Title: A Phase 3, Multicenter, Open-Label, Uncontrolled Study to Evaluate the Efficacy and Safety of Cx601 in the Treatment of Complex Perianal Fistulas in Adult Patients with Crohn's Disease

NCT Number: NCT03706456
Protocol Approve Date: 13-APR-2021

Certain information within this protocol has been redacted (ie, specific content is masked irreversibly from view with a black/blue bar) to protect either personally identifiable information or company confidential information.

This may include, but is not limited to, redaction of the following:

- Named persons or organizations associated with the study.
- Patient identifiers within the text, tables, or figures or in by-patient data listings.
- Proprietary information, such as scales or coding systems, which are considered confidential information under prior agreements with license holder.
- Other information as needed to protect confidentiality of Takeda or partners, personal information, or to otherwise protect the integrity of the clinical study.

If needed, certain appendices that contain a large volume of personally identifiable information or company confidential information may be removed in their entirety if it is considered that they do not add substantially to the interpretation of the data (eg, appendix of investigator's curriculum vitae).

TAKEDA PHARMACEUTICALS

PROTOCOL

<Title>

A Phase 3, Multicenter, Open-Label, Uncontrolled Study to Evaluate the Efficacy and Safety of Cx601 in the Treatment of Complex Perianal Fistulas in Adult Patients with Crohn's Disease

<Short Title>

Phase 3 Study of Cx601 in Subjects with Complex Perianal Fistulising Crohn's Disease

Sponsor:	Takeda Pharmaceutical Company Limited, 1-1, Doshomachi 4-Chome, Chuo-ku, Osaka-shi, Osaka, Japan		
Study Number:	Darvadstrocel-3002		
IND Number:	Not Applicable	**EudraCT Number:**	Not Applicable
Product:	Cx601 (INN: Darvadstrocel)		
Date:	13 April 2021	**Amendment Number:**	5

Amendment History:

Date	Amendment Number	Amendment Type	Region
13 April 2021	Amendment No.5	Non-substantial	All sites
6 October 2020	Amendment No.4	Non-substantial	All sites
9 October 2019	Amendment No.3	-	All sites
5 September 2018	Amendment No.2	-	All sites
27 July 2018	Amendment No.1	-	All sites
20 April 2018	Initial Protocol	Not applicable	All sites

Note: After the marketing approval of Cx601 is obtained in Japan, this study will be continued as a post-marketing clinical study. This protocol will be continuously used in the post-marketing clinical study by replacing the term "study" in this document with "post-marketing clinical study" as appropriate.

1.0 ADMINISTRATIVE INFORMATION AND PRINCIPLES OF CLINICAL STUDIES

1.1 Contacts and Responsibilities of Study-Related Activities

See the Annex

1.2 Principles of Clinical Studies

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonisation (ICH) E6 Good Clinical Practice (GCP): Consolidated Guideline.
- Good Clinical Practice for regenerative medical products (Ordinance 89 of the Ministry of Health, Labour and Welfare [MHLW], 2014)
- All applicable laws and regulations, including, without limitation, data privacy laws, clinical trial disclosure laws, and regulations.

After the marketing approval of Cx601 is obtained in Japan, this study will be continued as a post-marketing clinical study in accordance with Good Post-marketing Study Practice for regenerative medical products (Ordinance 90 of the MHLW, 2014) in addition to above.

SIGNATURES

The signature of the responsible Takeda medical officer (and other signatories, as applicable) can be found on the signature page.

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

PPD

1.3 Protocol Amendment No.5 Summary of Changes

This section describes the changes in reference to the Protocol Amendment No.5.

The primary purpose of this amendment is to add the procedures for the post-marketing clinical study when this study is continued as a post-marketing clinical study after the marketing approval of Cx601 is obtained in Japan.

A summary of the changes made in Amendment No.5 compared with Amendment No.4 is described below.

In this amendment, minor grammatical, editorial, formatting, and administrative changes not affecting the conduct of the study are included for clarification and administrative purposes only.

Changes in Amendment No.5:

Protocol Amendment No.5		
Summary of Changes		
Sections Affected by Change	**Description of Each Change and Rationale**	
Location	***Description***	***Rationale***
Cover page	Added the note that this protocol will be continuously used by replacing the term "study" with "post-marketing clinical study" as appropriate after the marketing approval of Cx601 in Japan.	To conduct this study as a post-marketing clinical study after the marketing approval of Cx601 is obtained in Japan.
Section 1.2 Principles of Clinical Studies	Added the description that this study will be conducted in accordance with Good Post-marketing Study Practice for regenerative medical products after the marketing approval of Cx601 in Japan.	To clarify applicable regulations to be complied with in the post-marketing clinical study.
Section 10.3.1 Safety Reporting to Investigators, IRBs and Regulatory Authorities	Added the procedure of safety reporting to investigators when an SAE which could affect the study continuation occurs after the marketing approval of Cx601 in Japan.	To define the optimal procedure for the post-marketing clinical study, taking into account the situation that all subjects in the study have completed administration of Cx601 and certain safety assessments have been conducted by the time marketing approval is obtained.
Section 12.2 Record Retention	Added the description that after the marketing approval of Cx601 in Japan, the term of record retention shall expire on the day when a reexamination or reevaluation of Cx601 is completed.	To clarify the term of record retention in the post-marketing clinical study.

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2.0 STUDY SUMMARY

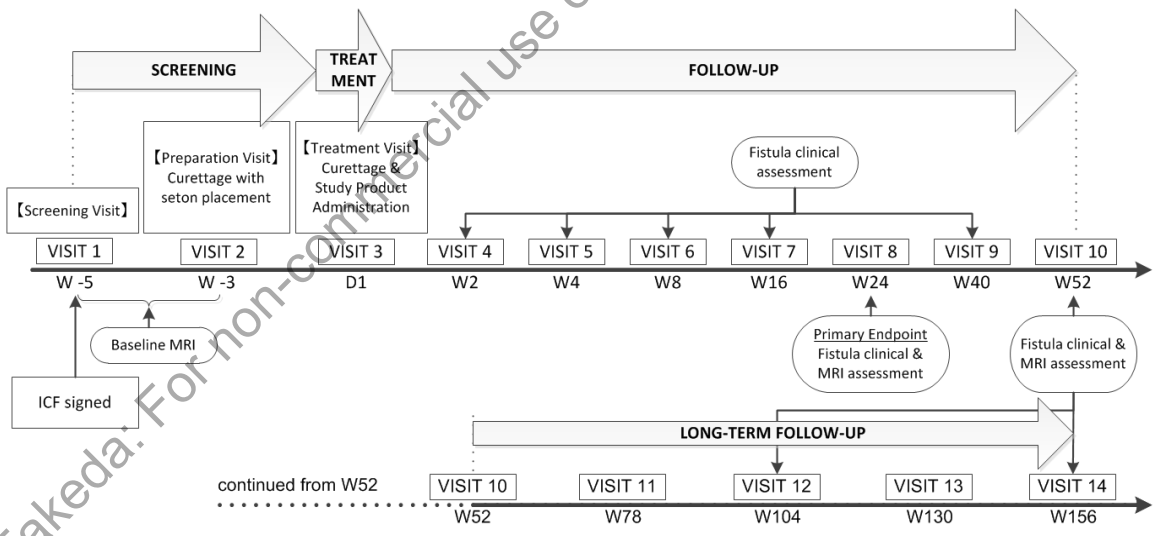
Clinical Study Sponsor(s): Takeda Pharmaceutical Company Limited	**Product:** Cx601	
Study Title: A Phase 3, Multicenter, Open-label, Uncontrolled Study to Evaluate the Efficacy and Safety of Cx601 in the Treatment of Complex Perianal Fistulas in Adult Patients with Crohn's Disease	**IND No.:** Not applicable	**EudraCT No.:** Not applicable
Study Identifier: Darvadstrocel-3002	**Phase:** 3	

Study Design:

This is a Phase 3, multicenter, open-label, uncontrolled study to evaluate the efficacy and safety of Cx601 in the treatment of complex perianal fistulas in adult patients with Crohn's disease. The study will permit continuation of baseline treatment for Crohn's disease in an add-on design (ie, biologics, immunosuppressants, etc.).

This study consists of the screening period (approximately 5 weeks prior to study product administration, including the screening visit and the preparation visit), the treatment period (the day of study product administration), the follow-up period (approximately 52 weeks after study product administration), and the long-term follow-up period (from Week 52 to Week 156). Cx601 will be administrated once on Day 1, and the efficacy and safety of Cx601 will be mainly evaluated in the subsequent 52-week follow-up period. The primary endpoint will be evaluated at Week 24. Additionally, in the long-term follow-up period after Week 52, a safety follow-up evaluation will be performed for all subjects every 26 weeks (6 months) until Week 156.

An overview of study design is illustrated below:



Primary Objective:

To evaluate the efficacy of Cx601 for the treatment of complex perianal fistulas in adult patients with Crohn's disease over 24 weeks.

Secondary Objectives:

To evaluate the efficacy of Cx601 for the treatment of complex perianal fistulas in adult patients with Crohn's disease over 52 weeks.

To evaluate the safety of Cx601 for the treatment of complex perianal fistulas in adult patients with Crohn's disease over 156 weeks.

<table>
<tr><td colspan="2">Subject Population:
The target population will consist of adult patients with Crohn's disease whose complex perianal fistulas were previously treated and refractory (inadequate response, loss of response or intolerance) to at least one of the following treatments: antibiotics, immunosuppressants or biologics. Treatment naïve subjects are excluded, and those who are refractory to antibiotics only must be less than 25% of all subjects enrolled.</td></tr>
<tr><td>Planned Number of Subjects:
20 subjects</td><td>Planned Number of Sites:
approximately 7 sites in Japan</td></tr>
<tr><td>Dose Level(s):
24 mL of cell suspension containing 120×10^6 cells of expanded allogeneic adipose-derived stem cells (eASCs) (5×10^6 cells/mL)</td><td>Route of Administration:
Intralesional injection
A half of the cell suspension will be injected into an adjacent site to the internal opening, and the other half into the tract wall along the fistula tracts through the external openings, with several micro-blebs forming inside the tract wall. If there are 2 internal openings, 6 mL will be injected into the adjacent site to each internal opening. The other half of the cell suspension (12 mL) will be portioned into all of the tract walls of the external openings.</td></tr>
<tr><td>Duration of Treatment:
Single dose</td><td>Study Length:
24 weeks for the primary endpoint evaluation, and 156 weeks for the safety follow-up for all subjects.</td></tr>
</table>

Main Criteria for Inclusion:

- The subject who was diagnosed with Crohn's disease at least 6 months prior to the screening period according to the Diagnostic Criteria for Crohn's Disease issued by the Research Group for Intractable Inflammatory Bowel Disease Designated as Specified Disease by the Ministry of Health, Labour and Welfare (MHLW) of Japan (revised January 2017).
- The subject is either a male or female outpatient, aged 18 years or older at the time of signing the informed consent form.
- The subject who has non-active or mildly active Crohn's disease defined by the Crohn's disease activity index (CDAI) ≤220 evaluated at any time between Visit 1 and Visit 2.
- The subject who has complex perianal fistulas with a maximum of 2 internal openings and a maximum of 3 external openings, confirmed by clinical assessment and magnetic resonance imaging (MRI). All of the external openings must connect to internal openings. Fistula must have been draining for at least 6 weeks prior to the screening. Complex perianal fistula is defined as the one that meets 1 or more of the following criteria:
  - High (ie, above the dentate line) inter-sphincteric or trans-sphincteric fistula, extrasphincteric fistula, or supra-sphincteric fistula.
  - Presence of ≥2 external openings (tracts).
  - Associated fluid collections.
- The subject whose perianal fistulas were previously treated and have shown an **inadequate response** (absence of closure of part or all fistula tract, or new fistula during induction treatment) or a **loss of response** (fistula relapse after complete closure of initial fistula, or fistula worsening after partial closure of initial fistula during maintenance treatment) while they were receiving either immunosuppressants or biologics, 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 approved or recommended doses during the minimum period mentioned;
  - Antibiotics (ciprofloxacin or metronidazole): 1 or more month treatment.
  - Immunosuppressants (azathioprine, 6 mercaptopurine or methotrexate): 3 or more months treatment.
  - Biologics (anti-TNFs, anti-integrin or anti-IL-12/23): 14 or more weeks (16 or more weeks for anti-IL-12/23) standard treatment for induction or maintenance.

Main Criteria for Exclusion:

- The subject whose CDAI is >220 at any time between Visit 1 and Visit 2, or who has active Crohn's disease requiring a new of escalating immediate therapy.
- The subject who has concomitant rectovaginal or rectovesical fistulas.
- The subject who has >2 internal openings or >3 external openings.
- The subject who is naïve to protocol required treatment for complex perianal fistulising Crohn's disease (ie, antibiotics, immunosuppressants or biologics).
- The subject who has an abscess or collections >2 cm.
- The subject who has rectal and/or anal stenosis and/or active proctitis, which would restrict the surgical procedure.
- The subject who underwent surgery other than drainage or seton placement for the treated fistula.
- The subject who has diverting stomas.
- The subject who was treated with systemic steroids within 4 weeks prior to study product administration.
- The subject receiving cytapheresis therapy.
- The subject who requires new treatment with immunosuppressants/biologics/non-tapered systematic steroids during the screening period.
- The subject who has known allergies or hypersensitivity to antibiotics (including but not limited to penicillin, streptomycin, gentamicin, aminoglycosides), human serum albumin (HSA), bovine-derived materials, local anesthetics or gadolinium (MRI contrast agent).
- The subject for whom MRI scan is contraindicated (eg, due to the presence of a pacemaker, a history of hip replacements, or severe claustrophobia).
- The subject who has received eASCs in a clinical study conducted in the past or as a therapeutic agent.

Main Criteria for Evaluation and Analyses;

- Primary Endpoint:

  Proportion of subjects with combined remission at Week 24 (combined remission is defined as the clinically confirmed closure of all treated external openings that were draining at the screening despite gentle finger compression, and absence of collections >2 cm in the treated fistulas confirmed by central MRI assessment).
- Secondary Endpoints:

  Efficacy analysis by Week 24:
  - Proportion of subjects with clinical remission at Week 24 (clinical remission is defined as the clinically confirmed closure of all treated external openings that were draining at the screening despite gentle finger compression)
  - Proportion of subjects with response at Week 24 (response is defined as the clinically confirmed closure of at least 50% of all treated external openings that were draining at the screening despite gentle finger compression).
  - Time to clinical remission by Week 24 (defined as the time from the study product administration to the first visit by which clinical remission is observed).
  - Time to response by Week 24 (defined as the time from the study product administration to the first visit by which response is observed).
  - Proportion of subjects with relapse at Week 24 in subjects with clinical remission at previous visit (relapse is defined as the clinically confirmed reopening of any of the treated external openings with active drainage, or the development of a collection >2 cm in the treated fistulas which is confirmed by the central MRI assessment).

– Time to relapse by Week 24 in subjects with clinical remission at previous visit (defined as the time from the first visit by which clinical remission is observed to the first visit by which relapse is observed).
– Perianal Disease Activity Index (PDAI) (including total score, discharge sub-score and pain sub-score) up to Week 24.
– CDAI score up to Week 24.
– Van Assche score up to Week 24.

Efficacy analysis by Week 52:

– Proportion of subjects with combined remission at Week 52.
– Proportion of subjects with clinical remission at Week 52.
– Proportion of subjects with Response at Week 52.
– Time to combined remission by Week 52 (defined as time from treatment start to the first visit combined remission is observed).
– Time to clinical remission by Week 52.
– Time to Response by Week 52.
– Proportion of subjects with relapse at Week 52 in subjects with combined remission at Week 24.
– Time to relapse by Week 52 in subjects with combined remission at Week 24.
– PDAI score (including total score, discharge sub-score and pain sub-score) up to Week 52.
– CDAI score up to Week 52.
– Van Assche score up to Week 52.

• Safety Endpoints:
– Adverse events (AEs), including serious adverse events (SAEs) and AEs of special interest.
– Product malfunctions.
– Physical examination findings.
– Vital signs (heart rate, blood pressure, body temperature).
– Clinical laboratory test results (serum chemistry, hematology and urinalysis).

Statistical Considerations:

The primary efficacy endpoint is proportion of subjects with combined remission at Week 24. Frequency distribution will be provided with proportion and the two-sided confidence intervals (confidence coefficient: 90% and 95%).

Sample Size Justification:

The planned sample size is 20 based on the feasibility. However, this study has at least 94% probability to show a proportion of combined remission of 35% or more given an expected proportion of subjects with combined remission of 50% based on Week 24 results in the study Cx601-0302.

3.0 STUDY REFERENCE INFORMATION

3.1 List of Abbreviations

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CDAI	Crohn's Disease Activity Index
eASC	expanded allogeneic adipose-derived stem cells
ECG	electrocardiogram
eCRF	electronic case report form
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	gamma-glutamyl transpeptidase
HBsAg	hepatitis B virus surface antigen
HBV	hepatitis B virus
hCG	human chorionic gonadotropin
HCV	hepatitis C virus
HSA	human serum albumin
ICH	International Conference on Harmonisation
IL	interleukin
INN	international nonproprietary name
INR	international normalized ratio
IRB	institutional review board
ITT	intention to treat
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
MHLW	Ministry of Health, Labour and Welfare
MHRA	Medicines and Healthcare products Regulatory Agency
mITT	modified intention to treat
MRI	magnetic resonance imaging
PCR	polymerase chain reaction
PDAI	Perianal Disease Activity Index
PMDA	Pharmaceuticals and Medical Devices Agency
PPS	per protocol set
SAE	serious adverse event
SAP	statistical analysis plan
SES-CD	Simple Endoscopic Score for Crohn's Disease
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent AE
TNF	tumor necrosis factor
ULN	upper limit of normal
WBC	white blood cell

3.2 Study Definition

Term	Definition
Combined remission	Defined as the clinically confirmed closure of all treated external openings that were draining at the screening despite gentle finger compression, and absence of collections >2 cm in the treated fistulas which is confirmed by the central MRI assessment.
Clinical remission	Defined as the clinically confirmed closure of all treated external openings that were draining at the screening despite gentle finger compression.
Response	Defined as the clinically confirmed closure of at least 50% of all treated external openings that were draining at the screening despite gentle finger compression.
Relapse	Defined as the clinically confirmed reopening of any of the treated external openings with active drainage, or the development of a collection >2 cm in the treated fistulas confirmed by central MRI assessment.
Time to combined remission/ clinical remission/response	The time from the study product administration to the first visit by which combined remission/clinical remission/response is observed.
Time to relapse [1)]	The time from the first visit which clinical remission is observed to the first visit by which relapse is observed.

1) Time to relapse by Week 52 in subjects who achieved combined remission at Week 24 is defined as "the time from the combined remission at Week 24 to the first visit by which relapse is observed."

4.0 INTRODUCTION

4.1 Background

4.1.1 Complex Perianal Fistulising Crohn's Disease

Crohn's disease is a granulomatous inflammatory disease, which occurs predominantly in young people. The lesion develops in any part of the gastrointestinal tract from mouth to anus, commonly in small intestine and large intestine, with characteristic pathological conditions (eg, intestinal stenosis due to edema or ulcer, and fistulas). Clinical symptoms mainly include gastrointestinal symptoms such as diarrhea and abdominal pain, systemic symptoms (eg, fever, weight loss, malnutrition) and complication-induced symptoms (eg, anemia, arthritis, iritis, skin lesions). The etiology of Crohn's disease has not been well elucidated yet, and Crohn's disease is designated as the specified rare and intractable disease in Japan. The number of the recipient certificates issued for specific disease treatment for Crohn's disease is increasing year by year[1]. The number of recipients of the specific medical expense for Crohn's disease (a designated intractable disease) in FY2016 is reported to be 42,789 patients[2].

Anal lesion is one of characteristic complications of Crohn's disease, and the cumulative incidence rates of the disease for 10 years after diagnosis is reported to be 74% to 80%[3]. In Japan, the frequency of anal fistula and perianal abscess is especially high[3], and the associated pain and drainage markedly deteriorate patients' quality of life (QOL). Futami et al. reported that, of 577 patients with Crohn's disease, 367 patients had anal fistula or abscess, of which 166 patients had complex fistulas with multiple external openings[4].

The prognosis of complex perianal fistula with Crohn's disease is poor. Similar to the intestinal lesion, recurrence/relapse is repeated. If repeated recurrence/relapse interferes with a patient's daily life, a surgical treatment may be selected. Also, a colostomy may be considered for severe cases. For simple anal fistula, a radical surgery may be selected, but it may invade the sphincter muscle and affect the postoperative anal function. Therefore, for complex perianal fistula, considering the high risk of relapse, the seton drainage is usually selected rather than radical surgery, in terms of preservation of anal function and alleviation of symptoms. However, the seton drainage often requires drainage for more than one year and deteriorate patients' QOL. Infliximab (recombinant), a biological product and anti-human tumor necrosis factor-α (TNFα) monoclonal antibody, was approved for the treatment of Crohn's disease with external fistula in 2002. However, in a clinical trial investigating the remission-maintaining effect of Infliximab on anal lesions (ACCENT II), the proportion of subjects who achieved fistula closure after 54-week treatment remains 36%[5], which suggests that Infliximab is not always effective in all patients. Furthermore, immunosuppressants and TNF antagonists may cause side effects associated with systemic immunosuppression. Therefore, there exists an unmet medical need in the treatment of complex perianal fistula.

4.1.2 Cx601

Cx601 (INN: Darvadstrocel) is a human cell-processed therapeutic product developed by the TiGenix S.A.U as a regenerative medical product for treatment of complex perianal fistulas with

Crohn's disease. By injecting a suspension of expanded allogeneic adipose-derived stem cells (eASCs) into the tract wall of the complex perianal fistula of Crohn's disease patients, eASCs are activated by inflammatory cytokines (mostly, IFN-γ). Then activated eASCs induce immunomodulative effects (eg, inhibition of inflammatory cytokine production, suppression of T-cell proliferation, and induction of regulatory T-cell), which may improve the complex perianal fistulas. Since Cx601 is a mesenchymal stem cell derived from adipose tissue, it is less immunoreactive and can be massively collected and cultured from adipose tissue, though it is allogenic.

Based on the results of 2 clinical studies conducted in Europe (studies Cx601-0101 and Cx601-0302, See Section 4.1.3), marketing authorization application of Cx601 was submitted to the European Medicines Agency (EMA) on March 2016. Thereafter the European Commission has approved Cx601 on March 2018, for the treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease, when fistulas have shown an inadequate response to at least one conventional or biologic therapy.

CCI [redacted] CCI an additional Phase 3 study (study Cx601-0303) is currently ongoing in Europe, Israel and North America (the U.S. and Canada).

4.1.3 Main Clinical Studies Results

The following 2 clinical studies of Cx601 have been conducted in Europe: Studies Cx601-0101 (Phase 1/2a, open-label, uncontrolled study) and Cx601-0302 (Phase 3, placebo-controlled study).

4.1.3.1 Study Cx601-0101 (Phase 1/2a, Open-Label, Uncontrolled Study)

Study Cx601-0101 is a Phase 1/2a, multicenter, open-label, uncontrolled study to evaluate the safety and efficacy of Cx601 in subjects with complex perianal fistulas with Crohn's disease. In this study, 24 subjects received cell suspension containing 20×10^6 cells of eASCs (Cx601). Of these subjects, 15 subjects failed to achieve complete fistula closure at Week 12, and received an additional 40×10^6 cells of eASCs. Continued standard treatments of Crohn's disease (other than TNF antagonists, tacrolimus and cyclosporine) were allowed during the study. The safety and efficacy of Cx601 were evaluated for 24 weeks after the initial dose.

The main results of study Cx601-0101 are shown below.

- Of the 24 subjects who received the study treatment, treatment-emergent adverse events (TEAEs) were reported in 13 subjects (54.2%), and most of the TEAEs were mild or moderate in intensity. Treatment-related TEAEs were reported in 5 subjects (20.8%), which included anal abscess (3 subjects), pyrexia (1 subject) and uterine leiomyoma (1 subject). Serious TEAEs were reported in 2 subjects (pyrexia and anal abscess observed in 1 subject each), and both were determined to be related to study treatment by the investigator.
- The proportion of subjects who achieved reductions of at least one draining fistula was 60.0% (12/20 subjects) at Week 12, and 69.2% (9/13 subjects) at Week 24. The proportion of

subjects who achieved complete closures of the external openings of the treated perianal fistula was 38.1% (8/21 subjects) at Week 12, and 56.3% (9/16 subjects) at Week 24[6].

4.1.3.2 Study Cx601-0302 (Phase 3, Placebo-controlled Study)

Study Cx601-0302 is a Phase 3, multicenter, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy and safety of Cx601 in subjects with complex perianal fistulas with Crohn's disease. The target subjects in this study were those with complex perianal fistulising Crohn's disease refractory to at least one of the existing treatments (eg, antibiotics, immunosuppressants or biologics). A total of 212 subjects were randomized, and single dose of Cx601 (120×10^6 cells of eASCs) or placebo (saline) were administered to 103 of 107 subjects in the Cx601 group and 102 of 105 subjects in the placebo group, respectively. The primary endpoint (proportion of subjects with combined remission) was evaluated at Week 24 and follow-up was conducted up to Week 104.

The main results of study Cx601-0302 up to Week 104 are shown below.

- The 24-week post-treatment evaluation was completed in 171 subjects (80.7%), which consisted of 88 subjects (82.2%) in the Cx601 group and 83 subjects (79.0%) in the placebo group. The main reasons for withdrawal from the study by Week 24 were adverse event, surgical procedure for other reason than fistulas, worsening of Crohn's disease requiring change in therapy. The 52-week post-treatment evaluation was completed in 131 subjects (61.8%), which consisted of 70 subjects (65.4%) in the Cx601 group and 61 subjects (58.1%) in the placebo group. The main reasons for withdrawal from the study during the period between Weeks 24 and 52 were the lack of efficacy against fistulas or worsening fistula, adverse events, surgical procedure for other reason than fistulas, worsening of Crohn's disease requiring change in therapy. The 104-week post-treatment evaluation was completed in 37 subjects (17.5%), which consisted of 23 subjects (21.5%) in the Cx601 group and 14 subjects (13.3%) in the placebo group. The main reasons for withdrawal from the study during the period between Weeks 52 and 104 were withdrawal of patient consent to participate in the study, or others.
- The proportion of subjects who achieved combined remission at Week 24, which is the primary endpoint, was significantly higher (p=0.024) in the Cx601 group (49.5%) than in the placebo group (34.3%). In addition, the secondary endpoints were also superior in the Cx601 group to the placebo group; the proportion of subjects who achieved clinical remission at Week 24 was 53.3% in the Cx601 group and 40.0% in the placebo group (p=0.064), and the proportion of subjects who achieved response at Week 24 was 66.4% in the Cx601 group and 53.3% in the placebo group (p=0.054).
- The proportion of subjects who achieved combined remission, clinical remission and response at Week 24 were all higher in the Cx601 group compared to the placebo group, and these trends were observed at Week 52 (the proportion of subjects who achieved combined remission at Week 52 [Cx601 vs Placebo] was 54.2% vs 37.1%, the proportion of subjects who achieved clinical remission at Week 52 [Cx601 vs Placebo] was 57.0% vs 40.0%, the

proportion of subjects who achieved response at Week 52 [Cx601 vs Placebo] was 63.6% vs 53.3%).

- In post hoc analyses of clinical remission at Week 104 on observed data, the proportion of subjects who achieved clinical remission at Week 104 (Cx601 vs Placebo) was 56.0% vs 40.0%, a greater proportion of patients in the Cx601 group achieved clinical remission compared with placebo group.
- Total Perianal Disease Activity Index (PDAI) decreased in both treatment groups at all visits up to Week 24 and Week 52 with the decrease being slightly greater in the Cx601 treatment group compared with the placebo group. Results at Week 104 are aligned. Mean Crohn's Disease Activity Index (CDAI) total scores at Week 104 were comparable to those at baseline, Weeks 24 and 52.
- The incidence of TEAEs up to Week 52 was 76.7% (79/103 subjects) in the Cx601 group and 72.5% (74/102 subjects) in the placebo group, which was comparable between both groups. In addition, the incidence of TEAEs that lead to discontinuation of the study was low (8.8% overall) and similar in both groups (8.7% in the Cx601 group and 8.8% in the placebo group). The majority of TEAEs observed by Week 52 were mild or moderate in intensity, and the incidence of severe TEAEs (10.7% overall) was similar in both groups (9.7% in the Cx601 group and 11.8% in the placebo group). Most of the TEAEs reported up to Week 52 were considered not related to the study treatment, and the treatment-related TEAEs were more frequently reported in the placebo group (26.5%) than in the Cx601 group (20.4%).
- The incidence of serious TEAEs up to Week 52 was slightly higher in the Cx601 group (24.3%) than in the placebo group (20.6%). The most common serious TEAE was anal abscess, the incidence was slightly higher in the Cx601 group (13.6%) than in the placebo group (7.8%). The number of subjects whose anal abscess was considered serious and treatment-related was similar in both groups (7 subjects in the Cx601 group, 5 subjects in the placebo group).
- The safety profile of Cx601 up to Week 104 were similar to that up to Week 52. Only 4 new serious TEAEs (3 in Cx601 group [fistula discharge, anal fistula/disease recurrence, anal abscess/pain/pyrexia] and 1 in placebo group [fistula discharge/anal fistula excision]) were reported among the 37 patients who entered the extended follow-up period after Week 52 up to Week 104. None were considered related to the treatment administered. No new suspected unexpected serious adverse reactions (SUSARs) were reported, thus no apparent new safety signals occurred up to 104 weeks follow-up extension.

4.2 Rationale for the Proposed Study

This will be the first study to evaluate the efficacy and safety of Cx601 in Japanese subjects with complex perianal fistulas with Crohn's disease.

The available evidences based on the efficacy and safety data from clinical studies conducted in Europe suggest that Cx601 was safe and effective in closing the fistula and the effect sustained

up to Week 52 in subjects with complex perianal fistula refractory to at least one of existing treatments (ie, antibiotics, immunosuppressants or TNF antagonists).

However, Cx601 has never been administered to Japanese, and no definite conclusion has been drawn from previous clinical studies on that there might be ethnic differences in the efficacy and safety of Cx601. Cx601 is allogeneic adipose tissue-derived mesenchymal stem cells, and the direct injection of Cx601 into the target lesion promotes the healing of the perianal tissue with fistula, so that there may be no ethnic differences in pharmacokinetics that may influence clinical outcome. There are no major differences in diagnosis and treatment of complex perianal fistulising Crohn's disease between Japan and foreign countries when comparing the Japanese guidelines for the treatment of perianal lesion of Crohn's disease (revised January 2016)[7], with the American Gastroenterological Association (AGA) guideline published in 2003[8], and the European Crohn's and Colitis Organization (ECCO) guideline published in 2010[9], there are no major differences between home and abroad on diagnosis and treatment of complex perianal fistulas with Crohn's disease. Considering the above, it is thought that Cx601 will be effective for Japanese population, similar to the result of the study Cx601-0302, and this study can be carried out safely by appropriate monitoring of safety during the study.

From the above, this study has been planned because Cx601 could be a new option for the treatment of complex perianal fistulas in Japanese patients with Crohn's disease as the current treatment options for the disease are limited.

5.0 STUDY OBJECTIVES AND ENDPOINTS

5.1 Objectives

5.1.1 Primary Objective

- To evaluate the efficacy of Cx601 for the treatment of complex perianal fistulas in adult patients with Crohn's disease over 24 weeks.

5.1.2 Secondary Objectives

- To evaluate the efficacy of Cx601 for the treatment of complex perianal fistulas in adult patients with Crohn's disease over 52 weeks.
- To evaluate the safety of Cx601 for the treatment of perianal fistulas in adult patients with Crohn's disease over 156 weeks.

5.1.3 Additional Objectives

- To evaluate the absence of clinically relevant alloreactivity.
- To evaluate the effects of Cx601 on Crohn's disease activity and quality of life.

5.2 Endpoints

5.2.1 Primary Endpoint

- Proportion of subjects with combined remission at Week 24.

5.2.2 Secondary Endpoints

Efficacy analysis using data up to Week 24:

- Proportion of subjects with clinical remission at Week 24.
- Proportion of subjects with response at Week 24.
- Time to clinical remission by Week 24.
- Time to response by Week 24.
- Proportion of subjects with relapse at Week 24 in subjects with clinical remission at previous visit.
- Time to relapse by Week 24 in subjects with clinical remission at previous visit.
- PDAI score (including total score, discharge sub-score and pain sub-score) up to Week 24.
- CDAI score up to Week 24.
- Van Assche score up to Week 24.

Efficacy analysis using data up to Week 52:

- Proportion of subjects with combined remission at Week 52.
- Proportion of subjects with clinical remission at Week 52.
- Proportion of subjects with response at Week 52.
- Time to combined remission by Week 52.
- Time to clinical remission by Week 52.
- Time to response by Week 52.
- Proportion of subjects with relapse at Week 52 in subjects with combined remission at Week 24.
- Time to relapse by Week 52 in subjects with combined remission at Week 24.
- PDAI score (including total score, discharge sub-score and pain sub-score) up to Week 52.
- CDAI score up to Week 52.
- Van Assche score up to Week 52.

5.2.3 Safety Endpoints

- Adverse events (AEs) including serious adverse events (SAEs) and AEs of special interest.
- Product malfunctions.
- Physical examination findings.
- Vital signs (heart rate, blood pressure, body temperature).
- Clinical laboratory test results (serum chemistry, hematology and urinalysis).

5.2.4 Additional Endpoints

- Presence/absence of anti-donor antibody.

6.0 STUDY DESIGN AND DESCRIPTION

6.1 Study Design

This is Phase 3, a multicenter, open-label, uncontrolled study to evaluate the efficacy and safety of Cx601 in the treatment of complex perianal fistulas in adult patients with Crohn's disease.

Subjects with Crohn's disease whose complex perianal fistulas were previously treated and refractory (inadequate response, loss of response or intolerance) to at least one of the following treatments: antibiotics, immunosuppressants or biologics (anti-TNFs, anti-integrin or anti-IL-12/23) will be included in the study (See Inclusion Criteria on Section 7.1). Note that those subjects who are refractory to antibiotics only will be must be less than 25% of all subjects enrolled.

The study will permit continuation of baseline treatments for Crohn's disease in an add-on design (ie, biologics, immunosuppressants, etc.). A total of 20 subjects are planned to be enrolled, and study product, cell suspension containing 120×10^6 cells of eASCs, will be intralesionally injected to all participants. Since this is the first study of Cx601 in Japanese subjects, the enrollment of at least the first 3 subjects will be adjusted not to be administered the study product at the same day.

This study consists of the screening period (approximately 5 weeks prior to study product administration, including the screening visit and the preparation visit), the treatment period (the day of study product administration), the follow-up period (approximately 52 weeks after study product administration), and the long-term follow-up period (from Week 52 to Week 156). Cx601 will be administrated once on Day 1, and the efficacy and safety of Cx601 will be mainly evaluated in the subsequent 52-week follow-up period. The primary endpoint will be evaluated at Week 24. Additionally, in the long-term follow-up period after Week 52, a safety follow-up evaluation will be performed for all subjects every 26 weeks (6 months) until Week 156.

In the screening period, a screening visit (Visit 1) to determine a subject's eligibility is scheduled during the period from Day -39 to the day before VISIT2. All the subjects eligible by the screening will receive fistula curettage and seton placement under anesthesia at the preparation visit (Visit 2: Day -21, done no later than Day -14). Seton(s) placed will be removed on the day of study product administration (Visit 3: Day 1), just before the administration of the study product. Appropriate training for the preparation and the administration will be implemented to standardize the procedures between study sites.

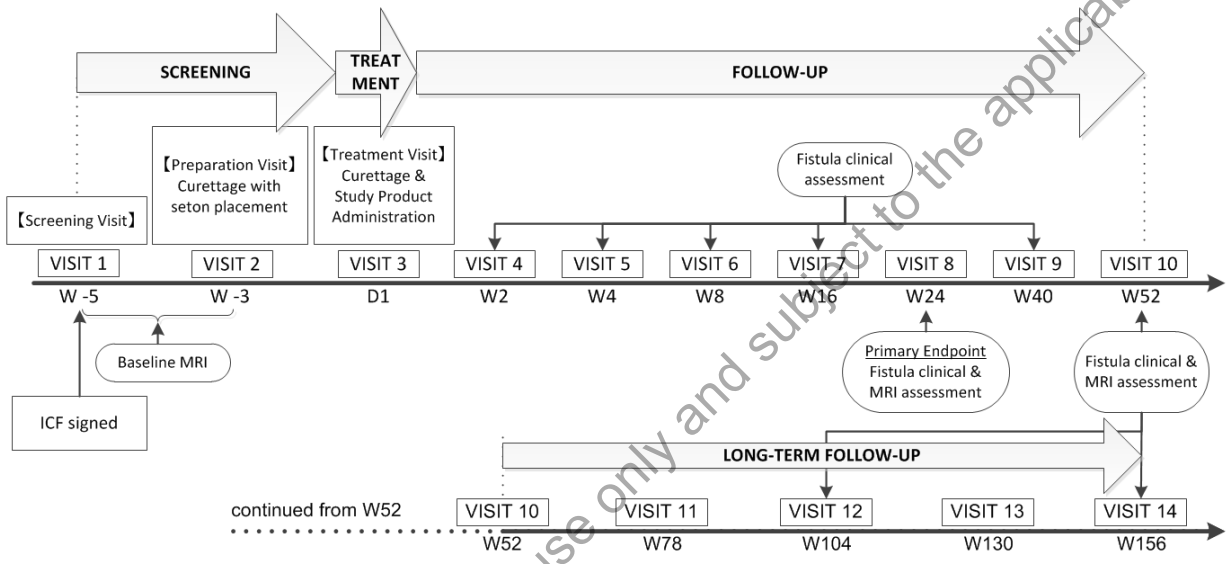
The subjects who meet all the eligibility criteria will visit the study site on Day 1, and receive fistula curettage under anesthesia and an intralesional injection of cell suspension containing 120×10^6 cells of eASCs (Cx601). Thereafter, in follow-up period, the subjects will visit the study site for the clinical assessments including fistula closure at Weeks 2, 4, 8, 16, 24, 40 and 52. Radiological assessments (MRI) of fistula closure will also be performed at Weeks 24 and 52.

Additionally, in the long-term follow-up period, the safety follow-up will be performed on all the subjects (where possible) every 26 weeks (6 months) from a visit in Week 52 through Week 156.

This study will be switched to a post-marketing clinical study if Cx601 is approved before the study completion.

A schematic study design is shown in Figure 6.a. A schedule of assessments is listed in Appendix A.

Figure 6.a Schematic Study Design



All or some parts of the study results may be used as the data for post-authorization safety studies conducted in Europe or long-term evaluation studies planned in the future.

6.2 Justification for Study Design, Dose, and Endpoints

(1) Objectives and target population

This study has been planned to evaluate the efficacy and safety of Cx601 for the treatment of complex perianal fistulas in Japanese patients with Crohn's disease as the current treatment options for the disease are limited.

In the Phase 3 placebo-controlled study conducted overseas (study Cx601-0302), the efficacy of Cx601 has been confirmed in subjects with complex perianal fistulising Crohn's disease refractory to at least one of existing treatments (ie, antibiotics, immunosuppressants or TNF antagonists). Since similar result is expected in the same sub-population in Japanese, the target population in this study is defined as Japanese subjects with complex perianal fistulising Crohn's disease refractory to at least one of existing treatments (ie, antibiotics, immunosuppressants or biologics) as well.

(2) Study design

Since the number of patients with complex perianal fistulising Crohn's disease is small in Japan, the study design with control-arm is considered to be very difficult to carry out. Considering the

feasibility of implementation in small number of subjects, the study is determined to be multicenter, open-label, uncontrolled design.

Considering that the target population is the subjects who failed to achieve complete closure of fistulas despite existing treatments and evaluation of complete closure of the fistulas could be performed objectively as well as study Cx601-0302, it is thought to be possible to suggest the clinical meanings of Cx601 without control group setting. Additionally, considering the results of study Cx601-0302, the efficacy and safety of Cx601 in Japanese population can be evaluated comprehensively.

(3) Dose and Regimen

In the open-label, uncontrolled study conducted overseas (study Cx601-0101), 24 subjects received a dose of at least 20×10^6 cells of eASCs (Cx601), of which 15 subjects with incomplete fistula closure at Week 12 received an additional 40×10^6 cells of eASCs. As a result, proportion of subjects with closure of the external opening of the treated perianal fistula at Weeks 12 and 24 was 38.1% (8/21 subjects) and 41.7% (5/12 subjects), respectively. Although a certain proportion of subjects achieved closure of the external opening by the first dosing of 20×10^6 cells, there was similar proportion of subjects who achieved closure of the external opening by additional dose of 40×10^6 cells after first dose. From this results, it is considered that the dose of 20×10^6 cells is not always sufficient for all patients.

Based on the above results, in the study Cx601-0302, considering that the subject has maximum 3 fistula tracts, 120×10^6 cells dose, 3-fold higher than 40×10^6 cells dose showing efficacy for 1 fistula tract in study Cx601-0101, was selected. And single dose were selected in order to avoid fistula curettage in subjects whose fistulas may have partially closed. In these dose and regimen settings, the efficacy in the Cx601 group was superior to the placebo group in study Cx601-0302.

Based on the results of study Cx601-0302, it is considered that a single dose of 120×10^6 cells could be also effective in Japanese, similar to study Cx601-0302.

(4) Study duration

Considering the comparability of the results in this study with study Cx601-0302, 24-week evaluation period for the primary endpoint, and 52-weeks evaluation period for secondary endpoints are defined in this study.

Furthermore, the evaluation up to Week 104 was included in the study Cx601-0302. Considering that this is the first study for Cx601 in Japanese population, and longer-term follow-up is important to establish the safety and efficacy profile of Cx601 in the small number of subjects, 156-week evaluation, exceeding the study Cx601-0302, is defined in this study for the purpose of safety follow-up.

(5) Endpoints

Considering the comparability of the results in this study with study Cx601-0302, combined remission with the same definition as the study Cx601-0302 is selected as the primary endpoint. In general, fistula closure (defined as no draining from external openings despite gentle finger compression) is a conventional endpoint to determine the response to treatment in clinical

studies[10], and it is also used in clinical practice[9]. However, no draining from external openings does not always reflect the state of complete closure of the fistula tract. If inflammation remains in the fistula tract, there are risks of perianal abscess and fistula recurrence[11]. Therefore, in addition to the confirmation of no draining from the external openings despite gentle finger compression, when absence of collections >2 cm are confirmed by MRI assessment is defined as combined remission, it is thought to be appropriate as the primary endpoint.

The other secondary endpoints are defined same as the study Cx601-0302 in order to compare the efficacy results of this study with those of study Cx601-0302. Because the presence or absence of draining is considered an important secondary endpoint in the clinical development guideline for the treatment of Crohn's disease published by EMA[12], clinical remission and response will be evaluated by confirming absence of draining from external openings despite gentle finger compression. In addition, PDAI[13] will be evaluated in this study because the above guideline recommends use of PDAI as a secondary endpoint as index of the severity of fistula in patients with Crohn's disease and study Cx601-0302 showed improvements in PDAI discharge sub-score and pain sub-score as well as PDAI total score. CDAI[14] will be evaluated because it is commonly used in clinical studies as an index of severity of Crohn's disease. Van Assche[15] will be evaluated because it is a useful to evaluate the severity of fistula in patients with Crohn's disease by MRI.

6.3 Premature Termination or Suspension of Study or Study Site

6.3.1 Criteria for Premature Termination or Suspension of the Study

The study will be completed as planned unless 1 or more of the following criteria are satisfied that require temporary suspension or early termination of the study.

- New information or other evaluation regarding the safety or efficacy of the study product that indicates a change in the known risk/benefit profile for the product, such that the risk/benefit is no longer acceptable for subjects participating in the study.
- Significant violation of GCP that compromises the ability to achieve the primary study objectives or compromises subject safety.

6.3.2 Criteria for Premature Termination or Suspension of Study Sites

A study site may be terminated prematurely or suspended if the study site (including the investigator) is found in significant violation of GCP, protocol, or contractual agreement, is unable to ensure adequate performance of the study, or as otherwise permitted by the contractual agreement.

6.3.3 Procedures for Premature Termination or Suspension of the Study or the Participation of Study Sites

In the event that the sponsor, an institutional review board (IRB) or regulatory authority elects to terminate or suspend the study or the participation of a study site, a study-specific procedure for

early termination or suspension will be provided by the sponsor; the procedure will be followed by applicable study sites during the course of termination or study suspension.

7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS

All entry criteria, including test results, need to be confirmed prior to study product administration.

7.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria prior to study product administration:

1. In the opinion of the investigator, the subject is capable of understanding and complying with the protocol requirements.
2. The subject or, when applicable, the subject's legally acceptable representative signs and dates a written, informed consent form and any required privacy authorization prior to the initiation of any study procedures.
3. The subject who was diagnosed with Crohn's disease at least 6 months prior to the screening period according to the Diagnostic Criteria for Crohn's Disease issued by Research Group for Intractable Inflammatory Bowel Disease Designated as Specified Disease by the MHLW of Japan (revised January 2017).
4. The subject is either a male or female outpatient, aged 18 years or older at the time of signing the informed consent form.
5. The subject who has non-active or mildly active Crohn's disease defined by the CDAI ≤220 evaluated at any time between Visit 1 and Visit 2.
6. The subject who has complex perianal fistulas with a maximum of 2 internal openings and a maximum of 3 external openings, confirmed by clinical assessment and MRI. All of the external openings must connect to internal openings. Fistula must have been draining for at least 6 weeks prior to the screening. Complex perianal fistula is defined as the one that meets 1 or more of the following criteria:
   - High (ie, above the dentate line) inter-sphincteric or trans-sphincteric fistula, extrasphincteric fistula, or supra-sphincteric fistula.
   - Presence of ≥2 external openings (tracts).
   - Associated fluid collections.
7. The subject whose perianal fistulas were previously treated and have shown an **inadequate response** (absence of closure of part or all fistula tract, or new fistula during induction treatment) or a **loss of response** (fistula relapse after complete closure of initial fistula, or fistula worsening after partial closure of initial fistula during maintenance treatment) while they were receiving either immunosuppressants or biologics, 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 approved or recommended doses during the minimum period mentioned;

- Antibiotics (ciprofloxacin or metronidazole): 1 or more month treatment.
- Immunosuppressants (azathioprine, 6-mercaptopurine or methotrexate): 3 or more months treatment.
- Biologics (anti-TNFs, anti-integrin or anti-IL-12/23): 14 or more weeks (16 or more weeks for anti-IL-12/23) standard treatment for induction or maintenance.

8. A male subject who is nonsterilized* and sexually active with a female partner of childbearing potential* agrees to use adequate contraception* from signing of informed consent up to Week 52 of the study.
9. A female subject of childbearing potential* who is sexually active with a nonsterilized* male partner agrees to use routinely adequate contraception* from signing of informed consent up to Week 52 of the study.

*Definitions and acceptable methods of contraception are defined in Section 9.1.9 and reporting responsibilities are defined in Section 9.1.10.

Justification for Inclusion Criteria

1 and 2: Are the basic criteria for conducting a clinical study.

3: Is the criterion for ensuring the diagnosis of Crohn's disease.

4: Is defined because Crohn's disease can occur in both men and women, and often in younger people. And it is also appropriate to enroll outpatients as the study subjects in consideration of the severity of Crohn's disease.

5 to 7: Are the criteria for identifying the pathological conditions of Crohn's disease and complex perianal fistulas in this study.

8 and 9: Are included in consideration of the risks of pregnancy.

7.2 Exclusion Criteria

Any subject who meets any of the following criteria will not qualify for entry into the study:

1. The subject whose CDAI is >220 at any time between Visit 1 and Visit 2, or who has active Crohn's disease requiring a new of escalating immediate therapy.
2. The subject who has concomitant rectovaginal or rectovesical fistulas.
3. The subject who has >2 internal openings of >3 external openings.
4. The subject who is naïve to protocol required treatment for complex perianal fistulising Crohn's disease (ie, antibiotics, immunosuppressants or biologics).
5. The subject who has an abscess or collections >2 cm.

6. The subject who has rectal and/or anal stenosis and/or active proctitis, which would restrict the surgical procedure.
7. The subject who underwent surgery other than drainage or seton placement for the to be treated fistula.
8. The subject who has diverting stomas.
9. The subject who was treated with systemic steroids in the 4 weeks prior to study product administration.
10. The subject receiving cytapheresis therapy.
11. The subject who requires new treatment with immunosuppressants/biologics/non-tapered systematic steroids during the screening period.
12. The subject who has renal impairment defined by creatinine clearance below 60 mL/min calculated using Cockcroft-Gault formula or by serum creatinine ≥1.5 × upper limit of normal (ULN).
13. The subject who has hepatic impairment defined by both total bilirubin ≥1.5 × ULN, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≥2.5 × ULN.
14. The subject who has history of abuse of alcohol or other addictive substances in the 6 months prior to the screening period.
15. The subject who has malignant tumour or who has a history of malignant tumour, including any type of fistula carcinoma.
16. The subject who has abnormal, severe, progressive, uncontrolled hepatic, hematological, gastrointestinal (except Crohn's disease), endocrine, pulmonary, cardiac, neurological, psychiatric, or cerebral disease, or the subject who developed any of the above diseases within 3 months prior to the screening period.
17. The subject who has congenital or acquired immunodeficiency, including subjects known to be HIV carriers.
18. The subject who has clinically significant chronically active hepatopathy of any origin, including hepatic cirrhosis, and subjects who is persistent positive for hepatitis B virus (HBV) surface antigen (HBsAg) and quantitative HBV polymerase chain reaction (PCR), or positive serology for hepatitis C virus (HCV) and quantitative HCV-PCR within 6 months prior to the screening period.
19. The subject who has known allergies or hypersensitivity to antibiotics (including but not limited to penicillin, streptomycin, gentamicin, aminoglycosides), Human Serum Albumin (HSA), bovine-derived materials, local anesthetics or gadolinium (MRI contrast agent).
20. The subject for whom MRI scan is contraindicated (eg, due to the presence of a pacemaker, a history of hip replacements, or severe claustrophobia).

21. The subject who has major surgery (eg, surgery under general anesthesia, laparotomy, thoracotomy, craniotomy) or severe trauma within 6 months prior to the screening period.
22. The female subject who is pregnant, or is lactating.
23. The subject who has received any investigational drug within 12 weeks (84 days) prior to the screening.
24. The subject who has received eASC in a previous clinical study or as a therapeutic agent.
25. The subject who needs perianal surgery other than fistulas preparation required by the protocol during the screening, or the subject who will receive a perianal surgery within 24 weeks after study product administration.
26. The subject for whom anesthesia is contraindicated.
27. The subject who received excluded medications or treatment listed in Section 7.3.

Justification of Exclusion Criteria

1 to 5: These subject were not the target population of this study.

6: These conditions could restrict the procedures for the study treatment.

7, 9, 10, 11, 21, 23 to 25, 27: These conditions could influence the evaluation of efficacy and safety.

8: This condition could make it impossible to evaluate perianal fistulas.

12 to 18: These conditions could influence the safety of the subjects, and because it could influence the evaluation of study product.

19, 26: These were set with respect to the safety of the subjects exposed to the ingredients/drugs, which are contained in study product or used for procedure prior to the administration of study product.

20: This condition could make it impossible to perform MRI scan required for the primary endpoint.

22: To ensure the safety of pregnant women and fetuses, or nursing infants.

7.3 Excluded Medications and Treatments

Subjects must be instructed not to take any medications including over-the-counter products, without first consulting with the investigator.

The details of allowed/excluded medications or treatments during the period from start of screening period to study product administration, and from the study product administration to Week 52 are summarized in Table 7.a. There are no particular restrictions for medications and treatments after Week 52, but follow the rules set before Week 52, as far as possible. In there are any questionable medications or treatments, the sponsor or its designee should be contacted.

If a subject received any excluded medications or treatments, the subject may continue to participate in the study as long as the risk is considered acceptable by the investigator.

Table 7.a Allowed/Excluded Medications and Treatments

Medications/Treatment	Period from start of screening period to study product administration	Period from study product administration to Week 52	Justification
Any investigational drugs or other local investigational treatments for the perianal region	**NOT Allowed** (Should be excluded from 84 days prior to start of screening period.)	**NOT Allowed**	These medications/treatments are excluded in consideration of any influences on the efficacy and safety evaluation of the study product.
Antibiotics (including but not limited to ciprofloxacin and/or metronidazole)	**Allowed** (Continuous use of antibiotics must be up to 4 weeks.)	**Allowed** (Continuous use of antibiotics must be up to 4 weeks, including the period before the study product administration.)	These medications are allowed with the restriction of dosing period because long-term use beyond clinical standard may jeopardize the safety of the subject.
5-aminosalicylic acid	**Allowed**	**Allowed** (A stable dose should be given after the study product administration. Only dose reduction from the initial dose will be allowed.)	These medications are allowed with the restriction to avoid any influences on the efficacy and safety evaluation of the study product.
Topical steroid Inhalatory steroids	**Allowed**	**Allowed**	These medications are allowed because they have less effect on the efficacy and safety evaluation of the study product.
Immunosuppressants (Azathioprine, 6-mercaptoprine, methotrexate, tacrolimus)	**Allowed** (Continuous use of immunosuppressants is allowed if it has been used at stable dose for more than 3 months before start of screening period.)	**Allowed** (A stable dose should be given after the study product administration. Dose reduction or discontinuation of immunosuppressants will be allowed only if adverse reactions associated with immunosuppressants are observed.)	These medications are allowed with the restriction to avoid any influences on the efficacy and safety evaluation of the study product.

Table 7.a Allowed/Excluded Medications and Treatments (Cont.)

Medications/Treatment	Period from start of screening period to study product administration	Period from study product administration to Week 52	Justification
Biologics (anti-TNF, anti-integrin, anti-IL-12/23)	**Allowed** (Continuous use of biologics is allowed if it showed no response to complex perianal fistulas despite at least 14-week treatment [16 weeks for anti-IL-12/23] prior to start of screening period, OR complex perianal fistulas recurred after achieving closure of fistulas.)	**Allowed** (A stable dose should be given after the study product administration.)	These medications are allowed with the restriction to avoid any influences on the efficacy and safety evaluation of the study product.
Systematic steroids Rectal steroids	**Allowed** (These medications should be tapered and discontinued within 4 weeks prior to study product administration.)	**NOT Allowed** (These medication are allowed as a rescue therapy only when Crohn's disease worsened. See Section 7.4.)	These medications are excluded in consideration of any influences on the efficacy and safety evaluation of the study product. Use for a rescue therapy is allowed in consideration of the safety of subjects.
Enteral nutrition	**Allowed**	**Allowed**	These medications are allowed because they have less effect on the efficacy and safety evaluation of the study product.
Central venous nutrition Enteral total nutrition	**NOT Allowed**	**NOT Allowed** (These treatments are allowed to use as a rescue therapy only when Crohn's disease worsened. See Section 7.4.)	These treatments are excluded in consideration of any influences on the efficacy and safety evaluation of the study product. Use for a rescue therapy is allowed in consideration of the safety of subjects.
Cytapheresis	**NOT Allowed**	**NOT Allowed**	These medications are excluded in consideration of any influences on the efficacy and safety evaluation of the study product.

Table 7.a Allowed/Excluded Medications and Treatments (Cont.)

Medications/Treatment	Period from start of screening period to study product administration	Period from study product administration to Week 52	Justification
Medications/treatments for Crohn's disease not including the above	**Allowed**	**Allowed**	These medications are allowed because they have less effect on the efficacy and safety evaluation of the study product.
Oral anticoagulants	**NOT Allowed** (If an anticoagulant has been used before screening period and required continuous use, it should switch to appropriate dose of low molecular weight heparin.)	**NOT Allowed** For 2 weeks after study product administration	These medications are restricted in consideration of the safety risk of possible bleeding in preparation and study product administration.
Medications/treatments for a disease other than Crohn's disease, not including the above	**Allowed**	**Allowed**	These medications are allowed because they have less effect on the efficacy and safety evaluation of the study product.

7.4 Rescue Therapy

If worsening of Crohn's disease is observed within 52 weeks after study product administration, the following treatments will be permitted as a rescue therapy. In the applicable case, the subject should continue the study according to the study schedule.

- A steroid course starting at 40 mg or less dose (as prednisolone or equivalent, 9 mg for Budesonide) and tapering over 12 weeks, or
- Central venous nutrition/enteral total nutrition (allowed until a flare improves).

If a subject receive an excluded medication or treatment for rescue (eg, commencement of a new dosing with an immunosuppressant/biologics, or a dose increase in a concomitant medications which has been used at the baseline of the study), the subject must be treated as a non-responder (See Section 13.1.3.3). Then, the subject may continue to participate in the study as long as the risk is considered acceptable by the investigator.

If a deterioration of the complex perianal fistulising Crohn's disease or a complication (such as abscess, sepsis, etc.) is observed, the subject should receive an appropriate treatment depending on the symptom. If a subject receive medications which could affect fistula closure directly, the subject must be treated as a non-responder (See Section 13.1.3.3).Then, the subject may continue to participate in the study as long as the risk is considered acceptable by the investigator.

7.5 Criteria for Discontinuation or Withdrawal of a Subject

The primary reason for discontinuation or withdrawal of the subject from the study should be recorded in the electronic case report form (eCRF) using the following categories. For subject failure, refer to Section 9.1.19.

1. Death. The subject died on study.

   Note: If the subject dies on study, the event will be considered as SAE. See Section 10.2.2 for the reporting procedures.

2. Adverse event (AE). The subject has experienced an AE that requires early termination because continued participation imposes an unacceptable risk to the subject's health or the subject is unwilling to continue because of the AE.

   • Liver Function Test Abnormalities.

   Appropriate clinical follow-up (including repeat laboratory tests, until a subject's laboratory profile has returned to normal/baseline status, See Section 9.1.8) will be conducted, if the following circumstances occur at any time during study treatment:

   – ALT or AST >8 × ULN, or

   – ALT or AST >5 × ULN and persists for more than 2 weeks, or

   – ALT or AST >3 × ULN in conjunction with elevated total bilirubin >2 × ULN or international normalized ratio (INR) >1.5, or

   – ALT or AST >3 × ULN with appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (>5%).

3. Protocol deviation. The discovery after the dose of study product that the subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unacceptable risk to the subject's health.

4. Lost to follow-up. The subject did not return to the clinic and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented in the subject's source documents.

5. Withdrawal by subject. The subject wishes to withdraw from the study. The reason for withdrawal, if provided, should be recorded in the eCRF.

   Note: All attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be recorded (ie, withdrawal due to an AE or lack of efficacy).

6. Study terminated by sponsor. The sponsor terminates the study.

7. Pregnancy. The subject is found to be pregnant, and continued participation imposes an unacceptable risk to the subject's health.

   Note: If the subject is found to be pregnant before Week 52, the subject must be withdrawn immediately. If the subject is found to be pregnant after Week 52, the subject may continue

to participate in the study as long as the risk is considered acceptable by the investigator. The procedure when a pregnancy occurs is described in Section 9.1.10.

8. Lack of efficacy. The investigator has determined that the subject is not benefiting from study treatment; and, continued participation would pose an unacceptable risk to the subject.
9. Other.

   Note: The specific reasons should be recorded in the "specify" field of the eCRF.

7.6 Procedures for Discontinuation or Withdrawal of a Subject

The investigator may discontinue a subject's study participation at any time during the study when the subject meets the study termination criteria described in Section 7.5. In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Should a subject's participation be discontinued, the primary criterion for termination must be recorded by the investigator. In addition, efforts should be made to perform all procedures scheduled for the Early Termination Visit.

8.0 CLINICAL TRIAL MATERIAL MANAGEMENT

This section contains information regarding all medication and materials provided directly by the sponsor, and/or sourced by other means, that are required by the study protocol, including important sections describing the management of clinical trial material.

8.1 Study Product and Materials

8.1.1 Dosage Form, Manufacturing, Packaging, and Labeling

The study product is a human cell-processed product defined as following:

Study product name:

Code name: Cx601

INN: Darvadstrocel

Product substance, excipients, strength and formulation

Cx601 is a cell suspension containing eASCs, which original allogeneic adipose tissue is obtained from a healthy donor and expanded ex vivo.

Cx601 is supplied as a white to yellowish homogeneous suspension of 5×10^6 eASCs per mL of Dulbecco Modified Eagle's Medium with 5% HSA.

Packaging, Labeling, Manufacturing

Study product is provided as 4 glass vials containing 6 mL of eASC suspension each, duly labelled, tightly closed with rubber stoppers and sealed with an aluminium cap. The disposable vials are packaged in a carton box with its corresponding labeling.

The labels will contain, but will not be limited the following: study product name, product identification number, protocol number, sponsor's name and address, and state that the drug is for study use only.

Study products are manufactured by TiGenix S.A.U.

Additional reference information can be found in the manual.

8.1.2 Storage

The study product will be shipped directly from the manufacturing site to each study site under temperature-controlled conditions, using an appropriate transportation method. The study product must be stored under the storage conditions specified in the manual, and be remained in the original container until dispensed.

8.1.3 Dose and Regimen

In this study, 24 mL of cell suspension containing 120×10^6 cells of eASC (5×10^6 cells/mL) will be given to all subjects as a single intralesional dose according to the study schedule.

A half of the cell suspension will be injected into an adjacent site to the internal opening, and the other half into the tract wall along the fistula tracts through the external openings, with several micro-blebs forming inside the tract wall. If there are 2 internal openings, 6 mL will be injected into the adjacent site to each internal opening. The other half of the cell suspension (12 mL) will be portioned into all of the tract walls of the external openings.

The details of procedures for study product administration are specified in the manual

Prior to the first administration of the study product, appropriate training will be implemented at each study site. Only personnel who completed the training can administer the study product.

8.1.4 Overdose

An overdose is defined as a known deliberate or accidental administration of study product, to or by a study subject, at a dose above that which is assigned to that individual subject according to the study protocol.

All cases of overdose (with or without associated AEs) will be recorded on an Overdose page of the eCRF, in order to capture this important safety information consistently in the database. Cases of overdose without manifested signs or symptoms are not considered AEs. AEs associated with an overdose will be recorded on an AE page of the eCRF according to Section 10.0.

SAEs associated with overdose should be reported according to the procedure outlined in Section 10.2.2.

8.2 Study Product Dispensing Procedures

The study products will be shipped from the manufacturing site upon request from the investigator according to the separately specified procedures, and delivered to the study site on the day of study product administration or the previous day. The investigator or the designee will record the product identification number on eCRF.

8.3 Accountability and Destruction of Sponsor-Supplied Products

The site designee will receive the procedure manual for handling, storage and management of study products created by the sponsor, according to which the site designee will appropriately manage the sponsor-supplied product. The investigator will also receive those procedures from the sponsor. The procedure manual includes those for ensuring appropriate receipt, handling, storage, management, dispensation of the sponsor-supplied product, and return of unused products to the sponsor or destruction of them. See the procedure manual for details of these procedures.

9.0 STUDY PLAN

9.1 Study Procedures

The following sections describe the study procedures and data to be collected. For each procedure, subjects are to be assessed by the same investigator or site personnel whenever possible. The Schedule of Study Procedures is located in Appendix A.

9.1.1 Informed Consent Procedure

The requirements of the informed consent are described in Section 15.2.

Informed consent must be obtained prior to the subject entering into the study, and before any protocol-directed procedures are performed.

A unique subject identification number (subject number) will be assigned to each subject at the time that informed consent is explained; this subject number will be used throughout the study.

9.1.2 Demographics, Medical History, and Medication History Procedure

Demographic information to be obtained will include date of birth, sex, and smoking classification of the subject.

Medical history to be obtained will include determining whether the subject has any significant conditions or diseases relevant to the target disease (complex perianal fistulising Crohn's disease) that stopped prior to signing of informed consent. Ongoing conditions are considered concurrent medical conditions (See Section 9.1.7).

Medication history information to be obtained includes any medication relevant to eligibility criteria and efficacy/safety evaluation stopped at or within 6 months prior to signing of informed consent. Previous treatment for Crohn's disease or associated complex perianal fistulas are included.

9.1.3 Physical Examination Procedure

A baseline physical examination (defined as the assessment prior to dose of study product) will consist of the following body systems: (1) eyes; (2) ears, nose, throat; (3) cardiovascular system; (4) respiratory system; (5) gastrointestinal system; (6) dermatologic system; (7) extremities; (8) musculoskeletal system; (9) nervous system; (10) lymph nodes; (11) other. All subsequent physical examinations should assess clinically significant changes from the assessment prior to dose examination.

9.1.4 Weight and Height

A subject should have weight and height measured while wearing indoor clothing and with shoes off. The Takeda standard for collecting height is centimeters without decimal places and for weight it is kilograms (kg) with 1 decimal place.

9.1.5 Vital Sign Procedure

Vital signs will include body temperature (axilla measurement), sitting blood pressure (resting more than 5 minutes), and heart rate (beats per minute).

When vital signs are scheduled at the same time as blood draws, the blood draw will take priority and vital signs will be obtained within 0.5 hour before or after the scheduled blood draw.

9.1.6 Documentation of Concomitant Medications

Concomitant medication is any drug given in addition to the study product. These may be prescribed by a physician or obtained by the subject over the counter. Agents to be used for fistulas preparation (9.1.17) are not included. At each study visit, subjects will be asked whether they have taken any medication (used from signing of informed consent through the end of the study), and all medication including vitamin supplements, over-the-counter medications, and oral herbal preparations, must be recorded in the eCRF.

9.1.7 Documentation of Concurrent Medical Conditions

Concurrent medical conditions are those significant ongoing conditions or diseases that are present at signing of informed consent. This includes clinically significant laboratory or physical examination abnormalities noted at start of screening period. The condition (ie, diagnosis) should be described.

9.1.8 Procedures for Clinical Laboratory Samples

All samples will be collected in accordance with acceptable laboratory procedures. Samples will be collected after fasting at least 8 hour, if possible. The maximum volume of blood collected for clinical laboratory tests at each visit is provided in the procedure manual.

The items of clinical laboratory tests are shown in Table 9.a.

Table 9.a Clinical Laboratory Tests

Hematology	Serum Chemistry	Urinalysis
Hemoglobin	C-reactive protein	pH
Hematocrit	ALT	Specific gravity
Red blood cells count	AST	Qualitative tests for glucose, protein, occult blood, ketone body, bilirubin, urobilinogen
Mean Corpuscular Volume	Alkaline phosphatase (ALP)	
Mean Corpuscular Hemoglobin	Gamma-glutamyl transpeptidase (GGT)	
Mean Corpuscular Hemoglobin Concentration	Total bilirubin	
White blood cells (WBC) count	Total protein	
differential WBC (neutrophils, basophils, eosinophils, lymphocytes, monocytes)	Glucose	
Platelet count	Creatinine	
	Creatine phosphokinase	
	Blood urea nitrogen	
	Potassium	
	Sodium	
	Chloride	

Tests performed only for eligibility assessment: Serum immunology tests	**Tests performed only for women of childbearing potential: Pregnancy Tests**
HBsAg, HBV-PCR, HCV antibody, HCV-PCR, HIV antigen/antibody	hCG (for pregnancy)*

*Serum hCG pregnancy test will be performed at Visit 1 (Screening), Visit 8 (Week 24), Visit 10 (Week 52), or Early Termination Visit in follow-up period. Urine hCG pregnancy test will be performed at Visit 3 (Day 1) before study product administration.

The central laboratory will perform laboratory tests for hematology, serum chemistry, urinalysis, serum immunology tests, and serum hCG pregnancy tests. Each study site will perform urine hCG pregnancy tests. The results of laboratory tests will be returned to the investigator, who is responsible for reviewing and filing these results.

If subjects experience ALT or AST >3 × ULN, follow-up laboratory tests (at a minimum, serum ALP, ALT, AST, total bilirubin, GGT, and INR) should be performed within a maximum of 7 days and preferably within 48-72 hours after the abnormality was noted.

(Please refer to Section 7.5 for discontinuation criteria, and Section 10.2.3 for the appropriate guidance on Reporting of Abnormal Liver Function Tests in relation to ALT or AST >3 × ULN in conjunction with total bilirubin >2 × ULN.)

If the ALT or AST remains elevated >3 ×ULN on these 2 consecutive occasions the investigator must contact the study monitor for consideration of additional testing, close monitoring, discussion of the relevant subject details and possible alternative etiologies. The abnormality should be recorded as an AE (please refer to Section 10.2.3).

The investigator will maintain a copy of the reference ranges for the laboratory used.

9.1.9 Contraception and Pregnancy Avoidance Procedure

From signing of informed consent up to Week 52 of the study, female subjects of childbearing potential (ie, nonsterilized, premenopausal female subjects) who are sexually active must use acceptable methods of contraception. Also from signing of informed consent up to Week 52 of the study, nonsterilized male subjects who are sexually active with a female partner of childbearing potential must use contraception. Such subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process, and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy from signing of informed consent up to Week 52 of the study. During the course of the study, regular serum/urine human chorionic gonadotropin (hCG) pregnancy tests will be performed, and subjects will receive continued guidance with respect to avoiding pregnancy as part of the study procedures (Appendix A).

In addition to a negative serum hCG pregnancy test at screening, subjects also must have a negative urine hCG pregnancy test on the day of the dose of study product, prior to receiving any dose of study product.

In addition, male subjects must be advised not to donate sperm from signing of informed consent up to Week 52 of the study.

9.1.10 Pregnancy

If a female subject is found to be pregnant before Week 52, the subject should be withdrawn. If a female subject is found to be pregnant after Week 52, the subject may continue to participate in the study as long as the risk is considered acceptable by the investigator. In addition, any pregnancies in the partner of a male subject during the study, should also be recorded following authorization from the subject's partner.

If the pregnancy occurs after the study product administration, eg, after Visit 3, the pregnancy should be reported immediately, using a pregnancy notification form, to the contact listed in Annex.

If the female subject and/or female partner of a male subject agrees to the primary care physician being informed, the investigator should notify the primary care physician that the subject/female partner of the subject was participating in a clinical study at the time she became pregnant and provide details of treatment the subject received.

All pregnancies in subjects on active study product will be followed up to final outcome, using the pregnancy form. The outcome, including any premature termination, must be reported to the sponsor. An evaluation after the birth of the child will also be conducted.

9.1.11 Rectosigmoidoscopy

Rectosigmoidoscopy will be performed to evaluate endoscopic disease activity in the rectum and sigmoid colon using the Simple Endoscopic Score for Crohn's Disease (SES-CD) score. SES-CD includes 4 variables: size of ulcers, ulcerated surface, affected surface, presence of

narrowings. Each variable can be assigned a score of 0 to 3. Each item score will be recorded in the eCRF.

Rectosigmoidoscopy will not be required in case that the SES-CD is evaluable by using the data of previous rectosigmoidoscopy performed within 4 weeks before start of screening period. Also in case of re-screening, both rectosigmoidoscopy and SES-CD evaluation will not be required.

9.1.12 Fistula Clinical Assessment

For the fistula tract treated or to be treated, the presence of spontaneous drainage from the external openings or the presence of drainage from the external openings after gentle finger compression will be confirmed. The same fistula tracts will be assessed throughout the study period. Number of external openings, location of each external opening, and presence or absence of drainage from each external opening will be recorded in the eCRF.

Appropriate training for gentle finger compression to confirm presence or absence of drainage will be implemented before the first assessment is performed to standardize the procedure between evaluators.

9.1.13 Fistula MRI Assessment

A pelvic MRI will be performed on the fistula tracts treated or to be treated at each study site. The MRI data will be evaluated by a designated MRI central laboratory and the investigator. MRI assessments include the confirmation of the presence or absence of collections >2 cm (3-dimensional evaluation) in the fistula tracts treated or to be treated, and the presence or absence of new fistula tracts. The results of these assessments made by the central laboratory will be provided to the sponsor directly, and the results of assessments made by the investigator will be recorded in eCRF.

The MRI results assessed at screening will also be used by a surgeon for the preparation and study product administration.

9.1.13.1 Van Assche Score

The Van Assche score represents the MRI-based severity of perianal lesion associated with Crohn's disease. Based on MRI data, the number, location and extension of fistula tracts, hyperintensity on T2-weighted images, presence or absence of collections (cavities >3 mm in diameter), and rectal wall involvement will be evaluated. A higher score means more severe disease. The Van Assche score will be assessed by the designated MRI central laboratory, and the results will be provided to the sponsor directly.

9.1.14 Perianal Crohn's Disease Activity Index (PDAI)

The PDAI is a scoring system to evaluate the severity of perianal lesion associated with Crohn's disease. It includes the following 5 items: (a) Discharge; (b) Pain; (c) Restriction of sexual activity; (d) Type of perianal disease; and (e) Degree of induration. Each item is graded on a 5-

point scale ranging from no symptoms (score of 0) to severe symptoms (score of 4); a higher score means more severe disease. Each item score will be recorded in the eCRF.

9.1.15 Crohn's Disease Activity Index (CDAI)

CDAI score is calculated from the following 8 items: (a) Number of liquid or very soft stools; (b) Abdominal pain; (c) General wellbeing; (d) Extraintestinal complications; (e) Antidiarrhoeal drugs; (f) Abdominal mass; (g) Hematocrit; and (h) Body weight. Scores of some items in CDAI are calculated based on patient diary.

In CDAI evaluation between Visit 1 and Visit 2 to determine subject eligibility, a hematocrit value which is tested at each study site on the day of CDAI evaluation will be used. In other CDAI evaluations, a hematocrit value obtained the most recently from the central laboratory will be used.

Each item score will be recorded in the eCRF.

9.1.15.1 Patient Diary

For CDAI evaluations, subjects will be required to complete the following 5 items each day before going to bed in principle: (1) Number of liquid or very soft stools; (2) Score of abdominal pain (0 = none, 1 = mild, 2 = moderate, 3 = severe); (3) Score of general wellbeing (0 = general well, 1 = slightly below par, 2 = poor, 3 = very poor, 4 = terrible); (4) Evening body temperature (axillary); (5) Use of antidiarrheal drug (yes/no).

Subjects will be instructed to record the diary every day until Week 52 (Visit 10) and bring it with them at the next visit. After Week 52, subjects will be instructed to record the diary for at least 10 days before CDAI evaluation visit and bring it with them at the visit.

9.1.16 Anti-donor Antibody Test

Presence/absence of anti-donor antibodies will be investigated. A 3 mL of whole blood sample will be collected from each subject at each time point. The procedures for handling and delivery of samples are provided in a separately created manual.

Anti-HLA antibodies in each blood sample will be determined by CCI [redacted] in the laboratory for anti-donor antibody test. For anti-HLA antibody-positive samples except for baseline sample, donor specific antibodies will be determined using CCI [redacted] CCI [redacted]

9.1.17 Fistula Preparation

As the fistula preparation, all subjects will receive examination under anesthesia, fistula curettage and seton placement at the preparation visit (Visit 2: Day -21, done no later than Day -14). At least 7-day treatment with antibiotics (ciprofloxacin and/or metronidazole are recommended unless intolerance to these drugs is observed or these drugs are contraindicated)

after the fistula curettage is mandatory. Seton(s) placed will be removed on the day of study product administration, just before the administration.

Appropriate training for the preparation procedure will be implemented before the first preparation is performed to standardize the procedures between study sites. A manual for fistula preparation will be provided to each study site. Only the personnel who completed the training will perform the preparation.

9.1.18 Study Product Administration

The study product will be given at Visit 3 (Day 1). Seton(s) placed will be removed on the day of study product administration, just before the administration. Subjects will receive fistula curettage under anesthesia and subsequently study product. See Section 8.1.3 for dose regimen.

Appropriate training for the administration procedures will be implemented before the first administration is performed to standardize the procedures between study sites. A manual for the administration procedures will be provided to each study site. Only the personnel who completed the training will perform the study product administration.

9.1.19 Documentation of Subjects Failure

Investigators must account for all subjects who sign informed consent.

If the subject is found to be not eligible before study product administration, the investigator should complete the eCRF.

The primary reason for subject failure is recorded in the eCRF using the following categories:

- Death.
- Adverse event.
- Screen failure (failed inclusion criteria or did meet exclusion criteria).
- Protocol deviation.
- Lost to follow up.
- Withdrawal by subject <specify reason>.
- Study terminated by sponsor.
- Pregnancy.
- Other <specify reason>.

Subject numbers assigned to subjects who fail screening should not be reused.

9.1.20 Documentation of Study Entrance

Only subjects who meet all of the inclusion criteria and none of the exclusion criteria are eligible for entrance into the treatment period.

If the subject is found to be not eligible for the treatment period, the investigator should record the primary reason for failure on the applicable eCRF.

9.2 Monitoring Subject Treatment Compliance

Treatment compliance will be assured because treatment will be directly given by site staff at Visit 3 (Day 1). The dates and times of study product administration, infused volume, and injection site will be recorded in the eCRF.

When injection of a study product was not completed, the reason for dose incompletion will be recorded in the eCRF.

9.3 Schedule of Observations and Procedures

The schedule for all study-related examinations/observations/assessments are shown in Appendix A. The examinations, observations and assessments listed below should be performed at scheduled visit/time point(s).

9.3.1 Screening period

9.3.1.1 Screening (Visit 1)

For the screening test (Visit 1), subjects will visit the study site during the period between Day -39 and the day before VISIT2, and the examinations, observations and assessments shown in Appendix A will be performed. Subject eligibility will be determined according to the inclusion/exclusion criteria shown in Section 7.0. The procedures for documenting subject failures during screening period including Visit 1 are shown in Section 9.1.19.

9.3.1.2 Preparation (Visit 2)

For fistulas preparation (Visit 2), subjects will visit the study site on Day -21 (no later than Day -14) and receive the examinations, observations and assessments shown in Appendix A. Subject eligibility will be determined according to the inclusion/exclusion criteria shown in Section 7.0. The procedures for documenting subject failures during screening period including Visit 2 are shown in Section 9.1.19.

9.3.1.3 Potential Re-Screening

For those subjects needing a re-screening due to an out-of-window of Visit 1 and/or Visit 2 caused by re-scheduling Visit 3 because of any problem on study product administration at Visit 3, Visit 1 and/or Visit 2 will need to be re-scheduled based on protocol timelines, and applicable examinations, observations and assessments will be repeated.

9.3.2 Treatment

For study product administration (Visit 3), subjects will visit the study site on Day 1 and receive the examinations, observations and assessments shown in Appendix A. Subject eligibility will be determined according to the inclusion/exclusion criteria shown in Section 7.0, and those who met

all of the inclusion criteria and none of the exclusion criteria will be enrolled in the study. The procedures for documenting subject failures before study product administration are shown in Section 9.1.19.

If there is any problem on study product administration at Visit 3, the visit will need to be re-scheduled. The seton(s) will be maintained until the re-scheduled Visit 3. The time frame for the re-scheduled Visit 3 should be a minimum of 2 weeks from the date of the original Visit 3, but if re-screening will be needed due to an out-of-window of Visit 1 and/or Visit 2, the re-scheduled Visit 3 should be after the re-screening. All procedures for Visit 3 will be repeated at the re-scheduled Visit 3.

9.3.3 Follow-up period

For the evaluation in the follow-up period (Visits 4 to 10), subjects will visit the study site according to the schedule shown in Appendix A, and receive examinations, observations and assessments scheduled for each visit.

9.3.4 Long-term Follow-up Period

For the evaluation in the long-term follow-up period (Visit 11 to Visit 14), subjects will visit the study site according to the schedule shown in Appendix A, and receive examinations, observations and assessments scheduled for each visit.

9.3.5 Final Visit or Early Termination

The final visit will be performed on Visit 14 (Week 156).

If a subject will withdraw from the study earlier than Visit 14 (Week 156), the examinations, observations and assessments for early termination shown in Appendix A will be performed immediately.

For all subjects who received study product administration, the investigator must complete the Subject Status eCRF page.

9.3.6 Post Study Care

After the study, subjects will return to their primary care physicians to receive the previous standard care.

10.0 ADVERSE EVENTS

10.1 Definitions

10.1.1 AEs

An AE is defined as any untoward medical occurrence in a clinical investigation subject who has signed informed consent to participate in a study; it does not necessarily have to have a causal relationship with this treatment or study participation.

An AE can therefore be any unfavorable and unintended sign (eg, a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the study participation whether or not it is considered related to the product or study procedures.

An AE includes any unfavorable and unintended sign, symptom, or disease temporally associated with the collection of cells or tissues for manufacturing the product.

10.1.2 Additional Points to Consider for AEs

An untoward finding generally may:

- Indicate a new diagnosis or unexpected worsening of a pre-existing condition. (Intermittent events for pre-existing conditions or underlying disease should not be considered AEs.)
- Necessitate therapeutic intervention.
- Require an invasive diagnostic procedure.
- Require discontinuation or a change in dose of study product or a concomitant medication.
- Be considered unfavorable by the investigator for any reason.

AEs caused by a study procedure (eg, a bruise after blood draw) should be recorded as an AE.

Diagnoses vs signs and symptoms:

- Each event should be recorded to represent a single diagnosis. Accompanying signs (including abnormal laboratory values or electrocardiogram [ECG] findings) or symptoms should NOT be recorded as additional AEs. If a diagnosis is unknown, sign(s) or symptom(s) should be recorded appropriately as an AE(s).

Laboratory values and ECG findings:

- Changes in laboratory values or ECG parameters are only considered to be AEs if they are judged to be clinically significant (ie, if some action or intervention is required or if the investigator judges the change to be beyond the range of normal physiologic fluctuation). A laboratory re-test and/or continued monitoring of an abnormal value are not considered an intervention. In addition, repeated or additional noninvasive testing for verification, evaluation or monitoring of an abnormality is not considered an intervention.

- If abnormal laboratory values or ECG findings are the result of pathology for which there is an overall diagnosis (eg, increased creatinine in renal failure), the diagnosis only should be reported appropriately as an AE.

Pre-existing conditions:

- Pre-existing conditions (present at the time of signing the informed consent form) are considered concurrent medical conditions and should NOT be recorded as AEs. The first evaluations after signing of informed consent (eg, laboratory tests, ECG, X-rays etc.) should NOT be recorded as AEs unless related to study procedures. However, if the subject experiences a worsening or complication of such a concurrent medical condition after informed consent is signed, the worsening or complication should be recorded appropriately as an AE. Investigators should ensure that the event term recorded captures the change in the condition (eg, "worsening of…").
- If a subject has a pre-existing episodic condition (eg, asthma, epilepsy) any occurrence of an episode should only be captured as an AE if the episodes become more frequent, serious or severe in nature, that is, investigators should ensure that the AE term recorded captures the change in the condition from Baseline (eg "worsening of…").
- If a subject has a degenerative concurrent medical condition (eg, cataracts, rheumatoid arthritis), worsening of the condition should only be captured as an AE if occurring to a greater extent to that which would be expected. Again, investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Worsening of AEs:

- If the subject experiences a worsening or complication of an AE after starting administration of the study product, the worsening or complication should be recorded appropriately as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").
- If the subject experiences a worsening or complication of an AE after any change in study product, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Changes in intensity of AEs:

- If the subject experiences changes in intensity of an AE that are not related to starting the study product or changing in the dose or regimen, the event should be captured once with the maximum intensity recorded.

Preplanned procedures (surgeries or therapies):

- Preplanned procedures (surgeries or therapies) that were scheduled prior to signing of informed consent are not considered AEs. However, if a preplanned procedure is performed early (eg, as an emergency) due to a worsening of the pre-existing condition, the worsening

of the condition should be captured appropriately as an AE. Complications resulting from any planned surgery should be reported as AEs.

Elective surgeries or procedures:

- Elective procedures performed where there is no change in the subject's medical condition should not be recorded as AEs, but should be documented in the subject's source documents. Complications resulting from an elective surgery should be reported as AEs.

Insufficient clinical response (lack of efficacy):

- Insufficient clinical response, efficacy, or pharmacologic action, should NOT be recorded as an AE. The investigator must make the distinction between exacerbation of pre-existing illness and lack of therapeutic efficacy.

Overdose:

- Cases of overdose with any medication without manifested side effects are NOT considered AEs, but instead will be recorded on an Overdose page of the eCRF. Any manifested side effects will be considered AEs and will be recorded on the AE page of the eCRF.

Product malfunctions:

- In cases of product malfunctions that caused AEs, the product malfunctions will be recorded on the Product malfunction page of the eCRF, and AEs will be recorded on the AE page of the eCRF.
- In cases of product malfunctions that would lead to any health injuries but actually no AEs occurred, the product malfunctions and assumed health injuries will be recorded on the Product malfunction page of the eCRF.

10.1.3 SAEs

A SAE is defined as any untoward medical occurrence in a clinical investigation subject who has signed informed consent to participate in a study:

1. Results in DEATH.
2. Is LIFE THREATENING.
    - The term “life threatening” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
3. Requires inpatient HOSPITALIZATION or prolongation of existing hospitalization.
4. Results in persistent or significant DISABILITY/INCAPACITY.
5. Leads to a CONGENITAL ANOMALY/BIRTH DEFECT.
6. Is an IMPORTANT MEDICAL EVENT that satisfies any of the following:
    - May require intervention to prevent items 1 through 5 above.

- May expose the subject to danger, even though the event is not immediately life threatening or fatal or does not result in hospitalization.
- Includes any event or synonym described in the Takeda Medically Significant AE List (Table 10.a).

Table 10.a 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
COVID-19-related disease*	Neuroleptic malignant syndrome/malignant hyperthermia
COVID-19 pneumonia*	Spontaneous abortion/stillbirth and fetal death

*Asymptomatic positive COVID-19 test is not considered as medically important event.

10.1.4 AEs of Special Interest

An AE of Special Interest (treatment-emergent only, serious or non-serious) is one of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to Takeda may be appropriate. Such events may require further investigation in order to characterize and understand them. How and when they should be reported to Takeda is described in Section 10.2.1.3.

AEs of special interest in this study include the following:

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

10.1.5 Intensity of AEs

The different categories of intensity are characterized as follows:

Mild:	The event is transient and easily tolerated by the subject.
Moderate:	The event causes the subject discomfort and interrupts the subject's usual activities.
Severe:	The event causes considerable interference with the subject's usual activities.

10.1.6 Causality of AEs to Study Product(s)

The causality of each AE to study product(s) will be assessed using the following categories:

Related:	An AE that follows a reasonable temporal sequence from administration of a product (including the course after withdrawal of the product), or for which possible involvement of the product is at least a reasonable possibility, ie, the relationship cannot be ruled out, although factors other than the product, such as underlying diseases, complications, concomitant drugs and concurrent treatments, may also be responsible.
Not related:	An AE that does not follow a reasonable temporal sequence from administration of a product and/or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant drugs and concurrent treatments.

10.1.7 Causality of AEs to Study Procedures

The causality of each AE to study procedures will be assessed.

The causality should be assessed as Related if the investigator considers that there is reasonable possibility that an event is due to a study procedure. Otherwise, the causality should be assessed as Not Related.

10.1.8 Start Date

The start date of the AE is the date that the first signs/symptoms were noted by the subject and/or the investigator.

The start date of AEs will be determined using the following criteria;

AEs	Start Date
Any signs/symptoms/diseases (diagnosis)	The date that the first signs/symptoms were noted by the subject and/or the investigator should be recorded.
Asymptomatic diseases	The date when examination was performed for diagnosis and diagnosis was confirmed should be recorded. The date when diagnosis was confirmed should also be recorded even when laboratory findings showed previous findings or the onset time can be estimated.
Worsening of concurrent medical conditions or any signs/symptoms/diseases before treatment from signing of informed consent	The date that a worsening of disease and symptom was noted first by the subject and/or the investigator should be recorded.

AEs	Start Date
The first examination after signing of informed consent or the examination right before the start of the study product showed abnormal findings	The date of examination when an abnormal value that was judged to be clinically significant was noted should be recorded.
The first examination after signing of informed consent or the examination right before the study product showed abnormal findings, and the subsequent examinations showed worsening of the symptoms	The date of examination when apparent elevation, reduction, increase or decrease was confirmed in medical judgment according to the trends in laboratory values should be recorded.

10.1.9 End Date

The stop date of the AE is the date at which the subject recovered, the event resolved but with sequelae or the subject died.

10.1.10 Pattern of Adverse Event

Episodic AEs (eg, vomiting) or those which occur repeatedly over a period of consecutive days are intermittent. All other events are continuous.

10.1.11 Action Taken with Study Treatment

- Drug withdrawn – a study product is stopped due to the particular AE.
- Dose not changed – the particular AE did not require stopping a study product.
- Unknown – only to be used if it has not been possible to determine what action has been taken.
- Not Applicable – a study product was stopped for a reason other than the particular AE eg, the study has been terminated, the subject died, dosing with study product was already stopped before the onset of the AE, the AE that occurred before the study product administration

10.1.12 Outcome

- Recovered/Resolved – Subject returned to first assessment status with respect to the AE.
- Recovering/Resolving – the intensity is lowered by one or more stages: the diagnosis or signs/symptoms has almost disappeared; the abnormal laboratory value improved, but has not returned to the normal range or to baseline; the subject died from a cause other than the particular AE with the condition remaining “recovering/resolving”.
- Not recovered/not resolved – there is no change in the diagnosis, signs or symptoms; the intensity of the diagnosis, signs/ symptoms or laboratory value on the last day of the observed study period has got worse than when it started; is an irreversible congenital

anomaly; the subject died from another cause with the particular AE state remaining "Not recovered/not resolved".

- Recovered/Resolved with sequelae – the subject recovered from an acute AE but was left with permanent/significant impairment (eg, recovered from a cardiovascular accident but with some persisting paresis.
- Fatal – the AEs which are considered as the cause of death.
- Unknown – the course of the AE cannot be followed up due to hospital change or residence change at the end of the subject's participation in the study.

10.1.13 Causality of AEs to Product Malfunctions

The causality of each AE to product malfunctions will be assessed.

If the AE is related to product malfunctions, the details of the product malfunction will be recorded on the Product malfunction page of the eCRF. The procedures for collection and reporting of product malfunction are described in Section 10.2.4.

10.2 Procedures

10.2.1 Collection and Reporting of AEs

10.2.1.1 AE Collection Period

Collection of AEs will commence from the time the subject signs the informed consent. Routine collection of AEs will continue until Visit 14 (Week 156) or Early Termination Visit.

10.2.1.2 AE Reporting

At each study visit, the investigator will assess whether any subjective AEs have occurred. A neutral question, such as "How have you been feeling since your last visit?" may be asked. Subjects may report AEs occurring at any other time during the study. Subjects experiencing a serious AE that occurs prior to the first exposure to study product must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline or there is a satisfactory explanation for the change. Non-serious AEs that occur prior to the first exposure to study product, related or unrelated to the study procedure, need not to be followed up for the purposes of the protocol.

All subjects experiencing AEs after the first exposure to study product, whether considered associated with the use of the study product or not, must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline or until there is a satisfactory explanation for the changes observed. All AEs will be recorded in the AE page of the eCRF, whether or not the investigator concludes that the event is related to the product treatment. The following information will be documented for each event:

1. Event term.

2. Start and end date.
3. Pattern.
4. Intensity.
5. Investigator's opinion of the causality between the event and administration of study product(s).
6. Investigator's opinion of the causality to study procedure(s), including the details of the suspected procedure.
7. Action taken with study treatment (not applicable for the AE that occurred before the study product administration).
8. Outcome of event.
9. Seriousness.
10. Treatment emergent.
11. Investigator's opinion of the causality between the event and product malfunction(s).

Patient diary will not be used as a primary means to collect AEs. However, should the investigator become aware of a potential AE through the information collected with this instrument, proper follow-up with the subject for medical evaluation should be undertaken. Through this follow-up if it is determined that an AE not previously reported has been identified, normal reporting requirements should be applied.

10.2.1.3 AEs of Special Interest Reporting

If an AE of special interest in this study (See section 10.1.4) occurs during AEs collection period, it should be reported by the investigator to the Emergency Reception Center for Safety Information (see annex) immediately or within 1 business day of first onset or subject's notification of the event. An AE of special interest Form or SAE Form should be completed, signed and/or sealed by the principal investigator, and reported to appropriate personnel in the separate contact information list within 10 calendar days.

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

The AEs of special interest in this study include the following. If any other AEs which may be specific to the product or program occur, consider handling of AE of special interest.

- Ectopic tissue formation:
  Evidence of new masses or new lesions with calcifications in the area of the fistula which were not present before study treatment will be collected.
- Hypersensitivity:
  Hypersensitivity or AEs related to hypersensitivity.

- Transmission of infectious agents:
  Any suspected transmission of an infectious agent via the study product or AEs related to transmission of infectious agents.
- Immunogenicity:
  The investigators will not be required to reported since the sponsor will evaluate based on anti-donor antibody test results. However, a suspected severe allergic reaction following the administration of the study product must be reported as hypersensitivity.
- Alloimmune reactions:
  The AEs related to Type III immune complex mediated reaction, immune-mediated adverse reaction, rash, pyrexia and anaphylactic reaction, which are suspected to be alloimmune reactions.
- Medication errors:
  Medication errors are defined as an unintended failure in the drug treatment process that leads to, or has the potential to lead to, harm to the patient
- Tumorigenicity:
  Occurrence of anal canal or colorectal malignancy will be collected.

10.2.2 Collection and Reporting of SAEs

When a SAE occurs through the AE collection period it should be reported according to the following procedure:

A SAE should be reported by the investigator to the Emergency Reception Center for Safety Information (see annex) within 24 hours after first onset or subject's notification of the event. The principal investigator should submit the completed SAE form within 10 calendar days. The information should be completed as fully as possible but contain, at a minimum:

- A short description of the event and the reason why the event is categorized as serious.
- Subject identification number.
- Investigator's name.
- Study product name.
- Product identification number.
- Causality assessment.

Any SAE spontaneously reported to the investigator following the AE collection period should be reported to the sponsor if considered related to study participation.

10.2.3 Reporting of Abnormal Liver Function Tests

If a subject is noted to have ALT or AST >3 × ULN and total bilirubin >2 × ULN for which an alternative etiology has not been identified, the event should be recorded as a SAE and reported as per Section 10.2.2. The investigator must contact the study monitor for discussion of the

relevant subject details and possible alternative etiologies, such as acute viral hepatitis A or B or other acute liver disease or medical history/concurrent medical conditions. Follow-up laboratory tests as described in Section 9.1.8 must also be performed.

10.2.4 Collection and Reporting of Product Malfunctions

All product malfunctions (eg, a functional failure in the study product), regardless of presence or absence of AEs, will be recorded in the Product Malfunction page of the eCRF. The following information will be documented for each event:

- Product identification number.
- Event term.
- Date observed by the investigator.
- Presence or absence of AEs due to the malfunction.
- Presence or absence of a potential risk of health injury due to the malfunction (not applicable if any AEs have already occurred)
- Presence or absence of a potential risk that would lead to deaths[1] or SAEs[2] due to the malfunction (If there is such a risk, assumed health injury name must be provided. It is not applicable if any AEs have already occurred)

If a SAE occurred due to product malfunction, the SAE and the product malfunction should be reported as per section 10.2.2 and the following procedure. If a product malfunction that may lead to death or SAE occurred without actual AE, it should be also reported according to the following procedure:

A product malfunction should be reported by the investigator to the Emergency Reception Center for Safety Information (see annex) within 1 business day of first onset or subject's notification of the event, such as SAE or product malfunction that may lead to death or SAE. The principal investigator should submit the completed report form within 10 calendar days. The information should be completed as fully as possible but contain, at a minimum:

- A short description of the event.
- Subject identification number.
- Investigator's name.
- Study product name
- Presence or absence of AEs

[1] Including deaths and life threatening cases
[2] Including: cases that would require inpatient hospitalization or prolongation of existing hospitalization; cases that would lead to a persistent or significant disability/incapacity; other serious cases second to these cases or deaths/life threatening cases; and cases that would lead to a congenital anomaly/birth defect in later generation.

The sponsors also evaluate whether there is a risk that would lead to deaths or SAEs due to the malfunction. As a result of the evaluation, the sponsor may ask the investigator for additional investigation. If additional investigation is required, the principal investigator should submit the completed report form within 10 calendar days after the sponsor's request.

10.2.5 Collection and Reporting of Other Special Situations

The following information, regardless of presence or absence of AEs, should be reported to the sponsor if it occurs:

- Breastfeeding: infant exposure from breast milk.
- Drug abuse, misuse or medication error: all information on medicinal product abuse, misuse, or medication error (potential or actual).
- Occupational exposure.
- Use of falsified medicinal product.

The investigator should contact the study monitor for the reporting of those cases immediately.

10.3 Follow-up of SAEs

If information not available at the time of the first report becomes available at a later date, the investigator should complete a follow-up SAE form or provide other written documentation and fax it immediately. Copies of any relevant data from the hospital notes (eg, ECGs, laboratory tests, discharge summary, postmortem results) should be sent to the addressee, if requested.

All SAEs should be followed up until resolution or permanent outcome of the event. The timelines and procedure for follow-up reports are the same as those for the initial report.

10.3.1 Safety Reporting to Investigators, IRBs and Regulatory Authorities

The sponsor will be responsible for reporting all SUSARs, any other applicable SAEs, and product malfunctions that have risks that would lead to deaths or SAEs to regulatory authorities, investigators and IRBs /the head of the study site in accordance with the applicable local requirements.

Relative to the first awareness of the event by/or further provision to the sponsor or sponsor's designee, SUSARs will be submitted to the regulatory authorities as expedited report within 7 days for fatal and life-threatening events and 15 days for other serious events, unless otherwise required by national regulations. The sponsor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of a study product/sponsor supplied product or that would be sufficient to consider changes in the study product/sponsor supplied product administration or in the overall conduct of the trial. The sponsor will also prepare an expedited report for product malfunctions in accordance with applicable regulations.

If a SAE reported from this study in accordance with Section 10.2.2 is considered as "The safety information that could affect study continuation" by the sponsor, the information will be reported to the investigators in this study within 24 hours after the sponsor's receipt of the information. Safety information that could affect study continuation in relation to a SAE reported from clinical studies of Cx601 conducted in foreign countries will also be reported to investigators in this study within 24 hours after the sponsor's receipt of the information. After the marketing approval of Cx601 is obtained in Japan, the timeframe of 24 hours after the sponsor's receipt of the information will not be applied, but the sponsor will be responsible for reporting the information to the investigators in this study in accordance with the applicable regulations.

The study site also will forward a copy of all expedited reports to his or her IRB.

11.0 STUDY-SPECIFIC COMMITTEES

No steering committee, data safety monitoring committee, or clinical endpoint committee will be used in this study.

12.0 DATA HANDLING AND RECORDKEEPING

The full details of procedures for data handling will be documented in the Data Management Plan. AEs and medical history including concurrent conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Drugs will be coded using the World Health Organization Drug Dictionary.

12.1 eCRFs

Completed eCRFs are required for each subject who signs an informed consent.

The sponsor or its designee will supply study sites with eCRFs. The sponsor will make arrangements to train appropriate site staff in the use of the eCRF. These forms are used to transmit the information collected in the performance of this study to the sponsor and regulatory authorities. eCRFs must be completed in English. Data are transcribed directly onto eCRFs.

After completion of the entry process, computer logic checks will be run to identify items, such as inconsistent dates, missing data, and questionable values. Queries may be issued by Takeda personnel (or designees) and will be answered by the study site.

Corrections are recorded in an audit trail that captures the old information, the new information, identification of the person making the correction, the date the correction was made, and the reason for change. Reasons for significant corrections should additionally be included.

The principal investigator must review the eCRFs for completeness and accuracy and must sign and date the appropriate eCRFs as indicated. Furthermore, the investigator must retain full responsibility for the accuracy and authenticity of all data entered on the eCRFs.

The following data will not be recorded into the eCRFs:

- Clinical laboratory test results (excluding urine pregnancy test results)
- Fistula MRI assessment results by the designated MRI central lab
- Van Assche scores
- Anti-donor antibody test results

After the lock of the clinical study database, any change of, modification of or addition to the data on the eCRFs should be made by the investigator with use of change and modification records of the eCRFs (Data Clarification Form) provided by the sponsor.

eCRFs will be reviewed for completeness and acceptability at the study site during periodic visits by study monitors. The sponsor or its designee will be permitted to review the subject's medical and hospital records pertinent to the study to ensure accuracy of the eCRFs. The completed eCRFs are the sole property of the sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate governmental health or regulatory authorities, without written permission of the sponsor.

12.2 Record Retention

The investigator and the head of the study site agree to keep the records stipulated in Section 12.1 and those documents that include (but are not limited to) the study-specific documents, the identification log of all participating subjects, medical records, temporary media such as thermal sensitive paper, source worksheets, all original signed and dated informed consent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent forms), electronic copy of eCRFs, including the audit trail, and detailed records of product disposition to enable evaluations or audits from regulatory authorities, the sponsor or its designees. Any source documentation printed on degradable thermal sensitive paper should be photocopied by the site and filed with the original in the subject's chart to ensure long term legibility. Furthermore, the investigator and the head of the study site are required to retain essential relevant documents until the day specified as 1) or 2) below, whichever comes later. After the marketing approval of Cx601 in Japan, the term of record retention shall expire on the day when a reexamination or reevaluation of Cx601 is completed, instead of the day specified as of 1) and 2). However, if the sponsor requests a longer time period for retention, the head of the study site should discuss how long and how to retain those documents with the sponsor.

1) The day on which marketing approval of the study product is obtained (or the day 3 years after the date of notification in the case that the investigation is discontinued.)
2) The day 3 years after the date of early termination or completion of the clinical study.

In addition, the investigator and the head of the study site should retain the essential relevant documents until the receipt of a sponsor-issued notification to state the retention is no longer required.

13.0 STATISTICAL METHODS

13.1 Statistical and Analytical Plans

A statistical analysis plan (SAP) will be prepared and finalized prior to database lock. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives. Statistical analyses will be performed using all subjects' data up to Week 24 after the data are locked. Furthermore, statistical analyses will also be performed at the time when the data are locked after all subjects complete Week 52 and Week 156, using all subjects' data up to Week 52 and overall data respectively.

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

13.1.1 Analysis Sets

Four kinds of analysis sets are defined in this study:

- Intention to treat (ITT):
  All subjects who have enrolled into treatment period.
- Modified intention to treat (mITT):
  All subjects who have received the study treatment and whose primary efficacy endpoint is evaluable.
- Per protocol set (PPS):
  All subjects who have completed the minimum protocol-specified procedures without any major protocol deviations.
- Safety analysis set:
  All subjects who have received the study treatment.

The sponsor will verify the validity of the definitions of the analysis sets as well as the rules for handling data, consulting a medical expert as needed. If necessary, the SAP will be supplemented and finalized with new handling rules that were not discussed at the planning stage.

13.1.2 Analysis of Demographics and Other Baseline Characteristics

Demographics and other baseline characteristics will be summarized using the ITT population.

13.1.3 Efficacy Analysis

13.1.3.1 Primary Endpoint and Analytical Methods

[Primary endpoint]

- Proportion of subjects with combined remission at Week 24.

[Primary analysis]

The following analysis will be performed for the ITT population.

For combined remission at Week 24, frequency distribution will be provided with proportion and the two-sided confidence intervals (confidence coefficient: 90% and 95%).

[Secondary analysis]

For sensitivity point of view, the same analysis as the primary analysis will be performed for the mITT population and PPS to confirm robustness of the results.

13.1.3.2 Secondary Endpoints and Analytical Methods

[Secondary endpoints]

Efficacy analysis using data up to Week 24:

- Proportion of subjects with clinical remission at Week 24
- Proportion of subjects with response at Week 24.
- Time to clinical remission by Week 24.
- Time to response by Week 24.
- Proportion of subjects with relapse at Week 24 in subjects with clinical remission at previous visit.
- Time to relapse by Week 24 in subjects with clinical remission at previous visit.
- PDAI score (including total score, discharge sub-score and pain sub-score) up to Week 24.
- CDAI score up to Week 24
- Van Assche score up to Week 24.

Efficacy analysis using data up to Week 52:

- Proportion of subjects with combined remission at Week 52.
- Proportion of subjects with clinical remission at Week 52.
- Proportion of subjects with response at Week 52.
- Time to combined remission by Week 52.
- Time to clinical remission by Week 52.
- Time to response by Week 52.
- Proportion of subjects with relapse at Week 52 in subjects with combined remission at Week 24.
- Time to relapse by Week 52 in subjects with combined remission at Week 24.

- PDAI score (including total score, discharge sub-score and pain sub-score) up to Week 52.
- CDAI score up to Week 52.
- Van Assche score up to Week 52.

[Analytical Methods]

For proportion of subjects with clinical remission at Week 24 and proportion of subjects with response at Week 24, the same analysis for the primary endpoint will be performed.

For other secondary endpoints, details will be described in the SAP.

13.1.3.3 Methods of Data Transformation and Handling of Missing Data

For the primary and secondary analyses of the primary endpoint, the last observation carried forward (LOCF) from the latest earlier post-baseline visit (including an Early Termination Visit prior to Week 24, if applicable) will apply in case of missing clinical assessment at Week 24. In case of missing MRI data at Week 24, LOCF from an Early Termination Visit prior to Week 24 will apply if applicable. In case of no MRI data by Week 24 or no post-baseline clinical assessment, non-response will be imputed.

If rescue therapy which is not allowed in section 7.4 or medications which could affect fistula closure directly occur prior to Week 24, non-response will be imputed overriding all other imputation conventions.

Details will be described in the SAP.

13.1.4 Safety Analysis

The following analysis will be performed for the safety analysis set.

13.1.4.1 AEs

A TEAE is defined as an adverse event whose date of onset occurs on or after receiving study treatment. TEAEs will be coded using the MedDRA dictionary. The frequency distribution will be provided using the system organ class and the preferred term as follows:

- All TEAEs.
- Study treatment-related TEAEs.
- Intensity of TEAEs.
- Intensity of study treatment-related TEAEs.
- Serious TEAEs.
- TEAEs of special interest.
- Product malfunction-related TEAEs.
- TEAEs over time.

13.1.4.2 Clinical Laboratory Test and Vital Signs

For continuous variables, the observed values and the changes from baseline will be summarized for each visit using descriptive statistics. Case plots will also be presented for the observed values.

For categorical variables, shift tables showing the number of subjects in each category at baseline and each post-baseline visit will be provided.

13.2 Interim Analysis and Criteria for Early Termination

There will not be any interim analysis. Note, the analysis using data up to Week 24 is the primary analysis for marketing application. Additionally, separate analyses using data up to Week 52 and overall data will be performed after all subjects complete Week 52 and Week 156 respectively.

13.3 Determination of Sample Size

The planned sample size is 20 based on feasibility. However, this study has at least 94% probability to show a proportion of subjects with combined remission of 35% or more given an expected proportion of subjects with combined remission of 50% based on Week 24 results in the Cx601-0302 study.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Study-Site Monitoring Visits

Monitoring visits to the study site will be made periodically during the study to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data recorded on the eCRFs. Source documents are defined as original documents, data, and records. The investigator and the head of the study site guarantee access to source documents by the sponsor or its designee and by the IRB.

All aspects of the study and its documentation will be subject to review by the sponsor or designee, including but not limited to the Investigator's Binder, study product, subject medical records, informed consent documentation, and review of eCRFs and associated source documents. It is important that the investigator and other study personnel are available during the monitoring visits and that sufficient time is devoted to the process.

14.2 Protocol Deviations

The investigator can deviate and change from the protocol for any medically unavoidable reason, for example, to eliminate an immediate hazard to study subjects, without a prior written agreement with the sponsor or a prior approval from IRB. In the event of a deviation or change, the investigator should notify the sponsor and the head of the study site of the deviation or change as well as its reason in a written form, and then retain a copy of the written form. When necessary, the investigator may consult and agree with the sponsor on a protocol amendment. If the protocol amendment is appropriate, the amendment proposal should be submitted to the head of the study site as soon as possible and an approval from IRB should be obtained.

The investigator should document all protocol deviations.

14.3 Quality Assurance Audits and Regulatory Agency Inspections

The study site also may be subject to quality assurance audits by the sponsor or designees. In this circumstance, the sponsor-designated auditor will contact the site in advance to arrange an auditing visit. The auditor may ask to visit the facilities where laboratory samples are collected, where the medication is stored and prepared, and any other facility used during the study. In addition, there is the possibility that this study may be inspected by regulatory agencies, including those of foreign governments (eg, the Food and Drug Administration [FDA], the United Kingdom Medicines and Healthcare products Regulatory Agency [MHRA], the Pharmaceuticals and Medical Devices Agency of Japan [PMDA]). If the study site is contacted for an inspection by a regulatory body, the sponsor should be notified immediately. The investigator and institution guarantee access for quality assurance auditors to all study documents as described in Section 14.1.

15.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (ie, subjects) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the ICH Harmonised Tripartite Guideline for GCP. Each investigator will conduct the study according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator" that are listed in Appendix B. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

15.1 IRB Approval

IRBs must be constituted according to the applicable local requirements of each participating region. The sponsor or designee will require documentation noting all names and titles of members who make up the respective IRB. If any member of the IRB has direct participation in this study, written notification regarding his or her abstinence from voting must also be obtained.

The sponsor or designee will supply relevant documents for submission to the respective IRB for the protocol's review and approval. This protocol, the Investigator's Brochure, a copy of the informed consent form, and, if applicable, subject recruitment materials and/or advertisements and other documents required by all applicable laws and regulations, must be submitted to a central or local IRB for approval. The IRB's written approval of the protocol and subject informed consent form must be obtained and submitted to the sponsor or designee before commencement of the study (ie, before study specific screening activity). The IRB approval must refer to the study by exact protocol title, number, and version date; identify versions of other documents (eg, informed consent form) reviewed; and state the approval date. The sponsor will notify site once the sponsor has confirmed the adequacy of site regulatory documentation. Until the site receives notification no protocol activities, including screening may occur.

Sites must adhere to all requirements stipulated by their respective IRB. This may include notification to the IRB regarding protocol amendments, updates to the informed consent form, recruitment materials intended for viewing by subjects, local safety reporting requirements, reports and updates regarding the ongoing review of the study at intervals specified by the respective IRB, and submission of the investigator's final status report to IRB. All IRB approvals and relevant documentation for these items must be provided to the sponsor or its designee.

Subject incentives should not exert undue influence for participation. Payments to subjects must be approved by the IRB and sponsor.

15.2 Subject Information, Informed Consent, and Subject Authorization

Written consent documents will embody the elements of informed consent as described in the Declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all applicable laws and regulations. The informed consent form describe the planned and permitted uses, transfers, and disclosures of the subject's personal and personal health information for purposes of conducting the study. The informed consent form further explain the nature of the study, its objectives, and potential risks and benefits, as well as the date informed consent is

given. The informed consent form will detail the requirements of the participant and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care.

The investigator is responsible for the preparation, content, and IRB approval of the informed consent form. The informed consent form must be approved by both the IRB and the sponsor prior to use.

The informed consent form must be written in a language fully comprehensible to the prospective subject. It is the responsibility of the investigator to explain the detailed elements of the informed consent form to the subject. Information should be given in both oral and written form whenever possible and in the manner deemed appropriate by the IRB.

The subject, or the subject's legally acceptable representative, must be given ample opportunity to: (1) inquire about details of the study and (2) decide whether or not to participate in the study. If the subject, or the subject's legally acceptable representative, determines he or she will participate in the study, then the informed consent form must be signed and dated by the subject, or the subject's legally acceptable representative, at the time of consent and prior to the subject entering into the study. The subject or the subject's legally acceptable representative should be instructed to sign using their legal names, not nicknames, using blue or black ballpoint ink. The investigator must also sign and date the informed consent form at the time of consent and prior to subject entering into the study.

Once signed, the original informed consent form will be stored in the investigator's site file. The investigator must document the date the subject signs the informed consent in the subject's medical record. Copies of the signed informed consent form shall be given to the subject.

All revised informed consent forms must be reviewed and signed by relevant subjects or the relevant subject's legally acceptable representative in the same manner as the original informed consent. The date the revised consent was obtained should be recorded in the subject's medical record, and the subject should receive a copy of the revised informed consent form.

15.3 Subject Confidentiality

The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject's source data will only be linked to the sponsor's clinical study database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes, such as sex, age, or date of birth may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires the investigator to permit its monitor or designee's monitor, representatives from any regulatory authority (eg, FDA, MHRA, PMDA), the sponsor's designated auditors, and the appropriate IRBs to review the subject's original medical records (source data or documents), including, but not limited to, laboratory test result reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports.

Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process (See Section 15.2).

Copies of any subject source documents that are provided to the sponsor must have certain personally identifiable information removed (ie, subject name, address, and other identifier fields not collected on the subject's eCRF).

15.4 Publication, Disclosure, and Clinical Trial Registration Policy

15.4.1 Publication and Disclosure

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 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.

The investigator needs to obtain a prior written approval from the sponsor to publish any information from the study externally such as to a professional association.

15.4.2 Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable laws, regulations and guidance, Takeda will, at a minimum register interventional clinical trials it sponsors anywhere in the world on ClinicalTrials.gov or other publicly accessible websites before start of study, as defined in Takeda Policy/Standard. Takeda contact information, along with the study sites' name and location (ie, city, state [for Americas investigators], country), and recruiting status will be registered and available for public viewing.

15.4.3 Clinical Trial Results Disclosure

Takeda will post the results of clinical trials on ClinicalTrials.gov or other publicly accessible websites, as required by Takeda Policy/Standard, applicable laws and/or regulations.

15.5 Insurance and Compensation for Injury

Each subject in the study must be insured in accordance with the regulations applicable to the study site where the subject is participating. If a local underwriter is required, then the sponsor or sponsor's designee will obtain clinical study insurance against the risk of injury to study subjects. Refer to the Study Site Agreement regarding the sponsor's policy on subject compensation and treatment for injury. If the investigator has questions regarding this policy, he or she should contact the sponsor or sponsor's designee.

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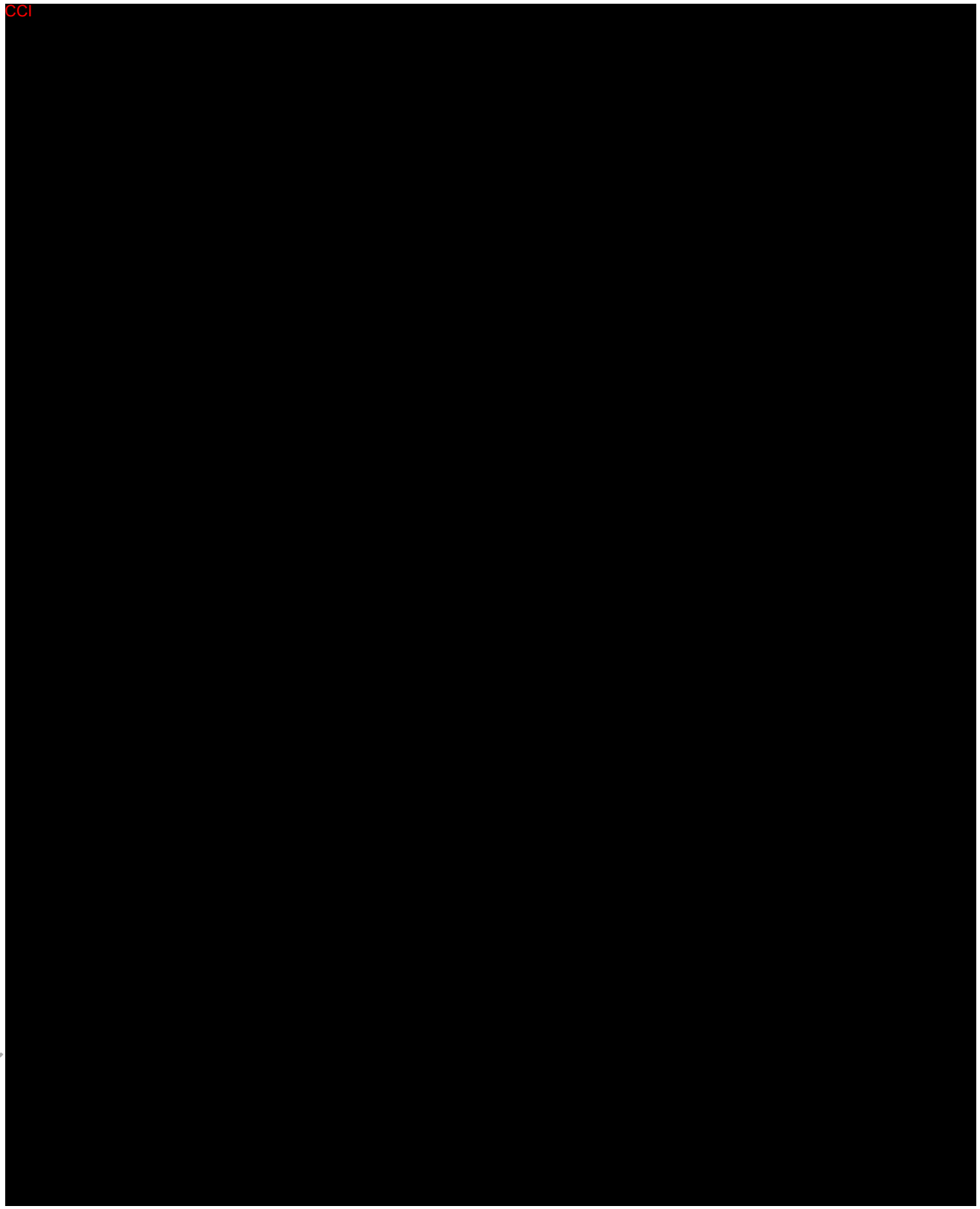

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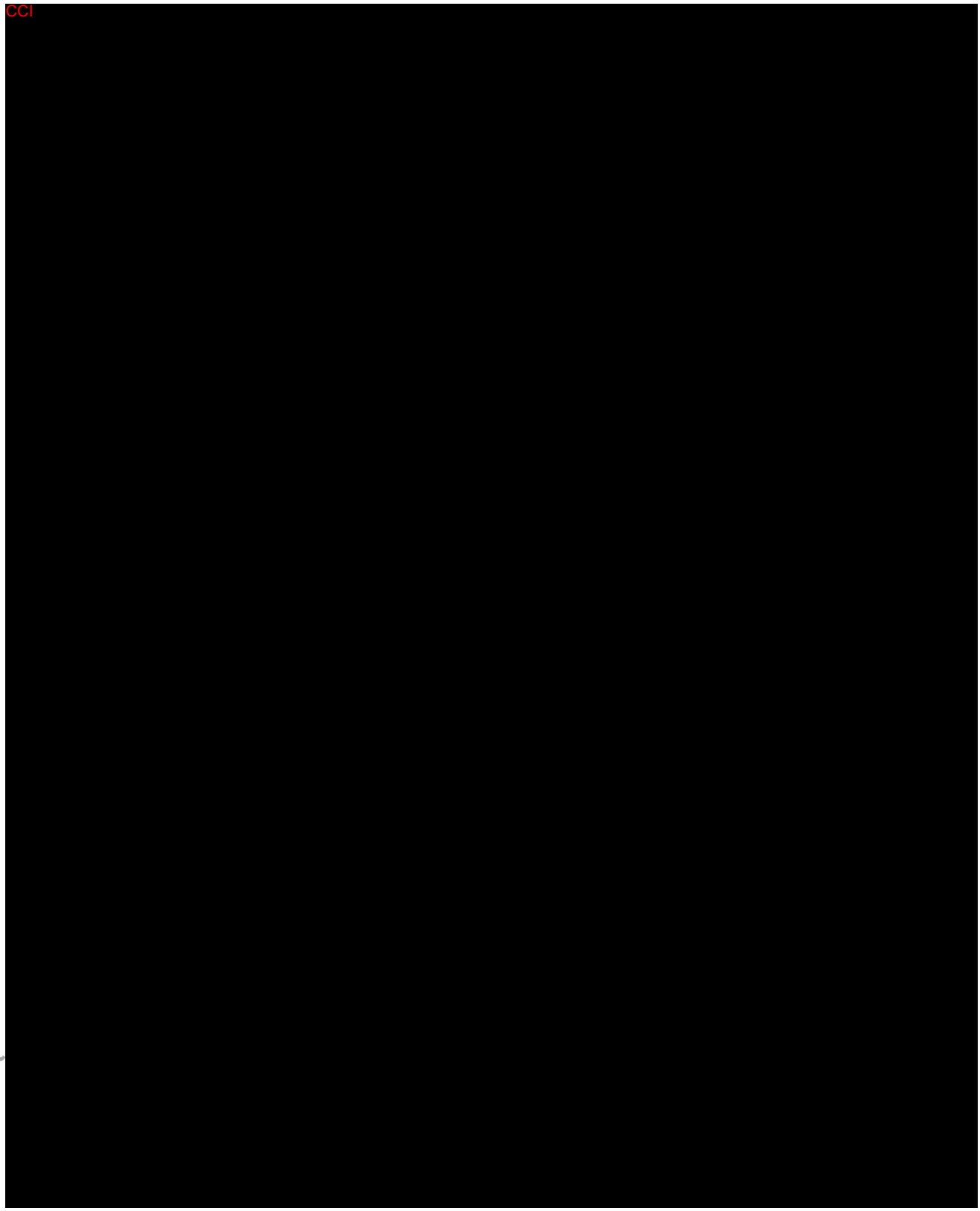

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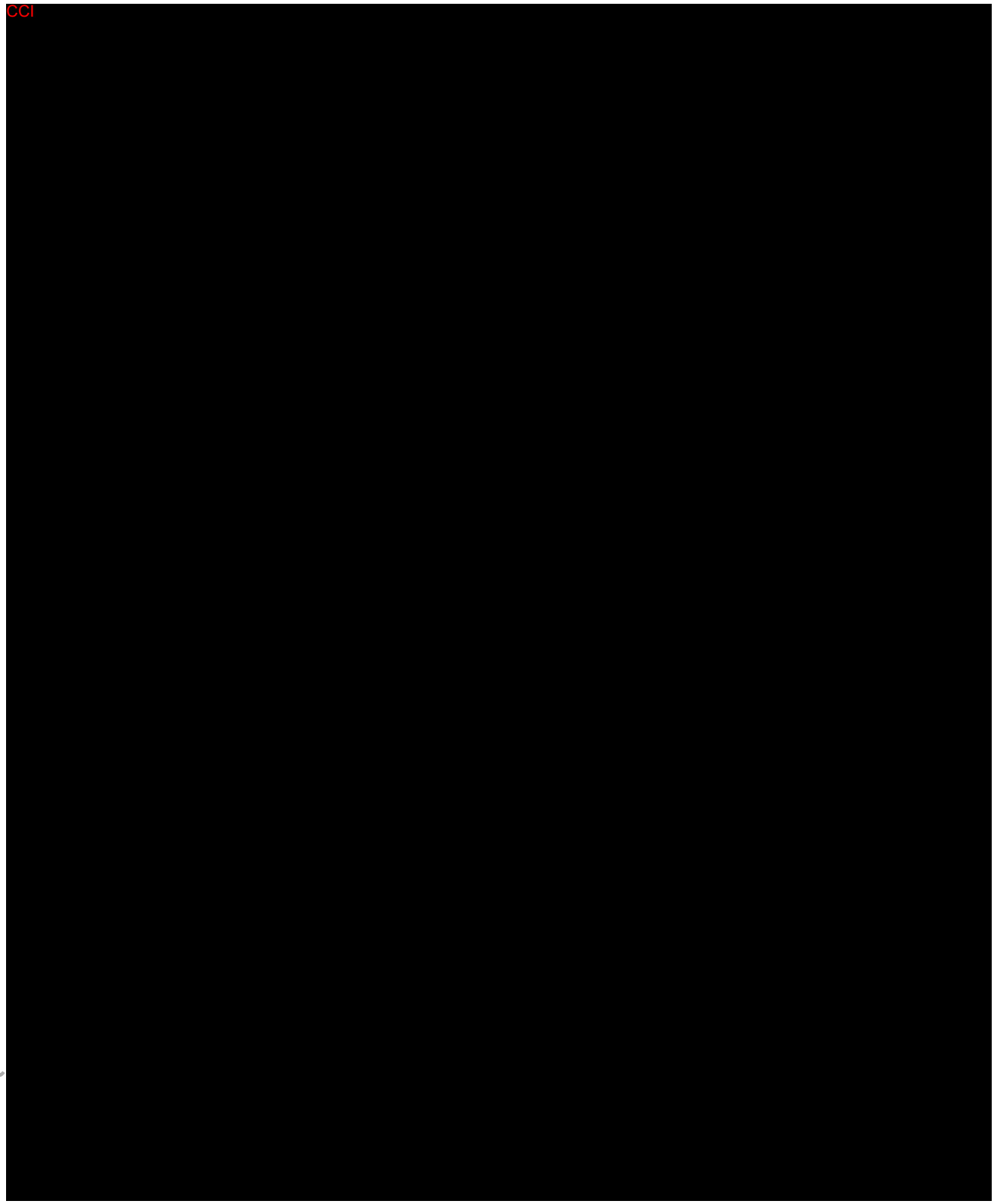

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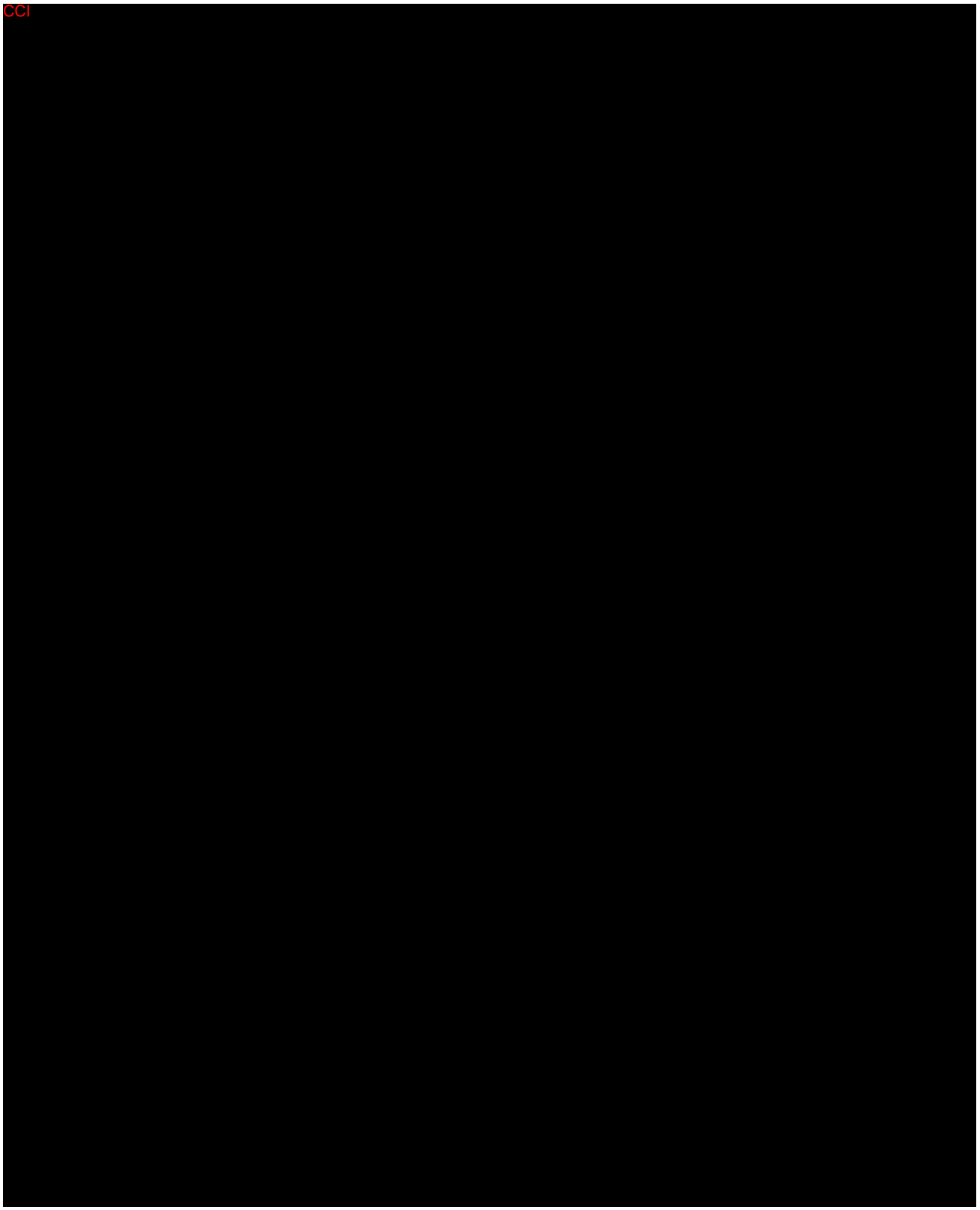

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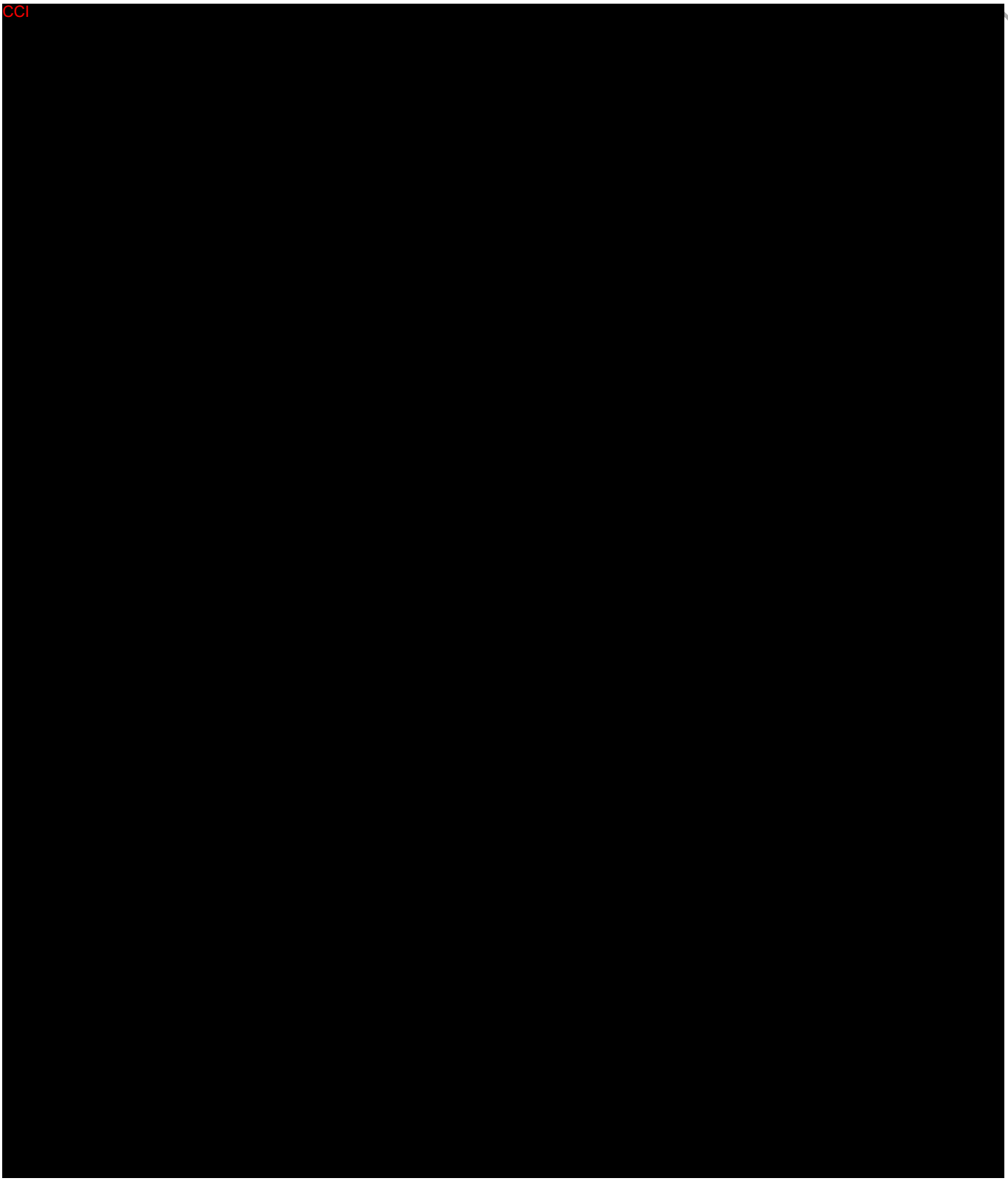

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