14 December 2017
EMA/CHMP/64055/2018 Corr.[1]
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Alofisel

International non-proprietary name: darvadstrocel

Procedure No. EMEA/H/C/004258/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

[1] Redaction of sensitive data on page 66

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List of abbreviations

AS	Active substance (also named drug substance DS)
CPA	Critical performance attributes
CQA	Critical quality attributes
DMEM	Dulbecco's Modified Eagle´s Medium
DNA	Deoxyribonucleic acid
eASC	Expanded Adipose Stem Cells
FACS	Fluorescence-activated cell sorting
Fce R1 α	(high-affinity IgE) Q23
FP	Finished product (also named drug product DP)
HSA	Human Serum Albumin
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon-gamma
IPC	In-process control
LAL	Limulus amebocyte lysate
LLOQ	Lower limit of quantification
MAH	Marketing Authorization Holder
MCS	Master cell stock (previously named MCB)
NAT	Nucleic acid amplification technique
QTPP	Quality Target Product Profile
PBMC	Peripheral blood mononuclear cell
Ph Eur	European Pharmacopoeia
PD	Population Doublings
PASS	Post Authorisation Safety Study
SVF	Stromal Vascular Fraction
TEAE	Treatment Emergent Adverse Event
vCJD	Variant Creutzfeldt-Jakob Disease

1. Background information on the procedure

1.1. Submission of the dossier

The applicant TIGENIX, S.A.U. submitted on 2 March 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Alofisel, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

Alofisel was designated as an orphan medicinal product EU/3/09/667 on 08 October 2009 in the following condition: treatment of anal fistula.

The applicant applied for the following indication: Alofisel is indicated for the treatment of complex perianal fistula(s) in adult patients with non-active/mildly active luminal Crohn's disease, when fistula(s) have shown an inadequate response to at least one conventional or biologic therapy.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Alofisel as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/Find medicine/Human medicines/European public assessment reports.

The legal basis for this application refers to:

Article 8(3) of Directive 2001/83/EC - complete and independent application composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies. The applicant indicated that darvadstrocel was considered to be a new active substance.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included EMA Decision(s) P/0253/2014 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-001561-PIP01-13 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's requests for consideration

New active Substance status

The applicant requested the active substance darvadstrocel contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product

previously authorised within the European Union.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 17 March 2011. The Protocol Assistance pertained to non-clinical and clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Lennart Åkerblom

Co-Rapporteur: Margarida Menezes-Ferreira

- The application was received by the EMA on 2 March 2016.
- The procedure started on 24 March 2016.
- The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 10 June 2016. The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 14 June 2016.
- The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 8 July 2016.
- During the meeting on 15 July 2016, the CAT agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 15 July 2016.
- The applicant submitted the responses to the CAT consolidated List of Questions on 13 December 2016.
- Three GCP inspections were requested by the CAT and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CAT and CHMP members on 30 January 2017.
- During the PRAC meeting on 9 February the PRAC agreed on a PRAC Assessment Overview and Advice to CAT/CHMP. The PRAC assessment Overview and Advice was sent to the applicant on 9 February 2017.
- During the CAT meeting on 17 February 2017, the CAT agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CAT List of Outstanding Issues on 14 September 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CAT and CHMP members on 16 October 2017.
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CAT and CHMP members on 27 October 2017.
- During the CAT meeting on 30 October 2017, outstanding issues were addressed by the applicant during an oral explanation before the CAT.
- During the meeting on 31 October 2017, the CAT agreed on a 2nd List of Outstanding Issues to be sent to the applicant. The List of Outstanding issues was sent to the applicant on 14 November 2017.

- The applicant submitted the responses to the CAT List of Outstanding Issues on 16 November 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CAT and CHMP members on 28 November 2017.
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CAT and CHMP members on 4 December 2017.
- During the meeting on 8 December 2017, the CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive draft opinion for granting a marketing authorisation to Alofisel.
- During the meeting on 11-14 December 2017, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive scientific opinion for granting a marketing authorisation to Alofisel on 14 December 2017.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Fistulas are common complications of Crohn's disease (CD) and include perianal fistulas that connect the anorectum with the perianal area and also fistulas between the gastrointestinal tract and an internal organ or the abdominal skin.

2.1.2. Epidemiology

In particular patients with CD involving the distal bowel are at increased risk of developing perianal fistulas. Results from population based studies indicate that perianal fistulas are the most common manifestation of fistulising CD and develop in approximately 20-30 % of patients along the course of the disease. Recurrences are observed in approximately 30 % of the cases (Hellers et al 1980, Schwartz et al 2002). The prevalence of anal fistulas beyond Crohn's diseases patients is expected to be higher.

2.1.3. Biologic features

Anal fistulas, typically present as fissures penetrating the intestinal lumen and perianal skin surface, and are characterised by local inflammation that is exacerbated by bacterial infection(s) and faecal contamination. In the inflamed area, there is lymphocyte activation and local release of inflammatory cytokines.

2.1.4. Clinical presentation, diagnosis

Perianal fistulas in CD are classified as simple or complex. A complex fistula is characterised as having an origin above the dentate line, to have multiple external openings or are associated with the presence of perianal abscess, or of a rectovaginal fistula . Further, a complex anal fistula could also meet the following criteria: have rectal stenosis and/or macroscopic proctitis. A complex fistula is more treatment resistant than simple fistulas. (Schwartz et al, 2015).

The main symptoms of perianal fistulas are pain, abdominal swelling, abscess formation, fever and drainage of faeces, pus and blood. Perianal disease is associated with high morbidity and has a negative impact on the quality of life for the affected patients.

Presently recommended treatments for perianal fistulising CD include drainage and immunosuppressive treatment. Antibiotics and thiopurines are considered as adjunct treatments and anti-TNF is considered the gold standard (Gecse KB et al, 2014). However, only infliximab has an approved indication for treatment of fistulising CD.

2.1.5. Management

About the product

Alofisel is an allogeneic somatic cell therapy medicinal product for the treatment of complex perianal fistula(s) in adult patients with Crohn's disease. Alofisel is a suspension for injection containing expanded human allogeneic mesenchymal adult stem cells extracted from adipose tissue (expanded adipose stem cells - eASC) presented as four glass vials, containing each a 6 mL suspension of 30 million of eASC in Dulbecco's Modified Eagle Medium (DMEM) (consisting of a mixture of amino acids, vitamins, salts and carbohydrates) and Human Serum Albumin (HSA).

The proposed mechanism of action for allogeneic eASC is based on immune modulatory and anti-inflammatory properties. Inflammatory cytokines, in particular IFN-γ produced by activated immune cells (i.e., lymphocytes), activate eASC in a mechanism which requires induction of indoleamine 2,3-dioxygenase expression. Once activated, eASC suppress proliferation of lymphocytes and inhibit the release of pro-inflammatory cytokines. This immunoregulatory activity reduces inflammation, which may allow the tissues around the fistula tract to heal.

The intended indication claim for Alofisel is: treatment of complex perianal fistula(s) in adult patients with non-active/mildly active luminal Crohn's disease, when fistula(s) have shown an inadequate response to at least one conventional or biologic therapy.

As conditioning of the fistula is a critical treatment step before applying Alofisel and to add clarification the approved indication was amended to: treatment of complex perianal fistula(s) in adult patients with non-active/mildly active luminal Crohn's disease, when fistula(s) have shown an inadequate response to at least one conventional or biologic therapy. *Alofisel should be used after conditioning of fistula, see section 4.2.*

The intended and approved posology of Alofisel is a single local administration of 120 million cells and the method of administration is for local injection in a surgical environment under anaesthesia (general or regional).

Type of Application and aspects on development

The clinical development programme for Alofisel for the treatment of complex perianal fistula(s) in adult patients with non-active or mildly active luminal Crohn's disease consisted of one pivotal Phase III study (study Cx601-0302) and one Phase I/IIa study (study Cx601-0101). The targeted population in both studies consisted of patients with complex perianal fistula(s) with inadequate response to at least one conventional or biologic therapy.

Relevant CHMP guidance includes the guideline on the development of new medicinal products for the treatment of Crohn's disease (CPMP/EWP/2284/99 Rev. 1, 24th July 2008).

Central scientific advice was issued by CHMP initially in 2005 (EMEA/H/SA/654/1/2005/PA/I). Follow-up protocol assistance was given in 2006 ((EMEA/H/SA/645/1/2006/PA/SME/I), 2009 (EMEA/H/SA/654/3/FU/1/2009/PA/SME/ADT/I) and 2011 (EMEA/H/SA/2069/1/2011/PA/ADT/SME/III). The advice given pertained to quality, nonclinical, and clinical aspects. Whereas the initial advices involved the autologous application of eASC, the 2011 advice is based on the proposed allogeneic application of eASC. Although quality aspects was only involved in the initial advices, the applicable recommendations have been implemented in the allogeneic program.

The CHMP has previously commented that the primary endpoint in the pivotal Phase 3 study in principle should be defined in accordance with CHMP/EWP/2284/99 Rev 1, i.e., "complete closure of fistulas and maintenance of a closed fistula without development of new fistulas". It was considered that the optimum endpoint for demonstrating remission at week 24 would be the combination of complete healing (no discharge after gentle pressing) with complete MRI based closure of the fistulae.

The primary efficacy endpoint utilized in the pivotal study deviates from this recommendation and respective guideline, because it was defined as clinical closure of external openings that were draining at baseline despite gentle finger compression and absence of collections >2 cm of the treated fistula confirmed by MRI images, at week 24. This is discussed in the chapter on clinical efficacy.

Alofisel is indicated for adult patients only. On 29 September 2014, the European Medicines Agency (EMA) adopted a decision (EMA Decision P/0253/2014) to grant a waiver in accordance with Article 11(1)(b) of Regulation (EC) No 1901/2006) for Alofisel for the condition Crohn's disease perianal fistula for the age subset 0 years to 3 years. Said decision also granted a deferral in accordance with Article 21 of Regulation (EC) No 1901/2006 until December 2020 for Alofisel for the condition Crohn's disease perianal fistula for the age subset 4 years to 17 years (procedure number EMEA-001561-PIP01-13).

Compliance with GCP

The Applicant declares that the clinical trials included in this submission were conducted in accordance with Good Clinical Practice standards and in accordance with applicable regulatory clinical requirements.

According to the Applicant, the clinical trials within this submission conducted outside the European Union (EU) meet the ethical requirements of Directive 2001/20/EC.

Three GCP inspections were conducted by EMA in relation to the MAA and comprised 5 inspection sites in total, including clinical investigator sites, CROs and the Sponsor. The detected departures from GCP and deficiencies related to trial management were overall considered by the inspection team to have no impact on the inspected trials' data reliability and validity.

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2.2. Quality aspects

2.2.1. Introduction

The finished product (FP) is presented as suspension for injection containing 5 million cells/mL of darvadstrocel, which are expanded human allogeneic mesenchymal adult stem cells extracted from adipose tissue as active substance (AS). The following abbreviation expanded adipose stem cells (eASC) is used hereafter for the active substance.

Other ingredients are: Dulbecco's Modified Eagle Medium (DMEM) (containing amino acids, vitamins, salts and carbohydrates) and human serum albumin.

The product is available as one treatment dose contained in 4 Type I glass vials. Each vial contains 6 mL of eASC suspension and is closed with a rubber stopper and a flip-off seal. The final dose consists of 4 vials with a total quantity of 120 million eASC in 24ml administration volume. The vials are placed inside a cardboard box.

2.2.2. Active Substance

General information, characterisation and process controls

Alofisel contains as active substance *in vitro* expanded human mesenchymal adult stem cells extracted from subdermal adipose tissue (eASC), which are formulated in a cell suspension.

Human eASC are obtained from donations of lipoaspirate extracted from human adipose tissue of subdermal origin.

The mechanism of action proposed for eASC involves immunomodulation through reduction of T cell proliferation and release of pro-inflammatory cytokines, decrease of proliferation, expression of activating receptors and cytotoxic activity of NK cells, decrease of immune cells migrating to inflamed tissues, induction of anti-inflammatory suppressor cells such as Treg cells and M2 macrophages, and increase of phagocytosis by macrophages and neutrophils.

Manufacture, characterisation and process controls

The manufacturer of the active substance is TiGenix S.A.U., with facilities located in Tres Cantos (Madrid), Spain. The manufacturing, control test for batch release and batch release of Alofisel active substance are carried out in accordance with current good manufacturing practice by TiGenix and by its contractors.

Manufacturing activities are performed in Grade B clean facilities with Grade A laminar flow cabinets in compliance with cGMP for biologic active substance. No terminal sterilisation, purification, viral removal and inactivation steps are performed.

The active substance is a cell suspension of eASC after *in vitro* expansion.

The starting material is obtained from a healthy allogeneic donor through liposuction (lipoaspirate) following the requirements for procurement and testing laid down in the European Tissues and Cells Directive 2006/17/EC implementing Directive 2004/23/EC. Each Alofisel AS batch is prepared from a single donation from an individual donor.

The manufacturing process of the active substance starts after the tissue procurement and consists of the following three main steps:

- Step 1: Isolation (extraction) of the stromal vascular fraction (SVF) cells from the adipose tissue and subsequent selection of adipose stem cells (ASC).

- Step 2: Cell Expansion to master cell stock (MCS) and cryopreservation which consists of the following steps: eASC culturing/expansion, eASC harvest, vials filling, MCS cryopreservation and storage. The MCS is the only intermediate in the active substance manufacturing process.

- Step 3: Cell expansion to AS and cryopreservation which consist of the following steps: MCS thawing, eASC culturing/expansion, eASC harvest, vials filling, cryopreservation and storage.

Detailed description of the intermediate MCS and AS manufacturing process has been provided and includes the identification of in-process controls as well as the respective acceptance criteria, which are considered to be adequate. Information on acceptable ranges for the number of population doublings has been requested and was provided and acceptably justified by the applicant. No reprocessing steps are defined.

The control of critical steps and intermediates has been established on a risk based assessment as per ICHQ11. The limits and ranges for in-process controls (IPC) for MCS and AS were established during development and include biological and microbiological parameters established based upon the quality target product profile (QTPP).

The IPC tests for the MCS and AS manufacturing process and respective criteria are provided and considered to be adequate. The mycoplasmas test applied in the IPC was validated as per ICH recommendations.

The release specifications proposed for the intermediate MCS are suitable for the control of this intermediate.

The batch analyses presented demonstrate compliance of all batches and for all parameters tested with MCS proposed release-specifications and indicate reproducibility and consistency in the MCS manufacturing process.

For the MCS stability studies, specifications/acceptance criteria applied include cell viability, potency, identity and sterility as these are relevant parameters to assess the quality of the MCS.

For stability studies, specifications/acceptance criteria applied included cell viability, potency, identity and sterility as these are relevant parameters to assess the quality of the MCS. Stability studies performed with MCSs that were used in clinical development generated supportive data. Primary stability studies applied to commercial MCS have been initiated. The primary stability program was considered to be acceptable.

Control of materials

A list of the raw and starting materials used in the manufacturing of intermediate MCS and AS is provided together with their respective function in the manufacturing process as well as specifications and acceptance criteria.

Certificates of analysis from representative suppliers are presented demonstrating compliance with specifications. Also compliance with Note for guidance EMEA/410/01 in respect to TSE/BSE was presented. Specifications for raw materials include confirmation of sterility, mycoplasmas and bacterial endotoxins. Animal derived products are tested for the presence of specific viruses.

Lipoaspirate starting material

The starting material (Lipoaspirate) is collected from healthy living female donors.

A valid certificate of Authorization for the Extraction of Adipose-Tissue-Derived Mesenchymal Cells has been issued by the relevant competent authority for the centre proposed for collection of adipose tissue. The procurement process to obtain the lipoaspirate starting material from healthy living female donors is carried out in agreement with the principles laid down in Directive 2006/17/EC on donation, procurement and testing of human tissues and cells as well as Directive 2006/86/EC on traceability of human tissues and cells.

Detailed information on which virus markers are tested on the donors of adipose tissue has been provided. Both immunological and NAT tests are used for all markers. It has been confirmed that only CE-marked test kits are used for donor testing. Information about the laboratory where the donor virus testing is performed has been provided.

Each donation (lipoaspirate) has a unique identification code to ensure donation traceability. This code is maintained through the complete manufacturing process and lifecycle.

Process validation

Validation of the AS manufacturing process was performed based on aspects that could impact the critical quality attributes (CQAs) of the only intermediate of the process, the MCS, and of the final AS.

Validation was performed for the steps starting from donation (starting material) to MCS and from MCS to AS. Aseptic conditions were monitored throughout the process as per cGMP. IPC sampling plan was provided and MCS and AS batches were also tested according to the proposed release specifications.

The results from relevant OC, IPC and release also demonstrate that the AS manufacturing process is also able to consistently produce a finished AS with the required quality namely in respect to patient safety (microbial contamination), yield, potency, purity and identity.

The transport kit is considered suitable to maintain the quality of the donated material from tissue collection establishment to AS manufacturer.

The stability of the lipoaspirate from extraction up to further processing was also studied. The Applicant's proposal for handling the lipoaspirate starting material is considered supported by the stability data provided.

Manufacturing process development

The steps taken during development of the manufacturing process were described. This was initially based on published data and the development of an autologous version of the product for which scientific advice was received from EMA. Relevant aspects for the process performance or the quality of the product were monitored through the development process and the critical steps were identified

In general the information provided is acceptable. Data provided confirmed process consistency. The control strategy implemented for MCS and AS manufacturing processes was based on ICH Q11v.

Characterisation

The characterisation of eASC was performed in accordance with the Guideline on Human Cell-based Medicinal Products (EMEA/CHMP/410869/2006) and the Reflection Paper on stem cell-based medicinal product (EMA/CAT/571134/2009) regarding structural (morphology, positive expression of surface markers, absence of surface markers from other cell types) and biological and functional features of the eASC (growth kinetics, viability, differentiation capacity, immune-regulation, immune-related proteins and proteins related with regenerative and reparative activity).

The approach taken for the characterisation of the eASC is adequate to assess the identity, purity, viability, functionality and potency of eASC.

Main impurities potentially present in the final AS are microbial contaminants, product-related or process-related impurities.

The approaches taken for the assessment of the structural, biological and functional features of the eASC as well as for the identification and control of potential impurities performed from the starting materials and reagents throughout the manufacturing up to MSC and AS are in general satisfactory to ensure proper AS characterisation and control. Additionally, it was adequately demonstrated that the manufacturing process developed is adequately capable of removing potential process impurities to undetectable levels at the level of AS.

Specification

The release and shelf life specification for the active substance have been provided by the Applicant.

The proposed release and end of shelf-life specifications for identity, purity and potency proposed for AS are considered adequate. Stated impurities have been studied in nonclinical and clinical studies.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

The principles and procedures of some of the analytical methods used are adequately described to allow for an exact identification and fit for purpose evaluation. The applicant provided additional information including details on assay procedure, system suitability, quality of reagents used and process parameters for the analytical methods. Early in the procedure a major objection was raised in relation to the potency assay. During the procedure the applicant provided additional data to support the suitability of the potency assay and the major objection is considered to be resolved.

Batch analysis

Batch analysis data of the active substance were provided. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

There is no reference material for this product.

Stability

A shelf-life of 24 months is proposed for commercial AS. On the basis of the submitted data, the proposed 24 months shelf-life for AS batches is considered to be acceptable.

A post-approval stability commitment has been provided confirming that an annual stability protocol will be undertaken. The applicant also confirmed that the post-approval annual stability protocol follows the protocol provided in the dossier.

Comparability Exercise for Active Substance

A comprehensive prospective comparability exercise between batches produced pre- and post-change has been presented. In summary, the available data support the prospective comparability exercise between batches produced for clinical trials and the commercial product.

2.2.3. Finished Medicinal Product

Description of the product

Alofisel finished product (FP) is stored in glass vials of 6 mL containing eASC combined with DMEM used as isotonic buffer and Human Serum Albumin (HSA) used a stabilizer. There are no novel excipients used in the finished product formulation. The final composition is included in Table 1.

Table 1. The final composition of Alofisel finished product

Component	Function	Quantity (per mL)		Quantity (per vial)		Quantity (per dose)		Quality Standard
eASC[a]	Active substance	5 million cells		30 million cells		120 million cells		In-house
DMEM[b]	Excipient (Isotonic buffer)	[redacted] (v/v)	*q.s.* 1 mL	[redacted] (v/v)	*q.s.* 6 mL	[redacted] (v/v)	*q.s.* 24 mL	In-house
HSA[c], 20%	Excipient (Stabiliser)	[redacted] (v/v)		[redacted] (v/v)		[redacted] (v/v)		Ph. Eur.

[a] Expanded Adipose Stem Cells
[b] Dulbecco´s modified Eagle´s medium
[c] Human Serum Albumin 20%

The container closure system for Alofisel FP is a 6 mL Type I glass vial (9 mL volume capacity) with a rubber stopper and an aluminium seal with a flip-off plastic cap. The final dose consists of 4 vials with a total quantity of 120 million eASC.

The description of the container closure system is given in sufficient detail. Specifications and schematic drawings were provided for the primary packaging components: 9 mL volume capacity Type I colourless borosilicate glass vial, chlorobutyl rubber stoppers and aluminium seal (not in contact with the product). The shipping container was also described. The suitability of the shipping container in the transport of Alofisel FP has been sufficiently justified in the dossier. The glass vials and rubber stoppers comply with the requirements of the Ph. Eur. 3.2.1 and 3.2.9, respectively. The primary container closure system has been shown to be compatible with the finished product.

Pharmaceutical development

The development of the final FP aimed to achieve a sterile cell suspension for injection of eASC which can be administered intralesionally in the anal fistula wall. Cells' stability and sterility were evaluated through the manufacturing, storage and administration of the final FP. Stability studies were performed using different formulations tested in different storage conditions: different eASC concentrations, excipients, and storage conditions.

The manufacturing process of FP is very simple consisting of three main steps: 1. Thawing of frozen AS and recovery of thawed eASC; 2. Formulation of the FP and 3. Filling, packaging and shipping of the FP. The selection of the culture flasks for the recovery of the thawed eASC, culture medium and conditions of incubation, seeding density and duration of the recovery phase was adequately performed based on data obtained in various studies where different conditions were tested.

The container closure system was selected based on its suitability with the dosage form and route of administration. Studies performed during the development of FP (including extractables testing, protection from microbial ingress, potential cell adhesion, tightness, and consistency of the manual vial filling) demonstrated that the container closure system maintains the proposed storage conditions during transportation of the FP until administration to patient.

Due to the cellular nature of the AS it is not possible to include any terminal sterilization, purification, viral removal or viral inactivation steps in the manufacturing processes of both AS and FP. As such, the safety of the

final FP with respect to adventitious agents relies strongly on a risk assessment and a number of measures undertaken from the collection of the starting material and throughout the manufacturing processes, namely donor selection and serology testing, selection of raw materials and excipients, maintenance of aseptic environment conditions during manufacturing, testing of adventitious agents at appropriate stages of production and protection from primary packaging.

The description of the pharmaceutical development is considered adequate.

Manufacture of the product and process controls

Alofisel FP is manufactured at TiGenix S.A.U. which is involved in the various steps of the manufacturing excluding sterility, mycoplasmas and genetic stability control tests for release which are carried out by four contractors. All these manufacturers comply with cGMP and the QP declaration.

The manufacturing process of Alofisel FP consists of the following steps: thawing of frozen AS and recovery of eASC, formulation of FP which includes harvesting and washing eASC and formulation with DMEM and 20%HSA as excipients and finally manual filling, packaging and shipping.

It is stated that all manufacturing steps are carried out as per cGMP and under aseptic conditions inside a Grade A Laminar flow biosafety cabinet to avoid any potential contamination and cross-contamination.

The Alofisel manufacturing process steps are controlled based on the risk assessment described for AS having the critical steps defined on the basis of the input and output parameters likely to affect the CQA with impact on the efficacy, safety and/or yield of the FP.

The control strategy proposed for Alofisel is based on control of CQAs by specification at release (critical microbiological control; identity, purity and potency only performed at AS), operational ranges for OCs, monitoring IPC (classification of each IPC as critical IPCs/CPA where cell count and microbiological control are included; and monitored attributes such as PD, cell viability or morphology), and raw material and excipients tight control. This is found to be acceptable.

The input and output parameters applied during manufacturing were defined by operation conditions and IPC established based on the experience gathered during development and from the manufacturing process.

There are no intermediates isolated during the manufacturing process of FP. No reprocessing steps are defined.

Due to the limited shelf-life of 48h, IPCs are performed during the last steps of the manufacture, immediately before and after FP formulation.

The excipients used for the Alofisel finished product formulation are Dulbecco's Modified Eagle's Medium (DMEM) and Human Serum Albumin (HSA) 20%.

Adequate information has been provided on the control of the excipients.

Non-product related contaminants to be controlled at the level of the FP.

Packaging information is provided including shipping conditions (up to 48h at 15-25ºC and immediate administration to patient) which were qualified by monitoring the temperature inside the shipping system. A batch numbering system ensures adequate traceability from donors through AS to FP and patient.

Process validation

The validation of the manufacturing process of the Alofisel finished product was performed at the cell therapy manufacturing plant of TiGenix in Tres Cantos (Madrid, Spain) and included the following aspects: a) Process

consistency and reproducibility (preliminary validation study); b) Process robustness (primary validation study); c) Aseptic process (preliminary validation study); and d) Suitability of container closure system and shipping system.

Manufacturing operations for Alofisel FP do not define an intermediate and therefore no holding time validation was performed.

The analytical procedures used for the validation of the various critical steps of the manufacturing process of the Alofisel FP, like all the QC testing, are to be performed as per AS release specification. The exception was the Gram staining testing applied to check for absence of bacterial contamination during manufacturing which is based on a visual inspection test. This is considered acceptable.

Process consistency and reproducibility were confirmed.

Robustness of the process was validated for the source of the starting material.

Aseptic processing was validated by environmental and microbiological monitoring (in-operation non-viable particle counting and viable particle counting) and media fill validation.

For the finished product packaging and shipping validation, the adequacy of the proposed shipping system was evaluated for the transport of Alofisel FP within 48h at 15-25ºC while maintaining its quality up to administration to patient(s).

Overall the performed process validation demonstrates that the Alofisel finished product manufacturing process is robust and consistently yields FP that meets the predetermined quality attributes which included its intended sterility.

Product specification

The agreed release and shelf life specifications for Alofisel finished product have been provided.

The release and shelf-life specifications include tests for identity, purity and potency are considered to be adequate.

A justification for specifications proposed for routine release and end of shelf-life was provided and considered to be adequate.

The parameters and limits proposed for release and end of shelf-life specifications have been based on process capabilities, manufacturing and testing experience, batch analysis, stability data and literature.

The acceptance criteria proposed have been provided and found to be acceptable.

Analytical methods

Whenever applicable, Ph. Eur. analytical procedures are used in the control of FP (i.e. cell concentration, cell viability, accumulated PD, sterility, mycoplasmas, and bacterial endotoxins). Non-compendial analytical methods have been validated according to ICH guidelines.

Batch analysis

Batch analysis data was provided for three consecutive batches of Alofisel FP of maximum size and for batches sourced from different donors and for three proposed batch sizes

A summary of the analytical data was provided against the release criteria accepted at time of release. In all cases compliance was observed

The results obtained indicate compliance with the specification proposed, showing reproducibility in the quality of the FP batches manufactured.

Reference materials

There is no reference material for the finished product.

Stability of the product

A shelf-life of 48 h is proposed for commercial finished product when stored between 15ºC and 25ºC in the container closure system. This is considered to be acceptable.

A primary stability study was performed to support the shelf-life of 48h and the storage conditions proposed based on the data obtained from the supporting stability study. In all cases compliance is observed with specification in particular for appearance, cell viability, potency and sterility, indicating that the Alofisel FP is stable up to 72h between 15ºC and 25ºC.

In addition a stability study has been performed covering a period of 96h.

The data obtained indicate that the Alofisel FP is stable up to 48h between 15ºC and 25ºC.

The post-approval stability commitment was provided referring to an annual stability protocol.

Adventitious agents

Materials of human and animal origin used in the production of Alofisel include the lipoaspirate starting material and the excipients Dulbecco's Modified Eagle's Medium (DMEM) as isotonic buffer and Human Serum Albumin (HSA) 20% as stabilizer.

Virus safety

A revised risk assessment has been provided upon request where more detailed information has been included on testing of donor, virus testing on MCS and active substance and any risk related to the type of tissue used. The revised adventitious agent risk assessment report is acceptable.

Regarding the lipoaspirate donor material, donors are tested according to requirements in directive 2006/17/EC. Detailed information regarding which virus markers are tested on the donors of adipose tissue has been provided. Both immunological and NAT tests are used for all markers. It has been confirmed that only CE-marked test kits are used for donor testing.

Method description and validation data has been provided for the virus testing performed on MCS and AS. These data are in general acceptable.

The Viral safety of Human Albumin 20 %, which is used as an excipient, has been sufficiently ensured. To minimise the risk of virus transmission and assure that the human albumin is of sufficient quality in line with current EU legislation, the Applicant selected an EU registered medical product to be used as excipient. A short summary of the bases for donor selection and viral testing strategy is provided. A detailed summary over the virus validation studies demonstrating sufficient safety margin for both enveloped and non-enveloped virus for Human Albumin 20 % has been included.

TSE safety

The testing of adventitious safety and the introduced safety measures for raw of biological origin is considered to be acceptable.

The animal materials have been adequately described and are deemed acceptable. The risk of transfer of vCJD from donor cells and measures taken to minimise this has also been adequately addressed by the Applicant.

Sterility is tested at several steps of the manufacturing process including the starting material, active substance and finished product according to Ph.Eur. 2.6.1.

Bacterial endotoxin testing according to Ph.Eur. 2.6.14 applying the limulus amoebocyte-lysate (LAL) assay method is performed as part of the starting material, active substance and finished product release testing.

Mycoplasma testing is performed during manufacture and as part of the starting material, active substance finished product

Due to the nature of the product and the short shelf life, the final tests for sterility and mycoplasma are only available after release. In case a positive sterility test result is identified after the product is released, the Applicant uses a batch coding system to trace the AS and MCS from which the contaminated final product was derived and notifies the treating physician. This proposal has been considered accepted also taking into account that thus far there have not been any cases of transmission of infectious agents after Alofisel administration.

In conclusion the adventitious agent safety evaluation and controls implemented at the level of the donor material and during manufacture and control of Alofisel are considered acceptable.

GMO

Not applicable.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

A number of deficiencies and points for clarification have been identified during the procedure and three major objections were raised.

The first major objection related to the stability of an intermediate product which was considered not adequately supported by stability data available so far. During the procedure the applicant presented new data and the concern related to the stability of the intermediate could be resolved.

Two additional major objections were related to a lack of information on the starting material and virus safety of the allogeneic donor material. During the procedure the Applicant provided additional information regarding testing of donors of adipose tissues (starting material) and the centre where this is performed. The Applicant has clarified the inspection status of the centre where the collection of adipose tissue takes place and was able to resolve the concern. In relation to virus safety, the Applicant performed an additional adventitious agent safety risk assessment and provided more details on donor testing and viral test on MCS and AS. The strategy for the risk assessment is considered acceptable.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

The CHMP endorse the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommends the following points for investigation:

1. The Applicant will undertake to review the data generated for the potency assay from clinical experience after suitable experience has been generated and to follow any recommendation that is issued following the review of the data.
2. The Applicant will submit additional data demonstrating the suitability of the microbial control test, Ph. Eur. 2.6.27, for lipoaspirate.
3. The Applicant will provide a summary of a cross-validation exercise for a requested analytical method.
4. Regarding the addition of a new irradiation site for a biological raw material, the applicant will provide a critical assessment on the functionality of the material demonstrating that the radiation per se does not raise any concerns.

The CHMP endorse the CAT assessment regarding the recommendations for future quality development as described above.

2.3. Non-clinical aspects

2.3.1. Introduction

An extensive program of nonclinical studies was conducted with eASC. In most studies, cell preparations were expanded with the method of manufacture and to the same population doubling level of the product for clinical use. Several routes of administration were investigated: a combination of perianal + intrarectal administration reflecting the intended clinical route, the intravenous route exemplifying a systemic administration and the intravaginal route to provide supportive information of safety, although it is important to highlight that rectovaginal fistulas are not intended to be treated and were excluded from clinical study protocols.

For the perianal + intrarectal and intravaginal routes of administrations the dose used in the non-clinical studies was the maximum feasible dose, based on: i) the maximum volumes that could be injected into the animal model (mouse or rat) by the specific route of administration; and ii) by the maximum concentration in which eASC could be formulated without affecting their viability. For the intravenous route, the dose used for the repeat-dose was the NOAEL derived from the acute-dose toxicological studies.

The applicant has chosen immunocompromised athymic rats to perform the biodistribution, toxicology and tumourigenicity studies. The applicant considers that this was done in order to minimize the immune recognition and potential rejection of human eASC in the experimental animal models and to extend Cx601 persistence

which should, in principle, enhance the likelihood to observe test-item related toxic effects. Data to support the notion of rejection was also observed when eASC cells were administered to immunocompetent rats.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The applicant investigated the role of eASC in modulation of the inflammatory response in induced colitis, as well as the clinical presentation of disease.

Colitic mice were treated with either 3 x 10^5 or 1 x 10^6 eASC (intraperitoneal, i.p.) 12 hours after intrarectal injection of TNBS. Dose dependent, statistically significant improvements in overall survival and body weight loss were associated with eASC treatment relative to TNBS control treatment. In addition, a significantly reduced infiltration of CD4+ and CD11b+ (commonly used as a marker for macrophages) cells ($p < 0.05$) was observed in eASC treated mice as compared to TNBS control mice. Importantly, eASC treatment reduced the concentration of inflammatory cytokines TNF-α, IFN-γ, IL-6, IL-1ß and IL-12 and chemokines RANTES (regulated on activation, normal T cell expressed and secreted) and macrophage inhibitory protein 2 (MIP-2), in comparison to untreated colitic mice. Increased expression of the anti-inflammatory cytokine, IL-10, was also associated with eASC treatment.

To verify the in vitro findings of an important role for eASC in induction of Treg cells, CD4+ T cells from eASC treated TNBS mice were purified ex vivo and administered i.p to colitic mice. Administration of T cells from eASC pre-treated mice resulted in favourable clinical presentation of colitic mice, with improvements in body weight and overall survival relative to the TNBS control. Treatment with either T cells isolated from TNBS mice that had not been primed with eASC treatment, or CD25-depleted T cells (depleting the CD4+CD25+ Treg population), abrogated the protective activity that had been afforded with administration of T cells from eASC pre-treated mice. Thus, the role for eASC in induction of Treg cells has also been shown in an inflammatory model in vivo.

Up to 5 x 10^6 eASC expanded to population doubling levels equal to levels of products for clinical use were intravenously infused twice at 2 week intervals to athymic nude rats (N = 20M/20F/dose level). A functional observational test battery (FOB) was performed and results compared to control animals receiving equal volumes of lactate ringer's solution. Animals were euthanised at 2 or 26 weeks after the final infusion. Deaths were reported at the low dose (0.2 x 10^6 cells, N = 1F [day 173]), medium dose (1 x 10^6 cells, N = 1M [day 196]) and high dose (5 x 10^6 cells, N = 1M [day 166], 1F [day 26]). Consistent with the rapid clearance of eASC reported, there was no indication that any death was treatment related. Treatment did not induce any adverse signs in the FOB, which included evaluations of motor activity, behavioural parameters and reflex responses.

Secondary pharmacodynamic studies

Secondary pharmacodynamic studies have not been performed for eASC which was considered acceptable by the CAT.

Safety pharmacology programme

Central Nervous System (FCI-07-03-FT)

Method

Human eASC preparations from six donors (3 men and 3 women) were expanded to population doubling levels equal to levels of product for clinical use. Cell suspensions for injection contained a mixture of the 6 eASC

preparations. Male and female athymic nude rats (Crl:NIH-Foxn1rnu; n=20 per sex per dose level) received two intravenous administrations (10-min infusion) with a 2-week interval of 1 ml cell suspension at a dose level of 0.2 x 10^6, 1 x 106 or 5 x 10^6 cells. Control animals received equal volumes of Lactate Ringers solution. Animals were euthanized at 2 or 26 weeks (n=10 per sex per dose level per time point) after the final infusion.

Results

4 animals died during study. None of these were considered to be related to treatment. eASC treatment did not affect any of the neurological parameters evaluated in the Functional Observational Battery Test, which included motor activity, behavioural parameters, coordination, and reflex responses.

Cardiovascular and respiratory systems

Cardiovascular or respiratory safety pharmacology studies were not performed for eASC which was considered acceptable by the CAT.

Pharmacodynamic drug interactions

Below relevant publications were submitted to evaluate the effect of commonly administered therapies in Crohn's disease on the activity of eASC.

Effect of anti TNFα on the immunomodulatory properties of eASC (De La Rosa et al 2009)

Method

The effect of anti-TNFα on the capacity of eASC to inhibit PBMC proliferation was investigated by adding anti-TNFα both in conditioned supernatants of the co-culture of eASC with activated PBMC and directly in the co-culture in transwell or contact conditions.

Results

TNF-α blocking had no effect over the inhibitory capacity of the eASC, whereas IFN-γ neutralization significantly blocked eASC-mediated inhibition in transwell conditions.

Effect of methotrexate (MTX) and azathioprine (AZA) on the immunomodulatory properties of eASC (Duijvestein et al., 2011 and Mancheño-Corvo et al., 2013)

Method

The effect of MTX and AZA on the capacity of eASC to inhibit PBMC proliferation was investigated by adding clinically relevant concentrations of MTX or AZA in co-cultures of eASC and activated PBMC.

Results

Results from MTT cell viability assays indicated that incubation with MTX did not significantly affect the viability and proliferation of eASC. Incubation with AZA did not affect viability and proliferation when used at clinically relevant concentrations, but was toxic at the high concentration (1000μM). In addition, MTX or AZA did not significantly modify the anti-proliferative effect of eASC indicating that no synergistic or antagonizing effect between eASC and MTX or AZA was apparent *in vitro.* Also, the capacity of eASC to generate Tregs *in vitro* was not affected by MTX or AZA.

2.3.3. Pharmacokinetics

A total of 3 biodistribution studies were performed in male and female athymic nude rats (rnu/rnu) with eASC, expanded to population doubling levels equal to levels of product for clinical use. Several routes of administration were applied: a combination of perianal + intrarectal administration reflecting the intended clinical route, the intravenous route, and the intravaginal route. The distribution of the injected eASC was monitored at different timepoints (day 1 or 2, day 14, day 90 or 91 and day 182). When cells were administered using the perianal + intrarectal route or the intravenous route, human genomic DNA was detected at day 1/2 and day 14. When cells were administered using the intravaginal route, cells were only detected at day 2. In conclusion, when administered locally human eASC is only persistent for a short period of time in rats.

The biodistribution of eASC injected using the perianal + intrarectal route was restricted to the rectum (high signal) and jejunum (low signal, mostly just above LOQ), which seems logical from an anatomical point of view when administering the cells intrarectally. When the intravaginal route was used, human DNA was detected at a very low level in the upper part of the uterus in 1 female. However, no detection of human DNA into the ovaries was reported, indicating that there is not migration of eASC into the ovaries, even when this route of administration is used.

When eASC were administered intravenously, human genomic DNA was detected at a high level in lungs of almost all treated athymic rats, which is to be expected when using this route. Some other tissues (including heart, liver, kidney, jejunum, ileum, colon and cecum) tested positive in very few animals.

2.3.4. Toxicology

Table 2 Overview of Alofisel non-clinical toxicity program.

Study type and duration	Route of administration	Species
Single-dose toxicity		
14-days	Intravenous	Rat/Crl:NIH-Foxn1^{rnu}
14-days	Subcutaneous	Rat/Crl:NIH-Foxn1^{rnu}
26-week (biodistribution study)	Perianal+intrarectal	Rat rnu/rnu
26-week (biodistribution study)	Intravenous	Rat rnu/rnu
26-week (biodistribution study)	Intravaginal	Rat rnu/rnu
Safety parameters in a Single Dose Biodistribution Study		
26-week	Perianal + Intrarectal	Rat rnu/rnu
26-week	Intravenous	Rat rnu/rnu
26-week	Intravaginal	Rat rnu/rnu
Repeat-dose toxicity		
2 doses with 2-week interval + up to 12 weeks post-dose	Perianal	Rat/Crl:NIH-Foxn1^{rnu}
2 doses with 2-week interval + up to 24 weeks post-dose	Perianal	Rat/Crl:NIH-Foxn1^{rnu} Rat/Crl:NIH-Foxn1^{rnu}
2 doses with 2-week interval + up to 26 weeks post-dose	Intravenous	
Genotoxicity		
No study		
Carcinogenicity		
In vitro	NA	NA
Karyotype analysis	NA	NA

Growth kinetics and senescence	NA	NA
Telomerase activity	NA	NA
C-Myc expression	NA	NA
Tumourigenic growth potential (soft agar assay)	NA	NA
In vivo		
Tumourigenicity test with eASC	Subcutaneous	Mouse/ Nu/Nu
Tumourigenicity test with eASC expanded to population doubling levels equal to levels of product for clinical use	Subcutaneous	Mouse/Swiss Nu/Nu Foxn-1
Reproductive and developmental toxicity		
No study		
Local Tolerance		
No study		
Other toxicity		
Immunogenicity of eASC	NA	NA
NK and eASC crosstalk (NK)	NA	NA
T cell recognition of eASC	NA	NA
Animal model development	NA	NA
Differentiation potential	NA	NA

Single dose toxicity

Single doses of 5 x 10^6 cells/rat and 10 x 10^6 cells/rat were well tolerated when administered via the intravenous or subcutaneous route. After perianal + intrarectal or intravaginal administration, the administered dose of 5 x 10^6 cells was also well tolerated. An intravenous dose of 10 x 10^6 cells was associated with mortality and moribund sacrifice during or immediately after dosing, related to pulmonary embolism. This is consistent with the noted thrombi in lungs on day 2. Thrombi decreased in incidence between day 2 and day 14 post-injection and were no longer observed at time-points ≥ 90 days. Despite this toxicity pattern specific of the intravenous route of administration at a dose of 10 x 106 cells in rats, there were no indications of systemic toxicity, but rather an inconsistent and variable toxicological profile, suggesting that the limited toxicity observed is likely incidental. Observations were confined to local inflammatory histopathological changes at the site of injection for the perianal + intrarectal route and to the observation of thrombi in lungs for the intravenous route. These microscopic observations decreased in incidence between day 1/2 and day 14 and showed full recovery within 90 days post-injection.

Repeat dose toxicity

Two repeat-dose toxicity studies were conducted with human eASC in male and female athymic nude rats (Crl:NIH-Foxn1rnu) via the perianal route, a study with a 3-month post-dose observation period and a study with a 6-month post-dose observation period. Moreover, one repeat-dose toxicity study was conducted with human eASC in male and female athymic nude rats (Crl:NIH-Foxn1rnu) via the intravenous route.

Repeat dose (two weeks apart) of 2.5 x 10^6 cells/rat were well tolerated when administered using the perianal route, and there was no treatment-related mortality. A repeat dose (two weeks apart) of 5 x 106 cells/rat resulted in some mortality due to pulmonary embolism during or immediately after dosing. Taken together, two administrations of eASC two weeks apart at doses up to 2.5 x 10^6 cells were well tolerated in athymic nude rats when administered via the perianal route. There were no indications for systemic toxicity. Apart from some

mortality explained by embolism of injected cells to pulmonary vasculature, intravenous repeat-dose studies at dose levels up to 5 x 10^6 cells/rat have likewise provided no evidence of systemic toxicity.

Genotoxicity

Genotoxicity studies are not applicable for the product, considering the cell-based nature of the product, which precludes interaction directly with DNA or other chromosomal material.

Carcinogenicity

The *in vitro* and *in vivo* studies performed to determine whether eASC could be tumourigenic. When cultured *in vitro* according to the procedure for the product, five of six eASC preparations reached a plateau in their growth curve between population doubling level 25 and 30 and the 6th preparation around population doubling level 40, marking the onset of senescence. The presence of senescent cells was confirmed by acidic ß-galactosidase staining. eASC preparations expanded to population doubling levels equal to levels of product for clinical use showed negligible telomerase activity and low c-myc expression. There was no increase in telomerase activity or c-myc expression during the process of expansion. Moreover, eASC preparations showed no anchorage-independent growth in the soft-agar test. Analysis revealed normal male or female diploid karyotypes. One eASC (expanded to population doubling levels equal to levels of product for clinical use) preparation showed increased structural aberrations but was not of clonal nature and therefore did not indicate a transformation. In addition, no elevated c-myc expression, no elevated senescence and no elevated telomerase activity were observed. In *in vivo* tumourigenicity tests, nude mice administered a subcutaneous injection of eASC at various population doubling levels, equal to levels of product for clinical use, all survived to scheduled sacrifice and no tumour-formation was observed.

Reproduction toxicity

Reproductive and developmental toxicity studies have not been performed for Alofisel because preclinical biodistribution studies indicated no migration and integration of eASC into reproductive organs following administration of eASC via different routes. This was considered acceptable by the CAT.

Local tolerance

Local tolerance evaluation was included in the single and repeat-dose toxicity studies for the product. In the repeat-dose toxicology studies in which cells were administered through the perianal route, a slight oedema/erythema lasting between 48-72 h post-injection was noted at the injection site of most animals including controls. Microscopically, aggregates of mesenchymal cells (presumably areas of injected cells) and focal or multifocal granulation tissue were noted in rectal submucosa and to a lesser extent in adventitia, suggesting a host reaction. Overall, the treatment shows a good local tolerance.

Other toxicity studies

Immunogenicity

Study, ID, GLP status	Test system	Findings
Immunogenic potential of eASC, Study CX-FSR-R&D-1	eASC were incubated with PBMCs, activated PBMCs or conditioned supernatants from activated or non-activated PBMCs. eASC were	No expression of costimulatory molecules is found in any of the conditions. However, cells are activated with the subsequent upregulation of MHCI/II when they are in contact with activated

, non-GLP	subsequently analyzed for cell-surface expression of MHC class I/II and co-stimulatory molecules.	PBMC or in the presence of conditioned supernatant of activated PBMC.
Natural Killer cells and eASC crosstalk, CX-SR-R&D-1407, CX-R&D-1412, non-GLP	The ligands expressed on eASC that might bind to the NK cell receptors have been assayed, including CD94/NKG2A-B-C, HLA I, MICA/B, ULBP-1/2/3, CD112, CD155, NKp30 and NKp46. PBMCs were grown for 5 days in supplemented RPMI 1640 and the natural killer (NK) cells were purified (90-95%) by FACS. eASC were used as target cells in a method correlated with NK cell cytotoxicity and in an assay which addresses IFNγ secretion by NK cells.	ASC expressed low levels of HLA class I molecule and also low levels of additional NK-cells activating ligands. NK-cells degranulation rates in response to eASC were very low and not statistically significant compared with unstimulated NK cells. HLA class I blocking was performed and results indicated that antibody mediated masking of HLA class I did not increase NK cell degranulation against eASC. When purified NK cells were cultured with eASC or K562 (positive control cell line susceptible to NK lysis) during 72 hours, IFN-γ production was observed.
T cell recognition of eASC, CX-SR-R&D-1404, CX-SR-R&D-1402, non-GLP	PBLs were activated with anti CD3/CD2/CD28 coated beads or left unstimulated and co-cultured in the presence of alloASCs for 7 days. After three days of resting with fresh medium, half of the cells were co-cultured with a second batch of alloASCs, the other half was left without ASCs. In both conditions anti CD3/CD2/CD28 coated beads were added for another 10 days. An aliquot of cells was harvested at days 0, 10 and 21 for phenotypic characterisation by FACS.	eASC were able to delay or block the maturation to the terminally differentiated effector stage. This modulation of T cell maturation is independent of being autologous or allogeneic. A second exposure to eASC is not sufficiently immunogenic for peripheral blood lymphocytes to generate terminally differentiated effector cells. No signals of generation of specific T cell memory against eASC were seen.

Studies in Support of the Choice of Animal Model

Method

A study was conducted in immunocompetent rats, to assess the potential rejection of human eASC (MG/0069/05). Three groups of female Sprague-Dawley rats (n=5 per group) randomly received a single intradermal injection of vehicle, eASC preparation at a dose level of 1 x 10^6 cells (100 µl cell suspension) or human keratinocytes which acted as the positive control.

Results

All rats injected with eASC or human keratinocytes showed oedema and erythema at the injection site. The occurrence of oedema/erythema was somewhat more pronounced in the group treated with keratinocytes. Microscopic examination of the injection site revealed intradermal and/or subcutaneous inflammation, mainly consisting of lymphocyte infiltrates. Granuloma formation was often observed in the central area of the inflammatory lesion. These findings were recorded in one of two eASC-treated females sacrificed on day 7 and in all eASC-treated females euthanised on day 14. Inflammation at the injection site was also noted in all human keratinocyte-treated females at the two time points.

Although the elimination of cells was not confirmed by immunohistochemical means, the inflammation at the injection site was considered to indicate potential rejection of the human cells by the immunocompetent rats.

Data generated in other toxicity studies show low potential for T-cell mediated eASC recognition, in vitro and delayed NK cell mediated elimination. In addition, capacity of eASC preparations to differentiate into osteocytes or adipocytes was shown to decline with an increase in population doubling level.

2.3.5. Ecotoxicity/environmental risk assessment

Alofisel is a suspension for injection containing expanded human allogeneic mesenchymal adult stem cells extracted from adipose tissue.

The environmental risk presented by darvadstrocel (eASC), due to their human origin that is not altered during the manufacturing process, the excipients and the potential impurities of Alofisel is considered as negligible.

The applicant has provided an ERA including the CV of the expert and provided adequate justification for not performing any studies.

2.3.6. Discussion on the non-clinical aspects

Alofisel has been developed to reduce inflammation in perianal fistulas and thus allowing repair and healing to take place. To this end, the in vitro pharmacology program has uncovered numerous immunosuppressive functions including, increased IDO activity, TLR/IFNγ activation and the induction of regulatory T cells. *In vivo*, no model for anal fistulas was used to provide proof-of-concept data. This was agreed by the CHMP during the 2011 scientific advice. Instead, an experimental model of colitis was used to show the effect of eASC in an inflamed gut and the data provide evidence for proof-of-concept in this model.

A total of 3 biodistribution studies were performed in male and female athymic nude rats. When cells were administered using the perianal + intrarectal route or the intravenous route, human genomic DNA was detected at day 1/2 and day 14. The biodistribution of eASC injected using the perianal + intrarectal route was restricted to the rectum (high signal) and jejunum. When eASC were administered intravenously, human genomic DNA was detected at a high level in lungs of almost all treated athymic rats, which is to be expected when using this route.

There is no information on the likely pathways of homing, migration and elimination of eASCs cells once they enter the systemic circulation in patients with Crohn's Disease. The perineum is a very vascular area and eASC cells may enter the circulation inadvertently through blood vessels & capillaries adjacent to the site of injection, despite the pull-back aspiration procedure used being negative. The potential distribution, homing & persistence is important to understand to determine the sites potentially at risk of serious adverse reactions such as persistence of cells with subsequent mutagenesis or tumourogenicity. Ectopic tissue formation and tumourigenicity has therefore been included in the RMP as important potential risk and will be followed up with the agreed post-authorisation safety study (PASS).

Reproductive and developmental toxicity studies have not been performed for Alofisel because preclinical biodistribution studies indicated no migration and integration of eASC into reproductive organs following administration of eASC via different routes.

Data from toxicology studies show that single doses of 5 x 10^6 cells/rat were well tolerated when administered via the intravenous or subcutaneous route. After perianal + intrarectal or intravaginal administration, the administered dose of 2.5 x 10^6 cells was also well tolerated. In one of the GLP single-dose toxicity study an intravenous dose of 10 x 10^6 cells was associated with mortality and moribund sacrifice during or immediately after dosing, related to pulmonary embolism. The risk for pulmonary effects is also strengthen by the biodistribution data which show that cells administered systemically have the ability to distribute to the lungs

however it is agreed with the applicant that these findings are of limited relevance to humans due to the local clinical administration. No findings indicate effects on central nervous system or cardiovascular functions.

The likelihood of interference of other commonly administered medications on the pharmacodynamics of eASC was investigated in vitro. Inhibition of TNF-α with infliximab did not alter the inhibitory effect of eASC on activated peripheral blood mononuclear cell (PBMC) proliferation. Incubation with MTX did not significantly affect the viability or proliferation of eASC. Furthermore, MTX and AZA did not affect eASC-mediated inhibition of PBMC proliferation.

It is recommended however that the treatment administration procedure be carried out under general or regional anaesthesia. Since Alofisel and local anaesthetics would be administered in close proximity and the effect of local anaesthetics on the injected cells is unknown, the proposed SmPC contains a statement that local anaesthesia is not recommended.

In vitro and *in vivo* studies were performed to determine whether eASC could be tumourigenic. None of these studies indicate a risk for tumourigenicity. However, the relevant use of human cells in immunocompromised animals can be questioned (especially since the cells do not appear to persist for very long) in relation to this risk. This data will be supplemented with long-term follow-up of tumour formation in patients administered Cx601 (Alofisel) under a Post Approval Safety Study (as described in the RMP) which will follow patients for up to five years post administration with primary outcomes of safety including tumourgenicity, ectopic tissue formation and immunogenicity which is endorsed.

In other safety studies eASC showed low potential for T-cell recognition and the capacity of eASC to differentiate into osteocytes or adipocytes were reduced with population doublings. The effect of *ex vivo* expansion on the genetic stability of cells has been assessed in vitro without any indication of carcinogenic potential.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.3.7. Conclusion on the non-clinical aspects

The pharmacology, pharmacokinetics, safety pharmacology and toxicology programs are considered sufficient for marketing authorisation. Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology and repeated dose toxicity. This application is approvable from a nonclinical point of view.

The CHMP endorse the CAT conclusions on the non-clinical aspects as described above.

2.4. Clinical aspects

2.4.1. Introduction

The assessment of efficacy in the sought indication is based on the results of one pivotal Phase III study (Cx601-0302) and one Phase I/IIa supportive study (study Cx601-0101). Patients in study Cx601-0302 were followed for up to 104 weeks. The initial submission included data up to Week 52.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 3: Clinical studies included in the development programme for perianal fistulas

Study ID	No. of study centres / locations	Design	Study Posology	Study Objective	Subjs by arm entered/ compl.	Duration	Gender M/F Mean Age (SD)	Primary Endpoint
Cx601-0302 Completed up to week 24	47 centres Austria Belgium, France, Germany, Italy Netherlands Spain Israel	Rand, Double-blind Parallel group Placebo-contr	$120x10^6$ cells Single dose	Efficacy Safety	Alofisel 107/88 Placebo 105/83	24 weeks	116/96 38 yrs (13.1)	Combined remission (clinical and MRI)
Cx601-0101 Completed	6 centres Spain	Single-arm Open-labelled	$20x10^6$ cells At week 12 additional $40x10^6$ cells if incomplete closure	Safety Efficacy	Alofisel 24/16	24 weeks	11/13 36 yrs (9.0)	Treatment related AEs

2.4.2. Pharmacokinetics

No clinical pharmacokinetic studies (absorption, distribution, metabolism and excretion) have been performed during the development program of Alofisel. Conventional studies of absorption, distribution, metabolism and excretion are not considered relevant in accordance with the CHMP guideline on human cell-based medicinal products (EMA/CHMP/410869/2006).

For results on biodistribution studies please refer to the non-clinical part of this assessment report.

2.4.3. Pharmacodynamics

Mechanism of action

The proposed mechanism of action for allogeneic eASC is based on immune modulatory and anti-inflammatory properties. Anal fistulas typically present as fissures penetrating the intestinal lumen and perianal skin surface, and are characterised by local inflammation that is exacerbated by bacterial infection(s) and faecal contamination. In the inflamed area, there is lymphocyte activation and local release of inflammatory cytokines. Inflammatory cytokines, in particular IFN-γ produced by activated immune cells (i.e., lymphocytes), activate eASC in a mechanism which requires induction of indoleamine 2,3-dioxygenase expression. Once activated, eASC suppress proliferation of lymphocytes and inhibit the release of pro-inflammatory cytokines. This immunoregulatory activity reduces inflammation, which may allow the tissues around the fistula tract to heal.

Primary and Secondary pharmacology

No conventional PD studies have been performed. Information regarding the mechanism of action of Alofisel has been obtained from nonclinical studies. A summary of the known information is provided below.

Mesenchymal stem cells are known to possess immunomodulatory properties and to regulate the function (proliferation, activation and effector function) of a broad number of immune cells, including B-lymphocytes, T-lymphocytes, natural killer cells, monocytes, macrophages, dendritic cells and neutrophils (Bernardo and Fibbe, 2013; Le Blanc, 2012).

In vitro studies that were conducted to elucidate the cellular and molecular mechanisms underlying the immunomodulatory effects of Alofisel showed the following:

– Co-incubation of activated peripheral blood lymphocytes and eASC resulted in inhibition of T-lymphocyte proliferation (both CD4+ and CD8+ subsets), in a dose-dependent fashion. eASC are activated by inflammatory mediators (principally interferon-gamma) produced by actively proliferating lymphocytes. In response to interferon-gamma, eASC induce the expression of the cytoplasmic enzyme, indoleamine 2,3-dioxygenase (IDO). IDO mediates the degradation of tryptophan and the accumulation of kynurenine. The depletion of tryptophan and the accumulation of kynurenine in the surrounding milieu affects T-lymphocytes (and other immune cells) and results in inhibition of T cell function and proliferation (De La Rosa et al., 2009; Menta et al., 2014).

-Regulatory T cells (CD4+CD25+Foxp3+) play a crucial role in the principle tenance of self-tolerance and in the prevention of autoimmune diseases. Regulatory T cells have been shown to inhibit T cell proliferation, cytokine production, autoantibody production and dendritic cell and monocyte/macrophage function (Pittenger et al., 1999; Ishimura et al., 2008).Exposure of activated peripheral blood mononuclear cells to eASC results in an inhibition of their proliferation together with an increase in the number of regulatory T cells through a mechanism that requires the enzymatic activity of IDO.

-When activated peripheral blood lymphocytes were co-cultured with eASC, production of pro-inflammatory cytokines (e.g. IFN-gamma and TNF-alpha) was reduced and production of anti-inflammatory cytokines (e.g. IL-10) was increased (De La Rosa et al., 2009).

An adequate animal model of fistulas for conduct of *in vivo* efficacy studies is not available. The efficacy of eASC (total intra-peritoneal doses of 0.3 million cells or 1 million cells) has been investigated, however, in the TNBS-induced colitis model (Gonzalez et al., 2009).The following was demonstrated:

– Reduced levels of inflammatory cytokines (TNF-alpha, IFN-gamma, IL-6, IL-1beta, and IL-12) and chemokines (RANTES and macrophage inhibitory protein 2) and increased levels of the anti-inflammatory cytokine, IL-10, were observed in the colons of Alofisel-treated mice in comparison with the colons of untreated mice.

– Increased numbers of regulatory T cells (CD4+ CD25+Foxp3+) were observed in the mesenteric lymph nodes of eASC-treated mice. Purification *ex vivo* of CD4+ T cells (comprising CD4+ inflammatory cells and regulatory T cells) from eASC-treated mice and injection into TNBS colitic mice, reduced the severity of colitis, showing that the regulatory T cells generated or activated during eASC treatment have therapeutic effect *in vivo.*

-A dose-dependent reduction in body weight loss was observed. Administration of eASC was also associated with increased survival relative to vehicle-treated mice.

2.4.4. Discussion on clinical pharmacology

Due to the nature of the product, and the topical route of administration, conventional pharmacokinetic studies of absorption, distribution, metabolism and excretion (ADME) were not performed which is considered acceptable.

It is not currently possible to track the biodistribution of eASC when topically applied in humans, the Applicant has provided a discussion with supporting preclinical studies & literature outlining the current evidence for the likely length of persistence of eASC in humans and the tumourigenicity and ectopic tissue formation are followed up as important potential risks in the RMP (please refer to discussion on non-clinical aspects).

In vitro interaction studies and data from the clinical trials to examine the potential effect of immunosuppressant treatment on viability and immunomodulatory properties of the cells in vivo did not indicate an effect.

The CHMP endorsed the CAT discussion on the Clinical pharmacology as described above.

2.4.5. Conclusions on clinical pharmacology

The marketing authorisation application is approvable from a pharmacology point of view.

The CHMP endorse the CAT assessment regarding the conclusions on the Clinical pharmacology as described above.

2.5. Clinical efficacy

2.5.1. Dose response study(ies)

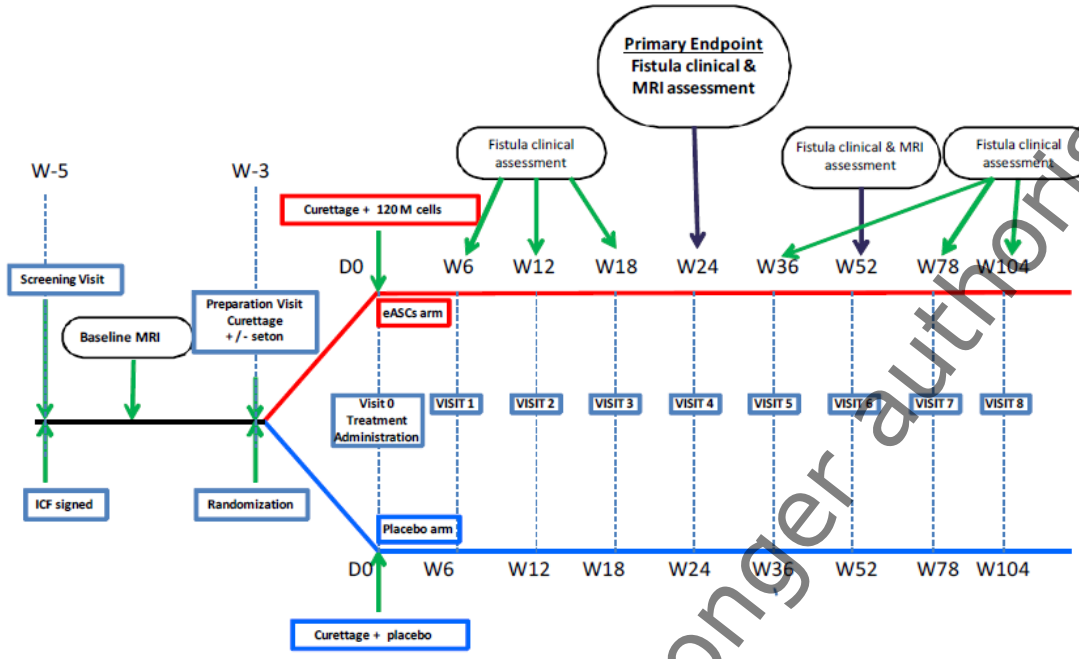
No separate dose response studies have been performed. The selected dose (120 x 10^6 cells) is based on data from the supportive Phase I/IIa study Cx601-0101 where one fistula per patient was treated. The initial dose of $20x10^6$ cells was considered sub-optimal since only 8 of 21 responded to the treatment. Following a second higher dose of $40x10^6$ cells the numbers of responding patients increased. Since up to three fistulas were intended to be treated in the pivotal study and the dose should be distributed between the areas, a three-fold increase of the dose was considered relevant. The chosen dose was administered to all patients independent of numbers and complexity/size of fistulas.

2.5.2. Main study

A phase III, randomised, double blind, parallel group, placebo controlled, multicentre study to assess efficacy and safety of expanded allogeneic adipose-derived stem cells (eASC) for the treatment of perianal fistulising Crohn's disease over a period of 24 weeks and an extended follow-up period up to 104 weeks (ADMIRE-CD study).

Methods

Figure 1: Overall study design



Study Participants

The included patient population had CD and complex perianal fistulas refractory to conventional therapy (antibiotics, immunosuppressants) or anti-TNFs. Patients that were naïve to specific treatment for perianal fistulising CD were excluded and the proportion of patients that was refractory to antibiotics was limited to 25%.

The main enrolment criteria were the following:

Inclusion criteria

1. Signed informed consent.
2. Patients with CD diagnosed at least 6 months earlier in accordance with accepted clinical, endoscopic, histological and/or radiologic criteria.
3. Presence of complex perianal fistulas with a maximum of 2 IOs and a maximum of 3 EOs, assessed by clinical assessment and MRI. Fistulas must have been draining for at least 6 weeks prior to inclusion.

A complex perianal fistula was defined as a fistula that met one or more of the following criteria during its evolution:

- High inter-sphincteric, trans-sphincteric, extra-sphincteric or supra-sphincteric.
- Presence of ≥ 2 EOs (tracts).
- Associated collections.

4. Non-active or mildly active luminal CD defined by a Crohn's Disease Activity Index (CDAI) score ≤ 220.
5. Patients of either sex aged 18 years or older.
6. Good general state of health according to clinical history and a physical examination.

7. Women of a childbearing age must have had a negative serum or urine pregnancy test (sensitive to 25 IU human chorionic gonadotropin [hCG]). Both men and women had to agree to use appropriate birth control methods defined by the investigator.

Further, for inclusion in the study, treatment failure to at least one of the following, antibiotics, immunosuppressants or anti-TNF agents, was defined as follows:

- Antibiotics (recommended treatments were ciprofloxacin and metronidazole): no therapeutic effect after one month
- Immunosuppressants (azathioprine (2-2.5 mg/kg), 6-mercaptopurine 1-1.5 mg/kg)): no response after 3 months
- Anti-TNF agents: no response either 12 weeks after initiation of induction treatment or loss of response after 12 weeks of maintenance treatment under a stable dose

Exclusion criteria (selected)

Patients were excluded for the following reasons:

1. Presence of dominant luminal active CD requiring immediate therapy.
2. CDAI > 220.
3. Concomitant rectovaginal fistulas.
4. Patients naïve to specific treatment for perianal fistulising CD including antibiotics.
5. Presence of an abscess or collections > 2 cm, unless resolved in the preparation procedure (Week -3 to Day 0).
6. Presence of > 2 internal fistula openings.
7. Presence of > 3 external fistula openings.
8. Rectal and/or anal stenosis and / or active proctitis, if this meant a limitation for any surgical procedure.
9. Patients who had undergone surgery for the fistula other than drainage or seton placement.
10. Patients with diverting stomas.
11. Patients with ongoing steroid treatment or treated with steroids in the last 4 weeks.
12. Renal impairment defined by creatinine clearance below 60 mL/min calculated using
13. Cockcroft-Gault formula or by serum creatinine ≥ 1.5 x upper limit of normal (ULN).
14. Hepatic impairment defined by both of the following laboratory ranges:
    - Total bilirubin ≥ 1.5 x ULN.
    - Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≥ 2.5 x ULN.
15. Known history of abuse of alcohol or other addictive substances in the 6 months prior to inclusion.
16. Malignant tumour or patients with a prior history of any malignant tumour, including any type of fistula carcinoma.

17. Current or recent history of abnormal, severe, progressive, uncontrolled hepatic, haematological, gastrointestinal (except CD), endocrine, pulmonary, cardiac, neurological, psychiatric, or cerebral disease.
18. Congenital or acquired immunodeficiencies.
19. Known allergies or hypersensitivity to antibiotics including but not limited to penicillin, streptomycin, gentamicin, aminoglycosides; HSA (human serum albumin); DMEM (Dulbecco Modified Eagle's medium); materials of bovin origin; local anaesthetics or gadolinium (MRI contrast).

Continuation of previous treatment was allowed and was to be maintained at stable doses (anti-TNFs, immunosuppressants (azathioprine, 6-mercaptopurine, methotrexate), 5-ASA, and medication for other diseases). Oral corticosteroids were required to be tapered and discontinued 4 weeks prior to Visit 0 (day of treatment with IMP).

Patients with flares of luminal CD were permitted to use an oral steroid starting with prednisone/prednisolone at 40 mg, or equivalent, and tapering over 12 weeks.

According to the study protocol, if the patient required a new immunosuppressant or new anti-TNF agent, or required higher doses of their existing immunosuppressant or anti-TNF medication compared to baseline, the patient was to be withdrawn and considered a treatment failure.

Treatments

All fistulas were treated (drainage, curettage and setons as clinically indicated) before treatment with Alofisel (week -3 and at baseline).

The patients received either:

- Alofisel 120 $x10^6$ cells (24 mL containing $5x10^6$ cells/mL) by intralesional injection
- Placebo (saline) by intralesional injection at a volume of 24 mL

The active and placebo products were administered and divided between the internal openings and external openings of the fistulas, with half of the suspension administered into the tissue surrounding the internal opening(s) and the other half administered along the walls of the fistula tract(s).

Objectives

The primary objective of the study was to evaluate the efficacy and safety of Alofisel compared to placebo for the treatment of perianal fistulising CD over 24, 52 and 104 weeks.

Outcomes/endpoints

The primary endpoint was defined as combined remission of perianal fistulising CD and absence of collections > 2 cm of the treated fistula confirmed by MRI images, at week 24. Remission was defined as clinical closure of external openings that were draining at baseline despite gentle finger compression. The MRI images were evaluated by a central laboratory that was blinded to the individual patients' treatment and visit. A collection > 2 cm was considered to be present if at least two out of the three dimensions assessed were > 2 cm. Collections larger than 2 cm that remain despite drainage at baseline are considered to be predictive of treatment relapses.

The MRI images were evaluated by a central laboratory. However, MRI investigations of presence of collections >2 cm were assessed both locally and centrally, although only the central reading was considered for the assessments of the primary outcome.

The assessment of the fistulas was performed by, not only the principal investigator, but also by a second gastroenterologist/surgeon that was blinded to the treatment (was not involved in the treatment and had no access to the patient's records). In case of divergent classifications, the opinion of the second gastroenterologist/surgeon was used.

Key secondary endpoints were:

- Clinical remission (closure of all external openings that were draining at baseline despite gentle finger pressure compression) at week 24
- Clinical response (closure of ≥ 50 % of external openings) at week 24, defined as one opening closed if the numbers of openings at baseline were one or two, and two openings closed if there were three openings at baseline

The clinical assessment of closure was performed as for the primary efficacy endpoint.

Secondary endpoints included time to clinical remission/response, relapse, time to relapse, changes in the Perianal Disease Activity Index (PDAI), IBDQ scores for quality of life evaluations, Crohn's Disease Activity Index (CDAI) and van Assche Score (MRI based score of perianal fistulas).

Sample size

The sample size calculation was based on a previous open-label Phase I/II trial (Cx601-0101) performed with Cx601 and published efficacy rates for placebo in clinical trials with anti-TNFs for the treatment of fistulas in patients with CD.

Placebo efficacy rates ranged from 23% to 26% (response) and from 13% to 19% (remission of perianal fistulising disease) and preliminary results of a previous phase II trial (Cx601-0101) showed that Cx601 showed a fistula closure rate between 53.3% and 56.3%.

With this information, the initial sample size was assumed with a minimum 25% efficacy rate for placebo; and a minimum 50% of efficacy rate for Cx601. However, it was difficult to estimate the placebo effect in this phase III study, because the standard curettage procedure performed for all patients was expected to increase the remission of perianal fistulising disease rate in the placebo group; and so the sample size approach was focused in a safer scenario covering for a potential higher placebo effect (efficacy rates symmetrically as close as possible to 50% while respecting the expected difference of 25%; i.e., 37.5% vs. 62.5% for placebo and Cx601, respectively).

Response rates were used for the sample size calculation of the study as there are no data available with respect to remission of perianal fistulising disease rates in previous studies with eASC. However, it was estimated that the difference between placebo and Cx601 would be maintained (either assessed by response or remission) because Cx601 would also benefit from the standard curettage procedure, and this maintenance of the difference related to remission rates had been observed in previous clinical trials with anti-TNFs.

In summary, the planned sample size to be screened was around 278 patients in order to randomize at least 208 patients (104 receiving Cx601 and 104 receiving placebo; assuming a screening failure rate of 25%). This sample size was considered statistically able to detect a minimum 25% difference in the percentage of patients

with remission of perianal fistulising disease confirmed by MRI, between Cx601 and placebo (α=0.025; β=0.20; power=80%), including a 20% of premature withdrawals.

Randomisation

Patients were randomised 1:1 to receive Cx601 or placebo. Treatments were automatically allocated to a treatment group by the eCRF according to a pre-established randomization list held by the Sponsor, using a stratified allocation with the following criteria:

- Concomitant anti-TNF treatment (yes / no).
- Concomitant immunosuppressants treatment (yes / no).

Blinding (masking)

Since there were visual differences between the active and placebo product a fully blinded study was not deemed practicable. Consequently, a plan for blinding was presented at each site. It was agreed and signed before patients were randomised. The double-blind was preserved by having one Investigator (surgeon) who administered the treatment and another Investigator who evaluated the fistula(s) in a blinded fashion. Surgeons were not permitted to share information about the treatment administered in the surgery procedure with the investigator(s) responsible for following up the patient, but they were to identify the treated fistula (with a drawing) in order to allow the investigator to assess the efficacy and safety during the study visits. Surgeons who administered the treatments were not allowed to participate in any clinical assessment of the same patient's fistula during the study.

Investigators responsible for the patient assessments and the patients remained blinded to the patients' treatment allocation up to the Week 52 analysis. The Sponsor was unblinded from the time-point of the primary efficacy analysis at Week 24. Individual patient's treatment allocation was unblinded to investigator site staff and patients after the Week 52 analysis.

Statistical methods

The following analysis populations were defined for the study analyses:

All Screened Patients

The All Screened Patients population included all patients who attended a screening visit. This population was used for summary of screening failures.

Safety Population

The Safety Population included all the patients who received the study treatment. The Safety Population is the primary analysis set for safety analyses. Safety analyses were performed with patients grouped according to the actual treatment received.

Intention-to-Treat Population

The Intention-to-Treat (ITT) Population included all randomised patients, regardless of their having received the study treatment or having any post-baseline measurements. The ITT Population was the primary analysis set for efficacy analyses. Efficacy analyses were performed with patients grouped according to the randomised treatment assigned.

Modified Intention to Treat Population

The Modified ITT (mITT) Population included all randomised patients who received the study treatment and for whom at least one post-baseline efficacy value was present, independent of the degree of adherence to the protocol. Analyses of all efficacy variables were repeated in the mITT Population for supportive purposes. Efficacy analyses were performed with patients grouped according to the randomised treatment assigned.

Per-protocol Population

The Per-protocol (PP) Population included all randomised patients who adhered to the protocol with no major deviations. The primary efficacy analysis (Combined Remission at Week 24) and the key secondary efficacy analyses (i.e., Clinical Remission and Response by Week 24) were repeated in the PP Population for supportive purposes. Efficacy analyses were performed with patients grouped according to the actual treatment received.

Two PP populations were defined, one based on patients without major deviations in clinical assessments and MRI evaluations and used for analysis of the primary efficacy endpoint and a second based on patients without major deviations in clinical assessments and used for analysis of the key secondary efficacy endpoints.

The final population definitions were agreed and documented at the blind data review meeting prior to unblinding.

Multiplicity

Inference was based on the primary endpoint only on the ITT analysis set. No adjustments for multiple comparisons were made for the primary endpoint in the others populations (e.g. mITT, PP). Multiplicity adjustments for analyses of the two key secondary endpoints for which the primary efficacy endpoint serves as the gatekeeper. The primary efficacy variable was assessed with statistical significance at a two-sided type I error (α) level of 0.025. For the key secondary variables a significance level of 0.05 was applied. Statistical testing on key secondary endpoints was only to be performed if the primary endpoint was significant by 2.5% level.

Analysis Methods

The Combined Remission percentage of perianal fistulising CD by Week 24 was compared between treatment groups using a stratified Cochran-Mantel-Haenszel test (CMH), adjusting for the randomisation strata.

The primary analysis was based on an ITT approach where any patient that recorded a missing value was imputed as a non-response. However, in case of missing clinical assessment by Week 24, the last observation carried forward (LOCF) from the latest earlier post-baseline visit (including an Early Termination Visit, if applicable) applied. In case of missing MRI data by Week 24, if there was an MRI at an Early Termination Visit prior to Week 24 then LOCF applied to this MRI.

If any of the rescue events specified below occurred, the primary and key secondary efficacy endpoints were imputed as a non-response, overriding all other imputation conventions. Rescue events were considered only for primary endpoint and key secondary endpoints at Week 24 in the following circumstances:

- Corticoids at 40 mg prednisone equivalent, for at least 12 weeks (non-response is imputed after 12 weeks of concomitant therapy)
- New anti-TNF (new compared to the baseline add-on therapy of the study) for at least 8 weeks (non-response imputed after 8 weeks of concomitant therapy).

- New immunosuppressant (new compared to the baseline therapy of the study) for at least 12 weeks (non-response imputed after 12 weeks of concomitant therapy).
- Surgical procedure of the treated fistula during the treatment period (non-response after the surgical procedure).

Results

Participant flow

	Cx601 n (%)	Placebo n (%)	Total n (%)
Screened			289
Randomised	107	105	212
Patient who completed the 24 weeks follow-up	88 (82.2)	83 (79.0)	171 (80.7)
Patient who discontinued study before Week 24	19 (17.8)	22 (21.0)	41 (19.3)
Reasons For Discontinuation from Study before Week 24 [1]			
Patient's Decision	0 (0.0)	4 (18.2)	4 (9.8)
Withdrawal of Patient Consent to participate in the study	1 (5.3)	1 (4.5)	2 (4.9)
Wrong Inclusion Criteria	0 (0.0)	2 (9.1)	2 (4.9)
Adverse Event / Serious Adverse Event	7 (36.8)	6 (27.3)	13 (31.7)
Significant Clinical Deterioration [2]	7 (36.8)	4 (18.2)	11 (26.8)
Fistula is not healing or worsening of fistula symptoms [3]	2 (10.5)	0 (0.0)	2 (4.9)
Need of new course of antibiotics for fistula or abscess	0 (0.0)	1 (4.5)	1 (2.4)
Need for a new surgery in perianal region [4]	5 (26.3)	3 (13.6)	8 (19.5)
Major protocol deviation during the 24 weeks study period	3 (15.8)	1 (4.5)	4 (9.8)
Worsening of Crohn's disease requiring change in therapy [5]	3 (15.8)	1 (4.5)	4 (9.8)
Other	1 (5.3)	4 (18.2)	5 (12.2)

Source: Table 14.1.1.1, Table 14.1.1.2.1
[1] Percentages for the reason for discontinuation are based on the number of patients who discontinued in each group.
[2] Significant clinical deterioration of perianal fistula (fistula treatment failure).
[3] The fistula is not healing (or appearance of new fistula) or worsening of fistula symptoms (including appearance or worsening of abscess).
[4] Need for a new surgery in perianal region (e.g. new seton placement).
[5] Worsening of luminal CD requiring change in therapy (new therapy or increase of doses).

A total 19.3 % of patients discontinued before week 24 and 15.6 % discontinued between week 24 and 52. The proportion of discontinuations was similar between the two arms up to week 24 and between week 24 and 52 there were more patients discontinuing from the placebo group as compared with the active group. The major reasons for discontinuations were AEs/SAEs, clinical deterioration and need for new perianal surgery.

Of screened patients, there were 77 that were considered screening failures, see Table 4.

Table 4: Screening failures

Overall
Overall

Screened (n)		289	
Screen Failures (n(%))		77	(26.6)
Reason For Screen Failures (n(%))	Patient's Decision	5	(1.7)
	Withdrawal Of Patient Consent To Participate In The Study	7	(2.4)
	Wrong Inclusion Criteria	23	(8.0)
	Wrong Exclusion Criteria	20	(6.9)
	Adverse Event / Serious Adverse Event	2	(0.7)
	Surgical Procedures For Other Reasons Than Fistulas	1	(0.3)
	Fistula Is Not Healing Or Worsening Of Fistula Symptoms	1	(0.3)
	Other	18	(6.2)

TiGenix S.A.U.: Cx601-0302/CIL-IS/Final/SCR01P.SAS
Produced: 17 November 2015, 10:27
Source: Listings 16.2.2.1, 16.2.2.2

Notes: [1] Table presents number and percentage of patients (n(%))
[2] Percentages are based on the number of patients screened in each centre.

Recruitment

First patient enrolled: 6 July 2012

Data cut-off for the 24 week presentation 27 July 2015

Conduct of the study

There have been five protocol amendments.

Amendment 1 (October 2012). Included clarifications of the study procedure and the length of the study was increased from 24 to 52 weeks.

Amendment 2 (May 2013). Update concerned visit procedures (additional immunological sampling for alloreactivity) and the informed consent process.

Amendment 3 (November 2013). This was an administrative change concerning prolongation of the enrolment period to allow for sufficient numbers of patients to be included.

Amendment 4 (June 2014). This amendment included further increases of the length of the enrolment period and clarification for reasons for study completion (eCRF).

Amendment 5 (December 2014). The follow-up period was increased to be 104 weeks. Further changes included clarification of statistical analyses.

Amendment 6 (May 2016). some points of the protocol might lead to potential misunderstandings and needed to be further clarified. Additionally, there were in the protocol some omissions that needed to be corrected for a correct understanding and compliance.

The final SAP (dated 22 July 2015) was finalised and signed before the 24 analyses.

Baseline data

Table 5: Baseline Demographic Characteristics (ITT Population)

	Cx601 N=107	Placebo N=105	Overall N=212
Gender, n (%)			
Male	60 (56.1)	56 (53.3)	116 (54.7)
Female	47 (43.9)	49 (46.7)	96 (45.3)
Age (years)			
Mean (SD)	39.0 (13.11)	37.6 (13.12)	38.3 (13.10)
Range	18.0, 74.0	19.0, 73.0	18.0, 74.0
Age Group			
≤65 years	104 (97.2)	101 (96.2)	205 (96.7)
66-75 years	3 (2.8)	4 (3.8)	7 (3.3)
76-85 years	0	0	0
≥85 years	0	0	0
Race			
White	100 (93.5)	96 (91.4)	196 (92.5)
Black	4 (3.7)	1 (1.0)	5 (2.4)
Asian	0 (0.0)	0 (0.0)	0 (0.0)
Other	0 (0.0)	1 (1.0)	1 (0.5)
Missing	3 (2.8)	7 (6.7)	10 (4.7)
Weight (kg)			
Mean (SD)	73.93 (15.006)	71.33 (14.922)	72.64 (14.985)

Source: Table 14.1.1.4.2
[1] Percentages are based on the number of patients in the respective group.

Table 6: Baseline disease and treatment history (ITT population)

	Cx601 N=107	Placebo N=105	Overall N=212
Time since diagnosis (years)			
Mean (SD)	12.1 (10.0)	11.3 (8.9)	11.7 (9.5)
Median	10.0	9.2	9.9
Range	0.5, 48.8	0.5, 36.4	0.5, 48.8
Any previous medications for the disease during the last 6 months?			
Yes, n (%)	98 (91.6)	99 (94.3)	197 (92.9)
Previous treatment with antibiotics for the disease			
Yes, n (%)	82 (76.6)	74 (70.5)	156 (73.6)
Previous treatment with immunosuppressants for the disease			
Yes, n (%)	89 (83.2)	77 (73.3)	166 (78.3)
Previous treatment with anti-TNFs for the disease			
Yes, n (%)	83 (77.6)	84 (80.0)	167 (78.8)

Source: Table 14.1.1.6.1 and Table 14.1.1.6.2
[1] Time since CD diagnosis (years) is defined as (Date of Consent – Date of CD Diagnosis)/365.25.
[2] Percentages are based on the number of patients in the respective group.
[3] Any systemic antibiotics, i.e. with route in oral, intravenous, intramuscular.
[4] Immunosuppressants with a dose range of 2-2.5 mg/kg for azathioprine and 1-1.5 mg/kg for 6-mercaptopurine.
[5] Any anti-TNF under stable dose.

A stratification allocation was applied in order to balance concomitant exposure to anti-TNF agents and immunomodulators between treatment groups.

Table 7: Distribution of internal and external openings (ITT population)

Patients with:	Cx601 N=107 n (%)	Placebo N=105 n (%)	Overall N=212 n (%)
0 IOs	0 (0.0)	1 (1.0)	1 (0.5)
1 IO	82 (76.6)	90 (85.7)	172 (81.1)
2 IOs	21 (19.6)	11 (10.5)	32 (15.1)
1 EO	58 (54.2)	73 (69.5)	131 (61.8)
2 EOs	37 (34.6)	25 (23.8)	62 (29.2)
>2 EOs	8 (7.5)	4 (3.8)	12 (5.7)
0 IO and 1 EO	0 (0.0)	1 (1.0)	1 (0.5)
1 IO and 1 EO	55 (51.4)	70 (66.7)	125 (59.0)
1 IO and 2 EOs	23 (21.5)	17 (16.2)	40 (18.9)
1 IO and 3 EOs	4 (3.7)	3 (2.9)	7 (3.3)
2 IOs and 1 EO	3 (2.8)	2 (1.9)	5 (2.4)
2 IOs and 2 EOs	14 (13.1)	8 (7.6)	22 (10.4)
2 IOs and 3 EOs	4 (3.7)	1 (1.0)	5 (2.4)

Source: Table 14.1.1.7.2
[1] Percentages are based on the number of patients in the respective group.

There were no major differences between the groups with regard to baseline demographic data or data related to Crohn's disease.

The numbers of included patients > 65 years was limited (n=7).

The majority of patients were receiving concomitant CD medication at baseline. However, approximately 24 % of the actively treated and 18 % of placebo treated patients did not receive concomitant treatment with either immunosuppressants and/or anti-TNF.

The majority of patients had 1 EO, 54 % in the Alofisel group and almost 70 % in the placebo group. Further, the proportion of patients with ≥2 external fistula openings was limited to overall 12 patients. There are three criteria for the definition of complex fistula of which presence of ≥ 2 EOs is one. Ninety-four percent of the patients fulfilled the criteria for fistula complexity.

Numbers analysed

The number of patients by analysis set is shown in Table 8.

Table 8: Analyses populations at Week 24

	Cx601 n (%)	Placebo n (%)	Total n (%)
All Screened Patients			289
Randomised Patients / ITT Population	107 (100.0)	105 (100.0)	212 (100.0)
Randomised Patients who did not receive treatment	4 (3.7)	3 (2.9)	7 (3.3)
Safety Population	103 (96.3)	102 (97.1)	205 (96.7)
mITT Population	103 (96.3)	101 (96.2)	204 (96.2)
PP Population	86 (80.4)	84 (80.0)	170 (80.2)
PP2 Population	99 (92.5)	95 (90.5)	194 (91.5)

Source: Table 14.1.1.1, Table 14.1.1.2.1, Listing 16.2.2.3

The mITT population included randomised patients that received study treatment and had at least one post-baseline efficacy value recorded.

The PP population included randomised, treated patients with post baseline MRI and fistula assessment, and who adhered to the protocol with no major deviations that affected the primary endpoint.

The definition of the secondary PP population (PP2) was similar to that of the PP population but patients had no major deviations that affected the key secondary endpoints.

Outcomes and estimation

Primary endpoint

Table 9: Combined Remission of Perianal Fistulising Crohn's Disease by Week 24 (ITT Population)

	Cx601 N=107	Placebo N=105
Combined Remission [1], n (%) (95% CI)	53 (49.5) (40.1, 59.0)	36 (34.3) (25.2, 43.4)
Difference in Remission Rate, % (97.5% CI) [2]	15.2 (0.2, 30.3)	
p-value [3]	0.024	

Source: Table 14.1.1.1.1
[1] Combined Remission: closure of all treated EOs that were draining at baseline despite gentle finger compression and absence of collections > 2 cm of the treated perianal fistulas confirmed by centrally blinded MRI assessment.
[2] Calculated using Wald's stratified asymptotic method.
[3] As determined by Cochran-Mantel-Haenszel test adjusted for randomisation strata (use of anti TNF agents or immunosuppressants at randomization).

There was a statistically significant difference between the numbers of patients in combined remission in the active and placebo groups at week 24. The estimated difference between the groups was 15.2 %.

Supportive analyses (post-hoc) of combined remission have been performed using the thresholds of collections > 1.2 cm, > 1.5 cm and > 1.7 cm after re-reading of the MRIs. The results show a consistent difference between the active and the placebo treatment irrespective of cut-off point used, see the table below.

Table 10

Q85 - Table 1 PrimaryAnd Supportive Analyses Of Combined Remission By Week 24 And Week 52 (ITT Population-LOCF) Using Absence Of Collections At Different Cut Offs (Central MRI Re-Reads)

Absence of Collection on Central MRI Cut off Size	Combined Remission (ITT population) n (%) Patients % Difference (97.5% CI); *p value*			
	W24		W52	
	Alofisel N= 107	Placebo N= 105	Alofisel N= 107	Placebo N= 105
>2cm	53 (49.5)	36 (34.3)	58 (54.2)	39 (37.1)
	15.2 (0.2 to 30.3); *p=0.024*		17.1 (3.9 to 30.3); *p=0.012*	
>1.7cm	51 (47.7)	34 (32.4)	56 (52.3)	37 (35.2)
	15.3 (0.4 to 30.2); *p=0.023*		17.1 (3.9 - 30.3); *p=0.012*	
>1.5cm	48 (44.9)	34 (32.4)	54 (50.5)	36 (34.3)
	12.5 (-2.4 to 27.3); *p=0.065*		16.2 (3.1 - 29.3); *p=0.016*	
>1.2cm	46 (43.0)	31 (29.5)	51 (47.7)	34 (32.4)
	13.5 (-1.2 to 28.1); *p=0.045*		15.3 (2.3 to 28.3); *p=0.023*	

- Combined Remission: closure of all treated external openings that were draining at baseline despite gentle finger compression and absence of collections > x cm of the treated perianal fistulas confirmed by centrally blinded MRI assessment
- Difference in Remission rate calculated using Wald's stratified asymptotic method
- For difference in Remission Rate (eASC - Placebo) the p-value from the Cochran-Mantel-Haenszel test, randomisation strata as stratification variable (use of anti TNF agents or immunosuppressants at randomization)
- LOCF rules applied. Treatment failure is imputed after rescue therapy.
- Central MRI readings based on re-read data

Sources: Q85 – Attachment 3 - Table EMA 2.54; Q85 – Attachment 4 - Table EMA 2.66; Q85 –Attachment 5 - Table EMA 2.139, Q85 – Attachment 6 - Table EMA 2.159, Q85 – Attachment 7 - Table EMA 2.141, Q85 – Attachment 8 -Table EMA 2.161, Q85 – Attachment 9 – Table EMA 2.140, Q85 – Attachment 10 – Table EMA 2.160.

The Applicant also performed supportive analyses to evaluate the impact of relapses (imputed as failures) on the clinical outcome. The results were supportive of the primary analyses.

A sensitivity analysis of the primary endpoint using the *local* assessment of the MRI has been performed that was supportive of the primary analysis where the central reading was used.

Secondary endpoints

Combined remission at Week 52

Table 11: Combined Remission of Perianal Fistulising Crohn's Disease by Week 52 (ITT Population)

	Cx601 N=107	Placebo N=105
Combined Remission [1], n (%) (95% CI)	58 (54.2) (44.8, 63.6)	39 (37.1) (27.9, 46.4)
Difference in Remission Rate, % (95% CI) [2]	17.1 (3.9, 30.3)	
p-value [3]	0.012	

Source: Table 14.2.2.1.1.1
[1] Combined Remission: closure of all treated EOs that were draining at baseline despite gentle finger compression and absence of collections > 2 cm of the treated perianal fistulas confirmed by centrally blinded MRI assessment.
[2] Calculated using Wald's stratified asymptotic method.
[3] As determined by Cochran-Mantel-Haenszel test adjusted for randomisation strata (use of anti TNF agents or immunosuppressants at randomization).

Clinical remission at Week 52

Table 12: Clinical Remission of Perianal Fistulising Crohn's Disease by Week 52 (ITT Population)

	Cx601 N=107	Placebo N=105
Clinical Remission [1], n (%) (95% CI)	61 (57.0) (47.6, 66.4)	42 (40.0) (30.6, 49.4)
Difference in Remission Rate, % (95% CI) [2]	17.0 (3.8, 30.3)	
p-value	0.016	

Source: Table 14.2.2.3.1.1, EMA 2.21 (p value)
[1] Clinical Remission: closure of all treated EOs that were draining at baseline despite gentle finger compression.
[2] Difference in Remission rate was calculated using Wald's stratified asymptotic method.

Clinical response at Week 52

Table 13: Response of Perianal Fistulising Crohn's Disease by Week 52 (ITT Population)

	Cx601 N=107	Placebo N=105
Response [1], n (%) (95% CI)	68 (63.6) (54.4, 72.7)	56 (53.3) (43.8, 62.9)
Difference in Response Rate [2], % (95% CI)	10.2 (-3.0, 23.4)	
p-value	0.0145	

Source: Table 14.2.2.5.1.1, EMA 2.21 (p value)
[1] Response: closure of at least 50% of all treated EOs that were draining at baseline despite gentle finger compression.
[2] Imputed as treatment failure, i.e. non-response, due to insufficient post-baseline data.
[3] Difference in Response rate was calculated using Wald's stratified asymptotic method.
[4] LOCF rules applied. Treatment failure is imputed after rescue therapy.

Data from week 52 show statistically significant differences between the groups in favour of Alofisel treated patients for combined remission, remission and response. The difference between the groups in combined remission and clinical remission was 17 % and for clinical response the difference was 10 %.

Time to combined remission by Week 24

Since MRIs was not scheduled until Week 24, this endpoint could not be properly analysed.

Combined remission at Weeks 24 and 52

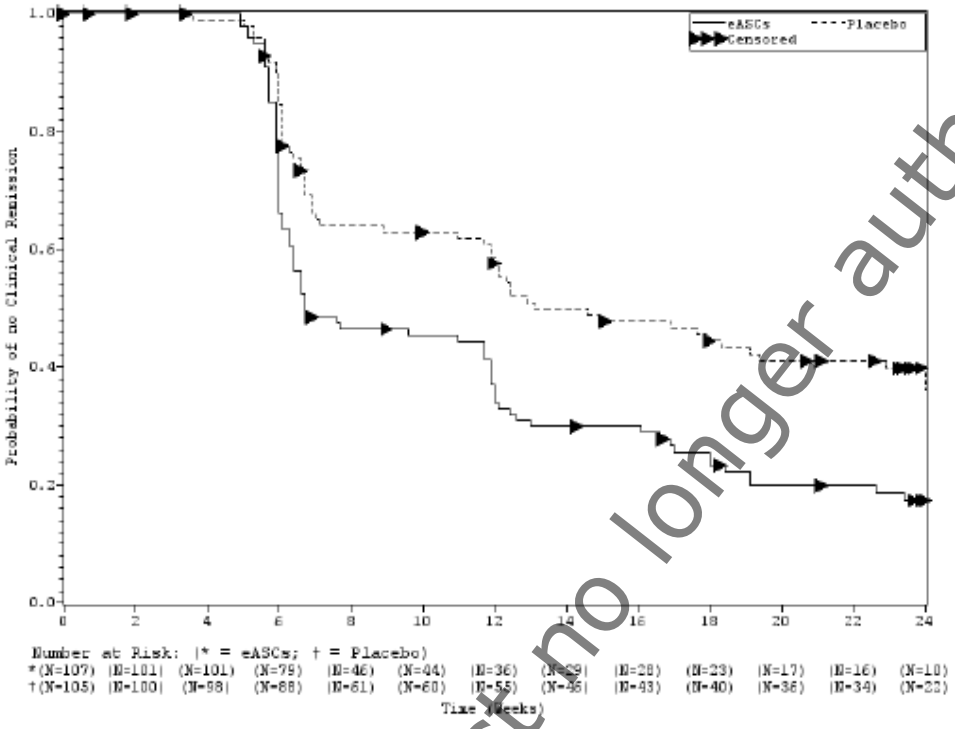
Table 14: Combined Remission of Perianal Fistulising Crohn's Disease at Week 24 and Week 52 (and Week 24 but not at Week 52) (ITT Population)

	Cx601 N=107	Placebo N=105
Combined remission at Week 24 and Week 52 [1]	45 (42.1)	27 (25.7)
Combined remission at Week 24 and not Week 52 [1]	8 (7.5)	9 (8.6)

Source: Table 14.2.2.7.1.1
[1] Combined Remission: closure of all treated external openings that were draining at baseline despite gentle finger compression and absence of collections > 2 cm of the treated perianal fistulas confirmed by centrally blinded MRI assessment. LOCF rules applied

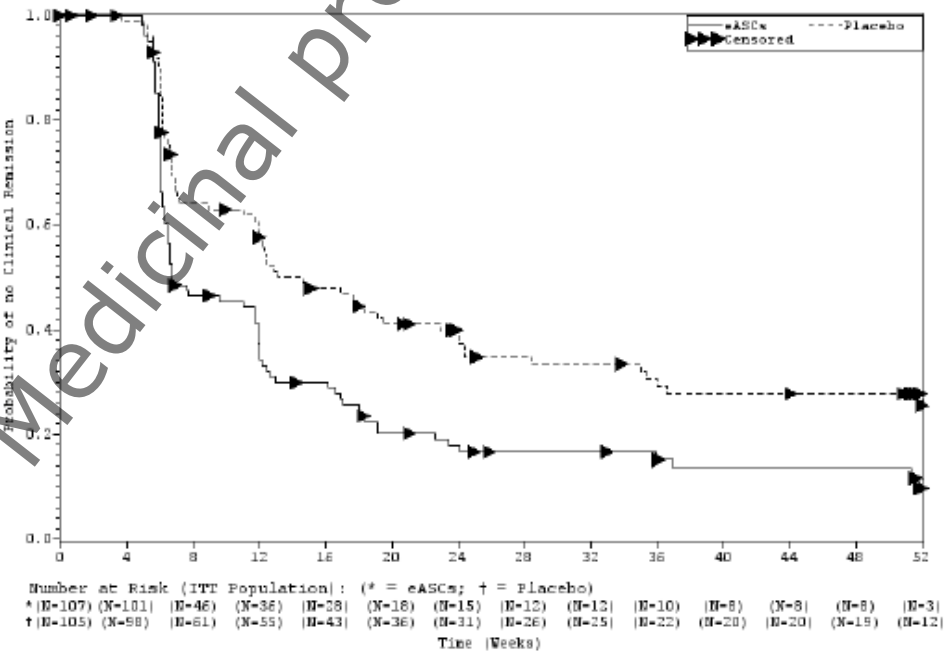
Time to clinical remission by Week 24

Figure 2: Kaplan-Meier Curve of Time to Clinical Remission of Perianal Fistulising Crohn's Disease by Week 24 (ITT Population)



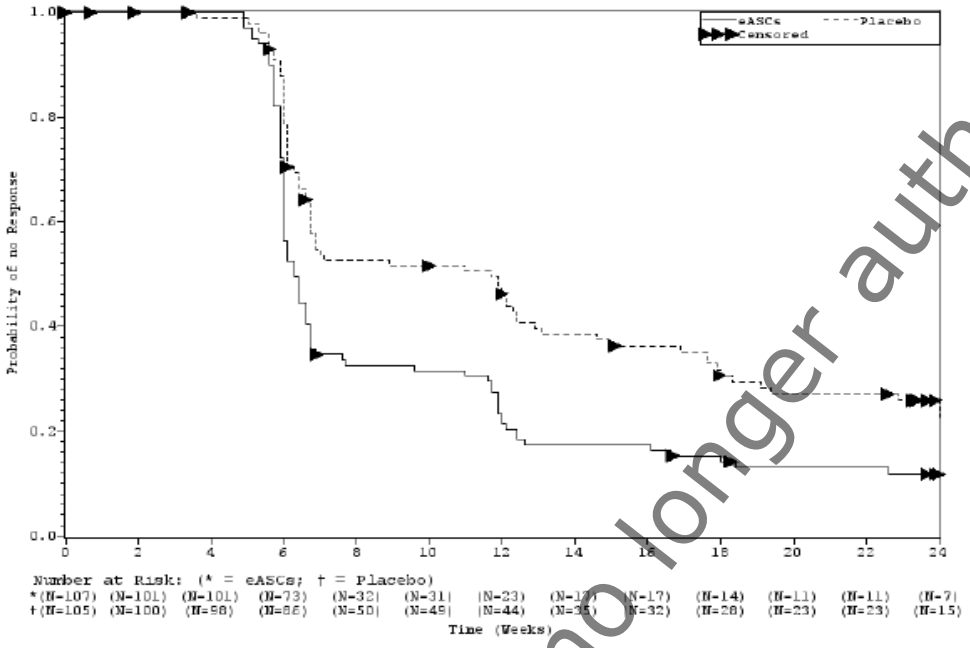
Time to clinical remission by Week 52

Figure 3: Kaplan-Meier Curve of Time to Clinical Remission of Perianal Fistulising Crohn's Disease by Week 52 (ITT Population)



Time to clinical response by Week 24

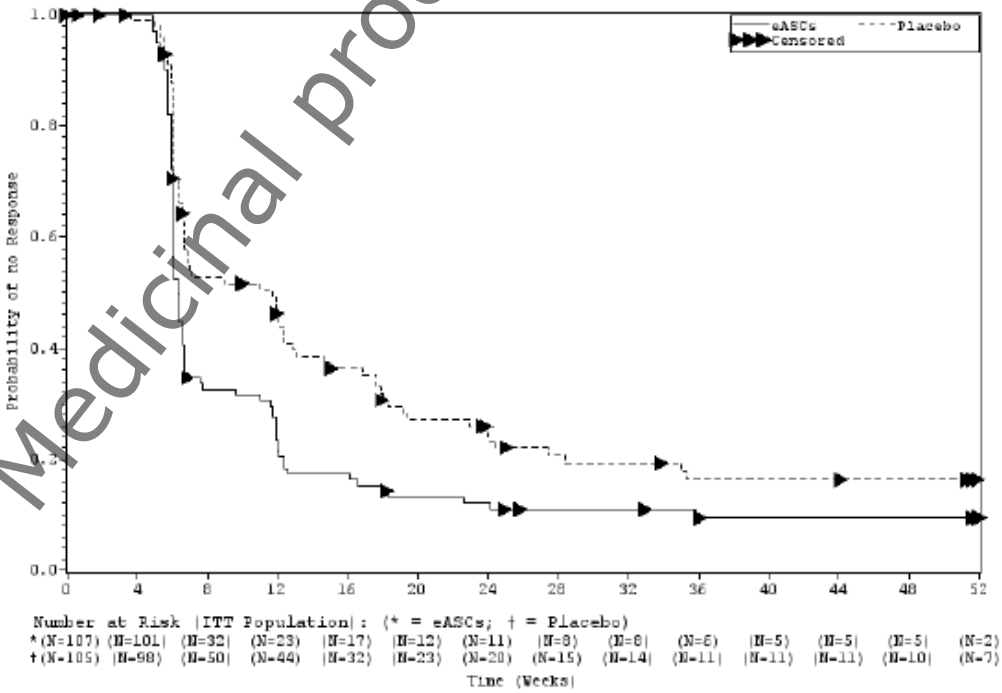
Figure 4: Kaplan-Meier Curve of Time to Response of Perianal Fistulising Crohn's Disease by Week 24 (ITT Population)



Source: Figure 14.2.2.1

Time to clinical response by Week 52

Figure 5: Kaplan-Meier Curve of Time to Response of Perianal Fistulising Crohn's Disease by Week 52 (ITT Population)



Relapse by Week 24

Table 15: Relapse of Perianal Fistulising Crohn's Disease by Week 24 (ITT Population)

	Cx601 N=107	Placebo N=105
Relapse, n (%) (95% CI)	30 (38.0) (27.3, 48.7)	28 (50.0) (36.9, 63.1)
Difference in Relapse Rate	-12.0 (-28.9, 4.9)	

Source: Table 14.1.4.6.1
[1] Relapse: reopening of any of the treated EOs with active drainage as clinically assessed, or development of a perianal collection > 2cm of the treated perianal fistulas confirmed by centrally blinded MRI assessment in patients with clinical remission at previous visit.
[2] Difference in Relapse rates was calculated using Wald's stratified asymptotic method.
[3] LOCF rules applied. Treatment failure is imputed after rescue therapy for Week 24 only.

Relapse by Week 52 in patients with combined remission at week 24

Table 16: Relapse of Perianal Fistulising Crohn's Disease at Week 52 in patients with Combined Remission at week 24 (no LOCF) (ITT Population)

	Cx601 N=52	Placebo N=34
Relapse, n (%) (95% CI) [1]	13 (25.0) (13.2, 36.8)	15 (44.1) (27.4, 60.8)
No relapse	39 (75.0) (63.2, 86.8)	19 (55.9) (39.2, 72.6)
Difference in Relapse Rate [2]	-19.1 (-39.5, 1.3)	-

Source: Table 14.2.2.7.1.1
[1] Relapse: reopening of any of the treated EOs with active drainage as clinically assessed, or development of a perianal collection > 2cm of the treated perianal fistulas confirmed by centrally blinded MRI assessment in patients with Combined Remission at week 24.
[2] Calculated using Wald's stratified asymptotic method.

Time to relapse by Week 24 in patients with Clinical remission

The median time to relapse was similar in the groups, 19 and 18 weeks in the active and placebo groups, respectively by week 24.

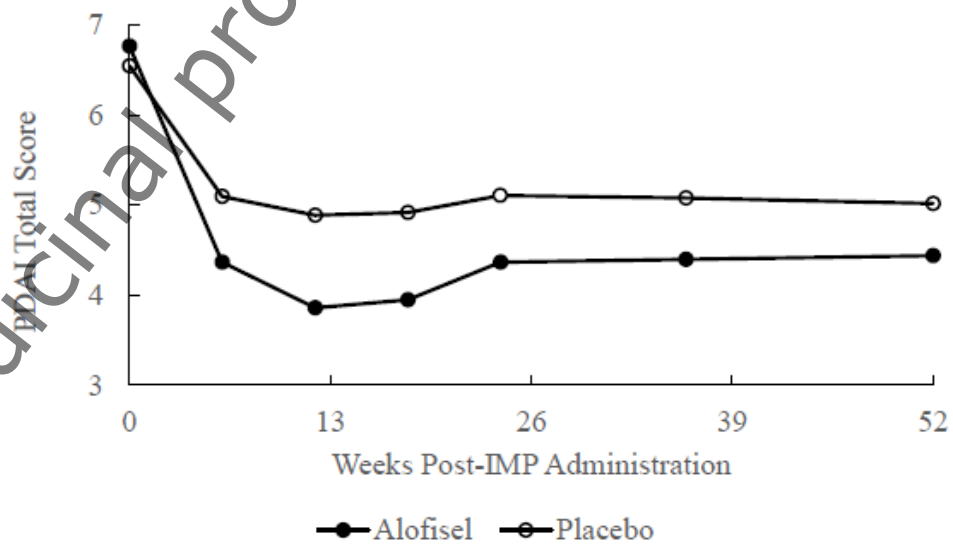
Perianal Disease Activity Index

Table 17: Perianal Disease Activity Index, Total Score, Change from Baseline to Week 24 and Week 52 (ITT Population)

	Cx601 N=107	Placebo N=105	Treatment Difference (95% CI)
Baseline			
N	107	105	
Mean (SD)	6.77 (2.475)	6.55 (2.919)	
Change at Week 24			
N	103	99	-0.840 (-1.844, 0.164)
Mean (SD)	-2.32 (3.846)	-1.34 (3.533)	
Baseline			
N	107	105	
Mean (SD)	6.77 (2.475)	6.55 (2.919)	
Change at Week 52			
N	103	99	-0.699 (-1.738, 0.339)
Mean (SD)	-2.25 (4.144)	-1.43 (3.739)	

Source: Table 14.1.4.9.1.1, Table 14.1.4.9.2.1, Table 14.2.2.9.1.1.1 and Table 14.2.2.9.1.2.1.
[1] Baseline is defined as the last value recorded prior to treatment administration.
[2] If treatment was administered then Early Termination Visit data are reassigned to appropriate visits based on relative day from treatment.

Figure 6: PDAI score up to week 52 (ITT population)



a In case of missing data, last-observation carried forward approach was applied
Abbreviations: ITT= intent-to-treat
Source: CSR-Week 52 Cx601-0302, Table 14.2.2.9.1.1.1

QoL up to Week 24 and Week 52 assessed by IBDQ

There were minor changes in IBDQ scores with a mean increase of 3.81 (25.53) from baseline in patients in the active group and 4.01 (25.56) for patients in the placebo group. The corresponding figures at week 52 were 2.14 (27.42) in the active group and 1.69 (25.01) in the placebo group.

CDAI score up to Week 24 and Week 52

The CDAI score remained similar to those observed at baseline in both groups at week 24. The mean increase (SD) from baseline at week 52 in the Alofisel group was 11.11 (80.54) and 7.62 (67.29) in the placebo group.

Van Assche Score up to Weeks 24 and 52

Mean total score and mean domain remained at similar levels in both groups at weeks 24 and 52.

Ancillary analyses

Comparison of Results in Sub-populations at Week 24

Subpopulation analyses were performed in the ITT patient population of clinical study Cx601-0302 to evaluate factors that may influence the efficacy of Alofisel. The following subpopulations were evaluated:

• Stratification factors: concomitant treatment at randomisation visit with anti-TNF agents only, or with immunosuppressants (azathioprine, methotrexate or 6-mercaptopurine) only, or with anti-TNF agents plus immunosuppressants or with neither anti-TNF agents nor immunosuppressants.

• Age: <65 years or ≥65 years.

• Gender: male or female.

• Race: Caucasian, Black, Asian, or other.

• Smoking status: current smoker, ex-smoker or non-smoker.

• Duration of antibiotic use between preparation visit and administration of IMP: <7 days or ≥ 7 days.

• Topography of external and internal openings: 1 internal opening plus 1 external opening or 1 internal opening plus ≥2 external openings or 2 internal openings (irrespective of number of external openings).

• Topography of external openings: 1 external opening or 2 external openings or 3 external openings.

• Topography of internal openings: 1 internal opening or 2 internal openings.

• Dose per external/internal opening: dose equal to 12 mL/external opening and any dose/internal opening, or dose equal to 6 mL/external opening and ≥12 mL/internal opening, or dose equal to 6 mL/external opening and ≤6 mL/internal opening, or dose equal to 4 mL/external opening and any dose/internal opening.

• Country: France, Spain, Italy or all other countries.

Analyses of baseline features and disease characteristics (including gender, sole immunosuppressant use, duration of prophylactic antibiotic use, country), as well as time since CD diagnosis (> 10 y / ≤10 y), the fistula type (Parks classification), any collections at screening > 2 cm (drained), CDAI at baseline (>150 /≤150), and concomitant medication) did not show a significant effect on the response to Alofisel treatment, based on the current dataset.

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit-risk assessment (see later sections).

Table 18: Summary of efficacy for pivotal study Cx601-0302

Title: A phase III, randomised, double blind, parallel group, placebo controlled, multicentre study to assess efficacy and safety of expanded allogeneic adipose-derived stem cells (eASC) for the treatment of perianal fistulising Crohn's disease over a period of 24 weeks and an extended follow-up period up to 104 weeks. ADMIRE-CD study.				
	Cx601-0302			
Study identifier Design	randomised, double blind, parallel group, placebo controlled			
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		24 weeks 5 weeks 28 and 80 weeks	
	Confirmatory: The Combined Remission percentage of perianal fistulising CD by Week 24 in the Cx601 group is equal to the Combined Remission percentage of perianal fistulising CD by Week 24 in the Placebo group			
Hypothesis Treatments groups	Alofisel		One injection $120x10^6$ cells, number randomized n=107	
	Placebo		One injection, saline, number randomized n=105	
Endpoints and definitions	Primary endpoint	Combined remission	Remission defined as clinical closure of external openings that were draining at baseline despite gentle finger compression, and absence of collections > 2 cm of the treated fistula confirmed by MRI images, at week 24.	
	Key Secondary endpoint	Clinical remission	Closure of all external openings at week 24	
	Key Secondary endpoint	Clinical response	Closure of ≥ 50 % of external openings at week 24	
	27 July 2015			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	Alofisel	Placebo	
	Number of subject	107	105	
	Primary endpoint n (%)	53 (49.5)	36 (34.3)	
	95 % CI	(40.1, 59,0)	(25.2, 43.4)	

	Key secondary remission n (%) 95 % CI	57 (53.3) (43.8, 62.7)	43 (41) (31.5, 50.4)	
	Key secondary response n (%) 95 % CI	71 (66.4) (57.4, 75.3)	56 (53.3) (43.8, 62.9)	

Effect estimate per comparison	Primary endpoint	Comparison groups	Alofisel, placebo
		Differences in combined remission %	15.2
		97.5 % CI	(0.2, 30.3)
		P-value	0.024
	Key secondary remission	Comparison groups	Alofisel, placebo
		Difference in remission %	12.3
		95 % CI	(-1.0, 25.7)
		P-value	0.064
	Key secondary response	Comparison groups	Alofisel, placebo
		Differences in response %	13.0
		95 % CI	(-0.1, 26.1)
		P-value	0.054
Notes	Sensitivity analyses of combined remission in the mITT, Safety and PP populations support a difference between the groups in the magnitude of 13-14 %.		

Supportive study

The supportive study Cx601-0101was an open-labelled pilot study to assess the safety and efficacy of Alofisel in the treatment of complex perianal fistulas in perianal Crohn's disease. The inclusion and exclusion criteria were appropriate. Patients in this supportive study could be naïve to fistula treatments and were not required to be refractory to antibiotics or immunosuppressants or to anti-TNF agents. During the study period patients were treated with standard care excluding infliximab or any other anti-TNF, tacrolimus or cyclosporine.

The patients received intralesional administration of Cx601 (eASCs) $20x10^6$ cells at day 0 and for patients with incomplete closure of the fistula at week 12, a second dose of Cx601 containing $40x10^6$ cells was administered. Injections followed surgery standards including curettage with special emphasis of intersphincteric tracts and closure of internal openings with a stitch.

The primary endpoint was the incidence of treatment emergent adverse events (TEAEs). Secondary endpoints included the following safety variables: serious TEAEs, treatment-related serious TEAEs, TEAEs leading to withdrawal, changes in laboratory parameters and vital signs. Evaluations at weeks 12 and 24 were performed to evaluate reduction of numbers of draining fistulas, increased numbers of closed fistula, closure of external openings, MRI fistula healing, luminal relapse, PDAI, CDAI and MRI Score of Severity.

Baseline demographic data are presented in the table below.

Table 19: Demographic and baseline characteristics. Safety/Full analysis population

Variable	eASCs N=24
Sex (male)[A]	11 (45.8%)
Age (years)[B]	36.00 (9.03)
Race (Caucasian)[A]	24 (100.0%)
Height (cm)[C]	166.00 (1.50, 1.90)
Weight (kg)[C]	72.00 (53.25, 84.60)
Number of fistula tracts[A]	
One	15 (62.5%)
Two	6 (25.0%)
Three	3 (12.5%)
Number of external openings[A]	
One	18 (75.0%)
Two	4 (16.7%)
Three	2 (8.3%)
Location[A]	
Extra-sphincteric	1 (4.2%)
Inter-sphincteric	5 (20.8%)
Supra-sphincteric	1 (4.2%)
Trans-sphincteric	17 (70.8%)
Extension[A]	
Infraelevator	19 (86.4%)
Supraelevator	3 (13.6%)
Rectum wall involvement	
Enlarged	1 (4.2%)
Normal	5 (20.8%)
Not involved	18 (75.0%)
Presence of ulcers[A]	
0	20 (83.3%)
1	4 (16.7%)
Ulcerated surface[A]	
0	20 (83.3%)
1	4 (16.7%)
Affected surface[A]	
0	18 (75.0%)
1	6 (25.0%)
Presence of narrowings[A]	
0	21 (87.5%)
1	2 (8.3%)
2	1 (4.2%)
Number of affected segments[A]	
0	19 (79.2%)
1	5 (20.8%)

A) n (%)
B) mean (s.d)
C) median (interquartile range)

The result of the efficacy analysis is presented in the table below.

Table 20: Reduction in the number of draining fistulas at 12 and 24 weeks. Per protocol and Safety/Full analysis populations

Per protocol population	Number	eASCs n=22
After 12 weeks	0	7 (36.8%)
	1	10 (52.6%)
	2	2 (10.5%)
After 24 weeks	0	4 (33.3%)
	1	7 (58.3%)
	2	1 (8.3%)
Safety/FA population	Number	eASCs n=24
After 12 weeks	0	8 (40.0%)
	1	10 (50.0%)
	2	2 (10.0%)
After 24 weeks	0	4 (30.8%)
	1	8 (61.5%)
	2	1 (7.7%)

All 24 patients received Alofisel at a dose of 20 million cells and 15 patients received a second dose. The second dose of Alofisel was 40 million cells for all patients except for one, who received a second dose of 20 million cells. In the evaluation of the efficacy of Alofisel following the second dose, this patient is included with the patients who received doses of 40 million cells.

Absence of collections >2 cm was reported in 100% of patients at 12 and 24 weeks, in both the per protocol and the full analysis populations.

At week 12, following a dose of $20x10^6$ cells, 38 % (8/21) had closure of the external opening (assessed clinically) and 29% (6/21) had an increase in the number of closed fistulas (assessed clinically and radiologically). Of the nine patients that received one dose only, closure of external openings were observed in 50 % (4/8) at week 12 and in 100 % (4/4) at week 24. Corresponding figures for patients receiving two doses were 31 % (4/13) week 12 and 42% (5/12) week 24.

No patients presented luminal relapse at 12 weeks in any of the two populations. At 24 weeks, 5 patients reported luminal relapse (22.7% and 20.8% of patients in the per protocol and full analysis populations respectively).

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The present application concerns the use of expanded human allogenic stem cells extracted from adipose tissue (Cx601, Alofisel) in the treatment of complex perianal fistula(s) in adult patients with non-active/mildly active luminal Crohn's disease, when fistula(s) have shown an inadequate response to at least one conventional or biologic therapy.

No formal dose response study has been performed. In support of efficacy, the results of one pivotal Phase III study (Cx601-0302) and one Phase I/IIa supportive study (study Cx601-0101) has been submitted.

The dose was based on the results from the supportive study where one fistula was treated. All patients received $20x10^6$ cells at day 0 and for patients with incomplete closure of the fistula at week 12; a second dose $40x10^6$

was administered. In the pivotal study, patients with up to three fistulas were to be treated and it was reasoned that three times the second dose $40x10^6$ used in the supportive study was expected to be sufficient.

The use of a fixed dose, regardless of numbers and tract size of fistulas has been further discussed. Since it is not feasible to measure the length of single fistula tracts, the dose selected is based on data from the phase II supporting study, although the number of patients was limited. Analyses have been presented of the outcome of the primary endpoint in subpopulations with different numbers of internal and external openings. No consistent differences are revealed in numbers with combined remission at week 24.

The *pivotal study* was a double-blind placebo-controlled study in patients with CD and complex fistulas refractory to conventional therapy. Following initial surgical treatment (including draining of collections, curettage and setons when appropriate), the patients were treated with intralesional injections with either Alofisel or placebo. Thereafter patients were clinically assessed every sixth week up to week 24 when also a MRI assessment was performed. The primary efficacy endpoint was combined remission that included clinical closure of external openings and absence of collection > 2 cm as visualized by MRI at week 24.

The primary endpoint was recommended by CHMP to be defined as complete closure of fistulas (complete healing with complete MRI based closure of the fistulas) and maintenance of closed fistulas without the development of new fistulas (EMA/CHMP/SAWP/240241/2011). The Applicant chose to use a cut-off i.e. MRI confirmed absence of collections > 2 cm rather than complete closure of fistula. The Company has clarified that there is no established criteria available defining complete fistula healing on MRI. A threshold of 2 cm is of relevance since in the absence of clinical symptoms, persistence of smaller collections should not be considered as failures. Further, inclusion of this criterion as part of the primary endpoint adds stringency to the endpoint, since in patients where drainage has been achieved persistence of large abscesses predicts long-term failures. Further, the minimum size of collections that can be reliably measured on the MRI is > 0.8 cm. Additional analyses have been presented of combined remission in the ITT population using cut-off points from 1.2 to 2 cm. The new analyses support that there is a statistically and clinically relevant consistent difference in combined remission between active and placebo treatment irrespective of cut-off point used, which is reassuring

Clarifications were requested regarding the appropriateness of the studied population in relation to fulfilment of the different inclusion criteria. With regards to the fulfilment of criteria for complex fistulas this has been further clarified. Complex fistulas are defined as high (high intersphincteric or high transsphincteric or extrasphincteric or suprasphincteric origin of the fistula tract), may have multiple external openings, may be associated with the presence of pain or fluctuation to suggest a perianal abscess, may be associated with the presence of a rectovaginal fistula, may be associated with the presence of an anorectal stricture, and may be associated with the presence of active rectal disease at endoscopy (Sandborn et al, Gastroenterology 2003). Data has been presented showing that ninety-four percent of the patients fulfilled the criteria for fistula complexity. Further, comparisons between the definition in the present study compares to the definition(s) presented in the literature and/or generally recognized and utilized within the profession has been presented showing that the definitions are comparable.

The appearance of the active and placebo products were different due to the cell content of the active product. Although it was stated that all efforts were made to keep the study blinded there were differences in exposure between the groups that could be a result from this difference in appearance. It has been clarified that the exposure errors concerned eight patients of which 7 were in the placebo group. However, the patients were included at 7 sites in 5 different countries and is agreed that the clustering of patients to the placebo group most likely occurred at random.

Three GCP inspections were conducted by EMA in relation to the MAA and comprised 5 inspection sites in total, including clinical investigator sites, CROs and the Sponsor. The detected departures from GCP and deficiencies related to trial management were overall considered by the inspection team to have no impact on the inspected trials' data reliability and validity.

Efficacy data and additional analyses

The results from the pivotal study showed that there was a statistically significant difference between the numbers of patients in combined remission in the active and placebo groups. The combined remission in the active group was 49.5 % (53/107) (95% CI, 40.1, 59.0) and the corresponding figures in the placebo group were 34.5 % (36/105) (25.2, 43.4). The difference between the groups was 15.2 % at week 24. Key secondary endpoints were clinical remission (closure of all external openings that were draining at baseline despite gentle finger pressure compression) and clinical response (closure of ≥ 50 % of external openings) at week 24. The result was supported by the key secondary and other secondary endpoints although differences were not statistically significant.

Updates for the pivotal study have been provided as part of the response to the Day 120 LoQ. This includes data up to Week 52. Patients were clinically assessed at weeks 36 and 52. At week 52 a MRI assessment was also performed. Presented data from week 52 were supportive of the primary analyses. Statistically significant effects in favour of Alofisel treatment is shown for combined remission, clinical remission and response. Further, the relapse rate in patients in clinical remission at any previous visit at week 24 was 38 % (30/79) in the active group and 50 % (28/56) in the placebo group. The corresponding figures at week 52 for patients with combined remission at week 24 were 25 % (13/52) and 44 % (15/34), respectively. Thus, the primary outcome is supported by data from the long-term follow-up to 52 weeks. Although there is a moderate difference between the treatment groups, the effect is considered to be clinically meaningful when other treatment options for fistulas have failed.

For patients that were not receiving concomitant CD treatment at baseline (n=26, 24.3 %) it was not clear if treatment failures were connected with their current fistula. Results of post-hoc analyses have been presented showing that there was no major difference in response between patients with or without concomitant medication..

Subgroup analysis was performed on subjects who were on immunosuppressive treatment at randomisation and subjects were only excluded from the analysis if they had received greater than 8-12 weeks rescue therapy. The Applicant has clarified that there were 13 patients requiring increased doses of immunosuppressants or anti-TNFs. Of these, four patients required an increased dose for less than 12 weeks and were not regarded as treatment failures. Analyses have been presented on combined remission and clinical remission at week 24 with treatment failure imputed after rescue medication. No obvious impact on the results was observed.

The current data did not detect a difference in relapse rate; active treatment did not result in fewer SAEs of fistulas/perianal abscess as compared with placebo, as might be anticipated. This most likely should be interpreted as a lack of efficacy in some patients. Analyses of various baseline and disease characteristics by the Applicant did not indicate any significant interactions, though the proportion of patients with multiple tract fistulas in the Alofisel group (44.8%) was noted to be numerically higher than in the Control group (29.6%), which might have somewhat disfavoured the active group.

The potential impact of SAEs of fistulas/perianal abscess has been further evaluated by imputing failure to treatment for all patients with TESAEs connected with fistulas and abscesses. Results for combined remission

and clinical remission at weeks 24 and 52 are supportive of the primary analyses showing statistically significant differences between the treatment groups.

The lack of data specifically on closure of internal fistula openings (IOs) was also questioned and it was clarified by the applicant that this relates to the limited sensitivity of the MRI and the surgical investigative approach of IOs that could interfere with healing. The explanation was considered acceptable by the CAT.

Considering week 24 data only there were uncertainties with regard to the clinical benefit of the treatment also considering that there was a minimal change in PDAI score and no observed difference in the QOL before and after treatment with Alofisel, which were measured as secondary endpoints of the study. The applicant could demonstrate that although the reductions in PDAI score are limited, the reductions remain at similar levels throughout week 52 with maintained differences between the groups. There was a reduction in both the active and placebo group, in the placebo group most likely due to the initial fistula treatment. Further, with regards to the QOL the Applicant is pointing out that the high scores at baseline are reflective of the patient populations with luminal disease in remission. Also, only two of the PDAI scores are reflective of signs and symptoms of fistulas. The questionnaire used for estimation of QoL was based on the Inflammatory Bowel Disease Questionnaire (IBDQ) that includes domains applicable to luminal disease and is not reflective of perianal fistulas. It was noted that there is no validated questionnaire for evaluation of QoL in connection with perianal fistulas.

Data from week 104 has been submitted. For patients that completed the week 52 follow-up and entered the 104 weeks follow-up (25 Alofisel, 15 placebo), the rate of clinical remission was 56 % and 40 % in the active and placebo group, respectively. However, data was limited and patient number small and unevenly distributed between groups.

The *supportive study* was an open-labelled pilot study with the primary aim to assess the safety of expanded adipose derived stem cells (Cx601) in the treatment of complex perianal fistulas in adult patients with CD (n=24). Secondary endpoints included clinical and MRI evaluations of fistulas at weeks 12 and 24. Patients were treated to obtain abscess drainage and reduction of symptoms followed by intralesional injections of Cx601. The inclusion and exclusion criteria deviated from those in the pivotal study i.e. patients were not required to be refractory to antibiotics, or immunosuppressants or to anti-TNF agents and there were different definitions of complex fistulas in the two protocols.

One third of patients responded to treatment at week 12. However, the numbers of patients receiving one dose (n=9) or two doses (n=15) are too low for any meaningful conclusions to be drawn also because patients with missing data were excluded from the analyses rather than being considered as failures.

Overall, there is insufficient data available on the effect and safety of repeated Alofisel administrations, whereas the need for additional treatment may be anticipated in a portion of the targeted population. This issue will be further evaluated in the planned PASS study which is considered appropriate. The Company has further presented a protocol for study Cx601-0303 that has been approved by the FDA. This is an ongoing pivotal Phase III study for the purpose of obtaining MA in the US. This study will include a patient population similar to that of the EU pivotal study and the main design features are also similar. The results will thus be of value in support of the efficacy of Alofisel in the treatment of fistulas.

The CHMP endorse the CAT discussion on clinical efficacy as described above.

2.5.4. Conclusions on the clinical efficacy

The difference between active and placebo treated groups in combined remission was approximately 15 % at week 24. Data has been presented from the long-term follow-up of the pivotal study up to week 104. Data are supportive of a consistent and statistically significant effect of Alofisel as compared with placebo. The observed effect size compared to control appears modest but is considered to be clinically meaningful as other treatment options for fistulas have failed. The clinical implications of the comparative incidence of anal abscess and fistula AEs between the groups do not appear to indicate a safety issue but rather lack of efficacy in some patients. Overall, the benefit-risk balance concluded to be positive from an efficacy point of view. However, considering the modest effect size together with the approval setting with a single pivotal study, confirmatory information on efficacy is considered of importance for the benefit-risk of the product and will be obtained from the ongoing Phase III study Cx601-303 (final CSR expected in 2022). Thus, the approval of Alofisel should be subject to the submission of study Cx601-303, which is similar in design to the pivotal study of this application, as an Annex II condition in line with Article 14 of Regulation (EC) No 1394/2007, as a post-authorisation follow-up of efficacy.

The CAT considers the following measures necessary to address issues related to efficacy:

Description	Due date
In order to follow-up on the efficacy of Alofisel, the MAH should submit the results of a Phase III randomised double-blind, placebo-controlled study *Cx601-0303* investigating a single administration of Cx601 for the treatment of complex perianal fistulas in Crohn's disease patients.	Final Report to EMA: 2Q/3Q 2022

The CHMP endorse the CAT conclusion on clinical efficacy as described above.

2.6. Clinical safety

Patient exposure

The Safety Population consisted of all included patients who received study treatment (Alofisel or placebo in the pivotal study Cx601-0302 or Alofisel in the supportive study Cx601-0101).

Overall, a total number of 236 patients were enrolled in the Alofisel clinical programme. Of these, 127 patients received Alofisel (103 in study Cx601-0302 and 24 in study, Cx601-0101).

In study Cx601-0302, all but one patient in the Alofisel group received the full dose of 24 mL (i.e., 120 million cells); one patient received 18 mL Alofisel. In the placebo group, 2 patients received a total of 20 mL of saline and 5 patients received 12 mL saline, instead of 24 mL. The median duration of follow-up (51.7 weeks and 52.0 weeks, respectively) was very similar for the treatment groups.

In study Cx601-0101, all 24 patients received an initial dose of 20 million cells. Of the 15 patients who received a second dose of Alofisel, 14 received the intended dose of 40 million cells and one patient received 20 million cells.

A summary of baseline characteristics of the pivotal Safety Population is provided in the table below. There were proportionally more patients in the Alofisel group with multiple-tract fistulas (46.6% in the Alofisel group versus 30.4% for placebo). Otherwise, the treatment groups were fairly well balanced.

The population of Study Cx601-0101 was similar but patients were not allowed to receive concomitant anti-TNF agents.

Table 21: Disease Characteristics at Baseline – Pivotal Study Cx601-0302 (Safety Population)

Characteristic	Alofisel (N=103)	Placebo (N=102)	Overall (N=205)
Time since CD diagnosis (years)			
Mean (SD)	11.8 (9.8)	11.4 (9.0)	11.6 (9.4)
Median	9.9	9.3	9.6
Range	0.5 – 48.8	0.5 – 36.4	0.5 – 48.8
Number of external openings			
Only 1 N (%)	58 (56.3)	73 (71.6)	131 (63.9)
2 or more N (%)	45 (43.7)	29 (28.4)	74 (36.1)
Number of internal openings			
Only 1[a] N (%)	82 (79.6)	90 (88.2)	172 (83.9)
2 or more N (%)	21 (20.4)	11 (10.8)	32 (15.6)
Openings combined			
1 external & 1 internal only N (%)	55 (53.4)	70 (68.6)	125 (61.0)
1 internal & 2 or 3 external or 2 internal & 1, 2 or 3 external N (%)	48 (46.6)	31 (30.4)	79 (38.5)
Previous Treatment for CD			
Within 6 months			
Yes N (%)	94 (91.3)	96 (94.1)	190 (92.7)
No N (%)	9 (8.7)	6 (5.9)	15 (7.3)
Antibiotics			
Yes N (%)	78 (75.7)	72 (70.6)	150 (73.2)
No N (%)	25 (24.3)	30 (29.4)	55 (26.8)
Immunosuppressants			
Yes N (%)	87 (84.5)	74 (72.5)	161 (78.5)
No N (%)	16 (15.5)	30 (29.4)	44 (21.5)
Anti-TNFs			
Yes N (%)	80 (77.7)	82 (80.4)	162 (79.0)
No N (%)	23 (22.3)	20 (19.6)	43 (21.0)
Concomitant Treatment at Randomisation			
Anti-TNFs only N (%)	36 (35.0)	32 (31.4)	68 (33.2)
IMSUP only N (%)	16 (15.5)	21 (20.6)	37 (18.0)
Anti-TNFs & IMSUPs N (%)	27 (26.2)	30 (29.4)	57 (27.8)
Neither N (%)	24 (23.3)	19 (18.6)	43 (21.0)

CD = Crohn's disease; IMSUP = immunosuppressant; SD = standard deviation; TNF = tumour necrosis factor
a Note that an internal opening was not identified at baseline for one patient in the placebo group
Source: CSR-Week 24 Cx601-0302; Table 14.1.1.5.1; Table 14.1.1.6.1b; Table 14.1.1.7.1

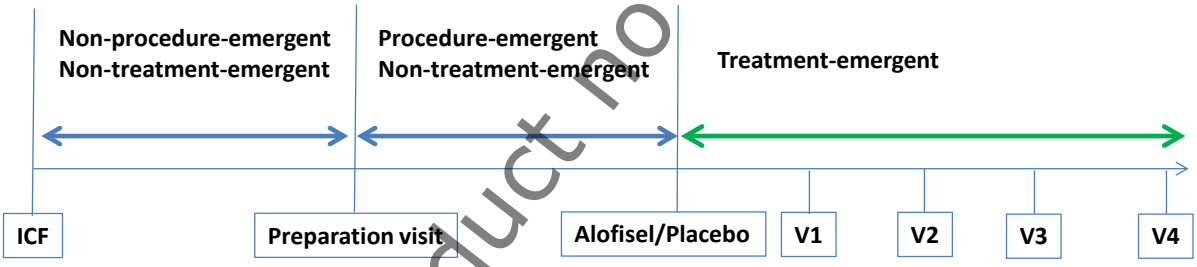
Adverse events

In both studies, an AE was defined as treatment-emergent if the onset date was on or after the date of administration of study treatment. In study Cx601-0302, non-treatment-emergent AEs (NTEAEs) were also collected. NTEAEs were further defined as procedure-emergent or non-procedure emergent.

Procedure-emergent NTEAEs started prior to study treatment but after the first (preparatory) curettage procedure that was performed 2 to 3 weeks prior to the day of study treatment administration; non-treatment nor procedure-emergent AEs started prior to the curettage procedure (see Figure 7 below).

NTEAEs were not collected in study Cx601-0101; patients underwent the preparatory curettage procedure on the same day as study treatment administration, just prior to study treatment.

Figure 7: Schematic of Non-Procedure-Emergent and Procedure-Emergent, Non-Treatment–Emergent Adverse Events (Pivotal Study, Cx601-0302)



An overall summary of TEAEs through to the Week 52 visit for study Cx601-0302 is presented in Table 22.

Table 22: Overall Summary of TEAEs: Pivotal Study Cx601-0302 (Safety Population)

	Up to Week 24		Up to Week 52*	
Category	**Alofisel (N=103) n (%)**	**Placebo (N=102) n (%)**	**Alofisel (N=103) n (%)**	**Placebo (N=102) n (%)**
All TEAEs	68 (66.0)	66 (64.7)	79 (76.7)	74 (72.5)
Severe TEAEs	10 (9.7)	10 (9.8)	10 (9.7)	12 (11.8)
Treatment-related TEAEs#	18 (17.5)	30 (29.4)	21 (20.4)	27 (26.5)
Serious TEAEs	18 (17.5)	14 (13.7)	25 (24.3)	21 (20.6)
TEAEs resulting in Discontinuation	5 (4.9)	6 (5.9)	9 (8.7)	9 (8.8)

n = number of patients with treatment-emergent adverse event; N = number of patients in analysis population; TEAE = treatment-emergent adverse event.
* Cumulative from treatment administration up to the Week 52 Visit
# The causality assessment of 9 TEAEs in 8 patients reported prior to Week 24 was changed by investigators after Week 24 tables had been produced. In the Alofisel group, 2 TEAEs of proctalgia in 1 patient changed from 'not assessable' to 'unrelated', 1 TEAE of nasopharyngitis was changed from 'not assessable' to 'unrelated', and 1 TEAE of genital fistula changed from 'missing' to 'unlikely'. In the placebo group, 1 TEAE of malaise changed from 'not assessable' to 'unrelated', 1 TEAE of fistula changed from 'not assessable' to 'possible', 1 TEAE of haematochezia changed from 'not assessable' to 'unlikely' and 1 TEAE of proctalgia changed from 'not assessable' to 'unrelated'
Source: CSR-Week 24 Cx601-0302; Table 14.1.6.1.1, Table 14.1.6.1.4 and CSR-Week 52 Cx601-0302; Table 14.2.3.1.1, Table 14.2.3.1.3.

Treatment-emergent AEs (TEAEs) occurring in ≥3 patients in either treatment group up to the Week 52 visit are presented by system organ class (SOC) and preferred term (PT) in Table 23 below.

Overall, the proportions of patients who experienced TEAEs were comparable between the treatment groups. The most common events were anal abscess and proctalgia, respectively. Some TEAEs occurred in a higher proportion of patients in the Alofisel group than the placebo group, such as proctalgia (Alofisel: 14.6% patients; placebo: 11.8%), anal fistula (Alofisel: 10.7%; placebo: 7.8%), and diarrhoea (Alofisel: 8.7%; placebo: 2.9%) in the Gastrointestinal Disorders SOC, and anal abscess (Alofisel: 19.4%; placebo: 13.7%) and nasopharyngitis (Alofisel: 10.7%; placebo: 4.9%) in the Infections and Infestations SOC.

The combined frequency of 'anal fistula' and 'fistula' TEAEs was however similar in both treatment groups (Alofisel: 13/103 [12.6%] patients; placebo: 13/102 [12.7%]).

Severe TEAEs were reported similarly.

In this study, procedural pain and proctalgia were reported as both procedure-emergent non-treatment emergent AEs and as treatment-emergent AEs, i.e. following the administration of IMP. These events resulted most likely from the conditioning of the fistula considering the similarity in incidence between the Alofisel and placebo treatment groups.

Treatment with Alofisel was not associated with fewer AEs or SAEs of fistula and/or abscess compared to placebo. In the Alofisel group, 15.0% of patients reported TESAEs of fistula/abscess up to Week 52, as compared to 9.5% in the control group. Such events appear to be related to lack of efficacy in individual patients. As there was a higher proportion of patients with multiple tract fistulas in the Alofisel group (44.8%) than in the Control group (29.6%), this could have somewhat disfavoured the Alofisel group. Other baseline features studied (gender, sole immunosuppressant use, duration of prophylactic antibiotic use and country) did not have a marked effect on the response treatment with Alofisel. An analysis of additional clinical

characteristics (time since CD diagnosis, fistula type, collections at screening >2cm, CDAI at baseline, and concomitant medication) did not show any interaction with treatment effect.

An additional analysis of combined remission, imputing patients presenting with TEAEs of fistula/abscess as failures, did not impact the primary efficacy analysis conclusions.

Table 23: TEAEs up to the Week 52 Visit in ≥ 3 Patients – Pivotal Study Cx601-0302 (Safety Population)

	Up to Week 24		Up to Week 52**	
System Organ Class Preferred Term	**Alofisel (N=103) n (%)**	**Placebo (N=102) n (%)**	**Alofisel (N=103) n (%)**	**Placebo (N=102) n (%)**
Number of Patients with TEAE(s)	68 (66.0)	66 (64.7)	79 (76.7)	74 (72.5)
Gastrointestinal Disorders#	32 (31.1)	39 (38.2)	44 (42.7)	46 (45.1)
Proctalgia	13 (12.6)	11 (10.8)	15 (14.6)	12 (11.8)
Diarrhoea	7 (6.8)	3 (2.9)	9 (8.7)	3 (2.9)
Abdominal pain	4 (3.9)	6 (5.9)	5 (4.9)	7 (6.9)
Anal fistula#	4 (3.9)	2 (2.0)	11 (10.7)	8 (7.8)
Crohn's disease*	2 (1.9)	3 (2.9)	4 (3.9)	8 (7.8)
Perianal erythema	2 (1.9)	1 (<1.0)	3 (2.9)	2 (2.0)
Vomiting	2 (1.9)	2 (2.0)	3 (2.9)	2 (2.0)
Nausea	2 (1.9)	0 (0.0)	3 (2.9)	0 (0.0)
Haemorrhoids	2 (1.9)	0 (0.0)	3 (2.9)	0 (0.0)
Constipation	1 (<1.0)	3 (2.9)	2 (1.9)	3 (2.9)
Anal haemorrhage	0 (0.0)	3 (2.9)	0 (0.0)	3 (2.9)

Infections and Infestations	32 (31.1)	40 (39.2)	46 (44.7)	45 (44.1)
Anal abscess	12 (11.7)	13 (12.7)	20 (19.4)	14 (13.7)
Nasopharyngitis	10 (9.7)	5 (4.9)	11 (10.7)	5 (4.9)
Infected fistula	2 (1.9)	3 (2.9)	4 (3.9)	4 (3.9)
Bronchitis	2 (1.9)	2 (2.0)	3 (2.9)	4 (3.9)
Urinary tract infection	2 (1.9)	2 (2.0)	3 (2.9)	3 (2.9)
Gastroenteritis	1 (<1.0)	1 (<1.0)	1 (<1.0)	3 (2.9)
Influenza	0 (0.0)	3 (2.9)	0 (0.0)	3 (2.3)
Sinusitis	0 (0.0)	1 (<1.0)	0 (0.0)	3 (2.9)
General Disorders and Administration Site Conditions	15 (14.6)	17 (16.7)	20 (19.4)	19 (18.6)
Pyrexia	5 (4.9)	5 (4.9)	6 (5.8)	5 (4.9)
Asthenia	2 (1.9)	2 (2.0)	3 (2.9)	3 (2.9)
Oedema peripheral	2 (1.9)	3 (2.9)	2 (1.9)	3 (2.9)
Musculoskeletal and Connective Tissue Disorders#	11 (10.7)	17 (16.7)	16 (15.5)	18 (17.6)
Arthralgia	4 (3.9)	3 (2.9)	6 (5.8)	4 (3.9)
Fistula#	3 (2.9)	6 (5.9)	2 (1.9)	5 (4.9)
Vascular Disorders	2 (1.9)	1 (<1.0)	5 (4.9)	2 (2.0)
Hypertension	2 (1.9)	0 (0.0)	4 (3.9)	0 (0.0)
Investigations	3 (2.9)	5 (4.9)	8 (7.8)	7 (6.9)
C-Reactive protein increased	1 (<1.0)	2 (2.0)	2 (1.9)	4 (3.9)
Nervous System Disorders	2 (1.9)	2 (2.0)	2 (1.9)	4 (3.9)
Headache	1 (<1.0)	2 (2.0)	1 (<1.0)	4 (3.9)

n = number of patients with treatment-emergent adverse event; N = number of patients in analysis population; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event

* Verbatim terms were mostly related to worsening of pre-existing Crohn's disease

** From treatment administration up to the Week 52 visit

One patient treated with Alofisel [redacted] and 1 patient treated with placebo [redacted]), had TEAEs recorded as PT 'fistula' for the period up to Week 24 which were subsequently changed to PT 'anal fistula' which is what is tabulated for the period up to Week 52

Source: CSR-Week 24 Cx601-0302; Table 14.1.6.2.1 and CSR-Week 52 Cx601-0302; Table 14.2.3.2.1

The safety data obtained from the supportive study Cx601-0101 did not reveal any additional safety concerns but were too limited to allow drawing firm conclusions on the safety of repeated administration.

Analysis of AEs of interest by organ system or syndrome

- **Hypersensitivity/immune reactions**

Hypersensitivity was recorded for 1 Alofisel-treated patient in study Cx601-0302. This TEAE (verbatim term 'allergy', no other details provided) occurred 110 days after Alofisel administration, was non-serious, of mild intensity and required no treatment, and was considered unrelated to study medication by the investigator. In addition, one placebo-treated patient in Study Cx601-0302 recorded a TEAE of 'hypersensitive reaction due to infliximab treatment' 135 days after receiving placebo that was considered unrelated to study medication. No cases of treatment-emergent hypersensitivity were reported in study Cx601-0101.

- **Tumourigenicity**

There were 4 cases of neoplasia (malignant plus benign) reported in Study Cx601-0302 (1 in the Alofisel group and 3 in the placebo group; none was considered related to study treatment). The one patient in the Alofisel group in study Cx601-0302 reported a TEAE of uterine leiomyoma (verbatim term, 'worsening of pre-existing condition myoma') 11 days after study treatment administration. The event was reported to be of mild intensity and was not considered related to study treatment as the leiomyoma had been diagnosed prior to preparatory surgery. In study Cx601-0101 a patient reported a TEAE of uterine leiomyoma 82 days after receiving the first study treatment administration. This case also seems likely a pre-existing condition considering the short time line.

- **Infection**

Infection was not listed by the Applicant as an AE of interest. The pivotal study results do not indicate an overall higher frequency of infections based on System Organ Class in patients treated with Alofisel. In total, 44.7% of patients in the Alofisel group experienced one or more AEs in the infections or infestations SOC as compared to 44.1% of patients in the placebo group. However, by PT, there were more events of anal abscess in the active treatment group up to Week 52 (19.4% of patients, 21 TEAEs in the Cx601 group, 13.7% of patients, 19 TEAEs in the placebo group).

- **Procedure-related adverse events**

In study Cx601-0302, for TEAEs, relationship to surgical procedure was not specifically collected. (Investigators were not requested to distinguish between the surgical [curettage] procedure on the day of treatment and IMP administration in assessing relatedness for regular AEs.) Thus, procedural pain and proctalgia were reported as both procedure-emergent non-treatment emergent AEs (so-called PENTAs) and as treatment-emergent AEs, i.e. following the administration of IMP.

However, TEAEs up to Week 52 that occurred following IMP administration that could be considered procedure-related include events of PT 'proctalgia' (Alofisel: 14.6%, 20 TEAEs; placebo: 11.8%, 17 TEAEs), 'procedural pain' (Alofisel: < 1.0%, 1 TEAE; placebo: 2.0%, 2 TEAEs) and 'post-procedural inflammation' (Alofisel: 1.9%, 2 TEAEs; placebo: 0.0%). Of the 37 TEAEs of proctalgia, a total of 8 were reported within 7 days of IMP administration and 6 of these were considered related to study treatment. All 3 TEAEs of procedural pain were considered related to study treatment (which could also have included the surgical procedure) and were reported on the same day or the day after IMP administration. The cases of post-procedural inflammation were reported by the investigator as either induration around the seton or swelling at the site of the seton placement and occurred between 6 weeks and 3 months after IMP administration; neither was considered related to study treatment. Considering the similarity in incidence between the Alofisel and placebo treatment groups e.g. for proctalgia a statement was included in section 4.4 and 4.8 that events of proctalgia and procedural pain have been reported as a result of the conditioning of the fistula.

Serious adverse event/deaths/other significant events

There were no treatment-emergent deaths reported during in study Cx601-0302, either up to the Week 52 visit or between the Week 52 visit and the data cut-off for SAEs of 30 June 2016. There were no deaths reported in study Cx601-0101. Treatment-emergent SAEs in study Cx601-0302 up to the Week 52 visit are summarised in the table below.

Table 24: Treatment-Emergent SAEs up to Week 52 in ≥2 Patients Overall - Pivotal Study Cx601-0302 (Safety Population)

	Up to Week 24		Up to Week 52*	
System Organ Class Preferred Term	Alofisel (N=103) n (%)	Placebo (N=102) n (%)	Alofisel (N=103) n (%)	Placebo (N=102) n (%)
Number of Patients with TESAE(s)	18 (17.5)	14 (13.7)	25 (24.3)	21 (20.6)
Infections and Infestations	11 (10.7)	9 (8.8)	16 (15.5)	10 (9.8)
Anal abscess	9 (8.7)	7 (6.9)	14 (13.6)	8 (7.8)
Gastrointestinal Disorders	2 (1.9)	5 (4.9)	5 (4.9)	8 (7.8)
Anal fistula	1 (<1.0)	1 (<1.0)	4 (3.9)	1 (<1.0)
Crohn's Disease	0 (0.0)	1 (<1.0)	0 (0.0)	3 (2.9)

n = number of patients with treatment-emergent serious adverse event; N = number of patients in analysis population; PT = preferred term; SOC = system organ class; TESAE = treatment-emergent serious adverse event.
* From treatment administration up to the Week 52 visit
Source: CSR-Week 24 Cx601-0302; Table 14.1.6.2.4 and CSR-Week 52 Cx601-0302; Table 14.2.3.2.3.

Up to the Week 52 visit, 46 patients (22.4%) experienced 57 TESAE. Most of these were experienced by one patient each. Anal abscess, anal fistula, and Crohn's Disease were the only TESAEs that were experienced by 2 or more patients in either treatment group.

Anal abscess was reported as a TESAE in more patients in the Alofisel group than the placebo group (Alofisel: 13.6% patients, 14 TESAEs; placebo: 7.8% patients, 9 TESAEs), as was anal fistula (Alofisel: 3.9% patients, 4 TESAEs; placebo: <1.0% patients, 1 TESAE).

The investigator's assessment of causal relationship to study treatment and causal relationship to the surgical procedure was not collected for treatment-emergent regular (nonserious) AEs but was collected for treatment-emergent SAEs on the SAE form.

Of the Alofisel-treated patients, 7/14 TESAEs of anal abscess were considered related to *study treatment* by the investigator and 4/14 were reported as severe. In the placebo group, 5/9 TESAEs of anal abscess were considered related and 4/9 were reported as severe.

In the Alofisel group, TESAEs considered by the investigator to be related to the *surgical procedure* were mostly anal abscess (6 of the 14 cases); an additional TESAE of groin abscess was also considered to be related. In the placebo group, TESAEs considered by the investigator to be related to the surgical procedure included 3 of the 9 cases of anal abscess, and one case each of anal inflammation, fistula, and liver abscess.

Most of the SAEs in the Alofisel group (10 out of 14 cases of anal abscess SAEs and all 4 cases of fistula SAEs) were reported to have occurred in the same fistula that was treated with Alofisel, in most cases a rather long time (~1-10 months) after the procedure. In the placebo group, SAEs of anal abscess (8), anal fistula or fistula (2) and/or anal inflammation (1) was reported to be in the same (treated) area in 7 of 11 reported cases.

All TESAEs other than anal abscess experienced by Alofisel-treated patients were considered by the investigator to be unlikely or unrelated to study treatment. Based on the submitted narratives this is agreed.

TESAEs reported between the Week 52 visit and the 30 June 2016 cut-off date for SAEs in Alofisel-treated patients were provided. These included 3 TESAEs, anal fistula and fistula discharge in the Alofisel group and

pregnancy in the placebo group. None of the events were considered causally related to treatment or the surgical procedure by the investigator.

Laboratory findings

There were no notable changes in laboratory assessments, vital signs or physical examinations in the clinical studies.

Safety in special populations

- Subgroup analyses

For study Cx601-0302, subgroup analyses of TEAEs by age (<65 years; ≥65 years) and gender and by the stratification factors assigned at randomization (immunosuppressants only, anti-TNFs only, both immunosuppressant and anti-TNF, or neither) did not reveal any conclusive trends of concern.

Based on the cell-based nature of Alofisel and its local route of administration, it is not anticipated that Alofisel would be associated with more risks in elderly as compared to the non-elderly population. However, the number of patients ≥65 years of age is too small to draw definite conclusions.

Hepatic and/or renal insufficiency is not expected to alter the distribution or clearance of the product although no specific studies have been conducted in these populations. The product information has been updated to reflect this.

The proportions of Alofisel-treated patients that reported TEAEs up to Week 24 was higher in patients with only one external opening compared to those with 2 or more external openings (46/58, 79.3% versus 22/45, 48.9%). This numeric difference persisted up to Week 52 (84.5% vs. 66.7%).

Overall the trans-sphincteric location of perianal fistulas was the most frequent fistula location in both treatment groups and consequently, the majority of related TEAEs were reported in patients with this type of fistula. The limited number of events pertaining to other fistula locations precluded drawing conclusions on whether fistula classification or location had any effect on risk of complication or adverse event rate.

- Use in pregnancy and lactation

Pregnant and breastfeeding women were excluded from participation in both clinical studies and no such patients were treated with Alofisel in either of the 2 clinical studies. There were no pregnancies reported following study treatment up to the Week 24 visit in study Cx601-0302 or during the 24-week follow-up period in study Cx601-0101. In study Cx601-0302, 3 pregnancies were reported up to the Week 52 visit and the date of clinical cut off of 30 June 2016 after receiving study treatment (1 in the Alofisel group and 2 in the placebo group). reported her pregnancy approximately 8 months after receiving Alofisel and had a healthy baby at term.

Two additional pregnancies occurred after completion of study Cx601-0101 (Sanz-Baro et al., 2015). In one case, a woman who had received 2 doses of Alofisel became pregnant 17 months later and gave birth to a baby who had (text redacted) but was otherwise healthy. In the second case, the woman experienced 2 consecutive first trimester spontaneous abortions starting 24 months after receiving 2 treatments with Alofisel. Neither case was considered causally related to Alofisel.

One further pregnancy occurred during an investigator-initiated study of Alofisel in patients with rectovaginal fistulas. The patient became pregnant 18 months after cell treatment (an initial 20 million cells followed by a

second treatment with 40 million cells). She had a normal pregnancy and gave birth by Caesarean to a normal healthy baby at 39 weeks.

Overall, experience with Alofisel during pregnancy and lactation is so far very limited and no conclusions can be drawn based on these few cases. The product information reflects this. Alofisel is not recommended during pregnancy and in women of childbearing potential not using contraception, and also that as a precautionary measure, Alofisel is not recommended for administration during breastfeeding.

- Use in children and adolescents

There is no experience in the Alofisel clinical development programme in the treatment of complex perianal fistula(s) in patients younger than 18 years old. The European Medicines Agency (EMA) has deferred the obligation to submit the results of studies with complex anal fistula in paediatric CD patients from 4 to less than 18 years of age, until efficacy and safety of Alofisel for the treatment of complex anal fistula(s) in CD patients has been assessed in adults. A waiver was granted for fistulas of CD origin in neonates (0-1 month), infants and toddlers (1 24 months) and in children from 2-3 years of age on the grounds of having no unmet therapeutic need, of safety concerns due to the need for anaesthesia and on the grounds that the disease does not occur (EMEA-001561-PIP01-13).

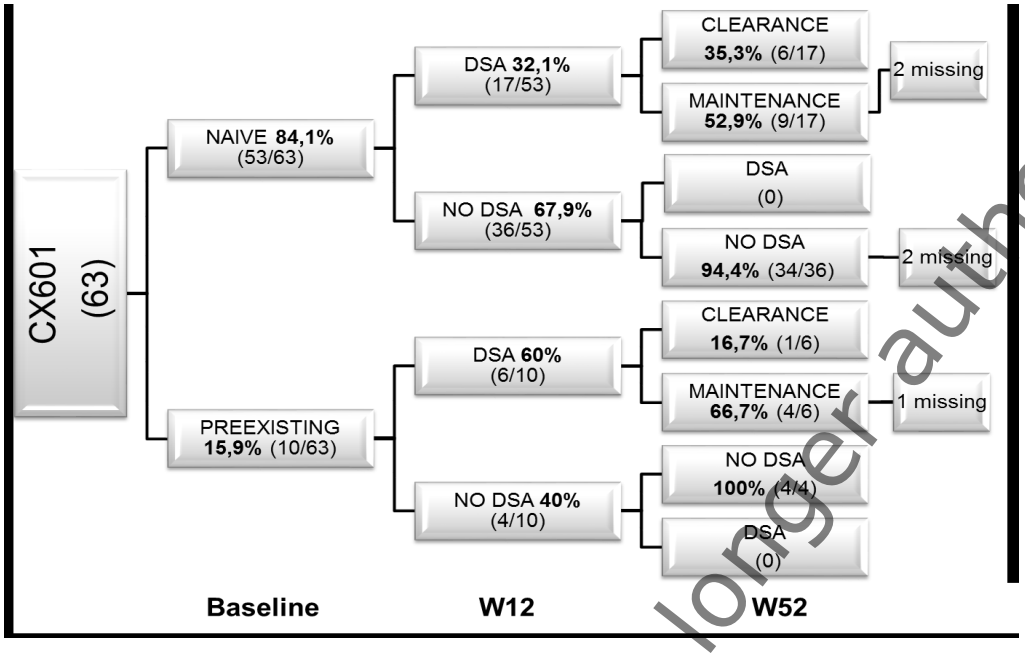
Immunological events

In a subset of 123 of the 212 randomized Crohn's disease patients in the pivotal study Cx601-0302 (63 Alofisel and 60 placebo), blood samples were analysed to assess the potential for generation of alloantibodies to Alofisel. Blood samples taken at baseline were analysed for IgG anti-HLA class I and II in order to assess pre-existing sensitisation (which could affect subsequent generation of antibody responses to Alofisel). The presence of IgG anti-HLA class I and II was re-tested at Week 12 and at Week 52 or early termination, and blood samples of patients with positive sera were further screened for donor-specific antibodies (DSA). All patients had samples collected at W12 (eASC: 63; placebo: 60). However, at W52, only 106 blood samples were collected (85.3%; (eASC: 58; placebo: 48).

One administration of 120 million allogeneic eASC induced DSA in 23 of 63 (36.5%) patients at W12. Of the 23 patients with DSA detected at W12, 17 (32.1%) were naïve (i.e. not pre-sensitized [no pre-existing anti-HLA antibodies]) at baseline and 6 (60%) were pre-sensitized.

Furthermore, of these 23 patients, 13 kept positivity for DSA at W52, whereas 7 cleared antibodies by W52 and 3 had missing samples at W52. No *de novo* generation of antibodies was detected from W12 to W52. Furthermore, none of the patients included in the placebo arm generated DSA.

Figure 8: Frequency of the population and panel of reactive antibodies in pivotal study Cx60-0302 (Alofisel group)



Safety related to drug-drug interactions and other interactions

No clinical studies investigating potential drug interactions with Alofisel were performed.

Results of the subgroup analyses did not show a clear difference in the safety profile of Alofisel with or without co-administered medications such as anti-TNFs or immunosuppressants.

Discontinuation due to adverse events

Overall, the proportion of patients who discontinued due to TEAE(s) up to the Week 52 visit was low (18/205, 8.8%) and similar in both treatment groups (8.7% Alofisel; 8.8% placebo).

The most common TEAE that resulted in withdrawal from the study was anal abscess, which led to the discontinuation of similar proportions of patients in both treatment groups (2.9%, Alofisel; 3.9% placebo). All 3 cases of anal abscess in the Alofisel group were reported as serious, and 2 of the 4 cases in the placebo group. All 7 cases of anal abscess (i.e., the 3 cases in the Alofisel group and the 4 cases in the placebo group) were considered to be related (possibly or probably) to study treatment.

Two of the 24 patients (8.3%) in study Cx601-0101discontinued due to TEAEs. The events included anal abscess, abscess drainage, and CRP increased in one patient (all non-serious and considered not related by the investigator) and anal abscess (serious, possibly related) in another patient. Both patients were withdrawn following their first treatment with study drug.

Post marketing experience

At submission of the application Alofisel was approved for use as a medicinal product. No post-marketing data are available.

2.6.1. Discussion on clinical safety

The primary safety data to support the targeted indication of Alofisel for treatment of complex perianal fistulas in patients with non-active or mildly-active Crohn's disease result from the single Phase III study Cx601-0302 in 212 patients with a planned follow-up to Week 104. The safety analyses in the current submission include the data up to the Week 52 visit, as well as SAEs that occurred and were entered between the Week 52 visit and the 30 June 2016 cut-off date.

In study Cx601-0302, up to Week 52, similar proportions of patients experienced TEAEs (76.7% Alofisel group; 72.5% placebo group). The most common events were proctalgia (Alofisel: 14.6% of patients; placebo: 11.8%) and anal abscess (Alofisel: 19.4%; placebo: 13.7%). Severe TEAEs were reported similarly (Alofisel: 9.7% patients, 10 TEAEs; placebo: 9.8% patients, 11 TEAEs).

In study Cx601-0302, procedural pain and proctalgia were reported as both procedure-emergent non-treatment emergent AEs and as treatment-emergent AEs, i.e. following the administration of IMP. Accordingly a statement was included in section 4.8 of the SmPC that these events have been reported as a result of the conditioning of the fistula. This is agreed as reasonable considering also the similarity in incidence between the Alofisel and placebo treatment groups e.g. for proctalgia. Although a significant proportion of treatment-emergent SAEs of perianal abscess, anal inflammation and/or fistula were considered related to the surgical procedure by the investigator, an analysis by the Applicant revealed this to be less plausible, considering the time to onset for most of the events.

The incidence of the TEAE anal abscess was similar between the treatment groups up to Week 24 (Alofisel: 12 patients; 11.7% vs. placebo: 13 patients; 12.7%), respectively. However, when patients were followed up to Week 52, anal abscess was reported more frequently in the Alofisel group (20 patients, 19.4%) vs. 14 patients (13.7%) placebo.

Similarly, the most common TESAE was anal abscess, which was reported in slightly more patients in the Alofisel group than placebo 14 (13.6%) versus 8 (7.8%). This was also the case for SAEs of anal fistula/fistula (Alofisel: 3.9%; placebo: 2%).

Most of the SAEs in the Alofisel group (10 out of 14 cases of anal abscess SAEs and all 4 cases of fistula SAEs) were reported to have occurred in the same fistula that was treated with Alofisel, in most cases a rather long time (~1-10 months) after the procedure. In the placebo group, SAEs of anal abscess (8), anal fistula or fistula (2) and/or anal inflammation (1) was reported to be in the same (treated) area in 7 of 11 reported cases. Thus, treatment with Alofisel was not associated with fewer events of serious abscess formation in the treated fistula as compared to placebo, and this most likely reflects a lack of efficacy in some patients. Analyses of baseline features and disease characteristics (including gender, sole immunosuppressant use, duration of prophylactic antibiotic use, country), as well as time since CD diagnosis (> 10 y / ≤10 y), the fistula type (Parks classification), any collections at screening > 2 cm (drained), CDAI at baseline (>150 /≤150), and concomitant medication) did not show a significant effect on the response to Alofisel treatment, based on the current dataset.

No treatment-emergent deaths were reported and there were no notable changes in clinical laboratory examinations, vital signs or physical examinations.

Subgroup analyses of TEAEs by age (<65 years; ≥65 years) and gender and by the stratification factors assigned at randomization (immunosuppressants only, anti-TNFs only, both immunosuppressant and anti-TNF, or neither) did not reveal any conclusive trends of concern. The number of patients ≥65 years of age was too small to draw conclusions regarding the safety of Alofisel in elderly. However, given the cell-based nature of Alofisel and its local administration route it is not expected that the benefit-risk profile of Alofisel in elderly

patients will differ from that observed in non-elderly patients. Experience in the elderly is included as missing information into the RMP which is considered acceptable.

The proportions of Alofisel-treated patients that reported TEAEs was higher in patients with only one external opening compared to those with 2 or more external openings (84.5% versus 66.7%), which might point towards possible dose/exposure-related effects. This may signify random variability, differences in local exposure to Alofisel between the groups, or other. The Applicant was unable to clearly explain this observation but pointed to the fact that the numeric differences between these subgroups were smaller when comparing only related, serious AEs. Considering also the relatively limited patient numbers it can be accepted that this issue is further followed and discussed anew in future safety reporting, including the planned PASS.

The supportive open-label Phase I/IIa study Cx601-0101included only 24 patients who received a lower dose (at 20 million cells) than the intended one for marketing. No additional safety concerns were identified in this limited study. Some patients received a second administration of Alofisel. The need for repeated injection in the clinical setting is foreseen in this patient population and appropriateness will be further studied by means of a PASS.

In study Cx601-0302, a subgroup of 63 Alofisel and 60 placebo patients were analysed for the presence of IgG anti-HLA antibodies at baseline and for the presence of donor specific antibodies (DSA) at Week 12 and Week 52, or at early termination; one administration of 120 million allogeneic eASC induced DSA in 23 of 63 (36.5%) patients at W12. Of the 23 patients with DSA detected at W12, 17 (32.1%) were naïve (i.e. not pre-sensitized [no pre-existing anti-HLA antibodies]) at baseline and 6 (60%) were pre-sensitized. Furthermore, of these 23 patients, 13 kept positivity for DSA at W52, whereas 7 cleared antibodies by W52 and 3 had missing samples at W52. No *de novo* generation of antibodies was detected from W12 to W52. None of the patients included in the placebo arm generated DSA. Overall, the results suggest that pre-sensitized eASC-treated patients may be more likely to generate DSA compared to patients who were not pre-sensitized (DSA generation at Week 12 by 60.0% of pre-sensitized patients and 32.1% of patients who were not pre-sensitized). Furthermore, of the patients whose DSA status at W12 and W52 is known, it appears that the pre-sensitized patients were somewhat more likely to maintain DSA positivity (67% maintained antibodies at W52) as compared to patients not pre-sensitized (53% maintained antibodies at W52).

However, few patients (~15% in both Alofisel and placebo groups) were pre-sensitized at baseline. This limits interpretability and it is unclear whether there could be any (additional) risk in terms of safety given that relatively more patients who were pre-sensitized as baseline developed DSA as compared to naïve patients.

The Applicant provided a summary of potential immune-related TEAEs (e.g. pyrexia, rash) but these events were not considered particularly associated with the presence of DSA or with pre-sensitisation (pre-existing IgG anti-HLA antibodies at baseline).

Overall, the data from this study do not indicate a clinically meaningful effect of DSA on the safety of Alofisel, but the data are limited, considering the modest patient numbers and lack of data on repeated dosing.

Whether repeated administration of Alofisel could be associated with increased generation of DSA and/or in any way with increased risk of allo-immune response will be further studied in a PASS, also to evaluate the need for any screening.

Bovine serum is used in the manufacturing process of the Alofisel. Also penicillin-G and streptomycin sulphate could potentially be present in drug product in trace amounts as impurities. This is sufficiently addressed in the SmPC.

No signs of opportunistic infections or tumour development have been reported in the Alofisel clinical studies to date. The conducted nonclinical in vitro and in vivo studies do not indicate a risk for tumour-formation. Similarly,

there is no evidence to suggest the risk of ectopic tissue formation. However, the biological relevance of using human eASC in immunocompromised animals can be questioned (especially since the cells do not appear to persist for very long) in relation to this risk. As the clinical database is too limited and would require a substantial longer follow up compared to the clinical data currently available it is agreed to add these concerns as important potential risks into the RMP.
Also the risk of transmission of infectious agents is viewed as an *important potential risk*. As outlined in the Quality sections, the testing of adventitious safety and introduced safety measures for raw and starting material of biological origin are now in essence considered acceptable. The final tests for sterility and mycoplasma are retrieved after release. The Applicant has devised a number of risk minimisation activities in case of microbial contamination is detected in post release testing (mycoplasma and sterility) such as reporting procedures to the treating physician and the development of educational materials to ensure a timely and appropriate treatment of the patient.
There is a potential risk on medication errors related to the surgical procedure such as the administration of the product (e.g. intravenous application), the manipulation of the product and the storage of the product. Medication errors could result in product contamination or incorrect administration, for example. Potential for medication errors is agreed to be included as an important potential risk in the RMP and educational material will be dispensed in order to provide information on how to correctly administer the product and to increase awareness about the potential transmission of infectious agents. Appropriate statements have also been included in the SmPC.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

The CHMP endorse the CAT discussion on clinical safety as described above.

2.6.2. Conclusions on the clinical safety

The safety database of Alofisel is considered limited but provides sufficient characterisation of the safety profile for marketing authorisation. Unfavourable effects and uncertainties are appropriately described in SmPC and RMP and post authorisation follow up will be carried out in particular on important potential risks (such as tumourigenicity and/or ectopic tissue formation, immunogenicity/alloimmunoreactions and the risk of transmission of infectious agents), repeated administration and long term safety by means of a post authorisation safety study.

The CHMP endorse the CAT conclusion on clinical safety as described above.

2.7. Risk Management Plan

The CAT received the following PRAC Advice on the submitted Risk Management Plan (RMP):

The PRAC considered that the risk management plan version 6.0 is acceptable.

Safety concerns

Table 25. Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	○ Tumourigenicity ○ Ectopic tissue formation ○ Hypersensitivity reactions ○ Transmission of infectious agents ○ Immunogenicity/allo-immunoreactions ○ Development of new anal fistula and/or anal abscess or relapse of treated fistula ○ Medication errors
Missing information	○ Long-term safety ○ Experience during pregnancy and lactation ○ Experience in the elderly ○ Repeated use in the patient

Pharmacovigilance plan

Table 26. Ongoing and planned studies in the PhV development plan

Activity/Study title (type of activity, study title category 1-3)*	**Objectives**	**Safety concerns addressed**	**Status (planned, started)**	**Date for submission of interim or final reports (planned or actual)**
Cx601-0303. Phase III (EudraCT No. 2017-000725-12) Category 1	To evaluate the efficacy and safety of Alofisel compared to placebo for the treatment of complex perianal fistula(s) in patients with Crohn's disease at Week 24 with a follow-up period up to 52 weeks.	Long-term safety up to 52 weeks.	Ongoing	Final Clinical Study Report: 2Q/3Q 2022
Post-authorisation safety study (PASS) Category 3	To evaluate the long term safety of Cx601 (Alofisel) in patients treated and retreated (i.e. repeated dosing and immunogenicity) and to assess the effectiveness of Cx601 (Alofisel) in patients treated and retreated (i.e. repeated dosing) in routine clinical practice (for treatment of complex perianal fistulas in	Long-term safety.	Planned	Submission of protocol: Q2-Q3 2018 Interim report 1: End of 2021 Interim report 2: End of 2023 Interim report 3: End of 2025 Interim report 4: End of 2027 Final report to EMA: March 2029

Activity/Study title (type of activity, study title category 1-3)*	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
	adult patients with non-active/mildly active luminal Crohn's disease, when fistulas have shown an inadequate response to at least one conventional or biologic therapy)			

Risk minimisation measures

Table 27. Summary table of additional Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Important potential risk – Tumourigenicity	- Routine risk minimisation reflects prescription and administration by specialist healthcare professionals	None proposed.
Important potential risk - Ectopic tissue formation	- Routine risk minimisation reflects prescription and administration by specialist healthcare professionals	None proposed.
Important potential risk - Hypersensitivity reactions related to impurities: bovine serum, benzylpenicillin , streptomycin and/or class related compounds	- Bovine serum: Information on contraindication is presented in section 4.3 (Contraindication) of the SmPC. - Benzylpenicillin and streptomycin: Information on warning is presented in section 4.4 (warnings and precautions for use) of the SmPC.	None proposed.
Important potential risk – Transmission of infectious agents	- Information on the potential for transmission of infection agents is provided in section 4.4 Special warnings and precautions for use in the SmPC	Healthcare Professional Educational Leaflet.
Important potential risk – Immunogenicity/alloimmunoreactions	- Routine risk minimisation reflects prescription and administration by specialist healthcare professionals - Information on the observed incidence of donor specific antibodies in section 5.1 Pharmacodynamic effect of the SmPC	None proposed.
Important potential risk – Development of new anal fistula and/or anal abscess or relapse of treated fistula	- Routine risk minimisation reflects prescription and administration by specialist healthcare professionals - -Information in Section 4.2 Posology and method of administration of the SmPC. - Information on the observed incidence of these AEs is	None proposed.

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	provided in section 4.8 (Undesirable effects) of the SmPC.	
Important potential risk – Medication errors	- Information in section 4.2 Posology and method of administration of the SmPC - Information in Section 4.4 Special warnings and precautions for use that Alofisel is for intralesional use	Educational materials explaining how to handle and administer the product will be provided to healthcare professionals.
Missing information - Long-term safety	- Routine risk minimisation reflects prescription and administration by specialist healthcare professionals	None proposed.
Missing information - Experience during pregnancy and lactation	- Information on pregnancy and lactation is provided in section 4.6 (Fertility, pregnancy and lactation) of the SmPC.	None proposed.
Missing information - Experience in the elderly	- Information on special populations is provided in section 4.2 (Posology and method of administration) of the SmPC.	None proposed.
Missing information – Repeat use	- Information on repeated administration is provided in section 4.2 (Posology and method of administration) of the SmPC.	None proposed.

AE: adverse event; MAH: Marketing Authorisation Holder; PhV: pharmacovigilance; SmPC: Summary of Product Characteristics.

The CHMP endorse the PRAC and CAT advice on the RMP.

2.8. Pharmacovigilance

Pharmacovigilance system

The CAT/CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant declared that darvadstrocel has not been previously authorised in a medicinal product in the European Union.

The CAT/CHMP, based on the available data, considers darvadstrocel to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Labelling exemptions

A request of translation exemption of the vial label in accordance with the third subparagraph of Article 63(1) of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

Alofisel is an ATMP which has a short shelf-life (48 hours from the start of the finished product manufacturing to the time of administration). The labeling of the vials happens during the manufacturing process, and the packaging and distribution consumes approximately 4 hours of the 48h shelf-life clock. Having a vial label in English only will therefore help reducing the complexity as much as possible to avoid additional packaging time being borrowed against the short shelf-life of the finished product.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language as agreed by the QRD Group.

A request of translation exemption of the outer carton label in accordance with the third subparagraph of Article 63(1) of Directive 2001/83/EC has been submitted by the applicant and has been found unacceptable by the QRD Group for the following reasons:

No consensus could be reached by the QRD Group. Some Member of the Group could not agree to have an outer carton in English only mainly due to safety consideration: the label contains important information for the safe handling of the product which should be understood by the trained staff at the hospital who do not speak English. Bilingual or multilingual outer carton was a preferred option.
Therefore, the applicant has been advised to submit this request separately to each National Competent Authority based on Art.63.3.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Alofisel (darvadstrocel) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Fistulas are common complications of Crohn's disease (CD) and include perianal fistulas connecting the anorectum and the perianal area and also fistulas between the gastrointestinal tract and an internal organ or the abdominal skin. In particular patients with CD involving the distal bowel are at increased risk of developing perianal fistulas. Results from population based studies indicate that perianal fistulas are the most common manifestation of fistulising CD and develop in approximately 20-30 % of patients along the course of the disease. Recurrences are observed in approximately 30 % of the cases (Hellers et al 1980, Schwartz et al 2002). Crohn´s disease perianal fistulas typically present as fissures or ulcers penetrating the anorectal wall and tracking till the perianal skin surface, and are characterised by local inflammation that is exacerbated by bacterial infection(s) and faecal contamination. In the inflamed area, there is lymphocyte activation and local release of inflammatory cytokines. Perianal fistulas in CD are classified as simple or complex. A complex fistula is characterised as having an origin above the dentate line, to have multiple external openings and are associated with perianal abscess, connection to the vagina or bladder. Further, a complex anal fistula could also meet the following criteria: have rectal stenosis and/or macroscopic proctitis. A complex fistula is more treatment resistant than simple fistulas. (Schwartz et al, 2015).

The main symptoms of perianal fistulas are pain, abdominal swelling, abscess formation, fever and drainage of faeces, pus and blood. Perianal disease is associated with high morbidity and has a negative impact on the quality of life for the affected patients.

3.1.2. Available therapies

Presently recommended treatments for perianal fistulising CD include drainage and immunosuppressive treatment. Antibiotics and thiopurines are considered as adjunct treatments and anti-TNF is considered the gold standard (Gecse KB et al, 2014). However, only infliximab has an approved indication for treatment of fistulising CD.

3.1.3. Main clinical studies

The clinical efficacy has been evaluated in one randomised, double blind, placebo-controlled, multicentre pivotal study. The study population included were patients with mild to moderate luminal Crohn's disease and with perianal fistulas. Fistulas had previously shown an inadequate response to antibiotics, immunosuppressants or anti-TNF agents. Patients were to have complex fistulas with a maximum of two internal fistula openings and three external openings. All patients received pre-study preparation treatment (curettage, antibiotics and seton placement when required). On the day of administration of Alofisel $120x10^6$ cells or placebo (saline), setons were removed and internal openings were closed with sutures.

One *supportive* open-labelled pilot study was performed including patients with CD and complex fistulas. Patients were not required to have failed previous fistula treatments. Preparation treatment was performed on the same day as the administration of the study compound.

3.2. Favourable effects

The primary efficacy endpoint for the pivotal study was defined as combined remission of perianal fistulising CD that included clinical closure of external openings that were draining at baseline despite gentle finger compression and absence of collections > 2 cm of the treated fistula confirmed by MRI images, at week 24. The results from this study show that there was a statistically significant difference between the numbers of patients in combined remission in the active and placebo groups at week 24.

The combined remission in the active group was 49.5 % (53/107) (95% CI, 40.1, 59.0) and the corresponding figures in the placebo group were 34.5 % (36/105) (25.2, 43.4).

The difference between the groups was 15.2 % (97.5 % CI 0.2, 30.3). This result was supported by the key secondary and other secondary endpoints although differences were not statistically significant. Key secondary endpoints were clinical remission (closure of all external openings that were draining at baseline despite gentle finger pressure compression) and clinical response (closure of ≥ 50 % of external openings) at week 24.

Other secondary endpoints were combined remission, clinical remission and clinical response at week 52. The results were supportive of the primary analysis and showed statistically significant difference between the active and placebo groups. The difference between the groups in combined remission was 17.1 % (95 % CI, 3.9, 30.3), for clinical remission 17.0 % (95% CI, 3.8, 30.3) and for clinical response the difference was 10.2 %.

The conducted sensitivity analyses of combined remission and key secondary endpoints in the mITT, Safety and PP are supportive of the primary analyses.

The proportion of patients with clinical remission at Week 52 was 59 % in the Alofisel group and 41 % in the control group (p=0.012) and corresponding figures for response were 66% and. 55% (p=0.114). These data are supportive of the results from week 24 and there was a consistent difference observed between active and placebo treatment groups although the effect is limited to a minor proportion of the included patients.

The results from the pilot study are also considered to be supportive although limited conclusions can be drawn due to the small number of patients included and the uncontrolled design of the study.

3.3. Uncertainties and limitations about favourable effects

There was no observed difference in the QoL of subjects before and after treatment with Alofisel. There was a very small improvement in PDAI (perineal disease activity index) in both groups that was maintained to week 52. However, the PDAI has limitations for determination of QoL for CD patients with fistulas.

Data from week 104 has been submitted. For those patients that completed the week 52 follow-up and entered the 104 weeks follow-up, the rate of clinical remission was 56 % and 40 % in the active and placebo group, respectively. However, the data are viewed as limited.

As a whole the treatment effect is considered to be of clinical relevance, as other treatment options for fistula have failed. The effect size appears modest and the application is based on a single pivotal study only. To provide confirmatory information on efficacy, the Company has presented a protocol for an ongoing Phase III study that is approved by the FDA, . Data from this study is expected to further increase the knowledge of the efficacy and safety of Alofisel in the treatment of fistulas. This study is considered key to the benefit-risk balance of the product, and thus, the approval of Alofisel should be subject to the submission of study Cx601-303 as an Annex II condition in line with Article 14 of Regulation (EC) No 1394/2007, as a post-authorisation follow-up of efficacy.

There is currently limited experience with the efficacy or safety of repeat administration of Alofisel and section 4.2 of the SmPC informs the prescriber accordingly. Furthermore the PASS will provide, next to long term safety data, data on the effectiveness of Cx601 (Alofisel) in patients treated and retreated (i.e. repeated dosing) in routine clinical practise as described in the RMP.

3.4. Unfavourable effects

Similar proportions of patients treated with Alofisel and placebo experienced TEAEs (76.7% and 72.5%, respectively) and most events were mild or moderate in intensity. Severe TEAEs were also reported similarly (9.7% vs. 11.8% of patients).

The most common TEAEs that occurred in a higher proportion of patients in the Alofisel group than the placebo group included proctalgia (Alofisel: 14.6% patients; placebo: 11.8%), anal fistula (10.7% vs. 7.8%) and anal abscess (19.4% vs. 13.7%) patients). The combined frequency of 'anal fistula' and 'fistula' TEAEs was however similar in both treatment groups (Alofisel: 13/103 [12.6%] patients; placebo: 13/102 [12.7%]).

Events of procedural pain (1-2% of patients) and also proctalgia seemed to (mainly) result from the conditioning of the fistula prior to Alofisel administration, considering the similarity in incidence between the Alofisel and placebo treatment groups and the fact that these AEs also were reported as non-treatment-emergent AEs during the initial surgical preparation visit (prior to randomization). Nasopharyngitis (10.7% vs. 4.9%) and diarrhea (8.7% vs. 2.9%) were reported as TEAEs by proportionally more patients in the Alofisel group than the placebo group but these events were typically considered as unrelated to treatment.

The most common TESAE was anal abscess, which was reported in slightly more patients in the Alofisel group than placebo up to Week 52 (13.6% versus 7.8%). This most likely reflects a lack of efficacy in some patients rather than a safety issue. Development of new anal fistula and/or anal abscess or relapse of treated fistula is addressed as important potential risk in the RMP and the safety profile is adequately described in section 4.8 of the SmPC.
All TESAEs other than anal abscess experienced by Alofisel-treated patients were reported in small numbers of patients and were considered unlikely related or unrelated to study treatment.

3.5. Uncertainties and limitations about unfavourable effects

The current safety analyses were largely based on a single pivotal study using data up to the Week 52 visit. While in the course of the assessment procedure additional follow-up data (up to Week 104) regarding the safety of Alofisel after single administration was provided, the overall scope of the safety database is still limited.

Most events of serious abscess formation (10 out of 14 cases of anal abscess SAEs) and all 4 cases of anal fistula SAEs in the Alofisel group occurred in the same fistula that was treated with Alofisel, in several cases a rather long time (~1-10 months) after the procedure. In the placebo group, SAEs of anal abscess (8), anal fistula or fistula (2) and/or anal inflammation (1) was reported to be in the same (treated) area in 7 of 11 reported cases. Thus, treatment with Alofisel was not associated with fewer events of serious abscess formation in the treated fistula as compared to placebo, as might be expected. This most likely reflects a lack of efficacy in some patients rather than a safety issue. However, as the follow-up time in the current submission is limited in terms of relapse risk and long-term maintenance of remission the risk of new anal fistula and abscess and/or the recurrence of previously treated fistulas was included as an important potential risk and will be followed up within the PASS as described in the RMP.

While treatment with Alofisel is proposed for single dose administration, the need for repeated treatment in the clinical setting seems foreseeable in the targeted patient population. Therefore the prescriber is informed in section 4.2 of the SmPC about the limited experience of repeat administration of Alofisel. The supportive open-label study Cx601-0101 explored the issue of retreatment but included only few patients and the doses were lower than intended for marketing. Whether repeated administration could be associated with increased generation of DSA and/or in any way with increased risk of allo-immune response will also be further studied in a PASS as described in the RMP.

Medication errors are possible in the handling and administration of Alofisel. Cell viability may be compromised if the fistula tract is washed through with hydrogen peroxide, methylene blue, iodine solutions or hypertonic glucose solutions before, during or after Alofisel administration. Cell viability may also be compromised if Alofisel is administered using thinner gauge needles than 22G, or if local anaesthesia is used for fistula conditioning (as the effect of local anaesthetic on the cells is unknown). Section 4.4 of the SmPC takes due account of this potential risk and educational materials as an additional risk minimisation measure will be provided to ensure healthcare professionals understand the correct procedures for Alofisel handling and administration.

As Alofisel contains living cells, the transmission of bacterial, viral, fungal or prion pathogens might potentially occur. In addition, the final tests for sterility and mycoplasma are retrieved only after release. There have been no cases of transmission of infectious agents after Alofisel administration so far. In case of a positive sterility test result is identified after the product is released, the company will utilize a batch coding system to trace the Drug Substance and MSC from which the contaminated final product was derived and urgently notifies the treating physician. The educational materials as an additional risk minimization measure will inform health care providers that the therapy could contain potentially infected biological material and that patients should be monitored for potential signs of infection after administration.

The risk of tumour formation is a key potential concern in cell based therapies. While nonclinical studies using human eASC in rodents suggest that the tumourigenicity risk of Alofisel is low, the relevance of these data for understanding the risk of tumourigenicity after prolonged engraftment of these cells in human is insufficiently understood. Another potential concern is whether there is any remaining differentiation potential of the eASC cells following administration in humans. Similarly, the nonclinical studies indicate a low potential for undesirable differentiation but how this translates to human risk is unknown. These concern would thus need to be clinically addressed and it is agreed with the applicant to include them as important potential risk into the RMP.

The endorsed PASS to address the remaining uncertainties is a prospective, multicentre, multinational single-arm exposure cohort of patients treated with Alofisel, to evaluate the long-term safety in patients treated and retreated (i.e. repeated dosing and immunogenicity) in the clinical setting. Primary safety outcome measures include new fistula development and/or anal abscess or relapse of treated fistula, immunogenicity/allo-immunoreactions and hypersensitivity reactions, transmission of infectious agents, ectopic tissue formation, tumourigenicity (anal canal and local colorectal malignancy and AEs (serious or nonserious) related to perianal Crohn's disease or related to Alofisel.

3.6. Effects Table

Table 28: Effects Table for Alofisel in the treatment of complex perianal fistulas (29 Feb 2016)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Combined remission Week 24	Closure of external openings and absence of collections > 2 cm confirmed by MRI images	%	49.5	34.3	Moderate evidence/ clinical relevance moderate in population with previous failed treatments	Pivotal study Cx601-302
Remission Week 24	Closure of all external openings	%	53.3	41.0	No statistical significance/ clinical relevance limited	
Response Week 24	Closure of ≥ 50 % of all external openings	%	66.4	53.3	No statistical significance/ clinical relevance limited	
Combined remission Week 52	Closure of external openings and absence of collections > 2 cm confirmed by MRI images	%	54.2	37.1	Moderate evidence/ clinical relevance moderate in population with previous failed treatments	
Reduction of draining fistulas					Limited evidence/clinical relevance uncertain due to study design	Pilot study Cx601-101
Unfavourable Effects						
Anal abscess	Incidence of anal abscess	%	19.4	13.7	Relatedness uncertain due to study population and study design	Pivotal study Cx601-302
Proctalgia	Incidence of proctalgia	%	14.6	11.8	Relatedness uncertain due to study population and study design	
Anal fistula, fistula	Incidence of anal fistula	%	12.6	12.7	Relatedness uncertain due to study population and study design	
Donor-specific antibodies (DSA)	Incidence of DSA	%	36.5%	0	Related to Alofisel only	
					No conclusions can be drawn on repeated administration due to the limited sample size and uncontrolled design	Pilot study Cx601-101

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Perianal fistulas are common in patients with Crohn's disease. Fistulas are associated with high morbidity and a number of patients suffer from scarring, persistent drainage and faecal incontinence. In the studies, patients included had complex fistulas that are more treatment resistant than simple fistulas. Patients were refractory to conventional therapy (antibiotics, immunosuppressants or anti-TNF agents).

This is an infrequent medical condition. There are few options for patients resistant to conventional therapy. Remaining options for this group of subjects would be last resort surgical treatment such as faecal diversion or proctectomy.

In support of the efficacy of Alofisel in the treatment of perianal fistulas in CD patients the results of one pivotal study only has been submitted. There was a statistically significant difference between the groups of 15 % at week 24. Results for key secondary endpoints were supportive but statistical significance was not achieved. However, additional efficacy data from week 52 provide further support in that a statistically significant difference between the treatment groups of 17 % was observed for combined remission and clinical remission. The beneficial effects of Alofisel based on the current data appear to be consistent although moderate. However, further data to support efficacy is expected to be available from the Cx601-0303 study.

The short-term safety profile of Alofisel is considered favourable. Approximately 77% of subjects treated reported a treatment emergent adverse event (vs. 73% in the placebo group) of which the majority were of mild intensity and approximately 10% are reported as severe. Serious safety concerns identified included anal abscess, anal fistula and proctalgia. These adverse events could be foreseen given the nature of the perianal disease and the procedures involved in the administration of the cells.

Treatment with Alofisel was not associated with fewer events of serious abscess formation in the treated fistula as compared to placebo, as might be expected. This most likely reflects a lack of efficacy in some patients rather than a safety issue. Additional post-hoc analyses of the primary outcome were performed in order to assess the potential impact of relapses on the clinical outcome (combined remission and remission) at week 24 and 52 in the ITT population in which TESAEs and TEAEs indicative of lack of efficacy (i.e. fistulas and abscesses) were imputed as treatment failure. These results confirm that fistula/abscess adverse events did not impact the primary efficacy analysis conclusions.

A specific risk associated with Alofisel use is the generation of Donor Specific Antibodies (DSA). Based on the current data, the generation of DSA does not appear to affect efficacy or safety after single treatment. However, there are uncertainties regarding repeated treatment. Also, the generation of such antibodies may have other implications for future transplant therapies.

While the current size of the safety database is acceptable for marketing authorisation additional data on the safety of Alofisel, in understanding the biological mechanisms, the safety and efficacy of repeated administration, the risk of differentiation and ectopic tissue formation, and the risk of tumourigenicity will be provided post marketing as described in the RMP. As Alofisel contains living, allogeneic cells, the collection of additional safety data is also important to further monitor and minimize the possible occurrence of transmission of infection and of immunogenicity/all-immunoreactions. These uncertainties will be addressed in the RMP.

3.7.2. Balance of benefits and risks

Overall, it is considered that the results from the single, pivotal phase III study provide sufficient evidence of efficacy. The results from the pivotal study showed that there was a statistically significant difference between the numbers of patients in combined remission in the active and placebo groups. The difference between the groups at week 24 for the primary outcome combined remission was 15.2 %. This was supported by the results of the key secondary endpoints clinical remission and clinical response although differences were not statistically significant. Other secondary endpoints i.e. combined remission and clinical remission at week 52 show statistically significant differences between treatment groups of 17 % and for clinical response at week 52 the difference was 10 %. Although the effect compared to control appears modest, is considered to be of clinical relevance as other treatment options for fistula have failed. Considering also the approval setting with a single pivotal study, confirmatory information on efficacy is considered as of importance for the benefit-risk of the product and will be obtained from the ongoing Phase III study Cx601-303 (final CSR expected in 2022). Thus, the approval of Alofisel should be subject to the submission of study Cx601-303 which is similar in design to the pivotal study of this application as an Annex II condition.

The safety database of Alofisel is considered limited but provides sufficient characterisation of the safety profile for marketing authorisation. Unfavourable effects and uncertainties are appropriately described in SmPC and RMP and post authorisation follow up will be carried out in particular on important potential risks, repeated administration and long term safety by means of a post authorisation safety study.

3.8. Conclusions

The overall benefit/risk balance of Alofisel is positive.

Divergent positions are appended to this report.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above.

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by majority decision that the benefit-risk balance of Alofisel is favourable in the following indication:

Alofisel is indicated for the treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease, when fistulas have shown an inadequate response to at least one conventional or biologic therapy. Alofisel should be used after conditioning of fistula, see section 4.2.

The CAT therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Based on the draft CHMP opinion adopted by the CAT and the review of data on quality, safety and efficacy, the

CHMP considers by majority decision that the risk-benefit balance of Alofisel in the treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease, when fistulas have shown an inadequate response to at least one conventional or biologic therapy. Alofisel should be used after conditioning of fistula, see section 4.2. is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to the launch of Alofisel in each Member State, the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities and any other aspects of the programme, with the National Competent Authority. The aim of the educational programme is to provide information on how to correctly administer the product in order to minimise the risk of medication errors and to increase awareness about the potential transmission of infectious agents.

The MAH shall ensure that in each Member State where Alofisel is marketed, all healthcare professionals who are expected to handle and administer Alofisel have access to the educational package for health professionals.

- **The educational material for health professionals** should contain:
  - The Summary of Product Characteristics
  - Guide for pharmacists with instructions on the appropriate reception and storage of Alofisel.
  - Guide in form of a video for surgeons and other health professionals involved in the preparation and administration of Alofisel
  - Guide for surgeons and other health professionals describing the method of administration

- Guide for health professionals providing information on potential for microbial information and advice on steps to follow in case a positive culture is identified

- These shall contain the following key elements:
  - Relevant information on the risk of medication errors and the potential for transmission of infectious agents and details on how to minimise these, including reception, storage and administration instructions (i.e. fistula conditioning, preparation and injection).
  - Instructions how to handle medication errors and transmission of infectious agents.

Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	**Due date**
In order to follow-up on the efficacy of Alofisel, the MAH should submit the results of a Phase III randomised double-blind, placebo-controlled study *Cx601-0303* investigating a single administration of Cx601 for the treatment of complex perianal fistulas in Crohn's disease patients.	Final Report to EMA: 2Q/3Q 2022

The CHMP endorse the CAT conclusion on the obligation to conduct post-authorisation measures as described above.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

Divergent positions to this majority recommendation are appended to this report.

New Active Substance Status

Based on the CAT review of the available data, the CAT considers that darvadstrocel is considered to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorse the CAT conclusion on the new active substance status claim.

APPENDIX

DIVERGENT POSITION DATED 14 December 2017

DIVERGENT POSITION DATED 14 December 2017

Alofisel EMEA/H/C/004258/0000

The undersigned members of the CHMP did not agree with the CHMP's positive opinion recommending the refusal of the granting of the marketing authorisation of Alofisel indicated for treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease, when fistulas have shown an inadequate response to at least one conventional or biologic therapy.

The reason for divergent opinion was the following:

As explained in the CHMP guideline for single pivotal trials, in the case of licensing based on one single pivotal trial, the results should be compelling. The currently available results are, however, modest.

Given the small effect size and given the history of negative studies with respect to mesenchymal stem cell treatment an additional confirmative study is needed to confirm the marginal efficacy at an acceptable safety profile for the proposed indication.

The company is currently performing an additional clinical study Cx601-0303 in the US and the EU. We consider that the benefit/ risk balance of Alofisel should be re-assessed when the final results of this additional study are available. Without confirmation of both efficacy and safety by results from an additional trial, the benefit/risk balance of Alofisel remains negative. Additionally, there remains the issue that repeated use of this product may be necessary when new fistulas open, but has never been investigated.

Alar Irs

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