EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 February 2013
EMA/250338/2013
Committee for Advanced Therapies (CAT)

Withdrawal assessment report

Oranera

(previously known as CAOMECS)

Multilayered cell-sheet of autologous oral mucosal epithelial cells

Procedure No. EMEA/H/C/002443/0000

Note

Day 180 Assessment report as adopted by the CAT with all information of a commercially confidential nature deleted.

This should be read in conjunction with the "Question and Answer" document on the withdrawal of the application: the Assessment Report may not include all available information on the product if the CAT assessment of the latest submitted information was still ongoing at the time of the withdrawal of the application.

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LIST OF ABBREVIATIONS

AE Adverse event

AM Amniotic membrane

CAOMECS Cultured Autologous Oral Mucosal Epithelial Cell-Sheet

CBMP Cell based medicinal product

CHMP The Committee for Medicinal Products for Human Use

EM(E)A European Medicines Agency

GCP Good Clinical Practice

K Keratin

LECs Limbal epithelial cells

LSCD Limbal stem cell deficiency

MUC Mucin

OCP Ocular cicatricial pemphigoid

OMEC Oral mucosal epithelial cell

PKP Penetrating keratoplasty

SA Scientific advice

SJS Stevens-Johnson-Syndrome

TR Temperature Responsive

1. RECOMMENDATION

Based on the review of the data on quality, safety and efficacy, the CAT considers that the application for OraNera in the treatment of limbal stem cell deficiency in adults, is not approvable since 'major objections' have been identified, which preclude a recommendation for marketing authorisation at the present time.

The information in the dossier precludes an evaluation of the benefit/risk, which is therefore negative. The clinical trial with the product allows no unequivocal conclusions on efficacy and the internal and external validity of these results and do not support the indication applied for by the applicant. Additionally the original product no longer exists, whereas an alternative production site has been closed as well. The closure of the new manufacturing site and therefore lack of a facility to produce a new product precludes the generation of new clinical data. This is required as the original data cannot be considered pivotal, only supportive at best provided there would be a comparable product. Furthermore there are doubts whether the intended Marketing Authorisation Holder is still viable and capable of adhering to its legally required post-authorisation responsibilities.

Questions to be posed to additional experts

N/A

Inspection issues

As agreed during the July CHMP, the France study has undergone GCP inspection. Serious and critical findings were reported, but as these did not invalidate the results from the trial it was decided to proceed with the assessment.

New active substance status

In the first round there was a question that the applicant should provide further evidence that the active substance contained in the medicinal product OraNera is to be qualified as a new active substance in itself (NAS). Based on the review of the data and the Applicant's response to the CHMP LoQ, the CAT consider that the claim for a NAS is justified.

2. EXECUTIVE SUMMARY

2.1. Problem statement

The applicant has proposed a new name for this product, OraNera. For textual consistency purposes and clarity, the name CAOMECS is used in the remainder of these Assessment Reports.

CAOMECS is a tissue graft intended to be used to replace the damaged corneal epithelium in patients with total unilateral or bilateral limbal stem cell deficiency (LSCD) with moderate or severe symptoms, caused by severe exogenous trauma or endogenous eye disease.

The concept of using autologous buccal mucosa biopsy material as a source for preparation of an epithelial cell sheet as a replacement of damaged corneal epithelium has several advantages to alternative methods used so far to obtain autologous grafts. Especially obtaining an autologous epithelial cell sheet with this method does not depend on the availability of healthy limbal stem cells e.g. from the other eye.

CAOMECS fulfils the definition of a tissue-engineered product as described in the EU regulation (EC) 1394/2007 article 1(1)b on advanced-therapy medicinal products.

In 2009 the company Cellseed applied for a Scientific Advice at EMA in which one of the questions was if their product (CAOMECS) could be eligible for a Conditional Marketing Authorisation. This approval would be based on data from 25 patients, which the Company said to be convincing. The Scientific Advice Working Party agreed.

When the company submitted the dossier, in June of 2011, it was found to be incomplete and the data generated a large number of questions, on all parts of the dossier. In addition, it became apparent that the company was in the process of transferring the production process to a new facility. Previously CAOMECS was produced in a laboratory of the Hospice Civils de Lyon (HCL).

The dossier contains one small clinical trial with the product from the original production site, with major GCP deficiencies and the applicant is not able to provide the underlying safety data from this study. Complicating factor is that the trial is also conducted with a product that no longer exists. Instead a product was being developed that has never been tested in a clinical trial and for which no comparability data with the original product are available. It was also unclear to the CAT how many (if any) clinical batches have actually been produced at the new facility. This situation made a (Conditional) Marketing Authorisation problematic.

The only option was to conduct a new pivotal trial with the new product to demonstrate benefit and risk. If comparability between the new and the original product was established, the existing clinical data might be assessed as supportive, but not as pivotal for the new product given the major flaws in this study. See also the benefit/risk.

This was discussed with the company in multiple clarification meetings. After the regular clock stop period of 6 months, the company requested and was granted an additional 9 month clock stop period

to solve their problems. However, this proved to be too short, and in October 2012 Cellseed requested an additional extension of the clock stop, this time with an extra 9 months, bringing the total clock stop close to 2 years. When asked, the company expected that, after these additional 8 months, production development would have proceeded far enough to be able to include up to 4 patients in a clinical trial. However, the French national regulatory agency (ANSM) has received a letter acknowledging that no batch of CAOMECS has ever been produced and indicating that due to the absence of activity the production site will be closed as of December 31st, 2012. Therefore neither a product nor a manufacturer is available. In its December 2012 meeting, the CAT decided that the discrepancy between what Cellseed expected to deliver and what the CAT requires for a positive licensing advice to CHMP, had become too large and declined this second extension.

Summarizing, the main issue with this application procedure is that there is no product available. In addition, if comparability between the old and a new product could be established, the already available clinical data would still be only supportive, not pivotal.

Additionally, it is unclear whether the applicant is still a viable company and able to fulfill their obligations as MAH. In its responses to the day 120 List of Questions, submitted on 20 December 2012, Cellseed again presented the original clinical data and provided a fragmented update to the quality dossier.

2.2. About the product

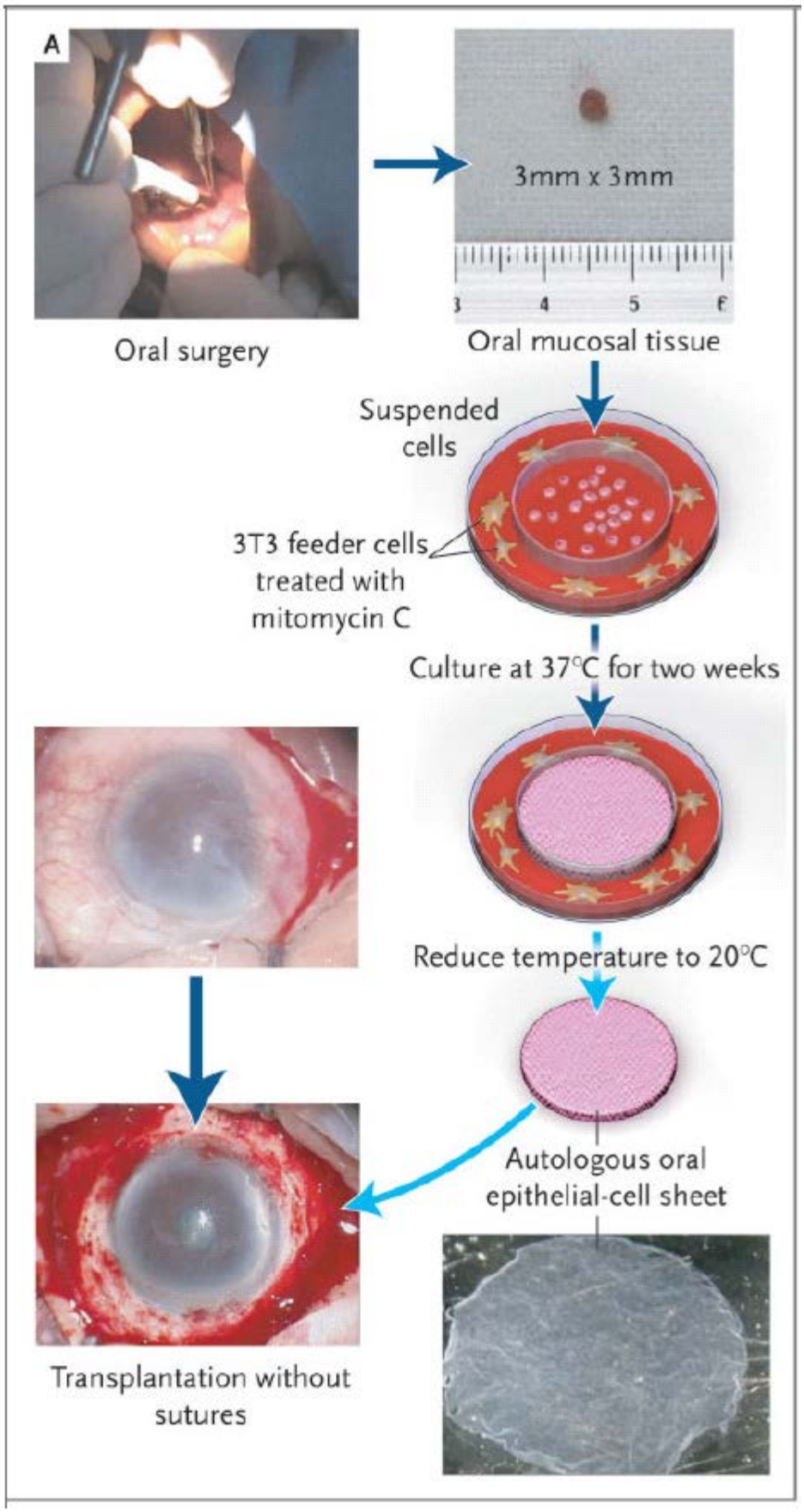
Cultured Autologous Oral Mucosal Epithelial Cell-Sheet (CAOMECS) is a multiplayer cell-sheet which is produced by culturing of oral mucosal epithelial cells obtained through biopsy from the patient's buccal mucosa. CAOMECS is intended to be used as tissue graft to replace damaged corneal epithelium in patients with LSCD caused by various etiological diseases.

The claimed indication is: Treatment of limbal stem cell deficiency in adults.

The cultivation process (2-3 weeks) is performed with the use of a temperature-responsive polymer-coated culture surface, on which the multilayer cell sheet is grown. As a result the epithelial cell-sheets can be obtained as an intact sheet from the temperature responsive membrane without destruction or damage of cell-cell junction and basal membrane components since enzymatic treatment is not necessary for harvesting the final product. Cells are generally cultured for two weeks.

The CAOMECS cell sheet can be grafted onto the corneal surface without need for sutures.

CAOMECS is also believed to provide a barrier function that protects the cornea from conjunctival epithelial growth and neovascularisation. These last two processes are stopped normally by the limbal stem cells present at the periphery of the corneal epithelium. It is claimed that persistence of conjunctivalisation and neovascularisation is hindered and that the corneal environment is improved and discomfort relieved. When the patients' stroma is not damaged, improvement of visual acuity can also be expected.



Source Nishida et al 2004

2.3. The development programme/Compliance with CHMP guidance/Scientific advice

The non-clinical development programme for CAOMECS included *in vitro* and *in vivo* primary pharmacodynamic studies, single-dose toxicity studies in rabbits with LSCD and studies to investigate the potential tumourigenicity of CAOMECS. The potential biodistribution of CAOMECS was evaluated by histopathological examination of selected organs within the tumourigenicity study in nude mice and the single-dose toxicity study with 4-week observation period in LSCD rabbits.

Scientific advice was given by the CHMP on several occasions. Non-clinical data were discussed in one scientific advice procedure in 2009 (EMA/H/SA/1272/1/2009/SME/ADT/III) and a follow up scientific advice procedure (EMA/H/SA/1272/1/FU/1/2009/SME/ADT/III) in 2010.

The clinical development plan consists of one small "proof-of-concept" study which included four patients with total LSCD who were treated with autologous OMECs expanded ex-vivo (Nishida et al in 2004) and one open, uncontrolled, single centre design in 25 patients with LSCD (France study 2009). Patients were grafted with tissue-engineered epithelial cell-sheets expanded ex-vivo from patients' OMECs.

An additional open, non-comparative, multi-centre, phase III study (Study CS001-EU01) in adult patients with total LSCD was planned to be initiated in autumn 2011, however, this has not happened and no new data are available.

The design and main endpoints of this study have been discussed with the European Medicines Agency (EMA) during a scientific advice.

It was underlined that primary endpoints should reflect the primary objectives of the study and reflect patient related outcomes. This goal would be achieved with primary endpoints able to show improvement of the corneal environment by:

1. creating a stable corneal epithelium by preventing conjunctivalisation
2. preventing neovascularisation

Such improved corneal environment over 12 months would allow either the patient to get prepared for further surgical measures (12 months or later following CAOMECS grafting) or to reach a stable disease status with improved visual acuity in those cases where the corneal stroma was not damaged.

2.4. General comments on compliance with GMP, GLP, GCP

Regarding the non-clinical data, only one study (4 weeks toxicity study) was performed in compliance with GLP. As clearly stated in a scientific advice procedure, a company should make every effort to carry out the non-clinical toxicology studies in a GLP facility. If that is not achievable, appropriate justification should be provided. In response to the day 120 LoQ the applicant provides a justification for the lack of GLP compliance of the safety studies in the marketing application dossier. According to the company, clinical data on general safety are already available which indicated no safety concerns and therefore superseed the GLP non-compliant animal data. The applicant indicated that the

tumorigenicity study will be repeated. It is assumed that this new study will be GLP compliant. This is acceptable from a non-Clinical point of view.

The 'France study' has undergone GCP inspection. This inspection was conducted by the Netherlands and France. The inspection revealed many deficiencies in the trial, including serious and critical findings.

In addition two GMP inspections have been conducted by ANSM.

a. The intended new facility responsible for the manufacture of the drug substance and drug product. A GMP inspection was conducted on 12-13 January 2012 and was found to be in general GMP compliance. However, this site is no longer in use, see above.

b. The intended laboratory responsible for mycoplasma testing of the drug product. A GMP inspection for the mycoplasma was carried out on 28-29 February 2012 and was found to be in general GMP compliance.

Type of application and other comments on the submitted dossier

- *Legal basis*

The application is made in accordance with article 8(3): full application.

The applicant applies for a 'prescription only' legal status, product subject to restricted prescription. In the SPC it is stated that CAOMECS should only be used by 'appropriately trained and qualified surgeon according to well-defined steps' and use is restricted to hospital use only.

- *Accelerated procedure*

N/A.

- *Conditional approval*

The applicant requests a marketing authorisation under conditional approval based on unmet medical need and the absence of another authorised product.

For conditional approval all three of the following criteria should be met: unmet medical need, a positive benefit/risk, that additional data will be generated to complete insufficiencies of the dossier.

It is agreed that there is an unmet medical need for the condition.

In the 2009 scientific advice it is stated a conditional approval may be possible based on one study. However, the clinical trial was conducted with a product that no longer exists. Additionally, the deficiencies in the dossier preclude a B/R assessment.

- *Exceptional circumstances*

N/A

- *Biosimilar application*

N/A

- *1 year data exclusivity*

N/A

- *Significance of paediatric studies*

N/A

3. SCIENTIFIC OVERVIEW AND DISCUSSION

The corneal epithelium is a tissue with a dynamic turnover. Its renewal is assured by corneal epithelial stem cells in the basal layer of limbal epithelium located at the transitional zone between the cornea and the bulbar conjunctiva. Moreover, the limbal epithelium serves as a barrier between corneal and conjunctival epithelia (Chen *et al.,* 1990, Dua *et al.,* 2000, Cauchi *et al.,* 2008).

Injury to the limbal epithelium either by endogenous eye disease by severe exogenous trauma results in partial or total LSCD (Chen *et al.,* 1991, Huang *et al.,* 1991, Dua *et al.,* 2003). Congenital causes include aniridia, erythrokeratodermia, dominantly inherited keratitis (characterized by absence of the palisades of Vogt), and ectodermal dysplasia. Acquired forms of LSCD can be due to chemical or thermal burns, radiation, cryotherapy, drug toxicity (e.g., corticosteroids, or ß-blockers, mitomycin-C), multiple surgeries, infection, or contact lens-induced keratopathy. Another group of acquired causes of LSCD are autoimmuno-inflammatory conditions such as Stevens-Johnson syndrome (SJS) or ocular cicatricial pemphgoid (OCP). LSCD can be diffuse or sectoral, and can affect one or both eyes.

Dysfunction or destruction of the corneal limbal stem cell population severely impairs corneal epithelial wound healing with in growth of the neighbouring conjunctival epithelium (conjunctivalisation). Replacement of corneal cells with conjunctival cells explains most of the clinical findings seen with LSCD. As conjunctival cells are held together by looser intercellular connections than corneal cells, leukocytes in the tear film are able to penetrate the corneal stroma, leading to chronic inflammation and corneal opacification. In addition, conjunctival cells, in contrast to corneal cells, are unable to secrete antiangiogenic factors, thus resulting in corneal neovascularisation. (Djalilian *et al.,* 2005, Shortt *et al.,* 2007, Hatch *et al.,* 2009).

Clinical symptoms include discomfort, recurrent episodes of pain or chronic pain, photophobia, dry eye or tearing, and blepharospasm. Non-healing epithelial defects may lead to scar formation, ulceration, or even perforation. The outcome is severe visual impairment and loss of vision (Puangsricharen *et al.,* 1995, Holland *et al.,* 1996, Dua *et al.,* 2000, Schlötzer-Schrehardt *et al.,* 2005).

The current treatment of total unilateral LSCD is a direct transplantation of autologous limbus containing LESCs from the contralateral normal eye to regain a stable corneal epithelium (Shortt *et al.,* 2007, Cauchi *et al.,* 2008). However, it requires a large piece of limbus, and that is not always available. Even when enough limbus is taken, there is a risk to damage the patient's healthy eye by depleting its stem cells reserves (Jenkins *et al.*, 1993). Recently, the transplantation of cultured autologous limbal epithelial cell-sheets has been performed, which requires minimal biopsy from a normal limbus, and favourable clinical results have been reported (Pellegrini *et al.*, 1997, Koizumi *et al.*, 2001, Nishida *et al.*, 2004b). However, the autologous limbal graft (applied either directly or after *ex vivo* expansion) cannot be obtained in cases where patient's ocular surfaces have been damaged or in cases of bilateral total LSCD.

When an autologous graft is not possible, the alternative treatment is currently the transplantation of allogeneic limbal epithelium in the form of a keratolimbal allograft from cadaveric tissue (Tsubota *et al*.,

1999, Samson *et al*., 2002 and Shimazaki *et al*., 2000, Rao *et al*., 1999) or living-related donors. Nevertheless, this treatment necessitates postoperative immunosuppression to avoid rejection, and even if the patients are controlled by systemic immunosuppression, the success rate of allograft tends to decrease gradually with time. Therefore, allograft is not considered as a standard treatment for LSCD.

Consequently, there is room for introduction of an alternative treatment approach for patients with total LSCD in order to avoid being visually impaired, when autologous limbal transplantation (with or without amplification) is not possible or not accepted by the patients afraid of damage to the healthy eye or in cases of bilateral LSCD.

3.1. Quality aspects

General information

CAOMECS fulfils the definition of a tissue-engineered product as described in the EU regulation (EC) 1394/2007 article 1(1)b on advanced-therapy medicinal products.

CAOMECS is a cell based product of human origin and consists of a transparent, smooth, contiguous, multilayer "Cultured Autologous Oral Mucosal Epithelial Cell-Sheet". These epithelial cells originate from an autologous buccal mucosa biopsy. The biopsy consists mainly of oral mucosal epithelial cells and contains a small percentage of epithelial stem cells and progenitor cells. These cells are grown to an autologous oral mucosal epithelial cell sheet. The company claims that CAOMECS contains highly proliferative potential cells that are putative stem cells or progenitor cells. CAOMECS will bring new epithelium by surgical procedure, but without suturing on stroma. In addition, CAOMECS aims to prevent current conjunctivalisation and neovascularisation. These cells are suggested to be key to providing a favourable long-term clinical outcome in the relief of discomfort to improve the "Quality of Life" of the patients. The function of CAOMECS is an autologous epithelial cell sheet for restoring ocular epithelial surface.

The initial quality dossier was considered premature. The Applicant has chosen to provide the responses to the CHMP D120 LoQ first and to provide an updated CTD dossier, including the Quality Modules 2&3, at a later stage. As such, it is as yet impossible to conclude on the completeness of the Quality sections within the MA dossier.

In addition, it has become clear that the intended commercial GMP manufacturing site stopped its services for ATMP production in December 2012, therefore the applicant is currently further investigating alternative facilities under GMP. A number of studies that were planned to be performed after final implementation of new control methods still remain to be conducted. These mainly include an additional stability study, a comparability study between the process used for the previous French clinical study (2007-A00270-53) and the current process under GMP, and a final process validation using three consecutive batches with all of the established specifications. As a consequence, there are significant uncertainties on the manufacturing site for CAOMECS commercial process, which preclude full assessment of many of the Applicant's responses.

As per request, the definitions for Drug substance and Drug Product have been revised and the updated documentation demonstrates a better knowledge and understanding of the product's nature and biological/physiological function has now been better defined.

In brief the CAOMECS manufacturing process consists of:

1. biopsy of the buccal mucosa at the hospital,
2. Transportation of the biopsy to the manufacturing site,
3. Preparation of single mucosal cells by dispase and trypsin treatment

4. Cell culturing in the presence of feeder cells, yielding a multi-layered cell sheet (Drug Substance)
5. Packaging of the cells-sheet (Drug Product)
6. Transportation to the hospital where the sheet is harvested and grafted.

Drug substance

The DS was redefined as the viable epithelial cell-sheet at the end of the culture period.

As per request, the quality of the biopsy used as starting material for the manufacture of CAOMECS has been further documented and is now considered acceptable. Quality controls on the biopsy are limited to visual inspection and verification of transport, patient and traceability documentation because it is not possible to perform analytical testing on the biopsy. Further quality control is done on epithelial cells isolated from the biopsy, with acceptance criteria defined for total viable cell number and identity. The biopsy is transported to the manufacturing site. An additional study was performed to address the stability of the biopsy during transport. Time specification and storage of the biopsy has been limited.

The biopsy is enzymatically treated with dispase and trypsin in order to isolate individual cells that will be used for drug product manufacturing. Whilst controls are envisaged for the dispase and trypsin digestion steps and operators are trained, these procedures have not yet been formally validated.

Besides the presence of epithelial cells, stem cells and progenitor stem cells the biopsy also contains other cell types that are considered cellular impurities. Removal of these cells is controlled using specific markers for different cell types. Generally the choice of these markers is sufficiently justified.

For the growth of epithelial stem cells to confluent cell sheets, cultivation in the presence of feeder cells is required. The feeder cells are treated with Mitomycin C. The Applicant has been requested to demonstrate that the contamination by the feeder cells is sufficiently controlled. A real-time quantitative PCR has been developed to detect such contamination in addition to rinsing steps in order to mitigate the risk of contamination but it is not validated yet and it has not been tested on validation batches. Due to the inconsistencies in the limit of detection of the PCR method it is not possible to conclude on the actual clearance of feeder cells.

On request the characterisation of the cells sheets was extended. Characterisation with respect to identity and purity of the isolated cells largely relies on the use of specific markers. For most proposed markers their specificity e.g. for mucosal epithelial cells and for stem cells has been established. Several CQAs have been proposed. However, the characterisation parameters have not been established in CAOMECS batches produced in the modified process, nor in a process that has been demonstrated to yield clinically efficacious cell sheets. Furthermore, the Applicant has not yet decided which of the additional characterisation parameters will be part of routine testing in batch release.

Regarding process-related impurities data on three batches have been provided. Generally it is concluded that these are removed to sufficiently low level and pose a low risk, except for hypersensitivities for bovine proteins (from foetal calf serum) and penicillin. Also for other impurities further justification of the limits is required. The experiments were not performed in the same conditions as for CAOMECS manufacturing, which does not allow drawing any conclusion.

Drug product

Drug Product has been redefined as the multi-layered cell-sheet of cultured autologous oral mucosal epithelial cells including stem and progenitor cells which is a result of cultivation on the insert.

Manufacturing of the DP comprises rinsing and primary packaging of the cell-sheets. The DP consists of two CAOMECS sheets, each of which is transported in a single-use cell culture vessel.

During product development the manufacturing process has been modified. The clinical trial material has been produced at a different site (HCL: Hospices Civils de Lyon) as the new GMP site indicated for the manufacturing of the commercial material. Furthermore some steps in the manufacturing process have been changed (e.g. biopsy method and feeder cell treatment). Also transportation of biopsy from and cell sheets to the hospital has been introduced. In addition also the control strategy of the process has been modified. However, no comparability data have been provided and comparability between the product used in the clinical trial and the commercial manufacturing process has not been demonstrated. In addition, it has become clear that the intended commercial GMP manufacturing site stopped its services for ATMP production in December 2012, therefore the applicant is currently further investigating alternative facilities under GMP.

Potency testing of the product is considered too limited. The correlation between potency testing, biological function and clinical efficacy has not been established. The potency assay is not sufficiently validated to represent the regenerative capacity of the sheet. The data presented to substantiate a relation between the results and clinical results in the pivotal clinical trial appear premature, the results were generated with an assay which was not validated, and the acceptance criterion does not ensure that only sufficiently potent batches will be released. Furthermore, this does not cover essential characteristics of the cell sheet. The applicant is still testing for other critical attributes of the cell-sheet. Identity and Potency testing should include all parameters relevant for the biological function. The applicant indicated that the final potency assay for CAOMECS has not been defined, because the main potency assay will be chosen after evaluation of the additional tests and production of process validation batches.

The production process is insufficiently validated, as it is not demonstrated that the manufacturing process is capable of reproducible commercial manufacture. The applicant has not produced validation data of a sufficient number of batches with a justified success rate in producing a DP meeting the specifications. In the initial application the validation study results were considered insufficient. Despite a request, no additional validation data have been provided.

The control strategy proposed is considered insufficient. E.g. in the batch data submitted for the 26 patients several batches did not meet the proposed specifications, demonstrating that the process may not be fully under control.

In response to concerns raised the control strategy has been revised considerably. Firstly, as a redefinition of Drug Substance and Drug Product. Secondly, the further characterisation studies lead to a better understanding of the essential characteristics. Several new markers and IPC have been proposed to be introduced. Because the manufacturing has not been resumed at the new GMP manufacturing site, no validation data are available for batches manufactured using the proposed control strategy. In the absence of these data the control strategy can not be considered demonstrated to be adequate.

Given the time needed for sterility and the shelf life of the product, the final results from sterility testing will not be available before grafting of the cell sheet. The strategy for microbiological controls is to use media fill test to validate the aseptic manufacturing. Furthermore, sterility testing is performed on medium samples of at the second medium change in order to ensure the availability of results at release where the specification is set to “negative to date”. Final product sterility results are only available after release. This could be acceptable however, as yet validation of the aseptic processing has to be ensured for the final commercial manufacturing site.

The container closure system consists of the insert and screw top container. Compatibility has been tested sufficiently.

No stability data have been provided from batches produced with the final commercial process and revised control strategy, and in the final manufacturing site.

3.2. Non clinical aspects

Pharmacology

The proof-of-concept study was performed in a rabbit model of LSCD (induced by keratectomy). While it is acknowledged that the rabbit is a widely used model for LSCD, information on the (dis)similarities between human and rabbit eye is lacking which hamper the interpretation of the observation in model. Information on this subject was asked but not provided. Rabbits develop LSCD within 4 to 6 weeks after chemical/mechanical removal of the corneal epithelium, and transplantation was performed 4 weeks following keratectomy. Therefore it is possible that in the presented studies not all limbal stem cells were gone at the time of transplantation.

Rabbit oral mucosal cells were used to produce an autologous CAOMECS product, which was then transplanted onto the injured ocular surfaces. The tissue engineering and grafting processes mimicked those used in the clinical setting. Prior to engraftment, immunochemistry and colony-forming assays have underlined the presence of putative progenitor/stem cells within the harvested cell-sheets. Batch data indicate that initial cell numbers and colony forming efficiency are similar between human and animal CAOMECS. Final batch characterisation of the animal cell sheets appears limited to the need for confluence and the day of confluence and expression of p63 for a limited number of parallel cultured cell sheets. The limited characterisation prohibits the use of non-clinical data in support of a potency assay. However as there is a change in the manufacture process/manufacturing site (see overarching MO 1) it is not clear how the data provided on clinical batches relates to new batches of human CAOMECS. Thus no adequate comparison between non-clinical and (future) clinical batches can be made.

Ophthalmological examinations revealed a reconstruction of the ocular surface in the CAOMECS transplanted animals, based on the observations of clarity and smoothness of the corneal surface. However, non-grafted LSCD rabbits displayed conjunctivalisation of the cornea. The applicant has stated that after CAOMECS transplantation, the cell-sheets inhibit vascularisation and inflammation of the cornea. However, this statement is in contradiction with the results highlighted in the single dose toxicology studies where corneal vascularisation severity was comparable between LSCD transplanted and control rabbits at up to 3 months post-transplantation.

Immunofluorescence assays were performed on the epithelial sheets at the time of biopsy, prior to and 4 weeks post-transplantation to investigate the cell phenotypic modulations. Data indicate that the transplanted cells maintain their oral mucosal phenotype. In the 4 weeks toxicity study, fluorescein dye staining test showed that there was no the hole and/or hiatus of epithelium in ocular surface. The expected function of CAOMECS is epithelial barrier against adventitious agents and conjunctival invasion. Because oral mucosal epithelium is more transparent than conjunctival epithelium, prevention of conjunctival invasion is conductive to ensure light path. Yet, no inhibition of conjunctival invasion was noted in the pharmacology and toxicology studies.

Secondary pharmacodynamics, safety pharmacology and pharmacodynamic drug interactions studies were not performed due to the nature of the product.

Pharmacokinetics

Conventional ADME studies have not been performed and are not considered to be relevant for cell-based medicinal products. This is consistent with the CHMP guideline on cell-based medicinal products (EMEA/CHMP/410869/2006). No data on absorption, metabolism, excretion and PK interactions were provided, which is agreed. The main objective of kinetics studies is to obtain information in the biodistribution, persistence, viability/growth and phenotype of this ATMP. Considering the two scientific advices given by the EMA prior to the submission of the MAA the applicant was expected to investigate biodistribution of cells grafted onto the cornea. To address the biodistribution the Applicant has conducted histopathological and immunohistological (re-)analysis of eyeball specimens obtained from GLP single dose toxicity study (Study No. C-10-007) to assess whether cell migration through the eye occur or not (Study No. X-11-009). However the study report on this re-analysis could not be found in the documentation. So the claimed lack of biodistribution cannot be verified. Furthermore, a relationship between CAOMECS treatment and occurrence of inflammatory lung lesions cannot be excluded. Therefore particular attention should be paid to these observations when biodistribution is re-evaluated.

Therefore there is no convincing evidence showing a lack of migration was provided while this was requested by the CHMP in a previous scientific advice procedure. This lack of data and also the discussion on the potential risks associated with unwanted distribution should be better addressed. Also the data on the persistence and longevity of the cells was limited and requires additional data.

Toxicology

Single dose toxicity

The applicant performed two toxicity studies in rabbits. LSCD was induced in the left eye, and treated animals were then transplanted with autologous CAOMECS while LSCD control rabbits remained untreated. In the first study (no.C-10-007), GLP-compliant, rabbits were kept for a 4-week post-transplantation observation period. In the second study (no.TNC-0068-1), non-GLP-compliant, the post-transplantation observation period reached 3 months. The Applicant was asked but has not supplied a proper justification for the lack of GLP of this study. There is also a main issue regarding the low number of animals included in each study which makes harder the interpretation of the observed findings. The low number of animals and lack of GLP severely hamper the non-clinical safety assessment of CAOMECS.

In general, no significant findings were reported at histopathological examination of tissues other than the transplantation site in both studies, with two exceptions, thymic involution was detected in both operated (sham and transplanted) groups in both studies and an increased incidence of lung lesions was observed in treated rabbits at 3-month post-transplantation (perivascular and endoarterial infiltration of eosinophils, microgranuloma, karyomegaly in alveolar epithelial cells). Based on the available data, a relationship between CAOMECS treatment and occurrence of inflammatory lung lesions cannot be excluded. Particular attention should be paid to these observations when biodistribution is re-evaluated.

In both studies, ophthalmological examinations highlighted corneal opacity, injury, and neovascularisation in transplanted LSCD rabbits. Compared with non-transplanted LSCD rabbits, the degree and/or incidence of corneal opacity and injury tended to decrease over time but were still observed at the end of the 4-week and 3-month observation periods. Due to the very small number of animals in the 3 month study and possible regeneration seen in 1 of the 2 control animals no conclusion on the effects of treatment on eye pathology can be drawn from this study. However, the statement that CAOMECS inhibits corneal neovascularisation appears not to be supported in the toxicity studies.

Histopathological examination highlighted the occurrence of eosinophilia and slight conjunctivalisation in reconstructed ocular surfaces. Treated patients will be exposed to CAOMECS for a period longer than 3 months; however there is currently no supporting non-clinical data reflecting the long-term use of CAOMECS in humans. The applicant indicates that a 3-month follow-up of transplanted rabbits is suitable to demonstrate the maintenance of the treatment effects since the renewal of the rabbit corneal epithelium is basically completed within 90 days. This justification is debatable since it relies on the process of corneal renewal in healthy rabbits (without limbal stem cell deficiency). As mentioned in the scientific advice issued in July 2009, this process is not considered to be identical for the oral mucosal cell sheet and its renewing potential. Nevertheless, at the present stage, an additional toxicity study with a longer post-transplantation observation period may not be necessary since the applicant may rely on the long-term data obtained in patients to evaluate the local adverse/beneficial effects of CAOMECS (maintenance of a smooth and clear corneal surface, neovascularisation and inflammation of the cornea).

Tumourigenicity

The tumourigenic potential of CAOMECS was investigated via in vitro and in vivo studies. In vitro studies included a (non-GLP) soft agar growth assay, karyotype analysis and an assay investigating epidermal-mesenchymal cell transition (EMT). The applicant states to have performed a clonogenicity assay, but the study report is lacking. Overall the provided data on the in vitro assays was limited and the results of the EMT assay are considered conflicting. The provided in vivo assay has major deficiencies and does not contribute to the assessment of the risk on tumourigenicity. Taken together the concern on potential tumourigenicity has not yet sufficiently been addressed in particular because the claimed lack of biodistribution has not been substantiated by data. When it has been shown that biodistribution does not occur the risk of tumour formation may be most suitably addressed in the clinic by closely monitoring the patients ocular surface. In the case of biodistribution rigid in vitro analysis of cell samples could give additional reassurance or an additional in vivo tumourigenicity study should be conducted.

Other studies

Conventional repeat-dose toxicity, genotoxicity, carcinogenicity, and reproduction toxicity studies are not required due to the nature of the product. The local tolerance of CAOMECS was evaluated as part of single dose toxicity studies.

Ecotoxicity / environmental risk assessment

CAOMECS is not considered to pose a significant risk to the environment.

Discussion on non-clinical aspects

The applicant has demonstrated the feasibility of the process to engraft CAOMECS onto keratectomized corneal surfaces in a rabbit model of LSCD rabbits. The Applicant states that the expected function of CAOMECS is to provide an epithelial barrier against adventitious agents and conjunctival invasion. Because oral mucosal epithelium is more transparent than conjunctival epithelium, prevention of conjunctival invasion is conductive to ensure light path. Yet, no inhibition of conjunctival invasion was noted in the pharmacology and toxicology studies.

Extrapolation of these data to the clinical situation is hampered by lack of information on the (dis)similarities between human and rabbit eyes and the transplanted cell sheets.

No specific biodistribution study has been performed and information on biodistribution. The data on biodistribution in the in vivo studies is severely limited. Furthermore inflammatory lung lesions were a toxicity studies and a relationship with CAOMECS treatment cannot be excluded. Taken together due to a lack of data no conclusion on the absence of migration can be concluded. The potential risks associated with unwanted distribution should be better addressed, with particular attention to the lung findings. Also the data on the persistence and longevity of the cells was limited and requires additional data.

Although the toxicity studies have been performed on few animals and do not represent the long-term use in humans, the applicant may rely on the long-term data obtained in patients to evaluate the local adverse / beneficial effects of CAOMECS (maintenance of a smooth and clear corneal surface, neovascularisation and inflammation of the cornea). However the current data on tumourigenicity is considered insufficient and additional information is required prior to a marketing authorisation.

Conclusion on non-clinical aspects

Several deficiencies in the dossier (lack of data, information, discussion) were noted. These should be addressed before a recommendation on registration can be made. A Marketing authorization cannot be granted since major deficiencies were identified.

3.3. Clinical aspects

Tabular overview of clinical studies

In table 1.a tabular overview of the two studies submitted is presented:

Table 1: Summary of study details.

Study ID	Design	Study Posology	Study Objective	Subjects by arm entered / completed	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
Nishida et al in 2004	Open label	One allograft transplantation	Proof of concept	4 transplanted	more than 1 year follow up	unknown	1 SJS 3 OCP	Corneal transparency; visual acuity
2007-A00270-53	Open label, uncontrolled , single arm	One allograft transplantation	Safety, tolerability, efficacy	26 transplanted	Follow up 1 year	16 M/ 10 F Median age 52 years	Total bilateral LSCD > 18 years	Success rate (composite of 5 criteria)

SJS= Steven Johnson Syndrome, OCP= Ocular Cicatricial Pemphigoid

Pharmacokinetic studies have not been performed. According to the guideline on human cell based medicinal products (CBMP, EMEA/CHMP/410869/2006), conventional studies on absorption, distribution, metabolism, and elimination are not relevant for human CBMP. As CAOMECS contain autologous mucosal cells in which the cell-cell junctions organize the cells in a cohesive tissue, it is not expected that the cells migrate within the host.

Studies on migration, biodistribution, and tumorigenicity were part of the non-clinical development program.

Conventional pharmacodynamic studies have not been performed. Structural/histological assays are recommended as potential pharmacodynamic markers (EMEA/CHMP/410869/2006), if the cell-based medicinal product is intended to be used to restore tissues, with an expected lifelong functionality. Slit-lamp microscopic examinations of the transplanted cell-sheets were part of the efficacy assessment.

A crucial question is to which extent the mucosal epithelial cells transform after CAOMECS graft. Three cell tissues are involved i.e. mucosal epithelium, conjunctival epithelium and corneal epithelium. It is recognised that the distinction between conjunctival and corneal epithelium is not straightforward and several cell markers have been tested for this purpose. A recent study described cytokeratin 12 and mucin-1 expression being useful markers to allow a reliable discrimination between cornea and conjunctiva (Barbaro V, et al. 2010). This has not been investigated by the Applicant, which is a deficiency since the conclusion that no conjunctival epithelium has been found after CAOMECS transplantation has not been substantiated by such evidence (see also comments in main clinical studies). In addition it is unclear whether the mucosa derived cells do secrete antiangiogenic factors as corneal epithelium does, in contrast to conjunctival cells, which do not secrete such factors.

Clinical efficacy

In support of the clinical efficacy the Applicant has submitted a clinical package including two studies: one small "proof-of-concept" study reported by Nishida et al in 2004 and one larger study (Study 2007-A00270-53) in 26 subjects which the Applicant has defined as pivotal. The latter is referred to as "the France study" throughout the dossier. Additional studies and case series reported in literature, in which a similar technique for the treatment of LSCD has been applied and in a similar patient population, have been briefly summarized in the summary of clinical efficacy.

In addition the Applicant has planned another open, non-comparative, multi-centre, phase III study (Study CS001-EU01) in adult patients with total LSCD. The intention was to initiate this study in the autumn of 2011. The Applicant has requested scientific advice at EMA and the details of this study have been discussed with the SAWP and in the CHMP.

Dose-response studies and main clinical studies

The Applicant has not presented data on dose-response studies with CAOMECS, which is a tissue-engineered product.

Conventional dose response studies are not expected for this product. It is recognized that there will be some variety in the percentage of putative stem cells in the starting material in the different patients/biopsies.

However "transplantation success" depends among others on the number of stem cells in the cell-sheet. Therefore information is needed regarding the minimum /optimum "cell load" for successful transplant survival.

The "proof of concept" study

One small study published study is submitted as proof of concept (Nishida et al in 2004). The study included four patients with total bilateral LSCD due to Steven Johnson Syndrome (SJS) (one patient) or ocular cicatricial pemphigoid (OCP) (three patients). Specimens of oral mucosal tissue were collected and tissue-engineered epithelial-cell-sheets were fabricated ex-vivo by culturing harvested cells for two weeks on temperature responsive (TR) cell-culture surfaces. After removal of conjunctival fibrovascular tissue CAOMECS were transplanted directly to the denuded corneal surfaces (one eye of each patient) without sutures. Corneal transparency was restored and visual acuity had improved in all patients (table 2).

Table 2: Summary of primary outcomes in "proof of concept" study.

Table 2. Surgical Outcome in Four Patients Who Received Transplants of Tissue-Engineered Autologous Oral Mucosal-Cell Sheets.

Patient No.	Best Corrected Visual Acuity in Damaged Eye		Corneal Opacity (Grade)*			Complication	Months of Follow-up
	Preoperative†	Postoperative	Preoperative	1 Month after Surgery	At Last Observation		
1	Counting fingers	20/100	3	2	1	None	15
2	20/2000	20/25	3	1	1	None	14
3	Hand motion	20/300	3	1	1	None	14
4	20/2000	20/50	3	1	1	None	13

* The extent of corneal opacity was graded by three masked observers on the basis of the slit-lamp examination with a previously described system[26] and modifications for ocular-surface diseases. Grade 0 indicates clear or trace haze, grade 1 mild opacity, grade 2 moderately dense opacity partially obscuring details of the iris, and grade 3 severely dense opacity obscuring details of the intraocular structure. Grading is based on the opacity observed in all corneal layers, including epithelium, stroma, and endothelium.

† The visual acuity of patients who could not read a visual-acuity chart at a distance of 0.5 m was assessed by asking whether they could see the number of fingers held up by the examiner. If they could not, visual acuity was assessed by the patient's ability to see hand movement by the examiner.

The Applicant considers this as a proof of concept study since it is the only one where the mucosal epithelial cells were cultured on a TR cell-culture surface (contrary to other publications where amniotic membranes were used).

It is noted that this study had a more appropriate selection of inclusion criteria and primary outcomes, i.e. visual acuity and corneal opacity, as compared to the France study that is considered 'pivotal' in this application.

Improvement in corneal opacity and visual acuity was observed. Maximally improved of visual acuity was obtained 6, 2, 10, and 8 weeks after transplantation for patients 1 through 4, respectively and was stable thereafter.

The France study

Design/methods

This study concerned a prospective, open-label, uncontrolled, single centre study performed in France in 2009. The study aimed to assess the safety, tolerability, and efficacy of the transplantation of CAOMECS in patients with total bilateral LSCD. Patient follow-up was 12 months including 6 visits after the CAOMECS grafting.

Several study objectives were defined, i.e. restoration of the corneal epithelium, reduction of functional signs such as pain, watering and photophobia, and/or improvement of visual acuity, and/or increasing chances for a successful corneal graft within a certain period of time. Unfortunately, study design, conduct and the outcomes do not completely comply with these objectives.

Ultimate goal of treatment of LSCD is to restore visual function, and/or reduce pain/discomfort due to keratopathy. A necessary prerequisite is that the epithelial sheet transplantation is successful which fits with the first objective. Restoration of visual functioning either directly (no need for corneal

transplantation) or indirectly by facilitation of cornea graft survival fits with the subsequent objectives. However, it seems that in the France study only the first objective was measured (see results later). It is questioned whether this is relevant since corneal epithelium stability without an effect on visual function or pain/discomfort is not clinically beneficial.

The lack of placebo is considered acceptable as there is no spontaneous improvement in total limbic cell LSD. However, since only one eye was treated, the contra lateral eye could have served, by lack of a better option and to a certain extent, as a control.

The open study design poses a problem because the evaluation by the single investigator and the patient is not blinded considering the subjective element in many assessments. This in combination with the fact that it is a single centre study raises doubts on the external as well as internal validity of the results.

The 12 months follow-up is justified by the Applicant with the argument that most corneal epithelium is renewed within one year. If so within this time frame an effect on vision, corneal opacity would be expected (see results later). In addition survival of the epithelial sheet, although a necessary condition for further success this is not the main treatment goal in LSCD. A longer follow up should have been performed to give indication on the condition of the patient in terms of vision and possibility/success of subsequent stromal corneal transplantation. In this respect the follow up time of 12 months is considered too short.

Inclusion criteria were male or female subjects aged 18 years with total bilateral limbal cell deficiency. How total bilateral limbal cell deficiency was operationally defined is unclear. In general punctate epithelial keratopathy and/or corneal epithelial defects may be used as criteria for LSCD although these criteria are not generally accepted and reproducibility of the quantitative scoring of mild/moderate/severe corneal keratopathy and/or epithelial defects is unknown. However, given the low score of corneal keratopathy/epithelial defects at baseline this does not appear to be a decisive criterion for total LSCD and thus inclusion (see later). More important visual function, corneal opacity, discomfort complaints (pain, watering and photophobia) were apparently not used as inclusion criteria. Hence additional information is requested from the Applicant on the methods for diagnosis of total LSCD, the staging system(s) applied and argumentation for exclusion of unilateral LSCD.

Exclusion criteria were a strictly unilateral ocular affliction, acute systematic infection: consultation by a physician in service and decision on the paraclinical parameters VS and CRP; history of acute phase of ocular inflammation in the previous year; history of neoplastic disease; glaucoma – defined by taking intra ocular pressure; total symblepharon: impossibility to open both eyes; history of hypersensitivity to antibiotics or serum; women who are or may be pregnant; any infectious disease (HBV, HCV, HIV, HTLLV-1 and syphilis); and patients who are otherwise ineligible for participation in the study in the opinion of the investigator. A clarification is requested about the exclusion criteria infectious disease, pregnancy and “otherwise not eligible according to the investigator”.

The study was conducted in 2009 in France and included 26 subjects (16 male and 10 female) with total bilateral LSCD. One of the eyes received the transplant. Data from 25 patients were available for efficacy assessment (follow up time 12 months). There was no randomisation, also not for the decision of which eye will be grafted. The study was open label.

Considering the incidence of the condition the small sample size is expected. However, since all subjects had bilateral LSCD a randomisation of eye could have been performed, to avoid doubts that in each patient the eye in a better condition has been selected for the application of CAOMECS. Additionally the non-treated eye could have been used as (although not ideally), some kind of a control.

The open label character of the study is less acceptable as patient's and investigator assessment do have subjective features. At least the pictures from the right/left eye of each visit could have been presented to external evaluators in an at random order and independently assessed. Although it is stated that photographs have been sent to external evaluator it is unclear whether the result presented are based upon these evaluations and whether the presentation was at random.

The treatment procedure consisted of a few steps. At the second visit a biopsy from the bucal mucosa was taken (3/3 mm) under local or general anaesthesia. The biopsy sample was rinsed in DMEM + antibiotics and stored in transport medium at 2 – 8 C. The biopsy had to be transported and processed by the laboratory within 24 hours. During the France study the surgical unit and the laboratory were at walking distance so transportation time was very short and the samples were further processed within 24 hours. These times would be significantly longer if the product would be marketed in the EU (increasing risk for contamination). This questions the external validity of the results of the France study and the feasibility of the product in the EU situation.

The cells from the biopsy (including stem or progenitor cells) were cultured for two to three weeks on a temperature-responsive cell culture surface.

At the third visit (21 days later) the procedure of placing the cell sheet took place. First the pathological corneal epithelium was dissected (under local or general anaesthesia) up to 3 mm outside the limbus to expose the stromal surface for application of the CAOMECS sheet. The graft CAOMECS sheet was applied onto the exposed stromal bed without sutures and covered with a soft silico-hydrogel permanent contact lens as a protective tool and to enhance adherence during healing. The transplantation lasted 40 – 60 minutes.

Postoperatively, topical antibiotics (0.3% ofloxacin) and steroids (0.1% betamethasone) were applied for 1 month and in addition the first week betamethasone (1 mg per day) was administered orally. After one month the 0.1% betamethasone was replaced with to 0.1% fluorometholone. It is unclear how long local corticosteroids could and have been given.

The procedure of dissecting the persisting corneal epithelium carries certain risks such as inflammation, infection, corneal perforation or poor adherence of the graft sheet due to sub epithelial haemorrhage, which have to be discussed further by the Applicant.

The primary efficacy endpoint was success defined based on 5 criteria, evaluated by the investigator using a slit lamp examination with and without fluorescence. The 5 criteria concerned the degree of epithelial defect; degree of punctate epithelial keratopathy (PEK); amount of conjunctival epithelium on cornea; and corneal vascularisation expressed in terms of number of vascular pediculi near the limbus in each screen and in terms of vessels activity. The treatment was considered as successful if, at Month 12, all five criteria either improved or remained stable provided that a criterion was normal at

inclusion. The treatment was considered as a failure if either at least one criterion worsened, or if at least one criterion remained stable while not being normal at inclusion.

The definition of success/failure and the criteria on which it is based upon raises two issues.

The criteria contain overlapping observations/clinical signs. Hence their relative importance is not very clear. Epithelial defect is an accepted variable and has clear operational criteria regarding its severity. “Punctate epithelial keratopathy (PEK)” is an accepted variable, but for this study it is unclear how this was scored i.e. operationally defined. “Conjunctival epithelium on cornea” is scored only in terms of absence/presence, not taking into account the localization (pheripheral/central cornea) which determines the clinical consequences. Moreover the distinction between corneal and conjunctival epithelium it is not always straightforward to define (particularly in cases of symblepharon). “Corneal vascularisation” included two criteria which are overlapping, and are not commonly used, and not the most relevant. The most relevant measure of neovascularisation is its degree and localization (covering the central cornea, or only periphery).

The criteria for success as chosen may indicate that the epithelial sheet transplantation is successful. However the relevance of this definition of success is questioned, because it does not incorporate functional and clinically relevant improvement for the patient either in visual functioning or discomfort. Measurements on visual acuity and corneal opacity should have been considered at least as key secondary endpoints.

Hence the validity of the criteria for definition of success and of success as defined by the applicant needs justification.

Secondary efficacy endpoints included functional symptoms (photophobia, watering, and pain), corneal opacification, and visual acuity.

Although the concept that any decrease in watering, photophobia and pain would be of clinical relevance for the patient is acknowledged, it is questionable how reliable the results are, since the patient was not blinded which is crucial for these subjective assessments.

Clinically relevant criteria for success of treatment of LSCD i.e. improvement of visual function as measured by visual acuity and corneal opacity were considered of secondary importance, while these should be given more weight.

As the treatment goal of LSCD is to improve functioning, improvement in visual acuity should have been considered as the most relevant one. In addition it is related to a certain degree of independent functioning of the patient. Moreover, it is a less subjective assessment. It is noted this is in accordance to the study objectives i.e. one of the objectives as defined in the study protocol was “to obtain sufficient transparency so that the patient feels sufficiently ameliorated and does not feel the need for a second surgery”. Hence visual acuity should have been considered a key endpoint.

Complications which could be expected from the procedure were defined by the Applicant as secondary endpoints and not as adverse events. Special attention was given to inflammation, haemorrhage under the epithelial sheet, perforation of the cornea and infection.

Results

Twenty six patients were recruited and assessed as eligible. The median age of the patients was 52 years. All received CAOMECS graft on one eye and 25 were evaluated. One subject was excluded from the analysis. This patient was treated first on one eye and subsequently on the other eye.

The most common cause of LSCD was corneal burns, followed by keratitis, Lyell syndrome, and aniridia. As different causes for LSCD might respond differently to the treatment this needs further evaluation.

Pre-operatively most patients had no or some mild epithelial defects, but all had some degree of PEK. Conjunctivalisation of the corneal surface was present in 18 patients. In most patients there were vessels with different diameter sizes and more than ten vascular pediculi. See table 3 below

Table 3 France study - ophthalmologic characteristics (N = 26)

	Number of patients
Persistent epithelial defect	
None	12
Mild	13
Moderate	1
Severe	-
Punctate epithelial keratopathy	
None	-
Mild	7
Moderate	8
Severe	11
Conjunctival epithelium on cornea	
Absent	8
Present	18
Vessel activity	
Inactive	-
Small diameter	1
Different size diameter	22
Large diameter and vasodilatation	3

Epithelial defect: none = no ulcer, mild = ulcer < 5mm, moderate =ulcer between 5 and 7 mm diameter, severe =ulcer over 7 mm.

Punctate epithelial keratopathy : none, mild, moderate, severe was not further specified.

Vessels activity : 0=blood absence, 1=vessels of small diameter, 2=vessels with different size of diameter, 3=vessels with large diameter and in the state of vasodilatation only

Source: Table 4, Clinical Study Report, Module 5.3.

The majority of patients presented with LSCD symptoms of photophobia (frequently winkling to light), dry eyes. Eight patients had no pain complaints and 16 occasional pain and 2 repetitive pain.

The visual acuity was under 1/20 (equivalent to 20/200) using the Snellen chart in the vast majority of patients (i.e. severe visual impairment). Opacity was mild in 9 patients, moderately dense in 14 patients, and severe in two patients.

In table 4 the efficacy results are presented. In addition to the result on the five criteria of the primary endpoint, the results on visual acuity and corneal opacification are presented, since they are considered at least as important by the CAT.

According to the predefined criteria for success, there were 16 subjects (64%) with successful treatment (95% CI 45% <> 80%) according to the Applicant. The Rapporteur's assessment leads to a different figure (see later).

Table 4. Summary of Efficacy for the France study - final analysis (N = 25) (by Rapporteur)

Variable s evaluated	**Number (%) of patients**			
'Success'- improvement or stable	**16/25 (64% $C_{i95\%}$ 45% ;80%)**			
	Improvement	**Steady state**	**Worsening**	**Missing[a]**
Persistent epithelial defect	11 (44)	12 (48)	-	2 (8)
Punctate epithelial keratopathy	19 (76)	4 (16)	-	2 (8)
Conjunctival epithelium on cornea	11 (44)	11 (44)	1 (4)	2 (8)
Number of vascular pediculi	22 (88)	1 (4)	-	2 (8)
Vessel activity	20 (80)	3 (12)	-	2 (8)
Visual acuity	8(32)	13(52)	2(8)	2(8)
Opacity	4(16)	17(68)	2(8)	2(8)

Source: Table 14, Clinical Study Report, Module 5.3.

[a] There were two patients with a serious adverse event. These patients were considered as failures.

However, for a better assessment the data from baseline and end of the study are presented in table 5 by Rapporteur.

Table 5: Shifts in efficacy criteria (by Rapporteur)

	Baseline		**12 months**	
	Persistent epithelial defect			
None	12		22	
Mild (<5 mm diameter)	13		1	
Moderate (>5 mm and <7mm diameter)	1		0	
Severe (>7 mm diameter)	0		0	
Not available	-		2	
	Punctate epithelial keratopathy			
None	0		8	
Mild	7		14	
Moderate	8		1	
Severe	11		0	
Not available	-		2	
	Conjunctival epithelium on cornea			
Absent	8		18	
Present	18		5	
Not available	-		2	
	Vascular pediculus			
0-2	Data not available		10	
3-5	Data not available		6	
6-8	Data not available		3	
10-15	Data not available		3	

20-40	Data not available		1	
Not available			2	
	Vessel activity			
Inactive	0		5	
Vessels with small diameters	1		14	
Vessels with different size diameters	22		4	
Vessels with large diameter with vasodilatation	3		0	
Not available			2	

* Percentages are not presented since with a such small number they are not indicative, e.g. 1 patient is 4%.

Visual acuity and corneal opacification remained unchanged in most patients. See table 6. However, according to the applicant an improvement of opacification cannot be expected from the grafting procedure. This is in contrast to the study objectives. Moreover in the proof of concept study an improvement in corneal opacity and visual acuity was observed within 10 weeks after transplantation and remained stable thereafter.

Table 6- change from baseline in visual acuity and corneal opacification (by Rapporteur)

	Baseline		**At 12 months**	
	Number of patients	**Missing**	**Number of patients**	**Missing**
Visual acuity				
Perception of light	26		23	2
Hand motion at 20 cm	25		23	2
Hand motion at 50 cm	23		22	2
Hand motion at 100 cm	21		22	2
Count fingers at 20 cm	16		23	2
Count fingers at 50 cm	15		23	2
Count fingers at 100 cm	12		23	2
Corneal opacification				
Clear or trace haze	1	-	Data not available	
Mild opacity	9	-	Data not available	
Moderate dense, partially obscuring detail	14	-	Data not available	
Severe dense, obscuring detail of intraocular details	2	-	Data not available	

The most common reason for failure was conjunctivalisation of the cornea, which was present in five patients (20%) at Month 12. Two patients presented with an SAE. Although not explicitly presented in the reasons for failure, in the safety section of the dossier it seems that two subjects received a stroma graft (one simultaneously with the CAOMECS graft and one 5 months later). In both cases there was a graft rejection meaning a treatment failure.

An overview of each patient's outcome for each of the five criteria is provided in table 7.

Table 7: Individual scores at baseline, month 3 and 12 for subjects in France study – data on five criteria (by Rapporteur)

	Persistent epithelial defect			Punctate epithelialkeratopathy			Corneal conjunctivalisation			Number of vascular pediculus			Vessel activity			SUM		
	Inclusion	Day 90	Day 360	Inclusion	Day 90	Day 360	Inclusion	Day 90	Day 360	Inclusion	Day 90	Day 360	Inclusion	Day 90	Day 360	Inclusion	Day 90	Day 360
pat01	1	1	0	1	1	1	0	0	0	10	1	0	2	1	0	4	3	1
Pat02	0	0	0	3	1	1	0	0	0	3	0	0	2	0	0	5	1	1
Pat03	3	0	0	1	1	1	0	0	0	4	3	3	2	1	1	6	2	2
Pat04	0	0	0	2	1	1	0	0	1	10	2	2	2	0	1	4	1	3
Pat05	1	1	.	2	2	.	1	1	.	20	20	.	2	2	.	6	6	.
Pat06	0	0	0	1	0	0	1	0	0	10	1	1	2	1	1	4	1	1
Pat07	1	1	0	2	0	0	1	0	0	5	0	1	3	1	1	7	2	1
Pat08	1	0	0	3	0	1	0	0	0	20	0	0	2	0	0	6	0	1
Pat09	1	0	0	2	1	0	1	0	0	20	2	2	2	1	1	6	2	1
Pat10	0	0	0	2	1	0	1	0	0	15	3	3	2	1	1	5	2	1
Pat11	1	0	0	3	1	1	0	0	0	12	0	0	2	0	0	6	1	1
Pat12	1	0	0	1	1	1	1	0	0	20	5	7	2	1	1	5	2	2
Pat14	1	0	0	3	1	1	1	0	1	20	3	4	2	1	1	7	2	3
Pat15	0	0	0	3	1	1	1	0	0	40	2	2	2	1	1	6	2	2
Pat16	1	0	0	3	1	1	1	0	0	30	3	3	3	2	2	8	3	3
Pat17	0	0	0	3	1	0	1	0	1	30	8	12	2	2	2	6	3	3
Pat18	1	0	1	1	1	0	1	0	0	20	0	10	2	0	1	5	1	2
Pat19	0	0	0	3	1	2	0	0	0	30	1	8	2	1	2	5	2	4
Pat20	0	0	0	3	0	1	1	0	0	20	2	3	2	1	1	6	1	2
Pat21	0	0	0	3	1	1	1	0	0	30	0	8	2	0	1	6	1	2
Pat22	1	0	0	2	0	0	0	0	0	4	0	0	1	0	0	4	0	0
Pat23	0	0	0	3	1	0	1	0	0	10	4	5	2	1	1	6	2	1
Pat24	1	4	.	2	1	.	1	0	.	20	10	.	2	2	.	6	7	.
Pat25	1	0	0	2	1	1	1	0	1	20	6	20	3	1	1	7	2	3
Pat26	0	0	0	1	0	1	1	0	1	30	12	12	2	2	2	4	2	4

Persistent epithelial effect: 0=no ulcer, 1=mild, 2= ulcer < 5mm, 3=moderate [ulcer between 5 and 7 mm diameter], 4=severe [ulcer over 7 mm])

Punctate epithelial keratopathy: 0 = none, 1=mild, 2=moderate, 3=severe

Conjunctival epithelium on cornea : 0=absence ; 1= presence

Corneal vascularisation: number of vascular pediculus

Corneal vascularisation: vessels activity : 0=blood absence, 1=vessels of small diameter, 2=vessels with different size of diameter, 3=vessels with large diameter and in the state of vasodilatation only

With respect to the lack of conjunctival growth over the corneal epithelium in 18/25 of the patients after 12 months it would be important to know whether stem cells (replacing functionally the limbal cells) are found in the epithelial sheet after 12 months and this is the reason for stopping the conjunctival overgrowth. Additional information is requested on the methods used to distinguish between conjunctival and corneal epithelium.

Ignoring the fact that the composite endpoint is not considered appropriate, if the criteria for success defined by the Applicant are applied, then subjects 01, 03 and 12 should be counted as treatment failure (remained stable on PEK which was mild, but not absent). This means that 13/25 (52%) had a success and 12/25 (48%) patients had a treatment failure, and not 16/25 (64%) and 9/25 (36%) correspondingly success and failure as reported by the Applicant. This discrepancy requires further discussion.

In addition for three other subjects there was an improvement at month 3 followed by worsening at month 12: Subject 20 worsened on 2 criteria – PEK (from no PEK to mild, and from 2 vascular pediculus to 3); subject 21 (from 0 pediculus at month 3 to 8 at month 12); subject 07 (from 0) pediculus at month 3 to 1 at month 12).

On the scoring of vessel activity and vascular pediculus there seems to be improvement in the majority of the patients. However the most relevant measure of neovascularisation is the degree and localization (covering the central cornea, or only periphery) of the new blood vessels. Unfortunately this has not been assessed/reported in the dossier.

With respect to visual acuity/corneal opacity it appears that the trial did not meet its objectives. The data on visual acuity are considered less subjective and of larger clinical relevance. Visual acuity remained stable in the majority of the subjects (13/25 – 52%), with a small proportion who showed improvement (8/25 – 32%). This is different from the findings in other published studies where an improvement in visual acuity has been reported. See also table 2. Data on visual functioning on baseline, at month 3 and 12 are requested, and it is relevant to assess what was the degree of improvement in those 8 subjects, who showed improvement.

Corneal opacification did not change in 17 subjects (68%), and improved only in 4 subjects (16%), which is less than what was reported in the proof of concept study (all four subjects) and in literature.

The discrepancy between the marginal effect on visual acuity and on corneal opacification in the France study and the more significant effect in publications requires further discussion, since these are relevant clinical outcomes.

The extent of improvement of vision (and corneal opacification) would depend on whether there is a deeper stromal opacification or not. Additional analyses are therefore needed to address this: The applicant should re-analyse visual acuity by type of baseline corneal opacification, i.e. superficial damage with only corneal epithelial involvement or opacification involving the corneal stroma as well as information of the localisation of the opacification (obscuring the visual axis).

Following a scientific advice post-hoc analyses using different definition of success were performed. The first was presence of a stable corneal epithelium (= no loss) and absence of conjunctivalisation in visual axis (= absence). Success as determined by these both measures combined was experienced by

17/25 patients (68.0%). The second analysis was if success was defined as reduction in corneal vascularisation: at Month 12 5/25 (20.0 %) patients had a 100% reduction; 14/24 patients (56.0%) had a 75% reduction; and 4/25 patients (16.0%) had a 50% reduction.

Absence of conjunctivalisation and stable epithelium might indicate a better prognosis in future grafting procedures and probably of better visual acuity. Lack of neovascularisation (20% with 100% reduction) especially when sparing the central cornea is a prerequisite towards a better visual function.

Concerning other secondary endpoints measuring LSCD symptoms, i.e. photophobia, watering and pain, there was a clear trend of improvement from baseline to month 12 (64%, 72%, and 56% of patients. With respect to these patient-reported outcomes the open label assessment of these subjective symptoms does not allow a firm conclusion on efficacy. It is noted that at baseline pain was mild (no-complaints in 8 subjects, occasionally in 16 subjects) hence there was not much room for improvement.

In total, bilateral LSCD with stromal involvement leading to opacification that obscures vision, the outcome after PKP is poor since there is no functional corneal epithelium to support the graft. Thus, there is a need for restoration of a corneal epithelium with regenerative capacities that will function and support a subsequent corneal graft. It is not known whether CAOMECS has a positive outcome on a future PKP. This will depend on whether there are sufficient epithelial cells with proliferative capacity to support the donor cornea after explantation of the patient's cornea. Therefore, the applicant must try to retrieve as many patients as possible to evaluate those undergone PKP and the outcome in these patients (compared to historical data in patients who have not received stem cells prior PKP). Together with this, the applicant should give recommendations regarding the time span between the two procedures.

Clinical studies in special populations

The Applicant has not performed studies in special populations in the conventional sense of this term. With regard to renal and hepatic impairment such studies would not be required since the product is locally applied and the preclinical data do not indicate systemic distribution and metabolism.

With regard to elderly subjects some possible differences in the application of this product in this age group could be expected on theoretical grounds. These could refer to successful preparation of the epithelial sheet (successful cell culture after biopsy) possibly related to lower cell proliferation potential and stem cell availability in elderly subjects. Another issue could be the success of the grafting itself in terms of clinical effect and adverse events.

It would not be feasible to request for separate studies in elderly but the Applicant should comment on the possible difference of applicability of the product (procedure) in this patient population.

All subjects in the France study were > 18 years (this was one of the inclusion criteria). The Applicant has submitted a PIP and has obtained a waiver for subjects from birth to 2 years and a deferral for the age category 2 – 18 years. A multicentre, open label study to evaluate the safety, tolerability and activity of Cultured Autologous Oral Epithelial Cell-Sheet graft in paediatric patients from 2 to less than 18 years of age and adults with LSCD is included in the PIP and should be completed by 2018.

For the time being no data in this population is available and this is reflected in the proposed SmPC in 5.1. The lack of data in this population should be mentioned in 4.2. as well (see comments SmPC).

Analysis performed across trials (pooled analyses AND meta-analysis)

N/A

Supportive studies

The Applicant has identified several studies and case series reported in the literature which also applied transplantation of cultivated cell-sheets derived from limbal epithelial cells and autologous OMECs expanded ex-vivo to treat total LSCD, however, using another culture methods (Table 14 Clinical Assessment Report).

Outcome measures to define success were not clearly defined. Parameters stated in the context of success included improvement in visual acuity, re-establishment of a stable and transparent epithelium, or resolution of conjunctivalisation, and reduction of signs of LSCD.

In four of the six studies with CAOMECs, a success rate of ≥95% was observed (table 8) and visual acuity was improved by more than two lines in most of the patients. However, in two studies the transplantation was followed by penetrating keratoplasty or conjunctivo-limbal autografting to further improve vision. The long-term outcome of CAOMECS transplantation was evaluated in the study of Nakamura et al, 2010. The conclusion was that a long survival of CAOMECS up to 7.5 years is possible, as well as a long-term benefit of CAOMECS transplantation with respect to the patients' clinical outcome.

Table 8: Literature data - success rates

Reference	No. of eyes/pts	Overall success rate (%)	Outcome details
Nakamura et al, 2004	6/4	100%	Successful outcome defined as: presence of a stable corneal surface and improved visual acuity
Inatomi et al, 2006a	15/12	67%	Successful outcome defined as: stable and transparent corneal surface without major complications and improved visual acuity
Inatomi et al, 2006b	2/2	100%*	Successful outcome defined as: absence of clinical complications, such as persistent epithelial defects, rejections, or recurrence of cicatrization
Ang et al, 2006	10/10	100% (based on successful engraftment) 90% (based on visual acuity)	Successful outcome defined as: stable corneal surface without major complications
Ma et al, 2009	5/5	na^*	No clear information on overall success. "all corneas became less inflamed, and corneal surface was completely reepithelialised in all but one patients. In all patients, superficial corneal blood vessels invariably developed, and to further improve vision, conjunctivo-limbal autografting (N=3) and/or penetrating keratoplasty (N=3) were performed subsequently. The vision of all patients showed substantial improvement after additional surgeries.
Nakamura et al, 2010	19/17	95 (79)[a]	Successful outcome defined as: improvement in best corrected visual acuity
Nishida et al, 2004	4/4	100%	Successful outcome defined as: restoration of corneal transparency, improved visual acuity, and absence of complications

There was not a priori definition of success in any of the studies. In the studies reported by Nakamura et al (2004), Ang et al, and Nishida et al success was evaluated at the last follow-up visit. In the studies reported by Inatomi et al (2006a) and Nakamura et al (2010) success was evaluated during the follow-up period and the time point was not explicitly specified. All other studies did not give information on the time point for success evaluation.

[a] BCVA was improved by 2 lines in 79% of eyes and by 1 line in 95% of eyes.

% Success rates were not explicitly stated and are therefore given based on the description of success.

^ The transplantation was followed by keratoplasty and it was not stated if success was based on the results before or after these subsequent surgery.

* Transplantation of CAOMECS was followed by penetrating keratoplasty or conjunctivo-limbal autografts.

BCVA = best corrected visual acuity, na = data not available, No. = number, pts = patients.

In the publication of Ma et al, 2009 "the vision of all patients showed substantial improvement after additional surgeries", the additional surgery consisted of conjunctivo-limbal autografting (N=3) and/or penetrating keratoplasty (N=3).

In the other publications, the definitions of treatment success are a mixture of absence of clinical complications, reduction in epithelial defects, less graft rejections, and/or improvement in vision. Definition of success in these studies suffers from the same problems as the France study - either vision was not incorporated or its contribution relative to the other determinants of success has been underemphasized.

Due to the above reasons, the evidence for efficacy provided from these studies is limited and can be considered at best as supportive only.

With regard to long term efficacy the observation period of 12 months in the France study is considered too short. Some beneficial effect from the CAOMECS grafting was observed at 3 months (which corresponds to the acute and sub acute phase post-grafting), and the fact that few parameters in some patients have deteriorated after that (between month 3 and month 12) is an indication for some stability of the transplant. Unfortunately in the France study patients were not followed after the first year and no analyses on the epithelium at the end of the study have been presented.

According to the results from the publication of Nakamura et al, 2010 there is some indication for maintenance of effect on conjunctivalisation, corneal opacification, neovascularisation and symblepharon formation. If the procedure leads to the formation of a clear demarcation zone between the conjunctival epithelium and the regenerated corneal-like epithelium, this might be promising in terms of long term efficacy and also possibility for further corneal graft if necessary.

The lack of long term efficacy data for CAOMECS is considered a deficiency. Since the France study (and the four patients in the Nishida et al, 2004 study) are the only ones in which this specific procedure has been applied to e.g. mucosal cell sheet grafting cultured on TR, long term data are of specific interest.

Discussion on clinical efficacy

Design and conduct of clinical studies

The claim for efficacy of CAOMECS is based upon data from one proof of concept study (Nishida et al) (n=4), and one small (n=26) open label single centre uncontrolled clinical study with one treatment arm in patients with bilateral total LSCD (the France study).

The outcome from the “proof of concept study” was published in New England Journal of Medicine.

The France study was conducted in 2009 in Hospices Civils de Lyon by one investigator. The results have been published /peer reviewed (Burillon et al., IVOS, 2012).

It is not clear what the inclusion criteria were for total LSCD which questions the representativeness of the patient population evaluated. Some exclusion criteria need further explanation as well i.e. unilateral LSCD, pregnancy and “otherwise ineligible … in the opinion of the investigator”.

The lack of placebo control may be accepted as in patients with total bilateral LSCD spontaneous improvement is not likely. However as all subjects had total bilateral LSCD and underwent treatment with CAOMECS of just one eye the other eye could have to some extend served as a kind of control. Moreover treatment of either the right or left eye could have been randomised in order to prevent selection of the better eye.

Another issue is the fact that the study was not blinded to the patients and the single investigator. In the study protocol it is mentioned that the photos of the eyes prior and after intervention were sent to two independent assessors, but it is not clear if the photographs of both eyes and visits were presented at random. Moreover it is not clear whether the results presented are based on the “blinded” or investigators’ evaluations.

The choice of primary outcome, definition of success, weight of the criteria was success was based upon, the lack of validity of the quantitative scoring do not allow a conclusion concerning efficacy. It indicates that the graft survived and a necessary prerequisite towards a better visual function is fulfilled. Outcomes of more relevant criteria for success i.e. improvement of visual function as measured by visual acuity and decreased corneal opacity were considered only of secondary importance and are so far not convincing. An increased success of subsequent corneal transplantation or the lack of need for such procedure have not been assessed in the study in contrast to the study objectives. Additionally the suggested by CHMP (during scientific advice) criterion for success i.e. intact epithelium with no evidence of neovascularisation has not been applied in the protocol as primary endpoint.

All these uncertainties together with the fact that this was a single centre (in fact one investigator and facilities nearby) open label and uncontrolled study questions the external and internal validity of the results from this study.

Efficacy data and additional analyses

Despite the limited number of transplanted eyes, the results from Nishida et al publication provide evidence for the proof of concept. Improvement in corneal opacity and visual acuity was observed. Maximally improvement of visual acuity was obtained 6, 2, 10, and 8 weeks after transplantation for patients 1 through 4, respectively and was stable thereafter. The France study was not able to replicate these findings.

Conclusions on clinical efficacy

The concept of using autologous buccal mucosa biopsy material as a source for preparation of a epithelial cell sheet has several, potential advantages to alternative methods. In contrast to a allogeneic graft, there is a lower probability of rejection and continuous immunosuppression is not necessary. In principle, if this procedure is successful, harvesting limbal stem cells form the healthy eye is not necessary. The temperature responsive polymer allows the growth of confluent cell cultures and their detachment from the supporting membrane without the use of proteolytic enzymes. This in theory should increase the chance for obtaining an intact cell layer with preserved cell-cell junctions which would be suitable for corneal epithelium replacement and should reduce the risk of infections.

Treatment of LSCD should target one or more of the following problems: visual impairment; corneal epithelium defects making the eye prone to infections; discomfort; preparation for a successful corneal graft.

In the proof of concept study improvement in corneal opacity and visual acuity was observed. Maximally improvement of visual acuity was obtained 6, 2, 10, and 8 weeks after transplantation for patients 1 through 4, respectively and was stable thereafter.

This could however not be reproduced in the France study - the most important study. The clinical evidence of efficacy is based on one single study which has serious methodological flaws, precluding any conclusion concerning the internal and external validity of the study results. These concern the

open label character of the study, the fact that the study was single centre and single investigator based, the representativeness of the patients included and the choice of primary endpoints.

In the France study treatment success was defined based on five criteria (epithelial defect, punctuate epithelial keratopathy, presence of conjunctival epithelium, number of vascular pediculi and vessel activity), which are not all widely used in ophthalmologic practice and not validated. Moreover the criteria overlap i.e. the same outcome would be measured twice. All in all the composite primary endpoint is not considered appropriate to measure the efficacy of CAOMECS. The outcome on these criteria may indicate that the CAOMECS graft is functioning, which however is not sufficient on its own to demonstrate clinical improvement.

Visual acuity remained stable in most subjects, which is different than the results in other published studies (where visual acuity had improved). Corneal opacity did not change in 68%, and improved only in 16%.
Reduction of discomfort (i.e. watering, pain and photophobia) is important for the patient; however, the open label design does not allow firm conclusions about the trend for improvement observed. Pain seems not to have been a large problem given the pain score at baseline.
Despite being one of the study objectives, success of subsequent grafting of cornea has not been discussed in the dossier. From the safety data as presented it seems that two subjects received a stroma graft (one simultaneously with the CAOMECS graft and one 5 months later). In both cases this ended with graft rejection. Although this might be a chance finding, the efficacy of CAOMECS i.e. increasing success of corneal transplantation cannot be accepted on face value.

Ignoring the critique for the primary endpoint, following the Applicant's definition of success, there was success in 64% of the patients, although this figure may need to be downgraded to 52% (according to the Rapporteur).

The discrepancies between the outcomes in the two studies require discussion, since improvement in vision and in corneal opacity are considered some of the most relevant clinical goals in LSCD.
In summary, the benefit of the application of CAOMECS as demonstrated in the France study is limited to survival of the CAOMECS epithelial cell transplant, with some indication of stabilisation of the corneal epithelium (less neovascularisation and conjunctival growth). Although this is a necessary condition for further improvement of visual function, it is not sufficient to draw conclusion with respect to clinical benefit. The latter could be achieved either by a direct improvement in corneal opacity and visual acuity or indirectly by enhancing chances for a successful cornea stroma transplantation. Unfortunately neither has been demonstrated, since visual acuity and corneal opacity remained more or less stable in the majority of cases and the two subjects who received CAOMECS and stroma graft ended with a graft rejection.

All together the evidence of efficacy of CAOMECS on clinically relevant endpoints is insufficient.

The discrepancies between the proof of concept study and France study emphasis the need for replication studies especially as these studies were single centre studies.

Clinical safety

Patient exposure

Patient exposure is presented in table 9:

need **Table 9: Patient exposure**

	Patients enrolled	Patients who received OMEC graft	Eyes grafted with OMECS	Patients with long term safety data(observed eyes) *	Follow up time (months)
Open uncontrolled study	26	26	26	25	12
Literature data		54	61	37 5 19	12-24 24-36 >48

* In general this refers to 6 months and 12 months continuous exposure data, or intermittent exposure.

The Applicant considers 6-12 months as long term exposure, but given the condition to be treated and evaluation of whether the procedure does not negatively affect subsequent cornea transplantation, long term safety data would need to be longer than 1 year. In this respect the long term efficacy, tolerability and safety of CAOMECS is not known.

The patient exposure data from literature is rather limited with different follow up times. The only long term data is from the 19 eyes/subjects in the Nakamura et al, 2010 study where subjects were followed for more than 4 years.

An overall summary of AEs observed in the France study is shown in table 10. A total of 17 AEs were reported by seven patients (27%). Two patients (8%) experienced a serious adverse event (SAE).

Table 10: France study - summary of adverse events (N = 26)

	Number (%) of patients
Number of patients with AEs	7 (27)
Number of AEs	17
Number of patients with SAEs	2 (8)
Number of SAEs	2

Source: Table 22, Clinical Study Report, Module 5.3.
AE = adverse event, N = number of patients.

Adverse events by patient are summarized in table 11. The investigator considered all AEs to be related to the underlying disease and unrelated to CAOMECS. Two AEs, eye inflammation and symblepharon that were ongoing at study termination, the other AEs resolved.

Table 11: France study - adverse events by patient

Patient	Adverse event LLT (MedDRA)	Comments	Outcome
004	Corneal neovascularisation	Due to hypoxia induced by wearing contact lenses for too long	Resolved
	Pain	-	Resolved
005	Corneal perforation	SAE	Resolved
	Eye inflammation (twice)	-	Resolved, ongoing
	Meibomian cyst	-	Resolved
	Sinusitis	-	Resolved
011	Intraocular pressure increased	Due to local corticosteroid treatment	Resolved
019	Amniotic membrane graft	Due to a vernal corneal ulcer caused by keratitis	Resolved
	Keratitis	Experienced during a renal dialysis	Resolved
	Pulmonary edema	-	Resolved
020	Conjunctival operation	Surgery for a small ridge of conjunctival epithelial not adhering to the cornea	Resolved
024	Corneal graft rejection	SAE. Not the CAOMECS graft.	Resolved
025	Graft complication		Resolved
	Inflammation	Related to corneal graft (not the CAOMECS graft) and to complications during surgery where an advanced cataract was found	Resolved
	Pain	-	Resolved
	Symblepharon	-	Ongoing

Source: Table 22, Clinical Study Report, Module 5.3.

CAOMECS = Cultured Autologous Oral Mucosal Epithelial Cell-Sheet, LLT = lowest level term, MedDRA = Medical Dictionary for Regulatory Activities, SAE = serious adverse event.

An overview of adverse events reported in published studies is shown in table 12.

Table 12 Literature - reported adverse events in the literature

Event	Number of patients (eyes)						
	Nakamura, 2004 6 (4)	**Inatomi, 2006a 12 (15)**	**Inatomi, 2006b 2 (2)**	**Ang, 2006 10 (10)**	**Ma, 2009 5 (5)**	**Nakamura, 2010 17 (19)**	**Nishida, 2004 4 (4)**
Epithelial defect	1 (2)	4 (5)	1 (1)[a]	5 (5)	1 (1)	na (7)	-
High IOP	-	-	1 (1)[b]	-	na	na (3)	-
Corneal infection	-	-	-	-	na	1 (1)	-
Microperforation	-	-	-	-	1 (1)	-	-

[a] AE occurred after PKP which was performed 5.5 months after the transplantation of CAOMECS.

[b] AE occurred after PKP which was performed six months after the transplantation of CAOMECS.

AE = adverse event, CAOMECS = Cultured Autologous Oral Mucosal Epithelial Cell-Sheet, IOP = intraocular pressure, na = data not available, PKP = penetrating keratoplasty.

The heterogeneity of the studies (e.g. different follow-up time, limited n of studies with subsequent corneal transplantation) makes it difficult to get a clear picture of safety based on these publications.

The data from literature indicates that epithelial defect is the most frequently observed AE, and it may occur shortly after the CAOMECS grafting or months later. Increased IOP, infections and

microperforations are seen as well. All these AEs could be expected in this condition and after such intervention.

Serious adverse events and deaths

No patient died during the France study and no deaths were reported in any of the studies reported in the literature.

In the France study, two patients experienced one SAE each.

Patient 05 presented a serious adverse event – corneal perforation. In this case, the investigator concluded that the inflammatory response suffering from a severe Lyell syndrome was responsible for the cornea perforation at 7 months event, because the contralateral non treated eye perforated spontaneously 1 year later. The surgery, but not CAOMECS itself, seemed to accelerate the evolution.

Patient 024 presented a massive graft rejection 4.5 months after the simultaneous surgery of CAOMECS and cornea grafts. An ulcer of the entire donor corneal graft surface with strong vascularisation led to a surgical management of this case.

Both SAEs are reported by the Applicant as resolved and not related to the CAOMECS graft.

In the study protocol analysis (France study) the conclusion is that it is recommended not to apply both CAOMECS and corneal stroma graft in the same surgical procedure: it is necessary to wait for the epithelium to grow in order to avoid conjunctival vessels proliferation on the cornea, and wait for a couple of months before performing a secondary donor cornea graft.

Laboratory findings

Since CAOMECS is a locally applied cell based product routine laboratory evaluations were not performed and are not expected.

Safety in special populations

No subgroup analyses were performed and safety data were not stratified by any special group of interest. It might be of interest to analyse the safety data according to underlying disease in order to detect if certain underlying conditions are more prone to AEs after the procedure.

The safety in paediatric subjects is not known and the product is not recommended for use in this population as reflected in the label. No elderly subjects were included in the studies and no safety data is available for this population.

If the efficacy results from the “pivotal” study were convincing extrapolation to special populations might be considered since this is a locally applied cell product and the condition itself does differ substantially in paediatric and elderly subjects.

Immunological events

Immunological events were not reported separately in the dossier, however the two cases of corneal graft rejection might be considered as an immunological event. No conclusions about the expected incidence of such event can be made from the limited safety database available.

Safety related to drug-drug interactions and other interactions

Not applicable.

Discontinuation due to AES

The two subjects who experienced a SAE - corneal perforation (patient 05) and massive graft rejection (patient 024) discontinued the study. In addition patient 013 has discontinued, since no data for efficacy is available according to the Applicant. The reasons for discontinuation of patient 013 should be provided by the Applicant.

Discussion on clinical safety

An overall picture of the safety of the application of CAOMECS graft is difficult to obtain due to the limited exposure with this specific product in terms of number of subjects, follow up time, and quality of the monitoring and reporting system.

Nevertheless, the number of adverse events reported in the France study is unexpectedly low. Most events are based on eye inspection by investigator. ~~P~~atient reported adverse events are rare, if any, which is unexpected. Given the condition and procedure applied, more patient reported adverse events such as pain/discomfort would have been expected.

In addition one of the two corneal transplant rejections was not reported as an adverse event.

Finally the conclusion of the investigator that all adverse events in the France study were related to the underlying disease and/or surgical procedure, but not to CAOMECS appears at least rather uncritical considering that adverse events caused by the surgical procedure for removal of the damaged epithelium in preparation for the CAOMECS sheet and the application of CAOMECS cannot be disentangled.

All in all the quality of the safety monitoring and reporting in the France study is seriously questioned.

A priori relevant adverse events are infections, corneal transplant rejections, epithelial defects, and neovascularisation.

No infections were reported in the France study. Apart form possible underreporting, this risk of infections might be higher if the procedure will be applied in different treatment centres and longer logistic lines.

PKP performed simultaneously or even months after the CAOMECS grafting seems to be related to additional risks. This leads to the question when is a suitable time for a PKP and does CAOMECS application contribute to a higher probability of successful grafting. The two cases of graft rejection in

the France study do support that role for CAOMECS. Although this might be a chance finding, the higher probability of corneal grafting after application of CAOMECS should not be taken at face value.

Graft rejection is an important AE. For patient 025 “an inflammatory reaction related to both a weak rejection against stromal graft is reported. This case requires further clarification – the timing of the stromal graft when the inflammatory reaction occurred and what “weak rejection” implies. Moreover this case further indicates deficiencies in monitoring and reporting of adverse events in the France study.

Neovascularisation is a relevant AE which occurs almost inevitably after all corneal epithelial grafting procedures, but with exception of the Nakamura et al, study, the extent (central/peripheral) was poorly addressed. Also in the France study, this is a major deficiency.

In conclusion the assessment of the risks of application of CAOMECS is based on limited data of apparently poor quality. No clear picture of the safety of the application of CAOMECS graft emerged.

Another uncertainty is related to the lack of data on long term exposure to the product. The Applicant considers 6-12 months as long term exposure, but for the condition to be treated and the procedure which is evaluated long term efficacy would mean longer than 1 year observation. In this respect the long term efficacy i.e. duration of graft survival, tolerability and safety of CAOMECS is not known.

Conclusions on clinical safety

In conclusion the assessment of the risks of application of CAOMECS is based on limited data of apparently poor quality. No clear picture of the safety of the application of CAOMECS graft emerged. Concern is expressed about a possible unfavourable interaction between the CAOMECS graft and subsequent corneal graft. Long term safety data will be required.

Pharmacovigilance system

The pharmacovigilance system is not implemented yet.

Risk management plan

The Applicant submitted the following risk management plan:

Safety concern	**Proposed Pharmacovigilance activities (routine and additional)**	**Proposed risk minimisation activities (routine and additional)**
Important identified risk: • None	Routine Pharmacovigilance Activities (PV) will be applied during post authorization surveillance	N/A
Important potential risk • Eye infections (viral, fungal or bacterial) • Sensitisation potential of reagents used during	Routine PV Activities Potential risks will be discussed as events of special interest in post marketing PSUR These events will be particularly	Standard procedures in the screening, harvesting, transport, manufacturing and release of product in order to diminish the potential risk of infections (testing, training, in-process and batch release control), as well as the other listed risks.

the manufacturing process in particular beta-lactam antibiotics	followed and evaluated in the planned phase III study	These procedures are part of the conditions of marketing authorization. The product is used in specialised centres by highly trained health care professionals and technicians: User manuals, the SmPC and label provide concise guidance on how to correctly use the product Labelling in section 4.4 of the SmPC The manufacturing process is controlled by GMP principles
Important missing information: • Long term safety and efficacy follow up • Use in paediatric patients	Routine Pharmacovigilance Activities Missing information will be followed up by an extension of the France study and the collection of efficacy and safety follow up for months 24 and 36 after transplant The applicant will perform a phase III study in LSCD including long term safety and efficacy follow up The applicant will conduct a paediatric development program (Procedure number: EMEA-001004-PIP01-10)	Missing information will be followed up by additional studies. If there were risks identified through these studies the applicant would re-evaluate the need of further measures.

The Applicant has not provided an updated RMP in response to the D120 LoQ.

Of great concern is that from the Applicant's response to clinical safety MOs 153, 155, and RMP MO 156 it appears that the Applicant has not performed source data verification (SDV) because the Applicant is currently still in negotiation with the clinical site in Lyon (HCL) waiting for their (legal) permission to provide narratives of all patients. This implies that AE relatedness assessment has not been systematically performed by the Applicant and is currently not available. The CAT is of the opinion that in absence of a complete AE-case-related risk assessment it is not possible to determine which of the risks are considered important identified or important potential risks.

In response to the D120 LoQ the Applicant provided the following additional documentation:

- *Draft risk analysis of ocular infectious diseases caused by oral micro-organisms*. The risks of the infection with the micro-organisms were evaluated by the flowchart, and classified in the risk level as 0 to 5.
- A comprehensive *Draft risk assessment of oral mucosa cells culture and oral autologous mucosa sheet used for treatment of severe limbal stem cell deficiency with Failure Modes and Effects Analysis FMEA method*. FMEA is a methodology for analyzing potential reliability problems in the product development cycle where it is easier to take actions to overcome these issues, thereby enhancing reliability throughout process and product design. FMEA is used to identify potential failure modes, determine their effect on the quality of the product, and identify actions to mitigate the potential failures. A crucial step is anticipating what might go wrong with a product. While anticipating every failure mode is not possible, the development team should formulate as extensive list of potential failure modes as possible.

These draft risk analyses, albeit useful, are considered a starting point to the Safety specification and the RMP. It is expected that these analysis are integrated into the RMP.

4. ORPHAN MEDICINAL PRODUCTS

An orphan designation has not been requested for this product.

5. BENEFIT RISK ASSESSMENT

Beneficial effects

The concept of using autologous buccal mucosa biopsy material as a source for preparation of a epithelial cell sheet, as a replacement of damaged corneal epithelium, and the technique used potentially has several advantages compared to alternative methods. In addition, it has several advantages to alternative methods used so far to obtain autologous grafts. Especially obtaining an autologous epithelial cell sheet with this method does not depend on the availability of healthy limbal stem cells e.g. from the other eye. In contrast to a allogeneic graft, there would be a lower probability of rejection whereas (continuous) immunosuppression may not be necessary.

Also, if effective, harvesting limbal stem cells from the healthy eye is not necessary. The temperature responsive polymer allows the growth of confluent cell cultures without scaffolds and their detachment from the supporting membrane is achieved without the use of proteolytic enzymes preventing tears and contamination. This in theory should increase the chance for obtaining an intact cell layer with preserved cell-cell junctions which could be suitable for corneal epithelium replacement.

The evidence for efficacy is based on the data from two open-label uncontrolled single-arm studies with 4 and 26 subjects respectively. The latter study, referred to as the France study, is considered decisive by the company.

At best, this study indicates some graft survival, absence of conjunctivalisation and stable epithelium. The open label assessment of subjective symptoms does not allow a firm conclusion on efficacy of these secondary endpoints.

However, several flaws in the study design and study conduct questions the external and internal validity of these results. Methodological flaws refer to lack of control, lack of randomisation open-label study design. External validity is questioned because the study was single-centre i.e. single-investigator based. Internal validity is questioned due to the population included, uncertain diagnostic criteria for LSCD, and appropriateness of the primary endpoint criteria and definition of success. Hence the validity of the results and there generalisation are questioned.

Uncertainty in the knowledge about the beneficial effects

The cellular dynamics of the CAOMECS graft is largely unclear. To which extend the mucosal epithelial cells deriving from the stem cells transform and/or replace the pathological corneal epithelial grafting,

the cellular interaction between the principle 3 cell tissues involved (i.e. graft mucosal epithelium, conjunctival epithelium and pathological corneal epithelium) is unclear. Additionally, the determination of minimum amount of stem cells needed for successful transplantation is missing. This knowledge is considered essential for successful grafting, lifelong functionality. Also the non-clinical data provided in this respect are not supportive and do not allow to obtain a clear view on the function of the epithelial sheet and the potential beneficial effect of CAOMECS.

A clinical benefit on visual function, opacity, less discomfort, pain, less infections, increased probability of subsequent successful corneal graft, is uncertain. Therefore, the submitted clinical data are considered insufficient to support the indication applied for by the company.

Whereas in the proof of concept study improvement of corneal opacity and visual acuity was observed in all 4 patients within 10 weeks and maintained, this has not been reproduced in the France study during the 12 months follow-up. This emphasises the caution needed before generalizing study results from single centre studies.

An additional deficiency is the fact that there is no comparison to alternative treatments and therefore the outcome of this study cannot be brought in perspective, which would have been very useful both for the benefit/risk assessment and later in clinical practice.

Risks

Unfavourable effects

An overall picture of the safety of the application of CAOMECS graft is difficult to obtain due to the limited exposure with this specific product in terms of number of subjects, follow up time, and quality of the monitoring and reporting system, and because of the lack of comparative arm.

The quality of the safety monitoring and reporting in the France study is questioned, because of the unexpectedly low number of adverse events, particularly patient reported ones, and because of other discrepancies. Similarly the data from published studies is rather heterogeneous with respect to procedure applied, follow up time and quality of the safety monitoring/reporting. Therefore the assessment of the risks of application of CAOMECS is based on limited data with several uncertainties.

Most common adverse events observed are related to the eye and it is difficult to determine to which extent the events can be attributed to the underlying condition, surgical procedure, co-medication or graft. Cornea epithelial defects, inflammation and infection, and an increased IOP may be considered identified risks. Neovascularisation is an adverse event which occurs almost inevitably after corneal epithelial grafting with might have consequences for vision if it occurs in the central cornea.

Uncertainty in the knowledge about the unfavourable effects

There are certain risks which can be predicted by performing the grafting procedure e.g. inflammation, haemorrhage under the epithelial sheet, corneal perforation and infections. The incidence and to which extent the transplant contributes to these AEs is uncertain.

An unfavourable impact on corneal opacification after CAOMECS application cannot be excluded as it is unknown how the multilayer cell sheet behaves in the long run e.g. forms too many layers which will reduce transparency or grows into the stroma. There might be a higher risk of contamination of the CAOMECS sheet and subsequent inflammation and infection of the eye, when the product will be used in clinical practice. This might be related to the fact that the transportation time will be longer than the one in the France study where the two units were at walking distance from each other. So it is questioned if the results from the France study can be extrapolated to different treatment centres.

Data on the long term maintenance, tolerability and safety of CAOMECS are missing and will not become available since the applicant has no longer access to the source data.

An unfavourable interaction between the graft and subsequent corneal transplant can not be excluded given the two cases of corneal graft rejection.

In addition, the low number of animals in the single dose toxicity studies (4/group) hampers the non-clinical safety assessment of CAOMECS. The applicant argues that the toxicity study was conducted to get the information from the use of a fewer animals and obtain significance for statistical analysis of data, and minimized sacrifice number in compliance with the guideline for care and use of laboratory animals. However, the number of animals is too small make a valid safety assessment.

It is also unclear whether the non-Clinical safety data obtained can be extrapolated to a product manufactured at another site. Therefore it may be necessary to generate additional non-clinical data, with a new product.

Balance

Importance of favourable and unfavourable effects

The lack of evidence of clinical benefit and potential unfavourable interaction between the CAOMECS graft and subsequent corneal transplant decrease the importance of the favourable effects mentioned and emphasis the potential unfavourable effect.

Benefit-risk balance

Next to the fact that the internal and external validity of the results is at stake, the benefit of the application of CAOMECS as demonstrated in the France study is limited to possible survival of the CAOMECS epithelial cell transplant, and some indications of stabilisation of the corneal epithelium. The clinical benefit for visual function is unclear.

The assessment of the risks of application of CAOMECS is based on limited data of apparent poor quality. No clear picture of the safety of the application of CAOMECS graft emerged and underlying data are lacking. Concern is raised regarding a possible unfavourable interaction between the CAOMECS graft and subsequent corneal graft. Long term safety data may be required.

Additionally the original product no longer exists, whereas an alternative production site has been closed as well. The closure of the new manufacturing site and therefore lack of a facility to produce a new product precludes the generation of new clinical data. This is required as the original data cannot

be considered pivotal support of the application, only supportive at best provided there would be a comparable product.

Discussion on the benefit-risk assessment

Based on the evidence for quality, efficacy and safety of CAOMECS in the treatment of LSCD the benefit/risk balance is considered negative. The limited amount of evidence of clinical benefit and doubts about its internal and external validity of the observed study results precludes a positive benefit risk assessment. This situation is not changed by the answers submitted by the applicant to the day 120 List of Questions.

The Applicant was not able to address the major clinical issues raised. An important argument used by the applicant is that due to lack of access to the source data, questions can not be answered.

However, it is doubtful that the main issues can be resolved in case access to the source data could have been obtained. Especially the discussion concerning the external and internal validity is relatively source data independent. In addition it is questioned, given the outcome of the inspection, whether the quality of the source data for both efficacy and safety, allow sufficient details for an adequate answer.

The question to which extend the mucosal epithelium transforms and/or replace the pathological corneal epithelium was not addressed. The applicant now appears to agree that a positive effect on vision would be a true benefit but this could not be the primary endpoint because CAOMECS does not restore epithelium. Instead it is suggested that that the procedure assumed to facilitate secondary transplantation, which is not supported by data. Moreover, it appears that the population in the France study is considered rather severe suggesting that this population was not optimal to evaluate the CAOMECS concept. The data obtained from this population does not support the indication applied for.

Hence even if access to the source data is obtained the results of this single centre, single investigator, single arm study, irrespective of GCP failures, is not considered sufficient for approval.

5.1. Conclusions

Based on the evidence for quality, efficacy and safety of CAOMECS in the treatment of LSCD the benefit/risk balance is considered negative. The data do not support the requested indication and there are many outstanding issues which can not be solved without generation of new data.