September 6, 2021
Medical Device Evaluation Division
Pharmaceutical Safety and Environmental Health Bureau
Ministry of Health, Labour and Welfare

Report on the Deliberation Results

Classification	Human cellular/tissue-based products 2. Human somatic stem cell-processed products
Non-proprietary Name	Darvadstrocel
Brand Name	Alofisel Injection
Applicant	Takeda Pharmaceutical Company Limited
Date of Application	February 10, 2021 (Application for marketing approval)

Results of Deliberation

In its meeting held on September 6, 2021, the Committee on Regenerative Medical Products and Biotechnology made the following decision and concluded that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product may be approved. The approval is not classified as a conditional and time-limited approval. The re-examination period is 10 years.

The following approval conditions must be satisfied.

Approval Conditions

1. The applicant is required to take necessary actions such as conducting seminars and disseminating the guidelines for proper use prepared in cooperation with relevant academic societies, to ensure that physicians with adequate knowledge and experience in complex perianal fistulas in patients with Crohn's disease acquire adequate skills in using the product and knowledge of complications associated with the procedures, and that the physicians use the product in compliance with the "Indication or Performance" and "Dosage and Administration or Method of Use" at medical institutions well equipped for providing medical care for complex perianal fistulas in patients with Crohn's disease.
2. The applicant is required to conduct a drug use-results survey covering all patients treated with the product after the market launch until data from a certain number of patients have been gathered, and to take appropriate measures as necessary.

Review Report

August 23, 2021
Pharmaceuticals and Medical Devices Agency

The following are the results of the review of the following regenerative medical product submitted for marketing approval conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Brand Name	Alofisel Injection
Classification	Human cellular/tissue-based products 2. Human somatic stem cell-processed products
Non-proprietary Name	Darvadstrocel
Applicant	Takeda Pharmaceutical Company Limited
Date of Application	February 10, 2021

Shape, Structure, Active Ingredients, Quantities, or Definition
The product is a human (allogeneic) somatic stem cell-processed product prepared by isolating and culturing mesenchymal stem cells derived from adult human subcutaneous adipose tissue.

Application Classification	(1-1) New regenerative medical product
Items Warranting Special Mention	Orphan regenerative medical product (Orphan Regenerative Medical Product Designation No. 10 of 2019 [*31 sai*]; PSEHB/MDED Notification No. 0313-1 dated March 13, 2019, by the Medical Device Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare)
Reviewing Office	Office of Cellular and Tissue-based Products

Results of Review
On the basis of the data submitted, PMDA has concluded that the product has a certain level of efficacy in the treatment of complex perianal fistulas in patients with non-active or mildly active Crohn's disease and that the product has acceptable safety in view of its benefits (see Attachment).

As a result of its review, PMDA has concluded that the product may be approved for the indication or performance as well as dosage and administration or method of use shown below, with the following conditions.

Indication or Performance

Treatment of complex perianal fistulas in patients with non-active or mildly active Crohn's disease who have had an inadequate response to at least 1 conventional drug.

Dosage and Administration or Method of Use

The usual adult single dose is 120×10^6 human mesenchymal stem cells (contained in 4 vials [24 mL]) administered to 1 or 2 internal openings and to up to 3 external openings after conditioning of the fistulas such as curettage.

Approval Conditions

1. The applicant is required to take necessary actions such as conducting seminars and disseminating the guidelines for proper use prepared in cooperation with relevant academic societies, to ensure that physicians with adequate knowledge and experience in complex perianal fistulas in patients with Crohn's disease acquire adequate skills in using the product and knowledge of complications associated with the procedures, and that the physicians use the product in compliance with the "Indication or Performance" and "Dosage and Administration or Method of Use" at medical institutions well equipped for providing medical care for complex perianal fistulas in patients with Crohn's disease.
2. The applicant is required to conduct a drug use-results survey covering all patients treated with the product after the market launch until data from a certain number of patients have been gathered, and to take appropriate measures as necessary.

Attachment

Review Report (1)

July 2, 2021

The following is an outline of the data submitted by the applicant and content of the review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Product Submitted for Approval

Brand Name	Alofisel Injection
Classification	Human cellular/tissue-based products 2. Human somatic stem cell-processed products
Non-proprietary Name	Darvadstrocel
Applicant	Takeda Pharmaceutical Company Limited
Date of Application	February 10, 2021

Shape, Structure, Active Ingredients, Quantities, or Definition

The product is a human (allogeneic) somatic stem cell-processed product prepared by isolating and culturing mesenchymal stem cells derived from adult human subcutaneous adipose tissue.

Proposed Indication or Performance

Treatment of complex perianal fistulas in patients with non-active or mildly active Crohn's disease

Proposed Dosage and Administration or Method of Use

The usual adult single dose is 24 mL of cell suspension containing 120×10^6 cells (contained in 4 vials) administered locally to the fistulas that have been conditioned.

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List of Abbreviations

See Appendix.

1. Origin or History of Discovery, Use in Foreign Countries, and Other Information

1.1 Outline of the proposed product

Alofisel is composed of human (allogeneic) expanded adipose stem cells (eASCs) obtained by isolating and cultivating mesenchymal stem cells derived from subcutaneous adipose tissue of healthy adults. It is a regenerative medical product intended to be administered locally to the inner wall of fistula tracts of complex perianal fistulas, to obtain a therapeutic effect through its pharmacological action in the same manner as a pharmaceutical product.

It is suggested that adipose stem cells (ASCs) exhibit, at the site of inflammation, immunomodulatory effects such as inhibition of release of inflammatory cytokines, inhibition of T cell growth, and induction of regulatory T cells. Alofisel is thus expected to have efficacy against complex perianal fistulas associated with Crohn's disease.

Alofisel was designated as an orphan regenerative medical product with the intended indication or performance for treatment of "complex perianal fistulas in patients with Crohn's disease" on March 13, 2019 (Orphan Regenerative Medical Product Designation No. 10 of 2019 [*31 sai*]).

1.2 Development history etc.

Crohn's disease is a granulomatous inflammatory disease of unknown cause characterized by inflammation/ulcer of small and large intestines, intestinal stenosis, fistula, etc. In Japan, the disease is designated as an intractable disease. Lesions may occur at any site in the intestinal tract from the oral cavity to the anus. The disease also causes various non-gastrointestinal complications related to blood, joints, skin, eyes, nutrient metabolism, etc. Patients with perianal lesion, one of the characteristic complications of Crohn's disease, show symptoms of perianal abscess and perianal fistula, such as pain, bleeding, pus discharge, pyrexia, and palpable protrusion.

Perianal fistula is generally divided into simple fistula and complex fistula depending on the number and course of fistula tracts (*Gastroenterology.* 2003;125:1508-30). The following symptoms or conditions are classified as a complex fistula: openings at a level higher than the dentate line; multiple external openings; pain or fluctuation suggesting a perianal abscess; a rectovaginal fistula; an anorectal stenosis; or active rectal disease confirmed by endoscopy.

The Japanese clinical practice guidelines for the perianal lesions of Crohn's disease state that treatment of fistula/abscess should be done according to pathological conditions. Mild disease (with symptoms not interfering with the activity of daily living) is treated by incisional drainage of fistula with administration of antibiotics, and moderate or severe disease (persistent pain, pus discharge) is treated by seton drainage. If necessary, drug therapy (immunomodulators, biological products such as infliximab, anti-tumor necrosis factor [TNF] agents) is introduced after the local infection focus has been controlled by drainage. If severe symptoms cannot be controlled by the above treatments, such as in case of complex fistula, colostomy is considered (FY2020 Partial Research Report, Diagnostic Criteria and Clinical Practice Guidelines for Ulcerative Colitis and Crohn's Disease [in Japanese]; a Grant-in-Aid for Intractable Gastrointestinal Diseases Research, Research on rare and intractable diseases [Suzuki group] funded by Health and Labour Sciences Research Grants).

There is a need for development of new treatment for complex perianal fistula in patients with Crohn's disease, for the following reasons:

(a) In Japan, the only drug approved for the treatment of perianal lesions associated with Crohn's disease is infliximab, which is indicated for external fistulas associated with Crohn's disease. Most of the other conventional therapies do not have sufficient evidence (e.g., *J Crohns Colitis.* 2020;14:4-22, *J Crohns Colitis*. 2020;14:155-168).
(b) The efficacy of infliximab is limited (*N Engl J Med*. 2004;350:876-85).
(c) Seton drainage requires a long treatment period.
(d) Colostomy reduces the quality of life (QOL) of patients.

Alofisel was developed by TiGenix in Belgium (currently Takeda Pharmaceutical Company Limited). In 2009, a phase I/IIa open-label, uncontrolled study (Study Cx601-0101) was initiated in Spain involving patients with complex perianal fistulas associated with Crohn's disease. Subsequently, a phase III, randomized, double-blind, placebo-controlled study (Study Cx601-0302) was started in 2012 in Europe involving patients with complex perianal fistulas associated with Crohn's disease. Based on the results of these clinical studies, Alofisel was approved in EU in March 2018 for the treatment of complex perianal fistulas in patients with non-active or mildly active Crohn's disease who have had an inadequate response to at least 1 conventional or biological therapy. Subsequently, Alofisel was approved in Switzerland, Israel, and Serbia, and it is marketed in 16 countries as of December 2020.

In Japan, a phase III open-label, uncontrolled study (Study Darvadstrocel-3002) was initiated by the applicant in March 2019 involving Japanese patients with Crohn's disease with complex perianal fistulas.

The applicant has submitted the application for the approval of Alofisel with data from Study Darvadstrocel-3002 and foreign studies (Studies Cx601-0302 and Cx601-0101) as pivotal studies.

2. Data Relating to Quality and Outline of the Review Conducted by PMDA

The component cells of Alofisel are eASCs obtained by isolating and culturing ASCs, which are mesenchymal stem cells derived from human subcutaneous adipose tissue.

2.1 Active substance

2.1.1 Manufacturing process

The manufacturing process of the active substance is comprised of receipt of human subcutaneous adipose tissue, [redacted], [redacted], [redacted], [redacted], [redacted] of ASCs, freezing/storage/testing of the master cell stock (MCS), thawing of MCS, [redacted] of eASCs, and freezing/storage/testing of the active substance.

All processes are handled as critical steps.

MCS, defined as the critical intermediate, is frozen for storage in [redacted]. MCS will be regenerated as needed [redacted].

The manufacturing process for the active substance has been validated on a commercial scale.

2.1.2 Safety evaluation of adventitious agents

2.1.2.1 Human subcutaneous adipose tissue

Human subcutaneous adipose tissue, the raw material of the active substance, was collected from healthy female donors in Spain, and conforms to requirements for the collection method and documentation defined in the Human Cell and Tissue Raw Material Standards under the Standard for Biological Ingredients (MHLW Public Notice No. 210 of 2003). The donors were interviewed (for evaluation of health condition by a physician, past history, history of infectious disease, and foreign travel history) and tested for infection by serological testing or nucleic acid amplification testing (hepatitis B virus [HBV], hepatitis C virus [HCV], human immunodeficiency virus [HIV]-1, HIV-2, human T-cell leukemia virus [HTLV]-1, HTLV-2, cytomegalovirus [CMV], Epstein-Barr virus [EBV], West Nile virus [WNV], Zika virus, parvovirus B19, severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], malaria plasmodium, toxoplasma, Treponema pallidum, and trypanosome).

2.1.2.2 Raw materials of biological origin other than human subcutaneous adipose tissue

Table 1 shows raw materials of biological origin other than human subcutaneous adipose tissue used in the manufacturing process of the active substance. All of the raw materials meet the Standards for Biological Ingredients (MHLW Public Notice No. 210 of 2003).

Table 1. Raw materials of biological origin other than human subcutaneous adipose tissue used in the manufacturing process of the active substance

Raw material	Origin	Derived from	Process
Peptone	Bovine	Skin, bone (excluding cranial bone and spinal column), and skeletal muscle	[redacted]
Casein	Bovine	Milk	[redacted]
FBS	Bovine	Blood	[redacted], [redacted]
Trypsin	Porcine	Pancreas	[redacted], [redacted]

2.1.3 Manufacturing process development (comparability)

The following are main changes made in the manufacturing process during the development of the active substance. Table 2 shows the manufacturing process of the active substance for the product used in nonclinical and clinical studies.

- Process A to Process B: [redacted]
- Process B to Process C: [redacted]
- Process C to Process D: [redacted]
- Process D to Process E: [redacted]
- Process E to Process F (proposed commercial process): [redacted]

With each change of the manufacturing process, the quality attributes of pre-change and post-change active substances were assessed and shown to be comparable.

Table 2. Manufacturing process of active substance contained in the product used in nonclinical and clinical studies

Manufacturing process	Nonclinical or clinical study
Process A	Nonclinical studies
Process B	Study Cx601-0101
Process C	Study Cx601-0101
Process D	Study Cx601-0302
Process E	Study Cx601-0302 and Study Darvadstrocel-3002
Process F (proposed commercial process)	-

2.1.4 Characterization

2.1.4.1 Structure and characteristics

The active substance was subjected to characterization shown in Table 3.

Table 3. Characterization of component cells

Structure and cytological characteristics	Cellular morphology (microscopic observation)
	Expression of [redacted]
	No expression of [redacted]
Biological and functional characteristics	Proliferation kinetics
	Cell viability
	Differentiation capacity (induction of differentiation: [redacted])
	Immunoregulation ([redacted])
	Immune-related proteins ([redacted])
	Proteins related with regenerative and reparative activity

2.1.4.2 Removal of process-related impurities

Antibiotics (penicillin G, streptomycin sulfate, and gentamicin), Impurity A, trypsin, Impurity B, and fetal bovine serum (FBS) were handled as process-related impurities. Impurities other than trypsin, FBS, and Impurity B have been shown to be adequately removed in the manufacturing process of the active substance. Evaluation of the residual amounts of trypsin, FBS, and Impurity B in the product has shown that they are adequately removed in the manufacturing process [see Section 2.2.5.1].

2.1.5 Control of active substance

The proposed specifications for the active substance include description, identification ([redacted]), purity ([redacted]), bacterial endotoxin, sterility, [redacted], cell viability, mycoplasma test, and potency ([redacted]). Also, the first batch of the active substance manufactured from the MCS batch derived from each donor is subjected to [redacted] and [redacted].

2.1.6 Stability of active substance

Table 4 shows the summary of the main stability studies for the active substance.

Table 4. Summary of the main stability studies for the active substance

	Manufacturing process	Number of batches	Storage condition	Study period	Storage form
Long-term	F	4	(°C)	months	cryopreservation vials

In the long-term testing, no clear changes were observed in the quality attributes throughout the study period.

Based on the above, the shelf life of months has been proposed for the active substance when stored (°C) in cryopreservation vials.

2.2 Product

2.2.1 Description and composition of product and formulation development

The product is a cell suspension containing 30×10^6 eASCs in each vial (solution volume of 6 mL) (4 vials [120×10^6 cells] per dose). The product contains Dulbecco's Modified Eagle's Medium (DMEM) and 20% human serum albumin (HSA) as excipients.

2.2.2 Manufacturing process

The manufacturing process for the product consists of thawing of the active substance, , , , , filling/testing, and secondary packaging.

All processes from the thawing of the active substance to the secondary packaging are defined as critical steps.

The manufacturing process for the product has been validated on a commercial scale.

2.2.3 Safety evaluation of adventitious agents

2.2.3.1 Raw materials of biological origin other than active substance

HSA is the only raw material of biological origin, other than the raw materials contained in the active substance used in the manufacturing process of the product.

HSA is derived from plasma collected from donors qualified in the US. Each plasma sample and the pooled plasma are tested for infection by serological testing or nucleic acid amplification testing (, HBV, HCV, HIV-1, HIV-2, and). During the manufacturing process of HSA, viruses are inactivated or removed by and , and HSA has been confirmed to meet the Standards for Biological Ingredients (MHLW Public Notice No. 210 of 2003).

Heparin may be added during the early stage of HSA manufacture. The heparin is derived from the intestinal mucosa of healthy pigs and is subjected to viral inactivation by and during the manufacturing process.

2.2.4 Manufacturing process development

The following are main changes made in the manufacturing process during the development of the product. Table 5 shows the manufacturing process of the product used in each clinical study.

- Process 1 to Process 2: [redacted]
- Process 2 to Process 3: [redacted]
- Process 3 to Process 4 (proposed commercial process): [redacted]

With each change of the manufacturing process, the quality attributes of pre-change and post-change products were assessed and shown to be comparable.

Table 5. Manufacturing process of product used in each clinical study

Manufacturing process	Clinical study
Process 1	Study Cx601-0101
Process 2	Study Cx601-0302
Process 3	Study Cx601-0302 and Study Darvadstrocel-3002
Process 4 (proposed commercial process)	-

2.2.5 Characterization

2.2.5.1 Process-related impurities

Trypsin, FBS, and Impurity B were defined as process-related impurities in the product. Evaluation of their residual amounts in the product has shown that they are adequately removed in the manufacturing process.

2.2.6 Control of product

The proposed specifications for the product include description, bacterial endotoxin, sterility, [redacted], cell viability, cellular concentration, [redacted], and mycoplasma test. Since the shelf-life of the product is only 72 hours [see Section 2.2.7], results of sterility and mycoplasma tests are unavailable at the time of shipping. Results of the mycoplasma test are available before administration of product, but results of the sterility test after administration. Sterility of the product at shipping is controlled by in-process control testing consisting of [redacted] and [redacted].

2.2.7 Stability of product

Table 6 shows the summary of the main stability studies of the product.

Table 6. Summary of main stability studies of product

Storage conditions	Manufacturing process of active substance	Manufacturing process of product	Number of batches	Evaluation time point	Storage form
[redacted]°C	F	4	6	[redacted] hours	[redacted] vial with [redacted] stopper
[redacted]°C	F	4	1	[redacted] hours	
	F	4	3	[redacted] hours	
[redacted]°C	F	4	6	[redacted] hours	
	F	4	1	[redacted] hours	

The stability studies ([redacted]°C and [redacted]°C) revealed no clear changes in the quality attributes throughout the study period. In the stability study ([redacted]°C), [redacted] of [redacted] batches failed to meet the specifications for [redacted] ([redacted]) at [redacted] hours.

To evaluate the stability before use at medical institutions, Alofisel was drawn out of the vial using a syringe and left standing for [redacted] hours [redacted], and then [redacted] and [redacted] were determined.

Results showed that the handling of the product before administration did not affect [redacted] or [redacted] of Alofisel.

Based on the above, the shelf life of 72 hours has been proposed for the product when stored at 15°C to 22°C in a [redacted] vial with [redacted] stopper.

2.R Outline of the review conducted by PMDA

Based on the data submitted and the results of the following review, PMDA concluded that the quality of the active substance and the product was controlled adequately.

2.R.1 TSE risk of the raw material

Human subcutaneous adipose tissue, the starting raw material of Alofisel, was collected in Spain, which is designated as a country of origin of variant Creutzfeldt-Jakob disease (vCJD) by "Enhancement of Quality and Safety of Drugs and Medical Devices Produced Using Raw Materials of Human Origin [in Japanese]" (PFSB Notification No. 1213002, dated December 13, 2005). PMDA therefore asked the applicant to explain (a) the risk of transmissible spongiform encephalopathy (TSE) caused by the raw material and (b) tolerance of TSE risk in view of the medical need of Alofisel.

The applicant's explanation:

(a) TSE risk associated with human subcutaneous adipose tissue:

The TSE risk of Alofisel has been reduced to an acceptable level, for the following reasons:

- Adipose tissue is classified as "tissues with no detectable infectivity" according to the World Health Organization (WHO) classifications of bovine spongiform encephalopathy (BSE) infectivity of tissues ("high-infectivity tissues," "lower-infectivity tissues," and "tissues with no detectable infectivity"). This suggests that there is only an extremely low possibility of TSE transmission through adipose tissue, the starting material of Alofisel (WHO, 2006; WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies).
- The following information suggests that the human subcutaneous adipose tissue collected in Spain has only a low risk of TSE:
  - According to the evaluation by U.S. Food and Drug Administration (FDA), the vCJD risk is highest in England, Ireland, and France, whereas the risk is relatively low in Spain (*Transfusion*. 2017;57:924-32).
  - The incubation period of vCJD mediated by food is approximately 10 years (ECDC, 2017; Facts about variant Creutzfeldt-Jakob disease). Given that the most recent case of vCJD in Spain was reported in 2008 (ECDC, 2019; Surveillance Atlas of Infectious Diseases), there is only an extremely low possibility of infection with dormant vCJD in Spain.
- Candidate donors of human subcutaneous adipose tissue were carefully screened against the risk of vCJD, including health history of family members to exclude the possible future risk of vCJD, and those with even the slightest risk of vCJD were excluded from donors.
- Since Alofisel is locally administered to the lesion within the large intestine, TSE transmission to brain tissue is unlikely.

(b) Tolerance of TSE risk in view of the medical need of Alofisel

Alofisel has a mechanism of action different from that of conventional drugs and has a lower invasiveness compared with surgical procedure. Therefore, the European guidelines state that Alofisel may provide a safe and effective treatment option for complex perianal fistulas associated with Crohn's disease (*J Crohns Colitis.* 2020;14:155-68). Accordingly, there is a medical need for Alofisel in Japan as is the case in Europe.

PMDA's view:

Given the explanation of the applicant, the risk of TSE caused by human subcutaneous adipose tissue collected in Spain cannot be completely excluded. Nevertheless, for the time being, there is no choice but to use the starting material collected in Spain, a country of origin of vCJD. PMDA instructed the applicant to, when regenerating MCS used for the manufacture of Alofisel, consider collecting the tissue in countries without vCJD specified in the "Partial revision of 'Enhancement of Quality and Safety of Drugs and Medical Devices Produced using Raw Materials of Human Origin [in Japanese]'" (PFSB Notification No. 1213002, dated December 13, 2005) and related notifications.

The applicant agreed to take actions accordingly, and PMDA accepted the applicant's response.

3. Primary Pharmacodynamics or Performance and Outline of the Review Conducted by PMDA

The applicant submitted the results from *in vitro* and *in vivo* studies as data relating to the primary pharmacodynamics or performance of Alofisel.

3.1 *In vitro* studies

Table 7 shows *in vitro* studies conducted.

Table 7. *In vitro* primary pharmacodynamic studies of Alofisel

Objective	Outline of study methods	Main results	Attached document CTD
Investigation of inhibition of lymphocyte proliferation	Alofisel was cultured in contact with or not in contact with PBMC, and growth inhibition against lymphocytes, CD4-positive T cells, and CD8-positive T cells was evaluated by flow cytometry.	Both under contact and non-contact conditions, Alofisel inhibited the growth of lymphocytes, CD4-positive T cells, and CD8-positive T cells.	4.2.1.1-1
Investigation of soluble factors involved in inhibition of lymphocyte proliferation	Alofisel and activated PBMCs were cultured in the presence of inhibitors or neutralizing antibodies of soluble factors potentially involved in inhibition of lymphocyte proliferation, and the inhibition of PBMC proliferation and concentrations of inflammatory cytokines were evaluated.	Alofisel inhibited the proliferation of activated PBMCs and release of inflammatory cytokines. Under the IFN-γ-neutralizing condition, Alofisel-associated inhibition of activated PBMC proliferation was suppressed.	4.2.1.1-1
Study of the mechanism of action of Alofisel to inhibit lymphocyte proliferation and decrease inflammatory cytokines	IDO expression after stimulation of Alofisel by IFN-γ for 24 hours was evaluated by RT-PCR and Western blotting, and kynurenine concentration by HPLC. Kynurenine concentration in the presence of anti-IFN-γ antibody or 1-MT (IDO inhibitor) was evaluated.	Stimulation of Alofisel by IFN-γ induced IDO gene expression, increased IDO protein expression, and increased kynurenine concentration. Kynurenine production decreased in the presence of anti-IFN-γ antibody or 1-MT.	4.2.1.1-1
	Cells constitutively expressing IDO and cells not expressing IDO were prepared by genetic engineering of Alofisel, and the effect of IFN-γ and anti-IFN-γ antibody on the inhibitory activity of these cells against PBMC proliferation was evaluated.	In the presence of cells constitutively expressing IDO, anti-IFN-γ antibody did not suppress the PBMC proliferation inhibition. In the presence of non-IDO-expressing cells, IFN-γ did not inhibit the proliferation of PBMC.	4.2.1.1-1
Induction of Treg by Alofisel	Alofisel was cultured in contact with or not in contact with PBMC, and Treg-induction was evaluated by flow cytometry.	When Alofisel was cultured in contact with PBMC, Treg induction was observed, whereas under the non-contact culture condition, no significant Treg induction was observed.	4.2.1.1-2 4.2.1.1-3 4.2.1.1-4 4.2.1.1-5
	Alofisel was cultured with PBMC or with PBMC cleared of Treg, and Treg induction was evaluated.	The number of Treg cells induced was similar regardless of the removal of Treg from PBMC,	
	(a) Cells constitutively expressing IDO in the presence of 1-MT or (b) non-IDO-expressing cells were cultured with PBMC, and Treg induction was evaluated.	Treg induction was reduced in both (a) cells constitutively expressing IDO in the presence of 1-MT and (b) non-IDO-expressing cells.	

3.2 *In vivo* study (CTD 4.3-2)

In vivo efficacy of Alofisel against colitis was evaluated in a mouse model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis caused by T cell-mediated immunoreaction against autoantigen, as is the case with Crohn's disease. The efficacy of Alofisel against complex perianal fistulas was not investigated because of the absence of the animal model of the disease.

Mouse models of TNBS-induced colitis were generated by administering 3 mg of TNBS into the colon of BALB/c mice. At 12 hours after administration of TNBS, Alofisel (3×10^5 or 1×10^6 cells) was administered intraperitoneally, and the animals were subjected to the following evaluations: (1) Changes in body weight over 10 days after administration of Alofisel, (2) survival period, (3) inflammatory cell infiltration into the colon, and concentrations of inflammatory cytokines (TNF-α, interferon-γ [IFN-γ], interleukin-6 [IL-6], interleukin-1beta [IL-1β], and interleukin-12 [IL-12]) in the colon and serum 3 days after administration. Compared with animals not receiving Alofisel, those receiving Alofisel showed a significant suppression of weight loss and a significant improvement in

the survival period, in a dose-dependent manner. Animals receiving Alofisel also showed a significant decrease in the inflammatory cell infiltration and the concentration of inflammatory cytokines in the colon, compared with those not receiving Alofisel.

Cluster of differentiation (CD)4-positive T cells containing and not containing regulatory T cells (Treg) were purified from the mesenteric lymph nodes of animals receiving and not receiving Alofisel, and were administered intravenously to another group of mice models of TNBS-induced colitis. Suppression of weight loss and improvement of survival period were observed only in mice that received CD4-positive T cells containing Treg derived from animals receiving Alofisel.

3.R Outline of the review conducted by PMDA

The applicant's explanation about the efficacy of Alofisel:

Results of nonclinical studies suggested that IFN-γ-activated Alofisel inhibits the proliferation of lymphocytes by increasing indoleamine 2,3-dioxygenase (IDO) activity. Results also suggested that Alofisel induces immunosuppressive Treg. These results suggest that Alofisel suppresses local inflammatory reactions by its immunomodulatory effects, thereby healing the tissue around the fistula.

PMDA's view:

The primary pharmacodynamic and performance studies did not provide direct evidence that Alofisel contributes to the closure of fistula, but the applicant's explanation about the mechanism of action of Alofisel against fistula is understandable.

4. Non-clinical Safety and Outline of the Review Conducted by PMDA

The applicant submitted the following data relating to the nonclinical safety of Alofisel: Single-dose and repeated-dose toxicity studies in nude rats, *in vitro* and *in vivo* tumorigenicity studies, and safety of excipients and impurities.

4.1 Single-dose toxicity

4.1.1 Single intravenous dose toxicity study in nude rats (CTD 4.2.3.1-1)

Alofisel (0.2×10^6 cells, 1×10^6 cells, 5×10^6 cells, or 10×10^6 cells) was administered intravenously to male and female nude rats. The dose of 5×10^6 cells did not show any toxicity, demonstrating favorable tolerability. The dose of 10×10^6 cells caused toxicities including pulmonary edema and death. The applicant explained that the death and toxicity findings such as pulmonary edema are not relevant to humans because, in clinical use, Alofisel is administered locally.

4.1.2 Single subcutaneous dose toxicity study in nude rats (CTD 4.2.3.1-2)

Alofisel (0.2×10^6 cells, 1×10^6 cells, or 10×10^6 cells) was administered subcutaneously to male and female nude rats. No Alofisel-related findings were observed.

4.1.3 Safety evaluation in single-dose biodistribution study (CTD 4.2.3.1-3 to 4.2.3.1-5)

In the biodistribution study administering Alofisel (5×10^6 cells) [see Section 5.1], animals were monitored for clinical signs, hematology and clinical chemistry, histopathology, etc. In the study administering Alofisel to perianal region and into the rectum, mild reactive changes (e.g.,

inflammation) were observed at the administration site from the next day of administration. The intravenous administration study showed findings suspected to be due to the embolization of Alofisel, with no death. The intravaginal administration study showed no findings related to Alofisel.

4.2 Repeated-dose toxicity

4.2.1 Three-month repeated-dose toxicity study in nude rats via the perianal route (CTD 4.2.3.2-1)

Alofisel (0.2×10^6 cells, 1×10^6 cells, or 2.5×10^6 cells) was administered twice, 2 weeks apart, to the perianal region of nude rats. No Alofisel-related findings were observed.

4.2.2 Six-month repeated-dose toxicity study in nude rats via the perianal route (CTD 4.2.3.2-2)

Alofisel (0.2×10^6 cells, 1×10^6 cells, or 2.5×10^6 cells) was administered to nude rats at the perianal region twice, 2 weeks apart. No local irritation or any other findings of toxicological significance were observed.

4.2.3 Six-month repeated intravenous dose toxicity study in nude rats (CTD 4.2.3.2-3)

Alofisel (0.2×10^6 cells, 1×10^6 cells, or 5×10^6 cells) was administered intravenously to nude rats twice, 2 weeks apart. No Alofisel-related findings were observed.

4.3 Tumorigenicity

The applicant explanation:

In vitro studies included karyotyping and soft agar colony formation assay, and *in vivo* studies included 2 tumorigenicity studies subcutaneously administering Alofisel to nude mice. No findings suggesting tumorigenicity were observed in any of the studies. In addition to the 2 subcutaneous tumorigenicity studies, a repeated-dose toxicity study administering to perianal region, the intended clinical route, was conducted. No changes suggesting tumorigenicity were observed either at the administration site or in any of the organs in the whole body [see Section 4.2.2]. Based on the above, Alofisel has only a low risk of tumorigenicity.

4.3.1 *In vitro* tumorigenicity study (CTD 4.2.3.4.1-1)

Alofisel obtained from 6 donors was subjected to karyotyping and to evaluation of anchorage-independent growth by soft-agar colony formation assay. The karyotyping revealed cells with numerical aberration in Alofisel derived from 1 donor, but this was not considered to be a finding suggesting genetic transformation because no same abnormality was observed in 2 or more cells. No other abnormalities were detected. Soft agar colony formation assay showed no colony formation in Alofisel from any donor, suggesting that the cells did not acquire the capacity of anchorage-independent growth.

4.3.2 Tumorigenicity study in nude mice (CTD 4.2.3.4.1-2)

Female nude mice received a subcutaneous single dose of Alofisel (10×10^6 cells), and were monitored for mortality (dead or alive) and clinical signs, and by palpation. After the 12-week observation period, histopathological examination was performed on the administration site, lung,

regional lymph nodes, and macroscopic lesion sites. There were no findings suggesting Alofisel-induced neoplastic lesion.

4.3.3 Tumorigenicity study in nude mice (CTD 4.2.3.4.1-3)

Male and female nude mice received a single subcutaneous dose of Alofisel (10×10^6 cells), and were monitored for mortality (dead or alive) and clinical signs, and by palpation. After the 12-week observation period, histopathological examination was performed on the administration site, lung, spleen, mesenteric lymph nodes, and macroscopic lesion sites. One animal showed nodes from 50 days after administration until necropsy, but histopathological examination of the site did not reveal any abnormality. No other abnormalities were detected. Thus, there were no findings suggesting the tumorigenicity of Alofisel.

4.4 Other safety profiles

4.4.1 Reproductive and developmental toxicity

In the biodistribution study administering Alofisel intravaginally, Alofisel was detected in the upper part of the uterus at 24 hours after administration, but not detected from 14 days after administration. In the 6-month repeated-dose toxicity study via the perianal route, histopathological examination showed no abnormal findings in the tissues of the reproductive system [see Section 4.2.2]. These results suggest that Alofisel has no particular effect on reproduction and development.

4.4.2 Safety evaluation of excipients

The applicant explanation:

Alofisel contains DMEM and 20% HSA as excipients. The safety of each excipient was evaluated based on the results of the toxicity studies and on clinical use experience, by taking account of the content of these excipients in Alofisel at the proposed clinical dose. The applicant considered that there was no safety concern.

4.4.3 Safety evaluation of impurities

The applicant explanation:

The final product may possibly contain the following impurities: Antibiotics (penicillin G, streptomycin sulfate, and gentamicin), Impurity A, trypsin, Impurity B, and FBS. The safety evaluation based on their residual amounts in Alofisel showed that these impurities do not pose a safety risk to humans.

4.R Outline of the review conducted by PMDA

Based on the submitted data, PMDA concluded that there were no particular concerns about the nonclinical safety of Alofisel.

5. Biological Disposition and Outline of the Review Conducted by PMDA

Three biodistribution studies were conducted to evaluate the distribution and engraftment following perianal/intrarectal, intravaginal, or intravenous administration of Alofisel.

5.1 Biodistribution studies

Alofisel (5×10^6 cells) was administered to athymic nude rats, and distribution of Alofisel was evaluated by quantitative polymerase chain reaction (PCR) of human Alu sequences in the organs/tissues of the whole body (Table 8).

Table 8. Biodistribution studies of Alofisel

Route of administration	Dose (cells/body)	Evaluation time point	Main results	Attached document CTD
Perianal/ intrarectal	2.5×10^6 each via perianal and intrarectal routes	Days 1, 14, 91, and 182	Human gDNA was detected in rectum (Days 1 and 14) and in jejunum (Day 1 only). On Day 14, human gDNA in rectum decreased substantially compared to the level on Day 1. On Day 91 and thereafter, human gDNA was undetectable in any of the tissues.	4.2.2.3-1
Intravenous	5×10^6	Days 2, 14, 91, and 182	On Days 2 and 14, human gDNA was detected in organs with many blood vessels (lung, heart, liver, kidney, gastrointestinal tract). On Day 91 and thereafter, human gDNA was undetectable in any of the tissues.	4.2.2.3-2
Intravaginal	5×10^6	Days 2, 14, 91, and 182	Alofisel was not distributed in ovary. On Day 2, human gDNA was detected in the upper part of the uterus. On Day 14 and thereafter, human gDNA was undetectable in any of the tissues.	4.2.2.3-3

5.2 Clinical biological disposition

It is technically difficult to differentiate human allogeneic ASCs from intrinsic cells. Also, isolating multiple tissue samples is unethical. Therefore, no biodistribution of Alofisel in humans was conducted.

5.R Outline of the review conducted by PMDA

Based on the submitted data, PMDA concluded that there were no particular concerns about the biological disposition of Alofisel.

6. Clinical Efficacy and Safety and Outline of the Review Conducted by PMDA

The applicant submitted the results from 3 clinical studies as efficacy and safety evaluation data: a Japanese phase III study, a foreign phase I/IIa study, and a foreign phase III study (Table 9).

Table 9. List of clinical studies on efficacy and safety

Data category	Region	Study	Phase	Population	No. of patients enrolled	Dosage regimen	Main endpoints
Evaluation	Japan	Darvadstrocel-3002	III	Patients with complex perianal fistulas associated with Crohn's disease	22	A single dose of Alofisel (containing 120×10^6 eASCs) was administered into the lesion.	Efficacy Safety
	Foreign	Cx601-0101	I/Iia	Patients with complex perianal fistulas associated with Crohn's disease	24	The half amount of Alofisel (containing 20×10^6 eASCs) was administered to the tissue surrounding the internal opening, and the other half into the fistula wall along the fistula tract from the external opening. If the fistula had not closed completely at Week 12, Alofisel (containing 40×10^6 eASCs) was administered again.	Safety
	Foreign	Cx601-0302	III	Patients with complex perianal fistulas associated with Crohn's disease	212	A single dose of Alofisel (containing 120×10^6 eASCs) or placebo was administered into the lesion.	Efficacy Safety

The outline of each clinical study is provided below.

6.1 Evaluation data

6.1.1 Japanese clinical study

6.1.1.1 Japanese phase III study (CTD 5.3.5.2-2 and 5.3.5.2-3; Study Darvadstrocel-3002 [ongoing since March 2019; data cut-off of February 1, 2021])

An open-label, uncontrolled study was conducted to investigate the efficacy and safety of Alofisel in Japanese patients with complex perianal fistulas associated with Crohn's disease (target sample size, 20 patients) at 9 study sites in Japan. Table 10 shows the main inclusion/exclusion criteria.

Table 10. Main inclusion/exclusion criteria

Inclusion criteria

- Patients aged ≥18 years diagnosed with Crohn's disease at least 6 months before the start of screening period according to the Diagnostic Criteria for Crohn's Disease issued by Research Group for "Research for Intractable Gastrointestinal Diseases" (revised January 2017).
- Patients who have complex perianal fistulas with ≤2 internal openings and ≤3 external openings (all connected to the internal openings), confirmed by clinical assessment and MRI. Fistula must have been draining for at least 6 weeks prior to screening. Complex perianal fistula is defined as a condition that meets 1 or more of the following criteria:
  - High (i.e., above the dentate line) inter-sphincteric, trans-sphincteric, extrasphincteric, or supra-sphincteric fistula.
  - Presence of ≥2 external openings (tracts).
  - Associated abscess.
- Patients with non-active or mildly active Crohn's disease (defined by CDAI ≤220 evaluated during the screening period).
- Patients whose complex perianal fistulas were previously treated and have shown an inadequate response (absence of closure of part or all fistula tracts, or new fistula during induction treatment), loss of response (fistula relapse after complete closure of initial fistula, or fistula worsening after partial closure of initial fistula during maintenance treatment) or intolerance (occurrence, at any time, of an unacceptable level of adverse drug reactions necessitating treatment discontinuation) while they were receiving at least 1 of the following drugs at the standard dose for a specified period:
  - Antibiotics (ciprofloxacin or metronidazole): ≥1-month treatment.
  - Immunomodulators (azathioprine, 6-mercaptopurine or methotrexate): ≥3-month treatment.
  - Biologics (anti-TNF agents, anti-integrin, or anti-IL-12/23): ≥14-week (≥16-weeks for anti-IL-12/23) standard treatment for induction or maintenance.

Exclusion criteria

- Patients with active Crohn's disease requiring new therapy immediately.
- Patients with concomitant rectovaginal or rectovesical fistulas.
- Patients with an abscess >2 cm.
- Patients with rectal and/or anal stenosis and/or active proctitis, which would restrict the surgical procedure.
- Patients who underwent surgery other than drainage or seton placement for the to-be-treated fistula.
- Patients with diverting stomas.
- Patients who were treated with systemic steroids in the 4 weeks prior to the study treatment.
- Patients who are receiving cytapheresis therapy.
- Patients who need perianal surgery (other than fistula conditioning required by the protocol) during screening, or patients who will receive a perianal surgery within 24 weeks after the study treatment.

The study consisted of a screening period (5 weeks prior to Alofisel administration), a treatment period (day of Alofisel administration), an observation period (52 weeks after Alofisel administration), and a follow-up period (52-156 weeks after Alofisel administration).

All patients who were considered eligible after screening were to undergo fistula conditioning consisting of fistula curettage and seton placement under anesthesia 21 to 14 days prior to administration of the study product.

The dosage regimen or method of use of Alofisel in this study:
Seton removal and fistula curettage were performed under anesthesia, and immediately after that, a single dose of Alofisel (containing 120×10^6 eASCs in a total volume of 24 mL) was injected into the lesions in divided portions. Half of Alofisel suspension was administered to the tissue surrounding the internal opening and the other half into the fistula wall along the fistula tract from the external opening, while forming several minute blebs. If there were 2 internal openings, 6 mL each was administered to the tissue surrounding each internal opening and, if there were 2 or 3 external openings, the cell suspension was evenly divided for each fistula tract.

A total of 22 patients enrolled in the study were included in the intention to treat (ITT) population, and the ITT population was handled as the efficacy analysis population. All 22 patients received Alofisel and were included in the safety analysis population. As of Week 52, 2 patients had discontinued the study due to lack of efficacy.

Table 11 shows the results of the primary efficacy endpoint, combined remission rate at Week 24 (which means the percentage of patients who achieved combined remission, i.e., clinically confirmed closure of all treated external openings that were draining at baseline despite gentle finger compression, and absence of abscess >2 cm in the treated fistulas confirmed by central MRI).

Table 11. Results of combined remission rate at Week 24 (Study Darvadstrocel-3002, ITT population)

	Alofisel
Combined remission rate (% [n/N])	59.1 (13/22)

* Missing values were imputed by last observation carried forward (LOCF). If there were no data after baseline or rescue treatment was given, the patient was handled as a non-remission case.

Table 12 shows the results of the main secondary endpoints.

Table 12. Results of main secondary endpoints[*1] (Study Darvadstrocel-3002 ITT population)

	Alofisel
Combined remission rate at Week 52 (% [n/N])	68.2 (15/22)
Clinical remission[*2] rate at Week 24 (% [n/N])	59.1 (13/22)
Clinical remission rate at Week 52 (% [n/N])	72.7 (16/22)
Improvement[*3] rate at Week 24 (% [n/N])	81.8 (18/22)
Improvement rate at Week 52 (% [n/N])	90.9 (20/22)
Time to clinical remission at Week 52 (days) (median [95% CI])	25.5 (14.0, 109.0)
Time to improvement at Week 52 (days) (median [95% CI])	18.0 (12.0, 30.0)
Relapse rate up to Week 52 in patients who had achieved combined remission at Week 24 (% [n/N])	23.1 (3/13)
PDAI score at Week 24 (mean ± SD)	2.4 ± 2.9
PDAI score[*4] at Week 52 (mean ± SD)	2.1 ± 2.1

*1 Missing values were imputed by LOCF. If there were no data after baseline or rescue treatment was given, the patient was handled as a nonresponder in calculating the rate.
*2 Clinically confirmed closure of all treated external openings that were draining at baseline despite gentle finger compression.
*3 Clinically confirmed closure of ≥50% of treated external openings that were draining at baseline despite gentle finger compression.
*4 Evaluated in 20 patients.

Adverse events were observed in 20 of 22 patients (90.9%) within 52 weeks after administration. Table 13 shows adverse events observed in ≥2 patients. Adverse events for which a causal relationship to Alofisel could not be ruled out were observed in 2 of 22 patients (9.1%). They were Crohn's disease, diarrhoea, and blood bilirubin increased.

Table 13. Adverse events observed in ≥2 patients (Study Darvadstrocel-3002, Week 52, safety analysis population)

	Alofisel (N = 22)
All adverse events	20 (90.9)
Gastrointestinal disorders	
Proctalgia	6 (27.3)
Anal fistula	4 (18.2)
Crohn's disease	2 (9.1)
Nausea	2 (9.1)
Infections and infestations	
Nasopharyngitis	5 (22.7)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	
Acrochordon	2 (9.1)

n (%)
Medical Dictionary for Regulatory Activities Japanese version (MedDRA/J) ver. 23.0

No death occurred. Serious adverse events were observed in 4 of 22 patients (18.2%). They were intestinal obstruction, intestinal anastomosis complication, calculus urinary, and Crohn's disease/tubulointerstitial nephritis. A causal relationship to Alofisel could not be ruled out for Crohn's disease in 1 patient. There was no adverse event leading to study discontinuation.

6.1.2 Foreign clinical studies

6.1.2.1 Foreign phase I/IIa study (CTD 5.3.5.2-1, Study Cx601-0101 [August 2009 to September 2010])

An open-label, uncontrolled study was conducted to investigate the safety and efficacy of Alofisel in non-Japanese patients with complex perianal fistulas associated with Crohn's disease (target sample size, 24 patients) at 6 study sites in Spain. Table 14 shows the main inclusion/exclusion criteria.

Table 14. Main inclusion/exclusion criteria

Inclusion criteria
- Patients aged ≥18 years with Crohn's disease diagnosed at least 12 months before the start of screening period in accordance with clinical, endoscopic, anatomopathological and/or radiologic criteria.
- Patients with complex perianal fistula with ≤3 fistula tracts assessed by MRI.
- Patients with persistent and active complex perianal fistula and non-active Crohn's disease defined by a CDAI ≤200. Complex perianal fistula is defined as a condition that meets 1 or more of the following criteria:
  - High (i.e., above the dentate line) inter-sphincteric, trans-sphincteric, extrasphincteric, or supra-sphincteric fistula.
  - Presence of ≤3 external openings.

Exclusion criteria
- Patients with concomitant rectovaginal, anal, or non-perianal rectocutaneous fistula.
- Patients with an abscess.
- Patients with severe proctitis (prominent mucosal friability, spontaneous bleeding, multiple erosions, deep ulcers) or dominant active luminal disease requiring immediate therapy, assessed by rectosigmoidoscopy.
- Patients who underwent surgery other than drainage or seton placement for the to-be-treated fistula.
- Patients with rectal and/or anal stenosis confirmed by rectoscopy or examination under anesthesia.
- Patients who have received infliximab or any other anti-TNF agent in the 8 weeks before the study treatment.
- Patients who have received tacrolimus or cyclosporine in the 4 weeks before the study treatment.

The study consisted of a screening period (1-3 weeks before Alofisel administration) and an observation period (24 weeks after Alofisel administration).

The dosage regimen or method of use of Alofisel in this study:
Alofisel (containing 20×10^6 eASCs) is administered to the lesion. If the fistula has not closed completely after 12 weeks, Alofisel (containing 40×10^6 eASCs) is administered again. Half of

Alofisel suspension is administered to the tissue surrounding the internal opening and the other half into the fistula wall along the fistula tract from the external opening.

All of the 24 patients receiving Alofisel were included in the safety analysis population and the full analysis set (FAS). A total of 22 patients were included in the per protocol set (PPS), and the remaining 2 patients were excluded (1 patient who used antimicrobial agents for ≥4 weeks and 1 patient who had not received pregnancy test at the screening and on the day of Alofisel administration). The FAS and PPS were subjected to efficacy analysis. Study discontinuation occurred in 8 patients, for reason of adverse events in 2 patients, protocol deviation in 1 patient, and relapse of Crohn's disease in 5 patients.

Table 15 shows the results of the main efficacy endpoints.

Table 15. Results of main efficacy endpoints (Study Cx601-0101)

			PPS (N = 22)	FAS (N = 24)
Reduction in the number of draining fistulas from screening	Week 12	0	7	8
		1	10	10
		2	2	2
		Missing data	3	4
	Week 24	0	4	4
		1	7	8
		2	1	1
		Missing data	10	11
Increase in the number of closed fistulas[a)]	Week 12	Fistulas closed	5	6
		Fistulas not closed	14	15
		Missing data	3	3
	Week 24	Fistulas closed	5	6
		Fistulas not closed	9	9
		Missing data	8	9
Number of closed external openings[b)]	Week 12	0	12	13
		1	7	8
		Missing data	3	3
	Week 24	0	7	7
		1	8	9
		Missing data	7	8

n

a) Clinically confirmed closure of all treated external openings without draining despite gentle finger compression, indicating completed re-epithelialization of the external openings. Absence of abscess >2 cm in the treated fistulas confirmed by MRI.

b) Clinically confirmed re-epithelialization of the external openings

Within 24 weeks after administration, adverse events were observed in 5 of 9 patients (55.6%) receiving a single dose of Alofisel and in 8 of 15 patients (53.3%) receiving 2 doses, with the incidence in the entire population being 54.2% (13 of 24) of patients. Table 16 shows the incidence of adverse events observed in ≥2 patients in the entire population. Adverse events for which a causal relationship to Alofisel could not be ruled out were observed in 2 of 9 patients (22.2%) receiving a single dose of Alofisel and in 3 of 15 patients (20.0%) receiving 2 doses, with the incidence in the entire population being 20.8% (5 of 24) of patients. Anal abscess (observed in 3 of 24 patients [12.5%]) was the only adverse event observed in ≥2 patients for which a causal relationship to Alofisel could not be ruled out. Anal abscess was observed in 2 of 9 patients (22.2%) receiving a single dose of Alofisel and in 1 of 15 patients (6.7%) receiving 2 doses.

Table 16. Adverse events observed in 2 patients in the entire population (Study Cx601-0101, Safety analysis population)

	Patients receiving a single dose of Alofisel (N = 9)	Patients receiving 2 doses of Alofisel (N = 15)	All patients (N = 24)
All adverse events	5 (55.6)	8 (53.3)	13 (54.2)
Gastrointestinal disorders			
Proctalgia	0	2 (13.3)	2 (8.3)
General disorders and administration site conditions			
Pyrexia	0	4 (26.7)	4 (16.7)
Infections and infestations			
Anal abscess	3 (33.3)	1 (6.7)	4 (16.7)
Anal fistula infection	1 (11.1)	1 (6.7)	2 (8.3)
Investigations			
C-reactive protein increased	2 (22.2)	1 (6.7)	3 (12.5)
Psychiatric disorders			
Anxiety	1 (11.1)	2 (13.3)	3 (12.5)

n (%)
MedDRA/J ver. 23.0

No death occurred. Serious adverse events were observed in 2 of 24 patients (8.3%) in the entire population. They were anal abscess in 1 patient receiving a single dose of Alofisel and pyrexia in 1 patient receiving 2 doses. Both events were considered to be adverse events for which a causal relationship to Alofisel could not be ruled out, and their outcomes were "recovered without sequelae." Adverse events leading to study discontinuation occurred in 2 of 24 patients (8.3%) in the entire population; both patients received a single dose of Alofisel. The events were anal abscess and anal abscess/C-reactive protein increased/abscess drainage.

6.1.2.2 Foreign phase III study (CTD 5.3.5.1-1, 5.3.5.1-2, and 5.3.5.1-3; Study Cx601-0302 [July 2012 to February 2017])

A randomized, double-blind,[1)] placebo-controlled study was conducted to investigate the efficacy and safety of Alofisel in non-Japanese patients with complex perianal fistulas associated with Crohn's disease (target sample size, 208 patients[2)]) at 47 study sites in 8 countries (Austria, Belgium, France, Germany, Italy, Spain, the Netherlands, and Israel). Table 17 shows the main inclusion/exclusion criteria.

1) Since the cell suspension containing eASCs (Alofisel) was visually distinguishable from placebo (physiological saline), the blindness of evaluation in this study was ensured by prohibiting, through the protocol, the staff in charge of preparation and administration of the study product from participating in the clinical evaluation during the study period.

2) By assuming that the combined remission rate at Week 24, the primary endpoint, to be 62.5% in the Alofisel group and 37.5% in the placebo group, the number of patients required to ensure the statistical power of 80% was calculated to be 208 (104 per group) at the two-sided significance level of 2.5%.

Table 17. Main inclusion/exclusion criteria

Inclusion criteria
- Patients aged ≥18 years with Crohn's Disease diagnosed at least 6 months before the start of screening period in accordance with clinical, endoscopic, histological and/or radiologic criteria.
- Patients who have complex perianal fistulas with ≤2 internal openings and ≤3 external openings, assessed by clinical assessment and MRI. Fistula must have been draining for at least 6 weeks prior to the screening. A complex perianal fistula is defined as a condition that meets one or more of the following criteria:
  - High (i.e., above the dentate line) inter-sphincteric, trans-sphincteric, extra-sphincteric, or supra-sphincteric fistula.
  - Presence of ≥ 2 external openings (tracts).
  - Associated abscess.
- Patients with non-active or mildly active Crohn's disease (defined by a CDAI ≤220 evaluated during the screening period).
- Patients with perianal fistulas who were refractory to at least 1 of the following: antibiotics, immunomodulators, or anti-TNF agents. Patients naïve to treatment for perianal fistulas were excluded.

Exclusion criteria
- Patients with active Crohn's disease requiring immediate therapy.
- Patients with concomitant rectovaginal fistulas.
- Patients with an abscess >2 cm, unless resolved by the conditioning of the fistula.
- Patients with rectal and/or anal stenosis and/or active proctitis, if this means a limitation for any surgical procedure.
- Patients who underwent surgery other than drainage or seton placement for the to-be-treated fistula.
- Patients with diverting stomas.
- Patients who were treated with systemic steroids in the 4 weeks prior to the study treatment.
- Patients who needs surgery in the perianal region at screening (except for the conditioning of the fistula specified in the protocol), or for whom such surgery is foreseen in the 24 weeks after the study treatment.

The study consisted of a screening period (5 weeks before the study treatment), a treatment period (day of study treatment), an observation period (24 weeks after study treatment), and an extended follow-up period (24 to up to 104 weeks after study treatment). All patients considered eligible by the screening were to undergo the conditioning of the fistula (curettage and, if necessary, seton placement) under anesthesia 21 to 14 days prior to the study treatment.

The dosage regimen or method of use of Alofisel in this study:
Seton removal and fistula curettage were performed under anesthesia, and immediately after that, a single dose of Alofisel (cell suspension containing 120×10^6 eASCs in a total volume of 24 mL) or placebo (physiological saline, total volume 24 mL) was injected into the lesions in divided portions. Half of Alofisel suspension was administered to the tissue surrounding the internal opening and the other half into the fistula wall along the fistula tract from the external opening, while forming several minute blebs. If there were 2 internal openings, 6 mL each was administered to the tissue surrounding each internal opening and, if there were 2 or 3 external openings, the cell suspension was evenly divided for each fistula tract.

All of the 212 randomized patients (107 in the Alofisel group, 105 in the placebo group) were included in the ITT population and handled as the primary efficacy analysis population. A total of 205 patients (103 patients, 102 patients) were included in the safety analysis population, and the remaining 7 patients (4 patients,[3)] 3 patients [4)]) were excluded because they discontinued the study without

3) Reason for not receiving the study product: Loss of internal information, consent withdrawal, treatment-associated adverse events in 2 patients (non-serious deep vein thrombosis, serious relapse of Crohn's disease).

4) Reason for not receiving the study product: Consent withdrawal, patient's request, inadequate randomization, failure to meet the inclusion criteria, and active luminal lesion.

receiving the study product after randomization. Study discontinuation occurred in 41 patients[5)] (19 patients, 22 patients) within 24 weeks after administration. Reasons for the discontinuation were "spontaneous discontinuation" in 4 patients (0 patients, 4 patients), "consent withdrawal" in 2 patients (1 patient, 1 patient), "failure to meet inclusion criteria" in 2 patients (0 patients, 2 patients), "adverse events/serious adverse events" in 13 patients (7 patients, 6 patients), "marked aggravation of clinical conditions" in 11 patients (7 patients, 4 patients), "protocol deviations" in 4 patients (3 patients, 1 patient), "other" in 5 patients (1 patient in the Alofisel group [loss of internal information], 4 patients in the placebo group [use of anti-TNF agents in 2 patients, difficulty in office visit in 2 patients]). Of 171 patients (88 patients, 83 patients) who had completed the 24-week observation period, 164 (84 patients, 80 patients) proceeded to the 52-week follow-up period, and the remaining 7 patients (4 patients, 3 patients) did not. A total of 33 patients (14 patients, 19 patients) discontinued the study between Week 24 and 52. Reasons for the discontinuation were "spontaneous discontinuation" in 4 patients (2 patients, 2 patients), "adverse events/serious adverse events" in 7 patients (4 patients, 3 patients), "surgical operation for diseases other than fistula" in 1 patient (0 patients, 1 patient), "marked aggravation of clinical symptoms" in 14 patients (7 patients, 7 patients), and "protocol deviations" in 7 patients (1 patient, 6 patients). Of 131 patients (70 patients, 61 patients) who had completed the 52-week follow-up period, 40 (25 patients, 15 patients) proceeded to the additional 104-week follow-up period, and the remaining 91 patients (45 patients, 46 patients) did not. Three patients (2 patients, 1 patient) discontinued the study between Week 52 and 104. Reasons for the discontinuation were "consent withdrawal" in 1 patient (Alofisel group) and "other" in 2 patients (1 patient in the Alofisel group [abscess], 1 patient in the placebo group [fistulotomy]).

Table 18 shows the results of the primary efficacy endpoint, combined remission rate at Week 24, which showed a statistically significant difference between the Alofisel group and the placebo group.

Table 18. Combined remission rate at Week 24[*1] (Study Cx601-0302, ITT population)

	Alofisel	Placebo
Combined remission rate (% [n/N])	49.5 (53/107)	34.3 (36/105)
Difference from placebo [97.5% CI]	15.2 [0.2, 30.3]	
P value[*2]	0.024	

*1 Missing values were imputed by LOCF. If there were no data after baseline or rescue treatment was given, the patient was handled as a non-remission case.

*2 Two-sided significance level of 2.5%. Cochran-Mantel-Haenszel test stratified by concomitant anti-TNF agents (yes/no) and concomitant immunomodulators (yes/no).

Table 19 shows results of the main secondary endpoints.

5) The 41 patients include 7 patients (4 in the Alofisel group, 3 in the placebo group) who were randomized but discontinued the study before receiving the study product.

Table 19. Results of main secondary endpoints[*1] (Study Cx601-0302, ITT population)

	Alofisel	Placebo
Combined remission rate at Week 52 (% [n/N])	54.2 (58/107)	37.1 (39/105)
Clinical remission rate at Week 24 (% [n/N])	53.3 (57/107)	41.0 (43/105)
Clinical remission rate at Week 52 (% [n/N])	57.0 (61/107)	40.0 (42/105)
Clinical remission rate at Week 104 (% [n/N])	56.0 (14/25)	40.0 (6/15)
Improvement rate at Week 24 (% [n/N])	66.4 (71/107)	53.3 (56/105)
Improvement rate at Week 52 (% [n/N])	63.6 (68/107)	53.3 (56/105)
Time to clinical remission at Week 52 (weeks) (median [95% CI])	6.7 (6.4, 11.9)	14.6 (11.9, 22.9)
Time to improvement at Week 52 (weeks) (median [95% CI])	6.3 (6.0, 6.6)	11.7 (6.7, 12.9)
Relapse rate up to Week 52 in patients who achieved combined remission at Week 24 (% [n/N])	25.0 (13/52)	44.1 (15/34)
Relapse rate up to Week 104 in patients who achieved combined remission at Week 52 (% [n/N])	31.6 (6/19)	33.3 (2/6)
PDAI score[*2] at Week 24 (mean ± SD)	4.37 ± 3.59	5.11 ± 3.92
PDAI score[*2] at Week 52 (mean ± SD)	4.44 ± 3.78	5.02 ± 4.03

*1 Missing values were imputed by LOCF. If there were no data after baseline or rescue treatment was given, the patient was handled as a nonresponder in calculating the rate.

*2 Evaluated in 103 patients in the Alofisel group and 99 patients in the placebo group. Data from patients who made an early termination visit after the study treatment, were adjusted for an appropriate visit based on the number of days after study treatment.

Up to Week 52, adverse events were observed in 79 of 103 patients (76.7%) in the Alofisel group and in 74 of 102 patients (72.5%) in the placebo group. Table 20 shows adverse events observed in ≥3 patients in either group. Adverse events for which a causal relationship to the study product could not be ruled out were observed in 21 of 103 patients (20.4%) in the Alofisel group and in 27 of 102 patients (26.5%) in the placebo group. Table 21 shows adverse events observed in ≥2 patients in either group and for which a causal relationship to the study product could not be ruled out.

Table 20. Adverse events observed in ≥3 patients in either group (Study Cx601-0302, Week 52, safety analysis population)

	Alofisel (N = 103)	Placebo (N = 102)
All adverse events	79 (76.7)	74 (72.5)
Gastrointestinal disorders		
Proctalgia	15 (14.6)	12 (11.8)
Anal fistula	11 (10.7)	8 (7.8)
Diarrhoea	9 (8.7)	3 (2.9)
Abdominal pain	5 (4.9)	7 (6.9)
Crohn's disease	4 (3.9)	8 (7.8)
Perianal erythema	3 (2.9)	2 (2.0)
Vomiting	3 (2.9)	2 (2.0)
Nausea	3 (2.9)	0
Haemorrhoids	3 (2.9)	0
Constipation	2 (1.9)	3 (2.9)
Anal haemorrhage	0	3 (2.9)
Anal fissure	0	3 (2.9)
General disorders and administration site conditions		
Pyrexia	6 (5.8)	5 (4.9)
Asthenia	3 (2.9)	3 (2.9)
Oedema peripheral	2 (1.9)	3 (2.9)
Infections and infestations		
Anal abscess	20 (19.4)	14 (13.7)
Nasopharyngitis	11 (10.7)	5 (4.9)
Infected fistula	4 (3.9)	4 (3.9)
Bronchitis	3 (2.9)	4 (3.9)
Urinary tract infection	3 (2.9)	3 (2.9)
Gastroenteritis	1 (1.0)	3 (2.9)
Tonsillitis	0	4 (3.9)
Influenza	0	3 (2.9)
Sinusitis	0	3 (2.9)
Investigations		
C-reactive protein increased	2 (1.9)	4 (3.9)
Musculoskeletal and connective tissue disorders		
Arthralgia	6 (5.8)	4 (3.9)
Fistula discharge	3 (2.9)	2 (2.0)
Fistula	2 (1.9)	5 (4.9)
Nervous system disorders		
Headache	1 (1.0)	4 (3.9)
Vascular disorders		
Hypertension	4 (3.9)	0

n (%)
MedDRA/J ver. 23.0

Table 21. Adverse events observed in ≥2 patients in either group and for which a causal relationship to the study product could not be ruled out
(Study Cx601-0302, Week 52, safety analysis population)

	Alofisel (N = 103)	Placebo (N = 102)
Adverse events for which a causal relationship to the study product could not be ruled out	21 (20.4)	27 (26.5)
Gastrointestinal disorders		
Proctalgia	5 (4.9)	8 (7.8)
Anal fistula	3 (2.9)	3 (2.9)
General disorders and administration site conditions		
Induration	0	2 (2.0)
Infections and infestations		
Anal abscess	8 (7.8)	9 (8.8)
Infected fistula	2 (1.9)	2 (2.0)
Injury, poisoning and procedural complications		
Procedural pain	1 (1.0)	2 (2.0)
Musculoskeletal and connective tissue disorders		
Fistula discharge	1 (1.0)	2 (2.0)

n (%)
MedDRA/J ver. 23.0

During the follow-up period from Week 52 to 104, only serious adverse events were collected. Up to Week 104, adverse events were observed in 81 of 103 patients (78.6%) in the Alofisel group and in 76 of 102 patients (74.5%) in the placebo group.

No death occurred up to Week 104. Serious adverse events were observed in 28 of 103 patients (27.2%) in the Alofisel group and in 22 of 102 patients (21.6%) in the placebo group. Serious adverse events observed in ≥2 subjects in either group were anal abscess in 23 patients (15 in the Alofisel group, 8 in the placebo group), anal fistula in 6 patients (5 in the Alofisel group, 1 in the placebo group), and Crohn's disease in 3 patients (placebo group). Serious adverse events for which a causal relationship to the study product could not be ruled out were observed in 7 of 103 patients (6.8%) in the Alofisel group and in 7 of 102 patients (6.9%) in the placebo group. They were anal abscess in 12 patients (7 in the Alofisel group, 5 in the placebo group), proctalgia in 1 patient (placebo group), anal inflammation in 1 patient (placebo group), and liver abscess in 1 patient (placebo group). The outcome of anal abscess in 7 patients in the Alofisel group for which a causal relationship to the study product could not be ruled out was "recovered" in all of them.

During the period from Week 52 to 104, serious adverse events were reported in 3 patients in the Alofisel group (anal abscess, anal fistula, and fistula discharge) and in 1 patient in the placebo group (fistula discharge). A causal relationship to the study product was ruled out for all of them.

Up to Week 104, adverse events leading to study discontinuation were observed in 9 of 103 patients (8.7%) in the Alofisel group and in 9 of 102 patients (8.8%) in the placebo group.

6.R Outline of the review conducted by PMDA

6.R.1 Clinical data package

6.R.1.1 Appropriateness of the design of the Japanese Study Darvadstrocel-3002 and of using the data from foreign clinical studies

The applicant's explanation:

Because of the extremely limited number of Japanese patients eligible for the treatment with Alofisel, Study Darvadstrocel-3002 to investigate the efficacy and safety of Alofisel in Japanese patients was conducted as an open-label, uncontrolled study in a small number of patients, considering the feasibility of the study. Although no statistical evaluation was conducted in this study, the efficacy and safety of Alofisel in Japanese patients were evaluated based on the results from this study and Study Cx601-0302, a foreign placebo-controlled, double-blind study to investigate the efficacy and safety of Alofisel.

The applicant considers that using data from the foreign clinical study is appropriate for evaluating the efficacy and safety of Alofisel in Japanese patients, for the following reasons:

- Diagnosis: The clinical practice guidelines for perianal lesions associated with Crohn's disease published in Japan (revised in January 2018) (Diagnostic Criteria and Clinical Practice Guidelines for Ulcerative Colitis and Crohn's Disease; FY2020 Partial Research Report [in Japanese], p.38-40) stipulate that local lesions should be examined under anesthesia (EUA) if necessary, in collaboration with an experienced surgeon and a proctologist, and should be examined by imaging such as endoscopy, fistula photography, computed tomography (CT), MRI, transanal ultrasonography. American Gastroenterological Association (ACG) Guidelines (*Am J Gastroenterol*. 2018;113:481-517) and European Crohn's and Colitis Organisation (ECCO) Guidelines (*J Crohn's Colitis.* 2017;11:3-25) also recommend evaluation of local lesions by EUA and imaging such as endoscopy, MRI. Thus, there is no significant difference in the diagnostic method of perianal legions between Japan and other countries.
- Treatment: The clinical practice guidelines for perianal lesions associated with Crohn's disease (revised in January 2018) recommend that seton drainage be performed in patients with anal fistula/abscess with moderate or severe symptoms (persisting pain, draining), and that drug therapy (immunomodulators, biological products), if deemed necessary, should be started after controlling the local infection focus by drainage. ECCO guidelines (*J Crohns Colitis.* 2020;14:4-22, *J Crohns Colitis*. 2020;14:155-68) do not specify treatments separately for each symptom, but strongly recommend the use of infliximab, an anti-TNF agent. The guidelines also state that surgical procedure such as EUA and seton drainage has an important role in controlling sepsis, and that there is evidence supporting the combination of drug therapy and surgical procedure. ACG Guidelines recommend seton drainage, antibiotics, immunosuppressants, anti-TNF agents, etc., for the treatment of perianal fistula while indicating no clear order of preference among them. The above facts suggest that there is no significant difference in the treatment method of anal fistula and abscess between Japan and other countries.
- Intrinsic and extrinsic factors: Table 22 shows the patient characteristics in Studies Darvadstrocel-3002 and Cx601-0302. The most frequent combination of the number of internal and external openings was "1 internal opening and ≥2 external openings" in Study Darvadstrocel-3002,

and "1 internal opening and 1 external opening" in Study Cx601-0302. No other significant difference was observed in the patient characteristics between these studies.

Table 22. Baseline patient characteristics (Study Darvadstrocel-3002, Study Cx601-0302, ITT population)

Patient characteristics		Study Darvadstrocel-3002	Study Cx601-0302	
		Alofisel (N = 22)	Alofisel (N = 107)	Placebo (N = 105)
Age	≤65	22/22 (100)	104/107 (97.2)	101/105 (96.2)
	>65	0	3/107 (2.8)	4/105 (3.8)
	Mean ± SD	36.4 ± 10.36	39.0 ± 13.11	37.6 ± 13.12
Sex	Male	14/22 (63.6)	60/107 (56.1)	56/105 (53.3)
	Female	8/22 (36.4)	47/107 (43.9)	49/105 (46.7)
Weight (kg)	Mean ± SD	68.33 ± 23.10	73.93 ± 15.01	71.33 ± 14.92
Duration of Crohn's disease (years)	Mean ± SD	11.3 ± 6.64	12.1 ± 10.0 (N = 106)	11.3 ± 8.9
PDAI score	Mean ± SD	4.8 ± 2.15	6.77 ± 2.48	6.55 ± 2.92
Number of internal and external openings	No internal opening, 1 external opening	0	0	1/105 (1.0)
	1 internal opening, 1 external opening	3/22 (13.6)	55/107 (51.4)	70/105 (66.7)
	1 internal opening, ≥2 external openings	13/22 (59.1)	27/107 (25.2)	20/105 (19.0)
	2 internal openings, 1 external openings	0	3/107 (2.8)	2/105 (1.9)
	2 internal openings, ≥2 external openings	6/22 (27.3)	18/107 (16.8)	9/105 (8.6)
Concomitant drugs	Biological product only	9/22 (40.9)	37/107 (34.6)	33/105 (31.4)
	Immunomodulator only	2/22 (9.1)	16/107 (15.0)	22/105 (21.0)
	Biological product and immunomodulator	7/22 (31.8)	28/107 (26.2)	31/105 (29.5)
	Neither	4/22 (18.2)	26/107 (24.3)	19/105 (18.1)

n (%)

Since Alofisel is administered directly into the lesion to promote the cure of the tissue surrounding the fistula, intrinsic ethnic factors are unlikely to affect the treatment. Also, there are no significant difference in the diagnosis and treatment of complex perianal fistulas associated with Crohn's disease between Japan and other countries, suggesting that extrinsic ethnic factors are unlikely to affect the treatment. Moreover, there was no significant difference in patient characteristics between the Japanese clinical study and the foreign clinical study, and the results of the efficacy and safety of Alofisel were similar in these studies [see Sections "6.R.2.2 Efficacy of Alofisel in Japanese patients" and "6.R.3.1 Safety profile of Alofisel and its difference between Japanese and non-Japanese patients]. Based on the above, the applicant considered that data from foreign clinical studies can be used for evaluating the safety and efficacy in Japanese patients.

PMDA's view:

The applicant's explanation about the following is understandable and acceptable:

(1) Study Darvadstrocel-3002 was conducted as an open-label, uncontrolled study.

(2) Data from foreign clinical studies of Alofisel were used in the evaluation of the efficacy and safety in Japanese patients.

6.R.2 Efficacy

6.R.2.1 Efficacy endpoints and results of evaluation

The applicant's explanation:

In Studies Darvadstrocel-3002 and Cx601-0302, the primary endpoint was the combined remission rate at Week 24. In general, closed fistulas (defined as no draining despite gentle finger compression of

external openings) have been used in clinical studies as an index for assessing the treatment effect (*N Engl J Med*. 1999;340:1398-405), and are also commonly used in clinical practice to assess patient condition (*J Crohns Colitis*. 2010;4:63-101). On the other hand, absence of drainage from external openings does not necessarily reflect the complete closure of fistula tracts, and persisting inflammation in fistula tracts, if any, may cause perianal fistulas or lead to relapse of fistulas (*Clin Imaging*. 2011;35:360-5). Based on the above, "no draining despite gentle finger compression of external openings and absence of abscess >2 cm confirmed by MRI" was defined as the combined remission, and the combined remission rate was used as the primary endpoint.

In Study Cx601-0302, the expected level of combined remission rate, the primary endpoint, was assumed to be 13% to 19% in the placebo group and 53.3% to 56.3% in the Alofisel group, with the between-group difference of 25%, based on the results of Study Cx601-0101 and publications on the treatment of perianal fistulas associated with Crohn's disease. The results showed that the combined remission rate at Week 24 was 49.5% (53 of 107 patients) in the Alofisel group and 34.3% (36 of 105 patients) in the placebo group, with the between-group difference [97.5% confidence interval (CI)] of 15.2% [0.2, 30.3]; the rate was statistically significantly higher in the Alofisel group than in the placebo group (P = 0.024). The combined remission rate in the Alofisel group was not significantly different from the expected level, but the between-group difference was smaller than expected. This was due to the higher-than-expected combined remission rate in the placebo group, presumably because of conditioning of the fistula before study treatment, such as (1) curettage given to both groups and (2) seton placement and suture of the internal opening performed as needed.

As for the clinical significance of the between-group difference in the combined remission rate observed in Study Cx601-0302, there is no generalized cut-off level for clinical significance specific to complex perianal fistulas associated with Crohn's disease. On the other hand, according to the International Organization for the Study of Inflammatory Bowel Diseases (IOIBD), a 15% difference between the active drug and placebo groups is considered to be clinically significant as the endpoint of clinical remission in clinical studies on inflammatory bowel diseases (IBD) (*Aliment Pharmacol Ther.* 2018;47:773-83). Taking account of the unmet needs for the treatment of complex perianal fistulas associated with Crohn's disease, the efficacy of Alofisel demonstrated by Study Cx601-0302 in patients with Crohn's disease who have an inadequate response to conventional treatments, is considered to be clinically significant.

Moreover, the combined remission rate at Week 52 was 54.2% (58 of 107 patients) in the Alofisel group and 37.1% (39 of 105 patients) in the placebo group with the between-group difference [95% CI] of 17.1% [3.9, 30.3], demonstrating the efficacy of Alofisel at Week 52 as well.

Tables 23 and 24 show the efficacy (combined remission rate at Week 24, clinical remission rate, and improvement rate) of Alofisel by patient characteristics (age, the number of the internal openings, the number of external openings, and types of concomitant drugs) in Study Darvadstrocel-3002 and Study Cx601-0302.

In Study Darvadstrocel-3002, the combined remission rate and the clinical remission rate tended to be lower in the following strata: Male, 2 internal openings, 3 external openings, and combination of 2 internal openings and ≥1 external opening, whereas the rates tended to be higher in patients who were using both a biological product and an immunomodulator at a randomized clinical visit. There are limitations to the interpretation of study results because of the limited number of patients and the varying numbers of patients in each strata, but the results showed that a certain percentage of patients achieved combined remission, clinical remission, or improvement in every stratum. This suggests patient characteristics have no clear effect on the efficacy. The effect of age on efficacy could not be evaluated because all patients were ≤65 years.

In Study Cx601-0302, the percentage of patients who achieved remission or improvement was higher in the Alofisel group than in the placebo group in all of the following strata: sex, number of internal openings and external openings, and concomitant drugs at a randomized clinical visit. The between-group difference of efficacy varied from stratum to stratum, presumably due to the small number of patients in each stratum. The effect of age on efficacy could not be evaluated because there were only 9 patients aged >65 years.

The above results suggest that patient characteristics have no clear effect on efficacy.

Table 23. Efficacy results by patient characteristics (Study Darvadstrocel-3002, Week 24, ITT population)

Patient characteristics		N	Combined remission rate*	Clinical remission rate*	Improvement rate*
Sex	Male	14	42.9 (6)	42.9 (6)	78.6 (11)
	Female	8	87.5 (7)	87.5 (7)	87.5 (7)
Number of internal and external openings	1 internal opening, 1 external opening	3	66.7 (2)	66.7 (2)	66.7 (2)
	1 internal opening, ≥2 external openings	13	69.2 (9)	69.2 (9)	92.3 (12)
	2 internal openings, ≥1 external opening	6	33.3 (2)	33.3 (2)	66.7 (4)
Concomitant drugs	Biological product only	9	44.4 (4)	44.4 (4)	66.7 (6)
	Immunomodulator only	2	50.0 (1)	50.0 (1)	100 (2)
	Biological product and immunomodulator	7	85.7 (6)	85.7 (6)	100 (7)
	Neither	4	50.0 (2)	50.0 (2)	75.0 (3)

* % (n)
Missing values were imputed by LOCF. If there were no data after baseline or rescue treatment was given, the patient was handled as a nonresponder in calculating the rate.

Table 24. Efficacy results by patient characteristics (Study Cx601-0302, Week 24, ITT population)

Patient characteristics		N		Combined remission rate		Clinical remission rate		Improvement rate	
		Alofisel	Placebo	Alofisel	Placebo	Alofisel	Placebo	Alofisel	Placebo
Sex	Male	60	56	55.0 (33)	30.4 (17)	58.3 (35)	37.5 (21)	68.3 (41)	51.8 (29)
	Female	47	49	42.6 (20)	38.8 (19)	46.8 (22)	44.9 (22)	63.8 (30)	55.1 (27)
Number of internal and external openings	1 internal opening[a)] and 1 external opening	55	71	49.1 (27)	38.0 (27)	54.5 (30)	47.9 (34)	54.5 (30)	49.3 (35)
	1 internal opening and ≥2 external openings	27	20	55.6 (15)	30.0 (6)	59.3 (16)	30.0 (6)	92.6 (25)	60.0 (12)
	2 internal openings and ≥1 external opening	21	11	52.4 (11)	27.3 (3)	52.4 (11)	27.3 (3)	76.2 (16)	81.8 (9)
Concomitant drugs	Biological product only	37	33	45.9 (17)	36.4 (12)	48.6 (18)	39.4 (13)	64.9 (24)	51.5 (17)
	Immunomodulator only	16	22	31.3 (5)	27.3 (6)	37.5 (6)	31.8 (7)	50.0 (8)	50.0 (11)
	Biological product and immunomodulator	28	31	64.3 (18)	45.2 (14)	67.9 (19)	58.1 (18)	75.0 (21)	64.5 (20)
	Neither	26	19	50.0 (13)	21.1 (4)	53.8 (14)	26.3 (5)	69.2 (18)	42.1 (8)

% (n)
a) The placebo group includes 1 patient in whom the internal opening was not identified at baseline.
Missing values were imputed by LOCF. If there were no data after baseline or rescue treatment was given, the patient was handled as a nonresponder in calculating the rate.

PMDA's view:
Although the applicant's explanation for using the combined remission rate at Week 24 as the primary endpoint is understandable, it is more appropriate to evaluate efficacy comprehensively by including other secondary endpoints in the evaluation.

In Study Cx601-0302, a statistically significant difference was observed in the combined remission rate at Week 24, the primary endpoint, between the Alofisel group and the placebo group. Although the between group difference [97.5% CI] of 15.2% [0.2, 30.3] was lower than the pre-specified expected between-group difference of 25%, the applicant's explanation that Alofisel has a clinical significance in the disease with limited therapeutic options is understandable. The combined remission rate at Week 52, a secondary endpoint, tended to be higher in the Alofisel group than in the placebo group. Also, the relapse rate tended to be lower in patients who achieved combined remission. These results show a certain level of efficacy of Alofisel.

6.R.2.2 Efficacy of Alofisel in Japanese patients

The applicant's explanation about the efficacy of Alofisel in Japanese patients:
The results of the combined remission rate at Week 24 in the Alofisel group in Study Darvadstrocel-3002 were similar to those in Study Cx601-0302. Also, there was no significant difference between the studies in the secondary endpoints: the clinical remission rate and improvement rate at Week 24, the combined remission rate, clinical remission rate, and improvement rate at Week 52, and other endpoints. This supports the similarity of the efficacy results between the studies.

PMDA's view:
The applicant's explanation (i.e., the results of the combined remission rate at Week 24, the primary endpoint and the secondary endpoints in Study Darvadstrocel-3002 in Japanese patients, were similar to those in Study Cx601-0302) is understandable. Accordingly, these results have demonstrated a certain efficacy of Alofisel in Japanese patients with complex perianal fistulas associated with Crohn's disease.

6.R.3 Safety

6.R.3.1 Safety profile of Alofisel and its difference between Japanese and non-Japanese patients

The applicant's explanation about the safety of Alofisel:

Tables 25 and 26 show the summary of safety in Study Darvadstrocel-3002 and Study Cx601-0302, respectively.

Table 25. Summary of safety (Study Darvadstrocel-3002: safety analysis population)

	Up to Week 24	Up to Week 52
	Alofisel (N = 22)	Alofisel (N = 22)
All adverse events	18 (81.8)	20 (90.9)
Adverse events for which a causal relationship to Alofisel could not be ruled out	1 (4.5)	2 (9.1)
Severe adverse events	2 (9.1)	2 (9.1)
Serious adverse events	3 (13.6)	4 (18.2)
Adverse events leading to death	0	0
Adverse events leading to study discontinuation	0	0

n (%)

Table 26. Summary of safety (Study Cx601-0302, safety analysis population)

	Up to Week 24		Up to Week 52		Up to Week 104*	
	Alofisel (N = 103)	Placebo (N = 102)	Alofisel (N = 103)	Placebo (N = 102)	Alofisel (N = 103)	Placebo (N = 102)
All adverse events	68 (66.0)	66 (64.7)	79 (76.7)	74 (72.5)	81 (78.6)	76 (74.5)
Adverse events for which a causal relationship to the study product could not be ruled out	18 (17.5)	30 (29.4)	21 (20.4)	27 (26.5)	20 (19.4)	27 (26.5)
Severe adverse events	10 (9.7)	10 (9.8)	10 (9.7)	12 (11.8)	10 (9.7)	13 (12.7)
Serious adverse events	18 (17.5)	14 (13.7)	25 (24.3)	21 (20.6)	28 (27.2)	22 (21.6)
Adverse events leading to death	0	0	0	0	0	0
Adverse events leading to study discontinuation	5 (4.9)	6 (5.9)	9 (8.7)	9 (8.8)	9 (8.7)	9 (8.8)

n (%)

* Only serious adverse events were collected from Week 52 to 104.

Common adverse events occurring after administration of Alofisel in Study Darvadstrocel-3002:

Adverse events with an incidence of ≥10% up to Week 24 were proctalgia in 6 patients (27.3%), nasopharyngitis in 4 patients (18.2%), and anal fistula in 3 patients (13.6%). Adverse events with an incidence of ≥10% up to Week 52 were proctalgia in 6 patients (27.3%), nasopharyngitis in 5 patients (22.7%), and anal fistula in 4 patients (18.2%).

In Study Cx601-0302, adverse events with an incidence of ≥10% up to Week 24 in the Alofisel group were proctalgia in 13 patients (12.6%) and anal abscess in 12 patients (11.7%), and these incidences were similar to those in the placebo group. In the Alofisel group, adverse events with an incidence of ≥10% up to Week 52 were anal abscess in 20 patients (19.4%), proctalgia in 15 patients (14.6%), nasopharyngitis in 11 patients (10.7%), and anal fistula in 11 patients (10.7%); all of these incidences exceeded those in the placebo group. As a whole, however, there was no significant difference between the Alofisel and placebo groups.

The applicant's explanation about the difference in the safety of Alofisel between Japanese and non-Japanese patients:
Comparison of the incidence of adverse events in patients receiving Alofisel between Study Darvadstrocel-3002 in Japanese patients and Study Cx601-0302 in non-Japanese patients up to Week 24 and 52, showed no significant difference in the trend of adverse events between the studies, except for the absence of anal abscess in Study Darvadstrocel-3002.

The applicant's explanation about the safety of Alofisel by patient characteristics:
Tables 27 and 28 show the summary of safety by patient characteristics in Study Darvadstrocel-3002 and Study Cx601-0302, respectively. There are limitations to the interpretation of some of the results because of the limited number of stratified patients in both studies. Nevertheless, no clear difference was observed in the incidence of adverse events between the strata compared.

Table 27. Summary of safety by patient characteristics
(Study Darvadstrocel-3002, safety analysis population, Week 52)

	Sex		Number of external openings	
	Male	Female	1	≥2
	Alofisel (N = 14)	Alofisel (N = 8)	Alofisel (N = 3)	Alofisel (N = 19)
All adverse events	12 (85.7)	8 (100)	3 (100)	17 (89.5)
Adverse events for which a causal relationship to Alofisel could not be ruled out	2 (14.3)	0	0	2 (10.5)
Severe adverse events	2 (14.3)	0	0	2 (10.5)
Serious adverse events	4 (28.6)	0	1 (33.3)	3 (15.8)
Adverse events leading to study discontinuation	0	0	0	0

	Concomitant drugs			
	Anti-TNF agent only	Immunomodulator only	Both	Neither
	Alofisel (N =9)	Alofisel (N = 2)	Alofisel (N = 7)	Alofisel (N = 4)
All adverse events	7 (77.8)	2 (100)	7 (100)	4 (100)
Adverse events for which a causal relationship to Alofisel could not be ruled out	1 (11.1)	0	1 (14.3)	0
Severe adverse events	1 (11.1)	1 (50.0)	0	0
Serious adverse events	3 (33.3)	1 (50.0)	0	0
Adverse events leading to study discontinuation	0	0	0	0

n (%)

Table 28. Summary of safety by patient characteristics
(Study Cx601-0302, safety analysis population, Week 52)

	Male		Female	
	Alofisel (N = 57)	Placebo (N = 54)	Alofisel (N = 46)	Placebo (N = 48)
All adverse events	40 (70.2)	36 (66.7)	39 (84.8)	38 (79.2)
Adverse events for which a causal relationship to the study product could not be ruled out	11 (19.3)	12 (22.2)	10 (21.7)	15 (31.3)
Severe adverse events	5 (8.8)	7 (13.0)	5 (10.9)	5 (10.4)
Serious adverse events	12 (21.1)	10 (18.5)	13 (28.3)	11 (22.9)
Adverse events leading to study discontinuation	4 (7.0)	2 (3.7)	5 (10.9)	7 (14.6)
	Age			
	<65 years		≥65 years	
	Alofisel (N = 98)	Placebo (N = 98)	Alofisel (N = 5)	Placebo (N = 4)
All adverse events	75 (76.5)	70 (71.4)	4 (80.0)	4 (100)
Adverse events for which a causal relationship to the study product could not be ruled out	21 (21.4)	27 (27.6)	0	0
Severe adverse events	9 (9.2)	12 (12.2)	1 (20.0)	0
Serious adverse events	22 (22.4)	20 (20.4)	3 (60.0)	1 (25.0)
Adverse events leading to study discontinuation	9 (9.2)	9 (9.2)	0	0
	Number of external openings			
	1 external opening		≥2 external openings	
	Alofisel (N = 58)	Placebo (N = 73)	Alofisel (N = 45)	Placebo (N = 29)
All adverse events	49 (84.5)	53 (72.6)	30 (66.7)	21 (72.4)
Adverse events for which a causal relationship to the study product could not be ruled out	15 (25.9)	19 (26.0)	6 (13.3)	8 (27.6)
Severe adverse events	8 (13.8)	7 (9.6)	2 (4.4)	5 (17.2)
Serious adverse events	17 (29.3)	14 (19.2)	8 (17.8)	7 (24.1)
Adverse events leading to study discontinuation	6 (10.3)	7 (9.6)	3 (6.7)	2 (6.9)
	Concomitant drugs			
	Anti-TNF agent only		Immunomodulator only	
	Alofisel (N = 38)	Placebo (N = 32)	Alofisel (N = 16)	Placebo (N = 21)
All adverse events	33 (91.7)	26 (81.3)	10 (62.5)	14 (66.7)
Adverse events for which a causal relationship to the study product could not be ruled out	9 (25.0)	13 (40.6)	4 (25.0)	5 (23.8)
Severe adverse events	5 (13.9)	4 (12.5)	1 (6.3)	3 (14.3)
Serious adverse events	12 (33.3)	11 (34.4)	3 (18.8)	2 (9.5)
Adverse events leading to study discontinuation	5 (13.9)	4 (12.5)	1 (6.3)	2 (9.5)
	Both		Neither	
	Alofisel (N = 27)	Placebo (N = 30)	Alofisel (N = 24)	Placebo (N = 19)
All adverse events	20 (74.1)	28 (93.3)	16 (66.7)	6 (31.6)
Adverse events for which a causal relationship to the study product could not be ruled out	3 (11.1)	7 (23.3)	5 (20.8)	2 (10.5)
Severe adverse events	2 (7.4)	5 (16.7)	2 (8.3)	0
Serious adverse events	7 (25.9)	8 (26.7)	3 (12.5)	0
Adverse events leading to study discontinuation	1 (3.7)	3 (10.0)	2 (8.3)	0

n (%)

PMDA's view:

In Studies Darvadstrocel-3002 and Cx601-0302, most of the adverse events were mild or moderate, and adverse events of concern specific to Alofisel did not occur. Also, the incidence of adverse events did not differ significantly between the Alofisel group and the placebo group in Study Cx601-0302. These findings show that there are no particular concerns about the safety profile of Alofisel. Because of the extremely limited number of Japanese patients enrolled in clinical studies, safety information should be collected continuously via post-marketing surveillance.

6.R.3.2 Individual events related to the safety profile of Alofisel

In the following sections, PMDA reviewed adverse events focusing on events suspected from the mechanism of action of Alofisel.

6.R.3.2.1 Hypersensitivity and alloimmune response

The applicant's explanation:

In Studies Darvadstrocel-3002 and Cx601-0302, the following adverse events were investigated: (1) Hypersensitivity-related adverse events (adverse events under "Hypersensitivity (narrow)" in Standardized MedDRA Query and adverse events coded to preferred terms (PTs) "Scratch," "Swelling," "Oedema peripheral," "Pruritus," "Acute respiratory failure," "Bronchial obstruction," "Chills," "Flushing," "Hypotension," "Stridor," or "Throat tightness"), and (2) alloimmune response-related adverse events (adverse events under "Hypersensitivity (narrow)" in Standardized MedDRA Query and adverse events coded to PTs "Nasopharyngitis," "Influenza like illness," "Oedema peripheral," "Pyrexia," "Hyperthermia," "Swelling," and "Autoimmune disorder").

In Study Darvadstrocel-3002, hypersensitivity-related adverse events were observed in 2 patients (drug eruption, pruritus) up to Week 52. Both events were mild and their causal relationship to the study product was ruled out. In Study Cx601-0302, hypersensitivity-related adverse events were observed in 10 patients (9.7%) in the Alofisel group and in 9 patients (8.8%) in the placebo group up to Week 104, showing a similar incidence in both groups. Events observed in ≥2 patients in the Alofisel group were oedema peripheral (2 patients [1.9%] in the Alofisel group, 3 patients [2.9%] in the placebo group) and eczema (2 patients [1.9%] in the Alofisel group, 2 patients [2.0%] in the placebo group). All events were mild or moderate, and their causal relationship to the study product was ruled out, except for eczema in 1 patient in the placebo group. There were no serious adverse events or adverse events leading to study discontinuation.

In Study Darvadstrocel-3002, alloimmune response-related adverse events were observed in 7 patients (nasopharyngitis in 5 patients [22.7%], drug eruption in 1 patient [4.5%], and pyrexia in 1 patient [4.5%]) up to Week 52. All of them were non-serious events of mild or moderate severity, and their causal relationship to the study product was ruled out. None of these adverse events led to study discontinuation, and the outcome of all events was "recovered." In Study Cx601-0302, alloimmune response-related adverse events were observed in 24 patients (23.3%) in the Alofisel group and in 15 patients (14.7%) in the placebo group up to Week 104. Alloimmune response-related adverse events with a higher incidence in the Alofisel group than in the placebo group were nasopharyngitis (11 patients [10.7%] in the Alofisel group, 5 patients [4.9%] in the placebo group) and pyrexia (7 patients [6.8%] in the Alofisel group, 5 patients [4.9%] in the placebo group). Most of these events were mild or moderate, and their causal relationship to the study product was ruled out, except for nasopharyngitis, pyrexia, eczema, and hyperthermia in 1 patient each in the placebo group. Serious adverse events were influenza like illness and pyrexia in 1 patient each in the Alofisel group. Their causal relationship to the study product was ruled out, with the outcome of "recovered without sequelae." There were no adverse events leading to study discontinuation.

The relationship between alloimmune response-related adverse events and donor-specific antibodies (DSA) production was investigated in Studies Darvadstrocel-3002 and Cx601-0302. No clear relationship was observed between the events and DSA production.

In Study Cx601-0101, DSA became positive in 10 of 18 patients (56%) with immunogenicity data. The study product had been administered once to 1 patient and twice to the remaining 9 patients. Of the DSA-positive patients, 1 patient receiving the study product twice had pyrexia after the second dose of study product. The pyrexia was moderate but serious, and was assessed to be possibly related to the study product. The outcome was "recovered without sequelae." As a whole, no clear relationship was observed between DSA production and adverse events, regardless of the number of doses of study product.

No hypersensitivity was reported in the foreign periodical benefit/risk evaluation report (data cut-off of September 22, 2020).

In the ongoing foreign phase III double-blind, placebo-controlled study (Study Cx601-0303), anaphylactic shock was reported in 1 patient. This event occurred 25 minutes after administration of the study product, necessitating intensive care such as mechanical ventilation, but the outcome was "recovered." The patient had a history of allergic reaction to dexketoprofen, suggesting that the adverse event was mainly due to dexketoprofen administered intravenously after administration of the study product, although the causal relationship to the study product cannot be ruled out currently.

Thus, results of the clinical studies and the post-marketing report did not reveal any clear relationship between the administration of Alofisel and the occurrence of hypersensitivity or alloimmune response. Subcutaneous adipose tissue of healthy human adults is used as the source material of Alofisel, and FBS, penicillin, and streptomycin, or gentamicin are used in the manufacturing process. Also, HSA is used as an excipient, and porcine intestinal mucosa-derived heparin is used in the manufacturing process of human serum albumin. Thus, hypersensitivity to the components of Alofisel (including source material, etc.) may possibly occur. Accordingly, the package insert will contain a precautionary statement that Alofisel should be used with care in patients with a history of drug hypersensitivity or with allergic predisposition. Incidences of hypersensitivity or alloimmune response will be monitored continuously after the market launch.

PMDA's view:
PMDA accepted the applicant's explanation that there is no clear relationship between administration of Alofisel and occurrence of hypersensitivity or alloimmune response based on the results of the clinical studies and post-marketing report on Alofisel. Because of the limited use experience of Alofisel, incidences of hypersensitivity should be monitored after the market launch.

6.R.3.2.2 Immunogenicity

The applicant's explanation about the immunogenicity of Alofisel:
In Study Darvadstrocel-3002, DSA production was evaluated in all of the 22 patients receiving Alofisel. Patients were screened for anti-human leukocyte antigen (HLA) antibody at baseline. They

were re-tested for anti-HLA antibody at Week 2, 4, 8, 16, 24, and 52, or at the early termination, and DSA was measured in patients positive for anti-HLA antibody.

At baseline, 16 of 22 patients (72.7%) were positive for anti-HLA antibody (pre-sensitized patients). Of the 16 pre-sensitized patients, 9 (56.3%) remained DSA-positive until any point up to Week 24, and 3 of the 9 patients remained DSA-positive even at Week 52. A total of 6 of 22 patients (27.3%) were negative for anti-HLA antibody at baseline (unsensitized patients). Of the 6 unsensitized patients, 3 (50.0%) turned DSA-positive at some point up to Week 24, and 1 of the 3 patients remained DSA-positive even at Week 52. Regardless of anti-HLA antibody status at baseline, all of the 10 patients who remained DSA-negative at all points up to Week 24 were DSA-negative at Week 52 as well.

In Study Cx601-0302, DSA production was evaluated in 63 patients in the Alofisel group and in 60 patients in the placebo group. Patients were screened for anti-HLA antibody at baseline. They were re-tested for anti-HLA antibody at Week 12, 52, or at the early termination, and DSA was measured in patients positive for anti-HLA antibody.

Anti-HLA antibody at baseline was detected (pre-sensitized) in 10 of 63 patients (15.9%) in the Alofisel group and in 9 of 60 patients (15.0%) in the placebo group. Of the 10 pre-sensitized patients in the Alofisel group, DSA was positive at Week 12 in 6 patients (60.0%) and, at Week 52, 4 of them remained DSA positive, and 1 turned negative. The test sample was unavailable from the remaining patient, precluding DSA evaluation at Week 52. None of the 9 pre-sensitized patients in the placebo group turned DSA-positive after the administration. Of the 53 patients in the Alofisel group who were negative for anti-HLA antibody at baseline (unsensitized patients), 17 (32.1%) were DSA-positive at Week 12 and, at Week 52, 9 of them were DSA-positive and 6 patients were negative. Of 36 patients negative for DSA at Week 12, none turned DSA-positive at Week 52. DSA status at Week 52 could not be evaluated in 2 DSA-positive patients and 2 DSA-negative patients at Week 12, because test samples could not be collected.

In order to investigate the possible effect of DSA production on the safety of Alofisel, the relationship between DSA production and the incidence of adverse events (including alloimmune response-related adverse events) was evaluated in DSA-evaluable patients in Studies Darvadstrocel-3002 and Cx601-0302 (Tables 29 and 30).

In Study Darvadstrocel-3002, the incidences of adverse events, serious adverse events, and alloimmune response-related adverse events up to Week 52 did not differ significantly between patients with and without DSA production up to Week 24 or at Week 52.

In unsensitized patients receiving Alofisel in Study Cx601-0302, the incidences of adverse events, serious adverse events, and alloimmune response-related adverse events up to Week 52 did not differ significantly between patients with and without DSA production up to Week 12 or at Week 52. The effect of DSA production on the safety could not be evaluated because of the small number of pre-sensitized patients in the Alofisel group.

Table 29. Incidence of adverse events in patients with and without DSA production (Study Darvadstrocel-3002, Week 52)

	Baseline anti-HLA antibody-negative (N = 6)		Baseline anti-HLA antibody-positive (N = 16)	
	DSA-positive up to Week 24 or at Week 52 (N = 3)	DSA-negative up to Week 24 and at Week 52 (N = 3)	DSA-positive up to Week 24 or at Week 52 (N = 9)	DSA-negative up to Week 24 and at Week 52 (N = 7)
All adverse events	3 (100)	3 (100)	7 (77.8)	7 (100)
Serious adverse events	0	1 (33.3)	3 (33.3)	0
Alloimmune response-related adverse events	1 (33.3)	0	4 (44.4)	2 (28.6)

n (%)

Table 30. Incidence of adverse events in patients with and without DSA production (Alofisel group in Study Cx601-0302, Week 52)

	Baseline anti-HLA antibody-negative (N = 53)		Baseline anti-HLA antibody-positive (N = 10)	
	DSA-positive at Week 12 or 52 (N = 17)	DSA-negative at Weeks 12 and 52 (N = 36)	DSA-positive at Week 12 or 52 (N = 6)	DSA-negative at Weeks 12 and 52 (N = 4)
All adverse events	15 (88.2)	29 (80.6)	5 (83.3)	4 (100)
Serious adverse events	5 (29.4)	6 (16.7)	2 (33.3)	2 (50.0)
Alloimmune response-related adverse events	5 (29.4)	9 (25.0)	0	2 (50.0)

n (%)

In Study Cx601-0101, anti-HLA antibody production at baseline and DSA production after Alofisel administration were evaluated in 18 of 24 patients. DSA was positive in 10 of 18 patients at either of the time points. One of the 10 patients had received a single dose of Alofisel whereas the remaining 9 patients had received 2 doses (thus, most of them had received 2 doses of Alofisel). Only 1 DSA-positive patient had an alloimmune response-related adverse event (pyrexia) for which a causal relationship to Alofisel could not be ruled out.

The possible effect of DSA production on the efficacy of Alofisel was evaluated as shown below.

Table 31 shows the results of efficacy (combined remission rate and clinical remission rate) in patients with and without anti-HLA antibodies (pre-sensitized or unsensitized) at baseline, classified by DSA production status up to Weeks 24 and 52. Combined remission and clinical remission were observed regardless of pre-sensitization or DSA production, although there are limitations to the interpretation of the results because of the limited number of patients analyzed. DSA production up to Week 24 did not have any clear effect on either the combined remission rate or the clinical remission rate at Week 24, in either pre-sensitized patients or unsensitized patients. Also, DSA production up to Week 52 did not have any clear effect either on the combined remission rate or on the clinical remission rate at Week 52, in either pre-sensitized patients or unsensitized patients.

Table 31. Efficacy by DSA production (Study Darvadstrocel-3002)

	Baseline anti-HLA antibody negative (N = 6)		Baseline anti-HLA antibody positive (N = 16)	
Week 24	DSA positive up to Week 24 (N = 3)	DSA negative up to Week 24 (N = 3)	DSA positive up to Week 24 (N = 9)	DSA negative up to Week 24 (N = 7)
Combined remission rate	33.3 (1)	66.7 (2)	55.6 (5)	71.4 (5)
Clinical remission rate	33.3 (1)	66.7 (2)	55.6 (5)	71.4 (5)
Week 52	DSA positive up to Week 52 (N = 3)	DSA negative up to Week 52 (N = 3)	DSA positive up to Week 52 (N = 9)	DSA negative up to Week 52 (N = 7)
Combined remission rate	33.3 (1)	66.7 (2)	77.8 (7)	71.4 (5)
Clinical remission rate	33.3 (1)	66.7 (2)	77.8 (7)	85.7 (6)

% (n)

Table 32 shows the results of efficacy (combined remission rate and clinical remission rate) at Week 24 and Week 52, classified by DSA production status at Week 12 and Week 52 in Study Cx601-0302. In unsensitized patients in the Alofisel group, DSA production status at Week 12 or 52 did not have any clear effect on the efficacy at Week 24 or 52. The effect of DSA production on efficacy in unsensitized patients in the Alofisel group could not be evaluated because of the limited number of patients.

Table 32. Efficacy in Alofisel group by DSA antibody production status (Study Cx601-0302)

		Baseline anti-HLA antibody negative (N = 49)			Baseline anti-HLA antibody positive (N = 9)		
		DSA positive only at Week 12 (N = 6)	DSA positive at Weeks 12 and 52 (N = 9)	DSA negative at Weeks 12 and 52 (N = 34)	DSA positive only at Week 12 (N = 1)	DSA positive at Weeks 12 and 52 (N = 4)	DSA negative at Weeks 12 and 52 (N = 4)
Week 24	Combined remission rate	83.3 (5)	66.7 (6)	55.9 (19)	0	25.0 (1)	50.0 (2)
	Clinical remission rate	83.3 (5)	66.7 (6)	61.8 (21)	0	25.0 (1)	50.0 (2)
Week 52	Combined remission rate	66.7 (4)	66.7 (6)	70.6 (24)	0	25.0 (1)	25.0 (1)
	Clinical remission rate	50.0 (3)	55.6 (5)	50.0 (17)	0	25.0 (1)	25.0 (1)

% (n)

In Study Cx601-0101, closure of the external openings was observed at Week 12 in 3 DSA-positive patients and in 2 DSA-negative patients out of 18 patients evaluated.

Thus, DSA production had no clear effect on the safety or efficacy in the clinical studies. However, since the possibility of such an effect cannot be completely ruled out, data will be collected continuously after the market launch.

PMDA accepted the applicant's explanation that although DSA production was observed after Alofisel administration, the production had no clear effect on the safety or efficacy of Alofisel.

6.R.3.2.3 Malignant transformation of anal fistula, tumorigenicity, and ectopic tissue formation

The applicant's explanation:

The risk of malignant transformation of anal fistula, tumorigenicity, and ectopic tissue formation at the site of Alofisel administration was investigated by analyzing the following events observed in clinical studies (Study Darvadstrocel-3002, Alofisel group in Study Cx601-0302, and ongoing Study Cx601-0303 [double-blind study]) and in the foreign post-marketing report (data cut-off of September 22, 2020).

- Risk of malignant transformation of anal fistula: Because there is no clear definition on events related to malignant transformation of anal fistula, they were extracted from a wider range of events (Medical Dictionary for Regulatory Activities Japanese version [MedDRA/J] [Ver. 23.1] SOC "Neoplasms benign, malignant and unspecified [incl cysts and polyps]," HLGT "Benign neoplasms gastrointestinal," or SMQ "Malignancies [broad]").
- Risk of tumorigenicity and ectopic tissue formation: Events related to tumorigenicity and ectopic tissue formation were extracted from those included in the Standardized MedDRA Query "Malignancies (broad)."

As a result, only 1 case (pseudopolyp) was extracted from the foreign post-marketing report. This event was reported as "rectal polyp" at the data cut-off. The event occurred approximately 1 year and 10 months after Alofisel administration. The effect of Crohn's disease, the primary disease, was suspected, and a causal relationship to Alofisel was ruled out by the reporting physician. Acrochordons observed in 2 patients in Study Darvadstrocel-3002 were non-serious, and their sites of occurrence were unknown; although they might have occurred at the site of Alofisel administration, the investigator ruled out the causal relationship between the events and Alofisel.

Thus, the clinical study data and the foreign post-marketing report of Alofisel have not revealed any clear information suggesting the relationship between Alofisel and malignant transformation of anal fistula, tumorigenicity, or ectopic tissue formation. However, attention should be paid to possible malignant transformation of anal fistula, because Alofisel may induce morphological and functional changes in cancer cells dormant in the administration site through the action of inflammatory mediators secreted from Alofisel, a human cellular product (*Gastroenterology.* 2011;141:1046-56, *Cancer Cell Int.* 2015;15:42, etc.). Also, the possible risk of tumorigenicity or ectopic tissue formation caused by Alofisel cannot be ruled out, given the capacity of Alofisel differentiating into various tissues. Therefore, information on malignant transformation of anal fistula, tumorigenicity, and ectopic tissue formation will be collected continuously after the market launch.

PMDA's view:

PMDA accepted the applicant's explanation that the clinical study data and the foreign post-marketing report of Alofisel have not revealed any clear information suggesting the relationship between Alofisel and malignant transformation of anal fistula, tumorigenicity, or ectopic tissue formation. However, because perianal lesion associated with Crohn's disease has a high risk of carcinogenesis, relevant information should be collected after the market launch of Alofisel.

6.R.4 Clinical positioning of Alofisel

The applicant's explanation about the clinical positioning of Alofisel:

Complex perianal fistulas associated with Crohn's disease are treated by drugs (antimicrobial agents, immunomodulators, and biological products) and by surgical therapy, depending on disease conditions. In Japan, the only drug approved for the treatment of perianal lesions associated with Crohn's disease is infliximab, which is indicated for Crohn's disease with external fistula. Most of the other conventional drugs lack sufficient evidence (*J Crohns Colitis.* 2020;14:4-22, *J Crohns Colitis*. 2020;14:155-168, etc.). In particular, there is scant evidence on the use of these drugs in the treatment of complex perianal fistulas that have relapsed after conventional therapies. In addition, long-time use of anti-TNF agents and immunomodulators have a risk of adverse drug reactions because of their systemic immunosuppressive effects. Also, depending on the operative method, surgical procedure has a high risk of relapse or a risk of anal sphincter invasion, and repeated surgeries may cause fecal incontinence.

Alofisel demonstrated a statistically significantly higher combined remission rate at Week 24, the primary endpoint, than placebo in the foreign phase III placebo-controlled, double-blind study (Study Cx601-0302) in patients with complex perianal fistulas associated with Crohn's disease who have an inadequate response to at least 1 conventional drug. Alofisel also tended to be superior to placebo in secondary endpoints. In Study Darvadstrocel-3002 (Japanese phase III open-label, uncontrolled study in Japanese patients with complex perianal fistulas associated with Crohn's disease who have an inadequate response to at least 1 conventional drug), the combined remission rate at Week 24, the primary endpoint, was similar to that observed in Study Cx601-0302, and the results of the secondary and other endpoints were not significantly different from those in Study Cx601-0302. These results suggest that Alofisel has efficacy in Japanese patients as well. Alofisel is approved and used in clinical practice in 16 counties including European countries as of December 2020. ECCO Guidelines state that Alofisel may be a safe and effective treatment of complex perianal fistulas associated with Crohn's disease (Evidence Level 2).

Based on the above, administration of a single dose of Alofisel is expected to provide a highly safe and effective treatment method for patients with complex perianal fistulas associated with Crohn's disease who have an inadequate response to at least 1 conventional drug.

PMDA accepted the applicant's explanation.

6.R.5 Indication or performance

The proposed "Indication or Performance" was "Treatment of complex perianal fistulas in patients with non-active or mildly active Crohn's disease."

PMDA's conclusion based on the review presented in Sections "6.R.2 Efficacy," "6.R.3 Safety," and "6.R.4 Clinical positioning of Alofisel":

"Indication or Performance" of Alofisel can be determined generally based on the results of the clinical studies. However, given that general or regional anesthesia, an invasive procedure, is required prior to the administration of Alofisel, the "Indication or Performance" section should states that

Alofisel should be administered only to patients with fistulas that have shown an inadequate response to at least 1 conventional drug (including a biological product), in order to avoid Alofisel administration to treatment-naïve patients.

Indication or Performance (Underline denotes addition.)
Treatment of complex perianal fistulas in patients with non-active or mildly active Crohn's disease who have fistulas that have shown an inadequate response to at least 1 conventional drug.

6.R.6 Dosage and administration or method of use

The proposed "Dosage and Administration or Method of Use" for Alofisel was as follows:
The usual adult single dose is 24 mL of cell suspension containing 120×10^6 cells (contained in 4 vials) administered locally to the fistulas that have been conditioned.

The applicant's rationale for the "Dosage and Administration or Method of Use" for Alofisel:
The dosage and administration or method of use for Alofisel was determined based on Studies Cx601-0101, Cx601-0302, and Darvadstrocel-3002.

In Study Cx601-0101, the study product was administered to only 1 fistula tract. All enrolled patients received local administration of a cell suspension containing 20×10^6 eASCs. If the treated fistula tract had not closed completely at Week 12, an additional dose of the cell suspension containing 40×10^6 eASCs was administered locally. Conditioning of the fistula such as curettage was performed immediately before Alofisel administration. According to the results of efficacy evaluation, the percentage of patients who showed a sign of cure of fistula at Week 24 after an additional dose of the cell suspension containing 40×10^6 eASCs, was similar to the percentage of patients who showed a sign of cure of fistula at Week 12 after receiving only a single dose of the cell suspension containing 20×10^6 eASCs. This suggests that the dose of 20×10^6 eASCs was insufficient for most of the patients receiving the additional dose. In Study Cx601-0101, only 1 fistula tract was treated, and inflammation and/or infection of untreated fistula tract(s) may have adversely affected the cure of the treated fistula tract. Accordingly, in Study Cx601-0302 and subsequent studies, the dose of 120×10^6 eASCs was used (which is 3 times the dose that showed a sign of efficacy in Study Cx601-0101 [40×10^6 eASCs per fistula tract]) to treat all of the $\leq$3 fistulous tracts. In these studies, a single-dose administration was employed to avoid curettage of fistula tracts that may have been partially closed; further, the conditioning of fistulas (i.e., seton removal and curettage under anesthesia) was performed immediately before Alofisel administration.

Results of Study Cx601-0302 demonstrated the efficacy and safety of Alofisel. Also, results of Study Darvadstrocel-3002 confirmed the efficacy and safety of the cell suspension containing 120×10^6 eASCs in Japanese patients as well. Based on the above, the proposed dosage and administration or method of use is considered to be appropriate.

Re-administration of eASCs suspension (containing 120×10^6 cells) was not evaluated in either Study Darvadstrocel-3002 or Study Cx601-0302, nor was there any report of re-administration of Alofisel in the foreign post-marketing report (data cut-off of September 22, 2020). In Study Cx601-0101, an

additional dose (40×10^6 eASCs to 14 patients, 20×10^6 eASCs to 1 patient) was administered to 15 patients 12 weeks after the administration of eASCs suspension containing 20×10^6 cells. Among patients receiving 2 doses, 60.0% (6 of 10) of patients had fewer draining fistulas at Week 24 after the initial dose than at baseline, and 33.3% (3 of 9) of patients had fewer draining fistulas at Week 24 after the initial dose than at Week 12. The safety profile in patients receiving 2 doses of Alofisel did not tend to be significantly different from that in patients receiving a single dose (Table 16).

Extremely limited information is available on the re-administration of Alofisel. Nevertheless, Study Cx601-0101 revealed no significant safety concern in patients receiving 2 doses compared with patients receiving a single dose, and showed the efficacy of Alofisel in some patients, although there are limitations to the interpretation of the results because the study evaluated a dose different from the proposed dose in a small number of patients. Accordingly, although re-administration of Alofisel is not strongly recommended, re-administration may be given at clinical practice if the attending physician considers the benefits of re-administration of Alofisel outweigh the risk. The package insert will include a caution statement that the efficacy and safety of re-administration of Alofisel have not been established, and the applicant will collect information on re-administration in the post-marketing surveillance.

PMDA's view:
The applicant's explanation is understandable. In Studies Darvadstrocel-3002 and Cx601-0302, 120×10^6 eASCs were administered to patients with ≤2 internal openings and ≤3 external openings. Therefore, the target number of internal and external openings for a single dose of Alofisel (120×10^6 eASCs in total) is an important information. Accordingly, this information should be included in "Dosage and Administration or Method of Use," and the wording should be as follows:

Dosage and Administration or Method of Use (Underline denote changes, and strikethrough denotes deletion.)
The usual adult single dose is ~~24 mL of cell suspension containing~~ 120×10^6 human mesenchymal stem cells (contained in 4 vials [24 mL]) administered ~~locally~~ to 1 or 2 internal openings and to up to 3 external openings after conditioning of the fistulas such as curettage ~~that have been conditioned~~.

Because of the long clinical course of this disease, re-administration will be necessary in a certain percentage of patients in clinical practice. Because of the limited experience of re-administration in clinical studies, information on the safety, etc., of re-administration should be collected after the market launch and provided to healthcare professionals in an appropriate manner.

7. Risk Analysis and Outline of the Review Conducted by PMDA

The applicant's explanation about the plan for the post-marketing surveillance on Alofisel:
In order to investigate the safety, etc., of Alofisel in clinical practice, the applicant plans to conduct post-marketing surveillance in patients with complex perianal fistulas associated with Crohn's disease treated with Alofisel.

The safety specification of this surveillance includes the following events expected to occur in post-marketing settings based on incidences of the adverse events reported in Studies Cx601-0302 and Darvadstrocel-3002: “Hypersensitivity,” “transmission of an infectious agent,” “occurrence or relapse of anal abscess and perianal fistula,” and “administration error.” In addition, missing information on Alofisel, “long-term safety” and “efficacy and safety in re-administration” were included.

The planned sample size is 275 patients by taking account of the incidence of “occurrence or relapse of anal abscess and perianal fistula” (an event included in the safety specification) in Study Cx601-0302.

The proposed observation period is 36 months for evaluating the long-term safety. Patients receiving re-administration of Alofisel will be followed up for an additional 36 months after the re-administration for data collection.

PMDA’s view:
Because of the extremely limited information available on the safety of Alofisel administration to Japanese patients, the surveillance should be conducted to collect data from all treated patients after the market launch, and safety information thus obtained should be provided to healthcare professionals without delay.

The planned sample size and the observation period proposed by the applicant are acceptable.

Details of the post-marketing use-results survey will be finalized, taking account of comments from the Expert Discussion on the safety evaluation of Alofisel.

8. Overall Evaluation during Preparation of the Review Report (1)

On the basis of the data submitted, PMDA has concluded that Alofisel has efficacy in the treatment of “complex perianal fistulas in patients with non-active or mildly active Crohn’s disease,” and that Alofisel has acceptable safety in view of its benefits. Accordingly, PMDA considers that making Alofisel available in clinical practice is meaningful because it offers a new treatment option for complex perianal fistulas in patients with non-active or mildly active Crohn’s disease.

PMDA has concluded that Alofisel may be approved if Alofisel is not considered to have any particular problems based on comments from the Expert Discussion.

Review Report (2)

August 23, 2021

Product Submitted for Approval

Brand Name	Alofisel Injection
Non-proprietary Name	Darvadstrocel
Applicant	Takeda Pharmaceutical Company Limited
Date of Application	February 10, 2021

List of Abbreviations
See Appendix.

1. Content of the Review

Comments made during the Expert Discussion and the subsequent review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below. The expert advisors present during the Expert Discussion were nominated based on their declarations, etc., concerning the product submitted for marketing approval, in accordance with the provisions of the Rules for Convening Expert Discussions, etc., by Pharmaceuticals and Medical Devices Agency (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

1.1 Efficacy

Based on review in Section "6.R.2 Efficacy" of the Review Report (1), PMDA has concluded that Alofisel was shown to have efficacy in patients with complex perianal fistulas associated with Crohn's disease.

This conclusion reached by PMDA was supported by the expert advisors at the Expert Discussion. The expert advisors commented that given the disease condition treatable with Alofisel, the Japanese disease name in "Indication or Performance" should be changed to *fukuzatsu jiro* (complex perianal fistula) [see Section "1.3 Clinical positioning, indication, or performance"].

1.2 Safety

As a result of the review in Section "6.R.3 Safety" of the Review Report (1), PMDA has concluded that there were no particular concerns about the safety profile of Alofisel.

This conclusion reached by PMDA was supported by the expert advisors at the Expert Discussion.

1.3 Clinical positioning, indication, or performance

PMDA's conclusion as a result of the review in Sections "6.R.4 Clinical positioning of Alofisel" and "6.R.5 Indication or performance" of the Review Report (1):

The “Indication or Performance” section should clearly states that Alofisel should be administered only to patients with fistulas that have shown an inadequate response to a conventional drug(s), as described in the relevant section of the Review Result (1). Details of patients enrolled in Studies Darvadstrocel-3002 and Cx601-0302 should be provided in the “Clinical Studies” section of the package insert. In addition, the “Precautions Concerning Indication or Performance” section should include a statement that eligible patients should be selected with a full understanding of the information presented in the “Clinical Studies” section.

The following comments were raised by the expert advisors at the Expert Discussion:

- PMDA’s conclusion is generally supportable. The proposed Japanese disease name in “Indication or Performance” is *komon shui fukuzatu roko* (complex perianal fistula), but the word *roko* means a condition of the disease, rather than the disease itself. Therefore the disease name should be changed to *fukuzatu jiro* (complex anal fistula).” (There is no change in English translation because both words are translated into the same English word, complex perianal fistula.)
- Surgical procedure such as drainage is usually considered as the first line therapy for complex perianal associated with fistulas Crohn’s disease. Also, in order to evaluate the eligibility for the treatment with Alofisel, it is necessary to control infection and inflammation at the site of treatment. Therefore the package insert should clearly state that surgical procedure of the complex perianal fistula must be performed before treatment with Alofisel.

Based on the above comments of the Expert Advisors, PMDA instructed the applicant to modify “Indication or Performance” and “Precautions Concerning Indication or Performance” sections as shown below. The applicant responded that they would take appropriate measures, and PMDA accepted the applicant’s response.

Indication or Performance:

Treatment of complex perianal fistulas in patients with non-active or mildly active Crohn’s disease who have had an inadequate response to at least 1 conventional drug.

Precautions Concerning Indication or Performance

- Before using Alofisel, the physician should confirm that the patient had underwent, before treatment with a conventional drug(s), an adequate draining procedure such as setons performed according to the guidelines, etc.
- Eligible patients should be selected with a full understanding of the efficacy and safety of Alofisel, and of the patient characteristics (e.g., prior treatments, condition of anal fistula) enrolled in clinical studies shown in the “Clinical Studies” section.

1.4 Dosage and administration or method of use

PMDA’s conclusion as a result of the review in Section “6.R.6 Dosage and administration or method of use” of the Review Report (1):

The condition of the treatable anal fistula should be clearly specified in the “Dosage and Administration or Method of Use” section, as described in the relevant sections of the Review Report (1).

This conclusion reached by PMDA was supported by the expert advisors at the Expert Discussion.

PMDA instructed the applicant to modify "Dosage and Administration or Method of Use" as shown below. As the applicant appropriately responded to the instruction, PMDA accepted the response.

Dosage and Administration or Method of Use
The usual adult single dose is 120×10^6 human mesenchymal stem cells (contained in 4 vials [24 mL]) administered to 1 or 2 internal openings and to up to 3 external openings after conditioning of the fistulas such as curettage.

1.5 Post-marketing surveillance plan (draft)

PMDA concluded that post-marketing surveillance should be conducted covering all patients receiving Alofisel, as described in Section "7. Risk Analysis and Outline of the Review Conducted by PMDA" of the Review Report (1).

This conclusion reached by PMDA was supported by the expert advisors at the Expert Discussion.

PMDA instructed the applicant to conduct post-marketing surveillance covering all patients receiving Alofisel. As the applicant appropriately responded to the instruction, PMDA accepted the response and concluded that the post-marketing surveillance as shown in Table 33 should be conducted.

Table 33. Outline of post-marketing surveillance plan (draft)

Objective	To evaluate the safety of Alofisel in clinical practice
Survey method	All-case surveillance
Observation period	36 months (Patients receiving re-administration of Alofisel will be followed up for an additional 36 months after the re-administration for data collection.)
Population	Patients with complex perianal fistula associated with Crohn's disease who have received Alofisel
Main survey items	Key survey items: Hypersensitivity, transmission of an infectious agent, anal abscess, occurrence or relapse of anal fistulas, administration error, long-term safety, and safety in re-administration

1.6 Other

1.6.1 Designation of specified regenerative medical product

Based on "Concept for designation of biological products and specified biological products as well as specified regenerative medical products" (PFSB/ELD Notifications No. 1105-1 and 1105-2 dated November 5, 2014), PMDA has concluded that Alofisel should be designated as a specified regenerative medical product because it is a regenerative medical product manufactured by using allogeneic cells as the starting material.

2. Results of Compliance Assessment Concerning the New Regenerative Medical Product Application Data and Conclusion Reached by PMDA

2.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The new regenerative medical product application data were subjected to a document-based compliance inspection and a data integrity assessment in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices. On the basis of the inspection and assessment, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

2.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The new regenerative medical product application data (CTD 5.3.5.2-2) were subjected to an on-site GCP inspection, in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices. On the basis of the inspection, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

3. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved after modifying the indication or performance and the dosage and administration or method of use as shown below, with the following approval conditions. Because the product is designated as an orphan regenerative medical product, the re-examination period should be 10 years. The product should be designated as a specified regenerative medical product.

Indication or Performance

Treatment of complex perianal fistulas in patients with non-active or mildly active Crohn's disease who have had an inadequate response to at least 1 conventional drug.

Dosage and Administration or Method of Use

The usual adult single dose is 120×10^6 human mesenchymal stem cells (contained in 4 vials [24 mL]) administered to 1 or 2 internal openings and to up to 3 external openings after conditioning of the fistulas such as curettage.

Approval Conditions

1. The applicant is required to take necessary actions such as conducting seminars and disseminating the guidelines for proper use prepared in cooperation with relevant academic societies, to ensure that physicians with adequate knowledge and experience in complex perianal fistulas in patients with Crohn's disease acquire adequate skills in using the product and knowledge of complications associated with the procedures, and that the physicians use the product in compliance with the "Indication or Performance" and "Dosage and Administration or Method of Use" at medical institutions well equipped for providing medical care for complex perianal fistulas in patients with Crohn's disease.

2. The applicant is required to conduct a drug use-results survey covering all patients treated with the product after the market launch until data from a certain number of patients have been gathered, and to take appropriate measures as necessary.

Appendix

List of Abbreviations

1-MT	1-Methyl-L-tryptophan
ACG	American Gastroenterological Association
Alofisel	Alofisel Injection
Application	Application for marketing approval
ASC	Adipose stem cells
BSE	Bovine spongiform encephalopathy
CD	Cluster of differentiation
CDAI	Crohn's disease activity index
CI	Confidence interval
CMV	Cytomegalovirus
Component cells	Cells contained in the product as the primary component
CT	Computed tomography
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DSA	Donor-specific antibodies
eASC	expanded Adipose Stem Cells
EBV	Epstein-Barr virus
ECCO	European Crohn's and Colitis Organisation
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
EUA	Examination under anesthesia
FAS	Full analysis set
FBS	Fetal bovine serum
FDA	U.S. Food and Drug Administration
gDNA	Genome DNA
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
HSA	Human serum albumin
HTLV	Human T-cell leukemia virus
IBD	Inflammatory Bowel Diseases
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon-γ
IL-12	Interleukin-12
IL-1β	Interleukin-1beta
IL-23	Interleukin-23
IL-6	Interleukin-6
Infliximab	Infliximab (genetical recombination)
IOIBD	International Organization for the Study of Inflammatory Bowel Diseases
ITT	Intention to treat
LOCF	Last observation carried forward
MCS	Master cell stock
MedDRA/J	Medical Dictionary for Regulatory Activities Japanese version
MRI	Magnetic-resonance imaging
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PDAI	Perianal disease activity index
PMDA	Pharmaceuticals and Medical Devices Agency
PPS	Per protocol set

QOL	Quality of life
SARS-CoV-2	Severe acute respiratory syndrome-associated coronavirus 2
SVF	Stromal Vascular Fraction
TNBS	2,4,6-Trinitrobenzene sulfonic acid
TNF	Tumor necrosis factor
Treg	Regulatory T cells
TSE	Transmissible spongiform encephalopathy
vCJD	variant Creutzfeldt-Jakob disease
WHO	World Health Organization
WNV	West Nile Virus