EMA/CHMP/352988/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report on the annual renewal of the conditional marketing authorisation

Procedure no.: EMEA/H/C/002450/R/0058

Invented name: Holoclar

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

Marketing authorisation holder (MAH): Holostem s.r.l.

Status of this report and steps taken for the assessment				
Current step[1]	**Description**	**Planned date**	**Actual Date**	**Need for discussion**[2]
☐	Start of procedure:	14 Aug 2023	14 Aug 2023	☐
☐	CAT and PRAC Rapporteurs Joint Assessment Report	14 Sep 2023	18 Sep 2023	☐
☐	PRAC CAT CHMP members comments	19 Sep 2023	27 Sep 2023	☐
☐	Updated CAT and PRAC Rapporteurs Joint Assessment Report	21 Sep 2023	28 Sep 2023 (due to late comment)	☐
☐	PRAC endorsed relevant sections of the assessment report[3]	28 Sep 2023	28 Sep 2023	☐
☐	CAT Request for Supplementary Information (RfSI)	06 Oct 2023	06 Oct 203	☐
☐	MAH re-submission deadline	08 Nov 2023	08 Nov 2023	☐
☐	MAH responses to (RfSI) received on	08 Nov 2023	08 Nov 2023	☐
☐	Re-start pf procedure	09 Nov 2023	09 Nov 2023	☐
☐	CAT and PRAC Rapporteurs' joint assessment report	28 Nov 2023	28 Nov 2023	☐
☐	PRAC Outcome	30 Nov 2023	30 Nov 2023	☐
	CHMP and CAT members comments	01 Dec 2023	30 Nov 2023	
☐	Updated CAT and PRAC Rapporteurs joint assessment report	04 Dec 2023	n/a	☐
☐	CAT Opinion	08 Dec 2023	08 Dec 2023	☐
☒	Opinion	14 Dec 2023	14 Dec 2023	☐

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1. Background information on the annual renewal

The European Commission issued on 17 February 2015, a conditional marketing authorisation (MA) for Holoclar This implied that, pursuant to Article 14-a of Regulation (EC) No 726/2004 and Article 5 of Commission Regulation (EC) No 507/2006, the marketing authorisation holder (MAH) has to complete ongoing studies, or to conduct new studies, as listed in Annex II.E of the MA, the so-called Specific Obligations (SOBs). These data form the basis of the renewal of the conditional MA.

Holoclar, was designated as an orphan medicinal product EU/3/08/579 on 07 November 2008.

The first approval of Holoclar was granted on 17 February 2015 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. Holoclar is approved with a unique dose which is 79,000 – 316,000 cells/cm² living tissue equivalent for treatment of adult patients with moderate to severe limbal stem cell deficiency (LSCD) (defined by the presence of superficial corneal neovascularization [CNV] 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.

In June 2020 the European Conditional Marketing authorization was transferred from the former MAH, Chiesi Farmaceutici, to the new one Holostem Terapie Avanzate s.r.l. with the European Decision C(2020) 4054 adopted on 12 June 2020. Holoclar is approved for treatment of adult patients with moderated to severe limbal stem cell deficiency (LSCD), defined by the presence of superficial corneal neovascularization [CNV] 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.

A conditional MA is valid for one year and may be renewed annually upon request by the MAH. Therefore, pursuant to Article 14-a of Regulation (EC) No 726/2004 and Article 6(2) of Commission Regulation (EC) No 507/2006, the MAH Holostem Terapie Avanzate s.r.l., submitted to the Agency on 26 July 2023 an application for renewal of the conditional MA for Holoclar. The expiry date of the MA is 19 February 2024.

The period covered by this annual renewal is 03 May 2022 to 02 May 2023 (data lock point: 12 July 2022).

The MAH informs that the Specific Obligation (SOB) has been fulfilled with submission of the final Clinical Study Report within this procedure. Changes to the Product information has been submitted concerning to SmPC sections 4.2, 4.4, 4.5, 4.7, 4.8, 5.1-3, 6.6, Annex II E, and PIL, accordingly.

In response to the RfSI, the MAH provided a new revised version of the SmPC. The changes made consider the results presented in the final CSR of the HOLOCORE-FU (PAM) – also submitted in the context of the present procedure - and are considered acceptable.

2. Overall conclusions and benefit-risk balance

Overall, the presented data are considered adequate to support the current positive risk-benefit profile of Holoclar for treatment of adult LSCD due to ocular burn.

2.1. Specific Obligations (SOBs)

Compliance of SOB data submitted

During the period covered by this annual renewal data on the SOBs have been submitted. According to Table 6 of the Clinical Overview, submitted for the 2020 renewal, figures are as follows:

Patients disposition

	Adult	**Paediatric**	**Overall**
Screened	91	4	95
Eligible/ Roll-in	76	4	80
1^{st} biopsy	76	4	80
2^{nd} biopsy	29	2	31
3^{rd} biopsy	2	0	2
First ACLSCT	69	4	73
12-months FU Visit after First ACLSCT	59	3	62
Early withdrawn after biopsy and before First ACLSCT	7		7
Discontinued* after First ACLSCT and before Second ACLSCT	27	1	28
Second ACLSCT	3	1	4
Discontinued after Second ACLSCT	-	-	-
12-months FU Visit after Second ACLSCT	1	-	1

According to Table 5 of the final CSR submitted for the current 2023 renewal procedure, figures are presented as follows:

Patient disposition

	Adult [n (%)]	Paediatric [n (%)]	Overall [n (%)]
Biopsy performed (at least one)	76 (100.0)	4 (100.0)	80 (100.0)
First ACLSCT performed	69 (90.8)	4 (100.0)	73 (91.3)
Second ACLSCT performed	6 (7.9)	1 (25.0)	7 (8.8)
Completed the study*	47 (61.8)	2 (50.0)	49 (61.3)
Discontinued the study*	29 (38.2)	2 (50.0)	31 (38.8)
Reasons for Discontinuation (withdrawal after biopsy)			
Adverse Event	4 (5.3)	---	4 (5.0)
Inclusion Criteria Not Met / Exclusion Criteria Met	---	---	---
Biopsy is not adequate / Three biopsies failed	4 (5.3)	1 (25.0)	5 (6.3)
Graft out of specifications	---	---	---
Contingency conditions which do not allow grafting	---	1 (25.0)	1 (1.3)
Lost to follow-up	3 (3.9)	---	3 (3.8)
Protocol violation	---	---	---
Death	---	---	---
Withdrawal of consent	11 (14.5)	---	11 (13.8)
Other	24 (31.6)	---	24 (30.0)

The initial data set provided for the annual renewal lead to the formulation of questions to the MAH regarding the long-term benefit of Holoclar for treatment of adult LSCD due to ocular burn. However, after the evaluation of the Applicant's responses, the CAT/CHMP supports the Applicant's proposal to remove the SOB, and to convert the conditional marketing authorization to full marketing authorization.

As part of this annual renewal the CAT/CHMP is of the opinion that the following obligation has been fulfilled, and therefore recommends its deletion from the Annex II:

SOB

Description	Due date
Multinational, multicentre, prospective, open-label, uncontrolled interventional study (HLSTM03 hereinafter referred as HOLOCORE or CCD-GPLSCD01-03) to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns	Last Patient Last Visit completed on 11 March 2022 Final CSR completed March 2023

Additionally, a final report of the PAM HOLOCORE-FU (originally due by March 2024, see below) was submitted in the context of this procedure and this PAM can also be removed.

PAM

Description	Due date
Study HLSTM03FU Long-term safety and efficacy follow-up after autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns. HOLOCORE-FU	Date for submission of interim of final reports March 2024

2.2. Benefit-risk Balance

During the period covered by this annual renewal, some new data have emerged.

The data are considered adequate to support the positive risk-benefit profile of Holoclar for treatment of adult with moderate to severe limbal stem cell deficiency due to ocular burn.

3. Recommendations

☒ Based on the review of the available information on the status of the fulfilment of Specific Obligations, the benefit-risk balance for Holoclar in its approved indication (see below) continues to be favourable. The specific obligations have been fulfilled; therefore, the granting of a marketing authorisation no longer subject to specific obligations is recommended.

'Treatment of adult patients with moderate to severe limbal stem cell deficiency (defined by the presence of superficial corneal neovascularisation in at least two corneal quadrants, with central corneal involvement, and severely impaired visual acuity), unilateral or bilateral, due to physical or chemical ocular burns. A minimum of 1 - 2 mm^2 of undamaged limbus is required for biopsy.'

Amendments to the marketing authorisation

The CAT/CHMP supports the conversion of the current conditional marketing authorization to full marketing authorization.

Conditions of the marketing authorisation

- ***Obligation to conduct post-authorisation measures***

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

Description	**Due date**
Post-authorisation Registry entitled "Long-term safety after Holoclar® implant for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns: observational study of routine clinical practice. HOLOSIGHT	Study is ongoing, date for the final report: December 2027

PSUR cycle

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

Based on the assessment of the data, the CAT/CHMP is of the opinion that the PSUR cycle should continue to follow a 6 monthly cycle.

4. EPAR changes

The table in the "Steps after" module of the EPAR will be updated as follows:

Scope

Renewal of conditional marketing authorisation

Summary

The CAT/CHMP, having reviewed the available information on the status of the fulfilment of Specific Obligations and having confirmed the positive benefit risk balance, is of the opinion that the quality, safety and efficacy of this medicinal product continue to be adequately and sufficiently demonstrated. Furthermore, the CHMP considered that, as all Specific Obligations have been fulfilled, there are no remaining grounds for the marketing authorizations to remain conditional and therefore recommends the granting of the MA no longer subject to Specific Obligations for Holoclar.

SmPC new text

In **4.4. Special warnings and precautions for use:**

[…]

Autologous use

Holoclar is intended solely for autologous use and must not, under any circumstances, be administered to other patients. Holoclar must not be administered if the information on the product labels and lot number do not match the patient's identity.

[…]

Transmission of an infectious agent

Holoclar could contain potentially infected biological material. Although Holoclar is tested for sterility and mycoplasma, a risk of transmission of infectious agents exists. Healthcare professionals administering Holoclar must, therefore, monitor patients for signs and symptoms of infections after treatment and treat appropriately, if needed. Although the risk is considered to be very low and routinely controlled in the manufacturing.

[…]

Precautions for use

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.

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. ~~When possible,~~ Concomitant eye problems should be corrected prior to Holoclar implantation.

At any of the steps of the treatment with Holoclar, topical lidocaine or anaesthetics containing adrenaline must be avoided.

In **4.5. Interaction with other medicinal products and other forms of interaction:**

The concomitant use of topical lidocaine or anaesthetics containing adrenaline must be avoided as they reduce the colony forming efficiency.

For more information, please refer to the Summary of Product Characteristics.

Annex: Rapporteurs' assessment comments on the renewal

PRAC input:

In this annual renewal,	**Yes**	**No**
- RMP submitted (If yes is ticked, discussion should be included in the Risk management plan section of the Annex)	☒	☐
- Outstanding SOB is a non-interventional PASS study (If yes is ticked, the relevant discussion should be included in the sub-section Outstanding Specific Obligations – status report for period covered of the Annex)	☐	☒
- There are issues originating from a parallel/recent PSUR or signal assessment to be flagged to the CHMP Rapporteur (If yes is ticked, the relevant discussion should be included in the Clinical safety section of the Annex)	☐	☒
- PhV inspections have been conducted/are ongoing with an impact on the MA under annual Re-Assessment (If yes is ticked, the relevant discussion should be included in the Pharmacovigilance inspections section of the Annex)	☐	☒

5. Specific Obligations

5.1. Specific Obligations adopted with the initial marketing authorisation

Table 1. Full list of SOBs as adopted with the initial marketing authorisation

Number	Description	Status
SOB 001	Multinational, multicentre, prospective, open-label, uncontrolled interventional study (HLSTM03 hereinafter referred as HOLOCORE or CCD-GPLSCD01-03) to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns	Last Patient Last Visit Completed on 11 March 2022 Final Clinical Study Report (CSR) March 2023
Periodic Safety Update Reports	Every 6 months	

Rapporteur comment
As the PRAC Rapporteur recommends the PSUR requirement should be kept as it is, which are currently every 6 months.

Since the granting of the conditional MA, the MAH has submitted the following SOBs:

5.2. Outstanding Specific Obligations – status report for period covered

SOB 001: Description

The interventional study HOLOCORE (ID: CCD_GLPSCD01-03) and its follow-up HOLOCORE-FU (ID: CCD-GLPSCD01-03-FU) are subjects to SOBs and both were ongoing during the reference period, while enrolment in the HOLOCORE study has been completed by 26th November 2018. On 6th of April 2021 an audit was performed in The Netherland at site 52801 by the Local Competent Authority for HOLOCORE, HOLOCORE FU and HOLOSIGHT (ID: CCD-GLPLSCD01-05), which is a non-interventional post-authorisation registry study (PASS) and not subject to SOBs. The reason of the inspection was the procurement licences extension. The inspector mainly focused on the biopsy and implants. During the audits, no critical issues and no major deviations were detected. The company confirms that according to the status of the trial, the final CSR to be available by March 2023.

Discussion

Rapporteur assessment/comment:

Based on the final CSR for the HOLOCORE study provided there are currently uncertainties, which need to be resolved.

5.3. Overall conclusion on Specific Obligations

During the period covered by this annual renewal, new data regarding SOB1 have emerged. The new data emerged are compliant in terms of adherence to deadlines and are not compliant in terms of acceptability of data submitted.

6. Additional scientific data provided relevant for the assessment of the benefit/risk balance

6.1. Quality

No specific obligations regarding quality have been raised for the conditional MA. Since granting of the MA, several changes had been introduced with regard to quality (see below). Furthermore, in May 2020, the MAH changed from Chiesi FARMACEUTICI S.p.A. to Holostem Terapie Avanzate S.r.l. /eCTD 0052). The manufacturer and the quality provisions remained not affected. In this cover letter, the MAH informs that Holostem Terapie Avanzate s.r.l. has started at the end of 2022 a liquidation process that involves the assignment to a third wholly owned legal entity of its business. As consequence, in parallel to the renewal procedure a request of Marketing Authorization Transfer for Holoclar MA (EU/1/14/987/001) from Holostem Terapie Avanzate s.r.l. to a new company set up for this purpose is going to be filed. Further information has not yet implemented in this renewal procedure.

The MAH included a comprehensive tracking table listing all changes in Modul 1, Annex 1. For the annual review, no new changes in Modul 3 have been declared. Variations regarding quality:

For the renewal period 2022-2023, eCTD sequence 0091, the following variations have been processed:

- Sequence 0090 (Type IA_{IN}): On 12th June 2023, the MAH filed a variation concerning the update of the PMF for fibrinogen and thrombin from the approved vendor. The updated EMA PMF was provided in Annex 5.21. The change raised no concern and was approved.

- Sequence 0088 (Type IA_{IN}): On 12th April 2023, the MAH filed a variation concerning the update of the PMF for fibrinogen and thrombin from the approved vendor. The updated EMA PMF was provided in Annex 5.21. The change raised no concern and was approved.

- Sequence 0087 (Type IA_{IN}): On 12th April 2023, the MAH filed a variation concerning the deletion of the ADP-ribosylation activity test procedure for the reagent Cholera Toxin by the vendor. Cholera toxin is used in the formulation of the culture media used in the manufacturing process of the active substance. To evaluate the activity of Cholera Toxin, two different tests were implemented: The "ADP ribosylating activity" and the "Haemoagglutination activity" tests. The existence of two activity assays was considered redundant, therefore it was proposed to keep only the "Haemoagglutination activity test" as specification parameter "activity" for the raw material. The new CoA was provided in Section 3.2.S.2.3. The change was approved.
- Sequence 0086 (Type IB, B.I.b.z): On 14th March 2023, the MAH filed a variation concerning the reduction of information for incoming goods testing performed by the active substance manufacturer

on receipt of batches of raw materials. The change affects information about the raw materials fibrinogen and thrombin from the approved supplier which are used to produce fibrin, the excipient constituting the matrix - based excipient of Holoclar. The change was approved.

- Sequence 0085 (Type IAIN): On 03th March 2023, the MAH filed a variation concerning the update of the PMF for fibrinogen and thrombin from the approved vendor. The updated EMA PMF was provided in Annex 5.21. The change was approved.

- Sequence 0084 (Type IA): On 13th Feb 2023, the MAH filed a variation concerning the replacement of secondary packaging manufacturer of the excipients Fibrinogen Powder and Thrombin Powder. The change was approved.

- Sequence 0083 (Type IA): On 24th Jan 2023, the MAH filed a variation concerning the update of EDQM TSE Certificate of Suitability for the reagent Foetal Bovine Serum from an already approved manufacturer. The Certificate was provided in section 3.2.R. Appendix 4. The change raised no concern and was approved.

Notification of administration of out of specification (OOS) products:

- For the renewal period 2022-2023, no OOS have been reported.

6.2. Non-clinical

N/A

6.3. Clinical pharmacology

N/A

6.4. Clinical efficacy

During the reference period, the clinical trial "HOLOCORE" was completed. The submitted final CSR is subject to this renewal and subject to the MAH'S request for fulfilment of the outstanding SOB. The follow-up clinical trial for the product (study "HOLOCORE-FU") was finalized in the reporting period (LPLV 31 March 2023, CSR planned by end of October 2023) and a non- interventional study (registry "HOLOSIGHT" of commercially transplanted patients) was ongoing. At the DLP of this renewal, which is 02 May 2023, the current status of HOLOCORE and HOLOCORE-FU was as follows:

HOLOCORE Interventional Study (CCD-GPLSCD01-03)

There is no change in the status of this trial since 2019. According to the MAH this trial was run over the whole critical time of the COVID pandemic. Therefore, the missing data are not negligible. Final results will be available in the HOLOCORE FOLLOW UP study and the registry-like HOLOSIGHT. For further details, please see the assessment below.

HOLOCORE-Follow-UP (CCD-GPLSCD01-03-FU)

A total of 47 subjects were enrolled (out of 68 who completed HOLOCORE study; 69%) and a total of 44 (65%) completed the study (one year follow-up), including two paediatrics. Three patients were early

terminated, 1 for SAE (mediastinum neoplasm resulted in death), 1 lost to follow- up and 1 for other reason. The LPLV for this study was carried out on 31 March 2023. No safety issues were reported in the clinical trial. The first patient was included on 13 December 2017. The LPLV was 31 March 2023 and the study was closed, with the statistical analysis currently ongoing, and CSR final planned by end October 2023. The database lock occurred on 28 June 2023 and key results provided on 03 July 2023. According to the MAH, no safety issues were reported in the clinical trial.

This study is part of the Additional Pharmacovigilance activities put in place for the product.

Total exposure in clinical trials

In total, 223 patients received the transplantation with Holoclar in HLSTM01, HLSTM02, HLSTM04 and HOLOCORE clinical trials. This is the largest cohort of patients with LSCD so far. Cumulatively, 14 patients received the second implantation with Holoclar.

Table 2: Cumulative Subject Exposure to "Holoclar" in all clinical trial by Age

		HLSTM01	HLSTM02	HLSTM04	HOLOCORE	TOTAL
Number of patients	Adults	96	20	12	63	191
	Paediatric patients	3	2	0	4	9
	Elderly (≥65 years)	7	7	3	6	23
Total		**106**	**29**	**15**	**73**	**223**
Number of treatments	Adults	103	20	12	68	203
	Paediatric patients	3	2	0	5	10
	Elderly (≥65 years)	7	7	3	7	24
Total		**113**	**29**	**15**	**80**	**237**

Source: Table 5 Clinical Overview Addendum

Holocore Study (CCD-GPLSCD01-03)

During the reference period this study has been completed. At the time of this report there are no patient ongoing enrolment as there is a temporary shortage of the product. The last 3 adult patients completed the study on March 2022 once they reached 6 month follow up after the 2 ACLSCT. The recruitment in HOLOCORE study was completed on 26 November 2018 for a total of 95 (including 4 paediatric patients) patients screened and 80 confirmed by the Independent Assessors evaluation based on 2D pictures (15 were assessed as screening failures after initial recruitment by Investigators).

Study design

Multinational, multicentre, prospective, open label, phase IV clinical trial

Main study objective

Primary objective

To demonstrate the efficacy of Holoclar at one year after the first treatment in patients suffering from moderate (at least two corneal quadrants, central corneal involvement resulting in severe visual impairment) to severe LSCD secondary to ocular burns, in terms of percentage of patients with a success of transplantation at approximately 12 months from the first Holoclar treatment.

Key Secondary Objectives

- To evaluate the efficacy of one or two treatments with Holoclar at one year after the last treatment.

Other important Secondary Objectives

- To evaluate the degree of corneal re-epithelialization during follow up.
- To evaluate the degree of severity of superficial corneal neo-vascularization during follow up.
- To evaluate the improvement in the presence and severity of clinical symptoms (pain, burning and photophobia) after last treatment with Holoclar during follow up.
- To evaluate the improvement in best corrected visual acuity (VA) after last treatment with Holoclar during follow up.
- To evaluate the improvement in patient's quality of life after last treatment with Holoclar during follow up.
- To evaluate the success of ACLSCT by number of Holoclar applications (either one or two).
- To evaluate the clinical safety profile of ACLSCT, including limbal biopsy, Holoclar transplantation procedure and post-transplantation treatment.

Investigational drug, dose and mode of administration

Holoclar consisted of a transparent circular sheet of 300,000 to 1,200,000 viable autologous human corneal epithelial cells (79,000 - 316,000 cells/cm^2), including on average 3.5% (0.4 to 16%) limbal stem cells, and stem cell-derived transient amplifying and terminally differentiated cells, attached on a supportive 2.2 cm diameter fibrin layer and maintained in the transport medium. After biopsy, ACLSCT included a single administration of Holoclar through a dedicated surgical procedure of corneal surface scraping and product application under local (para- or -retrobulbar) or general anaesthesia. The dose of Holoclar was 79,000 - 316,000 cells/cm^2, corresponding to 1 cm^2 of product/cm^2 of defect. Each preparation of Holoclar was intended as a single treatment. The treatment was repeated according to the physician's prescription, whereby each treatment consisted of a single application of Holoclar during ACLSCT. In case of failure during first transplant, a second transplantation with Holoclar was offered within 2- 6 months from declaration of failure after first Holoclar implantation, if eligibility was confirmed by the local Investigator (after re-check of inclusion and exclusion criteria) and if patients re- consented.

Table 3: Patient Disposition (Safety Population)

	Adult [n (%)]	Paediatric [n (%)]	Overall [n (%)]
Biopsy performed (at least one)	76 (100.0)	4 (100.0)	80 (100.0)
First ACLSCT performed	69 (90.8)	4 (100.0)	73 (91.3)
Second ACLSCT performed	6 (7.9)	1 (25.0)	7 (8.8)
Completed the study*	47 (61.8)	2 (50.0)	49 (61.3)
Discontinued the study*	29 (38.2)	2 (50.0)	31 (38.8)
Reasons for Discontinuation (withdrawal after biopsy)			
Adverse Event	4 (5.3)	—	4 (5.0)
Inclusion Criteria Not Met / Exclusion Criteria Met	—	—	—
Biopsy is not adequate / Three biopsies failed	4 (5.3)	1 (25.0)	5 (6.3)
Graft out of specifications	—	—	—
Contingency conditions which do not allow grafting	—	1 (25.0)	1 (1.3)
Lost to follow-up	3 (3.9)	—	3 (3.8)
Protocol violation	—	—	—
Death	—	—	—
Withdrawal of consent	11 (14.5)	—	11 (13.8)
Other	24 (31.6)	—	24 (30.0)

Source: Table 5 Final CSR

In the group of adults (n=76), 32 patients have undergone a second biopsy (6 of them to receive the second treatment, 26 due to manufacturing failure) and 2 patients had a third one. In the group of the paediatric patients (n=4), 2 had a second procedure of limbal biopsy and one underwent to the third biopsy. Overall, thirty-four (34) patients were biopsied twice and three (3) patients were biopsied three times (3 biopsies were the highest number of biopsies per patient allowed by the study protocol).

Results of the key primary and secondary efficacy analysis

Table 4: Successful Transplantation At 12 Months After First Holoclar Treatment By Independent Assessors (mITTa Population)

Parameter	Statistic	Overall (N=69) [n (%)]	P-value [2]
Success of transplantation [1]	n (%)	25 (36.2)	0.911
	95% CI	0.25-0.49	
Number of patients with Evaluable Results		66	
Success of transplantation [2]	n (%)	25 (37.9)	0.851
	95% CI	0.26-0.51	

Number of patients who performed 12 Months Visit and have Evaluable Results		61	
Success of transplantation [3]	n (%)	25 (41.0)	0.691
	95% CI	0.29-0.54	
Number of patients who performed 12 Visit up to and including Day 480	Months	66	
Success of transplantation [4]	n (%)	23 (34.8)	0.939
Parameter	**Statistic**	**Overall (N=69) [n (%)]**	**P-value [2]**
	95% CI	0.24-0.48	
Epithelial Defects (Investigator assessment)	None	51 (73.9)	
	Trace	11 (15.9)	
	Mild	——	
	Severe	2 (2.9)	
	Missing	5 (7.2)	
Number of corneal quadrants	0	11 (15.9)	
	1	14 (20.3)	
	2	14 (20.3)	
	3	8 (11.6)	
	4	14 (20.3)	
	Missing	8 (11.6)	
Central corneal involvement (CNV or opacity)	No	6 (8.7)	
	Yes	55 (79.7)	
	Missing	8 (11.6)	
Central corneal involvement: Neovascularisation	No	38 (55.1)	
	Yes	23 (33.3)	
	Missing	8 (11.6)	
Central corneal involvement: Opacity	No	6 (8.7)	
	Yes	55 (79.7)	
	Missing	8 (11.6)	

Class	None	11 (15.9)
	Mild	14 (20.3)
	Moderate	22 (31.9)
	Severe	14 (20.3)
	Missing	8 (11.6)

Source: Table 14 Final CSR

Table 5: Successful Transplantation At 12 Months After First Holoclar Treatment By Independent Assessors (mITTb Population)

Parameter	Statistic	Overall (N=65) [n (%)]	P-value [2]
Success of transplantation [1]	n (%)	22 (33.8)	0.955
	95% CI	0.23-0.47	
Number of patients with Evaluable Results		62	
Success of transplantation [2]	n (%)	22 (35.5)	0.917
	95% CI	0.24-0.49	
Number of patients who performed 12 Visit and have Evaluable Results	Months	57	
Success of transplantation [3]	n (%)	22 (38.6)	0.799
	95% CI	0.26-0.52	
Number of patients who performed 12 Visit up to and including Day 480	Months	62	
Success of transplantation [4]	n (%)	20 (32.3)	0.972
	95% CI	0.21-0.45	
Epithelial Defects (Investigator assessment)	None	47 (72.3)	
	Trace	11 (16.9)	
	Mild	—	
	Severe	2 (3.1)	
	Missing	5 (7.7)	
Number of corneal quadrants	0	11 (16.9)	
	1	11 (16.9)	
	2	14 (21.5)	

Parameter	Statistic	Overall (N=65) [n (%)]	P-value [2]
	3	8 (12.3)	
	4	13 (20.0)	
	Missing	8 (12.3)	
Central corneal involvement (CNV or opacity)			
	No	6 (9.2)	
	Yes	51 (78.5)	
	Missing	8 (12.3)	
Central corneal involvement: Neovascularisation			
	No	35 (53.8)	
	Yes	22 (33.8)	
	Missing	8 (12.3)	
Central corneal involvement: Opacity	No	6 (9.2)	
	Yes	51 (78.5)	
	Missing	8 (12.3)	
Class	None	11 (16.9)	
	Mild	11 (16.9)	
	Moderate	22 (33.8)	
	Severe	13 (20.0)	
	Missing	8 (12.3)	

Source: Table 15 Final CSR

Table 6: Successful Transplantation At 12 Months After Last Holoclar Treatment As Assessed By Independent Assessors (mITTa Population)

Parameter	Statistic	Overall (N=69) [n (%)]	P-value [2]
Success of transplantation [1]	n (%)	25 (36.2)	0.911
	95% CI	0.25-0.49	
Number of patients with Evaluable Results		66	
Success of transplantation [2]	n (%)	25 (37.9)	0.851
	95% CI	0.26-0.51	
Number of patients who performed 12 Months Visit and have Evaluable Results		58	

Success of transplantation [3]	n (%)	25 (43.1)	0.561
	95% CI	0.30-0.57	
Number of patients who performed 12 Months Visit up to and including Day 480		66	
Success of transplantation [4]	n (%)	23 (34.8)	0.939
	95% CI	0.24-0.48	
Epithelial Defects (Investigator assessment)	None	50 (72.5)	
	Trace	9 (13.0)	
	Mild	—	
	Severe	2 (2.9)	
	Missing	8 (11.6)	
Number of corneal quadrants	0	11 (15.9)	
	1	14 (20.3)	
	2	14 (20.3)	
	3	6 (8.7)	
Parameter	**Statistic**	**Overall (N=69) [n (%)]**	**P-value [2]**
	4	13 (18.8)	
	Missing	11 (15.9)	
Central corneal involvement (CNV or opacity)	No	6 (8.7)	
	Yes	52 (75.4)	
	Missing	11 (15.9)	
Central corneal involvement: Neovascularisation	No	38 (55.1)	
	Yes	20 (29.0)	
	Missing	11 (15.9)	
Central corneal involvement: Opacity	No	6 (8.7)	
	Yes	52 (75.4)	
	Missing	11 (15.9)	
Class	None	11 (15.9)	
	Mild	14 (20.3)	
	Moderate	20 (29.0)	
	Severe	13 (18.8)	

	Missing	11 (15.9)	

Source: Table 17 Final CSR

Table 7: Successful Transplantation At 12 Months After Last Holoclar Treatment As Assessed By Independent Assessors (mITTb Population)

Parameter	Statistic	Overall (N=65) [n (%)]	P-value [2]
Success of transplantation [1]	n (%)	22 (33.8)	0.955
	95% CI	0.23-0.47	
Number of patients with Evaluable Results		62	
Success of transplantation [2]	n (%)	22 (35.5)	0.917
	95% CI	0.24-0.49	
Number of patients who performed 12 Months Visit and have Evaluable Results		54	
Success of transplantation [3]	n (%)	22 (40.7)	0.687
	95% CI	0.28-0.55	
Number of patients who performed 12 Months Visit up to and including Day 480		62	
Success of transplantation [4]	n (%)	20 (32.3)	0.972
	95% CI	0.21-0.45	
Epithelial Defects (Investigator assessment)	None	46 (70.8)	
	Trace	9 (13.8)	
	Mild	—	
	Severe	2 (3.1)	
	Missing	8 (12.3)	
Number of corneal quadrants	0	11 (16.9)	
	1	11 (16.9)	
	2	14 (21.5)	
	3	6 (9.2)	
	4	12 (18.5)	
	Missing	11 (16.9)	
Central corneal involvement (CNV or opacity)	No	6 (9.2)	
	Yes	48 (73.8)	
	Missing	11 (16.9)	
Central corneal involvement: Neovascularisation	No	35 (53.8)	
	Yes	19 (29.2)	

	Missing	11 (16.9)	

Parameter	Statistic	Overall (N=65) [n (%)]	P-value [2]
Central corneal involvement: Opacity	No	6 (9.2)	
	Yes	48 (73.8)	
	Missing	11 (16.9)	
Class	None	11 (16.9)	
	Mild	11 (16.9)	
	Moderate	20 (30.8)	
	Severe	12 (18.5)	
	Missing	11 (16.9)	

Source: Table 18 Final CSR

Table 8: Successful Transplantation At 12 Months After Last Treatment By Number Of Holoclar Treatment (mITTa Population)

Parameter	Statistic	Overall	P-value [2]
One Holoclar treatment (N=63)	n (%)	25 (39.7)	0.764
	95% CI [1]	0.28-0.53	
Two Holoclar treatments (N=6)	n (%)	0	——
	95% CI [1]	——	

Source: Table 21 Final CSR

Table 9: Successful Transplantation At 12 Months After Last Treatment By Number Of Holoclar Treatment (mITTb Population)

Parameter	Statistic	Overall	P-value [2]
One Holoclar treatment (N=59)	n (%)	22 (37.3)	0.856
	95% CI [1]	0.25-0.51	
Two Holoclar treatments (N=6)	n (%)	0	——
	95% CI [1]	——	

Source: Table 22 Final CSR

Results of the key secondary and other important efficacy analysis

Table 10: Successful Transplantation At 12 Months After Last Holoclar Treatment As Assessed By Independent Assessors (mITTa Population)

Parameter	Statistic	Overall (N=69) [n (%)]	P-value [2]
Success of transplantation [1]	n (%)	25 (36.2)	0.911
	95% CI	0.25-0.49	
Number of patients with Evaluable Results		66	
Success of transplantation [2]	n (%)	25 (37.9)	0.851
	95% CI	0.26-0.51	
Number of patients who performed 12 Months Visit and have Evaluable Results		58	
Success of transplantation [3]	n (%)	25 (43.1)	0.561
	95% CI	0.30-0.57	
Number of patients who performed 12 Months Visit up to and including Day 480		66	
Success of transplantation [4]	n (%)	23 (34.8)	0.939
	95% CI	0.24-0.48	
Epithelial Defects (Investigator assessment)	None	50 (72.5)	
	Trace	9 (13.0)	
	Mild	—	
	Severe	2 (2.9)	
	Missing	8 (11.6)	
Number of corneal quadrants	0	11 (15.9)	
	1	14 (20.3)	
	2	14 (20.3)	
	3	6 (8.7)	

Parameter	Statistic	Overall (N=69) [n (%)]	P-value [2]
	4	13 (18.8)	
	Missing	11 (15.9)	
Central corneal involvement (CNV or opacity)	No	6 (8.7)	
	Yes	52 (75.4)	
	Missing	11 (15.9)	
Central corneal involvement: Neovascularisation	No	38 (55.1)	
	Yes	20 (29.0)	
	Missing	11 (15.9)	
Central corneal involvement: Opacity	No	6 (8.7)	
	Yes	52 (75.4)	
	Missing	11 (15.9)	
Class	None	11 (15.9)	
	Mild	14 (20.3)	
	Moderate	20 (29.0)	
	Severe	13 (18.8)	
	Missing	11 (15.9)	

Source: Table 17 Final CSR

Table 11: Successful Transplantation At 12 Months After Last Holoclar Treatment As Assessed By Independent Assessors (mITTb Population)

Parameter	Statistic	Overall (N=65) [n (%)]	P-value [2]
Success of transplantation [1]	n (%)	22 (33.8)	0.955
	95% CI	0.23-0.47	
Number of patients with Evaluable Results		62	
Success of transplantation [2]	n (%)	22 (35.5)	0.917
	95% CI	0.24-0.49	
Number of patients who performed 12 Months Visit and have Evaluable Results		54	
Success of transplantation [3]	n (%)	22 (40.7)	0.687
	95% CI	0.28-0.55	
Number of patients who performed 12 Months Visit up to and including Day 480		62	

Success of transplantation [4]	n (%)	20 (32.3)	0.972
	95% CI	0.21-0.45	
Epithelial Defects (Investigator assessment)	None	46 (70.8)	
	Trace	9 (13.8)	
	Mild	——	
	Severe	2 (3.1)	
	Missing	8 (12.3)	
Number of corneal quadrants	0	11 (16.9)	
	1	11 (16.9)	
	2	14 (21.5)	
	3	6 (9.2)	
	4	12 (18.5)	
	Missing	11 (16.9)	
Central corneal involvement (CNV or opacity)	No	6 (9.2)	
	Yes	48 (73.8)	
	Missing	11 (16.9)	
Central corneal involvement: Neovascularisation	No	35 (53.8)	
	Yes	19 (29.2)	
	Missing	11 (16.9)	
Parameter	**Statistic**	**Overall (N=65) [n (%)]**	**P-value [2]**
Central corneal involvement: Opacity	No	6 (9.2)	
	Yes	48 (73.8)	
	Missing	11 (16.9)	
Class	None	11 (16.9)	
	Mild	11 (16.9)	
	Moderate	20 (30.8)	
	Severe	12 (18.5)	
	Missing	11 (16.9)	

Source: Table 17 Final CSR

Table 12: Successful Transplantation At 12 Months After Last Treatment By Number Of Holoclar Treatment (mITTa Population)

Parameter	Statistic	Overall	P-value [2]
One Holoclar treatment (N=63)	n (%)	25 (39.7)	0.764
	95% CI [1]	0.28-0.53	
Two Holoclar treatments (N=6)	n (%)	0	——
	95% CI [1]	——	

Source: Table 21 Final CSR

Table 13: Successful Transplantation At 12 Months After Last Treatment By Number Of Holoclar Treatment (mITTb Population)

Parameter	Statistic	Overall	P-value [2]
One Holoclar treatment (N=59)	n (%)	22 (37.3)	0.856
	95% CI [1]	0.25-0.51	
Two Holoclar treatments (N=6)	n (%)	0	——
	95% CI [1]	——	

Source: Table 22 Final CSR

Table 14: Summary Of Epithelial Defects At Baseline And Each Post-Transplantation Visit After Last Holoclar Treatment (mITTa Population)

Parameter		Baseline (N=69) [n (%)]	Day 29 (N=69) [n (%)]	Day 90 (N=67) [n (%)]	Day 180 (N=67) [n (%)]	Day 270 (N=60) [n (%)]	Day 360 (N=61) [n (%)]
Epithelial Defects	None	48 (69.6)	35 (50.7)	39 (58.2)	54 (80.6)	50 (83.3)	50 (82.0)
	Trace	21 (30.4)	17 (24.6)	18 (26.9)	10 (14.9)	5 (8.3)	9 (14.8)
	Mild	——	3 (4.3)	5 (7.5)	2 (3.0)	2 (3.3)	——
	Severe	——	10 (14.5)	3 (4.5)	1 (1.5)	1 (1.7)	2 (3.3)
	Missing	——	4 (5.8)	2 (3.0)	——	2 (3.3)	——

Source: Table 23 Final CSR

Table 15: Summary Of Epithelial Defects At Baseline And Each Post-Transplantation Visit After Last Holoclar Treatment (mITTb Population)

Parameter		Baseline (N=65) [n (%)]	Day 29 (N=65) [n (%)]	Day 90 (N=63) [n (%)]	Day 180 (N=63) [n (%)]	Day 270 (N=56) [n (%)]	Day 360 (N=57) [n (%)]
Epithelial Defects	None	45 (69.2)	31 (47.7)	36 (57.1)	50 (79.4)	46 (82.1)	46 (80.7)
	Trace	20 (30.8)	17 (26.2)	17 (27.0)	10 (15.9)	5 (8.9)	9 (15.8)
	Mild	—	3 (4.6)	5 (7.9)	2 (3.2)	2 (3.6)	—
	Severe	—	10 (15.4)	3 (4.8)	1 (1.6)	1 (1.8)	2 (3.5)
	Missing	—	4 (6.2)	2 (3.2)	—	2 (3.6)	—

Source: Table 24 Final CSR

Table 16: Summary Of Ocular Symptoms Presence And Score At Baseline And Each Study Visit From 3 To 12 Months After Last Holoclar Treatment (mITTa Population)

Parameter		Baseline (N=69) [n (%)]	Day 90 (N=67) [n (%)]	Day 180 (N=67) [n (%)]	Day 270 (N=60) [n (%)]	Day 360 (N=61) [n (%)]
Presence of Burning	No	36 (52.2)	39 (58.2)	44 (65.7)	46 (76.7)	46 (75.4)
	Yes	33 (47.8)	28 (41.8)	23 (34.3)	13 (21.7)	15 (24.6)
	Missing	—	—	—	1 (1.7)	—
Burning Severity	None	36 (52.2)	39 (58.2)	44 (65.7)	46 (76.7)	46 (75.4)
	Mild	25 (36.2)	25 (37.3)	21 (31.3)	10 (16.7)	11 (18.0)
	Moderate	8 (11.6)	3 (4.5)	1 (1.5)	3 (5.0)	4 (6.6)
	Severe	—	—	1 (1.5)	—	—
	Missing	—	—	—	1 (1.7)	—
Presence of Photophobia	No	18 (26.1)	31 (46.3)	29 (43.3)	30 (50.0)	34 (55.7)
	Yes	51 (73.9)	36 (53.7)	38 (56.7)	29 (48.3)	27 (44.3)
	Missing	—	—	—	1 (1.7)	—
Photophobia Severity	None	18 (26.1)	31 (46.3)	29 (43.3)	30 (50.0)	34 (55.7)
	Mild	24 (34.8)	21 (31.3)	27 (40.3)	16 (26.7)	18 (29.5)
	Moderate	21 (30.4)	12 (17.9)	8 (11.9)	12 (20.0)	7 (11.5)
	Severe	6 (8.7)	3 (4.5)	3 (4.5)	1 (1.7)	2 (3.3)
	Missing	—	—	—	1 (1.7)	—

Presence of Pain	No	41 (59.4)	46 (68.7)	58 (86.6)	47 (78.3)	48 (78.7)
	Yes	28 (40.6)	21 (31.3)	9 (13.4)	12 (20.0)	13 (21.3)
	Missing	—	—	—	1 (1.7)	—
Presence of at least one Ocular Symptoms	No	9 (13.0)	18 (26.9)	17 (25.4)	20 (33.3)	27 (44.3)
	Yes	60 (87.0)	49 (73.1)	50 (74.6)	39 (65.0)	34 (55.7)
	Missing	—	—	—	1 (1.7)	—

Source: Table 26 Final CSR

Table 17: Summary Of Pain At Each Study Visit From 3 To 12 Months After Last Holoclar Treatment (mITTa Population)

Day		Overall (N = 69)	p-value [2]
Baseline	n	69	
	Mean (SD)	1.3 (2.0)	
	95% CI [1]	0.8 – 1.7	
Day 90	n	67	
	Mean (SD)	0.7 (1.5)	
	95% CI [1]	0.4 – 1.1	
Day 90 Change from Baseline	n	67	0.035
	Mean (SD)	-0.6 (2.2)	
	95% CI [1]	-1.1 – 0.0	
Day 180	n	67	
	Mean (SD)	0.4 (1.3)	
	95% CI [1]	0.1 – 0.7	
Day 180 Change from Baseline	n	67	<0.001
	Mean (SD)	-0.9 (2.0)	
	95% CI [1]	-1.4 – -0.4	
Day 270	n	59	
	Mean (SD)	0.4 (0.9)	
	95% CI [1]	0.1 – 0.6	
Day 270 Change from Baseline	n	59	<0.001
	Mean (SD)	-0.9 (1.6)	
	95% CI [1]	-1.3 – -0.4	
Day 360	n	61	
	Mean (SD)	0.5 (1.5)	

	95% CI [1]	0.2 – 0.9	
Day 360 Change from Baseline	n	61	0.006
	Mean (SD)	-0.7 (2.0)	
	95% CI [1]	-1.3 – -0.2	

Source: Table 27 Final CSR

Table 18: Summary Of Best Corrected Visual Acuity (BCVA) At Baseline And Each Study Visit From 3 To 12 Months After Last Holoclar Treatment (mITTa Population)

Day		Overall (N=69)	P-value [2]
Baseline	n	69	
	Mean (SD)	1.94 (0.49)	
	95% CI [1]	1.82 - 2.05	
	Median	1.90	
	Min - Max	1.0 - 2.7	
Day 90	n	66	
	Mean (SD)	1.53 (0.76)	
	95% CI [1]	1.34 - 1.72	
	Median	1.75	
	Min - Max	0.1 - 2.7	
Day 90 Change from Baseline	n	66	
	Mean (SD)	-0.39 (0.55)	
	95% CI [1]	-0.52 - -0.25	<0.001
	Median	0.00	
	Min - Max	-1.8 - 0.4	
Day 180	n	67	
	Mean (SD)	1.47 (0.83)	
	95% CI [1]	1.27 - 1.67	
	Median	1.51	
	Min - Max	0.1 - 2.7	
Day 180 Change from Baseline	n	67	
	Mean (SD)	-0.45 (0.57)	
	95% CI [1]	-0.59 - -0.31	<0.001

		Median	-0.40
		Min - Max	-1.8 - 0.4
Day 270	n	59	
		Mean (SD)	1.32 (0.91)
		95% CI [1]	1.08 - 1.56

Day		Overall (N=69)	P-value [2]
	Median	1.00	
	Min - Max	-0.1 - 2.7	
Day 270 Change from Baseline	n	59	
	Mean (SD)	-0.62 (0.69)	
	95% CI [1]	-0.80 - -0.44	<0.001
	Median	-0.60	
	Min - Max	-2.4 - 0.4	
Day 360	n	61	
	Mean (SD)	1.30 (0.91)	
	95% CI [1]	1.07 - 1.53	
	Median	1.00	
	Min - Max	-0.1 - 2.7	
Day 360 Change from Baseline	n	61	
	Mean (SD)	-0.63 (0.70)	
	95% CI [1]	-0.81 - -0.45	<0.001
	Median	-0.60	
	Min - Max	-2.4 - 0.4	

Source: Table 29 Final CSR

Post Marketing Experience

At this ACO DLP, the product is currently temporarily under partial shortage, as Holostem has not collected biopsies since December 8, 2022 in Austria, Belgium, Denmark, Germany, The Netherlands, Italy, Czech Republic and United Kingdom (UK).

According the MAH, the Holoclar production and distribution has been continued for all the biopsies already in house. These biopsies have been used as starting material to produce and distribute Holoclar in Europe and UK up to the 8 June 2023, date of the last implantation in UK. This shortage will be ceased

from 30 September, 2023, when the compulsory maintenance of the manufacturing site (due two times per year), shifted to July - August 2023, will be completed and the activities can be resumed again.

After approval of Holoclar, excluding the subjects in the HOLOCORE clinical trial (see below), additional n=105 patients started the treatment, with 110 biopsies for tissue procurement (5 patients underwent to 2 biopsies) conducted with the marketed product so far. Cumulatively, n=98 patients (88 included in HOLOSIGHT study) have been implanted with Holoclar according to clinical practice. Four (4) patients were treated with out of specification product (last chance), according to the section 11.5 of the "Guideline on Good Manufacturing practice specific to Advanced Therapy Medicinal Products" and two (2) patients received the sub-potent batches under exceptional circumstances on surgeon's request according to ATMP GMP guidelines.

Overall, considering patients included in the clinical trials (HLSTM01, HLSTM02, HLSTM04 and HOLOCORE; n=223) and patients treated in the commercial setting according to clinical practice (98), a total of 321 patients have been exposed to the medicinal product for a total of 336 transplantations of autologous cultivated limbal stem cells (Holoclar) so far.

Rapporteur assessment/comments/conclusion - clinical efficacy:

HOLOCORE Study

Due to the statistical analysis plan the study populations for efficacy parameters were defined as follows (see Table 7 Final CSR):

1. Modified Intention-to-Treat population A (mITTa): all adult patients (n=76) who underwent the ACLSCT procedure: n=69.

2. Modified Intention-to-Treat population B (mITTb): all adult patients (n=76) who underwent the ACLSCT procedure excluding patients treated with out of specification grafts (sub- potent batches): n=65.

3. Per-protocol population (PP): all patients from the mITTa population (n=76) without any major protocol deviations (i.e., wrong inclusions and non-permitted medications): n=64

The 4 pediatric subjects are not included in the efficacy analysis set which is supported.

Primary efficacy

A successful transplantation was calculated as the proportion of patients with less than 2 superficial neo-vascularization corneal quadrants with no central corneal involvement by CNV and absence of epithelial defects (none or trace) at Day 360 ±14 days after first Holoclar treatment in the mITTa population. According to Table 14 final CSR, evaluable results for a successful transplantation in accordance with the Independent Assessor Judgement was documented for n=25/n=69 subjects, which results in a percentage rate of 36,2 % (95% CI). In terms of the mITTb and PP populations, successful transplantation was reported as 33.8% (95% CI: 23%–47%) and 39.1% (95% CI: 27%–52%), respectively. Figures for success of transplant at month 12 after last Holoclar treatment at the end of roll-in phase in the mITTa and mITTb population slightly differ (see tables 19 and 20 of the final CSR). After one Holoclar treatment in the PP population, transplantation was judged to be successful in 43.1% (30%–57%). No patient in the PP population had a successful transplantation after two Holoclar treatments. Important to note is that the success rate was higher in patients with zero prior surgical treatment in the medical history (about 60% of the subjects had prior surgical procedures).

Key secondary and other secondary efficacy

Superficial neo-vascularisation after treatment with Holoclar: 25 (39.1%) patients who had severe superficial corneal neo-vascularisation at baseline showed either moderate (n = 12, 18.8%), mild (n = 8, 12.5%), or no (n = 5, 7.8%) neo-vascularization at 12 months. No change in the status of neo-revascularization was still reported in 24 (37.5%) patients with either severe (n = 14, 21.9%) or moderate (n = 10, 15.6%) neo-vascularization at baseline.

Central corneal class evaluation: There was a change from severe at baseline to moderate at month 12 after treatment with Holoclar in 24.4% in the mITT population and in 25% of the mITTb population. However, in 21.9% of the mITT population and in 21.7% of the mITTb population, the central corneal class continued to be rated as severe.

Epithelial defects: For the majority of the patients in the mITTa and mITTb population there were no epithelial defects reported at baseline and each post-transplantation visit after the last Holoclar treatment. The MAH states that for participants with observed traces of epithelial defects at baseline, there were no epithelial defects reported in these subjects at the post-transplantation visit. This supposed shift, however, is considered not clinically important.

Ocular symptoms and scores: As presented in Table 26 of the final CSR, at least one ocular symptom was reported for n=57 subjects (87.7%), which seems having been reduced to n=33 (57.9%) by Day 360 post-transplant.

BCVA: In the mITT population there was an improvement observed from baseline values at visits Day 90, Day 180, Day 270 and Day 360, which overall was judged as statistically significant ($p<0.001$; see Table 29 Final CSR).

Patient questionnaires (NEI VFQ-25, EQ-5D-3L, EQ-5D-3L VAS and HIS): The questionnaires used can be considered in accordance with clinical standard in the ophthalmology for the evaluation of the patients' perceptive of the benefit of a treatment on their life and daily routine. Overall, changes were reported for specific parameters in the relevant questionnaires between baseline and post -transplant at Day 360, indicating a benefit of Holoclar.

Safety profile (refer to the PRAC Rapporteur assessment for detailed evaluation)

A total of 131 TEAEs were reported in 44 (57.9%) patients. The number of serious and severe TEAEs were 16 and 25, respectively, reported in 12 (15.8%) patients and 16 (21.1%) patients, respectively. A total of 71 TRAEs were reported in 27 (35.5%) patients, of which the majority (59 TRAEs in 20 [26.3%] patients) were judged to be possibly related to the ACLSCT surgical procedure. Overall, 10 serious TRAEs were reported in 6 (7.9%) patients. The most commonly reported serious TRAEs were Eye Disorders, with 7 serious TRAEs in 4 (5.3%) patients. In these, the most common serious TRAEs was corneal epithelium defect (4 serious TRAEs in 3 [3.9%] patients). There were also two incidents of rise in intraocular pressure in 2 (2.6%) and one AESI of blepharitis. Due to narratives provided for the patients with SAEs or AESIs, causality unknown was reported for n=11 subjects, causality known, but not related to study and/or IMP for n=1 subjects, causality known as AESI blepharitis for n=1 subject, and for n=10 the causality was reported as related to the study procedure or the IMP (one subject experienced two related SAEs).

Long-term safety follow up data are available either retrospectively with HLSTM01, HLSTM02 and HLSTM04 studies and also prospectively with the observational study HOLO-UP (HLSTM07) and with the interventional study HOLOCORE FOLLOW-UP. Further long-term follow-up data collection is ongoing through the HOLOSIGHT PAS study. Follow-up safety 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).

Rapporteur conclusion:

There are no major concerns with regard to the acceptance of the data package presented for the annual renewal procedure. Though the HOLOCORE study is completed and the status of subjects enrolled and treated did not change since 2019 due to the COVID pandemic and IMP shortage as stated by the MAH, the presented data package on efficacy and safety criteria of Holoclar in the approved indication may be considered adequate for maintenance of the overall positive risk-benefit evaluation of the product. However, an interim efficacy analysis of subjects included in the HOLOCORE-FU study is required for a final conclusion.

Regarding the final CSR submitted for the HOLOCORE Study for fulfilment of the SOB, from a formal perspective the MAH's request may be acceptable. Where the content of the final study report is concerned, however the following aspects need to be taken into consideration for a final decision:

There is no doubt that the results for primary and secondary efficacy (and for safety) are presented in the final study report in a comprehensive way. However, there are no relevant changes in the numbers of subjects treated and results available since 2020 and the last renewal procedures, respectively, as no further patients were enrolled, and the trial is now completed. During the last renewal procedures, the MAH argued the recruiting process to be substantially negatively affected by the COVID pandemic. The regulatory agencies considered this for the first to be acceptable with regard the renewal procedures. For fulfilment of the SOB, however enrolment of more patients after the data lock point for the 2020 renewal was expected in order to have more solid results available in the final study report. It has to be noted that the primary efficacy analysis, i. e. the marketing authorisation is based on retrospective non-controlled case-series studies only. Thus, the HOLOCORE study as a prospective, multicentre, interventional study is important for an objective assessment of results gained by non-controlled retrospective trials and for re-evaluation of the marketing authorisation. From the Rapporteur perspective the SOB is not considered fulfilled with regard to the content. While the patents' safety is under constant surveillance, the available data on the long-term efficacy of the whole treatment procedure, comprising potential repeated biopsies and transplant procedures for an effective treatment are considered rather limited.

6.5. Clinical safety

Actions taken for safety reasons

There have been no safety related actions taken in relation to Holoclar during the reference period.

Changes to Reference Information

No significant safety related changes were made to the RSI during the reporting interval.

Exposure

Clinical trial exposure

80 patients (67 males and 13 females, 76 adults and 4 paediatrics) started the treatment with Holoclar (first biopsy received) during the study HOLOCORE.

With regards to the clinical trial "HOLOCORE-FU", forty-seven (47) patients previously participating to the HOLOCORE have been enrolled and forty-four (44) have completed the study. No exposure to Holoclar was foreseen during this clinical trial.

In total, 223 patients received the transplantation with Holoclar in HLSTM01, HLSTM02, HLSTM04 and HOLOCORE clinical trials. This is the largest cohort of patients with LSCD so far. Cumulatively, 14 patients received the second implantation with Holoclar.

Post-marketing exposure

After approval of Holoclar, excluding the patient enrolled in the HOLOCORE clinical trial, an additional one hundred and five (105) patients started the treatment, with 110 biopsies for tissue procurement (5 patients underwent to 2 biopsies) conducted with the marketed product so far.

Cumulatively, ninety-eight (98) patients (88 included in HOLOSIGHT study) have been implanted with Holoclar according to clinical practice. Four (4) patients were treated with out of specification product (last chance), according to the section 11.5 of the "Guideline on Good Manufacturing practice specific to Advanced Therapy Medicinal Products" and two (2) patients received the sub-potent batches under exceptional circumstances on surgeon's request according to ATMP GMP guidelines.

Overall, considering patients (223) included in the clinical trials (HLSTM01, HLSTM02, HLSTM04 and HOLOCORE) and patients treated in the commercial setting according to clinical practice (98), a total of 321 patients have been exposed to the medicinal product for a total of 336 transplantations of autologous cultivated limbal stem cells (Holoclar) so far.

PRAC Rapporteur´s comment:

80 patients have been exposed to Holoclar in the HOLOCORE study. Post-marketing 98 patients have been implanted with Holoclar. Long term safety is considered missing information in the Holoclar RMP.

Data in summary tabulations

Appendix 2 provides a cumulative summary tabulation of all serious adverse events (SAEs) in company-sponsored clinical from Development International Birth Date (DIBD) to the Data Lock Point (DLP) of this document.

Appendix 3 provides cumulative summary tabulations of adverse reactions from post-marketing data sources, from the International Birth Date (IBD) to the DLP of this document.

PRAC Rapporteur´s comment:

According to the cumulative summary tabulation in appendix 2 of the ACO (Addendum to Clinical Overview) there are a total of 23 SAEs from clinical trials. Of these, 12 are in the SOC 'eye disorders'. Ninety-eight (98) patients have been exposed to the commercial product with 10 serious ADRs reported from post-marketing sources cumulatively (of these, 8 are in the SOC 'eye disorders').

Summary of significant safety findings from clinical trials and non-interventional studies

Completed clinical trials

One clinical trial has been completed during the reporting interval, with the CSR finalised the 20th of March: 1) HOLOCORE; ID: CCD-GLPSCD01-03.

HOLOCORE; ID: CCD-GLPSCD01-03 involving eight (8) countries (Belgium, France, Germany, Italy, Poland, The Netherlands, Spain and United Kingdom) has been carried out in Europe. In the HOLOCORE study, a total of at least 68 patients (including at least 3 paediatrics, according to the EMEA-001082-PIP02-11-M02) was planned to be treated with Holoclar. The HOLOCORE study is the subject of the specific obligation of the Conditional MA of Holoclar.

At the end of the study, all consenting patients were eligible to enter the safety and efficacy long-term follow-up study (HOLOCORE-FU study).

At the time of this report there are no patient ongoing enrolment as there is a temporary shortage of the product. The last 3 adult patients completed the study on March 2022 once they reached 6 month follow up after the 2nd autologous cultivated limbal stem cell transplantation (ACLSCT). According to the current status of the HOLOCORE study, the final CSR was finalized by March 2023.

PRAC Rapporteur´s comment: The completed clinical trial HOLOCORE is a prospective, open-label, uncontrolled interventional study to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns, was implemented as a specific obligation to complete post-authorisation for the conditional marketing authorisation. As this is an interventional trial, assessment of the safety and efficacy results have been carried out by the CHMP Rapporteur. A discussion on the results of the trial is provided in section 5.

Ongoing clinical trials

- One clinical trial was ongoing and terminated during the reference period (CSR not finalized at the DLP): 1) HOLOCORE-FU; ID: CCD-GLPSCD01-03-FU. This follow-up clinical trial was planned as an extension study for patients terminating the HOLOCORE study (i.e., CCD-GPLSCD01-03; HLSTM03). This clinical trial is completed (LPLV 31MAR2023) and the statistical analysis as well as the CSR writing are ongoing. The objective of the follow up trial was to observe the patients included and treated in the main HOLOCORE study collecting safety and efficacy data in the long-term period and focusing on results of keratoplasties that the patients might receive after implantation with Holoclar to treat deep opacity. On 6 April 2017 this clinical trial was submitted to the Italian Competent Authority/Ethic Committees and was approved in June 2017. Overall, 47 patients (45

adults and 2 minors) have been enrolled and are included in the clinical database, and 44 have completed the study. Sixty-one (61) treatment emergent adverse events occurred in 20 patients (42.6%) overall. At study end two (2) SAEs were reported: one (1) SAE of suture rupture, and one (1) SAE of mediastinum neoplasm. Both events were considered not related to the administration of Holoclar.

PRAC Rapporteur´s comment: The Holocore-FU study is completed with the LPLV on 31 MAR 2023*. This study is part of the Additional Pharmacovigilance activities put in place for the product. No ADRs were reported during this renewal period. The final CSR is planned by October 2023 and the RMP milestones have been updated accordingly.*

Non-interventional studies

A PASS study (HOLOSIGHT) entitled "Long-term safety after Holoclar implant for restoration of corneal epithelium in patients with LSCD due to ocular burns: observational study of routine clinical practice" (HOLOSIGHT) is ongoing. The goal of this PASS is to enroll and observe the first hundred patients who receive Holoclar in a commercial setting. On 20 October 2016 the first patient was included in Italy. Biopsy was performed on the same day and the first implantation of Holoclar was made on 24 May 2017. At this report DLP, the study protocol was submitted in 9 European Countries (Austria, Belgium, Czech Republic, Denmark, France, Germany, Italy, The Netherlands, and United Kingdom) including a total of 28 sites evaluated. Among them, 14 sites are currently active (1 in Austria, 2 in Belgium, 1 in Czech Republic, 1 in Denmark, 3 in Germany, 3 in Italy, 1 in The Netherlands and 2 in UK). During the reporting interval, nineteen (19) new patients accepted to participate into the HOLOSIGHT study signing the Informed Consent. At the ACO DLP, ninety-six (96) subjects entered in the study. Among the 96 enrolled patients, 9 patients discontinued the study: 2 patients due to SAEs leading to study discontinuation; 3 patients withdrew the informed consent; 1 patient was lost to follow-up and the remaining for other causes (screen failures, excluded by the Investigator). Overall, 94 patients included in HOLOSIGHT were biopsied and 88 patients received Holoclar (20 in the reporting period). At this report DLP one patient completed the 5 years observation period after the implantation (primary endpoint). This study is ongoing. Two (2) candidates for receiving the treatment with Holoclar were never biopsied and resulted as screening failure. The enrolment is closed with the last patient treated in May 2023 and the final number of patients who started the treatment with Holoclar and included in the study is 94. No safety issues were reported in this study. This study is part of the Additional Pharmacovigilance activities put in place for the product.

PRAC Rapporteur´s comment: Enrolment into the above non-interventional study, HOLOSIGHT, is closed with the last patient treated in May 2023. The final number of patients who started the treatment with Holoclar and were included in the study is 94. One patient has completed the 5 years observation period after the implantation (primary endpoint).

Cumulatively, a total of 76 treatment-emergent adverse events (TEAEs) were reported in 42 patients. Overall, the majority of identified TEAEs and TRAEs have been classified within the SOC category "Eye disorder" with "corneal epithelium defect" being the most commonly reported event and they are typically related to "lack of efficacy". During the interval period, one serious TEAEs was registered (Trauma Left eye) and considered not related by Site Investigator. Based on the data provided, no further risk minimisation measures are considered warranted at this time.

Medication errors

No cases of medication error were received during the reporting interval.

Literature

None of the published articles during the reporting period revealed new safety or efficacy evidence that could impact the risk/benefit balance of Holoclar in the approved indications.

Overview of signals

No validated signals were identified, ongoing or closed for Holoclar during the reporting interval.

Late-breaking information

The following progresses occurred during the late breaking information reference period (03 May 2023-05 July 2023:

With reference to the commercial setting, 2 patients have been treated after 02 May 2023 (1 HOLOSIGHT and 1-Non-HOLOSIGHT) for a total of 99 commercial patients treated with Holoclar and 2 implanted with subpotent batches.

The scope of the HOLOSIGHT PASS was to include the first 100 commercial patients treated with Holoclar: 10 patients treated with Holoclar didn't accept to be enrolled in the HOLOSIGHT study or could not be collected for logistics reasons and they are not included in the study having not signed the ICF. The study enrolment is considered completed, the last patient signed the ICF on 14 November 2022. Of the 94 patients enrolled, after the DLP:

• 0 patients received the biopsy;

• 1 patients received the 1st implant on 23rd May 2023 (last implant in HOLOSIGHT).

Overall, 90 patients included in HOLOSIGHT have been treated with Holoclar, and 4 early terminated before implantation for any reason.

The HOLOCORE-FU study is closed (LPLV 31 March 2023) and the statistical analysis is ongoing. The database was locked on 28 June 2023 and 45 patients analysed.

PRAC Rapporteur´s comment: No significant late-breaking information, with reference to Holoclar safety has been received after the DLP.

Risk Evaluation

Summary of safety concerns at the beginning of the reporting interval:

Important identified risks	- Glaucoma - Lack of effect manifesting as corneal epithelium defect
e.g. Mi**ssing information**	- Pregnancy and lactation - Use in children - Use in elderly - Long term safety

PRAC Rapporteur´s comment:

No changes to the list of the safety concerns in the RMP were implemented during and after the reporting period of this renewal. Based on the data submitted with the renewal application, no changes are warranted.

6.6. Pharmacovigilance inspections

During the reference period of this annual renewal, the Company altogether (Corporate offices or affiliates) did not receive any Inspection of its Pharmacovigilance System from the Heath Authorities.

PRAC Rapporteur´s comment:

During the reference period, no PV inspection findings relevant to Holoclar were identified.

6.7. Discussion

The safety data reported in the renewal do not suggest any new safety concerns for Holoclar at this time. The completed clinical trial HOLOCORE is a prospective, open-label, uncontrolled interventional study to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns, implemented as a specific obligation to complete post-authorisation for the conditional marketing authorisation. As this is an interventional trial, assessment of the safety and efficacy results have been carried out by the CHMP Rapporteur. A discussion on the results of the trial is provided in section 5.

7. Risk management plan

PRAC Rapporteur´s comment: The MAH has submitted an updated RMP within the annual renewal procedure. Outlined in the table below are the detailed changes proposed by the MAH as part of this RMP update. The updated RMP was submitted primarily to include new data from the finalised study CCD-GPLSCD01-03 (HOLOCORE), which is a specific obligation of the conditional marketing authorisation. Updates have also been made throughout the document to align with the requirements and format of EMA/164014/2018 Rev.2.0.1 template. No changes to the list of safety concerns for the RMP have been proposed by the MAH. In addition, no new pharmacovigilance studies or additional risk minimisation measures have been proposed by the MAH. The updates to Part 1 and Part II of the RMP are primarily as a result of the completion of Study CCD-GPLSCD01-03 (HOLOCORE) and are considered acceptable. In PART II Module SVII Identified and Potential Risks, the MAH has included information on the important identified risk "Lack of effect manifesting as corneal epithelium defect" and the potential risk of "Medication errors" concerning the use of topical lidocaine or anaesthetics containing adrenaline, which should be avoided for any of the steps of the treatment with Holoclar, as they reduce the colony forming efficiency. This is in line with the information provided in the educational material in the annex of the RMP since granting of the CMA and also with the protocols used in studies (HLSTM01, HLSTM02, HLSTM03, HLSTM04) and is considered acceptable by the PRAC Rapporteur. Of note, this information has not been included in the product information of Holoclar and has been proposed to be included in the PI as part of this renewal procedure. The PRAC Rapporteur also considers that Annex IID of the product information should be updated to reflect that concomitant use of topical lidocaine or anaesthetics containing adrenaline must be avoided. As part of the RSI, the MAH has submitted an updated RMP to change the study GPLSCD01-03-FU (HOLOCORE Follow-up) from ongoing to completed, and to include data from the study. This study has been assessed by the CAT Rapporteur as part of the RSI. The significant changes to the RMP as a result to this update are highlighted further below.

Table 1

RMP Part/Module	RMP v12.1	RMP v12.2 (significant changes)
PART I PRODUCT(S) OVERVIEW	- Included number of Medicinal Products to which this RMP refers - Amended Paediatric population in Posology and route of administration in the EEA - Deleted the conditional marketing authorization from additional monitoring in the EU.	- Change the name of the MAH from Holostem Terapie Avanzate s.r.l. to Holostem s.r.l. (all of the document)
PART II SAFETY SPECIFICATION		
PART II Module SIII Clinical Trial Exposure	Update to include data of the first approval of Holoclar. Update to include data of Study CCD-GPLSCD01-03 (HOLOCORE)	Update to modify data regarding Study CCD-GPLSCD01-03-FU (HOLOCORE Follow-up)

PART II Module SIV Populations Not Studied in Clinical Trials	Update of section SIV.1 with the exclusion criteria of Study CCD-GPLSCD01-03 (HOLOCORE) Update of section SIV.2 to include data from the Study CCD-GPLSCD01-03 (HOLOCORE) Update of section SIV.3 with data from the Study CCD-GPLSCD01-03 (HOLOCORE) Deletion of section SIV.4 Conclusions on the populations not-studied and other limitations of the clinical trial development programme	Update of section SIV.2 to include data from the Study CCD-GPLSCD01-03-FU (HOLOCORE Follow-up)
PART II Module SV Post-Authorisation Experience	Updated the number of post-authorisation exposure	
PART II Module SVII Identified and Potential Risks	Section SVII.1: Correction of a mistake in the name of a studio: Change of HLSTM03 to CCD-GPLSCD01-03 (HOLOCORE). Section SVII.1: Update of the flow-chart of the logistics of Holoclar therapy with more details. Addition of more details regarding the risks related to interaction of the product and the patient and related to persistence of the product in the patient. Section SVII.2: correction of typos in the wording. Information unrelated to the update of safety concerns (past updates in educational material, reported TREAEs in HOLOCORE study and safety events in HOLOSIGHT) has been deleted. Section SVII. 3.1: Update data in the safety concerns with new the information from the accumulated data from clinical trials and post-marketing data from studies HOLOCORE, HOLOCORE-FU and HOLOSIGHT. Correction of typos and incorrect names of the studies. Addition of important risk "Lack of effect manifesting as corneal epithelium defect" new preventability information. Addition in potential risk of Medication errors the use of topical lidocaine or anaesthetics containing adrenaline.	Section SVII. 3.3 Update of "Missing information: Long-term safety" with data from Study CCD-GPLSCD01-03-FU (HOLOCORE follow-up).

PART III PHARMACOVIGILANCE PLAN (INCLUDING POST-AUTHORISATION SAFETY STUDIES)		
PART III.2 Additional Pharmacovigilance Activities	Update of the status of the completed and ongoing additional pharmacovigilance activities and dates of milestones.	Update of the status of the completed and ongoing additional pharmacovigilance activities and dates of milestones.
PART III.3 Summary Table of Additional Pharmacovigilance Activities	Planned and on-going studies: Deletion of Study CCD-GPLSCD01-03 (HOLOCORE) and update of the status of the ongoing additional pharmacovigilance activities and dates of milestones.	Planned and on-going studies: Deletion of Study CCD-GPLSCD01-03-FU (HOLOCORE follow-up) and update of the status of the ongoing additional pharmacovigilance activities and dates of milestones.
PART IV PLANS FOR POST-AUTHORISATION EFFICACY STUDIES	Deletion of Study CCD-GPLSCD01-03 (HOLOCORE) as it has been finalized.	
PART V RISK MINIMISATION MEASURES (INCLUDING EVALUATION OF THE EFFECTIVENESS OF RISK MINIMISATION ACTIVITIES)		
PART V.1 Routine Risk Minimisation Measures	Addition of routine risk communication activities in line with the changes performed in the updated SmPC. Addition of information of routine risk communication in the PIL. Addition of 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."	
PART V.2 Additional Risk Minimisation Measures	Inclusion of more details for Patient Information Guide.	
PART V.3 Summary of Risk Minimisation Measures	Deletion of the completed Study CCD-GPLSCD01-03 (HOLOCORE) in Pharmacovigilance activities.	Deletion of the completed Study CCD-GPLSCD01-03-FU (HOLOCORE Follow-up) in Pharmacovigilance
PART VI SUMMARY OF THE RISK MANAGEMENT PLAN		
II. Risks associated with the medicine and activities to minimise or further characterise the risks	Part IIB: Aligned the section according to changes performed in Part II: Module SVII.3, Part V.1 and Part V.3. of the document. Deletion of the completed Study CCD-GPLSCD01-	Part IIB: -Update of information in "Missing information: Long-term safety" according to

	03 (HOLOCORE) of Additional pharmacovigilance activities. Part IIC: Aligned the section according to changes performed in Part III of the document. Deletion of the completed Study CCD-GPLSCD01-03 (HOLOCORE) of Studies which are conditions of the marketing authorisation.	changes performed in Part II: Module SVII. -Deletion of the completed Study CCD-GPLSCD01-03-FU (HOLOCORE follow-up) of Additional pharmacovigilance activities according to the changes in Part V.3. of the document. Part IIC: Deletion of the completed Study CCD-GPLSCD01-03-FU (HOLOCORE follow-up) of Studies which are conditions of the marketing authorisation.

Safety concerns

Table 2. Summary of the Safety Concerns

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 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
Missing information	Pregnancy and lactation Use in children

Summary of safety concerns	
	Use in elderly Long-term safety

PRAC Rapporteur´s comment: Considering the data in the safety specification, the safety concerns listed by the MAH are appropriate. No changes to the current list of safety concerns has been proposed by the MAH. This is considered acceptable by the PRAC Rapporteur. There are ongoing PASS studies to further characterise the list of safety concerns as well as additional risk minimisation measures in place.

Pharmacovigilance plan

Table 3. On-going and planned studies in the post-authorisation pharmacovigilance development plan

Activity/Study title (type of activity, study title [if known] category 1-3)*	**Objectives**	**Safety concerns addressed**	**Status** (planned, started)	**Date for submission of interim or final reports** (planned or actual)
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				
~~**Study Holocore**~~ ~~Multinational, multicentre, prospective, open label, uncontrolled clinical study~~ ~~Patients from 2 years of age and adults will be included in the study.~~	~~To evaluate efficacy and safety of one or two Autologous Cultivated Limbal Stem Cell Implantation(s) (ACLSCT) in restoring a normal corneal epithelium in patients suffering from moderate-severe Limbal Stem Cell Deficiency (LSCD) secondary to ocular burns~~	~~-Glaucoma~~ ~~-Lack of effect (corneal implant failure)~~ ~~-Blepharitis~~ ~~-Safety profile in children under 18 years of age~~ ~~-Long-term safety~~	~~Annual interim reports.~~	~~December 2020~~
Category 3 – Required additional Pharmacovigilance activities				
~~Study HOLOCORE Follow-up Long-term safety and efficacy follow-up after autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns~~	~~All consenting patients from Study HOLOCORE are rolled-over into this study to evaluate the long-term safety and efficacy (visit every 6 months) and success after keratoplasty (whenever clinically indicated)~~	~~Long-term safety~~	~~NoneFinal study report~~	~~31/10January/2023~~

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."	Primary Objective To evaluate the long-term safety profile of patients treated with Holoclar during a 5-year follow-up period from first ocular implantation under routine clinical conditions, through the description of the occurrence of adverse events, adverse drug reactions, serious adverse events and adverse events of special interest. Adverse events of special interest are solicited and carefully monitored. Secondary objectives To describe demographic and clinical characteristics of patients undergoing one or more Holoclar implants including the occurrence of ocular grafts preceding the investigated implant. To describe the proportion of success, according to clinician's opinion, one year	-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 -Use in pregnancy and breast-feeding -Safety profile in children under 18 years of age -Long-term safety	~~The first patient enrolled on 20th October 2016 in Italy, The end of the data collection (including the follow-up period) is expected within January 2024, depending on the enrolment period necessary to collect data from at least one hundred patients. A study progress report will be submitted to EMAannually~~ Final study report	**31**/~~03~~**12/2028**

	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.				

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are Specific Obligations in the context of a marketing authorisation under exceptional circumstances under Article 14(8) of Regulation (EC) 726/2004 or in the context of a conditional marketing authorisation under Article 14(7) of Regulation (EC) 726/2004.
Category 3 are required additional PhV activity (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

PRAC Rapporteur´s comment:

The proposed changes to the post-authorisation pharmacovigilance development plan are considered acceptable. The PhV plan has been updated to remove Study CCD-GPLSCD01-03 (HOLOCORE) and update the status of the ongoing additional pharmacovigilance activities and dates of milestones. The final CSR for the HOLOCORE follow-up study was finalised by March 2023 and the date of submission for the final report is 31/10/2023. As part of the RSI, the MAH has provided the final CSR for the HOLOCORE follow-up study which has been assessed by the CAT Rapporteur. With regard to the PASS study, Holosight, the final study report submission date has been updated to 31/12/2028. The enrollment in this study is closed with the last patient treated in May 2023 and the final number of patients who started the treatment with Holoclar and included in the study is 94. One patient has completed the 5 years observation period after the implantation (primary endpoint).

Risk minimisation measures

Table 4. 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: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -Post-Authorisation safety study, registry -like (HOLOSIGHT)
2) Lack of effect manifesting as corneal epithelium defect	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 measures: Healthcare Professional Guide Patient information guide	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -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: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -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: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -Post-Authorisation safety study, registry -like (HOLOSIGHT):
5) Post-implant infection	Routine risk minimisation measures: 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	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -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: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -Post-Authorisation safety study, registry -like (HOLOSIGHT)
7). Off-label use Milder form of limbal stem cell deficiency than the proposed indication	Routine risk minimisation measures: SmPC section 4.1 SmPC section 4.2	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None

-Other aetiologies of limbal stem cell deficiency e.g. radiotherapy, aniridia, Stevens Johnson Syndrome and neurotrophic keratitis	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: Healthcare Professional Guide Patient information guide	Additional pharmacovigilance activities: -Post-Authorisation safety study, registry -like (HOLOSIGHT)
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: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -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: -Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) -Post-Authorisation safety study, registry -like (HOLOSIGHT)
11.) Long-term safety	None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None

		Additional pharmacovigilance activities: -Study CCD-GPLSCD01-03 FU

PRAC Rapporteur´s comment:

Part V of the RMP has been updated to include addition of routine risk communication activities in line with the changes proposed in the updated SmPC and PL as well as the addition of 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." Part V has also been updated to include more detail on the objectives and rationale for the Patient Information Guide. Part V of the RMP has also been updated to delete reference to the completed Study CCD-GPLSCD01-03 (HOLOCORE) and Study CCD-GPLSCD01-03 FU (HOLOCORE follow-up) in Pharmacovigilance activities. No new additional risk minimisation measures are proposed by the MAH which is considered acceptable. The risk minimisation measures are sufficient to minimise the risks of the product in the authorised indications.

Elements for a public summary of the RMP

The elements for a public summary of the RMP do not require revision following the conclusion of the procedure:

Annexes

The RMP annexes have been updated as follows:

Part VII Annexes to The Risk Management Plan

- Annex 2: Tabulated summary of planned, ongoing, and completed pharmacovigilance study program: Deletion of Study CCD-GPLSCD01-03 (HOLOCORE) and update of the status of the ongoing studies and dates of milestones.
Addition of the completed study CCD-GPLSCD01-03 (HOLOCORE).

Table 1 Annex II: Planned and on-going studies: Deletion of Study CCD-GPLSCD01-03-FU (HOLOCORE Follow up)

Table 2 Annex II: Completed studies: Addition of the completed study CCD-GPLSCD01-03-FU (HOLOCORE Follow up).

Annex 3: Protocols for proposed, on-going and completed studies in the pharmacovigilance plan

- Deletion of protocols of completed Study CCD-GPLSCD01-03 (HOLOCORE)
- Deletion of protocols of completed Study CCD-GPLSCD01-03-FU (HOLOCORE Follow-up)

Annex 5: Protocols for proposed and on-going studies in RMP part IV

- Deletion of protocol Study CCD-GPLSCD01-03 (HOLOCORE) Annex 6, 7 Educational material:
- Update in the training program to include more details on how is conducted.
- Changes in Healthcare professional information guide:

- Section 5.1.2 Assessment of concomitant diseases
- Section 6 to align the most common adverse reactions with the most updated version of the SmPC.
- Section 10.2: Deletion of a typographical error

PRAC Rapporteur´s comment: The changes made to the annexes of the RMP are primarily in line with completion of the Holocore study and Holocore FU study and considered acceptable. With regards to the educational material, no changes have been made to Annex IID. The changes proposed to Annex 6 and 7 educational materials are considered acceptable but must also be agreed by the relevant National Competent Authority in each Member State as necessary.

7.1. Overall conclusion on the RMP

☒ The RMP version 12.2 is acceptable.

The MAH is reminded that, within 30 calendar days of the receipt of the Opinion, an updated version of Annex I of the RMP template, reflecting the final RMP agreed at the time of the Opinion should be submitted to h-eurmp-evinterface@emea.europa.eu.

8. Changes to the Product Information

Changes to the Product information have been submitted to SmPC, sections 4.2, 4.4, 4.5, 4.7, 4.8, 5.1-3, 6.6, Annex II E, and PIL.

Changes to the Product Information (PI), based on the submitted data within the scope of this procedure, are introduced during the assessment of this renewal (see attached PI with comments).

Quality:

In Section 4.4, the special warning for transmission of an infectious agent has been extended. This is regarded acceptable.

Clinical:

Annex IID of the product information should be updated to reflect that concomitant use of topical lidocaine or anaesthetics containing adrenaline must be avoided.

Section 5: The figures for the key primary and secondary efficacy endpoints need to be presented in alignment with the data reported in the final CSR of the HOLOCORE Study. For example, evaluable results for a successful transplantation in accordance with the Independent Assessor Judgement was documented for n=25/n=69 subjects, which results in a percentage rate of 36,2 % (95% CI: 0.25-0.49) vs. 41% as currently reported in the SmPC.

With responses to RSI (eCTD 0096) Product Information has been revised accordingly. Please refer to the Attachment.

Additional monitoring

N/A

9. Request for Supplementary Information - RfSI

The MAH should provide the following supplementary information in response to Day 60 RfSI:

9.1. Major objections

Clinical

Specific obligations

1. The MAH is requested to provide a solid justification, supported by comparable scientific literature data to demonstrate the SOB being fulfilled not only from a formal perspective, but also by content (thus providing an overall comprehensive data package and justifying the granting of a standard MA instead of a renewal of the CMA), considering the following criteria inter alia:

a) N=93 subjects and at least n=87 adults were planned to be enrolled and treated in the HOLOCORE Study, which is subject to the SOB. However, results available on the efficacy endpoints did not change significantly since data lock point for the 2020 renewal; up to now, n=63 subjects have been treated in the HOLOCORE Study. Successful transplantation (primary efficacy endpoint) was reported as 33.8% in terms of the mITTb population. In terms of key secondary endpoints, central corneal evaluation at Day 360 remained severe in 22% of the participants, no change in the status of neo-revascularization was reported for 38% of the participants, and as the majority of participants presented with no epithelial defects at baseline, a potential shift could not be measured post-transplantation.

b) According to the SAP of the HOLOCORE Study, a proportion of successful transplantation of 66.7% was expected.

c) The high number of study procedure/IMP related TEAs and SAEs (please refer to the clinical OC);

d) No long-term efficacy data for the n=47 enrolled subjects enrolled in the HOLOCORE-Follow-Up study have been presented up to now (please refer to the corresponding OC below).

9.2. Other concerns

Clinical aspects

1. As the final study report the HOLOCORE-FU study (Chiesi ID CCD-GPLSCD01-03-FU) will be available to a later time Study procedure and/or IMP related serious adverse events have been reported up to now for n=10 subjects in the HOLOCORE study. The MAH is asked to provide a thorough discussion (apart from the narratives provided) on the underlying reasons for the occurrence of these SAEs and to evaluate the introduction of risk mitigation measures in order to avoid such events. This is considered particularly important as long-term safety is considered missing information (please see RMP assessment PRAC Rapporteur).

2. point according to the MAH (October 2023), the Applicant is asked to provide profound interim information on primary and secondary efficacy parameters for subjects included in this trial. The availability and acceptance of the results provided may have an impact on the acceptance of the SOB (see clinical MO).

3. The MAH is requested to submit a thorough presentation of post-marketing data for efficacy and safety.
In addition, the MAH should provide an evaluation of the impact of the product shortage since December 2022 in the EU and UK on the outstanding post-authorisation measures.

4. The SmPC needs to be revised in accordance with the points outlined in the assessment report; figures presented for the key primary and secondary efficacy endpoints below SmPC section 5 have to be in alignment with the data reported in the final CSR of the HOLOCORE Study.

10. Assessment of the MAH responses to the RfSI

10.1. Major objections

Specific Obligations

Clinical Question

Summary of the MAH's response

The response data package to the RfSI for the clinical MO and OCs contains:

1. The MAH's Response Document on the MO and the OCs, i. a. including the justification of the HOLOCORE study and the evaluation of the Holoclar treatment success and failures based on the trial primary composite endpoint evaluated by Independent Assessors using CNV in Quadrant Methodology on 2D pictures. The issues regarding the in 2020 identified little relevance of the key efficacy endpoints were mitigated by the new MAH Holostem with an amendment of the trial protocol (introduction of a blinded re-evaluation of all the 2D photos by the Independent Assessors), submitted to the Authorities.
2. A literature-based discussion on surgical options for the target population.
3. Further long-term efficacy and safety data, comprising:

a. The final study report of the non-pharmacological Phase 4 HOLOCORE-FU Study (CCDGPLSCD01-03-FU Version 1.0, 31 OCT 2023), a *'multinational, multicenter, prospective, long-term safety and efficacy follow-up study after ACLSCT for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns'*. The study was initiated on 13 Dec 2017 and completed on 31 Mar 2023. Overall, 47 patients (45 adults and 2 minors) have been enrolled, and 44 have completed the study, with a follow-up from 2 to 6 years after Holoclar implantation. Results are provided as pooled analysis.

b. Update on the Holosight Study, the ongoing long-term PAS study (final study report due date December 2027).

With regard to the MO part a) and b), the MAH argues, the SOB concerning the number of evaluated subjects is met, which has not been questioned per se. As per the HOLOCORE study protocol agreed with the EMA in the context of the granting a CMA, approximately 87 adult patients were planned to be included into the study, in order to have 65 adult patients treated with Holoclar, taking into consideration a possible drop-out rate of 25% before the Holoclar treatment. The final target was achieved: 80 patients were enrolled; they underwent the biopsy withdrawal for the product preparation, and out of 80, 73 (69 adults plus 4 paediatrics) received Holoclar implantation. Out of 73 patients, all the paediatrics completed the study (4 patients) while 64 adults completed the study (the remaining 5 are withdrawals before the 12-month final evaluation due to AE (2 patients), withdrawals of the informed consent form (2 patients) and withdrawal for other reasons (1 patient - transfer to another country). According to the MAH, the SOB is also fulfilled in content, as the results on mid- and long-term safety and efficacy presented confirm the Holoclar benefits and safety profile observed in previous retrospective studies. The overall success rate from a minimum of 51% in the Study HOLOCORE (measured according to Global Consensus standard guidelines) up to 77% (measured by ophthalmologists) with an overall improvement of 60% the population affected by LSCD since 153 months on average is sufficiently meaningful, as clinical assessments by ophthalmologist would be the most reliable parameter to define treatment success. The MAH also argues, in the long-term efficacy HOLO-UP study, conducted in 2017 (CCD-GPLSCD01-07) on 49 patients participating to the previous HLSTM01 study, with a mean follow-up of 153 months after Holoclar transplantation, the persistence of successful transplantations was judged as 50.0% (24 subjects; 95% CI: 0.35 to 0.65; p=0.443) according to the overall clinical judgement of the Investigator. The evidence of safety and efficacy of Holoclar gained by real world data would be the best approach for the marketing authorisation of the product, i. e. the conversion of the current CMA for Holoclar to a full approval. Moreover, the introduction of proper evaluation of 2D pictures by external independent assessors in alignment with LSCD Global Consensus Guidelines as mitigation action eliminates existing bias and ensures evidence based clinical assessment (for comparison between the trials see CSR Table 3).

Literature based discussion

According to the MAH, surgical options depend on patient selection:

- Conjunctival limbal autograft (CLAu), 40% of the limbus from the fellow healthy eye is taken. This approach requires one fully intact eye, and it is not well accepted by patients and by surgeons, as gives one chance only (Deng SX et al. 2020).

- Conjunctival limbal allograft (CLAL), in which stem cells are taken from a living, related donor or dead donor and transplanted into the diseased eye of the recipient. This approach requires a long-term immunosuppression as epithelia are strongly antigenic (Santos MS, 2005; Movahedan A, et al, 2017)

- Keratolimbal allograft (KLAL), transplants the entire limbus from a dead donor using the corneoscleral carrier to deliver a large number of stem cells to the recipient. This approach, even more invasive, requires a long-term immunosuppression as epithelia are strongly antigenic (Santos MS, 2005; Movahedan A, et al, 2017);

- Simple limbal epithelial transplantation (SLET), reduces the tissue withdrawal of CLAu, but it treats milder severity (superficial lesions only) than CLET (Holoclar). In the SLET procedure, re-epithelialization requires months (it takes at least 5–6 weeks) (Sangwan S.V., 2012; Swapna SS. 2019) with prolonged pain, and increased risk of inflammation and complications (Magrelli F.M 2020, Deng SX 2020).

- Cultured limbal epithelial transplantation (Holoclar) procedure can treat both eyes, it starts from a smaller amount of limbal tissue (1–2 mm^2), minimize the risk of injury to the healthy eye, if any. Holoclar does not require lifelong immunosuppression and in cases of failure, the treatment can be repeated multiple times without damage of the fellow eye, if any. In addition, the epithelialization is fast, as the full epithelium (with stem cells) quickly engrafts without suturing, with reduced risk of inflammation, infections and pain, burning photophobia (Magrelli F.M 202, Rama P 2010). The development of this technique has frequently resulted from collaboration between stem cell translational scientists and ophthalmologists. Direct transplantation of autologous limbal tissue from a healthy donor eye is regarded by scientific papers as the technique of choice, as it quickly restores the corneal limbal milieu (Deng SX, 2019, Sacchetti M. et al, 2018; Calonge M. et al, 2021, Guerin LP. et al, 2022).

HOLOCORE-FU final study report V1.0, 31Oct2023 (subject to PAM)

- Study design: Phase 4, non-interventional/pharmacological
- Inclusion criteria: adults and pediatric subjects who completed the Study HOLOCORE
- Exclusion criteria: no specific criteria
- Primary Objective: Long-term safety of one or two Holoclar treatments in patients with moderate/severe LSCD secondary to ocular burns in the HOLOCORE study (TEAEs; AESIs)
- Secondary objective: Long-term efficacy measured as percentage of patients defined as 'sustained success' by investigators based on the evidence of a degree of superficial CNV absent of at least invading not more than one quadrant without involvement of the central portion of the cornea AND absence of epithelial defects after staining with fluorescein; BCVA etc.)
- Study initiation: 31Dec2017. Study completion: 31Mar2023
- Study centers: Belgium: 1 (n=1 enrolled); France: 4 (n=7 enrolled); Germany: 3 (n=2 enrolled); Italy: 2 n=16 enrolled); Netherlands: 1 (n=2 enrolled); Poland: 2 (n=17 enrolled); Spain: 1 (n= 1 enrolled); United Kingdom: 2 (n=2 enrolled); *source: CSR Table 5*
- Study schedule: a minimum of 3 clinical visits (v1 to V3) were performed (*for details see CSR Table 2*):
  - Screening visit V1 ≙ final visit of the HOLOCORE study
  - 6-months visit V2 (Day 180±14 days)
  - 12-months final visit V3 (Day 360±14 days)
  - Every 6-months visit (Day 180±14 days from the previous visit)
  - Pts., who received keratoplasty (corneal transplantation) after Holoclar were followed acc. to a prespecified visit-schedule after surgery
- Number of patients (*CSR Table 6*):
  Planned: 70
  Enrolled: 47 (n=45 adults + n= 2 children)
  Completed: 44
  Analyzed for safety: 47 (45 adults ≙ adult safety population); n=18 (38,3%) received keratoplasty (≙ keratoplasty adult population; secondary study endpoint; *CSR Table 11*)
  Analyzed for efficacy: 45
  Planned: 70
- Demographic characteristics (adult safety population; *CSR Table 7*):
  - Median age (years): 46
  - Gender: n=38 male; n=7 female
  - Any pooled medical surgical history/concom. disease of the treated eye (*CSR Table 8 and Table 9*): n=32 (71.1%). For details on LSCD history *see CSR Table 10.*
- Estimation of the individual duration since inclusion in the HOLOCORE study:

Study duration		**HOLOCORE duration**		**HOLOCORE-FU duration**	**TOTAL duration**
		First ACLSCT	**Second ACLSCT**		
Shortest *[1] (i.e., last patient entering HOLOCORE who needed a second ACLSCT at 12 months. This patient determined the end of the HOLOCORE Follow-up for all patients)		19 months*[2]	19 months*[2]	12 months	50 months*[4]
Longest *[3] (i.e., first patient enrolled in HOLOCORE)	Only one ACLSCT needed	19 months*[2]	NA	57 months	76 months*[4]
	Second ACLSCT needed after 12 months	19 months*[2]	19 months*[2]	38 months	

*[1] In case the last enrolled patient did not need a second ACLCST (or needed it at an earlier follow-up stage), the last patient needing a second ACLSCT at 12 months drove the total study duration. In any case, the minimum duration included 12 months of Holocore Follow-Up study. *[2] Approximate time since inclusion, considering ~7 months from screening to ACLSCT. Time from screening to biopsy procurement, product manufacturing, and application varied for logistic and production reasons. *[3] Approximate time, assuming an enrollment period of 26 months, since first patient in and need for a second ACLSCT at 12 months in the last enrolled patient. *[4]In case the last enrolled patient underwent keratoplasty at the Last Visit of the Follow-up study, this consequently led to a prolongation of the study up to further 12 months for all patients; *Source: Final CSR*

Main Efficacy Results

Success of Transplantation by Visit (Adult Safety Population)

Visit	Parameter	Statistic	With keratoplasty (N=18)	Without keratoplasty (N=27)	Overall (N=45)
Day 1	Success	Non-missing values	15	21	36
		n (%)	9 (60.0)	8 (38.1)	17 (47.2)
		95% CI	0.32-0.84	0.18-0.62	0.30-0.65
	Success according to overall investigator's judgement	Non-missing values	15	21	36
		n (%)	15 (100.0)	14 (66.7)	29 (80.6)
		95% CI	0.78-1.00	0.43-0.85	0.64-0.92
Day 180	Success	Non-missing values	18	20	38
		n (%)	16 (88.9)	8 (40.0)	24 (63.2)
		95% CI	0.65-0.99	0.19-0.64	0.46-0.78
	Success according to overall investigator's judgement	Non-missing values	18	20	38
		n (%)	18 (100.0)	14 (70.0)	32 (84.2)
		95% CI	0.81-1.00	0.46-0.88	0.69-0.94
Day 360	Success	Non-missing values	14	14	28
		n (%)	12 (85.7)	5 (35.7)	17 (60.7)
		95% CI	0.57-0.98	0.13-0.65	0.41-0.78
	Success according to overall investigator's judgement	Non-missing values	15	15	30
		n (%)	14 (93.3)	11 (73.3)	25 (83.3)
		95% CI	0.68-1.00	0.45-0.92	0.65-0.94
Day 720	Success	Non-missing values	8	7	15
		n (%)	7 (87.5)	3 (42.9)	10 (66.7)
		95% CI	0.47-1.00	0.10-0.82	0.38-0.88
	Success according to overall investigator's judgement	Non-missing values	12	8	20
		n (%)	11 (91.7)	5 (62.5)	16 (80.0)
		95% CI	0.62-1.00	0.24-0.91	0.56-0.94
Day 1080	Success	Non-missing values	9	12	21
		n (%)	5 (55.6)	3 (25.0)	8 (38.1)
		95% CI	0.21-0.86	0.05-0.57	0.18-0.62
	Success according to overall investigator's judgement	Non-missing values	10	12	22
		n (%)	10 (100.0)	9 (75.0)	19 (86.4)
		95% CI	0.69-1.00	0.43-0.95	0.65-0.97
Day 1440	Success	Non-missing values	9	6	15
		n (%)	6 (66.7)	2 (33.3)	8 (53.3)
		95% CI	0.30-0.93	0.04-0.78	0.27-0.79
	Success according to overall investigator's judgement	Non-missing values	9	6	15
		n (%)	8 (88.9)	4 (66.7)	12 (80.0)
		95% CI	0.52-1.00	0.22-0.96	0.52-0.96
Day 1800	Success	Non-missing values	2	3	5
		n (%)	2 (100.0)	2 (66.7)	4 (80.0)
		95% CI	0.16-1.00	0.09-0.99	0.28-0.99
	Success according to overall investigator's judgement	Non-missing values	2	3	5
		n (%)	2 (100.0)	3 (100.0)	5 (100.0)
		95% CI	0.16-1.00	0.29-1.00	0.48-1.00

Notes: 1) Percentages are based on total number of non-missing values.2) Incidence of success at each visit is calculated as the proportion of patients with less than 2 superficial neo-vascularisation corneal quadrants involved, no Central corneal Involvement and absence of epithelial defects (none or trace) at that specific visit. 3) Only observed cases are presented. 4) If a Not-Permitted Medication was taken before the assessed visit, the Transplantation was considered as a 'Failure' at that specific visit; *Source: CSR Table 13*

Success of Transplantation by Post-Keratoplasty Visit (Keratoplasty Adult Safety Population)

Visit	Parameter	Statistic	Overall (N=18)
KDay 30	Success	Non-missing values	11
		n (%)	11 (100.0)
		95% CI	0.72-1.00
	Success according to overall investigator's judgement	Non-missing values	14
		n (%)	14 (100.0)
		95% CI	0.77-1.00
KDay 360	Success	Non-missing values	13
		n (%)	12 (92.3)
		95% CI	0.64-1.00
	Success according to overall investigator's judgement	Non-missing values	14
		n (%)	13 (92.9)
		95% CI	0.66-1.00
KDay 720	Success	Non-missing values	11
		n (%)	6 (54.5)
		95% CI	0.23-0.83
	Success according to overall investigator's judgement	Non-missing values	13
		n (%)	12 (92.3)
		95% CI	0.64-1.00
KDay 1080	Success	Non-missing values	8
		n (%)	6 (75.0)
		95% CI	0.35-0.97
	Success according to overall investigator's judgement	Non-missing values	8
		n (%)	8 (100.0)
		95% CI	0.63-1.00

Notes: 1) Percentages are based on total number of non-missing values.2) Incidence of success at each visit is calculated as the proportion of patients with less than 2 superficial neo-vascularisation corneal quadrants involved, no Central corneal Involvement and absence of epithelial defects (none or trace) at that specific visit. 3) Only observed cases are presented. 4) If a Not-Permitted Medication was taken before the assessed visit, the Transplantation was considered as a 'Failure' at that specific visit. *Source: CSR Table 14*

Degree of Neo-Vascularisation and Central Cornea Involvement by Visit (Adult Safety Population)

Visit	Parameter	Statistic	With Keratoplasty (N=18)	Without Keratoplasty (N=27)	Overall (N=45)
Baseline	Number of subjects at visit		18	27	45
	Number of corneal quadrants	0	—	—	—
		1	—	—	—
		2	—	1 (3.7)	1 (2.2)
		3	3 (16.7)	8 (29.6)	11 (24.4)
		4	15 (83.3)	18 (66.7)	33 (73.3)
		Missing	—	—	—
	Central Cornea (6 mm) involvement	Yes	18 (100.0)	27 (100.0)	45 (100.0)
		No	—	—	—
		Missing	—	—	—
Day 360	Number of subjects at visit		15	19	34
	Number of corneal quadrants	0	11 (73.3)	3 (15.8)	14 (41.2)
		1	1 (6.7)	2 (10.5)	3 (8.8)
		2	—	4 (21.1)	4 (11.8)
		3	—	3 (15.8)	3 (8.8)
		4	2 (13.3)	2 (10.5)	4 (11.8)
		Missing	1 (6.7)	5 (26.3)	6 (17.6)
	Central Cornea (6 mm) involvement	Yes	1 (6.7)	4 (21.1)	5 (14.7)
		No	13 (86.7)	10 (52.6)	23 (67.6)
		Missing	1 (6.7)	5 (26.3)	6 (17.6)

Day 720	Number of subjects at visit		12	8	20
	Number of corneal quadrants	0	5 (41.7)	2 (25.0)	7 (35.0)
		1	2 (16.7)	1 (12.5)	3 (15.0)
		2	—	1 (12.5)	1 (5.0)
		3	1 (8.3)	2 (25.0)	3 (15.0)
		4	—	1 (12.5)	1 (5.0)
		Missing	4 (33.3)	1 (12.5)	5 (25.0)
	Central Cornea (6 mm) involvement	Yes	1 (8.3)	2 (25.0)	3 (15.0)
		No	9 (75.0)	5 (62.5)	14 (70.0)
		Missing	2 (16.7)	1 (12.5)	3 (15.0)
Day 1080	Number of subjects at visit		10	12	22
	Number of corneal quadrants	0	4 (40.0)	1 (8.3)	5 (22.7)
		1	1 (10.0)	2 (16.7)	3 (13.6)
		2	1 (10.0)	7 (58.3)	8 (36.4)
		3	—	1 (8.3)	1 (4.5)
		4	3 (30.0)	1 (8.3)	4 (18.2)
		Missing	1 (10.0)	—	1 (4.5)
	Central Cornea (6 mm) involvement	Yes	1 (10.0)	3 (25.0)	4 (18.2)
		No	9 (90.0)	9 (75.0)	18 (81.8)
		Missing	—	—	—
Day 1440	Number of subjects at visit		9	6	15
	Number of corneal quadrants	0	6 (66.7)	1 (16.7)	7 (46.7)
		1	—	1 (16.7)	1 (6.7)
		2	1 (11.1)	2 (33.3)	3 (20.0)
		3	—	1 (16.7)	1 (6.7)
		4	2 (22.2)	1 (16.7)	3 (20.0)
		Missing	—	—	—
	Central Cornea (6 mm) involvement	Yes	2 (22.2)	2 (33.3)	4 (26.7)
		No	7 (77.8)	4 (66.7)	11 (73.3)
		Missing	—	—	—
Day 1800	Number of subjects at visit		2	3	5
	Number of corneal quadrants	0	2 (100.0)	1 (33.3)	3 (60.0)
		1	—	1 (33.3)	1 (20.0)
		2	—	1 (33.3)	1 (20.0)
		3	—	—	—
		4	—	—	—
		Missing	—	—	—
	Central Cornea (6 mm) involvement	Yes	—	—	—
		No	2 (100.0)	3 (100.0)	5 (100.0)
		Missing	—	—	—

Source: CSR Table 19

Degree of Re-Epithelialisation by Visit (Adult Safety Population)

Visit	Epithelial Defects	With Keratoplasty (N=18)	Without Keratoplasty (N=27)	Overall (N=45)
Baseline	Number of subjects at visit	18	27	45
	None	14 (77.8)	13 (48.1)	27 (60.0)
	Trace	4 (22.2)	14 (51.9)	18 (40.0)
	Mild	—	—	—
	Severe	—	—	—
	Missing	—	—	—
Day 360	Number of subjects at visit	15	19	34
	None	14 (93.3)	11 (57.9)	25 (73.5)
	Trace	—	1 (5.3)	1 (2.9)
	Mild	—	1 (5.3)	1 (2.9)
	Severe	—	1 (5.3)	1 (2.9)
	Missing	1 (6.7)	5 (26.3)	6 (17.6)
Day 720	Number of subjects at visit	12	8	20

	None	9 (75.0)	5 (62.5)	14 (70.0)
	Trace	1 (8.3)	—	1 (5.0)
	Mild	—	1 (12.5)	1 (5.0)
	Severe	—	1 (12.5)	1 (5.0)
	Missing	2 (16.7)	1 (12.5)	3 (15.0)
Day 1080	Number of subjects at visit	10	12	22
	None	7 (70.0)	11 (91.7)	18 (81.8)
	Trace	2 (20.0)	1 (8.3)	3 (13.6)
	Mild	—	—	—
	Severe	—	—	—
	Missing	1 (10.0)	—	1 (4.5)
Day 1440	Number of subjects at visit	9	6	15
	None	7 (77.8)	5 (83.3)	12 (80.0)
	Trace	1 (11.1)	1 (16.7)	2 (13.3)
	Mild	1 (11.1)	—	1 (6.7)
	Severe	—	—	—
	Missing	—	—	—
Day 1800	Number of subjects at visit	2	3	5
	None	2 (100.0)	3 (100.0)	5 (100.0)
	Trace	—	—	—
	Mild	—	—	—
	Severe	—	—	—
	Missing	—	—	—

Source: CSR Table 21

Clinical Symptoms by Visit (Adult Safety Population)

	Baseline (N=45) [n (%)]	Day 1 (N=37) [n (%]	Day 360 (N=34) [n (%)]	Day 720 (N=20) [n (%)]	Day 1080 (N=22) [n (%)]	Day 1440 (N=15) [n (%)]	Day 1800 (N=5) [n (%)]
Presence of Photophobia							
No	12 (26.7)	24 (64.9)	18 (52.9)	14 (70.0)	17 (77.3)	13 (86.7)	5 (100.0)
Yes	33 (73.3)	12 (32.4)	10 (29.4)	6 (30.0)	5 (22.7)	2 (13.3)	—
Missing	—	1 (2.7)	6 (17.6)	—	—	—	—
Photophobia Severity							
None	12 (26.7)	24 (64.9)	18 (52.9)	14 (70.0)	17 (77.3)	13 (86.7)	5 (100.0)
Mild	19 (42.2)	11 (29.7)	6 (17.6)	5 (25.0)	4 (18.2)	2 (13.3)	—
Moderate	10 (22.2)	1 (2.7)	3 (8.8)	1 (5.0)	1 (4.5)	—	—
Severe	4 (8.9)	—	1 (2.9)	—	—	—	—
Missing	—	1 (2.7)	6 (17.6)	—	—	—	—
Presence of Burning							
No	21 (46.7)	28 (75.7)	24 (70.6)	19 (95.0)	17 (77.3)	13 (86.7)	4 (80.0)
Yes	24 (53.3)	8 (21.6)	4 (11.8)	1 (5.0)	5 (22.7)	2 (13.3)	1 (20.0)
Missing	—	1 (2.7)	6 (17.6)	—	—	—	—
Burning Severity							
None	21 (46.7)	28 (75.7)	24 (70.6)	19 (95.0)	17 (77.3)	13 (86.7)	4 (80.0)
Mild	17 (37.8)	7 (18.9)	3 (8.8)	1 (5.0)	4 (18.2)	2 (13.3)	1 (20.0)
Moderate	7 (15.6)	1 (2.7)	1 (2.9)	—	1 (4.5)	—	—
Severe	—	—	—	—	—	—	—
Missing	—	1 (2.7)	6 (17.6)	—	—	—	—
Presence of Pain							
No	27 (60.0)	30 (81.1)	27 (79.4)	18 (90.0)	17 (77.3)	13 (86.7)	2 (40.0)
Yes	18 (40.0)	6 (16.2)	1 (2.9)	2 (10.0)	5 (22.7)	2 (13.3)	3 (60.0)
Missing	—	1 (2.7)	6 (17.6)	—	—	—	—
Presence of at least one Ocular Symptoms							

No	6 (13.3)	19 (51.4)	16 (47.1)	13 (65.0)	10 (45.5)	9 (60.0)	1 (20.0)
Yes	39 (86.7)	17 (45.9)	12 (35.3)	7 (35.0)	12 (54.5)	6 (40.0)	4 (80.0)
Missing	—	1 (2.7)	6 (17.6)	—	—	—	—

Notes: 1) Percentages are based on number of subjects per group. 2) The last non-missing value before the first ACLSCT is considered the Baseline value. *Source: CSR Table 23*

Summary of Pain Score by Visit (Adult Safety Population)

	Baseline (N=45)	Day 360 (N=34)	Day 720 (N=20)	Day 1080 (N=22)	Day 1440 (N=15)	Day 1800 (N=5)
Pain Score						
n	45	28	20	22	15	5
Mean (SD)	1.2 (2.0)	0.2 (1.1)	0.4 (1.1)	0.3 (0.6)	0.2 (0.6)	0.6 (0.5)
95% CI	0.6 – 1.8	-0.2 – 0.7	-0.2 – 0.9	0.0 – 0.5	-0.1 – 0.5	-0.1 – 1.3
Missing	0	6	0	0	0	0
Change from Baseline of Pain Score						
n		28	20	22	15	5
Mean (SD)		-0.9 (1.8)	-0.9 (1.9)	-0.2 (1.3)	0.1 (0.3)	-1.8 (2.5)
95% CI		-1.6 – -0.2	-1.8 – -0.0	-0.8 – 0.3	-0.1 – 0.2	-4.9 – 1.3
Missing		6	0	0	0	0

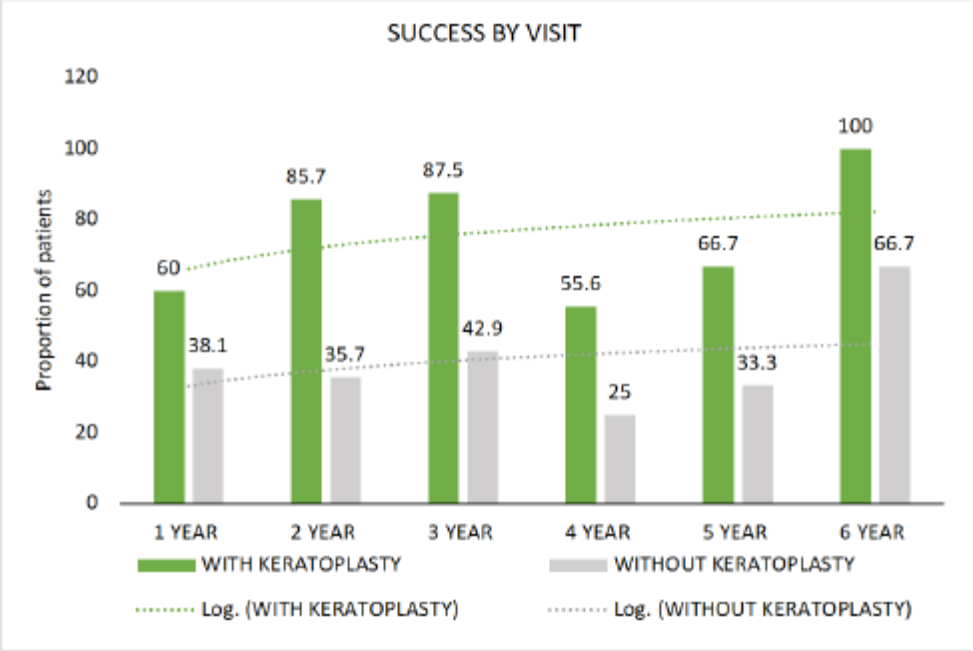
Source: CSR Table 28

Summary of Patients Stromal Scarring (Adult Safety Population)

Visit	Statistic	Stromal Scarring (N=34)	No Stromal Scarring (N=11)	Overall (N=45)
Day 360	Number of non-missing observations	21	7	28
	n (%)	15 (71.4)	6 (85.7)	21 (75.0)
	95% CI	0.48-0.89	0.42-1.00	0.55-0.89
Day 720	Number of non-missing observations	14	3	17
	n (%)	11 (78.6)	2 (66.7)	13 (76.5)
	95% CI	0.49-0.95	0.09-0.99	0.50-0.93
Day 1080	Number of non-missing observations	18	3	21
	n (%)	13 (72.2)	2 (66.7)	15 (71.4)
	95% CI	0.47-0.90	0.09-0.99	0.48-0.89
Day 1440	Number of non-missing observations	13	2	15
	n (%)	9 (69.2)	2 (100.0)	11 (73.3)
	95% CI	0.39-0.91	0.16-1.00	0.45-0.92
Day 1800	Number of non-missing observations	2	3	5
	n (%)	2 (100.0)	3 (100.0)	5 (100.0)
	95% CI	0.16-1.00	0.29-1.00	0.48-1.00

Source: CSR Table 33

Success of Transplantation with/without keratoplasty (Adult Safety Population)



Notes: 1) Figure is not a part of planned statistical analysis but is derived by HOLOSTEM from data available in Statistical Output. 2) Patients with missing data are excluded from denominator.

Source: CSR Figure 8

Main Safety Results

Summary of TEAEs of Follow-Up Study (Safety Population)

	Before Keratoplasty or in patients not candidate (N=47) [n (%) e]	After Keratoplasty (N=18) [n (%) e]	Overall (N=47) [n (%) e]
Number of TEAEs	11 (23.4) 18	13 (72.2) 43	20 (42.6) 61
Number of Serious TEAEs	1 (2.1) 1	1 (5.6) 1	2 (4.3) 2
Number of TRAEs	1 (2.1) 1	—	1 (2.1) 1
Number of Serious TRAEs	—	—	—
Number of TEAEs Leading to Study withdrawal	1 (2.1) 1	—	1 (2.1) 1
Number of TEAEs with Fatal Outcome	1 (2.1) 1	—	1 (2.1) 1
Number of Treatment Emergent AESIs	1 (2.1) 1	2 (11.1) 2	3 (6.4) 3
Related AESIs	—	—	—
Unrelated AESIs	1 (2.1) 1	2 (11.1) 2	3 (6.4) 3
Number of Patients withTEAEs by Worst Severity			
Mild	5 (10.6)	2 (11.1)	4 (8.5)
Moderate	4 (8.5)	8 (44.4)	12 (25.5)
Severe	2 (4.3)	3 (16.7)	4 (8.5)

Notes: 1) Percentages are based on number of patients per subgroup. 2) Overall is based on total patients in Safety Population. 3) If a patient has multiple events of the same severity, relationship, or outcome, he/she is counted only once in that severity, relationship or outcome. *Source: CSR Table 46*

TEAEs by System Organ Class and Preferred Term of Follow-Up Study (Safety Population)

System Organ Class	Preferred Term	Before Keratoplasty or in patients not candidate (N=47) [n (%) e]	After Keratoplasty (N=18) [n (%) e]	Overall (N=47) [n (%) e]
Any Treatment Emergent Adverse Events	---	11 (23.4) 18	13 (72.2) 43	20 (42.6) 61
Eye disorders	Total	8 (17.0) 11	11 (61.1) 22	16 (34.0) 33
	Blepharitis	1 (2.1) 1	2 (11.1) 2	3 (6.4) 3
	Corneal epithelium defect	1 (2.1) 1	2 (11.1) 2	3 (6.4) 3
	Ocular hypertension	---	3 (16.7) 5	3 (6.4) 5
Infections and infestations	Total	3 (6.4) 3	5 (27.8) 8	8 (17.0) 11
	Corona virus infection	2 (4.3) 2	1 (5.6) 1	3 (6.4) 3
Injury, poisoning and procedural complications	Total	---	3 (16.7) 6	3 (6.4) 6
	Suture related complication	---	3 (16.7) 3	3 (6.4) 3

Source: CSR Table 47

TRAEs possibly related to the Holoclar product by SOC and PT (Safety Population)

System Organ Class	**Preferred Term**	**First ACLSCT (N=69) [n(%)e]**	**Second ACLSCT (N=6) [n(%)e]**	**Overall (N=76) [n(%)e]**
Any TRAEs possibly related to Holoclar product (engineered living tissue)		5 (7.2) 6	—	5 (6.6) 6
Eye disorders	Total	5 (7.2) 6	—	5 (6.6) 6
	Corneal epithelium defect	3 (4.3) 3	—	3 (3.9) 3
	Ulcerative keratitis	2 (2.9) 2	—	2 (2.6) 2
	Corneal thinning	1 (1.4) 1	—	1 (1.3) 1

Source: CSR HOLOCORE Table 51

Assessor's evaluation of the presented results in the HOLOCORE-FU final CSR

Efficacy (secondary EPs):

Successful transplantation has been evaluated by two different methods: level of corneal neo-vascularisation based on the number of vessels invading the quadrants and the central cornea, and the Investigators judgement based on clinical eye observation at the slit lamp, which is a method of significant importance in the patient population without keratoplasty.

For the majority of patients (n=41/47; 87.2%) of the safety population the etiology of LSCD due to burn was chemical. Of the 45 patients in the adult safety population (N = 18, with keratoplasty; N = 27, without keratoplasty) 34 (N =15, with keratoplasty; N = 19, without keratoplasty), patients were assessed at Day 360 in the FU-study. The evaluation of the differences in outcome measurements in patients with/without keratoplasty was a focus of the HOLOCORE and HOLOCORE-FU studies, for which the MAH delivers comprehensive information in the HOLOCORE-FU Final CSR. The MAH's explanation for the difference in the evaluation of outcomes of patients without keratoplasty *vs* patients with keratoplasty is "the persistence of ghost non-active and stromal vessels when keratoplasty is not performed, which drives the classification to failure. The restoration of stroma by keratoplasty shows the absence of recurrence of those vessels, therefore, the real success rate by tissue function and wound healing capability without confounding elements from previous residual damage." The MAH's estimation provided is considered in accordance with scientific standard in the ophthalmology, based on available

literature in the on etiology and pathophysiology of ghost vessels, which are regressed vessels in the corneal stroma (e.g. *Powner et al., Investigative Ophthalmology & Visual Science, September 2016*).

Number of patients with keratoplasty after Holoclar (safety population)

		Adult N=45	Paediatric N=2	Overall N=47
Keratoplasty surgery	Yes	18 (40.0)	–––	18 (38.3)
	No	27 (60.0)	2 (100.0)	29 (61.7)
Onset study day of surgery	[0; 180)*	6 (13.3)	–––	6 (12.8)
	[180; 360)	5 (11.1)	–––	5 (10.6)
	[360; 540)	2 (4.4)	–––	2 (4.3)
	[540; 720)	2 (4.4)	–––	2 (4.3)
	>=720	2 (4.4)	–––	2 (4.3)

*1 patient had keratoplasty prior to day 0, so they are not included in the categories. Hence, the sum of the categories does not equal the number with Keratoplasty; *Source: CSR Table 11*

After adjustment of missing patients, the success of transplantation in the adult safety population at day 360 was calculated as 60.7% (n/N = 17/28) by corneal involvement/corneal neo-revascularisation (anatomical restoration) and 83.3% (n/N = 25/30) by overall investigator's judgement. Subsequently, 10/15 (66.7%), 8/21 (38.1%), 8/15 (53.3%) and 4/5 (80.0%) patients had success of transplantation at Day 720, 1080, 1440, and 1800, respectively. According to overall investigator's judgement, 16/20 (80.0%), 19/22 (86.4%), 12/15 (80.0%), and 5/5 (100%) patients had success of transplantation at Day 720, 1080, 1440, and 1800.

Taking specific subpopulations, the overall success rate at Day 360 was significantly higher in patients with the etiology of alkali burn, in patients with no previous surgical procedure and in patients, whose biopsy collection area was 11-12-1-5-6-7, respectively. For example, the transplantation success rate in the population, who underwent more than one surgery before Holoclar, was 28% only in the HOLOCORE main study. These 3 subpopulations, however, represented the minority of the trial participants; therefore, meaningful conclusions as regards potential limitation of the therapeutic indication and/or number of previous eye surgery are not possible.

With regard to the EP *neo-vascularisation and central cornea involvement after Day 360* in the adult safety population (see CSR Table 19), in the group with and without keratoplasty, n=3 (16.7%) had 3 corneal quadrants and n=15 (83.3%) 4 corneal quadrants, and n=8 (29.6%) and n=18 (66.7%) 4 corneal quadrants involved at baseline. Figures are available up to Day 1800 (Day 1440: n=15 evaluable patients; Day 1800: n=5 evaluable patients); they suggest that the results at least up to Day 1440 seem relatively stable compared to Day 360. Although the figures are very small, the number of corneal quadrants at Day 1800 was 0 in n=3/5 patients (60.0%), and n=5/5 patients (100%) had no central cornea involvement.

The proportion of patients with *no epithelial defects at Day 360* was 73.5% (n/N = 25/34) in comparison to 60.0% (n/N = 27/45) at baseline. The proportion of patients with or without keratoplasty with no epithelial defects was 93.3% (n/N = 14/15) and 57.9% (n/N = 11/19), respectively.

Regarding the EP *degree of re-epithelialisation,* 25/34 patients (73.5%) had no defects on Day 360. The figures at Days 1440 and 1800 are comparable: 12/15 (80%) and 5/5 (100%) respectively, however, as stated already, the number of evaluable subjects significantly reduces over the time.

The proportion of patients with *no clinical symptoms of photophobia, burning and pain at Day 360* seems increased to 52.9% (n/N = 18/34), 70.6% (n/N = 24/34), and 79.4% (n/N = 27/34), respectively, from 26.7% (n/N = 12/45), 46.7% (n/N = 21/45), and 60.0% (n/N = 27/45) at baseline. E. g., among patients who had moderate burning symptom at baseline, 5/34 (14.7%), 3/20 (15.0%), 0/22 (0.0%), 1/15 (6.7%), and 2/5 (40.0%) patients presented no symptom at Day 360, Day 720, Day 1080, Day 1440 and Day 1800, respectively.

Presence of *conjunctival inflammation* was reported in 31/45 (68.9%) patients at baseline. The incidence at subsequent annual visits was as follows: 14/34 (41.2%) patients at Day 360, 8/20 (40.0%) at Day 720, 13/22 (59.1%) patients at Day 1080, 8/15 (53.3%) patients at Day 1440 and 1/5 (20.0%) patients at Day 1800.

With regard to the EP *BCVA,* the following number of patients had BCVA improvement compared to baseline: 21/28 (75.0%), 13/17 (76.5%), 15/21 (71.4%), 11/15 (73.3%), and 5/5 (100.0%) at Day 360, Day 720, Day 1080, Day 1440, and Day 1800, respectively. The figures for BCVA improvement compared to baseline by keratoplasty were even higher: 14/14 (100.0%), 11/12 (91.7%), and 6/7 (85.7%), at KDay 360, KDay 720, and KDay 1080, respectively (see CSR Table 34).

Regarding the efficacy endpoint *corneal opacity and conjunctival sensitivity,* the number of patients with corneal opacity at baseline was 45/45 (100.0%), and at each subsequent annual visit: 19/34 (55.9%) at Day 360; 9/20 (45.0%) at Day 720; 17/22 (77.3%) at Day 1080; 12/15 (80.0%) at Day 1440 and 2/5 (40.0%) at Day 1800. Similar stability in improvement over the time is described for conjunctival sensitivity.

Safety (primary EP):

There was 1 adverse event of corneal opacity related to Holoclar reported in the HOLOCORE-FU study. Taking the pooled analysis of the HOLOCORE and HOLOCORE-FU studies a total of 175 AEs were reported in 33 (70.2%) patients. N=22 AEs have been reported in the pre-first transplantation phase in 18 (38.3%) patients. In Year 1, 81 AEs were reported in 28/47 (59.6%) patients, in Year 4 17 AEs in 8/45 (17.8%) and in Year 6 1 AE in 1/25 (4.0%) patient. A total of 2 serious TEAEs has been reported in 2 patients (4.3%): 1 TEAE was associated to injury and procedural complications, and 1 TEAE related to a mediastinal neoplasm with fatal outcome, not considered related to Holoclar or study procedures (see CSR Table 48 and Narratives). The narratives provided for the patient with the SAE *Suture Rupture Left Eye,* which required a surgery for re-suturing on 20 May 2020 indicates, the event being not judged related to study treatment, biopsy or ACLSCT by the investigator. The resolve of the event is reported on 06Aug2020. The most common cause of TRAEs was the ACLSCT surgical procedure; 41 TRAEs reported in 23.4% (n/N = 11/47) of the patients. Only 1 TRAE (corneal opacity) in 1/47 (2.1%) patient was deemed related to the Holoclar treatment. A total of 4 AESIs were reported in 8.5% (n/N = 4/47) of the patients and none were deemed to be related to the Holoclar treatment; the TEAESI *Blepharitis was* reported in 3/47 patients (6.4%). Overall, 6 TRAEs in 5 (6.6%) patients were judged to be related to Holoclar treatment. The reported TRAEs thought to be related to Holoclar were Eye Disorders: corneal epithelium defect (3 TRAEs in 3 [3.9%] patients), ulcerative keratitis (2 TRAEs in 2 [2.6%] patients) and corneal thinning (1 TRAE in 1 [1.3%] patient) that should be all considered as lack of efficacy. No other adverse effects caused by Holoclar were reported during the HOLOCORE study (*source: CSR Table 4 and Appendix 4*).

Assessor evaluation and conclusion of the MAH's response on the clinical MO 1a)-d)

The clinical assessors emphasized in previous reports on Holoclar renewal procedures major uncertainties with regard to the HOLOCORE main study, which the MAH also recognized. These uncertainties are related i. a. to the high number of recruiting centers (18) in 8 countries and assumed differences in clinical standards, evident by the number of major protocol variations (document Applicant Answers to the RfSI: "…the large majority of the centres (15/18; 83%) was involved…for the first time…learning curve…"), and the impacts of the COVID19pandemic on quality and consistency of the study results (document Applicant Answers to the RfSI: "Interruption of activities at Investigational sites during the COVID19pandemic …prevented to collect the proper images for the primary endpoint assessment…and decreased the number of evaluable subjects…"). The high number of adverse events are i. a. the result of a "learning curve of surgeons and treatment failures", a statement of the Applicant, which underlines the concerns of Authority described. This may explain the observation that the allover results on safety parameters during the studies HOLOCORE and HOLOCORE-FU indicate a clear decrease in the number of adverse events occurred over the time up to Day 360. The majority of TEAEs was attributed to the surgical procedure. With regard to the MO part b), the success rate of 66.7% had been calculated by the previous MAH (CHIESI), based on results of the clinical study HLSTM01. The HOLOSTEM company inherited the HOLOCORE study in 2020 and became aware that the protocol recommendations for the evaluation of the primary endpoint were not implemented, thus leading to "significant discrepancies" and "inconsistent judgment of successes and failures". Therefore, HOLOSTEM introduced mitigation measures, in order to ensure independent and evidence based clinical assessment of efficacy endpoints. The primary endpoint in the HOLOCORE Study was calculated based on 2D photos, evaluated by external independent assessors with quadrant methodology according to Global Consensus Criteria and focus on corneal neo-revascularisation.

The major objection regarding the reported rate on the primary endpoint successful transplantation of 33.8% in the mITTb population of the HOLOCORE study can be considered resolved based on the long-term results submitted in the HOLOCORE-FU final CSR for patients in the HOLOCORE main study, and interim results submitted for the HOLOSIGHT PASS for patients treated in the HLSTM01 study (see below). The figures on long-term efficacy and safety of once or twice treatment with Holoclar provided for the Study HOLOCORE-FU, the follow-up of subjects treated in the HOLOCORE main study comprise a mean follow-up period for patients of 3.3 years. The focus of this study was observation of the disease and to gather information on the efficacy and safety of Holoclar in subjects, candidate for keratoplasty, as the retrospective studies HLSTM01, HLSTM02 and HLSTM04 did not provide this information. Overall, the outcome of successful transplantation (irrespective of keratoplasty), i. a. judged by assessors in accordance with global standard criteria (based on 2-D pictures and assessed by CNV methods in quadrants), and the improvement of clinical important parameters such as BCVA and clinical symptoms reported to the HOLOCORE main study after treatment with Holoclar continued during the HOLOCORE study. Though the number of evaluable subjects decreased considerably from n=14 at Day 360 to n=2 at Day 1800, the long-term outcome of treatment with Holoclar in patients with LSCD appears to be comparable with e.g. patients, who received CLAu (*Eslani et al, Long-Term Outcomes of CLAU in Patients with LSCD, The Ocular Surface, 2019).* The potential major advantage of treatment with Holoclar may be the possibility of repeated use without necessity of accompanying immunosuppression, required in therapy methods with allografts. Ocular hypertension secondary to corticosteroid use is a known complication of allograft ocular stem cell transplantation.

The second follow-up study for patients treated with Holoclar is the HOLOSOGHT PAS study. The interim report was part of the data package of the original renewal, submitted on 26 July 2023, and the results

submitted for the efficacy endpoints in 56 participants, who completed the 1-year follow-up has been already assessed. In principle, the results at Year 1, calculated on non-missing data can be considered comparable with the data presented for the HOLOCORE-FU study with regard to the Ep *CNV (superficial and in central cornea*). However, an estimation of a shift of *severe epithelial defects* from baseline to Year 1 is not possible due to missing data at Year 1. According to the MAH the study is ongoing, and missing data (see Interim Report Table 33) are due to backlog in CRF completion sites.

There might be a potential impact on the endpoint evaluation in the HOLOCORE main study, however, difficult to judge, considering the following: The first subject screened in the HOLOCORE Study was on the 28 Oct 2015, and the last subject completed on 11 Mar 2022. When HOLOSTEM took the study over in 2020, patient enrolment, treatment and data acquisition for endpoints had been already carried out in accordance with trial protocol versions prior to the changes of the endpoint evaluation mentioned, even if the re-evaluation of patients in the study according to Global Consensus Criteria was done during the study, starting late 2020. In addition, considering certain subpopulations in the HOLOCORE main study such as patients with/without prior surgery and/or patients with LSCD due to chemical/alkali burn, there seem to be major differences in the efficacy outcome, which, at least for the patients with LSCD due to alkali burn is currently not understandable. To what extend those clinically relevant differences in subpopulations may be a rationale to request the conduction of a randomized clinical trial, is controversial based on the long-term efficacy and safety results presented up to Day 360 for a limited number of patients (n=14), however. Regarding the MAH's request on conversion of CMA to full approval, the critical point may be that there was no controlled clinical study performed with Holoclar up to now. The pivotal study for the original CMA was Study HLSTM01, an observational study including 106 LSCD patients (n=113 transplantations) from 2 centers in Italy, conducted between 1998 and 2007; the supportive study was HLSTM02, an observational study, including 29 LSCD patients (29 transplantations) from 7 Italian centers (EPAR, EMA/25273/2015).

From a regulatory perspective, a controlled clinical trial for treatment of a representative number of patients with LSCD due to ocular burn, may be preferred in order to eliminate existing uncertainties. However, there seems no reasonable comparative trial possible, evaluating the therapy concept with Holoclar and other similar ophthalmological surgery with exception maybe of conjunctival limbal autograft (CLAu). Furthermore, according to the literature data basis, long-term efficacy and safety data of Holoclar provided seem to be comparable with those reported in patients treated with CLAu.

Conclusion

The MO is resolved.

10.2. Other concerns

Clinical aspects

Question 1 and Question 2

Assessment of the MAH's response

The final CSR of HOLOCORE-FU study (Chiesi ID CCD-GPLSCD01-03-FU) is part of the data package submitted for the RfSI nad considered acceptable. For details, please see the assessment of the responses on the clinical MO.

Conclusion

The issues (clinical OC1 and OC2) are resolved.

Question 3

The MAH is requested to submit a thorough presentation of post-marketing data for efficacy and safety. In addition, the MAH should provide an evaluation of the impact of the product shortage since December 2022 in the EU and UK on the outstanding post-authorisation measures.

Summary of the MAH's response

Beyond patients who received the marketed product and participating to the HOLOSIGHT PAS study, additional 9 subjects were treated with Holoclar in EU and UK (*Ali E. Ghareeb. Majlinda Lako, Francisco C. Figueiredo,2020*). No pharmacovigilance notification nor product complaints, neither request for reimbursement (where applicable) were received from the treating centres so far.

Concerning the Holoclar shortage since December 2022, no impact occurred on the post-authorisation measures, specifically on the HOLOCORE, HOLOCORE FOLLOW-UP and HOLOSIGHT studies.

• HOLOCORE: the clinical trial was already closed before, and no treatment was outstanding at the □me of product shortage.

• HOLOCORE FOLLOW-UP: no further treatment was planned in the study.

• HOLOSIGHT (PASS): as described in the most recent Interim Report included in the ACO submitted on 28JUL2023, the enrolment is closed and treatment for all patients included were secured. The last treatment of the last patient was administered in MAY2023.

Assessment of the MAH's response

The answers provided are acceptable. The issue is resolved.

Question 4

The SmPC needs to be revised in accordance with the points outlined in the assessment report; figures presented for the key primary and secondary efficacy endpoints below SmPC section 5 have to be in alignment with the data reported in the final CSR of the HOLOCORE Study.

Summary of the MAH's response

Based on the discussion about the final data of the HOLOCORE study the Applicant revised the SmPC on the basis of the clarification presented in the present document.

The following sentence: "At the final visit, the 82% of patients who attended had no epithelial defects, 49.2% had normal limbal hyperaemia and 44.3% had normal corneal sensitivity" has been kept as these parameters provide evidence of the Holoclar efficacy in reconstructing the original corneal epithelium after conjunctival pannus removal.

Assessment of the MAH's response

The revised SmPC is considered acceptable. The issue is resolved.

Rapporteur overall conclusion after review of data provided on the RfSI:

The final results presented on long-term efficacy and safety data for patients treated with Holoclar might be considered not fully satisfactory in view of the number of patients and the conditions of the clinical trials. However, the data suggest that treatment with Holoclar seems to be safe and may provide a long-term benefit for adult patients with LSCD due to ocular burn when administered in professional clinical centres. Therefore, the Rapporteur supports the Applicant's proposal to remove the following SOB and PAM, and to convert the conditional marketing authorisation to full marketing authorisation:

SOB:

Description	Due date
Multinational, multicentre, prospective, open-label, uncontrolled interventional study (HLSTM03 hereinafter referred as HOLOCORE or CCD-GPLSCD01-03) to assess the efficacy and safety of autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns	Last Patient Last Visit completed on 11 March 2022 Final CSR completed March 2023

PAM:

Description	Due date
Study HLSTM03FU Long-term safety and efficacy follow-up after autologous cultivated limbal stem cells grafting for restoration of corneal epithelium in patients with limbal stem cell deficiency due to ocular burns. HOLOCORE-FU	Study is ongoing, date for submission of interim of final reports March 2024

11. Attachment

1. Product Information as submitted with the Responses to the RSI (eCTD 0096)