18 December 2014
EMA/25273/2015
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Holoclar

International non-proprietary name: Ex vivo expanded autologous human corneal epithelial cells containing stem cells

Procedure No. EMEA/H/C/002450/0000

Note

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

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Table of contents

List of abbreviations

ACLSCT	Autologous cultured limbal stem cell transplantation
ADR	Adverse Drug Reaction
AE	Adverse Event
ATMP	Advanced Medicinal product
BCVA	Best Corrected Visual Acuity
Bid	two times a day
CI	Confidence Interval
CBMP	Cell-Based Medicinal Products
CHMP	Committee for Medicinal Product for Human Use
COMP	Committee for Orphan Medicinal Products
cm^2	Square centimeters
CNV	Corneal Neovascularisation
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GPLSCD01	Company code for Holoclar used during product development
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
IOP	Intraocular pressure
ITT	Intent-to-Treat
K3	Cytokeratin 3
K12	Cytokeratin 12
K19	Cytokeratin 19
kg	Kilogram
LSCD	Limbal Stem Cell Deficiency
LSC	Limbal Stem Cells
MA	Marketing Authorisation
MAA	Marketing Authorisation Application
MCB	Master Cell Bank
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	MilliLiter
µg	Micogram
PD	Pharmacodynamics
PI	Product Information
PIP	Paediatric Investigation Plan

PK	Pharmacokinteics
PP	Per Protocol
SAE	Serious adverse event
SmPC	Summary of Product Characteristic
SOC	System Organ Class (MedDRA)
TEAE	Treatment-Emergent Adverse Event
Tid	three times a day
VA	Visual Acuity
WCB	Work Cell Bench

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Chiesi Farmaceutici S.p.A. submitted on 6 March 2013 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Holoclar, through the centralised procedure falling within the Article 3(1) and point 1 Annex of Regulation (EC) No 726/2004.

Holoclar was designated as an orphan medicinal product EU/3/08/579 on 7 November 2008. Holoclar was designated as an orphan medicinal product in the following indication: Treatment of corneal lesions, with associated corneal (limbal) stem cell deficiency, due to ocular burns.

The applicant applied for the following indication in adults:

"Treatment of patients with moderate-severe (superficial corneal neovascularisation in at least two quadrants) limbal stem cell deficiency, unilateral or bilateral with a minimum of 1-2 mm^2 of undamaged limbus, due to ocular burns."

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Holoclar as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website: ema.europa.eu/Find medicine/Rare disease designations.

The legal basis for this application refers to:

Article 8(3) of Directive 2001/83/EC - complete and independent application. The applicant indicated that the ex-vivo expanded autologous human corneal epithelial cells containing stem cells was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) EMA_001082-PIP02-11 on the agreement of a paediatric investigation plan and on the granting of a deferral and on the granting of a waiver.

At the time of submission of the application, the PIP P/0199/2012 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

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

Applicant's request for consideration

Conditional Marketing Authorisation

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of Regulation (EC) No 726/2004 based on the following claim(s):

Holoclar falls under the following categories regarding the scope of conditional marketing authorisation as laid down in Article 2 of Regulation (EC) No 507/2006:

- "medicinal products which aim at the treatment, the prevention or the medical diagnosis of seriously debilitating diseases or life-threatening diseases"
- "medicinal products designated as orphan medicinal products in accordance with Article 3 of Regulation (EC) No 141/2000"

The applicant considers that the requirements for conditional marketing authorisation as laid down in Article 4 of Regulation (EC) No 507/2006 are met:

(a) the risk-benefit balance of the medicinal product, as defined in Article 1(28a) of Directive 2001/83/EC, is positive;
(b) it is likely that the applicant will be in a position to provide the comprehensive clinical data;
(c) unmet medical needs will be fulfilled;
(d) the benefit to public health of the immediate availability on the market of the medicinal product concerned outweighs the risk inherent in the fact that additional data are still required.

New active substance status

The applicant requested the active substance ex-vivo expanded autologous human corneal epithelial cells containing stem cells contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

The applicant Chiesi Farmaceutici S.p.A. submitted on 27 Oct 2010 an application for Scientific recommendation on Classification to the European Medicines Agency (EMA) for Holoclar, which was designated as an Advanced Therapy Medicinal Product on 20 December 2010.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 25 June 2009, with follow-up or clarifications received on 25 September 2009, 23 June 2011, and 05 September 2011. The Protocol Assistance pertained to

quality, non-clinical and clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturer of the biological active substance

Holostem Terapie Avanzate S.R.L.
Via Glauco Gottardi,100, Modena, 41100, Italy

Manufacturer responsible for batch release

Holostem Terapie Avanzate S.R.L.
Via Glauco Gottardi,100, Modena, 41100, Italy

1.3. Steps taken for the assessment of the product

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

CAT Rapporteur: Egbert Flory

CHMP Coordinator (Rapporteur): Jan Mueller-Berghaus

CAT Co-Rapporteur: Paolo Gasparini

CHMP Coordinator (Co-Rapporteur): Daniela Melchiorri

CAT Peer reviewer: Marit Hystad

CHMP Peer reviewer: Karsten Bruins Slot

- The application was received by the EMA on 6 March 2013.
- The procedure started on 27 March 2013.
- The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 19 June 2013. The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 14 June 2013.
- PRAC RMP advice and assessment overview was adopted by PRAC on 17 July 2014.
- During the meeting on 19 July 2013, the CAT agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CAT consolidated List of Questions on 21 August 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CAT and CHMP members on 29 September 2014.
- PRAC RMP advice and assessment overview was adopted by PRAC on 9 October 2014.

- During the CAT meeting on 17 October 2014, the CAT agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CAT List of Outstanding Issues on 14 November 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CAT and CHMP members on 26 November 2014.
- PRAC RMP advice and assessment overview was adopted by PRAC on 4 December 2014.
- During the meeting on 11-12 December 2014, the CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Holoclar on 12 December 2014.
- During the meeting on 18 December 2014, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a conditional Marketing Authorisation to Holoclar.

2. Scientific discussion

2.1. Introduction

Problem statement

Limbal stem cells (LSC) are undifferentiated, long-lived progenitors of corneal epithelium cells, located in the narrow transitional zone of the ocular surface between the cornea and the bulbar conjunctiva. LSCs are important for corneal epithelial regeneration and wound healing. In the course of tissue regeneration, LSCs divide, creating daughter cells called transient amplifying cells, which migrate centripetally and after a high but limited number of mitoses, further differentiate into post-mitotic cells and finally into corneal epithelium. In addition to the regeneration of the corneal epithelium, the limbus acts as a barrier, ensuring separation of corneal and conjunctival/scleral components.

Damage to the limbus results in a reduction in the population of LSCs thereby impacting on the eye's ability for corneal healing and rejuvenation and compromising the limbal barrier function. As a result the conjunctiva overgrows the cornea. This conjunctivalisation of the cornea is associated with neovascularisation, which in turn is linked to the development of an unstable corneal epithelium. Further manifestations include ingrown fibrous tissue, corneal opacification, conjunctival scarring (symblepharon) and corneal ulceration. The clinical spectrum of limbal stem cell deficiency (LSCD) includes pain, photophobia, inflammation, corneal neovascularisation, and eventually, the reduction or complete loss of visual acuity. If left untreated, the condition may progress to a stage whereby persistent epithelial defects present with an associated high risk for the development of bacterial keratitis, corneal perforation and blindness.

Cases of LSCD can be distinguished as being of either primary or secondary origin. Primary LSCD is characterised by the absence of identifiable external factors and is related to an insufficient stromal microenvironment to support stem cell function. Secondary LSCD is caused by ocular surface diseases, such as Stevens-Johnson syndrome, chemical (e.g. acids, alkalis and solvents) and thermal burns, ultraviolet and ionising radiation, ocular cicatricial pemphigoid, severe microbial infections, contact lens wear and multiple ocular surgeries or cryotherapies.

According to Donisi (2003), alkali injuries are the main cause of LSCD due to chemical burns resulting in either moderate, severe or total LSCD, as alkali agents rapidly penetrate the ocular tissues causing extensive epithelial cell necrosis and stromal cell denaturation. General epidemiologic data relating to the prevalence of LSCD (due to ocular burn injury) were not available in the medical literature at the time of this report. Therefore, the prevalence of the condition in Europe was calculated using primary patient discharge data from hospital registries of four European countries: England, Germany, France and Italy. According to this information, the prevalence of the condition in the EU population was 0.3361 per 10,000 (95% confidence interval: 0.3321; 0.3401).

The management of LSCD depends on the extent of the damage and symptomatology and aims at achieving symptom relieve and increase in visual acuity or visual stabilisation. Asymptomatic patients with partial and peripheral conjunctivalization of the corneal surface may not require intervention. Patients with total LSCD, on the contrary, are indicated for corneal surface reconstruction with auto- or allo-transplantation, combined with or followed by keratoplasty in case of deep stromal injury. Donor tissue can be obtained from the fellow eye (autograft) in case of unilateral disease, or from a living related donor or a cadaver (allograft) in case of bilateral disease. Autologous cultivated stem cells have the advantage to permanently restore the corneal epithelium without the need of donor tissues and consequent chronic systemic immunosuppression. However,if the autologous stem cells are not cultivated, the transplantation of autologous limbal cells requires a large limbal graft from the healthy eye resulting in a risk of damaging integrity and function of a previously healthy eye. It is furthermore unsuitable for bilateral disease.

At the time of this report, no medicinal products had been approved in the European Union/European Economic Area (EU/EEA) for this indication and there was no gold-standard in treatment.

About the product

Holoclar consists of a transparent circular sheet of 300,000 to 1,200,000 viable autologous human corneal epithelial cells (79,000-316,000 cells/cm^2), expanded in cell culture and including on average 3.5% (0.4% to 10%) limbal stem cells in addition to stem cell-derived transient amplifying and terminally differentiated cells. The pharmaceutical form of the product thus is a living tissue equivalent on a transparent, circular sheet and the active substance is defined as ‘ex-vivo expanded autologous human corneal epithelial cells containing stem cells’.

Holoclar is proposed to be used for treatment of adult patients with moderate to severe LSCD due to ocular burns. In these patients, Holoclar is intended to be transplanted in the affected eye(s) after removal of the altered corneal epithelium. The recommended dose is 79,000 – 316,000 cells/cm^2, whereby the product contains a sufficient number of cells to cover the entire corneal surface of the patient’s affected eye.

Holoclar acts by replacing the damaged corneal epithelium and creating a reservoir of LSCs for the continuous regeneration of the epithelium. It is intended for use in ocular burn injuries both with and without preservation of deep stromal components. In case of deep stromal injury, patients may also require keratoplasty in order to restore visual function. However, in the absence of a healthy limbus, keratoplasty is associated with a very high failure rate, whereas restoration of limbal function with Holoclar aims at achieving long-term maintenance of normal corneal function. Holocar is not intended for use in case of genetic disorder or other inherent predisposition associated with LSCD, which are associated with a microenvironment unable to support LSC survival and proliferation.

LSCs are derived from a biopsy taken from a small area of undamaged limbus of the patient's eye. Following biopsy, the cells are grown in cell culture with the help of an irradiated 3T3-J2 mouse feeder cell line. The cell suspension is cryopreserved until a transplantation date is scheduled. Thawed cells are then used for subsequent manufacture of the final product. To this end, the expanded cells are again seeded on irradiated 3T3J2-feeder cells which have been previously grown on fibrin. When the patient cells have expanded and built a confluent layer, the product is formulated, packaged, shipped and administered to the patient. The finished product contains cells beginning to build a stratified epithelium, including stem cells. Depending on the individual patient's biopsy, the LSC population of the product varies within individual batches. Repeated manufacture of patient-specific grafts is possible.

2.2. Quality aspects

2.2.1. Introduction

The product responds to an unmet medical need for a rare disease. The design and manufacturing process presented by the Applicant reflects the finished product development and it is linked to the clinical data presented. The Applicant provides a clear distinction between active substance and finished product definition:

Holoclar active substance is defined as "*ex-vivo* expanded autologous human corneal epithelial cells containing stem cells". The active substance is manufactured from a biopsy taken from a small area of undamaged limbus of the patient's eye. After non-specific isolation of the cells, they are expanded in cell culture under specific culture medium conditions and are seeded on a layer of an irradiated 3T3-J2 mouse feeder cell line. Prior to active substance release, the expanded cell suspension is cryopreserved until a transplantation date is scheduled. The active substance is defined as a thawed suspension of a heterogeneous mixture of *ex vivo* expanded sub-confluent autologous human corneal cells in medium, with the potential to form a stratified epithelium. The active substance contains a minimum of small-sized limbal epithelial stem cells, as determined histochemically by expression of the phenotypic marker p63 bright at release ('holoclones'). Beside the p63++ cells, the keratinocyte culture is comprised of clonogenic transiently amplifying (TA) cells ('meroclones' and 'paraclones'), and terminally differentiated non-clonogenic corneal epithelial (K3+) cells. Other starting material-related cellular impurities are not analysed in detail at the active substance stage. The thawed cells are used for subsequent manufacture of finished product.

Holoclar is defined as autologous tissue-engineered product which consists of a transparent circular sheet of 300,000 to 1,200,000 viable autologous human corneal epithelial cells (79,000-316,000 cells/cm^2), expanded *ex vivo*, including on average 3.5% (0.4% to 10%) limbal stem cells, stem cell-derived transient amplifying and terminally differentiated cells, prepared from a limbus biopsy of the patient as starting material, and attached on a 2.2cm diameter fibrin support (manufactured from Ph. Eur. compliant human fibrin) and maintained in physiological transport medium (containing Dulbecco's modified eagle medium (DMEM), supplemented with L-glutamine). The proposed shelf life is 36 hours. The primary container is placed inside multiple layers of secondary packaging to protect the vulnerable product. The finished product presentation does not include a medical device.

The cellular components of the finished product are characterised to be qualitatively identical to the active substance with regard to cell types present, although quantitative differences in cellular populations between active substance and finished product are observed. This is expected and raises no concern.

Due to the inherent complexity, variability and autologous nature of the patient-specific corneal-derived *ex vivo* expanded cells, a noticeable degree of variability within cell identities and growth behaviour is intrinsic, making control and standardisation of the manufacturing process highly challenging. The need of the corneal keratinocytes for a direct feeder cell support for growth and stem cell maintenance adds another biological system, the irradiated 3T3 feeder cells, to the manufacturing process, leading to further variability. Keratinocyte culture obedience on feeder cells is a commonly recognised state-of-the-art practice. The properties of the excipient fibrin support further increase the overall complexity of the product and its manufacturing.

The Applicant therefore implemented the approach to control the manufacturing process by structuring the process into several process stages and sub-stages, which are monitored by a number of operational parameters, in-process controls (IPCs), and in-process monitoring measures.

2.2.2. Active Substance

The active substance is manufactured at Holostem Terapie Avanzate S.r.l (Modena, Italy). The site is also responsible for batch release testing of the active substance. The manufacture of the active substance is patient-specific.

The Applicant has implemented a training program which is mandatory for surgeons interested in treating patients with Holoclar, including training for collection of a biopsy sample including guidance on location and size of sample to collect.

As starting material, a limbus biopsy of 1-2 mm^2 in size from a patient's unaffected limbal eye area is procured at the hospital and transported to the manufacturing site in Modena, Italy. The starting material is thus of high value and of limited quantity. It is most critical to generate sufficient cells from the limited number of cells isolated from the small biopsy. In consequence, retaining testing samples from the starting material or from manufacturing intermediates is restricted. This is acknowledged.

The manufacturing process consists of the following steps:

1. Biopsy Processing
2. Primary Culture
3. Cryopreservation
4. Thawing

The cells are seeded on a cell layer of non-proliferating 3T3 J2 cells, a mouse cell line that provides growth factors for proliferation of the patient cells. When the keratinocytes reach the target confluency, they are detached with trypsin and prepared for cryopreservation.

Beside the Colony Forming Efficacy (CFE) test, with the CFE value as predictor for clonogenicity/proliferative capacity, no destructive IPCs on identity, purity, potency have been established. The progress of manufacture is mainly controlled by observing confluency of the cells via microscopy in a certain culture time.

The quantitative ratio of cell identities present in the active substance depends on the inherent variability of the patient's biopsy cells. The active substance contains p63bright positive limbal stem cells. A specified amount of them is needed for clinical success and therefore this marker is convincingly analysed within an active substance potency release test. The non-stem cells contribute to the forming of the epithelial-like structure during finished product manufacture, thereby harbouring the stem cells. Therefore, the need to define certain non-stem cell phenotypes as cellular impurities was not stressed.

Several raw materials have been exchanged during development. This includes the switch from non-irradiated to gamma-irradiated bovine sera, and the introduction of higher qualified reagents, as suggested during Protocol Assistance. These measures were endorsed as suitability of the new materials was sufficiently demonstrated.

One Major Objection was raised at Day 120 with respect to the murine 3T3 feeder layer, as non-proliferation of the cell line after irradiation was not sufficiently demonstrated. The Applicant was asked to use a validated and direct assay to demonstrate that irradiated 3T3 cells do not further proliferate. This concern was sufficiently addressed by validation of the irradiation method. Several methods were successfully employed to demonstrate that irradiated cells do not proliferate.

Overall, concerning manufacturing, characterisation and control of content, identity, potency, purity, impurities of the active substance, a number of other concerns were raised during the procedure. All concerns were sufficiently addressed by the Applicant.

2.2.3. Finished Medicinal Product

Holoclar finished product consists of living autologous human corneal epithelial cells, prepared from the active substance, supported on a fibrin support. The product's definition has been slightly adjusted during the marketing authorisation procedure, thus the definitions in the SmPC and in the dossier were also aligned accordingly. Holoclar finished product is manufactured at Holostem Terapie Avanzate S.r.l (Modena, Italy). The site is also responsible for batch release testing of the active substance.

Holoclar finished product is manufactured from an aliquot of Holoclar active substance. The expanded cells are again seeded on an irradiated 3T3J2-feeder cells that have been at this stage previously attached to fibrin. Cultivation ranges from 5 to 9 days. When the patient cells have expanded and built a layer reaching a target confluency, the product is trimmed to size, formulated, packaged in a specifically designed steel primary container, shipped and administered to the patient. Holoclar finished product is therefore presented as a finished product containing cells beginning to build a stratified epithelium, containing limbal stem cells (LSCs). The fibrin support is defined as an excipient of the finished product and is intended to rapidly dissolve *in situ* in order to release the cells. Depending on the amount of active substance cells and the cryopreservation period, a repeated manufacture of patient-specific finished product is possible.

The Applicant does not indicate any specific tri-dimensional organisation of the final product but only describes a semi-confluent epithelial sheet. It is assumed that the physical organisation is self-assured and that the different cell components will organise themselves once correctly grafted *in vivo*, a view that is actually supported by clinical data. Holoclar is supplied with a diameter of 2.2 cm, which has been demonstrated to be suitably large to ensure corneal replacement in all patients. The product is trimmed to match the exact size of the individual patient's cornea by the administering surgeon.

The manufacturing process of the finished product is comprehensively described (Figure 2). Certain refinements have been made mainly in the light of issues raised at Day 120 List of Questions, e.g. the qualification of a new feeder cell bank and the omission of antibiotics during final culture steps. In addition, adjustment of the specifications has been made, including the introduction of feeder cell impurity control. The Applicant performed a new study to confirm the validity of the refinements in the manufacture. Additional data were generated to support or amend the set limits for the following finished product parameters:

- Stability and transport for the finished product.

- Storage times for materials used in the process.
- Introduction of the additional control test for residual 3T3-J2 cells in the finished product.

Taking into account the type of the product, the manufacturing process can be regarded as standardised. This is supported by the data provided.

Regarding cell type characterisation and control, the Applicant defines the p63++ stem cell subset forming holoclones as main functional component of the product, since these cells are expected to mediate long-term efficacy. Potency is addressed by quantification of p63bright cells. Further differentiated cell populations are considered as supportive, but functionally contributing cells for short/medium-term efficacy. The argumentation was regarded comprehensible and valid. Omission of K19+ marker was justified as requested.

The Applicant was asked to consider the potential of the newly described ABCB5, PAX6 and WNT/A LSC markers in Holoclar characterisation and control. Because the markers are newly described and still under study, it was suggested to include this as part of the confirmatory study post-marketing authorisation. In their responses, the Applicant agreed on this proposal. Due to the short shelf life the product must be immediately released on the basis of macroscopic and microscopic appearance and results of intermediate control testing. As requested, the Applicant provided detailed information on the microscopic and macroscopic assessment. This visual control testing has been validated. To respond to the concern the Applicant extended the validation studies and provided acceptable levels of precision, accuracy and reliability on this visual control testing. The issue was considered solved.

A full dossier for the fibrin sealant was provided under Section 3.2.A.3. For Holoclar the specifications of the fibrin sealant finished product components thrombin and fibrinogen are relevant, which have been provided under 3.2.P.4.5 The Applicant does not perform itself tests on the excipients but states that thrombin, fibrinogen and aprotinin are accepted based on the suppliers' certificate of analysis. In the context of qualification and control of thrombin, fibrinogen, aprotinin, as well as on the manufacture and control of the matrix, a number of specific Other Concerns were raised. With their Day 120 responses, the Applicant was able to solve most concerns, with two minor remaining issues on the storage conditions of fibrinogen and thrombin bulk, which has been solved.

The specifications of the finished product serve to confirm routine quality, including tests for content, identity, potency, purity, impurities and contaminants

The final product has a proposed shelf life of only 36 hours and is vulnerable and sensitive to mechanical and temperature stress. An accurate and reliable stability evaluation, together with a strict and robust container closure system and a tight control of transport conditions was thus regarded crucial to ensure product quality. The Applicant was requested to provide further evidence on stability (physical integrity and viability of the product) and transport conditions.

Stability of Holoclar was sufficiently addressed by the Applicant. As requested, viability has been included in the stability studies and shows good results. The p63++ potency marker conforms to the specification. The established transport conditions are endorsed, taking into consideration the established risk minimisation activities. It is also acknowledged that no deviations on shipped batches manufactured at Modena have been reported. However, regarding the suitability of the container closure system, the temperature profile presented did not support an effective isolation and temperature compensation capacity of the outer packaging. The validation data presented were incomplete and supportive supplier's information was not provided. The chosen experimental environmental stress peak temperatures) appeared not appropriate for EU-wide transport under worst case low and high temperature peaks. The requested additional evidence for suitability of the container

closure system with respect to long-distance transport under challenging temperature conditions was thus not regarded fully convincing. As requested, the Applicant presented further measures by introducing additional risk minimisation strategies. Notably, the additional provisions target long distance transportation over under extreme climatic conditions. Thus, the Applicant implemented improvements beyond current standard pharmaceutical transport controls.

Regarding an ongoing stability program and a definition of the in-use-stability after opening the primary packaging, the Applicant will perform ongoing stability assessments on an annual schedule and perform additional in-use stability testing on 5 GMP conformant lots.

Adventitious agents safety evaluation

The control of adventitious agents for Holoclar is based on a risk management and mitigation approach. A summary of potential sources of adventitious agents and their control is presented. Various preventative actions have been applied for risk minimisation. An overview of the studies to investigate effectiveness of these risk minimisation measures was presented.

The Applicant adequately addressed concerns raised during the evaluation (see Sections 2.2.2 to 2.2.4). Adventitious agents safety including TSE have been sufficiently assured.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

A major concern was raised with respect to the murine 3T3 feeder layer, as non-proliferation of the cell line after irradiation was not sufficiently demonstrated. The Applicant was asked to use a validated and direct (e.g. biochemical, radiopharmaceutical, Colony Forming Efficacy) assay to demonstrate that irradiated 3T3 cells do not further proliferate. This Major Objection was sufficiently addressed by validation of the irradiation method. Several methods were successfully employed to demonstrate that irradiated cells do not proliferate. Therefore, this concern was considered solved.

Another Major Objection was raised on the microbiological control strategy. In their response, the Applicant proposed several measures. As requested, the Applicant implemented an appropriate in-process control (IPC) for microbial contaminations during primary culture and established a microbial control performed on samples collected before release of the finished product. A rapid detection method was validated for the intended sample matrices. The method was considered acceptable to improve safety, since information on potential microbial contamination may now be available before product administration. The antibiotics have been removed from the culture media after the thawing of the Intermediate Cell Bank (ICB). A new rapid sterility release test has been implemented on a sample taken from the beginning of the secondary culture, after thawing of the ICB. The test provides assurance that all original microbial contamination had been eliminated by this stage. The Applicant also satisfactorily addressed the minor outstanding issue regarding the use of filtration devices. In summary, all issues regarding control of microbiological safety are considered solved.

A full dossier for the fibrin support was provided under Section 3.2.A.3 For Holoclar the specifications the fibrin finished product components thrombin and fibrinogen are relevant and were provided under 3.2.P.4.5 The Applicant does not perform itself tests on the excipients but states that thrombin, fibrinogen and aprotinin are accepted based on the suppliers' certificate of analysis. In the context of qualification and control of thrombin, fibrinogen, aprotinin as well as on the manufacture and control of the matrix, a number of specific Other Concerns were raised at Day 120. With their responses, the Applicant was able to solve most concerns on the quality and safety of this raw material with only two minor topics remaining at Day 180. The Applicant also clarified that despite the fact that the strength of the fibrinogen and thrombin components may vary, their

concentration used to prepare the fibrin support remains constant, therefore not affecting the fibrin support properties.

Additional questions regarding description of the physicochemical properties, mechanical stability and degradation of the altered excipient were extensively addressed in the Applicant's Day 120 responses.

It was considered at Day 120 that stability of Holoclar finished product was not adequately demonstrated. The Applicant satisfactorily addressed this concern and will perform ongoing stability assessments on an annual schedule and additional in-use stability testing on GMP conformant lots. The Applicant also provided good evidence that the physical integrity of the product is preserved during anticipated transport conditions. The Applicant also confirmed that an adequate temperature monitoring system is in place.

The other outstanding issues raised at Day 180 are considered resolved.

The Applicant agreed to the post-authorisation measure to perform prospective study HLSTM03 (multi-national, multi-centre, prospective, open-label, uncontrolled clinical trial to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns) and the Applicant will include suitable markers in the product characterisation strategy.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall Quality of Holoclar is considered acceptable. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The manufacturing process of the active substance is adequately described, controlled and validated. The active substance is well characterised and appropriate specifications are set. The manufacturing process of the finished product has been satisfactorily described and validated. The quality of the finished product is controlled by adequate test methods and specifications. Adventitious agents safety including TSE have been sufficiently assured.

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

2.2.6. Recommendation for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical development programme of Holoclar, a transparent sheet of autologous human corneal epithelial cells including stem cells, comprised an evaluation of published studies from the scientific literature in conjunction with the proposed testing programme for production and release of the medicinal product. To justify the abridged development programme, reference was also made to the experience gained from clinical use of epidermal and limbal epithelial cells over more than 30 years and in particular with Holoclar which was used in clinical practice since 1998.

Conventional non-clinical studies were generally not considered appropriate for Holoclar as a cell-based medicinal product (CBMP). However, the applicant conducted *in vitro* non-clinical toxicology studies to evaluate the tumorigenic potential.

The non-clinical development programme is briefly summarised in sections 2.3.2. to 2.3.5. and further discussed in section 2.3.6.

2.3.2. Pharmacology

Considering the available evidence from the scientific literature supporting the proposed mechanism of action, no non-clinical studies have been conducted to assess the primary and secondary pharmacology of Holoclar. This was further justified by the applicant by the clinical experience originating from the use of the product since 1998 (see section 2.4. - 2.5. and the limited relevance of animal models for characterising the pharmacodynamic (PD) response to administration of Holoclar. In particular, support for the proof of principle was available from published non-clinical studies in rats which showed that ex vivo expanded limbal cells cultured on a fibrin support can be used to replace and regenerate corneal epithelium lost due to LSCD by creating a structural replacement, with the formation of a normal thickness corneal epithelial cell layer (Sacchetti et al., 2002; Talbot et al., 2006; Gimeno et al., 2007). The functionality of these types of grafts is further supported by the demonstration of clinical efficacy, as defined by a resolution of LSCD-associated symptoms (ocular burning and pain, photophobia, foreign body sensation) as well as improved visual acuity (Rama et al., 2001; Rama et al., 2010).

Reference was also made to the Part IV of Annex I to Directive 2001/83/EC, as amended by Directive 2009/120/EC, which acknowledges that the usual requirements for the pharmacological and toxicological testing of medicinal products may not always be appropriate for advanced therapy medicinal products (ATMPs) due to their unique and diverse structural and biological properties.

Finally, as the product is intended for administration to the eye where it remains locally at the site of application, conventional pharmacodynamic safety and drug interactions studies were not conducted.

2.3.3. Pharmacokinetics

In accordance with the Guideline on Human Cell-Based Medicinal Products (EMEA/CHMP/410869/2006), classical absorption, distribution, metabolism and excretion studies were not considered relevant for a human CBMP and thus these kind of studies have not been performed by the applicant.

The applicant furthermore argued that the proposed treatment was a single topical replacement treatment without systemic effect. Transplanted cells only colonise on the ocular surface. To support the lack of invasion of the cultured cells into basal ocular structures, the applicant provided data from an histological analysis of corneal sections derived from Holoclar-treated patients who underwent perforating keratoplasty. These data were generated as part of the clinical safety evaluation and relevant information for the PK/PD review is summarised in section 2.4.2. and 2.4.3. Moreover, the applicant referred to published data (Di Nunzio et al., 2008) in which the distribution of a similar cell sheet based on skin keratinocytes was analysed after subcutaneous transplantation in athymic mice. These data indicated that integrity of such a cell sheet is maintained after implantation.

2.3.4. Toxicology

The non-clinical toxicology assessment of Holoclar was limited and abridged, as was justified by the applicant by the already existing clinical experience with the product as well as the lack of relevant animal models due to differences in the ocular structure of most other mammals. Furthermore, complications may arise from the xenograft setting of such studies and due to ethical concerns with primate models.

The development programme focussed on the characterisation of the tumorigenic and carcinogenic potential of Holoclar. Other aspects of toxicology, such as antigenicity and microchimerism due to the presence of the irradiated 3T3-J2 feeder layer, were largely deduced from clinical findings (see sections 2.4. - 2.6.). However, in order to investigate the proliferative capacity of the 3T3-J2 feeder cells, the applicant performed *in vitro* karyotype analysis and soft agar assays.

Except for the analysis of the adhesion dependent growth, which was conducted following the general principles of Good Laboratory Practice (GLP), all studies have been performed in full compliance with GLP. This approach was considered acceptable by the CAT.

Tumorigenic potential

While conventional carcinogenicity studies have not been performed, the potential for transformation and formation of tumours by the human corneal epithelial cells in Holoclar was investigated in *in vitro* assays.

Karyotype analysis

The applicant provided data on chromosomal characterisation of the cells to detect genomic instability in order to ascertain whether culture and manipulations of the cell population during the manufacturing of the drug product induced chromosomal damage or abnormalities, including cell fusion. A worst case scenario was applied by performing the analysis subject to an additional cell passage step beyond that used to produce Holoclar.

The karyotype analysis included chromosomal counts and the frequency of hyperploidy, hypoploidy, polyploidy, breaks and structural abnormalities in 50 metaphasic cells in six batches cultured from the drug product. The analysis demonstrated a consistent karyotypic profile throughout the tested cell cultures. No evidence of chromosomal aberrations or minor chromosomal damage was detected, and the karyotypes were consistent with that of normal, unprocessed cells of human origin.

Growth factor dependence

The growth of cells extracted from Holoclar drug substance and drug product was evaluated both in the presence and in the absence of growth factors supplemented into the normal growth media, in order to investigate if manipulations during the manufacturing process give cell subpopulations a proliferative advantage. Two batches of pooled drug substance and drug product were cultured

- in the presence or absence of a feeder layer of lethally irradiated 3T3-J2 cells, and
- in the presence or absence of exogenous growth factor supplementation (insulin, epidermal growth factor, or hydrocortisone).

The cell populations were proliferative in the presence of fibrin support and cell growth was consistent independent of the source of the fibrin support. However, cell growth was either completely attenuated or markedly slowed in the absence of feeder cells and growth factor supplementation. In comparison, the positive cell growth control (squamous cell cancer line) showed proliferative capacity under all growth conditions independent of the presence of growth factors and/or a layer of feeder cells.

Soft agar assay

The adhesion dependence of Holoclar was assessed by evaluation of colony formation in soft agar gels. At the conclusion of the incubation period, the number of colonies (more than 40 cells) and bursts (less than 40 cells) were counted under light microscopy for both the positive control (human breast adenocarcinoma cell line) and test cells. Floating cut points were defined as 186 and 42 for bursts and colonies, respectively.

Holoclar human corneal epithelial cells showed growth and burst formation after 19 days of incubation. The number of bursts was always below the floating cut point and was therefore considered a negative result. The number of bursts was significantly lower than the MCF-7 positive tumour cell line control ($p<0.001$). No colonies of more than 40 cells were formed by human corneal epithelial cells, in contrast to MCF-7 cells. No effect of increased seeding concentration on the formation of bursts or colonies was evident, as seeding at a higher cell density did not manifest in increases in cell burst formation.

Other toxicity studies

Microchimerism

The risk of xenogeneic microchimerism arises from the presence of irradiated murine 3T3-J2 feeder cell layer in the composition of the drug product and the potential occurrence of viable 3T3-J2 cells and a lack of native immune clearance. The viability of 3T3-J2 cells following irradiation was investigated in the context of the validation of the manufacturing process confirming that the radiation dose applied to the feeder layer is sufficient to render them fully non-proliferative (see section 2.2.4.). Moreover, a karyotype analysis and soft agar assays were performed.

Karyotype analysis (3T3-J2 cells)

Chromosomal characterisation was conducted in both irradiated cells and non-irradiated cells, the latter of which were included in the analysis as a positive control. Initial analysis did not detect metaphase cells amongst the irradiated cells, which was considered consistent with treatment of normal eukaryotic cells with ionising radiation leading to G1 arrest. Re-testing however concluded that metaphase cells were present and a high degree of chromosomal damage was reported, with widespread chromosomal fragmentation evident in almost every metaphase cell observed. Detection of metaphasic cells was later considered to be an artefact reflecting the high degree of chromosomal damage that was elicited by the irradiation procedure.

Soft agar assay (3T3-J2 cells)

Soft agar assays were conducted to analyse the growth properties of three batches each of irradiated versus non-irradiated mouse 3T3-J2 cells from the Master and Work Cell Bench using an internal positive control. In addition, a fibroblastic cell line that does not exhibit anchorage-independent growth was employed as a negative control. Cell numbers of non-irradiated 3T3-J2 cells were shown to effectively remain stable until the 3-day time point of the assay, followed by a slight decrease at the 6-day time point, indicating that no anchorage-independent growth was occurring. For 3T3-J2 cells irradiated according to the Holoclar manufacturing protocol a decrease of the cell number was observed over the full timeframe of the assay, comparable to the negative control.

2.3.5. Ecotoxicity / environmental risk assessment

The CAT agreed that no environmental risk assessment (ERA) was required for Holoclar, as the cells of the product are not viable outside the laboratory or dangerous to the environment.

2.3.6. Discussion on the non-clinical aspects

Pharmacology

The general concept for the transplantation of allogenic or autologous limbal stems cells is well known and described in the scientific literature. The clinical efficacy of such therapies strongly depends on the underlying disease and their severity. In each case the injured eye has to be carefully diagnosed to make the decision on the optimal medical therapy. However, the clinical experience gained at the time of this report provide evidence for the general proof-of-concept and the benficial role of LSC in the treatment of LSCD.

Several publications were available on *ex vivo* expanded LSCs analysed in a homologous or heterologous LSCD rabbit model (including Ti et al. 2002; Talbot et al., 2006; Luengo et al., 2007). However, since the products used in studies described in the literature were not identical or sufficiently comparable to Holoclar, published non-clinical data were only considered supportive.

The anatomical and physiological differences between animal models and the human eye as discussed by the applicant were acknowledged by the CAT. In view of the species differences, a homologous model would be most suitable. However, confirmation of validity of this approach would be difficult and require an adequate bridging of the quality data of the animal cells to the intended human medicinal product Holoclar. Therefore, and in light of the availability of clinical data (see section 2.5.), *in vivo* studies in a homologous animal model were considered by the CAT not to be necessary and the absence of non-clinical PD data was accepted.

Pharmacokinetics

The applicant discussed the potential for invasion of the grafted cells beyond the area of transplantation. Possible risks associated with systemic distribution of cells derived from Holoclar are tumour formation, an accelerated immune response after application of a repeated graft or the transmission of adventitious agents. However, the risk of tumourigenicity was considered low (see discussion on toxicology below), no significant adaptive immune response was observed after repeated transplantation in the clinical setting (see section 2.5. and 2.6.) and transmission of adventitious agents was addressed by adequate quality control measures (see section 2.2.). Furthermore, some supportive evidence for a lack of cell migration was available from published data (Di Nunzio et al., 2008). Moreover, dissemination from the area of engraftment was considered unlikely due to the adhesion dependency of normal epithelial cells as well as other localised cell-type specific factors and the crossing of the basal membrane was considered a worst case scenario.

The main support for a lack of biodistribution, however, was provided by clinical data including an immunohistological analysis of corneal sections derived from Holoclar-treated patients who had perforating keratoplasty. These data are presented and discussed in sections 2.4.2. and 2.4.4. of this report. In light of the available clinical data, the CAT agreed that no additional non-clinical biodistribution data were needed. The CAT furthermore agreed that classical, non-clinical PK studies were not relevant for Holoclar.

Toxicology

One of the main concerns with the use of any cell therapy, in particular those comprising a heterogeneous population of cells including cells with proliferative potential, is the formation of tumours. The potential for

tumourigenicity of Holoclar drug substance and product was investigated in three different *in vitro* approaches, all of which pointed towards a low risk of tumour formation.

Regarding the risk of microchimaera formation by the murine feeder cells in the final drug product, available data suggested a low tumourigenic potential of the cells even without irradiation. Furthermore, irradiation as part of the manufacturing process resulted in lethal chromosomal damage rendering the cells non-proliferative (see section 2.2.4.). In light of these data in addition to the results from immunohistological analyses in a subset of patients treated with Holoclar and the absence of a significant adaptive immune response (see section 2.5. and 2.6.), the CAT considered that microchimera formation may not be expected.

Overall, the omission of classical toxicology animal studies, including single and repeat dose studies as well as immunogenicity studies, was considered by the CAT to be justified due to the lack of suitable animal models and in light of the available clinical safety data. As no systemic exposure was to be expected and since the product was autologous in nature, the risk for product-related toxicity to the reproductive system was considered to be negligible and therefore omission of reproductive toxicity testing was deemed acceptable.

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

2.3.7. Conclusion on the non-clinical aspects

In light of the supportive information from the scientific literature as well as the available clinical data and since due to the lack of suitable animal models, conventional non-clinical studies with Holoclar were considered not to be appropriate or feasible, the CAT considered the abridged non-clinical programme acceptable. No ERA was considered necessary.

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

2.4. Clinical aspects

2.4.1. Introduction

Good Clinical Practice (GCP)

No prospective clinical trials were conducted in support of this application. However, the applicant provided data from retrospective studies. Quality, integrity, and reliability of these data have been verified in GCP inspections performed at the two sites used for the pivotal study HLSTM01 as well as one site in HLSTM02. While ICH-GCP was not considered fully applicable in light of the retrospective, observational nature of the data collection, the inspections nevertheless confirmed that general GCP principles were complied with.

- Tabular overview of clinical studies

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Efficacy and Safety	HLSTM01	5.3.5.2	Long-term Efficacy and Safety	Retrospective case-series, non-randomised, non-controlled, multicentre	GPLSCD01, single treatment, surgically administered	Treatments: 113[1] Patients: 106	Moderate-severe limbal stem cell deficiency secondary to ocular burns	1-3 treatments[2]	Completed; Full
Safety	HLSTM02	5.3.5.2	Long-term Safety	Retrospective case-series, observational, non-randomised, non-controlled, multicentre	GPLSCD01, single treatment, surgically administered	29	Patients transplanted with GPLSCD01	Single treatment	Completed; Full

[1] A total of 106 patients who underwent 119 treatments were included in the study. Data was available for 113 treatment and all 113 treatment cases were included in the safety population, including 7 cases of repeated treatment.

[2] There were 12 cases of second treatments and one case of third treatment. Where data is available, patients with multiple treatments recorded in HLSTM01 database are counted multiple times for safety but only the last treatment is considered for efficacy.

2.4.2. Pharmacokinetics

Conventional PK studies have not been performed for Holoclar since, according to the Guideline on Human Cell-Based Medicinal Products (CBMP, EMEA/CHMP/410869/2006), such studies were not considered relevant. Holoclar consists of an autologous sheet of stratified epithelium, applied topically to the eye to act locally at the site of application. The cells are not expected to migrate beyond the ocular surface, or to produce systemic effects. Evaluation of the potential dissemination of the transplanted cells is difficult, due to an inability to distinguish engrafted autologous cells that by definition, lack unique identification markers.

However, some information on the potential for biodistribution was derived from data from an histological and morphological evaluation of corneal material collected from 26 patients who had undergone perforating keratoplasty 9 to 93 month (average 28 month) post limbal stem cell transplantation with Holoclar. Both central and paracentral cornea sections were obtained to analyse the expression of the human keratinocyte marker, keratin 3 (K3), and the murine 3T3-J2 cells marker, H2KQ. Samples were also tested for the conjunctival marker K19 and goblet cells. Expression of these markers was determined by an indirect immunohistochemical assay system (UltraView Universal DAB Detection Kit) using specific antibodies to the different markers.

Analysis of stained sections of the tissue samples showed that the cells formed a normal multilayer, stratified squamous epithelium on a continuous extracellular matrix, comparable to healthy corneal tissue. In all patient tissues, K3 expression was positive and H2kQ expression negative. However, no epithelial cells were detected in the underlying corneal stroma, indicating lack of migration beyond the location of treatment. Samples also contained a number of K19-positive cells, but no goblet cells. Hence, it resembled a normal control corneal epithelium.

2.4.3. Pharmacodynamics

Mechanism of action

The proposed mechanism of action of Holoclar is the replacement of LSC in LSC deficient regions of the cornea subsequent to removal of the impaired corneal epithelium in patients in whom the limbus has been irreversibly and extensively damaged as a result of an ocular burn. By re-establishing a reservoir of stem cells, normal physiological growth and repair of the corneal epithelium is enabled. As a result, corneal epithelial integrity as well as long-term maintenance and regeneration of the epithelium is achieved and the limbal barrier function is restored preventing new ingrowth of the conjunctiva.

Primary and Secondary pharmacology

Conventional pharmacodynamic studies have not been performed. Due to the nature of the product and related administration procedure, studies in healthy patients would have been unethical, while monitoring of the pharmacodynamic response in patients would have required additional eye biopsy(ies). Such invasive approach was considered inappropriate given that other measures of clinical success were available and in light of the available evidence in the scientific literature. In particular, the functionality of Holoclar grafts is supported by the demonstration of clinical efficacy, as defined by a resolution of LSCD-associated symptoms as well as improved visual acuity (Rama et al., 2001; Rama et al., 2010; see also section 2.5.2. for details). The demonstration of restoration of visual function is in accordance with the Guideline On Human Cell-Based Medicinal Products (EMEA/CHMP/410869/2006), which states that if the intended use of the CBMP is, for example, to restore the function of deficient cells/tissue (tissue regeneration), functional tests should be implemented to demonstrate that function is restored.

The guideline on human CBMP furthermore recommends structural or histological assays as potential pharmacodynamic markers for regenerative medicines. Such data were available from impression cytology performed on a subset of patients in the two main retrospective studies HLSTM01 and HLSTM02 (for a more detailed description of these studies see section 2.5.2 of this report) at baseline and 12 months (HLSTM01), or at least 3 months (HLSTM02) after transplantation.

In HLSTM01, the mean percentage of cytokeratin 3 (K3) expressing cells (corneal phenotype, K3+) increased from 14.0% (n=13) at baseline to 57.0% (n=15) post-treatment. The mean percentage of cytokeratin 19 (K19) expressing cells (conjunctival phenotype, K19+) decreased from 73.2% (n=14) at baseline to 20.4% (n=15) post-treatment. A subgroup analysis was conducted in patients for whom data was available from both pre- and post-treatment time points (n=12 for K3+; n=13 for K19+). In this subgroup, the mean percentage of K3+ cells increased from 13.9% to 64.6%. The mean percentage of K19+ cells decreased from 73.5% to 19.7%. These changes were statistically significant ($p<0.00001$).

In study HLSTM02, pre-treatment data were available with respect to K3+ cells and K19+ cells in 20 and 23 patients, respectively. Post-treatment data were available for 11 patients (for both K3+ and K19+ cells). At month 3, the mean percentage of K3+ cells was greater (32.3% vs. 21.7% pre-treatment), and the mean percentage for K19+ cells was lower (21.4% vs. 37.3% pre-treatment). Patients with both pre- and post-treatment impression cytology data were considered in a subgroup analysis (n=9 for K3+; n=10 for K19+). In this subgroup analysis, the percentage of K3+ cells increased slightly from 32.3% to 33.9%, and the percentage of K19+ cells decreased from 25.8% to 18.5% ($p=0.865$ and $p=0.417$, respectively).

The guideline on human CBMP also indicates that suitable pharmacodynamic markers, such as defined by microscopic, histological, imagine techniques or enzymatic activities, could be used. The pharmacodynamic marker of p63 has been selected for characterisation and control of the medicinal product. The potency of Holoclar and therefore the anticipated pharmacodynamic effect was defined by the percentage of $p63^{bright}$ cells, which are undifferentiated holoclones and considered the 'functional' component of the drug substance. Determination of $p63^{bright}$ is used as a test for potency in the release specification (see section 2.2.3.).

Finally, histological examination of the engrafted corneal epithelium (see section 2.4.2. for details) removed at the time of keratoplasty in a small group of patients showed formation of a stratified squamous epithelium with a cuboid basal layer, devoid of goblet cells and papillary structures, resembling a normal cornea and lying on an avascular stroma. The regenerated epithelium was uniformly stained for K3 and contained a number of K19-positive cells, but did not contain goblet cells. Hence, it resembled a normal control corneal epithelium. All sections were negative for the murine marker H2KQ.

2.4.4. Discussion on clinical pharmacology

With regards to PK, the CAT agreed that classical absorption, distribution, metabolism and excretion studies were neither necessary nor relevant. Data from the K3 immunohistochemical analysis of histological sections of the human cornea obtained from patients treated with Holocar who had perforating keratoplasty, showed no invasion of keratinocytes into basal ocular structures. Hence, no evidence for dissemination of cells beyond the intended treatment area was found. Overall, given the autologous nature of the cells and the highly restricted growth environment requirements, the risk for migration beyond the ocular surface into deeper tissue or systemic exposure was considered low.

The CAT furthermore considered that the lack of conventional PD studies to demonstrate the mechanism of action of Holoclar was acceptable. The results of impression cytology in a subset of patients in the pivotal studies, showed an increase of the percentage of keratinocytes and a decrease of the percentage of conjunctival cells after Holoclar treatment, thus providing evidence that Holoclar enables corneal type epithelialisation of the ocular surface and exerts a regenerative effect. Moreover, results from immunohistological analysis showed the establishment of a normal layer of stratified corneal epithelium by the transplanted stem cells. Additional support for the functionality of Holoclar grafts, as provided by the demonstration of clinical efficacy, is discussed in section 2.5. of this report.

Finally, no evidence of murine feeder cells was found in histological examinations of surgically removed corneas several months after Holoclar treatment, providing further support to the low risk of microchimaera formation derived from the available non-clinical data.

2.4.5. Conclusions on clinical pharmacology

The CAT considered the lack of a conventional clinical pharmacology development programme acceptable. Supportive evidence for the regenerative mechanism of action of Holoclar as well as lack of invasion of graft cells beyond the ocular surface was available from immunohistological analyses and impression cytology.

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

2.5. Clinical efficacy

At the time of this application, more than 200 patients had already been treated with Holoclar (GPLSCD01) in clinical practice from 1998 onwards. The application is based on retrospective analyses of these data.

2.5.1. Dose response study(ies)

Conventional dose-response studies for Holoclar have not been conducted.

The final product consists of a confluent sheet of 300,000 to 1,200,000 viable autologous human corneal epithelial cells (79,000-316,000 cells/cm2), including on average 3,5% (0.4% to 10%) limbal stem cells. The size of the graft is related to the size of the patient's cornea and the exposed corneal stroma after surgical excision of the fibrovascular pannus.

Cytological characterisation during the manufacturing process is used to determine the potency of the product linked to the presence of human keratinocyte stem cells. Expression of p63 is used to distinguish LSCs from transiently amplifying cells. Deductively, potency is addressed by quantification of $p63^{bright}$ cells. Release specifications were set at 2.5-10.0% $p63^{bright}$ cells which has been shown to be significantly related to clinical success (see section 2.2.).

2.5.2. Main study(ies)

To support this application for a conditional marketing authorisation, the applicant submitted clinical data from two retrospective, multicentre, case series based, non-randomised, and uncontrolled observational studies:

- Pivotal study **HLSTM01** including 106 patients from 2 centres in Italy with the diagnosis limbal stem cell deficiency (LSCD) who underwent at least one Holoclar transplantation during the time period from 1998 to 2007, with data provided for 113 transplantation events, and
- Supportive study **HLSTM02** including 29 LSCD patients from 7 Italian centres with 29 transplantation events.

Study HLSTM01 aimed at evaluating efficacy and safety of Holoclar treatment, and HLSTM02 evaluated the safety of the product, with supporting evidence for efficacy. The primary difference between patients evaluated in study HLSTM01 and HLSTM02 was the specific clinical centres involved. In HLSTM01, patients were included from two related, yet distinct clinical sites which used a standard treatment protocol (pre-treatment assessments, limbal biopsy procedures, cellular expansion, treatment application and subsequent patient follow-up), whereas HLSTM02 encompassed all other available patient data treated at a total of seven other sites. The strategy behind this approach was to generate a sufficiently homogeneous patient population in study HLSTM01 to enable merging of individual patient information into a single composite data set for efficacy assessment, whereas patients in HLSTM02 reflect a more heterogeneous participant population.

Of the total of 219 patients treated with Holoclar from 1998 to 2007, 82 patients have not been included in the two retrospective studies HLSTM01 and HLSTM02 because centres declined to release patient data. The overall evaluable efficacy population therefore comprised 133 patients (104 in HLSTM01 and 29 in HLSTM02, respectively).

Furthermore, in response to a CAT/CHMP request, the applicant submitted data from additional 15 patients with moderate to severe LSCD due to ocular burn injury who have been treated from 2008 to 2013 at three Italian

sites (retrospective study HLSTM04). These 15 patients accounted for 100% of patients treated from 2008 onward.

2.5.2.1. Study HLSTM01

Study title: Retrospective evaluation of the efficacy and safety of autologous cultivated limbal stem cells transplantation for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns

Study HLSTM01 was performed based on data from two Italian centres in Milan and Rome. These two centres treated the majority of all patients that received Holoclar from 1998 to 2007. Both centres used the same procedures for Holoclar treatment, post-transplantation patient care, and associated patient follow-up using a standardised and consistent parameter set. The eligibility criteria of the protocol of study HLSTM01 were modelled to this treatment protocol.

Methods

Study Participants

The study population included patients transplanted with Holoclar because of LSCD due to ocular burns.

Patients enrolled in this trial had to fulfil all the following inclusion criteria:

- Males or females of all ages;
- Moderate or severe LSCD secondary to ocular burns, unilateral or bilateral with minimum 1-2mm^2 of undamaged limbus to harvest for stem cells expansion in culture. LSCD severity grading was based upon the extent of superficial corneal neovascularisation as observed upon clinical examination and categorized according to a 4-point grading scale.

Table 1 – Criteria for LSCD severity grading

Superficial corneal neovascularization		
Score	Clinical meaning	Clinical observation
0	None	No vessel penetration
1	Mild	Vessel penetration up to 1 quadrant, without central cornea involvement.
2	Moderate	Vessel penetration of 2-3 quadrants, with or without central cornea involved.
3	Severe	Total vascularisation of the cornea

Patients who fulfilled any of the following exclusion criteria were not enrolled in the study:

- Compromised eyelid mobility and/or symblepharon;
- Tear secretion deficiency (Schirmer test < 5 mm);
- Corneal and conjunctival anaesthesia;
- Unable to stop the topic treatment(s) for the pathology;
- Active local or systemic infections;

- Positive to HIV-1 or HIV-2 test;
- Diagnosis of local or systemic neoplastic disease;
- Limbar deficiency due to radiotherapy;
- Aniridia;
- Steven-Johnson syndrome;
- Active ocular inflammation;
- Pterygium or pseudopterygium;
- Neurotrophic keratitis.

Treatments

All the patients received the same treatment in form of transplantation of autologous cultured limbal stem cells (ACLSCT).

Visits

Patients might have had more than 1 pre-surgical visit prior to limbal biopsy. Additional visits included the following:

- Transplantation: day 0
- Post-transplantation visit 1-5 at day 3, 14, 25 ± 5, 90 ± 15 and day 180 ± 21
- Post-transplantation visit 6: day 360 ± 30 (endpoint visit). In case of failure the patient can be enrolled for a new ACLSCT at this visit.
- Additional visits could be performed in the following years (up to 10 years from transplantation) to follow-up the patients' clinical outcome. If the patient needed to be visited in the period between two study visits, the Investigator could perform an additional visit reporting the reason of the visit, the clinical condition, the possible adverse event and the treatment prescribed or used in the CRF section 'Emergency/Not Planned Form'.

Procedure for biopsy collection

1. Topical anesthesia with Oxybuprocaine chlorhydrate 0.4% (Novesina) or para/retrobulbar anestesia with Carbocaine 1% without adrenalin. Avoid to use topical Lidocaine or other topical anesthetic different from Oxybuprocaine;

2. Preparation of the surgical field as preferred;

3. Detachment of the conjunctiva to expose 2-3 mm of superior limbus;

4. Removal of superficial limbal tissue (square 2-3 mm, 80-100 mμ deep). Do not cauterize the area of the biopsy to prevent damage to the donor-stem cells;

5. Insert the biopsy (with attention to maintain the sterility) in the vial with the transport medium (received in advance from the Coordinating Centre); prepare the box for sending the biopsy to the Laboratory as indicated in the instructions send with the medium before the biopsy;

6. Suture the conjunctiva with nylon 10/0; bandage is not required;

7. Topical antibiotic three times daily (tid) for 7 days.

Procedure for transplantation

1. Para/retrobulbar anesthesia with Marcaine or Naropine or general anesthesia;

2. Preparation of the surgical field as preferred;

3. Limbal perithomy with accurate cauterization. The conjunctiva should be undermined for 3-4 mm in order to create a pocket for the insertion of the fibrin sheet;

4. Excision of the corneal fibrovascular tissue trying to avoid keratectomy;

5. Place the fibrin-cultured epithelial sheet on the corneal-sclera surface and fit it under the undermined conjunctiva;

6. Remove the surplus of the fibrin sheet and cover the periphery suturing the conjunctiva using vicryl 8/0;

7. Close carefully the eyelids over the grafted cells and keep them closed with steril-strip band;

8. A systemic + topical treatment was chosen to avoid the potential side effects of topical medicaments: topical treatments were allowed only in case of contraindication to systemic drugs. The systemic treatment chosen and the topical treatment suggested are described here below:

Systemic treatment+ topical treatment:

- prophylactic systemic antibiotics for two weeks: doxycycline 100 mg two times a day (bid) or amoxicillin 500 mg bid;
- systemic prednisolone or prednisone (0.5 mg/kg/day) for two weeks and then tapered until suspension at day 30; an anti-ulcer (i.e. ranitidine) may be used for gastroprotection during the treatment with systemic corticosteroid;
- topical preservative-free methylprednisolone 05% or dexamethasone 0.1% tid for two weeks, starting two weeks after transplantation, and tapered in two weeks (BID one week and 1 daily for 1 week) or maintained in case of persistent inflammation.

Topical treatment only:

- preservative-free topical antibiotics (as preferred) tid for one week or longer in case of persistent epithelial defect;
- topical preservative-free methylprednisolone 0.5% or dexamethasone 0.1% tid;
- for one month and then tapered or maintained in case of persistent inflammation.

9. Eye bandage suggested for two weeks. Other antibiotics or corticosteroids molecules might be used. In such a case the antibiotic or corticosteroid, the dosage regimen and the duration of treatment have to be reported.

Criteria for retreatment

In the case of transplantation failure, the patients were allowed to receive additional limbal stem cell transplantations. The criteria for admittance to a new procedure were:

1. failure of the previous transplantation at the endpoint visit;
2. absence of active ocular inflammation;

3. absence of active local or systemic infections;
4. negative to HIV-1 or HIV-2 test;;
5. patient consent.

In the case of additional limbal stem cell transplantation the patients were followed up with the same trial schedule applied to the patients who received one transplantation.

Objectives

The primary objective of the study was to evaluate the success of transplantation based on a stable corneal epithelium without significant recurrence of neovascularisation at 12 months post-intervention.

The secondary objectives of the study were:

- To evaluate symptoms (pain, burning, photophobia) at baseline and 12 months post-intervention.
- To evaluate the inflammation at baseline and at 12 months post-intervention.
- To evaluate the superficial corneal neovascularisation in blinded fashion (i.e., not knowing the patient and the visit in which the picture was taken) by an independent assessor.
- To evaluate the improvement of visual acuity at 12 months versus baseline.
- To evaluate the impression cytology: percentage of CK3+, CK3-, CK12+, CK12-, CK19+, CK19-, presence of caliciform cells.
- To evaluate the number of previous limbal stem cell transplantations in each patient.
- To evaluate the number of successful keratoplasties after limbal stem cell transplantation.
- As part of the safety evaluation, any corneal material from patients undergoing keratoplasty was processed for histology and evaluated morphologically for abnormal differentiation, proliferation and cell transformation (also with specific markers).

Outcomes / endpoints

The **primary efficacy endpoint** of the study was a composite endpoint of the rate of patients with a successful transplantation at 12 months post-intervention, based on the co-presence of clinical signs as follows: (i) a superficial corneal neovascularisation classified as ‘None’ (no vessel penetration) or ‘Mild’ (vessel penetration 1 quadrant without central cornea involved), and (ii) epithelial defects classified as ‘None’ (no fluorescence staining) or ‘Trace’ (minimal superficial staining, pooling with light and/or late staining).

The success of the treatment was evaluated based on the assumption that a percentage of a positive outcome in more than 50% of transplantations is the minimal effect of clinical relevance in the management of patients.

Secondary endpoints:

- Change in symptoms (pain, burning, photophobia) from baseline to 12 months post-intervention. Symptoms were graded with categorical scales (0= None; 1=Mild; 2=Moderate; 3=Severe).
- Change in inflammation from baseline to 12 months post intervention. The assessment will be conducted using categorical scales for limbal and bulbar conjunctival hyperaemia. Inflammation yes: Limbal and/or bulbar hyperaemia= Mild or Moderate or Severe (Severe Limbal Hyperaemia). Inflammation no: Limbal and bulbar hyperaemia= None.

- Superficial corneal neovascularisation evaluated in blinded fashion by an independent assessor on photos of patients' eyes taken before and after the transplantation. The assessor will use the same categorical scale for superficial corneal neo-vascularisation used by the investigator (see primary efficacy variable).
- Change in visual activity from baseline to 12 months post-intervention. Improvement of visual acuity at 12 months versus baseline will be evaluated. Visual acuity will be measured as both natural and best refracted using the Snellen chart and values will be expressed according to tenth scale. Visual acuity lower than 1/20 will be evaluated as light perception or hand movements or finger count (from light perception to hand movements or from hand movements to finger count).
- Number of limbal stem cell transplantations in each patient.
- Number of successfull keratoplasties after limbal stem cell transplantation. The success was assessed based on the same criteria used to determine success of the limbal cell transplantation (primary endpoint), i.e. persisting success of limbal cell transplantation after keratoplasty, at the first visit at least 12 months after cornea transplantation.
- Evaluation of impression cytology: percentage of K3+, K3-, K12+, K12-, K19+, K19-, presence of caliciform cells

Sample size

The sample size was not based on any power calculation. All patients transplanted at the 2 Italian centres from 1998 to 2007 were included.

Randomisation

The study was uncontrolled and non-randomised.

Blinding (masking)

To provide an objective mechanism for assessment of efficacy of Holoclar, a retrospective blinded analysis of ocular photographs (before and after treatment) was performed by an independent investigator to confirm the results for corneal neovascularisation.

Statistical methods

The following populations were to be considered in data analysis:

- Intent-to-treat (ITT) population: all patients who underwent the cultivated limbal stem cells transplantation and had a control visit at least six months after transplantation. For patients who received two or more transplantations only the last documented one was considered for efficacy analysis. The demography and baseline characteristics and the analyses of efficacy were performed on the ITT population.
- Per Protocol (PP) population: all patients from the ITT population without any major protocol violations. Exact definition of major protocol deviation was discussed by the clinical team, case by case, before database lock. Primary efficacy analysis and analysis of the superficial neovascularisation evaluated on photos were performed also on the PP population.

- Safety population: all patients who underwent the cultivated limbal stem cells transplantation. Subjects who received two (or three) interventions were counted twice (or three times) and each intervention was considered independently. The safety analysis were performed on the safety population.

Protocol violations

Violations considered as potentially affecting the efficacy outcomes (e.g. significant violation from inclusion/exclusion criteria or absence of baseline measurement of the primary efficacy variable) were considered as major violations and led to the exclusion from the PP population. In the case of minor protocol violations that did not have a potential influence on efficacy outcomes, subjects were regularly included in the PP analysis.

Analysis of efficacy:

The analysis of the primary efficacy variable and of superficial neovascularisation evaluated on photos was carried out both on the ITT and PP population.

Primary variable:

An exact one-sided binomial test with 0.025 significance level was performed for the proportion of successful transplantations in order to test the null hypothesis H_0: $\pi \leq 50\%$ and therefore to check the success of the study. The 95% confidence interval (CI) for the estimated proportion of successful transplantations was to be calculated. The study was to be considered successfully if the lower bound of the 95% CI for the estimated proportion of successful transplantations was higher than 50%.

Secondary efficacy variables:

Comparison between proportions at baseline and at 12 months were be performed by means of McNemar's test for relevant endpoints.

The estimated proportion of patients with neovascularisation at baseline and 12 months post intervention, as evaluated in blind fashion by an independent assessor, was to be compared with that obtained from the evaluation of neo-vascularisation conducted by the investigator during the visit of the patient by means of the Cohen's kappa.

The number and percentage of patients with an improvement in visual acuity (VA) of at least one line was to be calculated. This analysis was to be conducted in two subsets of patients: the group without deep corneal opacity (no stromal scarring) and the group with deep corneal opacity (stromal scarring). Visual acuity lower than 1/20 was to be evaluated as light perception or hand movements or finger count. Visual acuity was considered improved if i) an increase by at least one line read without mistakes on the Snellen chart compared to baseline, or ii) a categorical change from light perception (LP) to hand movements (HM) or from HM to finger count (FC), or iii) 1/10 or 1/20 in best refracted conditions could be observed.

Handling of missing data

The following method was applied for the primary efficacy variable (successful transplantations) and for the superficial neo-vascularisation evaluated on photos: Zero imputation: In case of missing data for the endpoint visit the result of transplantation is considered a 'Failure'.

No replacement for missing data were to be performed for the other secondary efficacy variables.

Results

Recruitment

A total of 106 patients at the 2 selected centres in Rome (14, 100% of patients treated between 1998 and 2010) and Milan (92, 100% of patients treated between 1998 and 2010) underwent at least one ACLSC graft and were included in this study.

Conduct of the study

There were no amended versions of the protocol. However, a number of changes to the planned analyses were defined in the final statistical analysis plan dated 03/08/2010:

- Secondary endpoint definition:
    - The definition of 'Inflammation no' was changed in the sense that neither limbal nor bulbar hyperaemia should have been present;
    - In the definition of 'Failure of transplantation' the case that either superficial corneal neovascularization was 'moderate' or 'severe' or epithelial defects by fluorescein staining was 'dense coalescent staining up to 2 mm in diameter' (coded as 'mild' in the case report forms) or 'severe' was included;
- Statistical analysis:
    - The primary hypothesis was changed from a two-sided test to a one-sided test in order to cover the medical requirements;
    - The safety evaluation of any corneal material from patients undergoing keratoplasty regarding abnormal differentiation, proliferation, and cell transformation was not documented in the case report form by the investigators and therefore could not be evaluated;
    - The sensitivity analysis on the primary efficacy variable with the next observation carried backward technique was not performed;
- Inclusion criteria were clarified (due to inconsistencies/typing errors in the documentation):
    - confirmation that a moderate or severe LSCD was considered as inclusion criterion;
    - confirmation that the categorisation of the grade of severity of LSCD was based on corneal neovascularisation;
    - confirmation that a moderate degree of superficial corneal neovascularisation was defined as vessel penetration 2-3 quadrants (from 3 to 9 hours o'clock) without or with central cornea involved;
    - confirmation that the epithelial defects that defined a successful transplantation were: None (No staining) or Trace (Minimal superficial staining, pooling), whereas the defects that defined a failure of transplantations were: Mild (Dense coalescent staining up to 2 mm in diameter) or Severe (Dense coalescent staining greater than 2 mm in diameter).

Baseline data

The mean age was 46.8 ± 14.4 years (range 13.7-79.1). The study population included 80 males (76.9%) and 24 females (23.1%). Only limited data were available on patients younger than 18 years of age (3) and older than 65 years of age (7).

Table 2 - Demographic Profile of the Patient Population

		HLSTM01 (n=104) n (%)	HLSTM02 (n=29)[1)] n (%)	Total (n=133)[1)] n (%)
Age (years)	Mean (± SD)	46.8 ± 14.4	45.8 ± 17.4	46.1 ± 15.0
	Median	49.2	43.5	
Groups	Range	13.7 – 79.1	8.0 – 71.0	8 – 79
	< 18	3 (2.9%)	2 (7.1%)	5 (3.8%)
	18 – 39	31 (29.8%)	10 (35.7%)	41 (31.1%)
	40 – 64	63 (60.6%)	9 (32.1%)	72 (54.5%)
	65 – 75	5 (4.8%)	7 (25.0%)	12 (9.1%)
	> 75	2 (1.9%)	0 (0%)	2 (1.5%)
Gender	Female	24 (23.1%)	7 (24.1%)	31 (23.3%)
	Male	80 (76.9%)	22 (75.9%)	102 (76.7%)

1): Age data was available for 28 patients in HLSTM02 and 132 patients in total

Data regarding ethnicity of patients were not collected. Patients were assumed to be Caucasian of Italian descent.

Study HLSTM01 included patients with uni- or bilateral moderate to severe LSCD secondary to ocular burns. The diagnosis of LSCD was based on medical history (i.e. ocular burn injury) and clinical signs, in particular corneal neovascularisation (CNV) and corneal opacity. Patients presented pre-treatment with either moderate (55.8%) or severe (43.3%) superficial corneal neovascularisation (information missing in 1 case). About one third of patients had no epithelial defects at baseline as assessed by fluorescein staining, reflecting stabilisation of the ocular surface condition (e.g. pannus formation). Based on these clinical parameters, 99% of the patients were diagnosed with moderate to severe LSCD.

Deep stromal vascularisation was involved in 87.5% of the patients, and 90% had a severe loss in visual acuity. Information about corneal opacity and depth of corneal injury was recorded and used to classify two subsets of patients: i) those with stromal scarring (deep corneal opacity) and ii) those without stromal scarring (deep corneal opacity absent or superficial). Stromal scarring was present in 89 cases (86.4%), absent in 13 cases (12.6%), and information was missing in one case.

Table 3 – Outcome of examination of the involved eye at pre-surgery visit for HLSTM01 and HLSTM02

	HLSTM01 (ITT; n=104)	**HLSTM02 (n=29)**
Superficial corneal neovascularization:		
• None	0 (0%)	2 (6.9%)
• Mild	0 (0%)	5 (17.2%)
• **Moderate**	**58 (55.8%)**	**10 (34.5%)**
• **Severe**	**45 (43.3%)**	**12 (41.4%)**
• Missing information	1 (0.96%)	0 (0%)
Epithelial defects (fluorescein staining):		
• None	36 (34.6%)	9 (31.0%)
• Minimal superficial staining, pooling (trace defect)	57 (54.8%)	
• Dense coalescent staining up to 2 mm in diameter (mild)	6 (5.8%)	
• Severe (dense coalescent staining > 2 mm in diameter)	5 (4.8%)	
Epithelial defects:		17 (58.6%)
< 2 mm		1 (3.5%)
> 2 mm		1 (3.5%)
Occasional		2 (6.9%)
Recurrent		5 (17.2%)
Persistent		4 (13.8%)
< 2 mm + Recurrent		1 (3.5%)
< 2 mm + Persistent		1 (3.5%)
> 2 mm + Persistent		2 (6.9%)
Not available		3 (10.3%)
Corneal opacity:		
• None	0 (0%)	2 (6.9%)
• Superficial	12 (11.5%)	7 (24.1%)
• Deep	13 (12.5%)	8 (27.6%)
• Superficial & deep	78 (75.0%)	12 (41.4%)
• Missing information	1 (0.96%)	0 (0%)
Pain:		
• No	94 (90.4%)	21 (72.4%)
• Yes	7 (6.7%)	6 (20.7%)
- Mild	7 (6.7%)	
• Missing information	3 (2.9%)	2 (6.9%)
Burning:		
• No	71 (68.3%)	17 (58.6%)
• Yes	30 (28.8%)	10 (34.5%)
- Mild	19 (18.3%)	
- Moderate	11 (10.6%)	
• Missing information	3 (2.9%)	2 (6.9%)

	HLSTM01 (ITT; n=104)	HLSTM02 (n=29)
Photophobia:		
• No	66 (63.5%)	11 (37.9%)
• Yes	35 (33.7%)	15 (51.7%)
- Mild	20 (19.2%)	
- Moderate	14 (13.5%)	
- Severe	1 (0.96%)	
• Missing information	3 (2.9%)	3 (10.3%)
Inflammation: Limbal hyperaemia		
• No	78 (75.0%)	17 (58.6%)
• Mild	23 (22.1%)	7 (24.1%)
• Significant	3 (2.9%)	4 (13.8%)
• Severe		1 (3.5%)
Inflammation: Bulbar hyperaemia		
• No	72 (69.2%)	15 (51.7%)
• Slight diffuse	29 (27.9%)	8 (27.6%)
• Marked regional or diffuse	3 (2.9%)	4 (13.8%)
• Diffuse episcleral or scleral		2 (6.9%)
Corneal sensibility:		
• Normal	41 (39.4%)	
• Hypaesthesia	59 (56.7%)	
• Anaesthesia	4 (3.8%)	
Visus:		
• Finger count	44 (42.3%)	8 (27.6%)
• Hand movements	37 (35.6%)	4 (13.8%)
• Light perception	11 (10.6%)	4 (13.8%)
• Natural visus	9 (8.6%)	8 (27.6%)
• BCVA[1)]	10 (9.6%)	6 (20.7%)
Natural visus:		
• 0.5/10	1 (0.96%)	
• 1.5/10	1 (0.96%)	
• 2/10	5 (4.8%)	
• 4/10	1 (0.96%)	
• 5/10	1 (0.96%)	
Best Corrected Visual Acuity (BCVA):		
• 0.5/10	1 (0.96%)	
• 1/10	2 (1.92%)	
• 2/10	2 (1.92%)	
• 3/10	1 (0.96%)	
• 5/10	1 (0.96%)	
• 6/10	1 (0.96%)	
• 7/10	2 (1.92%)	

	HLSTM01 (ITT; n=104)	HLSTM02 (n=29)
CA examination:		
• Not executable	48 (46.2%)	6 (20.7%)
• Executable	55 (52.9%)	22 (75.9%)
• Missing information	1 (0.96%)	1 (3.4%)
Tonometry:		
• Executable	104 (100.0%)	21 (72.4%)
Conjunctiva:		
• Fornix regular	84 (80.8%)	24 (82.8%)
• Fornix shortening	13 (12.5%)	3 (10.3%)
• Scarring	7 (6.73%)	1 (3.5%)
• Missing information		1 (3.5%)
Eyelid margins:		
• Normal position	89 (85.6%)	28 (96.6%)
• Malposition	15 (14.4%)	1 (3.5%)
Symblepharon:		
• No	104 (100.0%)	19 (65.5%)
• Yes		7 (24.1%)
• Missing information		3 (10.3%)
Eye photo attached:		
• No	29 (27.9%)	
• Yes	75 (72.1%)	

The majority of patients (101, 97.1%) had a diagnosis of LSCD due to chemical or physical ocular burns which is the claimed target indication for Holoclar. Three patients were treated for LSCD due to one or more of the following:

- contact lens-associated damage (1 patient),
- ocular infections (2 patients),
- toxic pathology associated with medical drugs (1 patient), and
- post-surgery iatrogenic pathology (1 patient).

Information regarding the nature of the chemical burn (i.e., acid or alkali) had not been collected retrospectively, but some preliminary data have been published showing that 80% of the thermal/ chemical injuries leading to LSCD were alkali burns (Rama 2010).

The vast majority of patients experienced unilateral injuries; only one patients was treated in both eye with a gap of 3 years between surgeries.

The time span from burn injury to Holoclar treatment was very wide, ranging from a few months to more than seven decades (see Table 4).

Table 4 – Time elapsed since injury

Time since injury in months (years)	HLSTM01 (n=104)	HLSTM02 (n=29)
Mean	220.7 (18.4 yrs)	169.1 (14.1 yrs)
Median	123.0 (10.3 yrs)	107.5 (8.9 yrs)
SD	206.7 (17.2 yrs)	178.5 (14.9 yrs)
Range	7.0 - 866.0 (0.6 - 72.2 yrs)	10.0 - 600.0 (0.8 - 50 yrs)
Missing	1	1

Keratoplasty (40 patients, 38.5%), corneal transplant (39 patients, 37.5%) and conjunctival repair (30 patients, 28.8%) were the most common surgical procedures conducted in the involved eye prior to Holoclar treatment. Previous medications were reported for 96 patients (92.3%), while all 104 patients had concomitant medications. Sensory organs drugs were the most common previous medication (96 patients, 92.3%): ofloxacin was the most common active drug (87 patients, 83.7%). Among the concomitant medications, sensory organs (96 patients, 92.3%), anti-infectives for systemic use (91 patients, 87.5%) and anti-inflammatory agents (88 patients, 84.6%) were the most common drug classes. Dexamethasone was the most common active drug (79 patients, 76.0%).

Numbers analysed

A total of 106 patients underwent at least one ACLSC graft and were included in this study. Of these, 94 underwent only one graft procedure, 11 underwent two grafts, and one underwent three procedures, for a total of 119 ACLSCT performed. One patient had ACLSCT in both the left and the right eye (3 years after the left eye). Source data of 6 grafts were not available at the investigator sites (5 first transplantations and 1 second transplantation), and therefore they were not included in the study. As a result, a total of 113 transplantation cases were evaluated in this study. Of the 113 transplantation cases with recorded data, 106 had a complete post-transplantation visit 6 (Day 360 or Month 12).

All 113 cases received the transplantation and, therefore, were included in the safety population (including 7 cases with available records of repeated transplantations, for a final number of 106 patients).

A total of 104 patients were included in the ITT population, i.e. underwent the ACLSCT and had a control visit at least 6 months after transplantation.

Five (5) patients in the ITT population had major protocol violations and were excluded from the PP population, which thus included 99 patients. Major protocol violations consisted of active ocular inflammation in 3 patients, tear secretion deficiency in 1 patient and visit 6 outside of the specific range in 1 patient.

Outcomes and estimation

- **Primary endpoint: Success of transplantation**

Using a zero imputation method for handling missing values, treatment success, based on the composite efficacy endpoint of reduction in CNV and epithelial defects, was reported in 75 patients (72.1%) and failures were reported in 29 patients (27.9%), with an overall 95% confidence interval (CI) for success of 62.5 - 80.5% and a p-value<0.001. A sensitivity analysis performed for the observed cases only (i.e. without missing cases, n=99) confirmed these results. Furthermore, when analysing only patients with LSCD due to ocular burns, a success rate of 74.5% was observed, which is consistent with the ITT population. For patients with repeated Holoclar treatment, 91.7% of cases had a successful clinical outcome.

Table 5 – Primary efficacy analysis: successful transplantation at 12 months post-intervention (ITT and PP population)

	ITT population (N=104)	PP population (N=99)
Success	75 (72.1%)	72 (72.7%)
Failure	29 (27.9%)	27 (27.3%)
Exact one-sided binomial test 2.5% significance level:		
Proportion of patients with successful transplantation	72.1%	72.7%
95% Exact Confidence Interval	62.5%-80.5%	62.9%-81.2%

Table 6 and Table 7 depict the outcome of the evaluation for corneal neovascularisation (CNV) and epithelial defects, respectively, at baseline and month 12.

Table 6 – Superficial Corneal Neovascularisation at Baseline versus Month 12 (ITT)

Corneal Neovascularisation at Baseline	**Corneal Neovascularisation at Month 12**					
	None	Mild	Moderate	Severe	Missing	**Total**
Moderate	15 (14.4%)	33 (31.7%)	5 (4.8%)	1 (1.0%)	4 (3.8%)	**58 (55.8%)**
Severe	3 (2.9%)	25 (24.0%)	9 (8.7%)	7 (6.7%)	1 (1.0%)	**45 (43.3%)**
Missing	0	0	0	0	1 (1.0%)	**1 (1.0%)**
Total	**18 (17.3%)**	**58 (55.7%)**	**14 (13.5%)**	**8 (7.7%)**	**6 (5.8%)**	**104 (100%)**

Seventy-six (73.1%) of the 104 patients showed an improvement from moderate or severe CNV at baseline to mild or none CNV at month 12 post-transplantation, and thus fulfilled the definition of success for this component of the primary efficacy endpoint.

Table 7 – Epithelial Defect at Baseline versus Month 12 (ITT)

Epithelial Defect at Baseline	**Epithelial Defect at Month 12**					
	None	Trace	Mild	Severe	Missing	**Total**
None	33 (31.7%)	2 (1.9%)	1 (1.0%)	0	0	**36 (34.6%)**
Trace	33 (31.7%)	19 (18.3%)	0	2 (1.9%)	3 (2.9%)	**57 (54.8%)**
Mild	2 (1.9%)	1 (1.0%)	1 (1.0%)	1 (1.0%)	1 (1.0%)	**6 (5.8%)**
Severe	2 (1.9%)	1 (1.0%)	1 (1.0%)	0	1 (1.0%)	**5 (4.8%)**
Missing	0	0	0	0	0	**0**
Total	**70 (67.3%)**	**23 (22.1%)**	**3 (2.9%)**	**3 (2.9%)**	**5 (4.8%)**	**104 (100%)**

In the majority of patients (n=87; 83.6%), epithelial defects had remained as “none” or “trace” at 12 month post transplantation. In 6 patients (5.8%) with mild epithelial defects (dense coalescent fluorescein staining

<2 mm) or severe EDs (dense coalescent fluorescein staining ≥2 mm) at baseline, the condition had improved to “none” or “trace” at month 12.
Sixteen (16) patients treated with Holoclar who had no or only trace epithelial defects at baseline developed severe epithelial defects in the weeks following ACLSCT. This post-ACLSCT deterioration of corneal epithelial integrity was detected within the first month post treatment, i.e. at Visit 2 (Day 14) in 75% and at Visit 3 (Day 25) in 25%, respectively. In all but one case, the epithelial defects had resolved by one year post-ACLSCT. The occurrence of early severe corneal epithelial defects was usually associated with treatment failure. In 9 (56.3%) of the 16 cases, the outcome of ACLSCT was deemed a failure at the Month 12 efficacy endpoint assessment, and in 6 (37.5%) of the 16 patients, the outcome was judged successful, with information lacking for 1 case (6.25%).

- **Secondary endpoints:**

Symptoms

The number of patients with ocular symptoms significantly ($p<0.001$) decreased from the pre-surgical visit (40 patients; 38.5%) to the endpoint visit at 12 months post transplantation (12 patients; 11.5%).

Table 8 – Ocular Symptoms: Pain, Burning, Photophobia at Baseline and Month 12 (ITT)

	Pre-surgical visit			
Visit 6 (Month 12)	No[1)]	Yes[2)]	Missing	Total
No[1)]	52 (50.0%)	30 (28.8%)	3 (2.88%)	85 (81.7%)
Yes[2)]	4 (3.85%)	8 (7.69%)	0 (0%)	12 (11.5%)
Missing	5 (4.81%)	2 (1.92%)	0 (0%)	7 (6.73%)
Total	61 (58.7%)	40 (38.5%)	3 (2.88%)	104 (100.0%)

1): No symptoms (all 3 categories)

2): Mild, moderate or severe symptoms in at least one category (pain, burning, or photophobia)

Improvements were observed with respect to reductions in the intensity of ocular pain, burning, and photophobia. There were no reports of moderate or severe ocular pain at baseline. There were no reports of ocular pain after Holoclar transplantation.

Table 9 – Ocular Symptoms by Severity (ITT)

		Pre-surgical visit	**Visit 6 (month 12)**
Pain	No	94 (90.4%)	97 (93.3%)
	Mild	7 (6.7%)	-
	Moderate	0 (0%)	-
	Missing information	3 (2.9%)	7 (6.7%)
Burning	No	71 (68.3%)	89 (85.6%)
	Mild	19 (18.3%)	7 (6.7%)
	Moderate	11 (10.6%)	-
	Missing information	3 (2.9%)	8 (7.7%)
Photophobia	No	66 (63.5%)	89 (85.6%)
	Mild	20 (19.2%)	8 (7.7%)
	Moderate	14 (13.5%)	-

		Pre-surgical visit	Visit 6 (month 12)
	Severe	1 (< 1%)	-
	Missing information	3 (2.9%)	7 (6.7%)

Inflammation

The number of patients with inflammation did not change from the pre-surgical visit (32 patients; 30.8%) to the endpoint visit at 12 months post-treatment (33 patients; 31.7%) (p=0.732).

Two weeks after Holoclar transplantation, 3 patients without limbal hyperaemia at baseline showed moderate (n=2) or severe (n=1) limbal hyperaemia. The number increased to 4 patients with moderate and 1 patient with severe limbal hyperaemia 1 month post-transplantation.

In addition, 2 weeks after Holoclar transplantation, 6 patients without bulbar hyperaemia at baseline showed moderate (n=5) or severe (n=1) bulbar hyperaemia. One month post-transplantation, 4 patients had moderate, and 1 patient had severe bulbar hyperaemia, respectively.

The condition improved in all patients to no or only mild hyperaemia from Month 3 onwards.

Superficial corneal neovascularisation (CNV) based on photographic evidence evaluated by an independent assessor

Photographs from before and/or after treatment were available for 91 patients. The proportion of patients presenting any degree of superficial CNV decreased significantly (p<0.001) from baseline (93.8%; 95% CI: 88.6-99.1%) to 12 months after treatment (63.9%; 95% CI: 52.8-75.0%).

When testing the agreement between Investigator's evaluation and assessor's evaluation of photos regarding the grade of neovascularisation at the pre-surgical visit and at visit 6, several discrepancies between the clinical and photographic assessment were noted. At the endpoint visit, the Cohen's kappa coefficient was representative of a moderate agreement between the investigator's evaluation and assessor's evaluation of photographs regarding the grade of neovascularization (0.639; 95% CI: 0.497-0.781) across the four point scale for neovascularization. However, a sensitivity analysis for the composite primary endpoint performed in 46 patients with both baseline and month 12 photographic assessment showed a good consistency between the two assessments: Following photographic assessment, 31 out of the 46 cases (67.4%) were considered a treatment success compared to 75 patients out of 104 (72.1%) in the overall ITT population, resulting in a difference of approximately 5% for the point estimate.

Visual activity (VA)

An improvement of visual acuity was noted in 51 (49%) of the 104 patients. The proportion of patients with VA improvement was higher among those without stromal scarring (15 out of 18 patients, 83.3%) compared to those with stromal scarring (36 out of 81 patients, 44.4%).

Table 10 – Proportion of Patients with an Improvement of at least 1 Line in VA (ITT)

	Patients without stromal scarring N = 18	Patients with stromal scarring N = 81	All patients N = 104
Improvement in visual acuity			
No	2 (11.1%)	44 (54.3%)	46 (44.2%)
Yes	15 (83.3%)	36 (44.4%)	51 (49.0%)
Missing	1 (5.6%)	1 (1.2%)	7 (6.7%)
Proportion of patients with an improvement of at least one line	83.3%	44.4%	49.0%
95% Confidence Interval	66.1%-100.0%	33.6%-55.3%	39.4%-58.6%

Upon request of the CAT/CHMP, additional analyses for the change in VA were provided:

- Patients with off-chart vision at baseline (irrespective of deep stromal scarring)

A total of 92 patients out of 99 with baseline data (92.9%) had a pre-treatment VA below the limit measurable at the Snellen chart (off-chart). Of these, 47 improved after treatment according to the definition used in the secondary study endpoint, corresponding to 51.1% of all cases with not-measurable VA (including missing values). Moreover, 17 patients (16.4% including missing values) experienced a gain in vision sufficiently large to reach on-chart vision.

- Change of VA from baseline to month 12

To better quantify the relative change in VA from baseline to Visit 6 (month 12), the number of lines of VA improvement on the Snellen chart was calculated. In order to quantify the VA of patients with non-measurable VA, their categorical value was transformed in LogMAR values (Finger Count = 1.9, Hand Movement = 2.3, and Light Perception = 2.7). A large proportion of patients experienced a VA improvement after treatment and in 40 patients (38.5% including missing values) this improvement was more than 3 line equivalents.

- Impact of keratoplasty on VA outcome

A total of 5 patients had keratoplasty prior to the 12 months endpoint of the study. Of the 99 patients without cornea transplantation by month 12, 15 patients experienced a vision improvement from off-chart to on-chart vision (15.5% including missing values), which is in line with the findings in the ITT population.

Furthermore, in the group of 56 patients with at least one keratoplasty after the one-year follow-up visit, 32 patients (57.1% including missing values) had at least one line improvement in VA after the first keratoplasty while 18 patients did not improve (14 were stable and only 4 had a worsening in VA). Changes of 3 lines/categories or more were observed in 21 cases (corresponding to 65.6% of improvers and 37.5% of the overall group including missing values). In 6 patients, there was not sufficient data to assess change in VA.

Number of previous limbal stem cell transplantations

Of the 104 patients in the ITT population, 14 (13.5%) patients had one prior limbal cell transplantation and 2 (1.9%) patients had 2 previous limbal cell transplantation.

Number of successful keratoplasties after limbal stem cell transplantation

Overall, 57 patients underwent keratoplasty subsequent to Holoclar graft. Successful keratoplasty following Holoclar treatment was achieved in 24 patients (42.1%). In 13 (50%) of 26 patients, who had at least one previously failed keratoplasty, cornea transplantation subsequent to Holoclar transplantation was successful.

Table 11 – Proportion of Patients with Successful Keratoplasty after Holoclar treatment (ITT)

	ACLSCT N = 104 Failed keratoplasty before limbal cell transplantation					
	0	1	2	3	At least one	Any (zero included)
After limbal cell transplantation						
No. of pts who underwent at least one post-graft keratoplasty	31	17	6	3	26	57
No. of pts with at least one successful post-graft keratoplasty	11	7	5	1	13	24
Proportion of pts with at least one successful post-graft keratoplasty	35.5%	41.2%	83.3%	33.3%	50.0%	42.1%
95% Confidence Interval	18.6%-52.3%	17.8%-64.6%	53.5%-100.0%	0.00%-86.7%	30.8%-69.2%	29.3%-54.9%

Additional analyses were provided for the outcome of keratoplasty stratified by initial success of the ACLSCT (see Table 12).

Table 12 - Clinical Outcome of the First Keratoplasty Stratified by Initial Success of the ACLSCT Procedure

Keratoplasty outcome *Number* *% of total* *% of total (without missing)*	**ACLSCT outcome**		*Total*
	Success	**Failure**	
Success	**26** **(45.61 %)** **(72.22 %)**	**2** **(3.51 %)** **(5.56 %)**	*28* *(49.12 %)* *(77.78 %)*
Failure	4 (7.02 %) (11.11 %)	4 (7.02 %) (11.11 %)	*8* *(14.04 %)* *(22.22 %)*
Missing	17 (29.82 %) -	4 (7.02 %) -	*21* *(36.84 %)* -
Total	*47* *(82.46 %)* *(83.33 %)*	*10* *(17.54 %)* *(16.67 %)*	*57* *(100.00 %)* *(100.00 %)*

Analysis was done for all patients with available information on ACLSCT outcome and with at least one keratoplasty performed after Visit 6 (N=57). Keratoplasty outcome was assessed at the first follow-up visit with valid data occurred at least 6 months after the first keratoplasty executed after Visit 6. Keratoplasty was considered a success in presence of corneal neovascularization "none" OR "mild" AND epithelial defects "none" OR "trace" (as for HLSTM01 primary efficacy end-point). Data presented in this table are based on Table Q129-4 at the end of this document.

-: Not Applicable; ACLSCT: Autologous Cultivated Limbal Stem Cells Transplantation. **Bold text highlighted in grey**: positive keratoplasty outcome.

Evaluation of impression cytology

Relevant results are presented in section 2.4. of this report.

Ancillary analyses

Time since burn injury

Of the 101 patients with LSCD due to ocular burns, a total of 87 patients received Holoclar treatment ≥ 5 years after the injury occurred, while 14 patients had the injury within less than 5 years prior to ACLSCT. Upon request of the CHMP/CAT, the applicant analysed treatment success by time elapsed since burn injury. The results of this analysis showed a transplantation success rate of 75% or more in all patient groups that had the injury ≥ 5 years before Holoclar transplantation (5-10 years, 10-20 years, 20-30 years and > 30 years) and 50% in patients who had the ACLSCT less than 5 years prior to Holoclar treatment.

Follow-up and long-term efficacy

Patients in study HLSTM01 were followed for up to 10 years. Most treatment cases were followed for 1 to 2 years (28.3 %) and 2 to 3 years (22.1%), respectively. Fourteen treatments (12.5%) were followed for 5 years or more.

The follow-up time post transplantation was on average 3.6 years (median: 3.0 years), ranging from 1.4 to 9.9 years. Data were provided for the time points 2 and 3 years after Holoclar transplantation, and for the last available follow-up visit. At each of these time points, the transplantation success rate according to the primary endpoint was around 60-70% [37 of 60 patients (68.5%) with a visit at year 2; 22 of 41 patients (71.0%) with a visit at year 3; 46 of 89 patients (66.7%) at respective last available follow-up].

Treatment success, starting from year 1 post-transplantation, reached a plateau of 75% until year 5. After year 5, only 5 patients had long term follow-up, of which 4 were reported as continued treatment success. However, several patients underwent subsequent keratoplasty within the time period of 2 or 3 years after Holoclar transplantation.

Clinical studies in special populations

Due to the low number of paediatric patients and older patients, data are presented for both HLSTM01 and 02 (see section 2.5.2.2.).

Summary of main study(ies)

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

Table 13 – Summary of efficacy for trial HLSTM01

Title: **Retrospective evaluation of the efficacy and safety of autologous cultivated limbal stem cells transplantation for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns**			
Study identifier	HLSTM01		
Design	Retrospective, non-randomised, uncontrolled, case series-based observational study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 months not applicable not applicable
Hypothesis	Exploratory: Proportion of patients with successful transplantation is at least 50% (H_0: $\pi \leq 50\%$)		
Treatments group	Holoclar		ACLSCT with Holoclar, 106 patients
Endpoints and definitions	Primary endpoint	ACLSCT success	Composite endpoint: Presence of a stable corneal epithelium without significant recurrence of superficial corneal neovascularisation, defined as no or only trace epithelial defects AND no or only mild superficial corneal neovascularisation, at 12 months after Holoclar transplantation.
	Secondary endpoint 1	Symptoms	Presence/degree of ocular symptoms (pain, burning, photophobia) at 12 months after Holoclar transplantation.
	Secondary endpoint 2	Inflammation	Presence/degree of inflammation (limbal and bulbar conjunctival hyperaemia) at 12 months after Holoclar transplantation.
	Secondary endpoint 3	VA improvement	Improvement of visual acuity (VA) by at least one line read without mistakes on the Snellen chart (or change of category in case of off-chart vision) at 12 months after Holoclar transplantation.
	Secondary endpoint 4	Keratoplasty success	Number of successful keratoplasties following ACLSCT, at 6 months after cornea transplantation.
Database lock	06/08/2010 (Study data were collected over the period of 1998 – 2010)		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat (ITT): 104 patients Primary endpoint and secondary endpoints 1-3: 12 months after ACLSCT Secondary endpoint 4: 6 months after keratoplasty		
Descriptive statistics and estimate	Treatment group	Holoclar	p-value (month 12 versus baseline)

variability	Number of subject	104	
	Primary endpoint: **ACLSCT success** (rate %)	75 (72.1%)	n/a
	95% exact CI	62.5 – 80.5%	n/a
	Secondary endpoint 1: number of patients with **symptoms** at month 12 vs. baseline (percentage)	12 (11.5%) vs. 40 (38.5%)	p<0.001
	Secondary endpoint 2: number of patients with **inflammation** at month 12 vs. baseline (percentage)	33 (31.7%) vs. 32 (30.8%)	p=0.732
	Secondary endpoint 3: Number of patients with **VA improvement** (rate %)	51 (49.0%)	n/a
	Secondary endpoint 4: Number of patients with **successful keratoplasty** out of all patients with keratoplasty (percentage)	24 out of 57 (42.1%)	n/a

2.5.2.2. Analysis performed across trials (pooled analyses and meta-analysis)

Paediatrics

Five (5) paediatric patients were included in the two retrospective studies (n=3 in HLSTM01 and n=2 in HLSTM02, see section 2.5.2.3.) and all were boys. Four of the 5 boys were older than 12 years, and one was 8 years of age. In all 5 cases, LSCD was due to chemical or physical burns. In two children, the ACLSCT outcome was successful and accompanied by improvement in visual acuity and/or clinical symptoms (when present at baseline), while 3 paediatric patients (including the youngest 8-year-old boy) with severe corneal neovascularisation were considered as treatment failures.

Elderly

A total of 7 patients (6.7% of the ITT study population) with an age at baseline of > 65 years were included in the HLSTM01 study. For 5 (71%) of the 7 elderly patients, treatment with Holoclar was recorded as successful, whereas information was missing in the remaining 2 cases. Seven additional patients (24.1%) within this age range were included in HLSTM02 (see section 2.5.2.3.). Treatment success was reported in 5 cases, failure in 1 case, and information was missing for the remaining case. Although limited in terms of subject numbers, data from both studies show a success rate similar to that observed overall in treated patients.

2.5.2.3. Supportive study(ies)

2.5.2.3.1. Study HLSTM02

Title: Retrospective evaluation of the safety of autologous cultivated limbal stem cells transplantation for restoration of corneal epithelium in patients with limbal stem cell deficiency

Methods

At the 7 sites included in HLSTM02, Holoclar transplantation was performed according to the same surgical protocol as used in the centres of HLSTM01. Therefore, the methods described for study HLSTM01 largely also apply to study HLSTM02 unless stated otherwise.

Investigators in HLSTM02 followed local clinical practices for post-treatment patient management and follow-up.

Study Participants

Patients who refused to give their consent could not be enrolled. Patients enrolled in this trial had to be (i) Males or females (all ages) and (ii) have been transplanted with ex vivo expanded autologous limbal stem cells.

Treatments

No post-transplantation visit schedule was defined in the protocol due to the retrospective nature of the study and the heterogeneity distribution of the visits across participating centres. Furthermore, systemic and topical pharmacological treatment was applied according to the respective local practice in the clinical facility.

Objectives

The primary objective of the study was to evaluate the safety of the ACLSC transplantation both in terms of number of subjects that experienced adverse events (AEs) and of number of AEs.

The secondary objectives of the present study were:

- To evaluate the outcome of the ACLSC transplantation in terms of success or failure;
- To evaluate the number of ACLSC transplantations performed in each patient;
- To evaluate the number of successful keratoplasties after ACLSC transplantation;
- To evaluate the relative content of corneal and conjunctival epithelial cells by impression cytology.

Outcomes / endpoints

The primary efficacy endpoints of the study were (i) the number of subjects experiencing adverse events (AEs) and (ii) the number of AEs.

Secondary endpoints:

- Rate of ACLSC transplantation outcome recorded as “success” or “failure”, based on investigator’s judgement.

  The following definitions applied: Success: if no investigator judgement of transplantation outcome was reported as “failure” in any post transplantation visit at least 6 months after ACLSC transplantation; Failure: if at least one investigator judgement of transplantation outcome was reported as “failure” in any post transplantation visit at least 6 months after ACLSC transplantation; Missing: if no judgement was expressed in the source document at any visit occurred at least six months after transplantation.

- Number of ACLSC transplantations in each patient.
- Number of successful keratoplasties after ACLSC transplantation. The success/failure of a post-transplantation keratoplasty was defined based on the ACLSC transplantation outcome determined in the first available visit occurred ≥ 6 months after the keratoplasty.

Blinding (masking)

The study was uncontrolled and no blinding was applied.

Statistical methods

The safety population included all patients who underwent the limbal biopsy collection. The demography, secondary efficacy and safety analysis were performed on the safety population.

Secondary efficacy data were analysed as observed, without replacement for missing values.

Results

Recruitment and numbers analysed

The applicant traced all centres in Italy where the product had been used and contacted the investigators to formally request the clinical data for inclusion in the retrospective studies. Data for 106 of these patients treated in two centres in Rome and Milan had already been included in the pivotal study HLSTM01. Of the remaining 19 centres, only 7 agreed to participate in study HLSTM02 and to release patient data. From these 7 centres, data of all patients (31) receiving a Holoclar transplant up to 2007 were available including follow-up data until 2010. Data of the remaining 82 patients treated in the 12 centres refusing participation were not directly available to the applicant. However, two of the centres had published part of their data (see section 2.5.2.4.).

A total of 31 patients attended a pre-surgical visit. For 2 patients documentation of the biopsy and ACLSCT was missing and as a result 29 patients were included in the safety analysis.

Baseline data

The mean age was 45.8 ± 17.4 years (range 13.7-79.1). The study population included 22 males (75.9%) and 7 females (22.1%). See Table 2 for an overview of the demographic profile of study HLMST02. Data regarding the particular ethnicity of patients were not collected. Patients were assumed to be Caucasian of Italian descent.

The majority of patients (23, 79.3%) had a diagnosis of LSCD due to chemical or physical ocular burns which is the claimed target indication for Holoclar, while 6 patients were treated for LSCD due to one or more of the following reasons: contact lens-associated damage (1), ocular infections (3), radiation (1) toxic pathology associated with medical drugs (1), post-surgery iatrogenic pathology (2), or other (2).

Deep stromal vascularisation was involved in 69.0% of the patients in study HLSTM02, and 75% of the patients had a severe loss in visual acuity (see also Table 3). A total of 75.9% of patients showed moderate (34.5%) or severe (41.4%) superficial CNV at baseline and 93% had corneal opacity. Superficial CNV was absent or only mild in 6.95% and 17.2%, respectively. About one third of patients had no epithelial defects at baseline.

The time span from burn injury to Holoclar treatment ranged from 10 months to 50 years (mean 14.1 years, see Table 4).

Seventeen patients (58.6%) underwent at least one surgical procedure before inclusion. Keratoplasty (10 patients, 34.5%) and corneal operations (8 patients, 27.6%) were the most common surgical and medical procedures. All 29 patients had previous and/or concomitant medications. Sensory organs drugs were the most common previous medication (11 patients, 37.9%), with netilmicin as the most common active drug (6 patients, 20.7%).

Outcomes and estimation

Success of transplantation

Success according to the subjective, overall clinical judgment of the investigator, was reported in 19 out of 29 patients (65.5%) and failure in 6 patients (20.7%). Information was missing in 4 cases (13.8%). The 95% exact CI for the proportion of patients with successful transplantation was 48.2 to 82.8%. Analysis of patients with LSCD due to ocular burns only confirmed the results in the overall population.

Number of previous limbal stem cell transplantations

Only one patient (3.45%) had undergone an ACSLC transplantation before inclusion in the study.

Number of successfull keratoplasties after limbal stem cell transplantation

Six patients underwent one or more keratoplasty interventions after ACLSC transplantation. In 4 of them (66.7%) the intervention was successful in at least one attempt. Of the six patients, three had a history of one or more failed attempts before ACLSC graft; successful post-transplantation keratoplasty was reported in all 3 cases.

Evaluation of impression cytology

Relevant results are presented in section 2.4. of this report.

Follow-up and long-term efficacy

Long term efficacy data (>6 months) up to 8 years were available for 28 patients in study HLSTM02. Most of the subjects were followed for at least 1 year (24 patients, 82.8% of cases), with 5 patients (17.2%) followed for 5 years or more.

Of the patients with data at the time points 2 years (14 patients) and 3 years (9 patients) after Holoclar transplantation, and for the last available follow-up visit (29 patients, mean time of observation 34.3±26.2), 10 (71.4%), 5 (55.6%) and 21 (77.8%) were reported with a treatment success.

2.5.2.3.2. Study HLSTM04

Title: Retrospective evaluation of the safety and efficacy of Autologous Cultivated Limbal Stem Cells Transplantation for restoration of corneal epithelium in patients with Limbal Stem Cell Deficiency due to ocular burn.

In response to a request by the CHMP/CAT, the applicant presented the results of an ad-hoc retrospective, observational clinical study including all patients who underwent ACLSCT after the period covered by studies HLSTM01 and HLSTM02. From 2008 (end of collection period for the previous HLSTM01 and HLSTM02 studies) to 2013, 15 patients started Holoclar treatment procedure (i.e. underwent biopsy) at 3 Italian sites and consented to the retrospective data collection. These centres accounted for 100% of the patients treated with Holoclar since 2008 in Italy and data were collected for all the patients treated.

All patients were diagnosed with moderate to severe LSCD due to chemical or physical ocular burn. Diagnosis and severity grading of LSCD were based on clinical criteria as in study HLSTM01.

Data for all available visits after Holoclar application were collected, with evaluation of efficacy mainly at two time points: i) 3 months after ACLSCT and ii) last available follow-up visit. The clinical outcome was assessed using the same composite endpoint of superficial CNV and corneal epithelium integrity as in HLSTM01. The

definition of treatment success/failure as well as data collection particulars were modelled on study HLSTM01. Assessment of the presence/severity of inflammation and visual acuity was also aligned with study HLSTM01.

Results

Three-month data was available for all 15 patients including 14 males and 1 female (mean age: 46.5 years; range: 21-79 years). Follow-up duration and schedule varied by centre (median follow-up: 7.2 months; range: 3-26 months).

Twelve (80%) and 3 (20%) of the 15 patients included in study HLSTM04 were considered to have severe and moderate CNV at baseline, respectively. Most patient (13) had involvement of the central cornea and all but one of the patients had stromal scarring (deep corneal opacity).

ACLSCT resulted in a positive clinical outcome in 9 (60%) of the 15 patients at month 3 post-transplantation. The results were confirmed when using the last post-transplantation visit as endpoint. Superficial corneal neovascularization showed an improvement in 67% of the patients presenting either without CNV or with only 1 quadrant involved at both post-transplantation visits. In particular, 40% of patients had no vessel penetration at month 3 and thereafter. With regard to corneal epithelium integrity, the proportion of patients without epithelial defects increased from 40% (6) at the pre-surgical visit to 60% (9) at month 3, with a further increase to 73% (11) at the last visit post transplantation. Moreover, the proportion of patients with trace defects decreased from 47% (7) to 20% (3) at month 3 and 13% (2) at the last visit post transplantation, respectively.

Mild or moderate limbal hyperaemia was reported in 2 cases (13%) at the last visit post transplantation. These patients experienced blepharitis in conjunction with the inflammation.

Visual acuity (VA) had improved in 40% (natural vision) and 33% (best corrected VA) of patients with stromal scarring at month 3 post transplantation. This improvement was maintained at the last visit post transplantation.

2.5.2.4. Literature

Two publications were identified (Rama et al., Transplantations 2001 and Marchini et al., Clin Exper Ophthalmol 2011) reporting on 28 patients treated at clinical sites in Venice and Verona, which did not participate in the Holoclar studies.

Rama et al. (Transplantation 2001; 72: 1478-85)

This publication reports the results of ACLSCT in 18 patients (mean age 48 ±12 years) with moderate or severe LSCD secondary to chemical burns. Twelve of the 18 patients were transplanted in Venice and were not included in the retrospective studies. The remaining 6 patients were treated at the site in Rome included in study HLSTM01. ACLSCT was successful in 14 (77%) of the 18 patients with improvement of symptoms and establishment of a transparent and stable corneal epithelium. Visual acuity was not significantly improved at 1 year follow-up compared to the baseline value, mainly because of corneal stromal damage. In the subset of 12 patients transplanted in Venice (and excluded from the HLSTM02 study), ACLSCT was successful in 10 patients (83.3%).

Marchini et al. (Clin Exper Ophthalmol 2011; 40: 255-267)

This article reports results of a prospective, non-comparative, interventional case series including 16 patients (median age 47.5 years) suffering from LSCD due to chemical burn. Thirteen patients were from the Verona site. The other 3 patients were transplanted at the same site by the same investigator but could not be traced by the

applicant due to a change in the location of the manufacturing site. The clinical outcome after 12 months follow-up was successful in 10 patients (62.6%). Ocular pain and photophobia resolved in all patients with a successful or partially successful outcome. Visual acuity improved in 4 patients (25.0%) before keratoplasty. Three patients received 2 limbal stem cell transplants onto the same eye with partially successful outcome in both patients.

2.5.3. Discussion on clinical efficacy

To support the claim for efficacy of Holoclar in the intended indication of moderate to severe limbal stem cell deficiency (LSCD) due to ocular burns, the applicant submitted the results of two retrospective, uncontrolled and non-randomised studies, HLSTM01 and HLSTM02, involving a total of 133 patients and 142 transplantation events (including re-transplantations) at several centres in Italy during the period from 1998 to 2007. The efficacy evaluation relied primarily on the outcome of study HLSTM01. In comparison to HLSTM01, HLSTM02 patients were more heterogeneous with respect to the underlying cause of LSCD, post-transplantation patient care and follow-up. Therefore, efficacy data from HLSTM02 were only considered supportive. In the course of the review of this application, additional supportive data were presented in a separate retrospective analysis (HLSTM04) covering all patients (15) treated from 2008 to 2013. Supportive data were furthermore available from publications in the scientific literature including in particular Rama et al., 2001 and Marchini et al., 2011.

Design and conduct of clinical studies

No dose-response studies have been conducted. This was considered acceptable by the CAT since the size of the graft is being measured by the size of the patient's cornea and in light of the set specifications of the drug product regarding cell density and potency. These specifications were defined based on an analysis of clinical outcomes for product batches in study HLSTM01. However, the CAT noted that the minimum required proportion of LSC was rather low according to the specifications (0.4% to 10%). Therefore, future prospective data collection should further investigate the impact on efficacy of the amount of LSC in the final drug product.

Considering the low incidence of the condition, the sample size of the retrospective analyses was considered adequate by the CAT. The CAT furthermore acknowledged that the available clinical experience arising from the use of Holoclar since 1998 was considerable and that the retrospective analyses provided valuable support for this application. However, retrospective studies rely on the completeness and accuracy of the available data and are prone to bias threatening the validity of the results. In particular, as only a subset of all patients treated with Holoclar during the observation period (133 out of 219) was involved in the analyses, it could not be excluded that bias were introduced due to patient selection. However, the incomplete dataset was explained by the lack of willingness of several clinical centres to participate in the studies and their refusal to release patient data. It is noteworthy that for all sites included in the Holoclar studies, data of all patients treated at each site was available, thereby eliminating concerns of patient selection at centre level. In this context, the definition of the ITT population in study HLSTM01, leading to exclusion of 2 patients from the efficacy analysis, was not agreed by the CAT, as all patients who received a transplant should have been included. Nevertheless, a sensitivity analysis confirmed that the impact of exclusion of the 2 patients on the primary endpoint was negligible. Additional reassurance on the validity of the data was also provided by two publications (Rama et al., 2001 and Marchini et al., 2011) reporting efficacy outcomes consistent with the results of HLSTM01 and HLSTM02 for 28 patients treated at clinical sites not included in the Holoclar studies. Finally, the quality, integrity, and reliability of the data collection was verified in GCP inspections at selected study sites. The two centres involved in the pivotal study HLSTM01 applied a standard protocol for pre-treatment assessments, Holoclar treatment

and subsequent patient management, resulting in a harmonised dataset with a relatively low number of missing values suitable for research purposes.

The fact that the studies were uncontrolled and not randomised further added to the uncertainties of the validity of the dataset, but was considered inevitable due to the lack of a suitable comparator considering that there is neither an approved treatment for LSCD nor an ubiquitous accepted standard of care. Since this condition would not heal spontaneously, the single arm, un-controlled design was considered acceptable by the CAT. The CAT furthermore noted that efforts have been made towards a more independent evaluation of the study outcome by performing a review in a blinded fashion using photographic evidence of neovascularisation.

The main inclusion criterion for study HLSTM01 was moderate or severe LSCD (uni- or bilateral), secondary to ocular burns which is in line with the claimed indication for Holoclar treatment. Diagnosis of LSCD was based on medical history, i.e. ocular burns, as well as the main clinical signs, corneal neovascularisation and opacity, and the impairment in visual acuity. The severity of the ocular condition was graded based upon the clinically assessed degree of superficial corneal neovascularisation. Overall, the diagnostic and inclusion criteria were considered by the CAT to be adequate to define a patient population representative for the claimed indication. The CAT considered that all the diagnostic criteria for disease severity should be reflected in the indication, including central corneal involvement, and severely impaired visual acuity, which was not reflected in the initial proposal by the applicant. The indication was updated accordingly.

To evaluate the effect of Holoclar treatment, a composite primary efficacy endpoint was used, consisting of two components, corneal epithelial integrity and absence of significant corneal neovascularisation. With regard to the first component, the CAT noted that the majority of the patients showed no or only trace epithelial defects at baseline and thus presented already pre-transplantation with a finding in accordance with a successful treatment outcome. However, considering that LSCD is a condition with impaired ability to maintain/restore an intact corneal epithelium, maintenance of no or only trace epithelial defects over the follow-up period was considered of clinical relevance.

Efficacy data and additional analyses

The assessment of efficacy of Holoclar relied mainly on the outcome of study HLSTM01. Supportive data from HLSTM02, HLSTM04 and the scientific literature showed an efficacy of Holoclar of similar magnitude as that observed in the pivotal study HLSTM01.

With regard to the composite primary efficacy endpoint in HLSTM01, 72.1% (75/104) of the patients had a successful ACLSCT 12 months post- transplantation. The success rate was significantly higher than the pre-defined minimum effect of 50%. Available long-term follow up data up to 10 years after ACLSCT, though limited, supported persistence of treatment success beyond 12 months. Additional long-term efficacy data will be collected in the margins of a post-authorisation safety study to confirm this outcome. The CAT furthermore noted that the time span from burn injury to Holoclar treatment was very wide, ranging from a few months to more than seven decades. This means that the study population included patients in different phases of the natural history of the disease, which may have had an impact on the treatment response. Exploratory analyses of the impact of time to burn injury, however, suggested that once the clinical situation is stabilised, the outcome of Holoclar treatment is largely independent from the time elapsed since injury. Therefore, sufficient time should be allowed for the eye to reach steady-state and treatment is not recommended in the immediate post-burn phase (i.e. in acute or sub-acute phase).

Overall, 73.1% (76/104) of the patients showed an improvement of superficial corneal vascularisation from moderate or severe at baseline to mild or none. Evaluation based on photographic assessment showed acceptable consistency with the investigators' assessments.

Maintenance of an epithelial condition or improvement of an epithelial defect to no or only trace defects was observed in 89.4% of the patients [36/104 patients (34.6%) with no defects and 45/104 (54.8%) with trace defects]. Impression cytology performed in a subset of patients provided supporting evidence that Holoclar grafting enables corneal type epithelialisation (see section 2.4.3.). Occurrence of epithelial defects shortly after ACLSCT was observed in some patients and was considered a variable and highly individual phenomenon. From a pathophysiological perspective, it can be associated with the inflammation and temporary injury induced by the surgical procedure. In most of the cases, these epithelial defects reverted later, but the presence of early instability of the epithelium appeared to be associated with a greater probability of treatment failure.

No improvement with regards to the proportion of patients with inflammation was observed. While patients with active ocular inflammation were excluded from study participation, 29 patients presented with a mild form of inflammation at baseline and the vast majority of these showed an improvement of superficial corneal neovascularization at 12 months post-transplantation. However, some patients without signs of inflammation at baseline developed clinically significant hyperaemia during the period of 2 weeks to 1 month after surgery, which coincided with the switch from systemic to topical corticosteroid treatment. Despite the fact that inflammation improved later on, 4 of the 5 patients who developed early significant limbal hyperaemia were treatment failures. While active, uncontrolled inflammation is generally considered a negative prognostic factor for the success of Holoclar treatment due to its detrimental effect on the engraftment of limbal stem cells at the corneal surface, a mild degree of inflammation has not been associated with a compromised clinical outcome and local inflammatory pathways are in fact believed to be required for the healing process. Considering that, besides the intended anti-inflammatory and anti-scarring effect, topical application of corticosteroids has also been shown to delay corneal epithelial and stromal healing, the challenge may lie in achieving adequate control of local inflammation, but avoiding potential interference with the re-epithelialisation process.

The proportion of patients with ocular symptoms of pain, burning, or photophobia decreased significantly from 38.5% (40/104) at baseline to 11.5% (12/104) 12 months post-treatment. Specific improvements were also noted with respect to reductions in intensity of the symptoms.

Improvement of visual acuity was noted in 49% (51/104) of the patients, with a higher proportion among patients without deep stromal scarring (83.3%, 15/18) compared to those with stromal scarring (44.4%, 36/81). Post-hoc analyses showed that in a large proportion of the patients (38.5%, 40/104) vision improved to an equivalent of 3 lines on the vision chart. Furthermore, more than 50% (47/92) of patient with off-chart vision at baseline improved to on-chart vision after Holoclar treatment.

Overall, the CAT considered that it was important to differentiate between two patients groups, patients with and without stromal scarring as a result of the ocular burn injury. To achieve vision improvement in the first group of patients, limbal stem cell transplantation has to be combined with subsequent keratoplasty, whereby the success of the limbal stem cell transplantation provides the foundation for the subsequent cornea transplantation, reduces the risk of corneal graft rejection and enhances the chance of survival of the corneal graft which contains progenitor cells that have only limited longevity and regenerative capacity. A beneficial effect of Holoclar in this sub-population was demonstrated by the finding of successful keratoplasties in 42.1% (24/57) of patients who had at least one keratoplasty after ACLSCT. Furthermore, keratoplasty after Holoclar succeeded in half of all patients who had a failed corneal transplantation prior to ACLSCT. Subgroup analyses showed that a successful ACLSCT outcome was associated with a higher probability of a successful keratoplasty. Furthermore, 57% (32/56) of patients with keratoplasty after one year following Holoclar treatment, had at least one line improvement in visual acuity after the first post-Holoclar corneal transplant and clinically relevant changes equivalent to 3 lines or more on a vision chart were observed in 37.5% (21/56) of the patients.

In patients with moderate LSCD severity and no stromal damage, the main objective of treatment would be improvement in symptoms, as a small improvement in corneal neovascularisation (e.g. from moderate to mild) alone was considered to be of limited clinical relevance. Most of the patients showed a stable clinical picture at baseline, and only a limited number had ocular symptoms (pain, photophobia, burning, bulbar, or limbal hyperaemia). Treatment with Holoclar maintained the stable clinical picture or resulted in an improvement and/or resolution of manifestations, when present. The response appeared similar in the patient groups with moderate and severe CNV at baseline.

Only few paediatric patients (5) and patients >65 years (14) were included in the retrospective analyses of HLSTM01 and HLSTM02. Available data were considered insufficient to draw final conclusions for the paediatric and elderly populations. However, at least in the elderly, despite limited subject numbers, data from both studies showed a success rate similar to that observed in the overall population. Overall, the CAT considered that the adult population was a suitable target population and agreed that the indication should be restricted to adults. A specific commitment to acquire additional safety data in five patients between 3 and 17 years of age has been agreed in the approved Paediatric Investigation Plan.

Additional efficacy data needed in the context of a conditional MA

The evaluation of efficacy of Holoclar is based on retrospective analyses of a comparably large set of data given the rarity of the disease. Despite the disadvantages of such study design, overall, the data were considered by the CAT of sufficient quality to support establishment of a beneficial treatment effect, thereby enabling early availability to patients. Such early availability was considered by the CAT to be in the interest of public health given that LSCD is a serious debilitating disease for which no authorised treatment exists in the EU. Nevertheless, for a comprehensive clinical dataset, prospectively collected data are needed in order to confirm the treatment benefits observed in the retrospective analyses, in particular since it could not be excluded that bias have been introduced as a result of the retrospective study design. To this end, the applicant proposed to conduct a prospective, multinational, multicentre, open label, uncontrolled study in at least 65 patients (plus 5 paediatric patients) with moderate to severe LSCD.

2.5.4. Conclusions on the clinical efficacy

The CAT considered that the available data provided sufficient evidence to demonstrate a clinically relevant beneficial effect of Holoclar in the treatment of patients with moderate to severe LSCD. The data showed that Holoclar treatment enabled restoration of a stable, intact corneal epithelium with resolution of epithelial defects, regression of corneal vascularisation, and absence of conjunctivalisation in the majority of patients. Clinically relevant improvements in visual acuity were observed. Furthermore, ocular symptoms improved and the chance for subsequent successful keratoplasty in patients with deep stromal scarring increased.

However, the CAT considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

Multinational, multicentre, prospective, open-label, uncontrolled interventional study (HLSTM03) to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns.

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

2.6. Clinical safety

The safety assessment mainly relied on data from studies HLSTM01 and HLSTM02. Additional supportive data were available from study HLSTM04. Safety was reviewed taking into the account the therapeutic procedure as a whole, including surgery and use of concomitant treatments.

Further details on the methods applied are summarised in section 2.5.2.

Patient exposure

The clinical safety database used for safety assessment was based on 135 patients and 142 treatments, including

- 106 patients with 113 treatments in study HLSTM01, and
- 29 patients with 29 treatments in HLSTM02.

In study HLSTM01, 94 patients were treated once, 11 patients were treated twice (22 total treatments for this patient set), and one patient underwent three treatments. Complete data for six treatments was not available, or was missing at the treating clinical centre (five data sets for first patient treatment, and one data set for a second patient treatment), and were therefore, not included in the analysis. Consequently, 113 evaluable patient data sets were available for study HLSTM01. In study HLSTM02, no patient received more than one Holoclar treatment and documentation of the biopsy and treatment was available for all 29 patients receiving a single treatment with Holoclar.

Since several patients in HLSTM01 received multiple treatments with Holoclar, and each treatment event was considered to have a unique safety profile, the applicant presented safety analyses by treatments.

Patient follow-up data were available for up to 10 years in study HLSTM01 and for up to 8 years in study HLSTM02. In study HLSTM01 timing of follow-up visits were pre-determined, while in study HLSTM02 follow-up visits were conducted according to clinical practice, which resulted in less frequent monitoring. One year follow-up data were available for 93.8% of patients in study HLSTM01 and for 82.7% of patients in study HLSTM02. Three year follow-up data were available for 65% of patients in study HLSTM01 and for 55.2% of patients in study HLSTM02. The mean duration of treatment follow-up was 36.8 ± 23.0 months (HLSTM01) and 33.9 ± 25.9 months (HLSTM02).

An overview of the surgical history as well as preceding and concomitant medical treatments is provided in sections 2.5.2.1. (HLSTM01) and 2.5.2.3.1. (HLSTM02).

Additional supportive safety data were available from 15 patients analysed in study HLSTM04.

Adverse events (AE)

AEs prior to treatment with Holoclar

Pre-treatment AEs were reported to have occurred at a low percentage.

In study HLSTM01, 10 pre-treatment AEs were reported in 9 ACLSCD treatments (7.96%). None were considered to be of a serious or severe nature. The majority of pre-treatment AEs were of ocular nature (8 events; 6.2%) including blepharitis (5 patients; 4.4%), cataract (1 patient; 0.9%) and glaucoma (2 patients; 1.8%). One patient was diagnosed with both blepharitis and glaucoma prior to treatment. Additionally, one case of diabetes mellitus and one case of hypertension were recorded prior to treatment.

In HLSTM02, two adverse events were reported prior to treatment: mild viral flu and meibomitis of the right eye (1 patient, 3.4% each).

Treatment-emergent AEs (TEAEs) and ADRs

An overview of all TEAEs, i.e. adverse events that started after Holoclar transplantation, is provided in Table 14.

A total of 194 AEs were reported in association with 73 treatment events (64.6%) in study HLSTM01. A total of 46 AEs were reported in association with 19 treatment procedures (65.5%) in study HLSTM02. The most commonly observed AEs were eye-related disorders, occurring in 57% of the safety population (see Table 15). The second most common AEs belonged to the MedDRA system organ class (SOC) of Immune System Disorders, occurring in 17.6% of the treatments, followed by Infections and Infestations in 8.5%.

Table 14 - Summary of Treatment Emergent Adverse Events

N (%)	HLSTM01			HLSTM02	Total
	1 treatment N = 101	2 treatments N = 11	3 treatments N = 1	1 treatment N = 29	N=142
Total AEs	181	13	0	46	240
Total treatments with AEs	66 (65.3%)	7 (63.6%)	0	19 (65.5%)	92 (64.8%)
ADRs	21	1	0	21	43
Total treatments with ADRs	18 (17.8%)	1 (9.09%)	0	10 (34.5%)	29 (20.4%)
SAEs	6	0	0	5	11
Total treatments with SAEs	6 (5.9%)	0	0	3 (10.3%)	9 (6.3%)
Serious ADRs	0	0	0	3	3
Total treatments with serious ADRs	0	0	0	2 (6.9%)	2 (1.4%)
Severe AEs	10	0	0	10	20
Total treatments with severe AEs	10 (9.9%)	0	0	6 (20.7%)	12 (8.4%)

One treatment can have more than one adverse event.

Adverse events have been coded using the MedDRA dictionary. System Organ Class (SOC)

Percentages are calculated on number of treatments (N)

SAE = serious AE; ADR = adverse drug reaction.

Amongst the cases of Immune System Disorders corneal graft rejection and transplant rejection occurred in 13 and 9 treatments, respectively. The rate of Immune system disorders was higher in patients with prior keratoplasty and the incidence of transplant rejection (corneal graft rejection) increased with subsequent transplantations, from 3.85% and 9.09% to 66.67% following first, second and third keratoplasty in HLSTM01.

Overall, 12 patients were reported with infections, including keratitis herpetica, influenza and infections of the upper airway, and one case of corneal infection that was considered to be related to treatment.

One case each of metaplasia, subcutaneous haemorrhage, corneal infection, suture rupture, and vasovagal syncope was reported and was considered causally related to treatment.

AEs classified as severe occurred in a total of 9% of treatments in study HLSTM01 and in 20% of treatments in study HLSTM02. They consisted mainly in eye-related disorders including blepharitis (3), eye pain (2), corneal perforation (2), cornel epithelium defect, conjunctival hyperaemia, eye irritation, optic atrophy, retinal detachment and ulcerative keratitis (1 AE each).

One third of all AEs were experienced more than 1 year after treatment. Blepharitis and corneal epithelial defects occurred mainly in the first 3 months after transplantation. During this timeframe, events of blepharitis were reported in 10.5% of cases. They were judged to be related to treatment in a subset of cases. Seven cases of glaucoma were reported to occur within 3 months from Holoclar treatment, while the other cases of glaucoma (16 cases) occurred at least ninety days after ACLSCT, when the corticosteroid treatment was suspended. Notably, the reporting of glaucoma included events of increased intraocular pressure.

Table 15 – Summary of eye-related TEAEs

Body system / Adverse event	HLSTM01 N=113	HLSTM02 N=29	Overall N=142
Eye Disorders			
Instances per Treatment[1]	**128/65 (57.5%)**	**28/16 (55.2%)**	**156/81 (57.0%)**
Anterior Capsule Contraction	1 (0.9%)	0	1 (0.7%)
Blepharitis	38/37 (32.7%)	2 (6.9%)	40/39 (27.5%)
Cataract	19/18 (15.9%)	0	19/18 (12.7%)
Chemical burn of the eye	2 (1.8%)	0	2 (1.4%)
Conjunctival Adhesion	0	1 (3.4%)	1 (0.7%)
Conjunctival Granuloma	1 (0.9%)	0	1 (0.7%)
Conjunctival Haemorrhage	8 (7.1%)	0	8 (5.6%)
Conjunctival Hyperaemia	0	1 (3.4%)	1 (0.7%)
Conjunctivitis Allergic	1 (0.9%)	0	1 (0.7%)
Corneal Defect	3 (2.7%)	0	3 (2.1%)
Corneal Epithelium Defect	5 (4.4%)	5/3 (10.3%)	10/8 (5.6%)
Corneal Infiltrates	1 (0.9%)	0	1 (0.7%)
Corneal Oedema	0	3/2 (6.9%)	3/2 (1.4%)
Corneal Perforation	3 (2.7%)	1 (3.4%)	4 (2.8%)
Eye Haemorrhage	4 (3.5%)	0	4 (2.8%)
Eye Inflammation	1 (0.9%)	0	1 (0.7%)
Eye Irritation	0	1 (3.4%)	1 (0.7%)
Eye Pain	2 (1.8%)	5 (17.2%)	7 (4.9%)
Eyelid Ptosis	3 (2.7%)	0	3 (2.1%)
Glaucoma	20/19 (16.8%)	5/4 (13.8%)	25/23 (16.2%)
Keratitis	3 (2.7%)	0	3 (2.1%)
Macular Oedema	1 (0.9%)	0	1 (0.7%)
Ocular discomfort	1 (0.9%)	0	1 (0.7%)
Optic Atrophy	0	1 (3.4%)	1 (0.7%)
Photophobia	0	1 (3.4%)	1 (0.7%)
Retinal Detachment	1 (0.9%)	0	1 (0.7%)
Retinal Haemorrhage	1 (0.9%)	0	1 (0.7%)
Retinal Vein Occlusion	2 (1.8%)	0	2 (1.4%)
Strabismus	1 (0.9%)	0	1 (0.7%)
Trichiasis	3 (2.7%)	0	3 (2.1%)
Ulcerative Keratitis	2 (1.8%)	2 (6.9%)	4 (2.8%)
Vitreous Detachment	1 (0.9%)	0	1 (0.7%)

[1]One treatment may have more than one associated adverse event. The instances per treatment indicate the number of associated adverse events and the number of treatments. The percentage is calculated based on the number of treatments.

When not specified, the number of incidents equalled the number of treatments.

Adverse events have been coded using the MedDRA dictionary. System Organ Class (SOC) Preferred term (PT) are presented.

Adverse drug reactions (ADRs), i.e. AEs for which a relationship to Holoclar treatment (or associated procedures related to treatment) was judged as either possible, probable or definite, were reported in 20.4% of treatments (see Table 16).

A greater number of ADRs were reported for study HLSTM02 (34.5%) as compared to HLSTM01 (16.8%). The majority of ADRs were associated with eye disorders, whereby the number of reported ocular ADRs was greater in HLSTM02 than in HLMST01 (34.5% versus 15.0%). The most commonly experienced ADRs were conjunctival haemorrhage (7 reported reactions), corneal epithelial defects consistent with treatment failure (7), pain (4), and blepharitis (4). Other ADRs not related to the SOC of Eye Disorders included 1 case each of metaplasia, subcutaneous haemorrhage, corneal infection, suture rupture, and vasovagal syncope.

In principle, the relationship between an adverse drug reaction (ADR) with Holoclar and related procedures has been considered according to the procedures conducted within the timeframe from the biopsy to the last administration of the topical corticosteroid treatment, which was 45 days (3 months) after Holoclar treatment. Additional adverse events considered at least possibly related to Holoclar, which occurred after 3 months of Holoclar treatment, were conjunctival adhesion, corneal oedema, corneal perforation, corneal infection, metaplasia and suture rupture with onset more than 3 months after treatment. With the exception of these cases, the cut-off date of 3 months was used for the adverse reaction frequency calculations based on crude incidences, resulting in the following rates for adverse reactions observed in this time frame: Syncope vasovagal (1 case, 0.7%), blepharitis (15 cases, 10.56%), conjunctival haemorrhage (7 cases, 4.9%), eye haemorrhage (4 cases, 2.8%), corneal epithelium defect (5 cases, 3.52%), eye pain (4 cases, 2.8%), glaucoma (7 cases, 4.9%), ulcerative keratitis (2 cases, 1.4%), conjunctival hyperaemia (1 case, 0.7%), eye irritation (1 case, 0.7%), photophobia (1 case, 0.7%), haemorrhage subcutaneous (1 case, 0.7%), and metaplasia of the implant (1 case, 0.7%).

In HLSTM01, all ADRs were related to local complications associated with or attributable to:

a) surgical aspects of treatment administration (i.e. haemorrhage and inflammation),

b) administration of topical post-treatment agents (i.e. treatment of post-therapy emergent glaucoma), or

c) failure of the treatment procedure (i.e. epithelial defect or metaplasia): 20 reports of eye disorders in association with 17 treatments (15.0%).

No ADRs recorded were considered related to post-treatment prophylactic antibiotic administration performed up to one month post-treatment. AEs potentially related to post-treatment corticosteroid administration (i.e. occurring post-treatment in patients treated with corticosteroids and associated with known potential side effects of corticosteroids), reported up to 3 months after treatment, were recorded for 6 treatments (5.3%) including general eye disorders in 5 treatments (4.4%; all cases of glaucoma) and development of gastritis in the case of 1 treatment (0.9%). Only 1 of these cases (0.9%, glaucoma) was considered an ADR by the investigator.

In HLSTM04, 9 out of the 15 patients experienced a total of 14 AEs, the most frequent being eye and nervous system disorders. One SAE was observed in a 79-year old patient who had a stroke. None of the AEs including the SAE were considered related to Holoclar.

Table 16 – Number of ADRs reported by Treatments

Body system / Adverse event	HLSTM01 N = 113	HLSTM02 N = 29	Overall N = 142
Number of ADRs	**22**	**21**	**43**
Number of treatments with at least one ADR	**19 (16.8%)**	**10 (34.5%)**	**29 (20.4%)**
Eye Disorders			
Instances per Treatment[1]	**20/17 (15.0%)**	**18/10 (34.5%)**	**38/27 (19.0%)**
Blepharitis	4 (3.5%)	0	4 (2.8%)
Conjunctival adhesion	0	1 (3.5%)	1 (0.7%)
Conjunctival haemorrhage	7 (6.2%)	0	7 (4.9%)
Conjunctival hyperaemia	0	1 (3.5%)	1 (0.7%)
Corneal epithelium defect	2 (1.8%)	5/3 (10.3%)	7/5 (3.5%)
Corneal oedema	0	2/1 (3.5%)	2/1 (0.7%)
Corneal perforation	0	1 (3.5%)	1 (0.7%)
Eye haemorrhage	4 (3.5%)	0	4 (2.8%)
Eye irritation	0	1 (3.5%)	1 (0.7%)
Eye pain	0	4 (13.8%)	4 (2.8%)
Glaucoma	3 (2.6%)	0	3 (2.1%)
Photophobia	0	1 (3.5%)	1 (0.7%)
Ulcerative keratitis	0	2 (6.9%)	2 (1.4%)
General Disorders and Administration Site Conditions			
Instances per Treatment[1]	**1/1 (0.9%)**	**0**	**1/1 (0.7%)**
Metaplasia	1 (0.9%)	0	1 (0.7%)
Infections and Infestation			
Instances per Treatment[1]	**0**	**1/1 (3.5%)**	**1/1 (0.7%)**
Corneal infections	0	1 (3.5%)	1 (0.7%)
Injury, Poisoning and Procedural Complications			
Instances per Treatment[1]	**0**	**1/1 (3.5%)**	**1/1 (0.7%)**
Suture rupture	0	1 (3.5%)	1 (0.7%)
Nervous System Disorders			
Instances per Treatment[1]	**0**	**1/1 (3.5%)**	**1/1 (0.7%)**
Syncope vasovagal	0	1 (3.5%)	1 (0.7%)
Skin and Subcutaneous Tissue Disorders			
Instances per Treatment[1]	**1/1 (0.9%)**	**0**	**1/1 (0.7%)**
Subcutaneous haemorrhage	1 (0.9%)	0	1 (0.7%)

[1]One treatment may have more than one associated adverse event. The instances per treatment indicate the number of associated adverse events and the number of treatments. The percentage is calculated based on the number of treatments.

When not specified, the number of incidents equalled the number of treatments.

Adverse events have been coded using the MedDRA dictionary. System Organ Class (SOC)

ADR: adverse drug reaction

Relationship to treatment was categorised as "definite", "probable", "possible", "unlikely", and "not related". AEs classed as "definite", "probable", and "possible" are included in this table.

Preferred terms (PT) are presented.

Serious adverse event (SAE) / deaths / other significant events

Overall the rate of serious ADRs was low. Out of a total of 11 SAEs, three were judged as related to administration of Holoclar (serious ADRs). All three (corneal perforation, ulcerative keratitis and vasovagal syncope) were reported in study HLSTM02. The case of vasovagal syncope occurred the same day of the surgical procedure and together with an episode of acute eye pain (judged as non-serious ADR), representing the most probable cause of the vasovagal reaction, and therefore was considered to be possibly related to Holoclar treatment procedure as a whole. The second case was a child experiencing 2 serious ADRs of ulcerative keratitis and corneal perforation 7 months post-treatment. A causal relationship was considered probable.

The other SAEs included once case each of corneal oedema, corneal perforation, optic atrophy, retinal detachment and foot fracture.

In addition, there were 3 cases of death all relating to malignancies (progression of gastric carcinoma, brain tumour and lung cancer) in patients aged 53, 53 and 62 years, all occurring in study HLSTM01. None of the mortalities were considered related to the treatment procedure.

Laboratory findings

Standard clinical laboratory evaluations were not performed as local treatment with Holoclar was not considered likely to cause abnormalities in laboratory parameters, unless there was a post-operative complication, such as infection or immune system disorder. However, data for pre-treatment viral infections were available for the majority of patients in studies HLSTM01 and HLSTM02. Of the evaluated patients, three patients were positive for hepatitis B surface antigen and one patient was positive with respect to hepatitis C virus. All tested patients were negative with respect to the presence of anti HIV-1/HIV-2 antibodies.

Safety in special populations

Paediatric population

No AEs prior to treatment had been reported in the 5 paediatric patients included in the two retrospective studies. Post-treatment AEs were observed in two paediatric patients. One AE of glaucoma occurred in one patient in HLSTM01 and two AEs were observed in one patient in HLSTM02 (corneal perforation and ulcerative keratitis). Both boys were treatment failures.

Elderly patients

Fourteen patients (10.6% of the study population) were above 65 years of age and of these 2 were very elderly (75-84 years old). No AEs were reported in the elderly population prior to treatment in study HLSTM01. Two adverse events reported prior to treatment in HLSTM02 were detected in elderly patients.

The incidence of AEs in elderly patients was in the same range as the incidence in the overall adult population (71% versus 65% overall in study HLSTM01, and 57% versus 65% overall in study HLSTM02). The percentage of patients above 65 years of age with eye-related ADRs was comparable to the overall population in study HLSTM01 (14 versus 15% overall) and was slightly higher in study HLSTM02 with 43% versus 34% overall.

Use in Pregnancy and Lactation

There is no data available on safety of use during pregnancy or breast-feeding.

Overdose / drug abuse

Overdose and drug abuse of Holoclar was considered unlikely to occur due to the nature of the treatment. There was however a possibility of overdose and abuse of the concomitant anti-inflammatory and antibiotic medication administered systemically and topically after treatment. Reference is made to standard precautions for the use of the concomitant medications, as specified in the relevant product information. In addition, surgeons will receive training including a user manual.

Safety related to drug-drug interactions and other interactions

Formal studies on drug-drug or drug-food interactions were not conducted. As Holoclar is administered and engrafted locally, no interactions with systemically administered drugs were expected.

Holoclar treatment includes the use of concomitant anti-inflammatory and antibiotic medication administered systemically and topically after treatment. Surgeons will be appropriately trained to determine which treatment regimen is appropriate (see risk management plan, section 2.8.). With regards to drug-drug and drug food interactions of these concomitant medications, standard conditions of use as specified in the respective product information apply.

Finally, preservative-free eye drops were used in the study and thus the interaction with preservatives in topically administered eye drops has not been studied.

Discontinuation due to adverse events

Since there was no prospective intervention, there was no set study duration and therefore no definition for study withdrawal. For the purposes of the retrospective analyses, patients followed up for less than 6 months were considered study withdrawals. Patients who chose not to attend follow-up visits were documented as discontinuing the study.

In study HLSTM01, of the initial 113 treatments, 2 (1.8%) treatments resulted in study withdrawals and 9 (8.0%) additional treatments resulted in discontinuation of follow-up after 6 months. In the case of HLSTM02, one of the 29 patients on study was considered to have withdrawn as the last recorded visit was 3 days following treatment. There were 2 patients who experienced an ADR (eye pain and subconjunctival haemorrhage) and subsequently withdrew or discontinued. None of the patients who withdrew or discontinued, except for the patients who died, were reported to have experienced a SAE. Overall, the number of withdrawals and discontinuations was low, and did not raise concerns.

Post marketing experience

No post-marketing data were available.

2.6.1. Discussion on clinical safety

The clinical safety database consisting of 135 patients and 142 treatments and including long-term follow-up data up to 10 years was considered by the CAT in principle sufficient to support the safety evaluation of Holoclar, taking into account the rarity of the target disease. There were only limited data available in paediatric patients and patients of 65 years of age and older and thus no definite conclusions could be drawn in these populations. However, no safety concerns arose from the available data in either population and the incidence of adverse

events in elderly patients was in the same range as the incidence in the overall adult population. Furthermore, additional long-term data beyond 2 years of follow-up after ACLSCT were considered necessary including data from routine clinical practice in order to confirm the safety profile of Holoclar. These data should be collected post-authorisation.

Supportive safety data from study HLMST04 overall confirmed the safety profile observed in studies HLMST01 and HLMST02.

In general, the CAT agreed to the safety evaluations performed by the applicant. Consideration of the Holoclar treatment procedure as a whole including surgical intervention as well as the prophylactic anti-inflammatory and antibiotic regimen was considered adequate. The prophylactic use of both systemic and topical anti-inflammatory and antibiotic therapies was essential to ensure Holoclar treatment success, as surgical procedures are routinely associated with local ocular inflammatory sequelae or local infection, which may have a negative impact on the Holoclar graft.

The CAT pointed out that the primary safety evaluations should be performed on all patients receiving their first treatment with the product up to the time of any subsequent intervention, whereas repeat ACLSCTs and other subsequent surgical procedures should be assessed separately. Additional analyses were performed by the applicant and overall did not give rise to new concerns.

The absence of a control arm hampered the interpretation of the safety of the treatment. Comparison with other, though limited treatment options was difficult as outcome parameters varied between the studies reported in the scientific literature and due to the presence of various variables such as the underlying cause of LSCD, data collection, different study designs and surgical procedures as well as autologous or allogeneic type of LSC. Nevertheless, compared to allogeneic treatment options, the risk of graft rejection could be expected to be reduced with Holoclar due to the autologous nature of Holoclar LSCs. Furthermore, ex vivo expansion should help to reduce the invasiveness of the biopsy and, thus, the risk of iatrogenic induction of LSCD in the fellow, donor eye.

The majority of ADRs reported were related to eye disorders (conjunctival haemorrhage, eye haemorrhage, blepharitis, corneal epithelium defect, eye pain, glaucoma, ulcerative keratitis, conjunctival adhesion, conjunctival hyperaemia, corneal oedema, corneal perforation, eye irritation, and photophobia). Five additional ADRs were reported in other SOCs with one report each for corneal infection, vasovagal syncope, metaplasia of the implant, suture rupture, and subcutaneous haemorrhage. All ADRs have been listed in SmPC section 4.8.

The overall rate of serious AEs observed was low. For most of the reported SAEs no relationship to Holoclar treatment was considered by the investigator and only 3 events, corneal perforation, ulcerative keratitis and vasovagal syncope, observed in 2 patients and treatments were considered treatment-related.

Amongst the SAEs were 3 cases of death due to cancer. These cases were not considered causally related to treatment and although there was no evidence for an increased risk of tumorigenicity, neither from non-clinical nor from clinical data, the CAT requested that patients should be closely monitored to further elucidate a potential risk of tumour formation.

When comparing the two studies HLSTM01 and HLSTM02, a discrepancy in the reporting rates was observed, whereby a greater number of ADRs were reported for study HLSTM02 (34.5%) as compared to HLSTM01 (16.8%) and all three serious ADRs occurred in HLSTM02. An even higher rate of ADRs (50%) was reported in the publication by Marchini et al. (2011). This difference might have been partially due to chance in light of the smaller population size in HLSTM02 and Marchini et al. (2011). More importantly, however, the difference may be due to the limited experience with ACLSCT at the sites of HLSTM02 and Venezia and Verona (Marchini et al.,

2011), who conducted far less procedures compared to HLSTM01 study centers. Therefore, appropriate risk minimisation measures will be put in place including approaches to ensure adequate and comparable levels of experience across the centres that perform the ACLSCT (see also section 2.8.).

The CAT furthermore noted that reports relating to corneal epithelial defects may be interlinked to treatment failure of Holoclar as epithelial integrity was part of the composite primary endpoint of study HLSTM01. However, the incidence of corneal epithelial defect AEs was relatively low and did not necessarily compromise the clinical outcome. A clear distinction has to be made between stable or recurrent defects, which are usually also associated with other signs of chronic LSCD, and a single, self-limiting, temporary episode of epithelial defect, which could be due to a concomitant clinical condition or to a not yet stabilised epithelium after ACLSCT.

There were also several reports of blepharitis and cataract, all of which occurred more frequently in older age range. None of these events has been judged as related to treatment. Thirty-three adverse events of blepharitis were reported after 142 ACLSC treatments. Blepharitis is in fact a common condition of mainly older adults. While a reactivation of blepharitis due to surgery may be conceivable in individual cases, an induction of blepharitis by the Holoclar graft itself was considered unlikely. Overall, the reported frequency of blepharitis was below that reported in the scientific literature in patients with eye disorders (34% versus 37-47%, Lemp et al., 2009). Regarding the cases of cataract, these were mostly reported in patients of 65 years of age and older and a causal relationship to Holoclar product was considered unlikely. Although long-term use of corticosteroids is known to increase the risk of posterior subcapsular cataract formation, a causal relationship was considered unlikely, since according to Jobling et al. (2002), posterior subcapsular cataract only develops after patients have been on high dose steroid treatment for longer than one year, whereas those on doses less than 10mg/day of prednisone or equivalent were unlikely to develop lenticular changes. The dosage of corticosteroid therapy as defined in the Holoclar treatment protocol was a maximum of 0.5mg/kg per day for 2 weeks followed by a tapering of additional 2 weeks and thus has to be regarded as relatively low.

With regards to glaucoma, the reason for a higher prevalence of a glaucoma with increased age could be explained by structural changes affecting the outflow of aqueous humour as well as vascular alterations. Furthermore, older patients may be more sensitive to the influence of corticosteroid treatment. The frequency of glaucoma and increases in intraocular pressure (IOP) after corticosteroid treatment (7 out of 142 Holoclar treatments) was in line with literature reports (Armaly, 1965 and Becker, 1965). However, the risk of increased IOP and glaucoma associated with use of corticosteroid treatment was generally regarded to be rather low due to the limited exposure foreseen in the Holoclar treatment protocol, and provided no predispositions exist. While cases of increased IOP even have been described in the literature even for short-term use of corticosteroids, normally these are manageable and IOP returns to baseline after discontinuation of treatment. Surgery itself was considered unlikely to represent an additional risk factor, but it was noted that the risk to experience glaucoma may be altered in patients suffering from an increased IOP due to the concomitant therapy following keratoplasty. Notably, in the retrospective studies, the term glaucoma was used inclusively, including in addition to glaucoma increased IOP. Future prospective data collection (prospective study HLSTM03 and registry) will distinguish between these two term and thus enable to investigate the risk for IOP increased and glaucoma.

Regarding preceding keratoplasties, it could not be excluded that alloreactions and inflammatory conditions due to previous surgical procedures had an impact on the safety profile of Holoclar. In fact, more TEAEs associated with immune system disorders and transplant rejections were reported in patients with prior keratoplasty. As the transplanted cells in Holoclar are autologous, a possible allo-immunereaction would not be directed against the cells themselves. Due to the limited available information, a final judgement in view of the relation between the reported immune system disorders and Holoclar treatment could not be made. On the whole, a potential risk of antigenicity of the Holoclar draft was not supported by clinical findings, where repeated treatment of patients

did not lead to a subsequent rejection of the transplant and since the success rate of second or third transplants of the product was not worse than that of initial transplants. Furthermore, roughly half of the safety population had keratoplasties subsequent to Holoclar treatment. Rather than having been caused by a treatment failure of Holoclar, the need for corneal transplantation arose from deep stromal scarring in these patients and hence did not raise a safety concern.

As for the antibiotic prophylaxis, a lower grade of successful transplantations has been observed in the subgroup of patients who received gentamycin. Corneatoxicity of gentamycin has been shown in tissue culture and in animal models. In view of the small patient number, no clear conclusion could be drawn regarding a possible detrimental effect of gentamycin on Holoclar treatment outcome. Generally, a recommendation to avoid use of cytotoxic agents has been added to the product information, as these may affect cell growth and damage the newly-regenerated corneal epithelium. This also includes eye drops containing benzalkonium chloride or other preservatives, albeit not studied.

On the whole, side effects of the prophylaxis regimen were considered manageable.

Additional safety data needed in the context of a conditional MA

In line with the discussions on efficacy aspects in section 2.5.3. , for a comprehensive clinical dataset, prospectively collected data are required to confirm the safety profile of Holoclar. Centres included in the retrospective analyses applied different methods for data collection and in particular post-treatment patient management and follow-up and this may have affected the safety evaluation. The data from the required prospective trial will thus address some of the deficiencies of the safety database resulting from the retrospective study design and enrich the database both in size and with regard to long-term follow-up.

2.6.2. Conclusions on the clinical safety

The safety evaluation was based on a comprehensive analysis covering the time from biopsy procedure for limbal stem cell harvest through transplantation of Holoclar up to the end of follow up, with particular attention to a possible relation of adverse events with concomitant anti-inflammatory and antibiotic medications. Adverse reactions observed were mainly eye related and generally manageable. From the data submitted, no major safety concerns emerged.

Overall, the CAT was of the view that safety profile of Holoclar was acceptable.

However, the CAT considers the following measures necessary to address the missing safety data in the context of a conditional MA:

Multinational, multicentre, prospective, open-label, uncontrolled interventional study (HLSTM03) to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns.

The CAT furthermore considered the following measures necessary to address issues related to safety:

- Long-term safety and efficacy follow-up after autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns (HLSTM03-FU)
- Post-authorisation Registry entitled ‘Long-term safety after Holoclar implant for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns: observational study of routine

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

2.7. Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CAT and CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

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

The PRAC considered that the risk management plan version 4 would be acceptable provided the applicant would amend the category of study HLSTM03FU in the summary table of PhV activitities (i.e. instead of category 2, the study should be category 3). PRAC endorsed PRAC Rapporteur assessment report is annexed.

The CAT endorsed this advice without changes.

The applicant implemented the changes in the RMP as requested by PRAC and has submitted a updated RMP (version 6).

The CAT endorsed the Risk Management Plan version 6 with the following content:

Safety concerns

Important identified risks	• Glaucoma • Lack of effect manifesting as corneal epithelium defect
Important potential risks	• Blepharitis • Concomitant use of eye drops containing benzalkonium chloride • Post-implant infection • Medication errors (e.g. incorrect patient receives product, patient receives incorrect product, incorrect surgical technique) • Tumorigenicity • Off label use o milder form of limbal stem cell deficiency than the proposed indication (moderate-severe) o Off label use for other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis o Off label use in patients under 18 years
Missing information	• Pregnancy and breast-feeding • Children (limited data) • Elderly (limited data) • Re-administration of Holoclar • Long term safety and efficacy follow up

Pharmacovigilance plan

Study/activity Type, title and category (1-3)	**Objectives**	**Safety concerns addressed**	**Status (planned, started)**	**Date for submission of interim or final reports (planned or actual)**
Study HLSTM03 Multinational, multicentre, prospective, open label, uncontrolled clinical study to evaluate the efficacy and safety of autologous cultivated limbal stem cells grafting	To evaluate efficacy and safety of one or two Autologous Cultivated Limbal Stem Cell Implantation(s) (ACLSCT) in restoring a normal corneal epithelium in patients suffering from	• Missing information (children limited data) • Glaucoma • Lack of effect (corneal implant failure) • Blepharitis • Long-term safety and efficacy	Planned	December 2020

Study/activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns. Patients from 2 years of age and adults will be included in the study. (Category 2)	moderate-severe Limbal Stem Cell Deficiency (LSCD) secondary to ocular burns.			
Study HLSTM03FU Long-term safety and efficacy follow-up after autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns (Category 3)	All patients from Study HLSTM03will be rolled over into this study to evaluate the long term safety and efficacy (visit every 6 months) and success after keratoplasty (whenever clinically indicated)	Long-term safety and efficacy	Planned	January 2023
Post-authorisation Registry entitled"Long-term safety after Holoclar® implant for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns: observational study of routine clinical practice." (Category 3)	Primary Objective To evaluate the long-term safety profile of patients treated with Holoclar® during a 5-year follow-up period from first ocular implantation under routine clinical conditions, through the description of the occurrence of adverse events, adverse drug reactions, serious adverse events and adverse events of special interest. Adverse events of	Identified Risks Glaucoma Lack of effect manifesting as corneal epithelium defect Potential Risks Blepharitis Concomitant use of eye drops containing benzalkonium chloride Post-implant infection Medication errors (e.g. incorrect patient	Planned	Final report: June 2023 Interim reports will be provided after each year of the observation period.

Study/activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
	special interest will be solicited and carefully monitored. <u>Secondary objectives</u> To describe demographic and clinical characteristics of patients undergoing one or more Holoclar® implants including the occurrence of ocular grafts preceding the investigated implant. To describe the proportion of success, according to clinician's opinion, one year after implant, among patients undergoing one or more Holoclar® implants. To describe visual acuity during a 5-year follow-up from first implant. To describe quality of life, as measured by EuroQol-Five Dimensions (EQ-5D) and National Eye Institute 25-Item Visual Function Questionnaire (NEI VFQ-25), during a 5-year follow-up from first implant. To describe the administered post-implant surgical treatment, including keratoplasty.	receives product, patient receives incorrect product, incorrect surgical technique) Tumorigenicity Off label use in milder form of limbal stem cell deficiency than the proposed indication (moderate-severe). Off label use for other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis. Off label use in patients under 18 years. <u>Missing Information</u> Pregnancy and breast-feeding Children (limited data) Elderly (limited data) Re-administration of Holoclar Long term safety and efficacy follow up		

Study/activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
	Evaluation of the effectiveness of the risk minimisation measures in compliance with the Risk Management Plan for Holoclar®.			

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
1) Glaucoma	Patients should be warned of all possible adverse events to Holoclar and post-operative anti-inflammatory drugs during the consent process. Section 4.4 of the SmPC states "The procedure of Holoclar administration includes the use of antibiotics and corticosteroids. The physician is invited to consult the SPC of these concerned products." Glaucoma is included with a common frequency within section 4.8 of the SmPC. In addition, this section states that glaucoma (2.1%) was the most frequent adverse reaction related to the corticosteroid treatment.	Educational Manual for healthcare professionals and Patient Information Guide describe the required post-implant treatment with corticosteroids. Key safety information is provided for those products. Glaucoma is also listed as a common adverse reaction in both documents.
2) Lack of effect manifesting as corneal epithelium defect	The risk is listed in section 4.4 of the SmPC Special warning and precautions advising that where possible concomitant eye problems should be corrected prior to implantation. Section 4.3 of SmPC contraindicates patients with hypersensitivity to any of the excipients. Hypersensitivity to any of the residues from the manufacturing	Educational Manual for healthcare professionals has a section on exclusions and precautions that advises of eye conditions which should not be present or be corrected prior to Holoclar administration. Corneal epithelial defect is also

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	process such as bovine serum and murine 3T3-J2 cells is also a contraindication. Corneal epithelium defect is included with a common frequency in section 4.8 of the SmPC. Patients should be warned of all possible adverse events including implant rejection during the consent process. The product is only available as a Prescription only medicine. Section 4.2 of the SmPC clearly states that product must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Surgeons will only be allowed to use the product once they have been trained.	included as a common adverse reaction.
3) Blepharitis	Blepharitis is included with a common frequency within section 4.8 of the SmPC.	Educational manual for healthcare professionals and Patient Information Guide includes blepharitis/inflamed eyelid as an adverse reaction/side effect.
4) Concomitant use of eye drops containing benzalkonium chloride	Section 4.5 of the SmPC Interaction with other Medicinal Products and other forms of Interaction advises to avoid use contains the following 2 sentences. Eye drops containing benzalkonium chloride, and/or other preservatives must be avoided. Benzalkonium chloride (as well as other quaternary ammonium compounds) is cytotoxic and eye drops containing this preservative may damage the newly-regenerated corneal epithelium. Other cytotoxic agents must be avoided. Section 2 of the package leaflet warns patients not to use eye drops containing benzalkonium chloride.	Warnings to avoid the use of eye drops containing the preservative benzalkonium chloride are included in the Educational Manual for healthcare professionals and in the Patient Information Guide.

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	The product is only available as a Prescription only medicine. Section 4.2 of the SmPC clearly states that product must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.	
5) Post-implant infection	Section 4.2 of the SmPC states: Post-operative treatment Following implantation, an appropriate regimen of topical and systemic anti-inflammatory and prophylactic antibiotic treatment must be given. The following regimen is suggested: Doxacycline 100 mg tablets twice daily (or amoxicillin 500 mg twice daily) should be administered from the day of surgery for 2 weeks. Section 4.8 of the SmPC includes corneal infection as an uncommon adverse reaction. The product is only available as a Prescription only medicine. Section 4.2 of the SmPC clearly states that product must be administered by an appropriately trained and qualified qualified surgeon and is restricted to hospital use only.	Educational Manual for healthcare professionals and Patient Information Guide contain details about prophylactic post-operative treatment with antibiotics and the uncommon side effect of corneal infection/eye infection are included in both documents.
6) Medication errors (e.g. Incorrect patient receives product, Patient receives incorrect product, Incorrect surgical technique)	Section 4.1 of the SmPC clearly states the intended indication for Holoclar. Section 4.4 of the SmPC includes a paragraph advising of concomitant eye problems that should be corrected prior to Holoclar implantation. Section 4.2 of the SmPC describes the method of administration to be followed and states that Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.	The Educational Manual for healthcare professionals contains detailed instructions on who should receive Holoclar, the contraindications for its use and the correct surgical procedures to prevent medication errors.

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	Section 4.4 of the SmPC states: Holoclar is an autologous product and should under no circumstances be administered to anyone other than the donor patient. Section 6.6 of the SmPC Special precautions for disposal and other handling states that prior to implantation the patient's name should be carefully checked with the patient/donor identification on the shipment documentation and product container. The product is only available as a Prescription only medicine.	
7.) Tumorigenicity	None proposed.	None proposed.
8). Off-label use 1. Milder form of limbal stem cell deficiency than the proposed indication (moderate-severe) 2. Other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis 3. Patients under 18 years	Section 4.1 of the SmPC defines the indication as treatment of patients older than 18 years of age with moderate- to severe limbal stem cell deficiency (defined by the presence of superficial corneal neovascularisation in at least two corneal quadrants, with central corneal involvement, and severely impaired visual acuity), unilateral or bilateral, due to physical or chemical ocular burns. A minimum of 1-2 mm^2 of undamaged limbus is required for biopsy. Section 4.2 of the SmPC states that the product must be administered by an appropriately trained and qualified surgeon and it is restricted to hospital use only. Section 4.2 of the SmPC and section 2 of the package leaflet alert prescribers and patients that the safety and efficacy of Holoclar in children and adolescents aged 0 to 18 years has not yet been established. The product is only available as a Prescription only medicine.	The Educational Manual for healthcare professionals includes sections on how to select patients for treatment and how to assess the severity of the limbal deficit.

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
9). Pregnancy and breast-feeding	Section 4.6 of the SmPC states: Pregnancy There are no data for the use of Holoclar in pregnant women. Animal studies are not available with respect to reproductive toxicity. As a precautionary measure, since the requirement of the post-operative pharmacological treatment, it is preferable to avoid the use of Holoclar during pregnancy. Breast-feeding As a precautionary measure, Holoclar is not recommended for implant during breast-feeding. A cautionary warning is given in section 2 of the package leaflet not to use the product in pregnancy or breast-feeding. The product is only available as a Prescription only medicine. Section 4.2 of the SmPC clearly states that product must be administered by an appropriately qualified surgeon and is restricted to hospital use only.	None
10). Children (limited data)	Section 4.1 of the SmPC defines the indication as treatment of patients older than 18 years of age. Section 4.2 of the SmPC states: The safety and efficacy of Holoclar in children and adolescents aged 0 to 18 years has not yet been established. Section 4.8 of the SmPC states: Paediatric population There is no information on the safety of Holoclar in children up to 7 years of age and only limited information in patients 8 – 17 years of age. In the paediatric patients included in the studies	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	HLSTM01 (age 13, 14 and 16 years) and HLSTM02 (age 8 and 14 years) the profile of undesirable effects was not different from the adult population	
11). Elderly (limited data)	Section 4.2 of the SmPC states that data on the use in elderly populations are limited. Currently available data are described in 4.8 and 5.1 but no recommendation on a posology can be made. Section 4.8 of the SmPC states there is only limited information in elderly (n=12, >65 year old) and very elderly (n= 2, 75-84 year old) patients. The following paragraph is included in Section 5.1 of the SmPC. The HLSTM01 study enrolled a total of seven patients (6.2% of the study population) with an age at baseline of 65 years or above, and seven additional patients (25.0%) were included in HLSTM02. Although limited with regard to the number of subjects, data from both studies showed a success rate around 70% of treated cases in the elderly population. This level of efficacy is similar to that observed in the treated patients overall.	None
12). Re-administration of Holoclar	None	None
13.) Long-term safety and efficacy	None	None

The CHMP endorsed the PRAC and CAT advice on the RMP with a minor clarification of the wording of the key elements of the additional risk minimisation measures.

2.9. Product information

2.9.1. User consultation

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

However, as there were clarifications unavailable regarding recruitment, time aspects, procedural aspects,

interview aspects, evaluation of responses, and data processing for the report submitted by the applicant and since the product information has been amended substantially during the assessment of this application, the applicant is recommended to submit the results of an additional abridged user consultation with target patient groups on the package leaflet (10 patients) that meets the criteria for readability within 6 months after approval.

3. Benefit-Risk Balance

Benefits

Beneficial effects

The therapeutic approach of using autologous ex-vivo expanded limbal stem cells for treatment of LSCD offers several advantages compared to alternative methods for ocular surface reconstruction, such as limbal allografts with an associated risk of rejection requiring long-term systemic immunosuppression, or non-expanded limbal autografts from the healthy fellow eye which may lead to iatrogenic induction of LSCD in the donor eye. Successful reconstruction treatment is reflected by the restoration of a stable corneal epithelium with resolution of epithelial defects, regression of corneal vascularisation, and absence of conjunctivalisation.

For Holoclar transplants, treatment success has been shown in retrospective analyses of a total of 133 patients receiving 142 transplantation during 1998 to 2007 with additional supportive data provided for 15 patients treated from 2008 to 2013. Overall, in the pivotal study HLSTM01, 72% (75/104) of all analysed patients were considered a treatment success based on achievement of a stable corneal epithelium without significant recurrence of neovascularisation 12 months after surgery. Long-term data up to 10 years, although limited, suggested persistence of the effect. Overall, this was a convincing outcome considering that LSCD would not be expected to improve spontaneously.

Clinically meaningful outcomes for LSCD patients were furthermore the improvements of ocular symptoms and visual acuity. Most of the patients showed a stable clinical picture at baseline, and only a limited number had ocular symptoms (pain, photophobia, burning, bulbar or limbal hyperaemia). Treatment with Holoclar maintained the stable clinical picture or resulted in an improvement and/or resolution of manifestations as shown by a reduction of the number of patients with symptoms as well as a decrease in the intensity of ocular pain, burning, and photophobia. Furthermore, clinically relevant improvement of visual acuity of 3 lines on the vision chart was observed in 38.5% (40/104) of the patients and half of the patient with off-chart vision at baseline gained on-chart vision after Holoclar treatment.

Unsurprisingly, improvements in vision were achieved in more patients without stromal scarring compared to those with deep stromal injury. However, within the latter group of patients, vision improved to a similar extend [3 line gain in 37.5% (21/56) of patients] after corneal transplantation. It was furthermore found that Holoclar treatment increased the chance for subsequent successful keratoplasty, which was another therapeutically meaningful achievement in the group of patients with deep stromal scarring. Post-Holoclar keratoplasty was successful in 42% (24/57) of patients as well as in half of all patients who had a failed corneal transplantation prior to Holoclar.

Uncertainty in the knowledge about the beneficial effects.

With respect to the nature of the data provided in support of a beneficial effect of Holoclar, several uncertainties remained. Studies based on retrospective data collection are prone to numerous biases, including selection,

documentation and evaluation bias, thus weakening the evidence that can be obtained from such data. As the Holoclar studies did not enrol all patients treated with the product in the past, selection bias could not be excluded and may have contributed to an overestimation of the effect size. However, it was considered reassuring that those centres participating in the studies provided access to all available patient data and that additional data, collected retrospectively from 2008 to 2013 as well as from publications by two additional centres which did not participate in the Holoclar studies, confirmed the beneficial effects seen in the pivotal trial. Furthermore, an inspection of study sites confirmed the quality of the retrospective clinical data as being suitable for evaluation.

The uncontrolled, non-randomised and open-label design aspects, although considered unavoidable due to the lack of a suitable control group, added further to the uncertainties of the validity of the study outcome. Uncertainty was also created by the limited size of the study population and the limited long-term data. Thus, due to the small number of paediatric and older patients treated, no final conclusions could be drawn for these populations.

Available data in paediatric and elderly patients were considered insufficient to draw final conclusions in the populations. However, at least in the elderly, despite limited subject numbers, data from both studies showed a success rate similar to that observed in the overall population.

Furthermore, soon after surgery some patients experienced clinically significant hyperaemia, which later resolved. The data suggested that patients developing early significant limbal hyperaemia and epithelial defects are at higher risk of treatment failure. At the same time, some level of inflammation is required to support the normal healing process. Altogether, it was recommended to defer treatment in patients with acute, uncontrolled inflammation.

Finally, cell density and potency of the product were controlled by the specifications during the manufacturing process. However, the specifications for LSC were rather wide (0.4% to 10%) and the CAT considered that future prospective data collection should further investigate the impact on efficacy of the amount of LSC in the final drug product as well as explore new markers for the active substance.

Risks

Unfavourable effects

Eye-related disorders were the most commonly observed adverse events occurring in 57% of the safety population. The most commonly experienced ADRs were conjunctival haemorrhage, corneal epithelial defects consistent with treatment failure, eye pain and haemorrhage, and blepharitis.

The overall rate of serious ADRs with three cases in the entire study population was regarded as low. However, an imbalance in the reporting rates of adverse events and reactions was observed between studies. Far less adverse events occurred in study HLMST01, involving 2 experienced clinical sites, compared to other centres with less practice, analysed in study HLMST02 or reported by Marchini et al. (2011). Therefore, measures to ensure adequate and comparable levels of experience across treating centres were required.

Several adverse effects were related to the surgical intervention including conjunctival haemorrhage. The risk of local ocular inflammation or infection was mitigated by a prophylactic anti-inflammatory and antibiotic regimen combining both topical non-cytotoxic and systemic treatments. However, these concomitant treatments may cause adverse reactions themselves. In this context, events of glaucoma occurred within 3 months of ACLSCT and were considered related to the use corticosteroids.

Uncertainty in the knowledge about the unfavourable effects

Overall, the safety database, albeit limited due to the rarity of the target condition, was considered sufficient to assess the safety profile of Holoclar. However, no definite conclusions could be drawn with regards to the safety in children and patients of 65 years of age and older as too few data were available. Some information on the safety of Holoclar up to 10 years after ACLSCT were available, but additional data was needed to confirm the long-term safety profile. These data will be collected post-authorisation by means of a follow-up study of HLSTM03 as well as through a registry which will collect data from routine clinical practice.

As Holoclar is a cell-based medicinal product, a risk of tumorigenicity due to neoplastic transformations of the cells could not be excluded. Therefore, and although no evidence of tumor formation due to Holoclar treatment could be derived from non-clinical or from clinical data, close monitoring of patients was recommended.

Benefit-risk balance

Importance of favourable and unfavourable effects

Holoclar treatment resulted in the majority of patients in a successful ocular surface reconstruction, maintaining a stable clinical picture or resulting in an improvement and/or resolution of LSCD manifestations, including symptoms and visual acuity. The improvement of ocular symptoms was a relevant clinical outcome in particular for patients with moderate LSCD, where a small structural improvement by itself would be of limited clinical relevance. Furthermore, clinically relevant vision gains were achieved in a subset of patients including regaining of on-chart vision, which is of relevance in a population where the majority of patients are legally blind. Furthermore, Holoclar was shown to increase the likelihood for a successful subsequent keratoplasty in patients with deep stromal scarring. Albeit data were collected retrospectively and uncontrolled, these results were highly clinically relevant considering that moderate to severe LSCD is a condition that would not improve spontaneously. In this context, Holoclar addresses an unmet medical need considering that no treatments for LSCD had been approved for marketing in the EU at the time of this report. Furthermore, an ex-vivo expanded autologous product was considered advantageous compared to alternative, allogeneic treatment methods, requiring systemic immunosuppression.

Eye-related disorders were the most commonly observed adverse events, with the most commonly experienced adverse drug reactions comprising conjunctival haemorrhage, corneal epithelial defects consistent with treatment failure, eye pain and haemorrhage, and blepharitis. The majority of the adverse effects were manageable and the overall safety profile of the Holoclar treatment procedure was regarded acceptable.

Benefit-risk balance

Treatment with Holoclar allowed successful ocular surface reconstruction with improvements in symptoms and visual acuity in patients with moderate to severe LSCD. Clinically relevant outcomes were observed both in patients with and without deep stromal injury. These favourable effects were considered by the CAT to outweigh the risks of mainly ocular adverse reactions, which were generally manageable. By providing training to treating physicians including a detailed treatment protocol recommending effective anti-inflammatory and anti-infective prophylaxis, it is expected that risks of adverse reactions can be further reduced.

In conclusion, the benefit-risk balance for Holoclar in the treatment of adult patients with moderate to severe limbal stem cell deficiency (defined by the presence of superficial corneal neovascularisation in at least two corneal quadrants, with central corneal involvement, and severely impaired visual acuity), unilateral or bilateral, due to physical or chemical ocular burns and with a minimum of 1-2 mm^2 of undamaged limbus for biopsy, is considered favourable.

Discussion on the benefit-risk balance

The dataset provided in support of this application was considered unique and relatively large considering the rarity of the target condition. Despite the inherent disadvantages of a retrospective, uncontrolled and non-randomised study design, the quality of the dataset was considered adequate and the study results were compelling in demonstrating a clinically relevant benefit, considering that LSCD is a condition that would not spontaneously improve. Nevertheless, for a comprehensive clinical dataset, the retrospective data need to be supplemented by a set of prospectively generated results to confirm the benefit observed in the retrospective analysis and to enrich the clinical database.

Uncertainties also existed with regards to long-term safety and efficacy as well as in the paediatric population and patients of 65 years and older. However, no major concern arose from the available data in the paediatric and older patient groups, and for older patients similar effect sizes and safety profile compared to the overall population. Thus, use of Holoclar in adults patients is supported. Additional data for patients between 3 and 17 years of age should be collected in a future study.

The applicant applied for consideration of this application for a conditional marketing authorisation. Holoclar falls under the scope of conditional marketing authorisation as per Article 2 of Regulation (EC) No 507/2006 since Holoclar was granted an orphan designation in the claimed indication. Furthermore, moderate to severe forms of LSCD are serious debilitating if left untreated, leading to severe reduction or complete loss of vision. With regards to the acceptability of the request, the CAT considered the following points:

- The benefit-risk profile of Holoclar was judged positive. Despite uncertainties of the validity of the clinical data due to the retrospective design of the studies, the data were considered to be of sufficient quality to support establishment of a beneficial treatment effect as well as to conclude on an acceptable safety profile.
- It is likely that the applicant will be in a position to provide comprehensive clinical data. For a comprehensive clinical dataset, prospectively collected data are needed in order to confirm the treatment benefits observed in the retrospective analyses. To this end, the applicant will conduct a prospective multinational, multicentre, open label, uncontrolled study in patients with moderate to severe LSCD secondary to ocular burns, which was considered acceptable by the CAT. Fourteen countries in the EU/EEA were identified and given the experience from previous clinical use, an enrolment period of 12 months was considered feasible.
- Unmet medical needs will be fulfilled. At the time of this report, there were no approved medicinal products available in the EU/EEA for treatment of LSCD. Furthermore, the Holoclar treatment procedure was considered advantageous to alternative treatment methods including penetrating keratoplasty, which by itself cannot resolve the LSC deficiency, or allogeneic grafts, which require chronic systemic immunosuppression. In addition, ex-vivo expansion allows reduction of the biopsy size, which in turn reduces the risk of damaging the healthy eye.
- The benefit to public health of the immediate availability of Holoclar outweighs the risk inherent to the incomplete dataset. Holoclar has been shown to enable successful ocular surface reconstruction, thereby maintaining a stable clinical picture as well as restoring vision and improving symptoms in a subset of patients. A spontaneous healing and functional improvements in moderate to severe LSCD would not be expected without reconstruction therapy, leaving patients with significant clinical symptoms, such as pain, photophobia, and burning sensation, as well as impairment of visual function. At the time of this report, patients could not be treated with an approved product with an established favourable benefit-risk profile. Thus, despite the rarity of the condition, considering the serious

debilitation of patients with moderate to severe forms of LSCD, immediate availability of Holoclar was considered to benefit public health.

In conclusion, the CAT considered that all requirements for a conditional approval were met.

On the whole, the uncertainties, partially due to the need for additional data for a comprehensive clinical dataset, did not preclude a conclusion of a positive benefit-risk balance of Holoclar in the claimed indication. The applicant will perform a prospective clinical study suitable to confirm the benefit-risk profile and further address these uncertainties. The study will also further explore markers for the active substance and the relation between the amount of stem cells in the final drug product and treatment success.

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

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the risk-benefit balance of Holoclar in the treatment of adult patients with moderate to severe limbal stem cell deficiency (defined by the presence of superficial corneal neovascularisation in at least two corneal quadrants, with central corneal involvement, and severely impaired visual acuity), unilateral or bilateral, due to physical or chemical ocular burns with a minimum of 1-2 mm^2 of undamaged limbus is favourable and therefore recommends the granting of the conditional marketing authorisation subject to the following conditions described below.

Based on the draft CHMP opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Holoclar in the treatment of adult patients with moderate to severe limbal stem cell deficiency (defined by the presence of superficial corneal neovascularisation in at least two corneal quadrants, with central corneal involvement, and severely impaired visual acuity), unilateral or bilateral, due to physical or chemical ocular burns with a minimum of 1-2 mm^2 of undamaged limbus is favourable and therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription.

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

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

- **Risk Management Plan (RMP)**

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

An updated RMP should be submitted:

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

If the submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

- **Additional risk minimisation measures**

The following additional risk minimisation measures are necessary for the safe and effective use of the product:

Educational material for healthcare professionals to provide training on the appropriate use of the product and to minimise risks, addressing the key elements of:

- Patient selection
- Traceability of patients and use of identifiers
- Biopsy, implant and follow up care
- Avoiding use of eye drops containing benzalkonium chloride
- Risk of glaucoma and blepharitis
- Encouraging enrolment in the registry
- Reporting suspected side effects

The education material should also include both an Educational Manual and a training programme which will incorporate verification of physicians' comprehension of the training provided.

Educational material for patients and/or carers to address the following key elements:

- Avoiding use of eye drops containing benzalkonium chloride
- Side effects of post-transplant treatment with antibiotics and corticosteroids
- Inform patients of the registry
- Reporting suspected side effects

- **Obligation to complete post-authorisation measures**

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14(7) of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
Multinational, multicentre, prospective, open-label, uncontrolled interventional study (HLSTM03) to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns	Final CSR December 2020

The CHMP endorses the CAT conclusion on the specific obligation to complete post-authorisation measures for the conditional marketing authorisation as described above.

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

Not applicable.

These conditions fully reflect the advice received from the PRAC.

The CHMP endorses the CAT conclusion on the conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

New Active Substance Status

Based on the CAT review of data on the quality properties of the active substance, the CAT considers that 'ex vivo expanded autologous human corneal epithelial cells containing stem cells' is qualified as a new active substance.

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