EU Risk Management Plan (EU RMP) for Holoclar

RMP version to be assessed as part of this application:

RMP Version number: 12.3

Data lock point for this RMP: 26 July 2024

Date of final sign off: 13 September 2024

Rationale for submitting an updated RMP: Update the annexes of the RMP for Holoclar to align the body of the RMP already approved in the 2023 renewal granting the full marketing authorization.

Summary of significant changes in this RMP:

- Annex 3 – Protocols for proposed, on-going and completed studies in the pharmacovigilance plan: Inclusion of protocol of HOLOSIGHT study version 4.0. with update of study milestones and removal of mention of interim annual reporting to EMA after the full MA granting.
- Annex 7 - Other supporting data (including referenced material): Revision of the educational materials to align with the fact that HOLOSIGHT is no longer open for the registration of additional patients and correction of typographical errors in:
    - Annex 7.1 Healthcare professional information guide
    - Annex 7.2 Patient information guide
- Editorial changes across the sections for minor corrections.
- Harmonization with the exposure and adverse events data available at the DLP.

Other RMP versions under evaluation: Not applicable

Details of the currently approved RMP:

Version number: 12.2

Approved with procedure: EMEA/H/C/002450/R/0058
Date of approval (opinion date): 14 December 2023

QPPV name: Sonia López

QPPV oversight declaration: The content of this RMP has been reviewed and approved by the marketing authorisation holder's QPPV. The electronic signature is available on file.

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Part I: Product(s) Overview

Active substance (s) **(INN or common name)**	Ex vivo expanded autologous human corneal epithelial cells containing stem cells
Pharmacotherapeutic group (s) **(ATC Code)**	S01XA19 limbal stem cells, autologous
Marketing Authorisation Holder or Applicant	Holostem s.r.l. (hereinafter referred as Holostem)
Medicinal Products to which this RMP refers	1
Invented name(s) in the European Economic Area (EEA)	Holoclar
Marketing Authorisation procedure	Centralised
Brief description of the product including: • chemical class • summary of mode of action • important information about its composition (e.g. origin of active substance of biological, relevant adjuvants or residues for vaccines	A transparent circular sheet of 300,000 to 1,200,000 viable autologous human corneal epithelial cells (79,000 – 316,000 cells/cm^2), including on average 3.5% (0.4 to 10%) limbal stem cells, and stem cell-derived transient amplifying and terminally differentiated cells, expanded ex vivo from an autologous biopsy retained from the healthy eye or from an undamaged portion of the eye in case of bilateral injury, which are attached to a transparent supportive 2.2 cm diameter fibrin layer and maintained in the transport medium. The mechanism of action of Holoclar is the replacement of corneal epithelium and lost limbal stem cells in patients in which the limbus has been destroyed by ocular burns. During the corneal repair process, the administered stem cells are intended to partially multiply, differentiate and migrate to regenerate corneal epithelium, as well as maintaining a reservoir of stem cells that can continually regenerate the corneal epithelium.
Hyperlink to the Product Information	Product Information
Indication(s) in the EEA Current	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.

Posology and route of administration in the EEA Current	This medicinal product is intended for autologous use only. Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. <u>Posology</u> The amount of cells to be administered is dependent on the size (surface in cm^2) of the corneal surface. Each product contains an individual treatment dose with sufficient number of cells to cover the entire corneal surface. The recommended dose of Holoclar is 79,000 – 316,000 cells/cm^2, corresponding to 1 cm^2 of product/cm^2 of defect. Each preparation of Holoclar is intended as a single treatment. The treatment may be repeated according to the physician prescription. Special populations *Elderly* Data on the use of Holoclar in elderly populations are limited. No recommendation on posology can be made. *Hepatic and renal impairment* Data on the use of Holoclar in patients with hepatic and renal impairment are not available. *Paediatric population* The safety and efficacy of Holoclar in children and adolescents aged 0 to 18 is limited. No recommendation on posology can be made. <u>Method of administration</u> For implantation Full technical details on the procedures associated with the use of Holoclar are provided in the educational manual. *Biopsy* For the manufacture of Holoclar, a biopsy of 1-2 mm^2 of undamaged limbus is required. The biopsy is performed using topical anaesthesia. The eye is subjected to ocular surface lavage with commercially available sterile balanced salt solution for eye irrigation followed by detachment of the conjunctiva from the limbus to expose the sample collection site of the cornea. An incision of 2 x 2 mm is made to remove the biopsy. The biopsy is placed in the sterile test tube supplied containing transport medium. The biopsy must be received by the manufacturer within 24 hours from the procurement. <u>Post-biopsy treatment</u> Following the biopsy, an appropriate regimen of prophylaxis with an antibiotic treatment must be given.

	In some cases, it may be possible that the source limbal stem cells of the patient are not expandable or that the release criteria are not met, due to poor quality, patient characteristics, or manufacturing failure. Therefore, it can occur that Holoclar cannot be delivered. The surgeon will be informed as early in the process as possible and should hence select an alternative treatment for patient. *Implantation* Holoclar is intended solely for use in autologous limbal stem cell regeneration in line with approved therapeutic indication and should be administered under sterile conditions in conjunction with limbal peritomy, undermining of the conjunctiva and excision of the corneal fibrovascular tissue in preparation of the defect bed. Next, the insert is fitted under the undermined conjuntiva. The excess of insert is trimmed and the edge covered with the conjunctiva applying 2 or 3 stitches (sutures) of vicryl or silk 8/0 in order to form a physical seal of the lesion and to secure the implant. The eyelids are kept closed over the insert with a sterile-strip band. Holoclar is generally implanted under topical retrobulbar or parabulbar anaesthesia. Other anaesthesiology procedures may be followed at the discretion of the surgeon. Patients with acute ocular inflammation or infections should be deferred until recovery has been documented since inflammation may compromise treatment success. <u>Post-operative treatment</u> Following implantation, an appropriate regimen of topical and systemic anti-inflammatory and prophylactic antibiotic treatment must be given. The following regimen is suggested: Doxycycline 100 mg tablets twice daily (or amoxicillin 500 mg twice daily) and prednisone orally at a daily dose of 0.5 mg/kg (to a maximum dose of 25 mg) per day should be administered from the day of surgery for 2 weeks. After 2 weeks the systemic antibiotic administration should be stopped and the daily dose of prednisone should be tapered to 0.25 mg/kg (r maximum 12.5 mg) per day for 1 week, to 0.125 mg/kg (maximum 5.0 mg) per day for the following week and then stopped. Two weeks after surgery, a topical corticosteroid treatment should be started with preservative-free dexamethasone 0.1% eye drops, 1 drop three times per day for 2 weeks, then reduced to 1 drop twice daily for 1 week and 1 drop once daily for a further week. The topical corticosteroid can be maintained in case of persistent ocular inflammation. The implantation must be followed by an appropriate monitoring schedule.

Pharmaceutical form(s) and strengths Current	Living tissue equivalent. Transparent, circular sheet.
Is/will the product be subject to additional monitoring in the EU?	Yes New active substance

List of Abbreviations

ACLSC	Autologous Cultured Limbal Stem Cell
ADR	Adverse drug reaction
AE	Adverse event
ALSCT	Autologous limbal stem cell transplant
ALT	Allograft limbal transplantation
AMT	Amniotic membrane transplantation
DMEM	Dulbecco's Modified Eagle Medium
EU	European Union
HBCAb	Hepatitis B core antibody
HBSAg	Hepatitis B surface antigen
HCP	Healthcare professional
ITT	Intention to treat
LSCD	Limbal stem cell deficiency
LPLV	Last patient last visit
PKP	Penetrating keratoplasty
PSUR	Periodic safety update report
SAE	Serious adverse event
SmPC	Summary of Product Characteristics
SOC	System Organ Class

Part II: Safety specification

Part II: Module SI - Epidemiology of the indication(s) and target population

Indication

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.

The specific indication being sought for the product (corneal damage with Limbal Stem Cell Deficiency (LSCD) is a subgroup of ocular injury caused by chemical burns, in particular those which destroy more than 50% of the limbal epithelial stem cells. The LSCD is responsible for the abnormal corneal healing, which follow the injury, such as conjunctival epithelial ingrowth (pannus) **[27]** and corneal vascularisation **[19]**. Such eyes become chronically inflamed with recurrent and or persistent corneal epithelial defect, loss of corneal transparency and functional visual impairment or loss in the case of central cornea hazing **[27]**.

Incidence: No data for E.U. available

Prevalence: Estimated to be less than 0.33/10,000 persons in the EU.

Since epidemiologic data on the prevalence of LSCD due to ocular burn injury are unavailable in the medical literature, the prevalence of the condition in Europe was calculated using as data source the registries of primary diagnosis at hospital discharges in the period from 1999 to 2003 from 4 European countries: England, Germany, France and Italy. According to this, the prevalence of the condition in the EU population is: **0.3361 per 10,000 (**95% *C.I.* 0.3321 to 0.3401).

Moreover, the population with blindness due to chemical injury includes subjects not suitable for the proposed treatment. Patients with therapeutically uncontrolled chronic inflammation, absence of appropriate environment for corneal cell engraftment, decreased tear secretion, dry eye syndrome, massive conjunctival cells destruction and surface cicatrisation, are considered unsuitable for the corneal cell therapy procedure proposed in this application **[30]**.

Therefore, the relevant prevalence estimate for LSCD due to chemical burns will be even lower than the estimate of 0.33 per 10,000 persons because the population with blindness due to chemical injury will include those not suitable for this product.

Demographic of the population in the proposed indication and risk factors for the disease: Limbal stem cell deficiency might affect all ages and is not gender specific. It frequently arises due to chemical injury in the workplace (i.e. 16 - 65 years).

The main existing treatment options: There are no approved medicinal products in EU for the treatment of corneal lesions, with associated cornea (limbal) stem cell deficiency (LSCD), caused by ocular burns. In current medical practice a few treatments are considered. However, bilateral LSCD has no currently successful therapy ensuring long term restoration and unilateral lesions have been treated by suboptimal surgical

procedures. Severe ocular surface disease with LSCD due to chemical burns requires a complex approach of supportive therapy and surgical (often multiple) procedures **[31]**.

Various surgical corneal procedures have been used in the past to re-constitute the corneal surface. Simple excision of fibrous tissue and conventional keratoplasty are not sufficient to avoid recurrence of the fibrovascular pannus or tissue rejection, in cases characterized by limbal destruction. The most used surgical procedure in this disease has been until recently corneal transplant or penetrating keratoplasty (PKP). The outcome of PKP in such cases is poor. This is because the transient amplifying progenitor cells that were transferred onto the central corneal surface during PKP have a limited life span and limited proliferative potential. Moreover, corneal cells from donors are antigenic, and thus unable to restore the ocular surface epithelium on a long-term basis. So, even though keratoplasty is crucial for the reconstruction of damaged corneal stroma, it promotes true corneal re-epithelialisation only if residual autologous limbal epithelial stem cells are present in the injured eye **[23]**. Therefore, for total LSCD, limbal stem cell transplantation is required prior to the PK to give the best chance of vision. Although all techniques for limbal stem cell transplantation are in principle similar, the source of donor cells varies as does the carrier tissue **[6]**. The different treatment options are reviewed below:

Cadaveric keratolimbal allograft and conjunctival limbal allograft from live-related donors

Allograft surgery is generally reserved for bilateral ocular surface disease secondary to LSCD. It is also an alternative for unilateral disease where there is fear of damage to the healthy fellow eye. Recipients need to be selected carefully and preferably have minimal inflammation. Issues with this technique are potential lack of donor tissue and the requirement for long term systemic immunosuppression **[3]**. In addition, long term failure of allogeneic epithelial engraftment is described in the literature **[13]**.

Kerato-limbal allograft plus amniotic membrane transplantation (AMT)

Amniotic membrane has important properties such as induction of adhesion and migration of epithelial cells to reduce inflammatory reactions; it also appears to be immunologically inert. These properties have led to its application in ophthalmology with allograft limbal transplantation (ALT) to reconstruct the corneal surface in patients with severe dry eye **[37]**. The use of amniotic membrane transplantation (AMT) with and without allograft limbal transplantation was recently compared **[36]**. It was concluded that for partial limbal deficiency with superficial involvement, AMT alone is sufficient and hence superior to ALT because there is no need to administer immunosuppressants. For total limbal deficiency, additional ALT is needed, and AMT helps reconstruct the perilimbal stroma, with reduced inflammation and vascularisation, which collectively may enhance the success of ALT. Issues with this technique include long term failure of allogeneic engraftment, the potential lack of donor tissue and the requirement for long term systemic immunosuppression. Furthermore, there may be the problem of infectious agent transmission with the use of this tissue.

Autologous kerato-limbal transplantation

The Kenyon technique **[16]** involves grafting large limbal tissue fragments (perhaps 30-50 % of the total limbus) from the uninjured eye for transplantation to the damaged eye. In the series of 26 patients, 21 patients with follow up of 6 months or more consistently showed improved visual acuity, rapid surface healing and regression of corneal neovascularisation. Issues with this technique involve the amount of tissue required to be removed from the fellow healthy eye and the potential impact that may have on the vision of the previously healthy eye.

Autologous limbal stem cell transplantation using amniotic membrane as carrier

In this technique stem cells are cultured from the same patient and are transplanted with the amniotic membrane. The use of this method of cultivating the epithelium avoids allografting and immunosuppression. This technique has been used since 2001 and a success rate of approximately 70% has been reported with 3-year follow-up, but with lack of further long-term survival **[32]**. Issues with this technique include lack of demonstration of limbal stem cell preservation, depletion of stem cells in culture with formation of a well differentiated corneal epithelium **[5]**, selection and preparation of the amniotic membrane tissues are not standardised or validated and the use of explant culture means that one biopsy will provide material for only one transplantation.

Natural history of the indicated condition in the untreated population, including mortality and morbidity: Limbal stem cell deficiency due to ocular burns is not a fatal condition. However, the condition leads to chronic pain, increased risk of bacterial keratitis, corneal perforation and blindness.

Important co-morbidities: Patients with ocular burns may have other concomitant eye problems which should be corrected prior to implantation (concomitant eyelids malposition, conjunctival scarring with fornix shortening, corneal anaesthesia and/or conjunctival anaesthesia or severe hypoaesthesia, pterygium and severe dry eye). Thus, patient selection is critical in determining effectiveness.

The main important medical co-morbidities in the target population **(**Limbal stem cell deficiency due to ocular burns) are discussed below.

Co-morbidity 1	Co-morbidity arising from suspected adverse reactions arising from post-implant use of prophylactic antibiotics and anti-inflammatory drugs
Incidence	Unknown but prophylactic antibiotics and anti-inflammatory drugs are administered to all patients following treatment with Holoclar.
Prevalence	Unknown but prophylactic antibiotics and anti-inflammatory drugs are administered to all patients following treatment with Holoclar.
Mortality	No recognised risk of fatal adverse reaction from topical therapies
Co-prescribed medicinal products	Use of prophylactic antibiotic treatment is required post-implantation (e.g. doxycycline or amoxicillin). With reference to the SmPCs for Vibramycin (doxycycline) or Amoxil (amoxicillin), respectively, potential undesirable effects include but are not limited to: hypersensitivity reactions, severe cutaneous adverse reactions (e.g. erythema multiforme, Stevens- Johnson syndrome and toxic epidermal necrolysis), photosensitivity and candidiasis.

	Use of topical and systemic anti-inflammatory drugs is required post-implantation with reference to the use of corticosteroids (e.g. prednisone or dexamethasone), some of the potential undesirable effects are described in this section. Suppression of the inflammatory response and immune function increases the susceptibility to infections and their severity. Ophthalmic: increased intra-ocular pressure with development of glaucoma, papilloedema, posterior subcapsular cataracts, corneal and scleral thinning or perforation after prolonged use. Viral or fungal ophthalmic disease may be reignited or spread. Hypersensitivity.
Co-morbidity 2	Cataract (as pre-existing condition or as a result of surgery or concomitant use of topical prednisone)
Incidence	20.3% (Klein BE, Ophthalmology. 2008 115(3):477-82.)
Prevalence	4% **[39]**
Mortality	None
Co-prescribed medicinal products	Anti-inflammatory eye drugs such as prednisone
Co-morbidity 3	Glaucoma (as a pre-existing condition or as a result of surgery or concomitant use of topical prednisone)
Incidence	A retrospective longitudinal study performed in Southern Germany with 5 years of follow-up and 3,531 participants showed that the incidence rate of glaucoma as a main cause of blindness in the 40-59-year age group was 2.37 (95% CI: 1.93–2.81) per 100,000-person years **[35]**. A systematic literature review **[26]** identified a cross-sectional study from the European North of Russia that estimated the incidence of glaucoma at a level of 1.3 cases in 1,000 persons. Patients with ocular burns are more likely to have glaucoma. A retrospective, observational case series investigated 29 eyes (18 patients) with ocular chemical burns seen between 1997 and 2010 with a minimum of 3 months of follow-up at the University of Washington (Lin et al., 2012). Glaucoma after ocular chemical burns was associated with more severe burns: 16 (84%) of 19 eyes with Roper-Hall grade III or IV ocular chemical burns required long-term glaucoma medication. Only a small

	proportion of eyes that had initially low Intraocular Pressure (IOP) later demonstrated elevated IOP requiring glaucoma medications. Fifteen (83%) of 18 eyes that required long-term glaucoma treatment had elevated IOP within 1 week of presentation. Glaucoma is common following keratoplasty. A retrospective study was carried out in 228 patients who underwent penetrating keratoplasty from January 1995 to January 2000 at the Federal University of Uberlândia MG, Brazil **[9]**. Two hundred twenty-eight patients undergoing penetrating keratoplasty were evaluated and 49 (21.5%) developed glaucoma. Risk factors for developing glaucoma were bullous keratopathy [relative risk (RR) 2.1774), herpesvirus (RR 1.8979) and trauma (RR 1.0575). A consecutive series of penetrating keratoplasties performed in New Zealand for keratoconus were analysed retrospectively (Fan et al., 2009). The study included 57 eyes of 48 patients. Of these 18 eyes (32%) of 17 patients (35%) exhibited elevated IOP and 12 (21%) eyes exhibited moderate-to-severe elevation of IOP. IOP elevation occurred 3–6 months postkeratoplasty in 78% of eyes. All patients except one required reduction/cessation of corticosteroids to normalise IOP.
Prevalence	The following information has been extracted from a systematic literature review **[26]**. It has been estimated that 21.8% of European adults (including 18% of those over 50 years of age) have been diagnosed with glaucoma. According to recent epidemiological studies, Germany (14%) shows the highest prevalence of glaucoma in Europe followed by the European North of Russia (11.9%). The lowest prevalence of any type of glaucoma has been registered in France (3.4%) and the UK (3.3%).
Mortality	None

Part II: Module SII - Non-clinical part of the safety specification

The pre-clinical data are very limited since there are no relevant animal species, including the homologous models, to predict the toxicological effects of the human autologous limbal stem cells cultures and because of the limitation in the procurement of the autologous human cells for conducting the studies in animals.

The following are specific nonclinical safety concerns that have not been adequately addressed by clinical data or which are of unknown significance.

Key Safety findings (from non- clinical studies)	**Relevance to human usage**
Single and Repeat dose toxicity	Not applicable
Reproductive toxicity	Not applicable
Developmental toxicity	Not applicable
Nephrotoxicity	Not applicable
Hepatotoxicity	Not applicable
Genotoxicity	See below
Carcinogenicity	Non-clinical safety data were limited to the testing in vitro of tumorigenicity of the human autologous cell cultures. These tests included cell karyotype, cell growth in soft agar and growth factor-dependent proliferation. In vitro studies have revealed no evidence of anchorage-independent growth indicative of tumorigenic potential. These studies rule out carcinogenicity as a potential human risk.
Pharmacology/Pharmacokinetics migration and bio distribution data	Not applicable
Safety pharmacology	No applicable
Other toxicity-related information or data	Not applicable

Conclusions on non-clinical data: No safety concerns have been identified.

Part II: Module SIII - Clinical trial exposure

The medicinal product is a tissue engineered product of *ex vivo* expanded autologous human corneal epithelium containing stem cells. The product consists of a sheet of autologous corneal epithelium attached to a supportive fibrin layer in an aqueous nutrient medium (DMEM). The product is derived from expanded cells isolated from a biopsy of the undamaged area of the ipsi- or contra-lateral eye. The product has been developed for the treatment of corneal lesions with associated corneal (limbal) stem cell deficiency, resulting from ocular burns.

The product was initially considered clinically and scientifically acceptable in Italy as a therapy, and approved for reimbursement. Under this framework, the product has been used to treat over 219 patients in the EU from 1997 to 2007. Data for 135 of the patients treated during this period were included in the 2 retrospective studies described below (HLSTM01 and HLSTM02). However, data from a subset of approximately 84 patients from this treatment period were not available for inclusion in the retrospective studies. Although the clinicians involved with the treatment of this subset of patients were invited by the Company to participate in the retrospective study HLSTM02, they declined to participate and these patients were correspondingly excluded from any retrospective analyses performed.

With the advent of the ATMP Regulation (EC) No. 1394/2007, on April 2008, the Task Force on Emerging Therapies and Technologies of the European Medicines Agency (EMA) concluded that this cell-based therapy is to be considered as a medicinal product. This was confirmed by the Committee for Advanced Therapies (CAT) which considers the product to meet the definition of a Tissue Engineered Product (not combined).

The product was acknowledged as an orphan medicinal product, and the first approval of Holoclar was granted on 17th February 2015 in the European Union through Centralised Procedure. The product is therefore approved in the 27 European Member States plus Iceland, Liechtenstein and Norway. The product has been granted with a conditional MA in accordance with Article 14(7) of Regulation (EC) No 726/2004. In addition, during the reporting period, a marketing authorization approval was obtained for the United Kingdom on 14 June 2021.

The safety and efficacy of the product was evaluated in the following studies:

- Study HLSTM01 “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”: a retrospective case-series, non-controlled, multicentre observational clinical study in 106 patients of both genders, with moderate to severe limbal stem cell deficiency (LSCD). The study was conducted in two major clinical centres in Italy.

- Study HLSTM02, “Retrospective evaluation of the safety of autologous cultivated limbal stem cells transplantation for restoration of corneal epithelium in patients with limbal stem cell deficiency”, a retrospective, case-series, non-controlled, multicentre observational clinical study in 29 patients with limbal stem cell deficiency. The study was conducted in 7 clinical centres in Italy.

- Study HLSTM04, “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 burns"; a retrospective, case-series, non-controlled, multicentre observational clinical study in 15 patients with limbal stem cell deficiency from 3 clinical sites in Italy.

- Study CCD-GPLSCD01-03, "Multinational, multicentre, prospective, open-label, clinical trial to assess the efficacy and safety of autologous cultivated limbal stem cells transplantation (ACLSCT) for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns (HOLOCORE)". The study was conducted in 80 patients from 18 sites in 8 EU countries.

With reference to the above retrospective clinical studies:

- exposure figures by number of patients, and number of implantations are provided in Table 2
- follow-up duration post- ACLSC implant is provided in Table 3 and Table 4
- details of the year in which the implants took place are provide in Table 5
- exposure by gender and age is presented in Table 6.

Table 1: Exposure figures for clinical studies HLSTM01, HLSTM02, HLSTM04 and CCD-GPLSCD01-03 (HOLOCORE) – ACLSC implantations

Number of ACLSC implantations	Number of Patients				Number of ACLSC implantations with data available*				Total Number of ACLSC implantations with data available*
	Study HLSTM 01	Study HLSTM 02	Study HLSTM 04	Study CCD-GPLSCD 01-03	Study HLSTM 01	Study HLSTM 02	Study HLSTM 04	Study CCD-GPLSCD 01-03	
1	94	29	15	73	89	29	15	73	206
2	11	0	0	7	21	0	0	7	28
3	1	0	0	0	3	0	0	0	3
Total	106	29	15	80	113	29	15	80	237
* For HLSTM01 data were not available at the investigator sites (5 first implantations and 1 second implantation), and therefore were not included in the study. A total of 113 implantations were included in this study									

Table 2: Duration of follow-up post- ACLSC implantation for the clinical study HLSTM01 and HLSTM02

Duration of follow-up*	Number of ACLSC implantations n (%)	
	HLSTM01	HLSTM 02
< 1 year	7 (6.19%)	5(17.2%)
≥ 1 and < 2 years	32 (28.3%)	8(27.6%)
≥2 and < 3 years	25 (22.1%)	4(13.8%)
≥ 3 and < 4 years	16 (14.2%)	5(17.2%)
≥ 4 and < 5 years	19 (16.8%)	2(6.90%)
≥ 5 and < 6 years	6 (5.31%)	3(10.3%)

≥ 6 and < 7 years	2 (1.77%)	2(6.90%)
≥ 7 and < 8 years	3 (2.65%)	5
≥ 8 and < 9 years	1 (0.88%)	-
≥ 9 and < 10 years	2 (1.77%)	-
Total ACLSC implants	**113 (100%)**	**29(100%)**
HLSTM01: * Duration of follow-up calculated as (Date of last available visit - Date of implantation)/365.25 Last available visit is the last visit performed by the patient after implantation, including unscheduled visits (if any). Source: Table T29.2 Clinical study report no.: CCD-0917-CSR-0037 (15 Feb 2012) SAS Program ran 07 Dec 2010 HLSTM02: * Duration of follow-up calculated as (Date of last available visit - Date of implantation)/365.25 Last available visit is the last visit performed by the patient after implantation Source: Table T21.2 clinical study report no.: CCD-0918-CSR-0040 (15 Feb 2012) SAS Program ran 21 Jan 2011		

HLSTM04: The mean of the follow up for HLSTM04 study was 10.72 ± 7.99 months (2.83 – 25.9 months)
Source: clinical study report no.: CCD-GPLSCD01-04 DATE: 07 Aug 2014

CCD-GPLSCD01-03 (HOLOCORE): Seventy-three (73) patients received the treatment with Holoclar (including 4 paediatric patients). Sixty-eight (68) patients completed the 12-months follow-up after first treatment with Holoclar and forty-seven (47) among them accepted to continue in the Long-term Follow-up study (HOLOCORE-FU) from a minimum of additional 12 to a maximum of 57 months of follow-up after Holoclar implantation.

Table 3: Year of ACLSC implantation for the clinical studies HLSTM01 and HLSTM02

Year of implant	Number of ACLSC implantations	
	HLSTM01	HLSTM02
1998	1	0
1999	4	0
2000	1	0
2001	12	0
2002	3	10
2003	2	0
2004	26	6
2005	24	5
2006	10	4
2007	30	4
Total ACLSC implants	**113**	**29**
Source: Listing 01: Disposition of patients (HLSTM01) Program date 07 DEC 2010 Source: Listing 01: Disposition of patients (HLSTM02) Program date 21 JAN 2011		

Patients enrolled in HLSTM04 study have been transplanted between 2011 and 2014.

SIII.2 Age group and gender

Table 4: Exposure by age group and gender for the clinical studies HLSTM01, HLSTM02, HLSTM04 and CCD-GPLSCD01-03 (HOLOCORE)

	Age group and Gender	HLSTM01 N = 113 N (%)	HLSTM02 N = 29[1] N (%)	HLSTM04 N = 15 N (%)	CCD-GPLSCD01-03 N = 73 N (%)	Total N=230[2] N (%)
Age group (years)	< 18	3 (2.7%)	2 (7.1%)	0	2 (2.7%)	7 (3.0%)
	18 - 39	34 (30.1%)	10 (35.7%)	7 (46.7%)	2 (2.7%)	53 (23.0%)
	40 - 64	69 (61.1%)	9 (32.1%)	5 (33.3%)	63 (86.3%)	146 (63.5%)
	65 - 75	5 (4.4%)	7 (25.0%)	3 (20%)	4 (5.5%)	19 (8.3%)
	> 75	2 (1.8%)	0 (0%)	0	2 (2.7%)	4 (1.7%)
Gender	Females	25 (22.1%)	7 (24.1%)	1 (6.7%)	12 (16.4%)	45 (19.6%)
	Males	88 (77.9%)	22 (75.9%)	14 (93.3%)	61 (83.6%)	185 (80.4%)

[1]Age data was available for n=28 patients in HLSTM02

[2]Patient demographics are presented at the age of treatment. As patient age changed over the course of the studies for patients treated more than once demographic data are presented per treatment event.

SIII.3 Dose

Not applicable

SIII.4 Ethnic origin

Although data for ethnicity were not specifically collected as part of the two retrospective studies HLSTM01 and HLSTM02, all patients were treated in Italy and therefore, it can be assumed that the vast majority of patients exposed to the product were Caucasians of Italian descent.

Table SIII.4: Ethnic origin for CCD-GPLSCD01-03 (HOLOCORE)

		Number of subjects		
		Adult	**Paediatric**	**Total**
		N = 69 N (%)	**N = 4 N (%)**	**N = 73 N (%)**
Race	White	55 (79.3%)	4 (100%)	59 (80.8%)
	Asian	2 (2.9%)	-	2 (2.7%)
	Black	-	-	-
	Other	2 (2.9%)	-	2 (2.7%)
	Not collected	10 (14.5%)	-	10 (13.7%)

Part II: Module SIV - Populations not studied in clinical trials

SIV.1 Exclusion criteria in pivotal clinical studies within the development programme

Exclusion criteria initial studies HLSTM01, HLSTM02 and HLSTM04		
Criteria	**Reason for exclusion criterion**	**Included as important risk /missing information yes/no (rational if no)**
• Compromised eyelid mobility and/or symblepharon • Tear secretion deficiency (Schirmer test < 5 mm) • Corneal and conjunctival anaesthesia • Active local or systemic infections • Active ocular inflammation • Pterygium or pseudopterygium	Severe anatomical disruption of the eye, local active infection, inflammation, excessive dryness or local anaesthesia makes it much less likely that a limbal stem cell implant will succeed as the environment is not apt to receive stem cells to enable differentiation into clinically useful cells. Different underlying pathologies such as systemic inflammatory causes of corneal blindness, means that disease may recur.	No These conditions are listed in the warnings and precautions section of the SmPC (section 4.4): Concomitant eyelids malposition, conjunctival scarring with fornix shortening, corneal anaesthesia and/or conjunctival anaesthesia or severe hypoaesthesia, pterygium and severe dry eye are potential complicating factors. Where possible, concomitant eye problems should be corrected prior to Holoclar implantation. Different underlying pathologies such as inflammatory causes of corneal blindness, means that disease may recur within a limbal stem cell implant because of the systemic nature of the inflammatory disease.
• Diagnosis of local or systemic neoplastic disease • Limbal deficiency due to radiotherapy • Aniridia • Stevens-Johnson syndrome (SJS) • Neurotrophic keratitis • Unable to stop the topical treatment(s) for the pathology	The product is indicated in patients with LSCD due to physical or chemical ocular burns so other aetiologies e.g. SJS, neurotrophic keratitis would be off-label use.	Yes
• Positive to HIV-1 or HIV-2 test	Due to ethical reasons these patients were excluded from the clinical research. Patients with these criteria were not included as these would	No (the safety profile is not expected to be different in patients positive to HIV-1 or HIV 2)

Exclusion criteria initial studies HLSTM01, HLSTM02 and HLSTM04		
	make it unsafe for the subjects and study personnel to participate.	

Exclusion criteria Study CCD-GPLSCD01-03 (HOLOCORE)		
Criteria	**Reason for exclusion criterion**	**Included as important risk /missing information yes/no (rational if no)**
• LSCD of mild degree (i.e., below 2 quadrants of vascularization invasion), due to a recent burn (less than 24 months before screening for adults and 12 months for minors), or secondary to medical conditions other than burns (i.e., radiotherapy • Diagnosis of local or systemic neoplastic disease; • Congenital diseases (i.e., aniridia); • Bilateral inflammatory diseases (i.e., Stevens-Johnson syndrome, pemphigoid); • A pre-existing blindness precluding a functional recovery;	The product is indicated in patients with moderate to severe LSCD due to physical or chemical ocular burns so other severities or aetiologies e.g. SJS, neurotrophic keratitis would be off-label use.	Yes (important potential risks)
• Severe ocular inflammation according to the Efron Grading Scale for Contact Lens Complications. Patient was re-screened after appropriate treatment; • Presence of eyelids malposition; • Conjunctival scarring with fornix shortening;	Severe anatomical disruption of the eye, local active infection, inflammation, excessive dryness or local anaesthesia makes it much less likely that a limbal stem cell implant will succeed as the environment is not apt to receive stem cells to enable differentiation into clinically useful cells. Different underlying pathologies such as systemic inflammatory	No These conditions are listed in the warnings and precautions section of the SmPC (section 4.3 and 4.4): Concomitant eyelids malposition, conjunctival scarring with fornix shortening, corneal anaesthesia and/or conjunctival anaesthesia or severe hypoaesthesia, pterygium and severe dry eye are potential complicating factors. Concomitant eye problems should be corrected prior to Holoclar implantation.

Exclusion criteria Study CCD-GPLSCD01-03 (HOLOCORE)		
• Severe tear secretion deficiency, determined by Schirmer's test type I (<5 mm/ 5 min); • Corneal anaesthesia and conjunctival anaesthesia; • Active local or systemic infections at the time of screening*. Patient were re-screened after appropriate treatment;	causes of corneal blindness, means that disease may recur.	Different underlying pathologies such as inflammatory causes of corneal blindness, means that disease may recur within a limbal stem cell implant because of the systemic nature of the inflammatory disease.
• Allergy, sensitivity or intolerance to concomitant drugs or excipients (Hypersensitivity to any of the excipients listed in section 6.1 or to bovine serum and murine 3T3-J2 cells); • Contraindications to the local or systemic antibiotics and/ or corticosteroids foreseen by the protocol; • Contraindications to the surgical procedure; • Clinically significant or unstable concurrent disease or other clinical contraindications to stem cell transplantation based upon Investigator's judgment or other concomitant medical conditions affecting grafting procedure (i.e., use of contrast medium) or any other invasive procedure which could have affected the integrity of the epithelium;	Allowance of these conditions would confound assessment of safety and efficacy. These conditions would impair the success of the treatment.	No These conditions are listed in the contraindications, warnings and precautions section of the SmPC (section 4.3. and 4.4):

SIV.2 Limitations of ADR detection common to clinical trial development programmes

Ability to detect adverse reactions	Limitation of trial programme	Discussion of implications for target population
Uncommon and rare adverse drug reactions unlikely to have been detected	There were only 230 patients exposed over the whole clinical trial programme.	Rare and unexpected ADRs might occur
Due to prolonged exposure	A total of 237 implantations with data are available.	No evidence of long latency ADRs but numbers exposed too small to reach a final conclusion
Due to cumulative effects	There are a limited number of patients who have received more than one administration of Holoclar in the retrospective studies. Fourteen patients had a second ACLSC implant.	The success of multiple implants following previous failed attempts in the retrospective studies does not support a risk of antigenicity. Prospective study information will be required to confirm this finding.
Which have a long latency	The long-term follow up data is limited due to the retrospective nature of the clinical trials. Follow-up data are available for ≥ 1-year duration in 93.8% of ACLSC implants (106 of 113 implants) in Study HLSTM01. In the same study 43.4% (49 of 113 implants) have follow-up data pertaining ≥ 3 years. From Study HLSTM02, 82.8% (24 of 29 implants) included follow-up data covering a ≥ 1-year period while 41.4% (12 of 29 implants) covered durations of ≥ 3 years. In the clinical trial Study CCD-GPLSCD01-03 (HOLOCORE) sixty-eight (68) patients completed the 12-months follow-up after first treatment with Holoclar. Forty-seven (47) among them continued in the Long-term Follow-up study (HOLOCORE-FU) from a minimum of additional 12 to a maximum of 40.557 months of follow-up.	There is no evidence from the retrospective studies to suggest that long term use is associated with a safety concern. In vitro studies have confirmed localisation of the implant without migration of the epithelial cells into basal ocular structures. Furthermore, the risk of tumour formation related to 3T3-J2 feeder cells proliferative capacity has been demonstrated to be low following irradiation. Prospective study information will be required to confirm this finding.

SIV.3 Limitations in respect to populations typically under-represented in clinical trial development programmes

Table SIV.2: Exposure of special populations included or not in clinical trial development programmes

Type of special population	Exposure
Pregnant women	Not included in the clinical development program. Animal studies are absent with respect to reproductive toxicity. Conventional reproductive and developmental toxicity studies are not considered relevant, given the nature and the intended clinical use of the autologous tissue-engineered product. As a precautionary measure, since the requirement of the post-operative pharmacological treatment, it is preferable to avoid the use of Holoclar during pregnancy. Holoclar is not recommended during pregnancy and in woman of childbearing potential not using contraception from the biopsy to the conclusion of post-operative pharmacological treatment. As a precautionary measure, Holoclar is not recommended for implant during breast-feeding.
Breastfeeding women	
Patients with relevant comorbidities: • Patients with hepatic impairment • Patients with renal impairment • Patients with cardiovascular impairment • Immunocompromised patients • Patients with a disease severity different from inclusion criteria in clinical trials	***Patients with hepatic impairment*** There were no exclusion criteria relating to hepatic impairment in the two retrospective clinical studies HLSTM01 and HLSTM02. In the Intention To Treat Population (N=104) from study HLSTM01 three patients were noted to have had hepatic disorders (2 HBSAg +ve, 1 HBCAb+ve). Amongst the 29 patients from study HLSTM02, there was one case of hepatic steatosis (HBSAg +ve). No patient with hepatic impairment was treated during the studies HLSTM04 and HOLOCORE. No adverse events related to hepatic impairment were reported. No specific concerns related to autologous human limbal stem cells are anticipated in populations with hepatic impairment. However, post-operative treatment with systemic anti-inflammatory and prophylactic antibiotic treatment may be required and appropriate precautions will need to be applied when

	administering these medications to patients with hepatic impairment. ***Patients with renal impairment*** There were no exclusion criteria relating to renal impairment in the two retrospective open studies. In the Intention To Treat Population (N=104) from study HLSTM01 three patients were noted to have concomitant urinary system disorders. No patient with hepatic impairment was treated during the studies HLSTM04 and HOLOCORE. An adverse event related to renal disorders (Dysuria) was reported post-implantation for study HLSTM01 only. The adverse event reported was Dysuria. No specific concerns related to autologous human limbal stem cells are anticipated in populations with renal impairment. However, post-operative treatment with systemic anti-inflammatory and prophylactic antibiotic treatment may be required and appropriate precautions will need to be applied when administering these medications to patients with severe renal impairment. ***Patients with other relevant co-morbidity*** Patients with cardiac impairment were not excluded from the two retrospective open studies. In the Intention To Treat Population (N=104) from study HLSTM01 there were 14 instances of cardiovascular disorders. Amongst the 29 patients from study HLSTM02, there were 6 cases of hypertension and 1 arrhythmia. Adverse events related to cardiac disorders were reported post-implantation for study HLSTM01 only. The adverse events reported were Hypertension 1, Hypotension 1 and Atrial fibrillation 1. No specific concerns related to autologous human limbal stem cells are anticipated in populations with cardiac impairment.

	Patients with a disease severity different from the inclusion criteria in the clinical trial population The indication is treatment of 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. This is consistent with the inclusion criteria for the retrospective clinical studies HLSTM01. Any use for less severe limbal stem cell deficiency or limbal stem cell deficiency due to aetiologies other than ocular burns (e.g. Stevens Johnson Syndrome) would be considered off label use.
Population with relevant different ethnic origin	Data for ethnicity were not specifically collected as part of the two retrospective clinical studies. All patients were treated in Italy and therefore, can be assumed that the vast majority of patients exposed to the product were Caucasians of Italian descent. In the clinical trial Study CCD-GPLSCD01-03 (HOLOCORE) 80 patients were enrolled from 18 sites in 8 EU countries: - 62 subjects (77.5%) were White; - 2 subjects (2.5%) were Asian; - 1 subjects (2.5%) were Black; - 2 subjects (2.5%) were Other races; - In the remaining 13 subjects the race was missing;
Subpopulations carrying relevant genetic polymorphisms	Not applicable.
Other : • Children • Elderly	***Children*** There were no exclusion criteria relating to age in the two retrospective clinical studies HLSTM01 and HLSTM02. However, there is limited information on the safety of autologous human limbal stem cells in children Cumulatively, 7 paediatric patients were exposed to autologous human limbal stem cells. In the two retrospective clinical studies HLSTM01 and HLSTM02 there were 4 patients (12-17-year-old) and

	1 patient (5-11-year-old). In the development program on the product CCD-GPLSCD01-03 (HOLOCORE study), there were 2 patients (12-17-year-old) and 2 patients (5-11-year-old). Adverse reactions in the paediatric included in studies were not different from those seen in the adult population. ***Elderly*** 14 patients 65-year-old or over were included in the two retrospective clinical studies HLSTM01 and HLSTM02. Three (3) elderly patients were treated in HLSTM04 study and 6 additional patients were treated in CCD-GPLSCD01-03 (HOLOCORE study).

Part II: Module SV - Post-authorisation experience

SV.1 Post-authorisation exposure

SV1.1 Method used to calculate exposure

Not applicable.

SV1.2 Exposure

Up to 26 July 2024, one hundred and one (101) patients received the treatment with the marketed product so far, with 115 biopsies overall collected for tissue procurement in 110 patients (5 of them repeated the biopsy). Cumulatively, ninety-nine (99) patients (90 included in HOLOSIGHT safety study, registry -like) have been implanted with Holoclar since the commercial availability of the product. All implanted patients were adults. One patient was classified as a paediatric patient at the time of screening (17 years of age) but was an adult (18 years of age) at the implantation. Six (6) patients were treated with out of specification product (subpotent batches), according to the section 11.5 of the "Guideline on Good Manufacturing practice specific to Advanced Therapy Medicinal Products".

Part II: Module SVI - Additional EU requirements for the safety specification

Potential for misuse for illegal purposes

Addiction: Not applicable

Other: Not applicable

Effect of device failure: Not applicable

Part II: Module SVII - Identified and potential risks

ATMP version

SVII. 1 Identification of safety concerns in the initial RMP submission

SVII.1.1 Risk not considered important for inclusion in the list of safety in the RMP

Reason for not including an identified or potential risk in the list of safety concerns in the RMP:

- Potential for harm from overdose:

There is no risk of overdose with autologous human limbal stem cells. As an autologous limbal stem cell product Holoclar is not a conventional medicine and conventional dose-response and dose-dependent toxicity are not applicable for this type of product including overdose. The amount of Holoclar administered is dependent on the size (surface in cm^2) of the corneal surface. The product is controlled at product release and only released with a dose of 79,000 – 316,000 cells/cm^2 which corresponds to 1 cm^2 of product per cm^2 defective area. With stringent controls applied during the manufacturing process and at product release the risk of an 'overdose' by a trained physician is negligible. Furthermore, physicians are advised to trim the product prior to implantation both in the SmPC and the healthcare professional Educational Manual. Physicians are required to undertake an approved training process prior to biopsy or implantation.

Theoretically if the administered implant contained a higher concentration of cells per cm^2 and/or if the implant covered an area that was greater than the defective area it is not anticipated that the patient would experience an adverse reaction relating to overdose. In vitro studies have confirmed localization of the implant without migration of the epithelial cells into basal ocular structures. Of note no cases of overdose have been reported to date as specified in section 4.9 (overdose) of the draft SmPC.

- Risk of cataract:

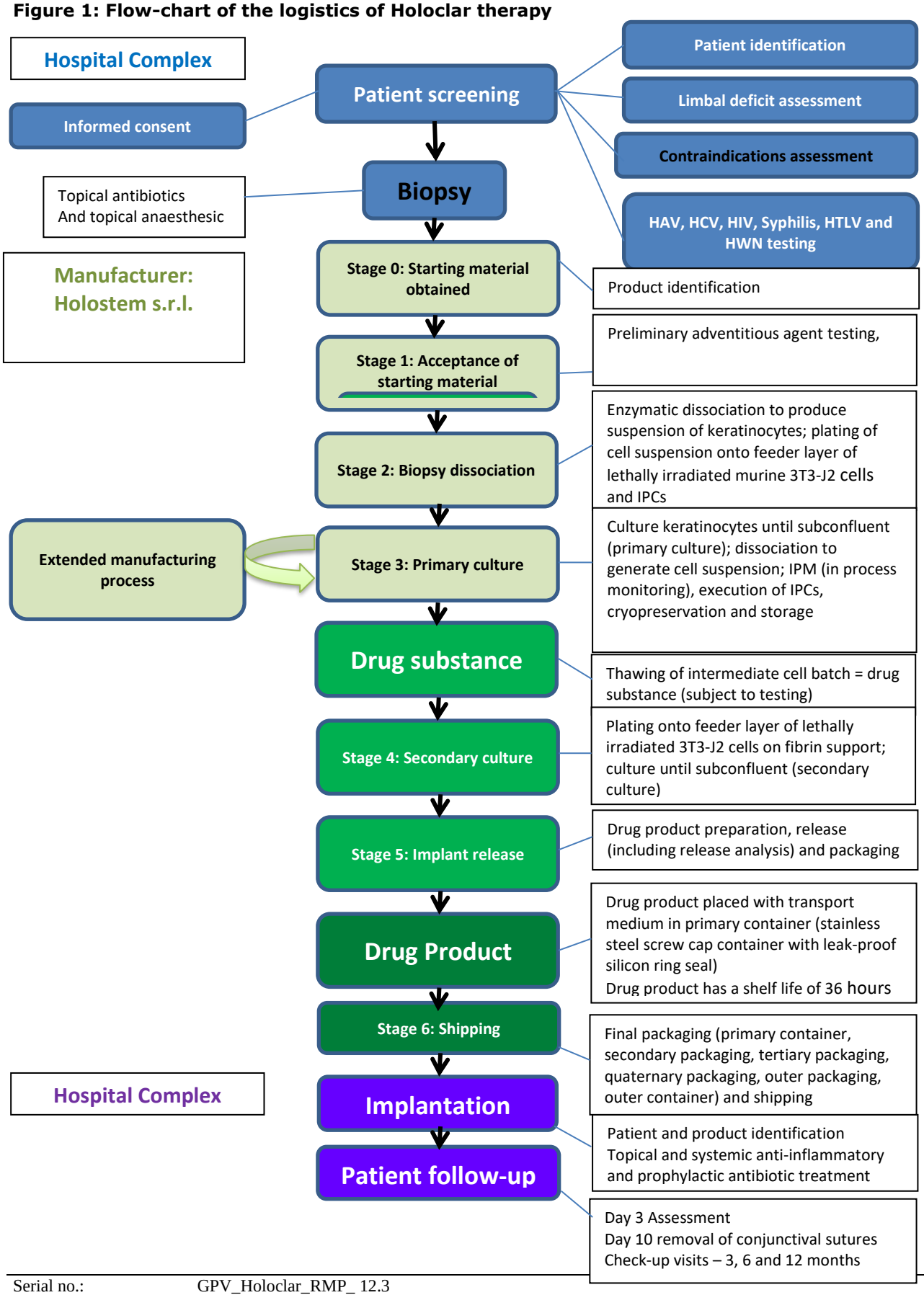
Although cataract was identified as an adverse event at a comparable frequency to glaucoma, causality assessment is confounded by its background co-morbidity. Cataracts are common in people over 65 years old. Other risk factors for cataracts include family history of cataracts, smoking, over exposure to sunlight, taking steroid medications for a prolonged time and less commonly diabetes, eye injuries and eye conditions such as uveitis. The diagnosis of cataract in a patient with ocular burns is virtually impossible. Usually diagnosis of cataract is based mainly on impaired sight and opacity of the crystalline lens. Patients with ocular burns will have impaired vision due to the limbal stem cell deficiency and the superficial opacity due to LSCD may impair the visual inspection of the crystalline lens. In addition, the eye injury itself and the potential previous long-term exposure to corticosteroids may cause a cataract that can remain unnoticed before Holoclar is given. Following the administration of Holoclar and the restoration of superficial corneal transparency it becomes possible to diagnose a cataract which may have been pre-existing. Post-implant treatment with corticosteroids is of a relatively short duration and so the likelihood of a cataract forming in association with this treatment is low. In light of the confounding factors outlined above, the Applicant considers that cataracts are co-morbidities and does not propose to include cataract as a potential risk in the RMP.

Specific risks in relation to ATMPs

- **Flow-chart of the logistics of Holoclar therapy**

A flow chart depicting the logistics of Holoclar treatment is provided in Figure 1 overleaf.

Figure 1: Flow-chart of the logistics of Holoclar therapy



- **Traceability**

Throughout the process outlined in Figure 1 the patient and related sample is identified by a patient identification which is unique to the patient. The patient identification will be assigned by the manufacturer Holostem s.r.l. and is specified on every document and record (paper or electronic) that is used throughout the patient management from pre- and post-biopsy and implantation procedures, including follow-up. The patient identification is also used throughout the manufacturing process.

The Educational Manual (Annex 7) provides guidance on the procedures to be undertaken by hospital staff and the MAH to ensure patient and product traceability during biopsy and implantation. Patients treated with Holoclar will always be identified by their unique patient identification comprising their first name, surname and date of birth. If there is any inconsistency in any of the three data points the surgeon must contact the MAH to clarify such discrepancy. At the initial identification stage, the full name and date of birth are checked to confirm that the hospital does not have another individual with the same name and date of birth having a biopsy processed on the same day. Once a patient has been identified as eligible for receiving a Holoclar implant through the patient selection process a Biopsy Request form is sent by the healthcare professional to the MAH to confirm details for the biopsy including the patient's first name, surname, date of birth and gender, details of the surgery, the eye involved, name of the surgeon, proposed date of the surgery and hospital details. The MAH returns a Confirmation form and once the surgery is confirmed, informed consent is sought from the patient for the surgery according to local hospital procedures that apply and in line with national healthcare system and legislation. A unique patient identification is assigned that will not be re-used even if the patient withdraws from the treatment regimen for any reason. Any additional treatments on the same patient will be traced with a new patient identification, having no relationship or link with the previously assigned one. During the manufacturing process the batch number (identical to the patient identification) is checked at a number of key steps throughout the process. SOP HS/PL/00-04 (Procedures for Biopsy Shipment, Transportation and Receipt) provides detailed guidance on checking that the codes on the labels of the product concur with recorded documentation including the batch number/patient identification and the hospital at several stages including checks by the Logistics manager and QC delegate, both authorised to access patient's personal data.

Healthcare professionals are expected to follow the guidance set out in Section 6.6 (Special precautions for disposal and other handling) of the draft SmPC "Holoclar is intended solely for autologous use. Prior to implantation the patient's name should be carefully checked with the patient/donor identification on the shipment documentation and product container." In addition, the healthcare professional Educational Manual sets out that it is the responsibility of the lead healthcare professional to confirm that the patient identification is correct prior to implantation and concurs with the patient identification on the shipment documentation. The surgeon or another appropriate healthcare professional must contact the MAH to clarify any discrepancies before proceeding with the procedures.

- **Risks to living donors**

Not applicable. Human Limbal Stem Cells are for autologous implantation.

- **Risks to patients in relation to quality characteristics, distribution and storage**

In some cases, it may be possible that the source limbal stem cells of the patient are not expandable or that the release criteria are not met, due to poor biopsy quality, patient characteristics or manufacturing failure Therefore, it can occur that the product cannot be delivered. The surgeon will be informed as early in the process as possible and should hence select an alternative treatment for the patient concerned.

The limbal biopsy should be transported from the hospital to Holostem s.r.l. within 24 hours for the manufacturing process. Following the manufacturing process, the product must not be refrigerated, frozen or irradiated and should be stored between 15°C – 25°C. The shelf life of the product is 36 hours (as per section 6.3 of the SmPC). If the storage time exceeds the shelf life the product will be discarded. Any delays in transportation could result in the patient not receiving the implant.

Due to the 36-hour shelf life, microbiological testing of the product will not be available until after implantation. The results are communicated to the hospital centre when they are available and appropriate action will be taken if necessary. A potential risk of post-implant infection due to microbiological contamination cannot be excluded. Details of the microbiological safety and sterility assurance system of Holostem s.r.l. in place to minimise the risk of microbial contamination are provided in Annex 7.

- **Risks related to interaction of the product and the patient**

The product may contain traces of bovine serum and lethally-irradiated murine 3T3 fibroblast cells. On the basis of the HOLOCORE trial and previous data all together we cannot consider the cornea as an immunopriviledged site as in our selection of patients, it is fully covered by blood vessels. However, the Applicant believes it is extremely unlikely that this xenogeneic tissue would provoke an implant rejection or inflammatory response that would compromise treatment outcome: the analysis performed on transplanted patients revealed absence of any engrafted 3T3 “ghosts” and absence of immunologic reaction: indeed patients transplanted twice or three times did not develop immunologic reactions and improved their previous clinical condition. This was evident also from the absence of such reactions in Study HLSTM01 and Study HLSTM02. It is noteworthy that multiple implants did not lead to a subsequent rejection of the implant, as the success rate of second or third implants has been similar to or higher than initial implants which lends support to the principle that implant failure is not related to an immunological response to the implant. As such an interaction between the product and patient is extremely unlikely. Holoclar is an autologous product and so the likelihood of an immunological reaction occurring is negligible.

Corneal implant rejection due to an active immune process is not considered to be an important risk as discussed above. However, it is recognised that Holoclar may not be effective in all patients as indicated by the need for repeat implantations in a small number of patients. Lack of effect manifesting as corneal epithelium defect is considered an important identified risk in the RMP and this risk more accurately describes the situation with respect to implant failures. As outlined in Part III.1 of this RMP, additional pharmacovigilance activities for this risk will include root cause analyses of corneal implant failures. In addition, the prospective clinical study CCD-GPLSCD01-03 (HOLOCORE) will look at efficacy outcomes and evaluate any common risk factors that may be attributed to implant failure.

Risks related to scaffolds, matrices and biomaterials

Not applicable.

- **Risks related to persistence of the product in the patient**

Patient follow-up data are available for up to 10 years in study HLSTM01 and for up to 8 years in study HLSTM02. One-year follow-up data are available for 93.8% of patients in study HLSTM01 and for 82.8% of patients in study HLSTM02. Furthermore 65% of patients have 2-year follow-up data in study HLSTM01 and this applies to 55.2% of patients in study HLSTM02. Summary tabulations of the duration of exposure are provided in Part II Module SIII.2 of this RMP.

Holoclar is applied topically to the eye and the product is expected to act locally at the application site. It is not expected to either migrate beyond the ocular surface or to produce systemic effect. Long term data support localisation of the implant without migration of the epithelial cells into basal ocular structures. This was demonstrated using histological sections of corneas obtained from patients who underwent perforating keratoplasty 1 to 3 years following Holoclar implantation. The stem cells from Holoclar formed a normal multilayer stratified epithelium on a continuous extracellular matrix. No epithelial cells were detected in the underlying corneal stroma.

The risk of tumour formation related to 3T3-J2 feeder cells proliferative capacity was evaluated using karyotype analyses, capacity for replicative senescence of exposed epithelial cells, soft agar assay to assess contact-independent growth, and growth factor dependency of the cells. The Applicant applied an irradiation procedure to prevent the proliferative capacity of the 3T3-J2 feeder cells and used these in vitro studies to demonstrate that the risk for tumorigenicity is not proven. This is supported by clinical data where there is a lack of evidence that Holoclar induces tumorigenicity.

Furthermore, data from multiple implants do not support the risk of antigenicity, as the second or third implants were observed to have a similar success rate or even a higher rate than first implants. Overall there is no evidence to suggest that patients exposed to Holoclar for longer time intervals are at greater risk of any ADR than those exposed for shorter time periods. As detailed above, there are substantial data available on long-term use for an orphan product with no risk identified that relates to persistence of the product in the patient. To date there has been no need to remove the implant for any reason and this covers long-term data on patients up to 10 years post-implantation.

- **Risks to healthcare professionals, caregivers, offspring and other close contacts with the product or its components, or with patients**

A patient may have a viral infection at the time of performing a biopsy. Biopsies from patients with viral infections are processed in different rooms with dedicated equipment. Standard Biohazard procedures will be implemented as will Good Laboratory Practice.

SVII.1.2 Risks considered important for inclusion in the list of safety concerns in the RMP

SVII.1.2.1 Important identified risks

Important Identified risks	Identification and benefit-risk impact

Glaucoma	The most commonly reported Adverse Drug Reactions after implantation in the two retrospective studies were in the Eye Disorders SOC. Those ADRs reported in 3 or more of the 142 implants across the two retrospective studies were Conjunctival haemorrhage (7), Corneal epithelium defect (5), Blepharitis (4), Eye haemorrhage (4), Eye pain (4) and Glaucoma (3). One of the implants reported glaucoma related to the use of corticosteroids within 3 months after implantation. Apart from glaucoma, these ocular Preferred Terms are non-specific and may be related to a variety of underlying pathologies. This makes it difficult to suggest specific risk mitigation measures. When reviewing the clinical developmental experience, glaucoma is one of the main identified important risks for autologous human limbal stem cell grafting, which can be mitigated by monitoring and early treatment. The risk of glaucoma increases with age and it is a common condition in patients over 40 years of age in the general population. Other risk factors for glaucoma include myopia, positive family history and diabetes. Patients with ocular chemical burns have an increased risk of glaucoma with the most severe injuries carrying the greatest risk **[20].** Fifteen (83%) of 18 eyes that required long-term glaucoma treatment in this observational study had elevated IOP within 1 week of presentation. Glaucoma is also common following penetrating keratoplasty **[9]**. In the study by Fan et al, IOP elevation occurred 3–6 months post-keratoplasty in 78% of the eyes. All patients, except one, required reduction/cessat. Glaucoma is a chronic disease in which life-long treatment and follow-up is required, and it is a condition which ultimately may progress to blindness. This risk is considered as important identified risk for the purpose of this RMP.
Lack of effect manifesting as corneal epithelium defect	Corneal implant rejection due to an active immune process is not considered to be an important risk. However, it is recognised that Holoclar may not be effective in all patients as indicated by the need for repeating implantations in a small number of patients. Lack of effect manifesting as corneal epithelium defect is considered an important identified risk in the RMP and this risk more accurately describes the situation with respect to implant failures.

SVII.1.2.2 Important potential risks

Important Potential risks	**Identification and benefit-risk impact**

Blepharitis	Blepharitis is a common condition in which the margins of the eyelids are inflamed. It can be caused by staphylococcal infection, seborrhoeic dermatitis, meibomian gland dysfunction, or any combination of these. Blepharitis is more common in older adults with a mean age of onset ranging from 42 years for staphylococcal blepharitis to 50 years for seborrhoeic and meibomian blepharitis but it can occur at any age. Blepharitis is usually a chronic condition and once it develops it can cause repeated episodes. Of the 39 adverse events of blepharitis reported in the 2 retrospective clinical studies 4 were classified as adverse reactions. Although there is no clear evidence, it is possible that the surgical intervention associated with the administration of Holoclar could reactivate blepharitis but there is no rationale to suggest that Holoclar by itself induces blepharitis. Given the association between the blepharitis observed in the retrospective studies and Holoclar is not clear it will be included as an important potential risk in the RMP.
Concomitant use of eye drops containing benzalkonium chloride	As preservative-free eye drops were used in the Holoclar studies, interactions with preservatives in topically administered eye drops has not been studied. However, the preservative benzalkonium is known to be cytotoxic (**[2]; [8]; [12]; [18]; [7]**) and the use of eye drops containing this preservative is considered to be a potential risk possibly causing a damage of the newly-regenerated corneal epithelium thereby reducing the effectiveness of the implant. Toxicity studies have tended to be carried out using relatively high concentrations of benzalkonium chloride but damage to the tear film and corneal-conjunctival surface have been noted in patients receiving regular long-term treatment for glaucoma with eye drops preserved with benzalkonium chloride in usual concentrations (eye drops typically contain 0.002% to 0.01% benzalkonium chloride). Benzalkonium is cytotoxic it may impair the corneal repair process and reduces effectiveness of the implant. Therefore, it is considered as important potential risk for the purpose of this RMP.

Post-implant infection	Due to the 36-hour shelf life, microbiological testing of the product will not be available until after implantation. A potential risk of infection due to microbiological contamination cannot be excluded. As with all surgical procedures there is a risk of bacterial infection post-implant. Treatment with antibiotics is recommended for 2 weeks following implantation to reduce the risk of eye infection. The experience from clinical studies with Holoclar to date indicates that infection of the eye post-implant is uncommon. Since the use of prophylactic antibiotic treatment (e.g. doxycycline or amoxicillin) and of topical and systemic anti-inflammatory drugs (e.g. prednisone or dexamethasone) is required post-implantation, potential for co-morbidities arising from suspected adverse reactions from the use of these drugs should be taken into consideration. With reference to the SmPC for Vibramycin (doxycycline) or Amoxil (amoxicillin), respectively, potential undesirable effects include but are not limited to: hypersensitivity reactions, severe cutaneous adverse reactions (e.g. erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis), photosensitivity and candidiasis. With reference to the use of corticosteroids (e.g. prednisone or dexamethasone), some of the potential undesirable effects are: suppression of the inflammatory response and immune function increases the susceptibility to infections and their severity. Ophthalmic ADRs are increased IOP with development of glaucoma, papilloedema, posterior subcapsular cataracts, corneal and scleral thinning or perforation after prolonged use. Viral or fungal ophthalmic disease may be reignited or spread. Hypersensitivity can also occur.
Medication errors (e.g. incorrect patient receives product, patient receives incorrect product, incorrect surgical technique)	The main potential for error concerns incorrect selection of patient for autologous limbal stem cell implant because of concomitant eye problems and underlying incorrect diagnosis. Product distribution errors may result in the wrong patient receiving wrong product. In addition, there may be errors in surgical technique implanting Holoclar. Finally, inappropriate prescription of eye drops containing benzalkonium chloride or other preservatives may affect outcome (see above). Holoclar is intended solely for autologous use. The risk of medication errors is included as an important potential risk within the RMP. The main concern is potential maladministration to a patient from whom the limbal stem cells did not originate which could result in an adverse immunological reaction which is not recognised to occur when the limbal stem cells are implanted to a patient from whom the cells originated. It is fundamental that the patient receives the implant that was

	harvested and grown from their own limbal stem cells taken at biopsy. Holostem is committed to minimise the risk of such errors with full traceability of the product during the manufacturing process and during the clinical procedures.
Tumorigenicity	There were 3 reports of adverse events with fatal outcome that concerned neoplasms in HLSTM01. Gastric carcinoma was judged as "unlikely related" to study treatment, whereas brain tumour and lung cancer were considered "not related". Lung cancer was recorded as pre-existing at the pre-surgical visit but no relevant information was recorded for the 2 other patients. The latency for the brain tumour is not supportive of a causal relationship because the patient died just 4 months after receiving Holoclar. The patient with gastric carcinoma died approximately 6 years after receiving Holoclar. The patient was 53 years old at the time of the pre-surgical visit. Risk factors for gastric carcinoma include male gender, > 55 years old, smoking, high salt diet, *Helicobacter pylori* infection, family history, type A blood group and hypogammoglobulinaemia **[**40**]**. unknown if this patient had any of these risk factors. The estimated age standardised incidence rate for gastric cancer in the EU for 2012 was 10.7 per 100,000 (both sexes) and 15.2 per 100,000 (males) **[**39**]**. The corresponding mortality rate was 7.3 per 100,000 (both sexes) and 10.4 per 100,000 (males). Evidence from non-clinical *in vitro* studies suggests that the risk of tumorigenicity is low as outlined in the following section concerning risks related to persistence of the product in the patient. Human keratinocytes forming stratified epithelia give rise to either basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) **[**33**]**. Melanocytes, which also populate some stratified epithelia give rise to melanoma. When either BCC, SCC or melanoma cells are injected into other body sites of laboratory animals, they form ectopic BCC, SCC or melanoma **[**29**]** but not other types of cancer. Accordingly, the primary tumour in humans can usually be inferred from the histology of the metastasis. Gastric cancer originates from glandular epithelium of the gastric mucosa. Histologically, there are two major types of gastric adenocarcinoma: intestinal type or diffuse type. In both cases these cancers, as well as their metastasis, are clearly identified at histological level. There have been no reports of SCC developing in the stomach. It can thus be excluded that the reported gastric cancer case could have arisen from limbal cultures.

	Although there is currently no evidence of tumour development directly related to the use of Holoclar (and skin keratinocytes) from long-term follow up of patients in the retrospective studies, the number of patients exposed to Holoclar is insufficient to formally exclude the theoretical possibility of the development of such tumours. For this reason, tumorigenicity is included as a potential risk in the RMP.
Off label use -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	Potential for off label use would include use in patients with a milder form of limbal stem cell deficiency than the proposed indication (moderate-severe), use in limbal stem cell deficiency due to aetiologies other than physical or chemical ocular burns (e.g. radiotherapy, aniridia, Stevens-Johnson syndrome and neurotrophic keratitis) and use in patients less than 18 years old.

SVII. 1. 2. 3 Missing information

Missing information	Identification and benefit-risk impact
Pregnancy and breast-feeding	No pregnant or breast-feeding patients were included in the retrospective clinical studies. As a result, there are no data for the use of Holoclar in pregnant women. Animal studies are insufficient with respect to reproductive toxicity. Conventional reproductive and developmental toxicity studies are not considered relevant, given the nature and the intended clinical use of the autologous tissue-engineered product. As a precautionary measure, since the requirement of the post-operative pharmacological treatment, it is preferable to avoid the use of Holoclar during pregnancy. Holoclar is not recommended during pregnancy and in woman of childbearing potential not using contraception from the biopsy to the conclusion of post-operative pharmacological treatment. As a precautionary measure, Holoclar is not recommended for implant during breast-feeding.
Children (limited data)	There were no exclusion criteria relating to age in the retrospective clinical studies HLSTM01, HLSTM02. No paediatric patients were included in HLSTM04. However, there is no information on the safety of autologous human limbal stem cells in children up to 7 years of age

	and only limited information in patients aged 8 – 17 years. The profile of adverse reactions in five paediatric patients included in the studies HLSTM01 (age 13, 14 and 16 years) and HLSTM02 (age 8 and 14 years) was not different from the adult population. In view of the limited safety and efficacy data in the paediatric population, Holoclar is indicated for adults only and section 4.2 of the SmPC states that the safety and efficacy of Holoclar in children and adolescents aged 0-18 years has not yet been established.
Elderly (limited data)	Fourteen (14) patients included in the two retrospective clinical studies HLSTM01 and HLSTM02 were 65 years old or older. Three (3) elderly patients were treated in HLSTM04 study. As such the data are limited in this population. No meaningful differences in AE profile between elderly and adult population were seen.
Re-administration of Holoclar	Eleven patients had a second ACLSC implant and one patient underwent a third implant. No specific risks related to re-administration of the product have been observed. However, as the amount of data is limited concerning re-administration this has been added as missing information
Long term safety and efficacy follow up	The long-term follow up data is limited due to the retrospective nature of the clinical trials. Follow-up data are available for ≥ 1-year duration in 93.8% of ACLSC implants (106 of 113 implants) in study HLSTM01. In the same study 43.4% (49 of 113 implants) have follow-up data pertaining ≥ 3 years. From study HLSTM02, 82.8% (24 of 29 implants) included follow-up data covering a ≥ 1-year period while 41.4% (12 of 29 implants) covered durations of ≥ 3 years. A limited follow-up was available for study HLSTM04 (10.72 ± 7.99 months). There is no evidence from the retrospective studies to suggest that long term use is associated with a safety concern. *In vitro* studies have confirmed localisation of the implant without migration of the epithelial cells into basal ocular structures. Furthermore, the risk of tumour formation related to 3T3-J2 feeder cells proliferative capacity has been demonstrated to be low following the lethally irradiation. Prospective study information will be required to confirm this finding.

SVII.2 New safety concerns and reclassification with a submission of an updated RMP.

RMP version 9.1 (26 October 2018):

1) The list of safety concerns included in previous versions of the RMP have been revised based on the definition of important identified/potential risks and missing information given in the GVP Module V (Rev.2).

- **The potential risk** << *Tumorigenicity* >> has been removed from the list of safety concerns as neither the product information is advising on specific clinical actions to be taken to minimise the risk, nor additional risk minimisation activities are part of the pharmacovigilance plan in this RMP. Nevertheless, all information on << *Tumorigenicity* >> will be collected and evaluated as part of the long-term safety profile which is considered as a missing information.

  In addition, in the clinical trials programme, there were 3 reports of adverse events with fatal outcome that concerned neoplasms (in HLSTM01). Gastric carcinoma was judged as "unlikely related" to study treatment, whereas brain tumour and lung cancer were considered "not related". Lung cancer was recorded as pre-existing at the pre-surgical visit but no relevant information was recorded for the 2 other patients. The latency for the brain tumour is not supportive of a causal relationship because the patient died just 4 months after receiving Holoclar.

  The patient with gastric carcinoma died approximately 6 years after receiving Holoclar. The patient was 53 years old at the time of the pre-surgical visit. Risk factors for gastric carcinoma include male gender, > 55 years old, smoking, high salt diet, *Helicobacter pylori* infection, family history, type A blood group and hypogammoglobulinaemia **[**40**]**. However, it is unknown if this patient had any of these risk factors. The estimated age standardised incidence rate for gastric cancer in the EU for 2012 was 10.7 per 100,000 (both sexes) and 15.2 per 100,000 (males) **[**39**]**. The corresponding mortality rate was 7.3 per 100,000 (both sexes) and 10.4 per 100,000 (males).

  Evidence from non-clinical *in vitro* studies suggests that the risk of tumorigenicity is low as outlined in the following section concerning risks related to persistence of the product in the patient. Human keratinocytes forming stratified epithelia give rise to either basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) **[**33**]**. Melanocytes, which also populate some stratified epithelia give rise to melanoma. When either BCC, SCC or melanoma cells are injected into other body sites of laboratory animals, they form ectopic BCC, SCC or melanoma **[**29**]** but not other types of cancer. Accordingly, the primary tumour in humans can usually be inferred from the histology of the metastasis. Gastric cancer originates from glandular epithelium of the gastric mucosa. Histologically, there are two major types of gastric adenocarcinoma: intestinal type or diffuse type. In both cases these cancers, as well as their metastasis, are clearly identified at histological level. There have been no reports of SCC developing in the stomach. It can thus be excluded that the reported gastric cancer case could have arisen from limbal cultures.

  Although there is currently no evidence of tumour development directly related to the use of Holoclar (and skin keratinocytes) from long-term follow up of patients in the retrospective studies, the number of patients exposed to Holoclar.

- Rewording and reclassification of the following important potential risk <<Off label use>>:

- *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*

The PT <<off label use>> has been split and re-worded as follow:

-Off label use (*milder form of limbal stem cell deficiency than the proposed indication or use in 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 has been merged and reworded as follow:* <<Safety profile in children under 18 years with moderate to severe limbal stem cell deficiency when used off label >> will be reclassified in <<missing information>> as follows <<Use in children>>.

➢ The missing information << re-administration of Holoclar >> has been removed from the list of safety concerns:

In line with the new RMP concept of GVP V revision2, this safety concern as missing information has no significant impact on the benefit-risk of the medicinal product for which authorisation is applied for. In addition, there are no risk minimisation activities and no advise on risk management in the product information. All risks related to the re-administration of Holoclar will be monitored in the long-term safety profile which remains a missing of information in this RMP.

List of proposed 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) • Off label use o milder form of limbal stem cell deficiency than the proposed indication o Off label use for other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis
Missing information	• Use in pregnancy and lactation • Use in children Use in elderly • Long-term safety

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SVII.3 Details of important identified risks, important potential risks and missing information

SVII. 3.1 Presentation of important identified risks and important potential risks

Important Identified risk 1	**Glaucoma**
MedDRA PT Term (code)	SMQ (20000146)
Potential mechanism	With increased age glaucoma 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
Evidence source and strength of evidence	In clinical trials (HLSTM 01, HLSTM02, HLSTM04) glaucoma was reported in 14.7% of treated patients. No cases of glaucoma were collected in the HOLOCORE study.
Characterisation of the risk	Seriousness/outcomes: Might have serious outcome
	Frequency: 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 **[**17**]**. Data from clinical trial HOLOCORE, HOLOCORE-FU and study HOLOSIGHT: Cumulatively, eight (8) cases were received reporting adverse events of glaucoma, intraocular pressure increase or hypertension. In the clinical HOLOCORE trial, two (2) SAEs of intraocular pressure increased were received. In the post-marketing HOLOSIGHT study two (2) cases reporting AEs of "Ocular hypertension" / "Glaucoma", and four (4) cases reporting the AE of "Glaucoma" were reported.
	Background incidence/prevalence: A retrospective longitudinal study performed in Southern Germany with 5 years of follow-up and 3,531 participants showed that the incidence rate of glaucoma as a main cause of blindness in the 40-59-year age group was 2.37 (95% CI: 1.93–2.81) per 100,000-person years **[**35**]**. A systematic literature review **[**26**]** identified a cross-sectional study from the European North of Russia that estimated the incidence of glaucoma at a level of 1.3 cases in 1,000 persons. Patients with ocular burns are more likely to have glaucoma. A retrospective, observational case series investigated 29 eyes (18 patients) with ocular chemical burns seen between 1997 and 2010 with a minimum of 3

Important Identified risk 1	Glaucoma
	months of follow-up at the University of Washington (Lin et al., 2012). Glaucoma after ocular chemical burns was associated with more severe burns: 16 (84%) of 19 eyes with Roper-Hall grade III or IV ocular chemical burns required long-term glaucoma medication. Only a small proportion of eyes that had initially low Intraocular Pressure (IOP) later demonstrated elevated IOP requiring glaucoma medications. Fifteen (83%) of 18 eyes that required long-term glaucoma treatment had elevated IOP within 1 week of presentation. Glaucoma is common following keratoplasty. A retrospective study was carried out in 228 patients who underwent penetrating keratoplasty from January 1995 to January 2000 at the Federal University of Uberlândia MG, Brazil **[9]**. Two hundred twenty-eight patients undergoing penetrating keratoplasty were evaluated and 49 (21.5%) developed glaucoma. Risk factors for developing glaucoma were bullous keratopathy [relative risk (RR) 2.1774), herpesvirus (RR 1.8979) and trauma (RR 1.0575). A consecutive series of penetrating keratoplasties performed in New Zealand for keratoconus were analysed retrospectively (Fan et al., 2009). The study included 57 eyes of 48 patients. Of these 18 eyes (32%) of 17 patients (35%) exhibited elevated IOP and 12 (21%) eyes exhibited moderate-to-severe elevation of IOP. IOP elevation occurred 3–6 months post keratoplasty in 78% of eyes. All patients except one required reduction/ cessation of corticosteroids to normalise IOP. The following information has been extracted from a systematic literature review **[26].** It has been estimated that 21.8% of European adults (including 18% of those over 50 years of age) have been diagnosed with glaucoma. According to recent epidemiological studies, Germany (14%) shows the highest prevalence of glaucoma in Europe followed by the European North of Russia (11.9%). The lowest prevalence of any type of glaucoma has been registered in France (3.4%) and the UK (3.3%).
Risk groups or risk factors	Glaucoma is one of the main identified important risks for autologous human limbal stem cell grafting. The risk of glaucoma increases with age and it is a common condition in patients over 40 years of age in the general population. Other risk factors for glaucoma include myopia, positive family history and diabetes. Patients with ocular chemical burns have an increased risk of glaucoma with the most severe injuries carrying the greatest risk **[20]**. Fifteen (83%) of 18 eyes that required long-term glaucoma treatment in this observational study had elevated IOP within 1 week of presentation. Glaucoma is also common following penetrating keratoplasty **[9]**.

Important Identified risk 1	**Glaucoma**
	In the study by Fan et al, IOP elevation occurred 3–6 months post-keratoplasty in 78% of the eyes. All patients, except one, required reduction.
Preventability	Not applicable
Impact on the risk-benefit balance of the product	The impact on benefit risk balance even is not expected to be significant, it remains uncertainties concerning nature and causality to be elucidate. Glaucoma might be a risk factor for failure of the implant and therefore considered an important identified risk for the product.
Potential public health impact of safety concern	Not known.

Important Identified risk 2	**Lack of effect manifesting as corneal epithelium defect**
MedDRA PT Term (code)	Lack of efficacy/effect (SMQ) 20000032
Potential mechanism	Not known
Evidence source and strength of evidence	Clinical trials (HLSTM 01, HLSTM02, HLSTM04 and HOLOCORE) Corneal epithelial defects were identified as ADRs in patients treated in HLSTM01, HLSTM02 and HOLOCORE and were potentially related to treatment failure.
Characterisation of the risk	Seriousness/outcomes: Not known
	Frequency: In clinical trials HLSTM 01, HLSTM02 and HLSTM04, 6 cases (3, 2%) of epithelium defect with onset between 1 week and 3 months after treatment have collected. Three (03) adult patient experienced epithelium defect reported (N=34) in HOLOCORE study during the reference period. Data from clinical trial HOLOCORE, HOLOCORE-FU and study HOLOSIGHT: Two cases coding events of engraft failure were reported from HOLOSIGHT study. Cumulatively, seventeen (17) AEs under HLTs Corneal structural change, deposit and degeneration (10011049) or Eye injuries NEC (10027674) were reported from the HOLOCORE trial and HOLOSIGHT study, eleven (11) of them serious and six (6) non-serious. The AEs were reported in sixteen (16) cases occurring in eleven (11) different patients.

Important Identified risk 2	Lack of effect manifesting as corneal epithelium defect
	Background incidence/prevalence: Not known
Risk groups or risk factors	Not known
Preventability	A thorough evaluation of the patient should be done taking into consideration not only the clinical need of the candidate, but also the biological and pathophysiologic alterations in the wound bed environment, to define the timing of any procedure and allow the proper engraftment and growth of the stem cells of the living tissue that constitute Holoclar. Concomitant surgeries should be excluded and anticipated or deferred to Holoclar implantation. For any of the steps of the treatment with Holoclar, topical lidocaine or anaesthetics containing adrenaline must be avoided as they reduce the colony forming efficiency.
Impact on the risk-benefit balance of the product	Not known
Potential public health impact of safety concern	Not known.

SVII. 3.2 Important potential risks

Important potential risk 1	Blepharitis
MedDRA PT Term (code)	Ocular infections (SMQ) 20000183 Periorbital and eyelid disorders (SMQ) 20000179
Potential mechanism	Blepharitis is a common condition in which the margins of the eyelids are inflamed. It can be caused by staphylococcal infection, seborrhoeic dermatitis, meibomian gland dysfunction, or any combination of these. Blepharitis is more common in older adults with a mean age of onset ranging from 42 years for staphylococcal blepharitis to 50 years for seborrhoeic and meibomian blepharitis but it can occur at any age. Blepharitis is usually a chronic condition and once it develops it can cause repeated episodes.

Important potential risk 1	**Blepharitis**
Evidence source and strength of evidence	Of the 39 adverse events of blepharitis reported in the 2 retrospective clinical studies 4 were classified as adverse reactions. Although there is no clear evidence, it is possible that the surgical intervention associated with the administration of Holoclar could reactivate blepharitis but there is no rationale to suggest that Holoclar by itself Induces blepharitis. Given the association between the blepharitis observed in the retrospective studies and Holoclar is not clear it will be included as an important potential risk in the RMP. Of the 39 adverse events of blepharitis reported in the 2 retrospective clinical studies 4 were classified as adverse reactions. Although there is no clear evidence, it is possible that the surgical intervention associated with the administration of Holoclar could reactivate blepharitis.
Characterisation of the risk	Seriousness/outcomes; Might be serious
	Frequency: Clinical trials Data from clinical trial HOLOCORE, HOLOCORE-FU and study HOLOSIGHT Cumulatively, three (3) SAEs have been reported: one (1) SAE of Keratitis and one (1) SAE of Ulcerative Keratitis in the HOLOCORE trial (both of them assessed as not related to the administration of Holoclar), and one (1) SAE of Blepharitis in the HOLOSIGHT study (assessed as possible related to the administration of Holoclar) and one non-serious AE of blepharitis (assessed as possibly related to Holoclar) in the HOLOSIGHT study. All three SAEs were resolved while the non-serious case of blepharitis outcome was reported as unknown.
	Background incidence/prevalence: As with any surgery, there is a recognised risk of infection which mandates post-operative topical and systemic antibiotic and anti-inflammatory treatment. By using this regimen, the incidence of treatment-related infections was observed to be low in the two retrospective clinical studies (12 infections reported in study HLSTM01 including herpetic keratitis, influenza and upper airway infections; three patients developed infections in study HLSTM02 but only one of these, corneal infection, was considered related to treatment).
Risk groups or risk factors	Not known
Preventability	Patients with acute ocular inflammation or infections should be deferred until recovery has been documented since inflammation may compromise treatment success. Following implantation, an appropriate regimen of topical and systemic anti-inflammatory and prophylactic antibiotic treatment must be given.

Important potential risk 1	**Blepharitis**
	The following regimen is suggested: Doxycycline 100 mg tablets twice daily (or amoxicillin 500 mg twice daily) and prednisone orally at a daily dose of 0.5 mg/kg (to a maximum dose of 25 mg) per day should be administered from the day of surgery for 2 weeks. After 2 weeks the systemic antibiotic administration should be stopped and the daily dose of prednisone should be tapered to 0.25 mg/kg (r maximum 12.5 mg) per day for 1 week, to 0.125 mg/kg (maximum 5.0 mg) per day for the following week and then stopped.
Impact on the risk-benefit balance of the product	The impact on the benefit risk balance is not expected to be significant, nevertheless the risk needs to be monitored due to unpredictable character of the infection and its complications. Infection might be a risk factor for failure of the implant and therefore considered an important potential risk for the product.
Potential public health impact of safety concern	Limited.

Important potential risk 2	**Concomitant use of eye drops containing benzalkonium chloride**
MedDRA PT Term (code)	Injury corneal 10022120
Potential mechanism	Benzalkonium chloride is a cationic surface-acting agent belonging to the quaternary ammonium group. It has three main categories of use: as a biocide, a cationic surfactant, and phase transfer agent in the chemical industry. The mechanism of action is thought to be due to disruption of intermolecular interactions. This can cause dissociation of cellular membrane bilayers, which compromises cellular permeability controls and induces leakage of cellular contents. Other biomolecular complexes within the bacterial cell can also undergo dissociation. Enzymes, which finely control a plethora of respiratory and metabolic cellular activities, are particularly susceptible to deactivation. Critical intermolecular interactions and tertiary structures in such highly specific biochemical systems can be readily disrupted by cationic surfactants. As benzalkonium is cytoxic it may impair the corneal repair process.
Evidence source and strength of evidence	Benzalkonium chloride is one of the most disruptive ophthalmic additives to the stability of the lipid film and to corneal epithelium membranes; toxicity studies have tended to be carried out using relatively high concentrations of benzalkonium chloride but damage to the tear film and corneoconjunctival

Important potential risk 2	Concomitant use of eye drops containing benzalkonium chloride
	surface have been noted in patients receiving regular long-term treatment for glaucoma with eye drops preserved with benzalkonium chloride in usual concentrations (eye drops typically contain 0.002% to 0.01% benzalkonium chloride). Corneal toxicity has also been reported in patients inadvertently exposed to benzalkonium as a preservative in viscoelastic material during cataract surgery.[1], [2], [12], [18], [7].
Characterisation of the risk	Seriousness/outcomes: Might be serious
	Frequency: Not applicable Data from clinical trial HOLOCORE, HOLOCORE-FU and study HOLOSIGHT: Cumulatively, neither SAEs from clinical trials nor suspected ADRs from the post-marketing experience were received.
	Background incidence/prevalence: Not applicable
Risk groups or risk factors	Not Applicable
Preventability	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.
Impact on the risk-benefit balance of the product	Reduces effectiveness of the implant and therefore considered an important potential risk for the product.
Potential public health impact of safety concern	Not applicable

Important potential risk 3	Post-implant infection
MedDRA PT Term (code)	Implant site infection 10059650
Potential mechanism	As with all surgical procedures there is a risk of bacterial infection post-implant.

Important potential risk 3	**Post-implant infection**
Evidence source and strength of evidence	The experience from clinical studies with Holoclar to date indicates that infection of the eye post-implant is uncommon.
Characterisation of the risk	Seriousness/outcomes: Might be serious
	Frequency: The experience from clinical studies with Holoclar to date indicates that infection of the eye post-implant is uncommon. Data from clinical trial HOLOCORE, HOLOCORE-FU and study HOLOSIGHT: Cumulatively five (5) AEs have been reported related to this risk: one (1) SAE of Ulcerative Keratitis and one (1) SAE of Keratitis occurred in the HOLOCORE trial and one (1) SAE of Blepharitis, one (1) non-serious AE of Blepharitis and one (1) non-serious AE of Eye inflammation occurred in HOLOSIGHT study. SAEs of Ulcerative keratitis, Keratitis and Blepharitis were assessed as not related to the administration of Holoclar while non-serious AEs of Blepharitis and Eye inflammation were assessed as possible related. All of the AEs resolved except for the non-serious AE of Blepharitis, the outcome of which is unknown.
	Background incidence/prevalence: Not known
Risk groups or risk factors	Not applicable
Preventability	Treatment with antibiotics is recommended for 2 weeks following implantation to reduce the risk of eye infection. The experience from clinical studies with Holoclar to date indicates that infection of the eye post-implant is uncommon. Since the use of prophylactic antibiotic treatment (e.g. doxycycline or amoxicillin) and of topical and systemic anti-inflammatory drugs (e.g. prednisone or dexamethasone) is required post-implantation, potential for co-morbidities arising from suspected adverse reactions from the use of these drugs should be taken into consideration.
Impact on the risk-benefit balance of the product	The impact on the benefit risk balance is not expected to be significant, nevertheless the risk needs to be monitored due to unpredictable character of the infection and its complications. Infection might be a risk factor for failure of the implant and therefore considered an important potential risk for the product.
Potential public health impact of safety concern	Not applicable

Important potential risk 4	**Medication errors (e.g. incorrect patient receives product, patient receives incorrect product, incorrect surgical technique)**
MedDRA PT Term (code)	Medication errors (SMQ) 20000224
Potential mechanism	The main potential for error concerns incorrect selection of patient for autologous limbal stem cell implant because of concomitant eye problems and underlying incorrect diagnosis. Product distribution errors may result in the wrong patient receiving wrong product. In addition, there may be errors in surgical technique implanting Holoclar. Finally, inappropriate prescription of eye drops containing benzalkonium chloride or other preservatives may affect outcome. The concomitant use of topical lidocaine or anaesthetics containing adrenaline must be avoided as they reduce the colony forming efficiency.
Evidence source and strength of evidence	Holoclar is intended solely for autologous use. The main concern is potential maladministration to a patient from whom the limbal stem cells did not originate which could result in an adverse immunological reaction which is not recognised to occur when the limbal stem cells are implanted to a patient from whom the cells originated. It is fundamental that the patient receives the implant that was harvested and grown from their own limbal stem cells taken at biopsy. Cumulatively no medication errors associated with Holoclar have been reported.
Characterisation of the risk	Seriousness/outcomes: Not known
	Frequency: Not known
	Background incidence/prevalence: Not applicable
Risk groups or risk factors	Not applicable
Preventability	To minimise the risk of such errors with full traceability of the product during the manufacturing process and during the clinical procedures. For any of the steps of the treatment with Holoclar, topical lidocaine or anaesthetics containing adrenaline must be avoided.
Impact on the risk-benefit balance of the product	Might be a risk factor for reducing the effectiveness of the implant and therefore considered an important potential risk for the product.
Potential public health impact of safety concern	Not known.

Important potential risk 5	**Off label use:** -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
MedDRA PT Term (code)	10053762
Potential mechanism	No known
Evidence source and strength of evidence	The product is indicated in patients with LSCD due to physical or chemical ocular burns so other aetiologies e.g. SJS, neurotrophic keratitis or moderate form limbal stem cell deficiency would be off-label use. Cumulatively, neither events from the clinical trials nor from the post-marketing experience were received.
Characterisation of the risk	Seriousness/outcomes: Not known
	Frequency: Not known.
	Background incidence/prevalence: Not known
Risk groups or risk factors	Not applicable
Preventability	Not applicable
Impact on the risk-benefit balance of the product	The effectiveness of implant might be reduced or absent.
Potential public health impact of safety concern	Not known.

SVII. 3.3 Presentation of the missing information

Missing information: Use in pregnancy and lactation

Evidence source: No patients included in clinical trials were pregnant or breast-feeding. As a result, there are no data for the use of Holoclar in pregnant women. Animal studies are insufficient with respect to reproductive toxicity. Conventional reproductive and developmental toxicity studies are not considered relevant, given the

nature and the intended clinical use of the autologous tissue-engineered product. As a precautionary measure, since the requirement of the post-operative pharmacological treatment, it is preferable to avoid the use of Holoclar during pregnancy. Holoclar is not recommended during pregnancy and in woman of childbearing potential not using contraception from the biopsy to the conclusion of post-operative pharmacological treatment. As a precautionary measure, Holoclar is not recommended for implant during breast-feeding.

Population in need of further characterisation: Based on the limited data this risk is in need for further characterisation.

Missing information: Use in children

Evidence source: There were no exclusion criteria relating to age in the two retrospective clinical studies HLSTM01 and HLSTM02. However, there is no information on the safety of autologous human limbal stem cells in children up to 7 years of age and only limited information in patients 8 – 17 years of age. The profile of adverse reactions in the paediatric patients included in the studies HLSTM01 (age 13, 14 and 16 years), HLSTM02 (age 8 and 14 years) and study CCD-GPLSCD01-03 (HOLOCORE) (age 6, 8, 12 and 13) was not different from the adult population.

Cumulatively, in the HOLOCORE study, a total of fourteen (14) AEs have been reported in two out of four paediatric participants, all of them non-serious. Two pre-treatment AEs (none related to the biopsy) were reported in 1 patient (25.0%). In total 12 treatment emergent adverse events (TEAEs) were reported in the paediatric population after Holoclar implantation, affecting 2 patients (50.0%). Two TEAEs were classified as possibly treatment-related adverse events (TRAEs) to the ACLSCT surgical procedure. Additionally, five (5) non-serious TEAEs occurred in the HOLOCORE-FU study (PTs: Eyelid rash, Eye discharge, Corona virus infection and Eye irritation [2 events]) in the same patient. All events were considered mild and not related to treatment. One (1) non-serious TEAE has been collected in the HOLOSIGHT study (PT: Blepharitis) in patients below 18 years of age so far.

Population in need of further characterisation: In view of the limited safety and efficacy data in the paediatric population, Holoclar will be indicated for adults only and section 4.2 of the SmPC will state that the safety and efficacy of Holoclar in children and adolescents aged 0-18 years has not yet been established. Based on the limited data this risk is in need for further characterisation.

Missing information: Use in elderly

Evidence source: Fourteen (14) patients included in the two retrospective clinical studies HLSTM01 and HLSTM02 were 65 years old or older. Three (3) elderly patients were treated in HLSTM04 study and six (6) in the HOLOCORE study. As such the data are limited in this population. No meaningful differences in AE profile between elderly and adult population were seen.

Population in need of further characterisation: Based on the limited data this risk is in need for further characterisation.

Missing information: Long-term safety

Evidence source: The long-term follow up data is limited due to the retrospective nature of the clinical trials. Follow-up data are available for ≥ 1 year duration in 93.8% of ACLSC implants (106 of 113 implants) in study HLSTM01. In the same study 43.4% (49 of 113 implants) have follow-up data pertaining ≥ 3 years. From study HLSTM02, 82.8% (24 of 29 implants) included follow-up data covering a ≥ 1 year period while 41.4% (12 of 29 implants) covered durations of ≥ 3 years. A limited follow-up was available for study HLSTM04 (10.72 ± 7.99 months). From study CCD-GPLSCD01-03 (HOLOCORE), sixty-eight (68) patients completed the 12-months follow-up after first treatment with Holoclar and forty-four (44) among them completed the Long-term Follow-up study. Individual patient duration for HOLOCORE Follow-Up varied from a minimum duration of 12 months, for the last patient entered, to up to potentially 57 months for the first enrolled patient.

In HOLOSIGHT study (ongoing) data covering a 1 year period has been collected for 56 implants, data covering a 2 year period has been collected for 31 implants and data covering a 3 or 4 year period has been collected for 30 implants.

There is no evidence from the retrospective studies, HOLOCORE and HOLOCORE FOLLOW-UP to suggest that long term use is associated with a safety concern. *In vitro* studies have confirmed localisation of the implant without migration of the epithelial cells into other ocular structures and below the basal lamina. Furthermore, the risk of tumour formation related to 3T3-J2 feeder cells proliferative capacity has been demonstrated to be absent following the lethal irradiation. The final conclusion of the prospective study (HOLOSIGHT PASS) will confirm these findings.

Population in need of further characterisation: Based on the limited data this risk is in need for further characterisation.

Part II: Module SVIII - Summary of the safety concerns

Table SVIII.1: Summary of 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) • 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
Missing information	• Pregnancy and lactation • Use in children • Use in elderly • Long-term safety

Part III Pharmacovigilance Plan (including post-authorisations safety studies)

III.1 Routine Pharmacovigilance activities

Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None.

III.2 Additional pharmacovigilance activities

Completed studies

1. **Study CCD-GPLSCD01-03 (HOLOCORE) summary:**

   Study short name and title: CCD-GPLSCD01-03; Multinational, multicentre, prospective, open-label, uncontrolled clinical trial to assess the efficacy and safety of autologous cultivated limbal stem cells transplantation (ACLSCT) for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns (HOLOCORE)

   Rationale and study objectives: To reproduce the established treatment protocol (including repeated transplantation whenever needed and post-operative therapies) and to confirm the observed efficacy and safety of ACLSCT in restoring a normal corneal epithelium in patients suffering from moderate to severe LSCD secondary to ocular burns. To further investigate the safety concerns of glaucoma, Lack of effect (corneal implant failure), blepharitis and safety profile in children under 18 years of age. Patients from 2 years of age and adults will be included in the study.

   Study design: Multinational, multicentre, prospective, open label, uncontrolled clinical study to evaluate 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.

   Study population: Adult male and female patients (≥18 years old); four paediatric patients aged 2 to 17 years were also planned to be enrolled for safety purposes only with LSCD secondary to unilateral or bilateral physical or chemical ocular burns*, with at least 1-2 mm2 of undamaged limbus to harvest stem cells for expansion in culture. LSCD was considered for inclusion in presence of superficial neo-vascularisation invading at least two corneal quadrants with evidence of central corneal (central 6 mm diameter) involvement (including central corneal neo-vascularisation or corneal opacity).

   Milestones: The study was authorized by Health Authorities according to the legal requirements in each participating country. Selection of the subjects did not start before the approval of the Ethics Committees had been obtained and the study authorized by to Health Authorities. During the trial interim reports were prepared and submitted to EMA annually according to the agreement with the Agency. The Interim Reports were not to be intended as Interim Analyses. In the interim report were included descriptive data on accrual, baseline characteristics, adverse events, primary endpoint and study conduct and compliance, and were prepared and submitted to EMA annually according to the agreement with the Agency. CSR was completed in March 2023.

2. **Study CCD-GPLSCD01-03-FU (HOLOCORE follow-up) summary:**

Study short name and title: CCD-GPLSCD01-03-FU; Multinational, multicenter, prospective, long-term safety and efficacy follow-up study after Autologous Cultivated Limbal Stem Cells Transplantation (ACLSCT) for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns (HOLOCORE-FU).

Rationale and study objectives: To demonstrate the long-term safety of one or two ACLSCT(s) with Holoclar in patients suffering from moderate to severe LSCD secondary to ocular burns.

Study design: Multinational, Multicenter, Prospective, Long-term Safety and Efficacy Follow-up Study.

Study population: Patients included and treated in the main HOLOCORE study.

Milestones: The study was notified to the Health Authorities (or authorized by) according to the legal requirements in each participating country.

Selection of the subjects did not start before the approval of the Ethics Committee/Institutional Review Board was obtained and the study notified to Health Authorities (or authorized by). The HOLOCORE FOLLOW-UP study is closed. LPLV was 31 March 2023. CSR was completed in October 2023.

Ongoing studies

3. Post-authorisation Safety Study, registry -like (HOLOSIGHT) summary:

Study short name and title:

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 (HOLOSIGHT).

Rationale and study objectives: In order to expand the body of data and experience so far collected, a PASS is planned as a part of the Risk Management Plan for Holoclar®. The study is conducted in the early post-authorization phase of Holoclar®, with the primary aim of evaluating the long-term safety profile of the first 100 EU patients treated with Holoclar® according to clinical practice during a 5-year follow-up period. Secondary aims include long-term patient management, treatment success, visual acuity and quality of life. Moreover, the safety study, registry -like collects data useful for the evaluation of the effectiveness of the risk minimization measures in compliance with the Risk Management Plan for Holoclar®. Therefore, this safety study, registry -like is an organized system that uses observational methods to collect data on specified outcomes in patients undergoing Holoclar®.
Study design: Observational, multinational, multicenter, prospective cohort safety study, registry -like.

Study population: Patients treated with Holoclar® in the routine clinical practice setting who agree to participate in the study. The first 100 commercial patients treated with Holoclar were planned to be included in the safety study, registry -like.

Milestones: The study enrollment is considered completed. The end of the data collection (including the follow-up period) is expected within May 2028. Final study report is planned by December 2028.

III.3 Summary Table of additional Pharmacovigilance activities

Table Part III.1: On-going and planned additional pharmacovigilance activities

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 – Imposed mandatory additional Pharmacovigilance activities which are conditions of the marketing authorisation				
None	None	None	None	None
Category 2 – Imposed mandatory additional Pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None	None	None	None	None
Category 3 – Required additional Pharmacovigilance activities				
None	None	None	None	None
Post-authorisation Safety Study (Holosight) "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."	<u>Primary Objective</u> 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 special interest are 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	-Glaucoma -Lack of effect manifesting as corneal epithelium defect -Blepharitis -Posi implant infection -Concomitant use of eye drops containing benzalkonium chloride -Medication errors -Off label use	Final study report	31/12/2028

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
	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. Evaluation of the effectiveness of the risk minimisation measures in compliance with the Risk Management Plan for Holoclar.	-Use in pregnancy and breast-feeding -Safety profile in children under 18 years of age -Long-term safety		

Part IV: Plans for post-authorisation efficacy studies

No imposed post-authorisation efficacy studies are planned or on-going. Study CCD-GPLSCD01-03, which was a condition of the marketing authorisation, has been finalised and the final study report was completed in March 2023.

Part V: Risk minimisation measures (including evaluation of the effectiveness of risk minimisation activities)

V.1 Routine Risk Minimisation Measures

Table Part V.1: Description or routine risk minimisation measures by safety concern

Safety Concern 1	**Routine risk minimisation activities**
Glaucoma	Routine risk communication: *SmPC section 4.4* *SmPC section 4.8* *PIL section 4* Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.

Safety Concern 2	**Routine risk minimisation activities**
Lack of effect manifesting as corneal epithelium defect	Routine risk communication: *SmPC section 4.2* *SmPC section 4.3* *SmPC section 4.4* *SmPC section 4.5* *SmPC section 4.8* Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 3	**Routine risk minimisation activities**
Blepharitis	Routine risk communication: *SmPC section 4.8* *PIL sections 2 and 4*

	Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.

Safety Concern 4	**Routine risk minimisation activities**
Concomitant use of eye drops containing benzalkonium chloride	Routine risk communication: *SmPC section 4.2* *SmPC section 4.5* *PIL section 2* Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 5	**Routine risk minimisation activities**
Post-implant infection	Routine risk communication: *SmPC section 4.2* *SmPC section 4.4* *SmPC section 4.8* *PIL sections 2 and 4* Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 6	**Routine risk minimisation activities**
Medication errors (e.g. incorrect patient receives product, patient receives incorrect product, incorrect surgical technique)	Routine risk communication: *SmPC section 4.1* *SmPC section 4.2* *SmPC section 4.4* *SmPC section 4.5* *SmPC section 6.6*

	Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 7	**Routine risk minimisation activities**
Off label use 1. milder form of limbal stem deficiency than the proposed indication, 2. other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson syndrome and neurotrophic keratitis	Routine risk communication: *SmPC section 4.1* *SmPC section 4.2* *PIL section 1* Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 8	**Routine risk minimisation activities**
Use in pregnancy and lactation	Routine risk communication: *SmPC section 4.2* *SmPC section 4.6* *PIL section 2* Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 9	**Routine risk minimisation activities**
Use in children	Routine risk communication: *SmPC section 4.1* *SmPC section 4.2* *SmPC section 4.8* *PIL section 2*

	Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 11	**Routine risk minimisation activities**
Use in elderly	Routine risk communication: *SmPC section 4.2* *SmPC section 4.8* *SmPC section 5.1* Other routine risk minimisation measures beyond the Product Information: Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only

Safety Concern 12	**Routine risk minimisation activities**
Long-term safety	None proposed

V.2 Additional Risk Minimisation Measures

Educational Manual for healthcare professional

Objectives:

To increase the healthcare professional understanding about the required treatment procedure by providing full technical details and on measures to be taken in order to minimise risks related to the treatment.

Rationale for the additional risk minimisation activity:

For some risks related to Holoclar, routine risk minimisation measures might be not sufficient and additional risk minimisation measure such Educational material deemed necessary to increase the knowledge on risks related to the product and how to prevent them.

Target audience and planned distribution path:

All surgeons intending to use Holoclar are subject to extensive training to promote a comprehensive understanding of Holoclar and the possible risks associated with the product, and to provide clear guidance on biopsy and implant techniques and post-implantation therapy. Training of healthcare professionals is referred to in the Educational Manual for healthcare professionals which includes the clinical protocol for the screening and treatment of pre- and post-operative patients undergoing an autologous implant of the corneal epithelium using Holoclar.

Plans to evaluate the effectiveness of the interventions and criteria for success:

The effectiveness is measured through the PASS <<Holosight>>. All-important risks and missing information will be analysed on ongoing basis.

Patient Information Guide

Objectives: To supplement the package leaflet and to provide additional guidance in lay terminology outlining what Holoclar is, the treatment process, and guidance on use of concomitant medications.

Rationale for the additional risk minimisation activity: For some risks related to Holoclar, routine risk minimisation measures might be not sufficient and additional risk minimisation measure such Educational material deemed necessary to increase the knowledge on risks related to the product and how to prevent them.

Target audience and planned distribution path: All patients intending to be treated with Holoclar. Importantly the Guide highlights what the patient can expect to occur post-implantation including possible side effects of Holoclar, the requirement for post-implantation concomitant therapies including post-operative antibiotics and anti-inflammatory medicines and their potential side effects, and details of what action should be taken if a patient experiences an adverse reaction.

Plans to evaluate the effectiveness of the interventions and criteria for success: The effectiveness is measured through the safety study, registry -like <<HOLOSIGHT>>.

V.3 Summary table of Risk Minimisation Measures

Safety concern	**Risk minimisation measures**	**Pharmacovigilance activities**
1) Glaucoma	Routine risk minimisation measures: SmPC section 4.4 SmPC section 4.8 PIL section 4 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation measures: Healthcare Professional Guide Patient information guide	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
2) Lack of effect manifesting as corneal epithelium defect	Routine risk minimisation measures: SmPC section 4.2	Routine pharmacovigilance activities beyond adverse

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	SmPC section 4.3 SmPC section 4.4 SmPC section 4.5 SmPC section 4.8 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation measures: Healthcare Professional Guide Patient information guide	reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
3) Blepharitis	Routine risk minimisation measures: SmPC section 4.8 PIL sections 2 and 4 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation measures: Healthcare Professional Guide Patient information guide	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
4) Concomitant use of eye drops containing benzalkonium chloride	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.5 PIL section 2 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation measures: Healthcare Professional Guide Patient information guide	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT):
5) Post-implant infection	Routine risk minimisation measures:	Routine pharmacovigilance activities beyond adverse

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	SmPC section 4.2 SmPC section 4.4 SmPC section 4.8 PIL sections 2 and 4 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation measures: Healthcare Professional Guide Patient information guide	reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
6) Medication errors (e.g. Incorrect patient receives product, Patient receives incorrect product, Incorrect surgical technique)	Routine risk minimisation measures: SmPC section 4.1 SmPC section 4.2 SmPC section 4.4 SmPC section 4.5 SmPC section 6.6 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation measures: Healthcare Professional Guide Patient information guide	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
7). Off-label use Milder form of limbal stem cell deficiency than the proposed indication -Other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis	Routine risk minimisation measures: SmPC section 4.1 SmPC section 4.2 PIL section 1 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation measures:	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	Healthcare Professional Guide Patient information guide	
8). Use in pregnancy and lactation	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.6 PIL section 2 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
9). Use in children	Routine risk minimisation measures: SmPC section 4.1 SmPC section 4.2 SmPC section 4.8 PIL section 2 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
10) Use in elderly	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.8 SmPC section 5.1 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
10.) Long-term safety	None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities:

Safety concern	Risk minimisation measures	Pharmacovigilance activities
		-Post-Authorisation safety study, registry -like (HOLOSIGHT)

Part VI: Summary of risk management plan for Holoclar® (*Ex vivo* expanded autologous human corneal epithelial cells containing stem cells)

This is a summary of the risk management plan (RMP) for Holoclar® (*Ex vivo* expanded autologous human corneal epithelial cells containing stem cells). The RMP details important risks of Holoclar®, how these risks can be minimised, and how more information will be obtained about Holoclar's risks and uncertainties (missing information).

Holoclar's summary of product characteristics (SmPC) and its package leaflet give essential information to healthcare professionals and patients on how Holoclar® should be used.

This summary of the RMP for Holoclar® should be read in the context of all this information including the assessment report of the evaluation and its plain-language summary, all which is part of the European Public Assessment Report (EPAR).

Important new concerns or changes to the current ones will be included in updates of Holoclar® 's RMP.

I. The medicine and what it is used for?

Holoclar® is authorised for the treatment of adult patients with moderate to severe limbal stem cell deficiency, unilateral or bilateral, due to physical or chemical ocular burns. Holoclar® consists of a layer of your own cells which have been grown (e*x vivo* expanded) from a sample of limbal cells taken from the eye during a biopsy. Each preparation of Holoclar® is made individually and is for a single treatment only, although treatments can be repeated. The cells used to make Holoclar® are known autologous limbal cells. Holoclar® must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.

Further information about the evaluation of Holoclar®'s benefits can be found in Holoclar®'s EPAR, including in its plain-language summary, available on the EMA website, under the medicine's webpage https://www.ema.europa.eu/en/medicines/human/EPAR/holoclar.

II. Risks associated with the medicine and activities to minimise or further characterise the risks

Important risks of Holoclar®, together with measures to minimise such risks and the proposed studies for learning more about Holoclar's risks, are outlined below.

Measures to minimise the risks identified for medicinal products can be:

• Specific information, such as warnings, precautions, and advice on correct use, in the package leaflet and SmPC addressed to patients and healthcare professionals;
• Important advice on the medicine's packaging;
• The authorised pack size — the amount of medicine in a pack is chosen so to ensure that the medicine is used correctly;
• The medicine's legal status — the way a medicine is supplied to the patient (e.g. with or without prescription) can help to minimise its risks.
Together, these measures constitute *routine risk minimisation* measures.

In the case of Holoclar®, these measures are supplemented with *additional risk minimisation measures* mentioned under relevant important risks, below.

In addition to these measures, information about adverse reactions is collected continuously and regularly analysed, including PSUR assessment so that immediate action can be taken as necessary. These measures constitute *routine pharmacovigilance activities*.

If important information that may affect the safe use of Holoclar® is not yet available, it is listed under 'missing information' below.

II.A List of important risks and missing information

Important risks of Holoclar® are risks that need special risk management activities to further investigate or minimise the risk, so that the medicinal product can be safely taken. Important risks can be regarded as identified or potential. Identified risks are concerns for which there is sufficient proof of a link with the use of Holoclar®. Potential risks are concerns for which an association with the use of this medicine is possible based on available data, but this association has not been established yet and needs further evaluation. Missing information refers to information on the safety of the medicinal product that is currently missing and needs to be collected (e.g. on the long-term use of the medicine).

List of important risks and missing information	
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) • Off label use ○ milder form of limbal stem cell deficiency than the proposed indication

	○ off label use for other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis
Missing information	• Use in pregnancy and breast feeding • Use in children • Use in elderly • Long-term safety

II.B Summary of important risks

Important identified risk: **Glaucoma**	
Evidence for linking the risk to the medicine	In clinical trials (HLSTM 01, HLSTM02, HLSTM04) glaucoma was reported in 14.7% of treated patients. No cases of glaucoma were collected in the HOLOCORE study.
Risk factors and risk groups	Glaucoma is one of the main identified important risks for autologous human limbal stem cell grafting. The risk of glaucoma increases with age and it is a common condition in patients over 40 years of age in the general population. Other risk factors for glaucoma include myopia, positive family history and diabetes. Patients with ocular chemical burns have an increased risk of glaucoma with the most severe injuries carrying the greatest risk. Fifteen (15) of eighteen (18 eyes) that required long-term glaucoma treatment in this observational study had elevated intraocular pressure (IOP) within 1 week of presentation. Glaucoma is also common following penetrating keratoplasty. In the study by Fan et al, IOP elevation occurred 3–6 months post-keratoplasty in 78% of the eyes. All patients, except one, required reduction.
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.4 SmPC section 4.8 PIL section 4 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation: -Healthcare professional guide -Patient information guide
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)

	See section II.C of this summary for an overview of the post-authorisation development plan.

Important identified risk: **Lack of effect manifesting as corneal epithelium defect**	
Evidence for linking the risk to the medicine	Clinical trials (HLSTM 01, HLSTM02, HLSTM04 and HOLOCORE) Corneal epithelial defects were identified as ADRs in patients treated in HLSTM01, HLSTM02 and HOLOCORE and were potentially related to treatment failure.
Risk factors and risk groups	Not known
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.3 SmPC section 4.4 SmPC section 4.5 SmPC section 4.8 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation: -Healthcare professional guide -Patient information guide
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT): See section II.C of this summary for an overview of the post-authorisation development plan.

Important potential risk: **Blepharitis**	
Evidence for linking the risk to the medicine	Of the 39 adverse events of blepharitis reported in the 2 retrospective clinical studies 4 were classified as adverse reactions. Although there is no clear evidence, it is possible that the surgical intervention associated with the administration of Holoclar could reactivate blepharitis but there is no rationale to suggest that Holoclar by itself Induces blepharitis. Given the association between the blepharitis observed in the retrospective studies and Holoclar is not clear it will be included as an important potential risk

	in the RMP. Of the 39 adverse events of blepharitis reported in the 2 retrospective clinical studies 4 were classified as adverse reactions. Although there is no clear evidence, it is possible that the surgical intervention associated with the administration of Holoclar could reactivate blepharitis.
Risk factors and risk groups	Not known
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.8 PIL sections 2 and 4 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation: -Healthcare professional guide -Patient information guide
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT): See section II.C of this summary for an overview of the post-authorisation development plan.

Important potential risk: **Concomitant use of eye drops containing benzalkonium chloride**	
Evidence for linking the risk to the medicine	Benzalkonium chloride is one of the most disruptive ophthalmic additives to the stability of the lipid film and to corneal epithelium membranes; toxicity studies have tended to be carried out using relatively high concentrations of benzalkonium chloride but damage to the tear film and corneoconjunctival surface have been noted in patients receiving regular long-term treatment for glaucoma with eye drops preserved with benzalkonium chloride in usual concentrations (eye drops typically contain 0.002% to 0.01% benzalkonium chloride). Corneal toxicity has also been reported in patients inadvertently exposed to benzalkonium as a preservative in viscoelastic material during cataract surgery
Risk factors and risk groups	Not Applicable
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.5 PIL section 2 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.

	Additional risk minimisation: -Healthcare professional guide -Patient information guide
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT) See section II.C of this summary for an overview of the post-authorisation development plan.

Important potential risk: **Post-implant infection**	
Evidence for linking the risk to the medicine	The experience from clinical studies with Holoclar to date indicates that infection of the eye post-implant is uncommon.
Risk factors and risk groups	Not known
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.8 PIL sections 2 and 4 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation: -Healthcare professional guide -Patient information guide
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT) See section II.C of this summary for an overview of the post-authorisation development plan.

Important potential risk: **Medication errors (e.g. Incorrect patient receives product, Patient receives incorrect product, Incorrect surgical technique)**	
Evidence for linking the risk to the medicine	Holoclar is intended solely for autologous use. The main concern is potential maladministration to a patient from whom the limbal stem cells did not originate which could result in an adverse immunological reaction which is not recognised to occur when the limbal stem cells are implanted to a patient from whom the cells originated. It is fundamental that the patient receives the implant that was harvested and grown from their own limbal stem cells taken at biopsy. Cumulatively no medication errors associated with Holoclar have been reported.

Risk factors and risk groups	Not applicable
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.1 SmPC section 4.2 SmPC section 4.4 SmPC section 4.5 SmPC section 6.6 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation: -Healthcare professional guide -Patient information guide
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT) See section II.C of this summary for an overview of the post-authorisation development plan.

Important potential risk: Off-label use: -milder form of limbal stem cell deficiency than the proposed indication, -other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis	
Evidence for linking the risk to the medicine	The product is indicated in patients with LSCD due to physical or chemical ocular burns so other aetiologies e.g. SJS, neurotrophic keratitis or moderate form limbal stem cell deficiency would be off-label use. Cumulatively, neither events from the clinical trials nor from the post-marketing experience were received.
Risk factors and risk groups	Not known
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.1 SmPC section 4.2 PIL section 1 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only. Additional risk minimisation: -Healthcare professional guide -Patient information guide
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)

	See section II.C of this summary for an overview of the post-authorisation development plan.

Missing information: **Use in pregnancy and breast feeding**	
Evidence for linking the risk to the medicine	No patients included in clinical trials were pregnant or breast-feeding. As a result, there are no data for the use of Holoclar in pregnant women. Animal studies are insufficient with respect to reproductive toxicity. Conventional reproductive and developmental toxicity studies are not considered relevant, given the nature and the intended clinical use of the autologous tissue-engineered product. As a precautionary measure, since the requirement of the post-operative pharmacological treatment, it is preferable to avoid the use of Holoclar during pregnancy. Holoclar is not recommended during pregnancy and in woman of childbearing potential not using contraception from the biopsy to the conclusion of post-operative pharmacological treatment. As a precautionary measure, Holoclar is not recommended for implant during breast-feeding.
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.6 PIL section 2 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT) See section II.C of this summary for an overview of the post-authorisation development plan.

Missing information: **Use in children**	
Evidence for linking the risk to the medicine	There were no exclusion criteria relating to age in the two retrospective clinical studies HLSTM01 and HLSTM02. However, there is no information on the safety of autologous human limbal stem cells in children up to 7 years of age and only limited information in patients 8 – 17 years of age. The profile of adverse reactions in the paediatric patients included in the studies HLSTM01 (age 13, 14 and 16 years) and HLSTM02 (age 8 and 14 years) was not different from the adult population. Cumulatively, in the HOLOCORE study, a total of fourteen (14) AEs have been reported in two out of four paediatric participants, all of them non-serious. Two pre-treatment AEs (none related to the biopsy) were reported in 1 patient (25.0%). In total 12 treatment emergent adverse events (TEAEs) were reported in the paediatric population after Holoclar

	implantation, affecting 2 patients (50.0%). Two TEAEs were classified as possibly treatment-related adverse events (TRAEs) to the ACLSCT surgical procedure. Additionally, five (5) non-serious TEAEs occurred in the HOLOCORE-FU study (PTs: Eyelid rash, Eye discharge, Corona virus infection and Eye irritation [2 events]) in the same patient. All events were considered mild and not related to treatment. One (1) non-serious TEAE has been collected in the HOLOSIGHT study (PT: Blepharitis) in patients below 18 years of age so far.
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.1 SmPC section 4.2 SmPC section 4.8 PIL section 2 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT) See section II.C of this summary for an overview of the post-authorisation development plan.

Missing information: Use in elderly	
Evidence for linking the risk to the medicine	Fourteen (14) patients included in the two retrospective clinical studies HLSTM01 and HLSTM02 were 65 years old or older. Three (3) elderly patients were treated in HLSTM04 study and six (6) in the HOLOCORE study. As such the data are limited in this population. No meaningful differences in AE profile between elderly and adult population were seen.
Risk minimisation measures	Routine risk minimisation measures: SmPC section 4.2 SmPC section 4.8 SmPC section 5.1 Holoclar must be administered by an appropriately trained and qualified surgeon and is restricted to hospital use only.
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT) See section II.C of this summary for an overview of the post-authorisation development plan.

Missing information: **Long-term safety**	
Evidence for linking the risk to the medicine	The long-term follow up data is limited due to the retrospective nature of the clinical trials. Follow-up data are available for ≥ 1 year duration in 93.8% of ACLSC implants (106 of 113 implants) in study HLSTM01. In

	the same study 43.4% (49 of 113 implants) have follow-up data pertaining ≥ 3 years. From study HLSTM02, 82.8% (24 of 29 implants) included follow-up data covering a ≥ 1 year period while 41.4% (12 of 29 implants) covered durations of ≥ 3 years. A limited follow-up was available for study HLSTM04 (10.72 ± 7.99 months). From study CCD-GPLSCD01-03 (HOLOCORE) sixty-eight (68) patients completed the 12-months follow-up after first treatment with Holoclar and forty-four (44) among them completed the Long-term Follow-up study. Individual patient duration for HOLOCORE Follow-Up varied from a minimum duration of 12 months, for the last patient entered, to up to potentially 57 months for the first enrolled patient. There is no evidence from the retrospective studies, HOLOCORE and HOLOCORE FOLLOW-UP to suggest that long term use is associated with a safety concern. In vitro studies have confirmed localisation of the implant without migration of the epithelial cells into other ocular structures and below the basal lamina. Furthermore, the risk of tumour formation related to 3T3-J2 feeder cells proliferative capacity has been demonstrated to be absent following the lethal irradiation. The final conclusion of the prospective study (HOLOSIGHT PASS) will confirm these finding.
Risk minimisation measures	No risk minimisation measures
Additional pharmacovigilance activities	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT) See section II.C of this summary for an overview of the post-authorisation development plan.

II. C. Post-authorisation development plan

II.C.1 Studies which are conditions of the marketing authorisation

There are no studies which are conditions of the marketing authorisation or specific obligation of Holoclar®.

II.C.2 Other studies in post-authorisation development plan

Post-authorisation safety study, registry -like (HOLOSIGHT)

Purpose of the study: In order to expand the body of data and experience so far collected, a safety study, registry -like is planned as a part of the Risk Management Plan for Holoclar®. The study is conducted in the early post-authorization phase of Holoclar®, with the primary aim of evaluating the long-term safety profile of patients treated with Holoclar® according to clinical practice during a 5-year follow-up period. Secondary aims include long-term patient management, treatment success, visual acuity and quality of life. Moreover, the safety study, registry -like collects data useful for the evaluation of the effectiveness of the risk minimization

measures in compliance with the Risk Management Plan for Holoclar®. Therefore, this safety study, registry -like is an organized system that uses observational methods to collect data on specified outcomes in patients undergoing Holoclar®.

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Part VII: Annexes

Table of contents

Annex 1: Annex 1 to 3 excluded from publication

Annex 4: Specific adverse drug reaction follow-up forms

None.

Annex 5: Excluded from publication

Annex 6: Details of proposed additional risk minimisation measures

The key elements of the educational material for healthcare professionals include:

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

A training program that qualifies the ophthalmic surgeon and the other healthcare professionals to perform the procedure is conducted. According to internal SOP the training is performed both theoretically and on the job by dedicated Medical Service staff. The training program includes periodical surgeons re-training in case of inactivity periods.

The training intends to ensure adequate and comparable levels of experience across the centres that perform the ACLSCT.

The key elements of the educational material for patients and/or carers (Patient Information Guide) include:

- Contraindicated 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

A mock-up of the Educational Manual for the Screening and Treatment of Pre and Post-Operative Patients Undergoing an Autologous Transplant of the Corneal Epithelium Reconstructed from Stem Cells and a mock-up of the Patient Information Guide are included in Annex 7.

Annex 7: Excluded from publication