain 0=none, 1=mild, 2=moderate, 3=severe									x 5 =	
									PRO2 TOTAL=	

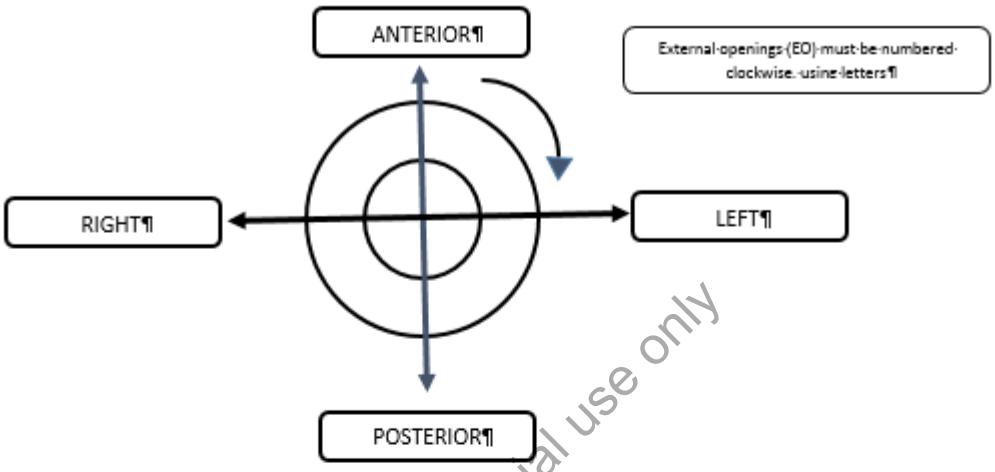
APPENDIX 2

Appendix 2 is shown below. Note that the anal clock is represented in a lithotomy position (gynecological).

APPENDIX·2·TO·PROTOCOL·Nº·Cx601-0303¶

VISIT·nº.·_____·· → → → → → VISIT·DATE:·_____¶
PATIENT·NUMBER:¶

¶



External·Opening·#A¤	DIRECTION¤	LATERAL¤	DRAINING·¤
¤	Anterior¤	Left¤	Yes·····□¤
	Posterior¤	Right¤	No·····□¤
¤	Middle·¤	Middle¤	¤

External·Opening·#B¤	DIRECTION¤	LATERAL¤	DRAINING¤
NA*··□¤	Anterior¤	Left¤	Yes·····□¤
	Posterior¤	Right¤	No·····□¤
¤	Middle¤	Middle¤	·¤

External·Opening·#C¤	DIRECTION¤	LATERAL¤	DRAINING¤
NA*··□¤	Anterior¤	Left¤	Yes·····□¤
	Posterior¤	Right¤	No·····□¤
	Middle¤	Middle¤	¤

New·Opening·#__**¤	DIRECTION¤	LATERAL¤	DRAINING¤
NA*··□¤	Anterior¤	Left¤	Yes·····□¤
	Posterior¤	Right¤	No·····□¤
	Middle¤	Middle¤	¤

*NA·(not·applicable)·to·be·checked·if·there·is·no·additional·External·Opening·present·¶

**·The·numbering·for·the·new·External·Openings·should·start·from·letter·D.·¶

Investigator·Name·_____··¶
¶
Signature:·_____¶

APPENDIX 3

The events listed below must be reported as an SAE. Please note that this list represents the broad medical concepts to be considered as "important medical events satisfying SAE reporting requirements, and may or may not be related to the particular medicinal product under study."

Takeda Medically Significant AE List

Term	
Acute respiratory failure/acute respiratory distress syndrome	Hepatic necrosis
Torsade de pointes / ventricular fibrillation / ventricular tachycardia	Acute liver failure
	Anaphylactic shock
Malignant hypertension	Acute renal failure
Convulsive seizure	Pulmonary hypertension
Agranulocytosis	Pulmonary fibrosis
Aplastic anemia	Confirmed or suspected endotoxin shock
Toxic epidermal necrolysis/Stevens-Johnson syndrome	Confirmed or suspected transmission of infectious agent by a medicinal product
	Neuroleptic malignant syndrome / malignant hyperthermia
	Spontaneous abortion / stillbirth and fetal death

APPENDIX 4

Detailed Description of Amendments to Text

The primary sections of the protocol affected by the changes in Amendment 02 are indicated. The corresponding text has been revised throughout the protocol.

Change 1: Sentence added to state when marketing approval for Cx601 was received.

The primary change occurs in Section 2.1 Treatment Rationale

Initial wording:	This efficacy data was initially submitted to the European Medicine Agency (EMA) in March 2016 to support Marketing Approval Authorization (MAA) as demonstrates that Cx601 treatment effectively induce fistula closure, an effect that is maintained for up to 52 weeks, in adult patients with complex perianal fistulas that have shown an inadequate response to at least one conventional or biologic therapy.
Amended or new wording:	This efficacy data was initially submitted to the European Medicine Agency (EMA) in March 2016 to support Marketing Approval Authorization (MAA) ~~as~~**to** demonstrate that Cx601 treatment effectively induce**s** fistula closure, an effect that is maintained for up to 52 weeks, in adult subjects with complex perianal fistulas that have shown an inadequate response to at least one conventional or biologic therapy. **Marketing approval was received from the EMA in March 2018.**

Rationale for change: Sentence added to reflect current status of Cx601.

Change 2: Editorial change to the secondary objectives.

The primary change occurs in Section 2.3.2 Secondary Objectives

Initial wording:	To evaluate the efficacy and safety of Cx601 as compared to placebo in clinical parameters and time-related endpoints at Weeks 24 and 52, and to evaluate how many patients with Combined Remission at Week 24 have sustained remission at Week 52 and those who relapse by Week 52.
Amended or new wording:	• **To evaluate the efficacy of Cx601 as compared to placebo in clinical remission at Week 24 and in time to clinical remission (weeks).** • **To evaluate the efficacy and safety of Cx601 as compared to placebo in other clinical and time-to-event related endpoints at Weeks 24 and 52.**

Rationale for Change: For completeness and improved clarity in the definition of the secondary objectives.

The summary section also contains this change.

Change 3: Editorial change to the exploratory objectives and addition of an exploratory objective related to microbiome diversity.

The primary change occurs in Section 2.3.3 Exploratory Objectives:

Initial wording:	Exploratory endpoints will also include ePROs assessment through the entire study period, as well as radiological (MRI) and immunological parameters at specific time-

points and their possible correlation with the clinical outcome if any.

Amended or new wording:

- **To evaluate the efficacy of Cx601 as compared to placebo as measured by exploratory endpoints related to patient-reported outcomes, radiological measurements (MRI), cytokine, immune and inflammation-associated markers.**
- **To characterize microbiome diversity.**
- **To characterize the immunogenicity of Cx601 (donor-specific antibodies [DSA]) and the impact of immunogenicity on safety and clinical response.**

Rationale for Change: Editorial changes were made for clarity, transparency, and completeness. The microbiome will be characterized to explore if microbial diversity impacts fistula healing.

The summary section also contains this change.

Change 4: Wording updated to reflect the planned increase in the study sample size from 326 subjects (specified in Version 3.0 of the protocol) to 554 subjects.

The primary change occurs in Section 3.1 Overall Study Design and Plan: Description

Initial wording:

This will be a phase III, randomized, double-blind, parallel group, placebo controlled, international and multicentre, study to assess the efficacy and safety at 24 weeks and with a follow-up period up to 52 weeks after the administration of a new therapy with eASC (Cx601) for the treatment of complex perianal fistulas in patients with Crohn's disease.

The study will follow an add-on design: patients receiving any ongoing concomitant medical treatment for Crohn's disease (CD) at stable doses at the time of Screening visit, will be allowed to continue it throughout the study. A total of approximately 436 patients are planned to be screened in order to randomise at least 326 patients in a 1:1 ratio to receive either a local injection of Cx601 (120 million cells) or matching placebo.

Amended or new wording:

This will be a phase III, randomized, double blind, parallel group, placebo controlled, ~~international~~ **global** and multicentre study to assess the efficacy and safety at 24 weeks and with a follow-up period up to 52 weeks after the administration of a new therapy with eASC (Cx601) for the treatment of complex perianal fistulas in subjects with **CD**~~Crohn's disease~~.

The study will follow an add-on design: patients receiving any ongoing concomitant medical treatment for ~~Crohn's disease (~~**CD**~~)~~ at stable doses at the time of Screening visit~~,~~ will be allowed to continue it throughout the study. A total of approximately ~~436 patients~~ **740 subjects** are planned to be screened in order to randomise at least ~~326 patients~~ **554 subjects** in a 1:1 ratio to receive either a local injection of Cx601 (120 million cells) or matching placebo.

Rationale for change: The assumptions made to calculate the original sample size of 326 patients were based on the estimate of 15.2% of the true treatment difference in the primary endpont from the completed phase III study (ADMIRE-CD) and provided at least 80% power for the primary efficacy analysis. However, an estimate of 12.2% was obtained from an ADMIRE-CD post hoc subgroup analysis using a subgroup of subjects who are more similar to the targeted patient population in ADMIRE-II. Therefore, an updated sample size of 554 subjects that is based on an assumption of 12.2% for the true difference and provides approximately 85% power for the

primary analysis was considered appropriate to ensure that ADMIRE-CD II is an adequate and well-controlled pivotal trial.

Change 5: Wording added to clarify study design.

The primary change occurs in Section 3.1 Overall Study Design and Plan: Description

Initial wording:	All local MRIs will be assessed centrally by the clinical research organization (CRO) MRI central lab in a treatment-blinded approach for eligibility and both treatment- and sequence-blinded for efficacy assessments.
Amended or new wording:	All local MRIs will be assessed centrally by the clinical research organization (CRO) MRI central lab in a treatment-blinded approach for eligibility and both treatment- and sequence-blinded for efficacy assessments **at Week 24 and treatment-blinded for efficacy at Week 52.**

Rationale for change: Timepoints included to clarify when MRI assessments will be assessed for efficacy.

Change 6: Addition of text for consistency with the summary section.

The primary change occurs in Section 3.1 Overall Study Design and Plan: Description

Initial wording:

[…]

Training on the surgical procedure and study treatment administration and technical support and assistance will be implemented for all participating sites by means of an unblinded sponsor representative available during the surgical procedure of treatment administration to first subject at each site and some of the subsequent treatment administrations if required. They will also assess the quality and integrity of the administration procedure at each site. Central reading of local pelvic MRIs will be performed. All local MRIs will be assessed centrally by the clinical research organization (CRO) MRI central lab in a treatment-blinded approach for eligibility and both treatment- and sequence-blinded for efficacy assessments at Week 24 and treatment-blinded for efficacy at Week 52. Study population will consist of subjects with complex perianal fistula(s) draining at Screening visit despite previous standard medical treatment, with up to 2 internal openings and a maximum of 3 external openings based on clinical assessment; a central reading of a locally performed contrast enhanced (gadolinium) pelvic MRI will be performed to confirm location of the fistula and potential associated perianal abscess(es). In addition, clinically controlled, non active or mildly active CD during the last 6 months prior to Screening visit will be confirmed with:

1. a PRO-2 score less than 14 at Screening,
   AND
2. a colonoscopy documenting the absence of ulcers larger than 0.5 cm in the colonic mucosa:

   […]

Amended or new

[…]

wording: Training on the surgical procedure and study treatment administration and technical support and assistance will be implemented for all participating sites by means of an unblinded sponsor representative available during the surgical procedure of treatment administration to first subject at each site and some of the subsequent treatment administrations if required. They will also assess the quality and integrity of the administration procedure at each site. Central reading of local pelvic MRIs will be performed. All local MRIs will be assessed centrally by the clinical research organization (CRO) MRI central lab in a treatment-blinded approach for eligibility and both treatment- and sequence-blinded for efficacy assessments at Week 24 and treatment-blinded for efficacy at Week 52. Study population will consist of subjects with complex perianal fistula(s) draining at Screening visit despite previous standard medical treatment, with up to 2 internal openings and a maximum of 3 external openings based on clinical assessment; a central reading of a locally performed contrast enhanced (gadolinium) pelvic MRI will be performed to confirm location of the fistula and potential associated perianal abscess(es). In addition, clinically controlled, non active or mildly active CD during the last 6 months prior to Screening visit will be confirmed with:

1. A PRO-2 score less than 14 at Screening.
   The investigator will instruct the subject to complete (at the beginning of the screening period) daily the intensity of abdominal pain (from 0 to 3) and the number of liquid stools per day during a complete week by using the PRO-2 diary (which will be provided as a separate document). PRO-2 scores will be calculated by the investigators based on subject's diary and according to the document provided in APPENDIX 1, **AND**
2. A colonoscopy documenting the absence of ulcers larger than 0.5 cm in the colonic mucosa:
   […]

Rationale for change: Consistency change to match wording in the summary section.

Change 7: Editorial update to inclusion criterion.

The primary change occurs in Section 3.2 Selection of Study Population

Initial wording: (7) Women of childbearing potential (WCBP) must have negative serum pregnancy test at Screening (sensitive to 25 IU human chorionic gonadotropin [hCG]). Both WCBP or male subjects participating in this study, with a WCBP as partner, must agree to use an adequate method of contraception during the entire duration of the study. An adequate method of contraception is defined as complete, non-periodic sexual abstinence, single-barrier method, vasectomy, adequate hormonal contraception (to have started at least 7 days prior to Screening visit), or an intra-uterine device (to have been in place for at least 2 months prior to Screening visit).

A WCBP, for the purposes of this study, is a sexually mature female; who is not surgically sterile by means of a prior hysterectomy, bilateral

oophorectomy, or bilateral tubal ligation; and has not been naturally postmenopausal for at least the last 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months)

Amended or new wording:

(7) Women of childbearing potential (WCBP) must have negative serum pregnancy test at Screening (sensitive to 25 IU human chorionic gonadotropin [hCG]). Both WCBP or male subjects participating in this study, with a WCBP as partner, must agree to use an adequate method of contraception during the entire duration of the study. An adequate method of contraception is defined as complete, non-periodic sexual abstinence **(refraining from heterosexual intercourse)**, single-barrier method, vasectomy, adequate hormonal contraception (to have started at least 7 days prior to Screening visit), or an intra-uterine device (to have been in place for at least 2 months prior to Screening visit).

A WCBP, for the purposes of this study, is a sexually mature female; who is not surgically sterile by means of a prior hysterectomy, bilateral oophorectomy, or bilateral tubal ligation; and has not been naturally postmenopausal for at least the last 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

Sexual abstinence for the purposes of this study, is considered a highly effective method of contraception only if defined as refraining from heterosexual intercourse during the entire period of the study duration.

Rationale for change: Wording added to meet MHRA approval criteria.

The summary section also contains this change.

Change 8: Assessments added to Screening visit.

The primary change occurs in Section 3.3.1.1.1 Screening Visit

Initial wording:

- Informed consent: a written informed consent will be obtained from the patient before any study procedure is performed.

  […]

- Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total bilirubin is above the ULN), potassium, sodium, chloride.

- Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification). Fistula must have been draining for at least 6 weeks prior to Screening visit. The clinical assessment will consist of a physical examination of the fistula(s) by a blinded investigator to evaluate the presence of drainage spontaneously or after gentle finger compression through the external openings. The tracts and external openings must be clearly identified in the eCRF in order to ensure the same tracts are assessed during the study period.

	[…]
	• Pelvic MRI performed locally (number of fistulas, location, type, collection(s) measured in 3 dimensions, if any, with fistula location). A quality copy will be sent within 24 hours from acquisition to the Central Imaging Lab for immediate blinded central MRI reading as detailed in the specific manual (i.e. the Image Acquisition Guidelines; as per this guidelines turnaround is 5 days assuming images are adequately acquired according). Blinded central MRI results (number of fistulas, location, type, collection(s) measured in 3 dimensions, if any, with fistula location) will be communicated to the investigator and the surgeon prior to Preparation visit to guide the surgeon through the preparation process. • […] • Review and record previous medication within 2 years prior to the Screening visit day, including but not limited to the treatment of the Crohn's disease, the treatment of perianal fistulas, anal abscesses. The following information regarding previous medication will also need to be reviewed and recorded: inadequate response, loss of response or intolerance to immunosuppressive agents (azathioprine, mercaptopurine, methotrexate), TNF antagonists, vedolizumab or uztekinumab.
Amended or new wording:	• Informed consent: a written informed consent will be obtained from the subject before any study procedure is performed. • **A separate informed consent(s) pertaining to the voluntary exploratory endpoints (obtaining tissue/fluids from the fistula curettage procedure and microbiome sampling) must be obtained prior to any assessment procedures and is included as part of the main ICF (where applicable). The provision of this consent is optional and independent of consent to the other aspects of the study.** • […] • Serum biochemistry: CRP, urea, creatinine, glucose, AST, ALT, albumin, total bilirubin (direct bilirubin if total bilirubin is above the ULN), potassium, sodium, chloride. • **Blood sample for soluble factors studies.** • Clinical evaluation of the fistula(s) in terms of drainage at the external opening(s) after gentle finger compression, anal pain, suspicion of perianal abscess (fistula identification). Fistula must have been draining for at least 6 weeks prior to Screening visit. The clinical assessment will consist of a physical examination of the fistula(s) by a blinded investigator to evaluate the presence of drainage spontaneously or after gentle finger compression through the external openings. The tracts and external openings must be clearly identified in the eCRF in order to ensure the same tracts are assessed during the study period. • […] • Pelvic MRI performed locally (number of fistulas, location, type, collection(s)

measured in 3 dimensions, if any, with fistula location). A quality copy will be sent within 24 hours from acquisition to the Central Imaging Lab for immediate blinded central MRI reading as detailed in the specific manual (i.e.**,** the Image Acquisition Guidelines; as per ~~this~~**these** guidelines**,** turnaround is 5 days assuming images are adequately acquired according **to image acquisition guidelines**). Blinded central MRI results (number of fistulas, location, type, collection**[**~~(~~s**]**~~)~~ measured in 3 dimensions, if any, with fistula location) will be communicated to the investigator and the surgeon prior to Preparation visit to guide the surgeon through the preparation process.

- […]
- Review and record previous medication within 2 years **before**~~prior to~~ the Screening visit day, ~~including but not limited to~~ **medication related to** the treatment of Crohn's disease, the treatment of perianal fistulas, **and** anal abscesses. The following information regarding previous medication will also need to be reviewed and recorded: inadequate response, loss of response or intolerance to immunosuppressive agents (azathioprine, mercaptopurine, methotrexate), TNF antagonists, vedolizumab or uztekinumab. **Within the last 6 months before the Screening visit, all blood products, steroids, and intravenous medications will need to be recorded; all other prior medications administered within 1 month prior to the screening visit should also be recorded**.

Rationale for change: Text added to clarify the optional informed consent for the voluntary exploratory endpoints. Blood sample assessment for soluble factors studies added to assess immunogenicity. Timeframe for the collection of blood products, steroids, and intravenous medication and all other prior medications before screening has been added for completeness. Wording included to clarify MRI acquisition requirements for completeness.

The following sections also contain this change:

- 3.3.1.1.4 Week 6 Follow-up/Visit 1 (Day 42 ±8)
- 3.3.1.1.5 Week 12 Follow-up/Visit 2 (Day 84 ±8)
- 3.3.1.1.7 Week 24 Follow-up Visit/Visit 4 (Day 168 ±8)
- 3.3.1.1.9 Week 52 Follow-up Visit/Visit 6 (Day 364 ±15)
- 3.3.1.1.10 Early Termination Visit (±15 days of early termination):

Change 9: Assessment added for collection of microbiome sample.

The primary change occurs in Section 3.3.1.1.1 Screening Visit

Initial wording:

[…]

- Schedule Preparation visit, transfer the Screening local MRI to the surgeon prior to Preparation visit and check that the results of the central blinded MRI are available to be communicated and review by the surgeon prior/during the Preparation visit.
- Assess and record AE at the time of Screening visit after the ICF has been signed

by the patient

Amended or new wording:

[…]

- Schedule Preparation visit, transfer the Screening local MRI to the surgeon prior to Preparation visit and check that the results of the central blinded MRI are available to be communicated and review by the surgeon prior/during the Preparation visit.
- Assess and record AE at the time of Screening visit after the ICF has been signed by the subject
- **For microbiome collection (optional), kits for at home fecal sample collection will be provided to the subject. Additional details will be provided in the Laboratory Manual.**
- […]

Rationale for change: Update made in line with exploratory endpoint.

This change also occurs in:

- Section 3.3.1.1.2 Preparation Visit
- Section 3.3.1.1.3 Study Treatment Administration/Visit 0 (Day 0)
- Section 3.3.1.1.4 Week 6 Follow-up/Visit 1 (Day 42 ±8)

Change 10: Inclusion of new exploratory assessments.

The primary change occurs in Section 3.3.1.1.2 Preparation Visit.

Initial wording:

[…]

- Fistula preparation, consisting on EUA, curettage and seton placement for ALL patients by the surgeon according to the Surgery Procedure Manual (provided as a separate document). This must be done at least 2 weeks and a maximum of 3 before the study treatment administration day.
- Mandatory antibiotics coverage will be administered during at least 7 days following the fistula curettage (ciprofloxacin and/or metronidazole are recommended unless documented previous intolerance)

[…]

Amended or new wording:

[…]

- Fistula preparation, consisting on EUA, curettage and seton placement for ALL patients by the surgeon according to the Surgery Procedure Manual (provided as a separate document). This must be done at least 2 weeks and a maximum of 3 before the study treatment administration day.
    - **Collection of curettage material (optional), details will be provided in the Laboratory Manual.**
    - **Microbiome collection (optional) from the fistula, details will be**

provided in the Laboratory Manual.

- Mandatory antibiotics coverage will be administered during at least 7 days following the fistula curettage (ciprofloxacin and/or metronidazole are recommended unless documented previous intolerance)
- **Extra whole blood sample for cell responses and immunological tests:**
  - **Presence/absence of anti-donor antibodies**
  - **Cell responses: study of cellular and soluble factors**

Rationale for change: Optional fistula curettage and microbiome samples have been included to better characterize the subjects and the fistula which will enhance the understanding of disease pathogenesis, with a minimal increase in the sampling burden on subjects.

The following sections also contain this change:

- Section 3.3.1.1.3 Study Treatment Administration/Visit 0 (Day 0)
- Section 3.3.1.1.4 Week 6 Follow-up/Visit 1 (Day 42 ±8)
- Section 3.3.1.1.7 Week 24 Follow-up Visit/Visit 4 (Day 168 ±8)

Change 11: Language added on hypersensitivity management.

The primary change occurs in Section 3.3.1.1.3 Study Treatment Administration/Visit 0 (Day 0)

Initial wording:	2. Study treatment administration […] All setons must be withdrawn; fistula(s) curettage should be done, placing a stitch on each internal opening according to the Surgery Procedure Manual (provided as a separate document). […]
Amended or new wording:	[…] All setons must be withdrawn; fistula(s) curettage should be done, placing a stitch on each internal opening according to the Surgery Procedure Manual (provided as a separate document). **Subjects will be observed after their surgical procedure until full recovery, with special attention to signs and symptoms of potential allergic reactions.** […]

Rationale for change: Hypersensitivity is a potential risk of Cx601, therefore language has been included to mitigate any potential allergic reactions during the procedure.

Change 12: Editorial changes to the description of the primary endpoint.

The primary change occurs in Section 3.3.2.1 Primary Endpoint.

Initial wording:	The primary endpoint at Week 24 is defined to be the Combined Remission of complex perianal fistula(s), defined as the clinical assessment at Week 24 of closure

of all treated external openings that were draining at baseline despite gentle finger compression, and absence of collection(s) >2 cm (in at least 2 dimensions) of the treated perianal fistula(s) confirmed by blinded central MRI assessment at Week 24.

Amended or new wording:

Proportion of subjects who achieve combined remission at Week 24 after IMP administration, where combined remission is defined as:

- **The closure of all treated external openings that were draining at baseline despite gentle finger compression**
  AND
- **Absence of collection(s) >2 cm (in at least 2 dimensions) of the treated perianal fistula(s) confirmed by blinded central MRI assessment.**

Rationale for change: For improved clarity in the definition of the primary endpoint.

The summary section also contains this change.

Change 13: Editorial changes to the description of the secondary efficacy endpoints and safety endpoints. In addition, one of the key secondary endpoints was changed from 'clinical response' to 'time to clinical remission'.

The primary change occurs in Section 3.3.2.2 Secondary Endpoints

Initial wording:

Efficacy at Week 24

- KEY: Clinical Remission at Week 24, defined as closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed at Week 24.
- KEY: Response at Week 24, defined as closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed at Week 24.
- Time to Clinical Remission by Week 24 (time from treatment start to first visit with closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed).
- Time to Response by Week 24 (time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed).

Efficacy at Week 52

- Combined Remission of perianal fistula(s), defined as the clinical assessment at Week 52 of closure of all treated external openings that were draining at baseline, despite gentle finger compression, and absence of collection(s) >2 cm (in at least 2 dimensions) confirmed by blinded central MRI assessment at Week 52.
- Clinical Remission defined as closure of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed at Week 52.
- Response defined as closure of at least 50% of all treated external openings that

were draining at baseline, despite gentle finger compression, as clinically assessed at Week 52.

- Time to Clinical Remission by Week 52 (time from treatment start to first visit with closure of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed).
- Time to Response by Week 52 (time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed).
- Relapse by Week 52 defined in patients with **Combined Remission by Week 24**, as reopening of any of the treated external openings with active drainage as clinically assessed or the presence of collection(s) >2 cm (in at least 2 dimensions) of the treated perianal fistula(s) confirmed by blinded central MRI assessment by Week 52.

Safety throughout the study

- Safety evaluations including physical examination, vital signs, and laboratory (biochemistry, hematology) assessments will be performed.
- The incidence of adverse events throughout the study will be monitored in all treated patients (Safety population).

Amended or new wording:

Key Secondary Efficacy ~~at Week 24~~

- ~~KEY: Response at Week 24, defined as closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed at Week 24.~~ **Proportion of subjects who achieve clinical remission at Week 24 after IMP administration, where clinical remission is defined as the closure of all treated external openings that were draining at baseline despite gentle finger compression.**
- ~~KEY: Clinical Remission at Week 24, defined as closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed at Week 24.~~ **Time to clinical remission (weeks) assessed at Week 24, defined as the time from treatment start to first visit with closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical remission by Week 24 will be censored at that visit.**

Other Secondary Efficacy Endpoints

- ~~Time to Clinical Remission by Week 24 (time from treatment start to first visit with closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed).~~
- **Proportion of subjects who achieve clinical response at Week 24 after IMP administration, where clinical response is defined as closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression.**
- **Time to clinical response (weeks) assessed at Week 24, defined as the time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical response by Week 24 will be censored at that visit.**

~~Efficacy at Week 52~~

- ~~Combined Remission of perianal fistula(s), defined as the clinical assessment at Week 52 of closure of all treated external openings that were draining at baseline, despite gentle finger compression, and absence of collection(s) >2 cm (in at least 2 dimensions) confirmed by blinded central MRI assessment at Week 52.~~

- **Proportion of subjects who achieve combined remission at Week 52 after IMP administration, where combined remission is defined as:**
  - **a) The closure of all treated external openings that were draining at baseline despite gentle finger compression,**
    AND
  - **b) Absence of collection(s) >2 cm (in at least 2 dimensions) confirmed by blinded central MRI assessment.**

- ~~Clinical Remission defined as closure of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed at Week 52.~~

- **Proportion of subjects who achieve clinical remission at Week 52 after IMP administration, where clinical remission is defined as closure of all treated external openings that were draining at baseline despite gentle finger compression.**

- ~~Response defined as closure of at least 50% of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed at Week 52.~~

- **Proportion of subjects who achieve clinical response at Week 52 after IMP administration, where clinical response is defined as closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression.**

- ~~Time to Clinical Remission by Week 52 (time from treatment start to first visit with closure of all treated external openings that were draining at baseline, despite gentle finger compression, as clinically assessed).~~

- **Time to clinical remission (weeks) assessed at Week 52, defined as the time from treatment start to first visit with closure of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical remission by Week 52 will be censored at that visit.**

- ~~Time to Response by Week 52 (time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed).~~

- **Time to clinical response (weeks) assessed at Week 52, defined as the time from treatment start to first visit with closure of at least 50% of all treated external openings that were draining at baseline despite gentle finger compression, as clinically assessed. Subjects who do not achieve clinical response by Week 52 will be censored at that visit.**

- ~~Relapse by Week 52 defined in patients with **Combined Remission by Week**~~

~~**24**, as reopening of any of the treated external openings with active drainage as clinically assessed or the presence of collection(s) >2 cm (in at least 2 dimensions) of the treated perianal fistula(s) confirmed by blinded central MRI assessment by Week 52.~~

- **Proportion of subjects with a relapse from Week 24 combined remission response, where a relapse is defined as:**
  - **a) Reopening of any of the treated fistula(s) external openings with active drainage as clinically assessed in subjects who were in combined remission,**

    OR
  - **b) The development of a perianal fluid collection >2 cm of the treated perianal fistulas confirmed by centrally read magnetic resonance imaging (MRI) assessment.**

Safety ~~throughout the study~~ **Endpoints**

- ~~Safety evaluations including physical examination, vital signs, and laboratory (biochemistry, hematology) assessments will be performed.~~
- ~~The incidence of adverse events throughout the study will be monitored in all treated patients (Safety population).~~
  - **Incidence of treatment-emergent AEs (TEAEs).**
  - **Incidence of treatment-emergent SAEs.**
  - **Incidence of adverse events of special interest (AESIs).**
  - **Vital signs.**
  - **Laboratory parameters.**

Rationale for Change: Editorial changes made for completeness and improved clarity in the definition of the secondary endpoints. For the change to the key secondary endpoint, 'clinical response' is defined as 50% healing of fistulas which were draining at baseline, and while clinically important, is likely less relevant to the patient than 'time to clinical remission' which is defined as the time to 100% healing of fistula.

The following sections also contain this change:

- Summary Section
- Section 6.1.3.2 Secondary Efficacy Analyses.

Change 14: Editorial changes to the description of the exploratory endpoints and addition of exploratory endpoints.

The primary change occurs in Section 3.3.3 Exploratory Endpoints.

Initial wording:

- Change in severity of the perianal disease assessed with the Perianal Disease Activity Index (PDAI) and the PRO-2 (defined as average daily stool frequency and average daily abdominal pain) at Week 6, 12, 18, 24, 36, and 52.
- Change from baseline in blinded central MRI Van Assche score at Weeks 24 and 52.
- Change from baseline at Weeks 24 and 52 in blinded central MRI analysis of

	hyperenhancement in T1 sequence, and hyperintensity in T2 sequence.
	• Change from baseline in electronic Patient Reported Outcomes (PRO) at Weeks 12, 24 and 52:
	  • Visual Analogue Scale (VAS) from 0 to 10 for perianal pain while standing, sitting, and defecating along last 2 weeks prior to the visit,
	  • CDAI symptoms diary,
	  • Number of pads used per day along last 2 weeks prior to each visit,
	  • Work Productivity and Activity Impaiment Questionnaire (WPAI),
	  • EQ-5D[47],
	  • SF36,
	  • Health Resources Utilization (HRU).
	• Cell responses upon Cx601 treatment at Week 6, 12, and 24.
	• Immunological tests of anti-donor responses up to Week 52.
	The results of the above immunological tests at baseline will also be used to understand whether it is possible to predict the efficacy of the treatment in advance.

Amended or new text:	• Change from baseline in total ~~in severity of the perianal disease assessed with the~~ Perianal Disease Activity Index (PDAI) ~~and the PRO-2 (defined as average daily stool frequency and average daily abdominal pain)~~ at Week**s** 6, 12, 18, 24, 36, and 52.
	• **Change from baseline in total PRO-2 score (defined as average daily stool frequency and average daily abdominal pain) at Weeks 6, 12, 18, 24, 36, and 52.**
	• Change from baseline in blinded central MRI Van Assche score at Weeks 24 and 52.
	• **Change from baseline in modified Van Assche blinded central MRI score at Weeks 24 and 52.**
	• **Change from baseline in the Magnetic Resonance Novel Index for Fistula Imaging for Crohn's disease (MAGNIFI-CD) score at Weeks 24 and 52.**
	• Change from baseline at Weeks 24 and 52 in blinded central MRI analysis of hyperenhancement in T1 sequence, and hyperintensity in T2 sequence.
	• Change from baseline in electronic Patient Reported Outcomes (PRO) **listed below**, all measured through Weeks 12, 24**,** and 52:
	  • Visual Analogue Scale (VAS) from 0 to 10 for perianal pain while standing, sitting, and defecating along last 2 weeks prior to the visit,
	  • CDAI **items score** ~~symptoms diary~~,
	  • Number of pads used per day along last 2 weeks prior to each visit,
	  • Work Productivity and Activity Impaiment Questionnaire (WPAI),
	  • EQ-5D[47],
	  • SF36,

- Health Resources Utilization (HRU).

- ~~Cell responses upon Cx601 treatment at Week 6, 12, and 24.~~
- ~~Immunological tests of anti-donor responses up to Week 52.~~
  ~~The results of the above immunological tests at baseline will also be used to understand whether it is possible to predict the efficacy of the treatment in advance.~~
- **Immunogenicity responses as measured by donor-specific antibodies (DSA) levels.**
- **Change from baseline in cytokines, immune- and other inflammation-associated markers at Weeks 6, 12, 24, and 52.**
- **Change from baseline in the microbiome diversity at Week 6.**

Rationale for Change: For completeness and improved clarity in the definition of the exploratoy endpoints. The exploratory endpoint of microbiome diversity has been included to evaluate the exploratory objective related to microbiome diversity. The microbiome will be characterized to explore if microbial diversity impacts fistula healing.

The following sections also contain this change:

- Summary Section
- Section 3.3.1.1.3 Study Treatment Administration/Visit 0 (Day 0)
- Section 6.1.4 Exploratory Efficacy Analyses.

Change 15: Consistency update to treatment administration.

The primary change occurs in Section 3.5.1 Treatments Administered

Initial wording:	Cx601 is a 24 mL suspension of human expanded adipose-derived stem cells (eASC) of allogeneic origin in aseptic buffered human albumin solution presented in disposable vials with no preservative agents. The cells will be given at a dose of 120 million cells (5 million cells / mL) for local injection in the fistula. Placebo (saline solution) will be given also for local injection in the fistula at the same quantity (volume: 24 mL) and following the same procedure described for the eASC. Throughout this protocol the treatment administrated in the study meaning Cx601 or matched-placebo will be referred to as IMP.
Amended or new wording:	Cx601 is a 24 mL suspension of human expanded adipose-derived stem cells (eASC) of allogeneic origin in aseptic buffered human albumin solution presented in disposable vials with no preservative agents. The cells will be given at a dose of 120 million cells (5 million cells / mL) ~~for local injection in the fistula~~ **locally injected into the tissue around the internal openings and into the walls of the fistula tracts**. Placebo (saline solution) will be **locally injected into the tissue around the internal openings and into the walls of the fistula tracts** ~~given also for local injection in the fistula~~ at the same quantity (volume: 24 mL) and following the same procedure described for the eASC. Throughout this protocol the treatment administrated in the study meaning Cx601 or matched-placebo will be referred to as IMP.

Rationale for Change: To clarify the IMP site of administration and to align with wording in the CCDS v2.0.

The Summary section also includes this change.

Change 16: Text added to provide instruction on where information on study medication storage conditions can be found.

The primary change occurs in Section 3.5.2.3 Storage and Disposition of Study Drug

Initial wording:	Cx601 product, and matching placebo, will be shipped under temperature controlled conditions, using appropriate transport for biological samples. Shipping material is also duly labelled and has an attached package content list and instructions for use. Specific instructions will be provided within a separate study manual.
Amended or new wording:	Cx601 product, and matching placebo, will be shipped under temperature controlled conditions, using appropriate transport for biological samples. Shipping material is also duly labelled and has an attached package content list and instructions for use. Specific instructions will be provided within a separate study manual. **The study medication must be stored under the storage conditions specified in the manual**.

Rationale for Change: To make it clear to study personnel where study medication storage conditions can be found.

Change 17: Inclusion of text to clarify where information on the IMP can be found.

The primary change occurs in Section 3.5.2.4 Handling of Study Medication

Initial wording:	Study medication will be shipped by specialised couriers to the hospital pharmacy or the corresponding operating room where the study treatment administration will be performed, according to local practice and/or regulations.
Amended or new wording:	Study medication will be shipped by specialised couriers to the hospital pharmacy or the corresponding operating room where the study treatment administration will be performed, according to local practice and/or regulations. **See the label on the investigational product vial for expiration date and time.**

Rationale for Change: For completeness, it has been clarified where further details can be found to look for details related to the IMP.

Change 18: Text added on steps to maintain the study blind.

The primary change occurs in Section 3.5.4 Blinding

Initial wording:	[…] Additionally, surgeons who administer the study treatment and any ancillary personnel involved in study treatment administration will not be allowed to participate in any clinical assessment of the fistula(s) during the study. The emergency unblinding is available 7 days a week, 24h through the IWRS. Details are described in the IWRS manual. The double blind will be kept throughout the study (Week 52), i.e. for investigators and patients. The primary efficacy analysis will be conducted at Week 24.

Amended or new wording:

[…]

Additionally, surgeons who administer the study treatment and any ancillary personnel involved in study treatment administration will not be allowed to participate in any clinical assessment of the fistula(s) during the study.

The emergency unblinding is available 7 days a week, 24h through the IWRS. Details are described in the IWRS manual.

The primary outcome of this study is determined based on the results of the efficacy analyses at Week 24. To evaluate long-term safety and efficacy of Cx601 compared to placebo, the study will continue in a blind fashion up to Week 52. Therefore, individuals unblinded to subject-level treatment allocation at Week 24 readout will not be directly involved in study conduct after they are unblinded. In order to be able to minimize bias for the treatment comparison of efficacy and safety at Week 52, study conduct during this period will be handled by a separate team who will remain blinded to subject level treatment allocation at all times during the conduct of the study.

Rationale for Change: For completeness and transparency related to the conduct of the study analyses and the study.

Change 19: The serious adverse events list was updated to reflect the addition of the Takeda Medically Significant AE List as a new appendix.

The primary change occurs in Section 4.1.4 Serious Adverse Events

Initial wording:

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital abnormality/birth defect, or
- Is a medically significant event or requires intervention to prevent at least one of the outcomes listed above,
- Is a suspected transmission of an infectious agent.

Amended or new wording:

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital abnormality/birth defect, or
- Is a medically significant event**, including any event or synonym described in the Takeda Medically Significant AE List** (APPENDIX 3)**,** or requires

intervention to prevent at least one of the outcomes listed above, **or**

- Is a suspected transmission of an infectious agent.

New text: **Appendix 3:**

The events listed below must be reported as an SAE. Please note that this list represents the broad medical concepts to be considered as "important medical events satisfying SAE reporting requirements, and may or may not be related to the particular medicinal product under study."

Takeda Medically Significant AE List

Term	
Acute respiratory failure/acute respiratory distress syndrome	**Hepatic necrosis**
Torsade de pointes/ventricular fibrillation/ventricular tachycardia	**Acute liver failure**
Malignant hypertension	**Anaphylactic shock**
Convulsive seizure	**Acute renal failure**
Agranulocytosis	**Pulmonary hypertension**
Aplastic anemia	**Pulmonary fibrosis**
Toxic epidermal necrolysis/ Stevens-Johnson syndrome	**Confirmed or suspected endotoxin shock**
	Confirmed or suspected transmission of infectious agent by a medicinal product
	Neuroleptic malignant syndrome/ malignant hyperthermia
	Spontaneous abortion/stillbirth and fetal death

Rationale for Change: As TiGenix is now a wholly owned subsidiary of Takeda, the recording of adverse events will now also consider items on the Takeda medically significant AE list.

The change also occurs in APPENDIX 3

Change 20: A new section defining adverse events of special interest has been added to reflect editorial changes to safety endpoints.

The primary change occurs in Section 4.1.6 Adverse Events of Special Interest

Added text: **An AE of Special Interest (serious or non-serious) is one of scientific and medical concern specific to the compound or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them and would be described in protocols and instructions provided for investigators as to how and when they should be reported to the sponsor.**

AEs of special interest will include:

- **Tumorigenicity**
- **Ectopic tissue formation**
- **Hypersensitivity**
- **Transmission of infectious agents**
- **Immunogenicity/alloimmune reactions**
- **Medication errors**

Rationale for Change: In order to adequately evaluate the potential risks of Cx601, the AE definition section has been updated to include a new section defining adverse events of special interest to allow collection and reporting of AEs that are of scientific or medical concern specific to the program.

Change 21: Consistency change to list of AEs exempt from reporting.

The primary change occurs in Section 4.5.1 Recording of Adverse Events

Initial wording: […]

All AEs must be reported regardless of whether or not they are considered related to IMP administration or surgical procedure, with the exception of:

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline "Medical History" eCRF module
- Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents
- Medical or surgical procedures (e.g. endoscopy, appendectomy); however, the condition leading to these procedures, rather than the procedure itself, should be considered as AE.
- The expected fluctuations of any disease(s) pre-existent, ongoing, or detected at study start (e.g. worsening of the luminal Crohn´s disease).

Amended or new wording:

[…]

All AEs must be reported regardless of whether or not they are considered related to IMP administration or surgical procedure, with the exception of:

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline "Medical History" eCRF module
- Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents
- Medical or surgical procedures (e.g. endoscopy, appendectomy); however, the condition leading to these procedures, rather than the procedure itself, should be considered as AE.
- The expected fluctuations of any disease(s) pre-existent, ongoing, or detected at study start (e.g. worsening of the luminal Crohn´s disease).
- **For the purpose of this study, drainage of the treated fistula and abscesses, defining the primary endpoint, will not be captured as AEs unless there is evidence suggesting a causal relationship between the IMP or the administration procedure.**

Rationale for Change: The last bullet point was added for completeness and transparency of the AE reporting requirements in line with Section 4.1.1.

Change 22: The SAE reporting process has been clarified and updated, including the recipient of the SAE form.

The primary change occurs in Section 4.5.2 Serious Adverse Event Reporting

Initial wording:

All SAEs will be reported by the investigator to the monitor responsible for the clinical trial and to the PV department of PAREXEL. Such report will be made using the appropriate SAE form/Pregnancy form/eCRF, as applicable.

The SAE information, duly completed, should be made available within 24 hours via SAE form/eCRF.

Reporting time for the phone communication: 24 hours

Reporting time for the complete report: 24 hours

The PV department of PAREXEL will acknowledge and inform to PV TiGenix as per the safety plan agreed for the study.

Initial minimum information for reporting an adverse event should include the following:

- AE and onset date
- Patient ID, sex, and age (or date of birth)

- Information on treatment received for unexpected and reportable SUSARs
- Name and address of the reporting physician
- Causal relationship to the study treatment or surgical procedure

The complete SAE form/eCRF containing all information will be made available to PAREXEL within the next 2 business days. If the SAE is still active at the time of reporting or further information is obtained after initial communication, this information must be updated accordingly.

Amended or new wording:

All SAEs will be reported by the investigator to the ~~monitor responsible for the clinical trial and to the PV department of PAREXEL~~ **sponsor or its representative within 24 hours of the investigator becoming aware of the event,**~~. Such report will be made~~ using the appropriate SAE form/~~P~~**p**regnancy form/eCRF, as applicable.

The SAE information, duly completed, should be made available within 24 hours via SAE form/eCRF.

~~Reporting time for the phone communication: 24 hours~~

~~Reporting time for the complete report: 24 hours~~

~~The PV department of PAREXEL will acknowledge and inform to PV TiGenix as per the safety plan agreed for the study.~~

Initial minimum information for reporting an ~~adverse event~~**AE** should include the following:

- AE and onset date
- Subject ID, sex, and age (or date of birth)
- Information on treatment received for unexpected and reportable SUSARs
- Name and address of the reporting physician
- Causal relationship to the study treatment or surgical procedure

The complete SAE form/eCRF containing all information will be made available to ~~PAREXEL within the next 2 business days~~ **the contact listed in the contact list table at the start of the protocol within 24 hours**. If the SAE is still active at the time of reporting or further information is obtained after initial communication, this information must be updated accordingly.

Rationale for Change: The SAE reporting process has been updated to reflect the current recipient of the SAE Form and administrative structure for the study.

Change 23: Addition of adverse event of special interest reporting requirements.

The primary change occurs in Section 4.5.3 Adverse Event of Special Interest Reporting

New wording:

If the AE of special interest/abnormality, which occurs during the study, is considered to be clinically significant based on the criteria below, it should be recorded in an AE of Special Interest Name Form or an SAE Form if the AESI meets the seriousness criteria. The SAE Form should be completed and reported to the sponsor or its representative within 24 hours of the investigator becoming aware of the event.

The investigator should submit the original copy of the AE of Special Interest Name Form or the SAE Form to the sponsor.

Special interest AE/abnormality criteria include:

- **Immunogenicity/alloimmune reactions.**
- **Hypersensitivity.**
- **Ectopic tissue formation.**
- **Medication errors.**
- **Tumorigenicity.**
- **Transmission of infectious agents.**

AEs of special interest must be recorded as AEs in the eCRF. An evaluation form along with all other required documentation must be submitted to the sponsor.

Rationale for Change: The reporting procedure for adverse events of special interest was added in the protocol due to the inclusion of a new section defining adverse events of special interest to allow collection of AEs that are of scientific or medical concern specific to the program.

Change 24: The statistical analytical plans section was updated to reflect revised current statistical plans.

The primary change occurs in Section 6.1 Statistical and Analytical Plans

Initial wording:

The statistical analysis will be performed using SAS® Version 9.1.3 or later.

Statistical significance will be assessed at a two-sided type I error (α) level of 0.05. For the primary efficacy analysis, no multiplicity adjustment is necessary. For analysis of key secondary efficacy endpoints, multiplicity adjustment will be employed.

Quantitative variables will be described showing their number of available and missing observations, mean, median, standard deviation, the range (minimum and maximum) and the first and third quartiles. Frequency and percentage will describe qualitative variables. Missing values will be tabulated but will not be included in the calculation of percentages.

Full details of the statistical analysis will be given in the corresponding SAP.

In order to avoid any delay in the assessment of the Cx601-0303 study, the follow-up period data, up to 52 weeks will be analysed separately from the 24 weeks primary analysis of results. The list of codes will not be revealed to the investigators / patients until the results of the follow up period, up to 52 weeks, are analysed.

Amended or new wording:

~~The statistical analysis will be performed using SAS® Version 9.1.3 or later.~~

~~Statistical significance will be assessed at a two-sided type I error (α) level of 0.05. For the primary efficacy analysis, no multiplicity adjustment is necessary. For analysis of key secondary efficacy endpoints, multiplicity adjustment will be employed.~~

~~Quantitative variables will be described showing their number of available and missing observations, mean, median, standard deviation, the range (minimum and maximum) and the first and third quartiles. Frequency and percentage will describe qualitative variables. Missing values will be tabulated but will not be included in the calculation of percentages.~~

~~Full details of the statistical analysis will be given in the corresponding SAP.~~

~~In order to avoid any delay in the assessment of the Cx601-0303 study, the follow-up period data, up to 52 weeks will be analysed separately from the 24 weeks primary analysis of results. The list of codes will not be revealed to the investigators / patients until the results of the follow-up period, up to 52 weeks, are analysed.~~

A statistical analysis plan (SAP) will be prepared and finalized before database unblinding. This document will provide further details regarding the derivation of analysis variables and analysis methodology to address all study objectives.

A targeted data review will be conducted before database lock. This review will assess the accuracy and completeness of the study database, subject evaluability, and appropriateness of the planned statistical methods.

Rationale for Change: The statistical analysis section was shortened by removing some details on the statistical methods that will be presented in the SAP instead (i.e., the SAP was considered to be the more appropriate location for this information).

Change 25: Editorial changes and addition of the per-protocol set.

The primary change occurs in Section 6.1.2 Demographic and Other Baseline Characteristics

Initial wording:

Baseline characteristics will be summarised descriptively for the ITT, mITT, and safety analysis sets.

Categorical data will be summarised with absolute and relative frequencies (percentages), and continuous data will be summarised with the number of subjects, mean, median, standard deviation, the range (minimum and maximum) and the first and third quartiles.

Amended or new wording:

Baseline characteristics will be summarized descriptively for the ITT, mITT, safety, and **PP** safety analysis sets.

Categorical data will be summarized with absolute and relative frequencies (percentages), and continuous data will be summarized with the number of subjects, mean, median, standard deviation, the range (minimum and maximum) and the first and third quartiles.

Rationale for Change: For clarity and completeness, a PP population set was added as one of the analysis sets for summarization of baseline characteristics.

Change 26: Editorial changes and addition of the per-protocol set, and removal of safety analysis

set. The population set for the analysis of the exploratory efficacy variables updated to ITT from mITT.

The primary change occurs in Section 6.1.3 Efficacy Analyses

Initial wording:	The analyses the primary and key secondary endpoints will be performed on ITT, mITT, and safety analysis sets, with the ITT as the primary analysis set. All exploratory variables will be analyzed on the mITT using all available data. Sensitivity analyses for the primary and the key secondary endpoints with respect to missing values and the use of rescue medication or surgery will be detailed in the SAP. Efficacy analyses will be performed with patients grouped according to the randomized treatment assigned. Statistical significance will be assessed at a two-sided type I error (α) level of 0.05, unless otherwise noted.
Amended or new wording:	The analyses the primary and key secondary endpoints will be performed on ITT, mITT, and ~~safety~~ **PP** analysis sets, with the ITT as the primary analysis set. All exploratory variables will be analyzed on the **ITT analysis set** ~~mITT using all available data~~. Sensitivity analyses for the primary and the key secondary endpoints with respect to missing values and the use of rescue medication or surgery will be detailed in the SAP. Efficacy analyses will be performed with ~~patients~~ **subjects** grouped according to the randomized treatment assigned. Statistical **hypothesis testing will be 2-sided, and** significance will be assessed at **the 5% level** ~~a two-sided type I error (α) level of 0.05~~, unless otherwise noted.

Rationale for Change: For clarity and completeness, a PP population set was added as one of the analysis sets for the primary and key secondary endpoints as a sensitivity analysis. The safety analysis set was removed as one of the analysis sets for the primary and key secondary endpoints as a sensitivity analysis. To make the analysis set for exploratory efficacy variables consistent with that for primary and key secondary endpoints, it was updated to ITT analysis set from mITT analysis set. In addition, editorial changes were made for improved clarity and completeness.

Change 27: Editorial changes to the description of the primary efficacy analysis.

The primary change occurs in Section 6.1.3.1 Primary Efficacy Analysis

Initial wording:	The primary efficacy analysis at Week 24 will consist of the Combined Remission percentage of patients with complex perianal fistula(s) by Week 24 being compared between the eASC and placebo treatment groups using a stratified Cochran-Mantel-Haenszel test, adjusting for the randomization strata, with statistical significance at a two sided type I error (α) level of 0.05. Primary efficacy analysis will be performed on a intention-to-treat set (ITT) approach defined as all randomized patients regardless of being treated or not. In the case of any missing data, either no MRI or no post-baseline clinical assessment at Week 24, the evaluation of Combined Remission will not be possible and a non-response will be imputed (single imputation). Special circumstances where concomitant medication

and other medical interventions will affect the evaluability of the patients (see Section 3.2.4) , then the status of the Combined Remission will be considered as "Treatment Failure" i.e. non-response at all subsequent visits/time-points following the need of rescue.

Amended or new wording:

~~The primary efficacy analysis at Week 24 will consist of the Combined Remission percentage of patients with complex perianal fistula(s) by Week 24 being compared between the eASC and placebo treatment groups using a stratified Cochran-Mantel-Haenszel test, adjusting for the randomization strata, with statistical significance at a two sided type I error (α) level of 0.05.~~

~~Primary efficacy analysis will be performed on a intention-to-treat set (ITT) approach defined as all randomized patients regardless of being treated or not. In the case of any missing data, either no MRI or no post-baseline clinical assessment at Week 24, the evaluation of Combined Remission will not be possible and a non-response will be imputed (single imputation). Special circumstances where concomitant medication and other medical interventions will affect the evaluability of the patients (see Section 3.2.4) , then the status of the Combined Remission will be considered as "Treatment Failure" i.e. non-response at all subsequent visits/time points following the need of rescue.~~

The primary efficacy analysis comparing Cx601 and placebo with respect to the primary endpoint (proportion of subjects with combined remission rate at Week 24) will be performed using a stratified Cochran-Mantel-Haenszel test, adjusting for the randomization strata. The point estimate of the treatment difference (Cx601 – placebo) in the combined remission rates along with the 95% CI will be provided.

The primary efficacy analysis will be performed on an intention-to-treat (ITT) population that includes all randomized subjects regardless of what treatment (if any) they received.

Handling of missing data and treatment failure:

A subject will be classified as a non-responder at Week 24 (single imputation) in any of the following situations:

- **a) Missing data, including no MRI or no clinical assessment at Week 24, evaluation of Week 24 combined remission is not possible; OR**
- **b) Treatment failure is documented for a subject if they require rescue medication or procedure as defined in Section 3.2.4.3.**

Other sensitivity analyses to assess the impact of missing data and analysis populations on the analysis of the primary endpoint will be detailed in the SAP.

Rationale for Change: For purposes of improved clarity, completeness, and transparency.

The Summary Section also contains this change.

Change 28: Editorial changes to the description of the secondary efficacy analysis. In addition, section updated to align with the change to one of the two key secondary endpoints.

The primary change occurs in Section 6.1.3.2 Secondary Efficacy Analyses

Initial wording:

To address the issue of multiplicity, the key secondary endpoints identified below will be grouped into a Week 24 short-term family, with a gatekeeping procedure [33], utilizing Hochberg's testing procedure[34] to control the overall type I error.

The primary efficacy endpoint of Combined Remission of perianal fistula(s) by Week 24 will serve as the gatekeeper for the Week 24 short-term family of key secondary endpoints. Therefore, the p-values for the key secondary efficacy analysis tables will be subject to inference only if the primary efficacy analysis is significant.

The key secondary endpoints will be analysed in the same way as that for the primary endpoint.

In the case of missing Clinical data at Week 24, a non-response will be imputed (single imputation) for the 2 key secondary endpoints.

If a patient needs rescue medication, or change in dose or switching medications due to flare in CDs, or need for antibiotics for at least three consecutive weeks, or rescue surgery for the fistula(s) in the perianal region, then the status of the Clinical remission/Clinical response will be considered as " non-response" at all Weeks following the need of rescue.

The analysis of Combined remission, Clinical remission and Clinical response at Week 52 will be performed similarly as for Week 24.

Amended or new wording:

~~To address the issue of multiplicity, the key secondary endpoints identified below will be grouped into a Week 24 short term family, with a gatekeeping procedure [33], utilizing Hochberg's testing procedure[34] to control the overall type I error.~~

~~The primary efficacy endpoint of Combined Remission of perianal fistula(s) by Week 24 will serve as the gatekeeper for the Week 24 short term family of key secondary endpoints. Therefore, the p-values for the key secondary efficacy analysis tables will be subject to inference only if the primary efficacy analysis is significant.~~

~~The key secondary endpoints will be analysed in the same way as that for the primary endpoint.~~

~~In the case of missing Clinical data at Week 24, a non response will be imputed (single imputation) for the 2 key secondary endpoints.~~

~~If a patient needs rescue medication, or change in dose or switching medications due to flare in CDs, or need for antibiotics for at least three consecutive weeks, or rescue surgery for the fistula(s) in the perianal region, then the status of the Clinical remission/Clinical response will be considered as " non response" at all Weeks following the need of rescue.~~

~~The analysis of Combined remission, Clinical remission and Clinical response at Week 52 will be performed similarly as for Week 24.~~

The key secondary endpoint of clinical remission at Week 24 will be analyzed with the same methodology that was used for the primary efficacy endpoint outlined above, including the handling of missing data and treatment failures.

The key secondary endpoint of time to clinical remission, assessed at Week 24,

will be analyzed using the stratified log-rank test for comparing CX601 and placebo. The Cox proportional hazards model will be used to obtain estimates of the hazard ratio and the associated CIs. Kaplan-Meier curves will be presented along with median survival times.

Multiplicity adjustment: To address multiplicity, a gate-keeping approach will be used. First the primary efficacy endpoint, proportion of subjects with combined remission at Week 24, will be tested. If the primary efficacy analysis statistically significant, the Hochberg procedure will then be used to test the pool of the following 2 key secondary endpoints.

- **Proportion of subjects with clinical remission at Week 24**
- **Time to clinical remission (weeks) assessed at Week 24**

Additional details on the multiplicity adjustment will be provided in the SAP.

Other secondary efficacy analysis: All the binary endpoints will be analyzed using the same methodology as used for the primary efficacy endpoint described above, including the handling of missing data and treatment failures.

Time-to-event endpoints will be analyzed using the same methodology as used for the key secondary endpoint (time to clinical remission) as described above.

Categorical data will be summarized with absolute and relative frequencies (percentages) and continuous data will be summarized by the number of subjects, mean, standard deviation, minimum, first quartile, median, third quartile, and maximum.

Rationale for Change: For improved clarity, completeness and transparency to the secondary efficacy analysis plan. Additional language added to align with the change to the key secondary endpoint from 'clinical response' to 'time to clinical remission'.

The Summary Section also contains this change.

Change 29: The subgroup analysis section was updated to add a subgroup analysis for each of the two stratification factors, concomitant treatment (4 levels), and Region with USA and Non-USA as the subgroups of interest. In addition, levels of colonoscopy status at baseline were further clarified.

The primary change occurs in Section 6.1.3.3 Subgroup Efficacy Analyses

Initial wording: Subgroup analyses for the primary and key secondary efficacy endpoints, using the ITT analysis set, will performed for the following subgroups (details will be given in the SAP):

- Gender
- Race
- Age group
- Donor
- Region (Europe + Israel vs North America)
- Smoking status (current/former/never)
- Full colonoscopy performed at baseline, or previously available within 1 to 3

months prior to screening, or within >3 and 6 months prior to screening

- Absence of any colonic large ulcers larger than 0.5 cm with improvement or no worsening in abdominal pain and/or in diarrhea and no initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs dose regimen, since the last endoscopy up to Screening visit, versus Absence of any colonic large ulcers larger than 0.5 cm without improvement or worsening in abdominal pain and/or in diarrhea sustained for one week or more or initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs therapy dose regimen, since the last endoscopy up to Screening visit
- Prior treatment with either immunosuppressants or biologics (i.e.: anti-TNF or anti-integrin or anti-IL12/23) or both
- By level of combination of the following stratification factor (at randomization):
    - Current concomitant immunosuppressant or biologics as single agent or as a combination or no ongoing concomitant treatment at Screening visit
    - External opening(s) (1 or >1)

Amended or new wording:

Subgroup analyses for the primary and key secondary efficacy endpoints, using the ITT analysis set, will performed for the following subgroups (details will be given in the SAP):

- Gender
- Race
- Age group
- Donor
- Region (Europe + Israel vs North America)
- **Region (USA, Non-USA)**
- Smoking status (current/former/never)
- ~~Full colonoscopy performed at baseline, or previously available within 1 to 3 months prior to screening, or within >3 and 6 months prior to screening~~ **Colonoscopy status at baseline (full colonoscopy performed at baseline vs colonoscopy performed prior to baseline)**
- ~~Absence of any colonic large ulcers larger than 0.5 cm with improvement or no worsening in abdominal pain and/or in diarrhea and no initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs dose regimen, since the last endoscopy up to Screening visit, versus Absence of any colonic large ulcers larger than 0.5 cm without improvement or worsening in abdominal pain and/or in diarrhea sustained for one week or more or initiation or intensification of treatment with corticosteroids, immunosuppressants or mAbs therapy dose regimen, since the last endoscopy up to Screening visit~~
- Prior treatment with either immunosuppressants or biologics (i.e.: anti-TNF or anti-integrin or anti-IL12/23) or both
- **Concomitant treatment (3 levels):**
    - **Concomitant immunosuppressant (IS) or monoclonal antibodies**

(mAbs) (i.e., anti-tumor necrosis factor (anti-TNF) or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab) as single agent

- **Concomitant IS or mAbs (i.e., anti-TNF or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab)) as combination treatment,**
- **No ongoing concomitant IS or mAbs treatment at time of randomization**

- **External opening(s) (1 or > 1)**
- **Concomitant treatment (4 levels):**
  - **Concomitant immunosuppressant (IS) as a single agent**
  - **Monoclonal antibodies (mAbs) (i.e., anti-tumor necrosis factor (anti-TNF) or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab) as single agent**
  - **Concomitant IS or mAbs (i.e., anti-TNF or anti-integrin (i.e., vedolizumab) or anti-interleukin (IL)12/23 (i.e., ustekinumab)) as combination treatment**
  - **No ongoing concomitant IS or mAbs treatment at time of randomization**
- By level of combination of the following stratification factor (at randomization):
  - Current concomitant immunosuppressant or biologics as single agent or as a combination or no ongoing concomitant treatment at Screening visit
  - External opening(s) (1 or >1)

Rationale for Change: The subgroup analysis based on the two stratification factors, concomitant treatment (4 levels), and region (USA and non-USA) was included to evaluate the clinical benefit of the treatment within each subgroup. Additionally, language of colonoscopy status at baseline subgroup was updated to clearly define 2 categories of interest.

Change 30: Editorial change to the description of exploratory efficacy analyses.

The primary change occurs in Section 6.1.4 Exploratory Efficacy Analyses

Initial wording:

[…]

All continuous variables will be analyzed on the basis of a repeated mixed model with treatment, stratum, and visit (if data collected at multiple post-treatment visits) as fixed factors with the inclusion of the time by treatment interaction (if appropriate); if available, the baseline value will be used as covariate. The covariance matrix will be assumed of the unstructured type. Within-group and between-group changes from baseline at each visit will be obtained by using the appropriate contrasts; 95% CI will be provided together with the appropriate descriptive statistics.

Descriptive statistics will be provided on MRI exploratory variables such as hyperenhancement in T1 sequence and/or hyperintensity in T2 sequence at each visit.

Descriptive Statistics of the above exploratory endpoints at Weeks 24 and 52 will be

presented separately for the subgroup of patients with Combined remission at the same Week.

Anti-donor antibody kinetics will be described in available patients samples.

Amended or new text:

[…]

All continuous ~~variables~~ **endpoints** will be analyzed ~~on the basis of~~ **using** a ~~repeated~~ mixed model **for repeated measures (MMRM)** with treatment, stratum, and visit (if data collected at multiple post-treatment visits) as fixed factors with the inclusion of the time by treatment interaction (if appropriate); if available, the baseline value will be used as covariate. ~~The covariance matrix will be assumed of the unstructured type. Within-group and between-group changes from baseline at each visit will be obtained by using the appropriate contrasts; 95% CI will be provided together with the appropriate descriptive statistics.~~ **The treatment comparisons and corresponding 95% CIs will be presented for each scheduled visit.**

All binary endpoints will be analyzed using the same methodology as used for the primary efficacy endpoint described above, including the handling of missing data and treatment failures.

~~Descriptive statistics will be provided on MRI exploratory variables such as hyperenhancement in T1 sequence and/or hyperintensity in T2 sequence at each visit.~~

~~Descriptive Statistics of the above exploratory endpoints at Weeks 24 and 52 will be presented separately for the subgroup of patients with Combined remission at the same Week.~~Antidonor antibody kinetics ~~will be described in available patients samples~~ **will be summarized by treatment group. The results will be reported in a separate report.**

Rationale for Change: For purposes of improved clarity, completeness, and transparency.

The Summary Section also contains this change.

Change 31: Editorial changes were made to the description of the safety analysis section.

The primary change occurs in Section 6.1.5 Safety Analyses

Initial wording:

Safety measurements will include:

- Incidence of all events reported since treatment administration (Treatment Emergent Adverse Events-TEAEs)
- Incidence of events "related" since treatment administration (TEAEs related to study treatment)
- Incidence of SAEs since treatment administration (Treatment Emergent Serious Adverse Events - TESAEs)
- Incidence of SAEs "related" since treatment administration (Treatment Emergent Serious Adverse Events - TESAEs related to study treatment)
- Incidence of TEAEs and TESAEs according to their intensity/severity
- Incidence of TEAEs or TESAEs leading to study withdrawal
- Incidence of NTEAEs related or not to surgical procedure(s) during fistula(s)

preparation visit up to the to study treatment administration (if related, then, Procedure Emergent-Non Treatment Emergent adverse event – PENTE)

- Incidence of TEAEs that could be related or not the surgical procedure(s) following treatment administration and within up to two weeks since the last procedure
- Incidence of deaths (Fatal SAEs)
- Incidence of clinical findings on physical examination, vital signs or laboratory tests
- Immunological humoral response by safety

All reported adverse events will be recorded as per reporter verbatim and the applicable coded terms. Safety data (including physical examination, vital signs and laboratory tests) will be summarised using descriptive statistics. The number of patients experiencing each AE category will be summarized by body system and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) terminology.

Safety analysis will be performed on the Safety analysis set. Categorical data will be summarised with absolute and relative frequencies (percentages), and continuous data will be summarised with the number of patients, mean, median, standard deviation, the range (minimum and maximum) and the first and third quartiles.

Non-treatment emergent adverse events (during the Screening period) will be collected and reported separately.

Amended or new wording:

Detailed evaluation and ~~Safety measurements~~ **summaries of safety endpoints** will include:

- Incidence of ~~all events reported since treatment administration (~~Treatment Emergent Adverse Events **(**TEAEs)
- Incidence of ~~events "related" since treatment administration (~~TEAEs related to study treatment~~)~~
- **Incidence of AESIs**
- Incidence of ~~SAEs since treatment administration (T~~treatment-~~E~~**e**mergent ~~S~~**s**erious ~~A~~**a**dverse ~~E~~**e**vents ~~–~~**(**TESAEs)
- Incidence of ~~SAEs "related" since treatment administration (Treatment Emergent Serious Adverse Events –~~TESAEs related to study treatment~~)~~
- Incidence of TEAEs and TESAEs according to their intensity/severity
- Incidence of TEAEs or TESAEs leading to study withdrawal
- Incidence of ~~events reported since Informed Consent signature up to the treatment administration (Non Treatment Emergent Adverse Events –~~ NTEAEs~~)~~**, whether or not related to surgical procedure(s), during fistula(s) preparation visit up to the to study treatment administration (if related, then, procedure-emergent non–treatment-emergent adverse event)**
- ~~Incidence of NTEAEs related or not to surgical procedure(s) during fistula(s) preparation visit up to the to study treatment administration (if related, then, Procedure Emergent Non Treatment Emergent adverse event – PENTE)~~
- **Incidence of serious NTEAEs and serious TEAEs related to the procedure**

or treatment administration

- Incidence of TEAEs ~~that could be related or not~~**, whether or not related to** the surgical procedure(s)**,** following treatment administration and within up to **2**~~two~~ weeks since the last procedure
- Incidence of deaths (fatal SAEs)
- Incidence of clinical findings on physical examination, vital signs, or laboratory tests
- Immunological humoral response by safety

~~All reported adverse events will be recorded as per reporter verbatim and the applicable coded terms.~~ Safety data (including ~~physical examination,~~ vital signs and laboratory tests) will be summarized using descriptive statistics. The number of subjects experiencing each AE category will be summarized by ~~body~~ System **Organ Class** and Preferred Term using Medical Dictionary for Regulatory Activities (MedDRA) terminology.

Safety analysis will be performed on the Safety analysis set. Categorical data will be summarized with absolute and relative frequencies (percentages), and continuous data will be summarized with the number of subjects, mean, ~~median,~~ standard deviation, ~~the range (~~minimum ~~and maximum)~~, ~~and~~ the first quartile, **median,** the third quartile~~s~~**, and maximum**.

Non–treatment-emergent adverse events (**serious and non-serious events that occur** during the Screening period) will be collected and reported separately.

Rationale for Change: For purposes of transparency, completeness and improved clarity.

Change 32: Text to describe the sample size rationale for the planned increase in the total sample size to 554 subjects from the originally planned sample size of 326 patients was added.

The primary change occurs in Section 6.3 Determination of Sample Size.

Initial wording: The sample size calculation for this study is based on the results from the previous phase 3 ADMIRE-CD study (Cx601-0302) performed in Europe and Israel in a similar patient population and study design. Briefly, 212 patients were randomized, 107 patients to a Cx601 treatment arm and 105 to a control of matching placebo: 1) the placebo-arm combined remission rate at Week 24 was approximately 34% which increased to 50% in the Cx601 containing-arm thus a 15.2% absolute difference; 97.5%CI: 0.2-30.3%; p=0.024 on intention-to-treat (ITT) analysis. Accordingly, a sample size of 326 patients (163 patients per arm) will be needed with at least 80% power to statistically detect a minimum 16 per cent points difference in the percentage of patients achieving Combined Remission of perianal fistula(s) as clinically and radiologically assessed with absence of collections >2 cm (in at least 2 dimensions) confirmed by blinded central MRI assessment by Week 24 between Cx601 and placebo (two-sided α=0.05; Chi-square test). Accordingly and assuming an estimated 25% of screening failures as per previous phase III study, approximately 436 patients will be screened in order to randomise, on a 1:1 ratio 326 eligible patients. No allowance for premature withdrawals is made since all randomised patients will be included in the analysis, although 10% premature withdrawals are accounted for in the difference to be detected).

Amended **The primary endpoint of the study is the proportion of subjects with combined**

or new wording:

remission at Week 24. The following assumptions are made in the sample size calculations:

- **True combined remission rates at Week 24 of 42.2% and 30% in the CX601 and placebo groups, respectively (based on estimates from a similar population in the Phase 3 ADMIRE-CD study).**
- **The family-wise Type-1 error rate will be controlled at 0.05 for the analyses of the primary and key secondary efficacy endpoints at Week 24.**

Based on the above assumptions, a total sample size of 554 subjects (277 per treatment arm) will provide at least 85% power for the primary efficacy endpoint analysis at Week 24.

~~The sample size calculation for this study is based on the results from the previous phase 3 ADMIRE CD study (Cx601 0302) performed in Europe and Israel in a similar patient population and study design. Briefly, 212 patients were randomized, 107 patients to a Cx601 treatment arm and 105 to a control of matching placebo: 1) the placebo arm combined remission rate at Week 24 was approximately 34% which increased to 50% in the Cx601 containing arm thus a 15.2% absolute difference; 97.5%CI: 0.2-30.3%; p=0.024 on intention to treat (ITT) analysis. Accordingly, a sample size of 326 patients (163 patients per arm) will be needed with at least 80% power to statistically detect a minimum 16 per cent points difference in the percentage of patients achieving Combined Remission of perianal fistula(s) as clinically and radiologically assessed with absence of collections >2 cm (in at least 2 dimensions) confirmed by blinded central MRI assessment by Week 24 between Cx601 and placebo (two sided α=0.05; Chi square test). Accordingly and assuming an estimated 25% of screening failures as per previous phase III study, approximately 436 patients will be screened in order to randomise, on a 1:1 ratio 326 eligible patients. No allowance for premature withdrawals is made since all randomised patients will be included in the analysis, although 10% premature withdrawals are accounted for in the difference to be detected).~~

Rationale for Change: The assumptions made to calculate the original sample size of 326 patients were based on the estimate of 15.2% of the true treatment difference in the primary endpont from the completed phase III study (ADMIRE-CD) and provided 80% power for the primary efficacy analysis. However, an estimate of 12.2% was obtained from an ADMIRE-CD post hoc subgroup analysis using a subgroup of subjects that are more similar to the targeted patient population in ADMIRE-II. Therefore, an updated sample size of 554 subjects that is based on an assumption of 12.2% for the true difference and provides approximately 85% power for the primary analysis was considered appropriate to ensure that ADMIRE-CD II is an adequate and well-controlled pivotal trial.

The following sections also contain this change:

- Summary Section
- Section 3.1 Overall Study Design and Plan: Description.

Change 33: Editorial changes to the section describing the handling of dropouts or missing data were made for improved clarity. Language that was not applicable to the section was removed.

The primary change occurs in Section 6.4 Handling of Dropouts or Missing Data. This section was Section 6.3 in the previous version.

Initial wording:

All efforts will be developed to keep the anticipated percentage of missing data not above the 10%, especially related to the outcome values meaningful for the analysis of the primary and key secondary endpoints.

In particular, the following measures[41, 42] will apply:

Missing data due to discontinuation of study treatment due to, e.g., adverse events or lack of efficacy will be avoided as patients receiving a rescue medication or procedure will be followed during the study (see Section 3.4.1.2), and investigators are exhorted to consider any patient's withdrawal by doing all reasonable efforts to keep the patient in the study for further assessment of efficacy and/or safety to study medication whenever is applicable (see Section 3.4.1).

Missing data because of lacking MRI due to waiting time for this assessment is anticipated to be minimal considering the North America top ranking in the number of MRI machines per-capita; the selection of investigational sites will be based on this key feasibility assessment globally.

Patients will be provided a reminder of next study visit at each visit throughout the study. Investigators will be trained on the importance of avoiding missing data.

Monitoring of adherence by patients, especially those who cannot tolerate or do not adequately respond to treatment will be performed.

The analyses of the primary and key secondary efficacy endpoints will be performed on ITT, mITT, and per protocol anlaysis sets, with the ITT as the primary analysis set. In ITT analysis, for the non- treated patients or those treated patients who have missing value for their primary or key secondary efficacy assessments or have taken any rescue medication or go through a rescue surgery (for details see also Section 6.1.3) at any time before Week 24 visit during the study, for both primary and key secondary endpoints will be imputed as a non-response: i.e. non-Combined Remission, non- Clinical Remission and non-Response, respectively.

If any of the rescue medications or surgery occurred after the Week 24 but before the Week 52, the non-response will be imputed from the documented date of occurrence up to Week 52 but not retrospectively if any efficacy data is available at Week 24.

If a patient's endpoint(s) is imputed as an overriding non-response at any point during the study, then that endpoint will necessarily be imputed as an overriding non-response at all subsequent time points throughout the remainder of the study, regardless of whether the patient has any actual non- missing data at any subsequent time point.

Reasons for treatment failure (TF) imputation will be explored and categorized.

Sensitivity analyses for the primary and the key secondary endpoints with respect to missing values and the use of rescue medication or surgery will be detailed in the SAP.

New wording:

The following approach for imputation of missing data will be used in the Week 24 primary efficacy analysis:

A subject will be classified as a non-responder at Week 24 (single imputation) in any of the following situations:

a) Missing data, including no MRI or no clinical assessment at Week 24, evaluation of Week 24 combined remission is not possible; OR

b) Treatment failure is documented for a subject if they require rescue medication or procedure as defined in Section 3.2.4.3.

Other sensitivity analyses to assess the impact of missing data and the analysis populations on the analysis of the primary endpoint will be detailed in the SAP. The analysis of the secondary and other efficacy responder rate endpoints will use a similar approach as described above for handling of dropouts or missing data. Under this approach, a subject will be classified as a non-responder at a given visit in any of the following situations:

a) Missing data at the visit of interest; OR

b) Treatment failure is documented for a subject before the visit of interest if they require rescue medication or procedure as defined in Section 3.2.4.3

Rationale for Change: For purposes of transparency, completeness and improved clarity.

Change 34: Editorial change to data quality assurance section.

The primary change occurs in Section 8.4 Data Quality Assurance

Initial wording:

[…]

The database quality will be assessed before the database closure; and only when the SAP is approved and the analysis sets are determined, the database will be locked and provided to Statistics to perform the statistical analysis and prepare the Clinical Study Report at Week 24, then Week 52. The blinding will be kept throughout the study for investigators and patients. Treatment codes will not be revealed to participating investigators and patients until the results of the follow up period, up to 52 weeks, are analysed. There will be no interim analysis. The primary and key secondary efficacy analysis will be performed when all randomized patients have their assessment done at Week 24.

Amended or new wording:

[…]

The database quality will be assessed before the database **lock** ~~closure~~; and only when the SAP is approved and the **subject evaluability (i.e. protocol deviations)** ~~analysis sets~~ are determined., ~~the database will be locked and provided to Statistics to perform the statistical analysis and prepare the Clinical Study Report at Week 24, then Week 52. The blinding will be kept throughout the study for investigators and patients. Treatment codes will not be revealed to participating investigators and patients until the results of the follow up period, up to 52 weeks, are analysed. There will be no interim analysis. The primary and key secondary efficacy analysis will be performed when all randomized patients have their assessment done at Week 24~~.

Rationale for Change: For purposes of transparency, completeness and improved clarity.

The following sections also contain this change:

- Summary Section
- Section 3.1 Overall Study Design and Plan: Description

Change 35: Update to publication policy.

The primary change occurs in Section 9.1 Publication Policy

Initial wording:

All study information provided by Takeda in relation to this study and not previously published is considered confidential information. Such information comprises the clinical protocol, any workbooks if applicable, eCRFs, assessment methods, sponsor technical methods, and basic scientific data. This confidential information will be the property of Takeda, must not be disclosed to third parties without prior written consent from the sponsor, and must only be used for the study purposes.

Information collected during the conduct of this clinical study is also considered to be confidential. Such information can be disclosed to the extent considered necessary by Takeda.

In order to allow use of information derived from this study and to ensure compliance with the applicable regulations, the investigator is committed to provide Takeda all examination results and all data collected in this study. Except as required by law, information obtained during the study can only be provided to physicians and regulatory authorities by Takeda.

Takeda undertakes publishing the study results and report them at scientific meetings. The list of authors will be based on study authorship considering the involvement in trial design, oversight, analysis of data, randomization of a significant number of subjects (among the top randomized subjects contributors, first signing centre will be the one randomizing the greatest number of subjects), results interpretation in the context of state-of-the-art, and preparation of the manuscript. The study will only be published once it has been completed and Takeda has made the final analysis.

Amended or new text:

~~All study information provided by Takeda in relation to this study and not previously published is considered confidential information. Such information comprises the clinical protocol, any workbooks if applicable, eCRFs, assessment methods, sponsor technical methods, and basic scientific data. This confidential information will be the property of Takeda, must not be disclosed to third parties without prior written consent from the sponsor, and must only be used for the study purposes.~~

~~Information collected during the conduct of this clinical study is also considered to be confidential. Such information can be disclosed to the extent considered necessary by Takeda.~~

~~In order to allow use of information derived from this study and to ensure compliance with the applicable regulations, the investigator is committed to provide Takeda all~~

~~examination results and all data collected in this study. Except as required by law, information obtained during the study can only be provided to physicians and regulatory authorities by Takeda.~~

~~Takeda undertakes publishing the study results and report them at scientific meetings. The list of authors will be based on study authorship considering the involvement in trial design, oversight, analysis of data, randomization of a significant number of subjects (among the top randomized subjects contributors, first signing centre will be the one randomizing the greatest number of subjects), results interpretation in the context of state of the art, and preparation of the manuscript. The study will only be published once it has been completed and Takeda has made the final analysis.~~

The investigator is obliged to provide the sponsor with complete test results and all data derived by the investigator from the study. During and after the study, only the sponsor may make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the study site agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of the sponsor.

The sponsor may publish any data and information from the study (including data and information generated by the investigator) without the consent of the investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the study site agreement. In the event of any discrepancy between the protocol and the study site agreement, the study site agreement will prevail.

Rationale for Change: The publication policy was updated in line with sponsor policy.

Change 36: Editorial change to anal clock in APPENDIX 2.

The primary change occurs in APPENDIX 2.

Rationale for Change: For purposes of completeness and improved clarity.

Change 37: Editorial change to schedule of assessment footnote.

The primary change occurs in Schedule of Assessments

Initial wording:	†† Pelvic MRI performed locally (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location). A quality copy will be sent to the Central Imaging Lab within 24h from acquisition for immediate blinded central MRI reading as detailed in the specific manual. Blinded central MRI results (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location) will be communicated to the investigator and the surgeon prior to Preparation visit. A turnaround of 5 days for Central Reading is needed (considering adequate images have been sent). At V4 and V6 or Early termination (if applicable), copies of the MRIs performed locally will be sent to the Central

	Imaging Lab for central MRI reading, blinded to treatment and sequence at W24 and blinded to treatment at W52. Results at Weeks 24 and 52 will include assessment of collections >3 mm in three axes and directly related to the fistula tracts treated, and any new tracts that might appear. MRIs will also be assessed for Van Assche score, hyperenhancement in T1 sequence, and hyperintensity in T2.
Amended or new wording:	†† Pelvic MRI performed locally (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location). A quality copy will be sent to the Central Imaging Lab within 24h from acquisition for immediate blinded central MRI reading as detailed in the specific manual. Blinded central MRI results (number of fistulas, location, type, collection in 3 dimensions, if any, with fistula location) will be communicated to the investigator and the surgeon prior to Preparation visit. A turnaround of 5 days for Central Reading is needed (considering adequate images have been sent). At V4 and V6 or Early termination (if applicable), copies of the MRIs performed locally will be sent to the Central Imaging Lab for central MRI reading, blinded to treatment and sequence at W24 and blinded to treatment at W52. Results at Weeks 24 and 52 will include assessment of collections >3 mm in three axes and directly related to the fistula tracts treated, and any new tracts that might appear. MRIs will also be assessed for Van Assche score, hyperenhancement in T1 sequence, and hyperintensity in T2. **MRI at Week 24 might be repeated if images do not have adequate quality to assess the primary endpoint.**

Rationale for Change: For purposes of transparency, completeness and improved clarity.

Change 38: The schedule of assesments table was updated to include optional samples of fistula curettage, fistula exudate, fistula swab for microbiome analysis and fecal sample for microbiome analysis.

The primary change occurs in the schedule of assessment table.

Rationale for Change: Optional fistula curettage and microbiome samples have been included to better characterize the subjects and the fistula which will enhance the understanding of disease pathogenesis, with a minimal increase in the sampling burden on subjects.

Change 39: Update to schedule of events footnote

The primary change occurs in footnote 8 of the schedule of assessment table.

Rationale for Change: To clarify assessment timings for PBMC blood samples.

ELECTRONIC SIGNATURES

Signed by	Meaning of Signature	Server Date (dd-MMM-yyyy HH:mm 'UTC')
[redacted]	Biostatistics Approval	28-Oct-2019 14:34 UTC
[redacted]	Clinical Approval	28-Oct-2019 15:36 UTC